Expression of Muscle Fat-Related Genes in Common Carp (*Cyprinus carpio*)

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Abstract

The fat content of muscle tissue is considered an important phenotypic trait in fish and thus affects the nutritional value and commercial quality of the fish. Therefore, the current research aims to identify the genes responsible and associated with the fat content of common carp muscles using reverse transcription quantitative PCR (RT-qPCR) for the ***fjx1***, ***tanc2***, and ***ankrd10a***, along with an internal control gene ***18s rRNA*** gene. Furthermore, it will also evaluate the fat content of muscles using samples from the dorsal abdominal muscle regions to compare with the gene expression of the studied genes. The current study showed that the expression of the three genes (***fjx1***, ***tanc2*** and ***ankrd10a***) are likely to be associated with the fat content of abdominal muscles in fish (MFdo), which play an important role in fat metabolism. RNA concentration extracted from the samples ranged from 74-124 ng/µL, with a purity of 1.65-1.96 for all the samples. The ***18s rRNA*** gene was used as a reference or control gene. Threshold cycle (CT) values were used to measure gene expression. The results showed the highest gene expression for the ***Tanc2*** gene, reaching 14.6, and the lowest value for the ***ankrd10a*** gene, reaching 6.6 in fish with high fat content compared to fish with low fat content. This research may provide insights into the complex genetic basis of fat metabolism and accumulation in fish muscles, these results will eventually help develop breeding strategies for improving flesh quality in common carp.

Keywords: ***ankrd10a***, Common carp, fat content, ***fjx1***, gene expression, muscle t***anc2***.

Introduction

Common carp (*Cyprinus carpio*) is considered one of the most widely cultivated fish species in the world, with a rich cultivation history spanning thousands of years (Wu *et al*., 2024). The global production of this species exceeded 4.16 million tons in 2012 (Zheng *et al*., 2016). This species plays a pivotal role not only due to its substantial economic value

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but also because of its remarkable adaptability and nutritional profile (Jeney and Jian, 2009). It is the model species for many scientific studies, including toxicology, ecology, physiology, nutrition, and evolution (Osamah *et al.*, 2021).

Considering the escalating demand for health-conscious food options, recent scientific endeavours have increasingly focused on improving the nutritional value of the common carp (Infante-Villamil *et al.*, 2021). Fish meat traits such as fat content, fatty acids, meat colour, and texture are important indicators of quality, flavour, and nutritional value (Johansson *et al.*, 2000). The muscle tissue of the common carp is highly nutritious and enriched with both essential and non-essential amino acids (Jeney and Jian, 2009). The fat content in common carp muscle can vary widely depending on diet and genetics, ranging from low levels (around 1% in white dorsal muscle) to high levels (up to 15% or more in the abdominal wall). Despite this variation, carp muscle is a rich source of healthy unsaturated fatty acids, particularly omega-3 fatty acids, including Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Wang *et al.*, 2024). These fatty acids are particularly beneficial for heart health, can reduce the risk of cardiovascular diseases (Zhang *et al.,* 2019) and play a significant role in brain health and vision development (Ghasemi Fard *et al.*, 2019). The genetic enhancements have proven effective for augmenting phenotypic traits in the common carp. Through selective breeding, researchers have developed carp varieties with faster growth rates and greater adaptability (Hu *et al.*, 2018). Nevertheless, the specific molecular response mechanisms regulating the nutritional deposition of carp remain unclear. Investigating the key genes and signaling pathways regulating muscle fat content in common carp is essential for improving this economically important trait. Such studies will enhance our understanding of nutrient deposition mechanisms and support the genetic improvement of common carp and other aquacultured species, contributing to the sustainable development of the aquaculture industry.

Materials and Methods

The current study was conducted in the Tigris River southern of Baghdad-Iraq for the period from September 2021 until March 2022 The site stretches approximately 4 km north and 4 km south of the point where the Diyala River meets the Tigris River (Fig. 1).

Samples collection

A total of 130 specimen of Common Carp (*Cyprinus carpio*) (weight 1- 1.5 kg , length 39-42 cm), were collected from Tigris River in the early morning using gill nets, then specimens were placed in ice box and transported directly to the Department of Animal & Fish Resource/ Agriculture Research Center/ Scientific Research Commissionin Al-Zaafarania City for analysis. Fish samples were caught with a special net from different areas of the Tigris River in Baghdad, consisting of 150 fish weighing between 1 and 1.5 kg. The fish were anaesthetised using MS222, and the growth-related traits, including length, weight, and age, were measured. Blood samples were then collected from the caudal vein for RNA extraction and stored at -20°C until use.

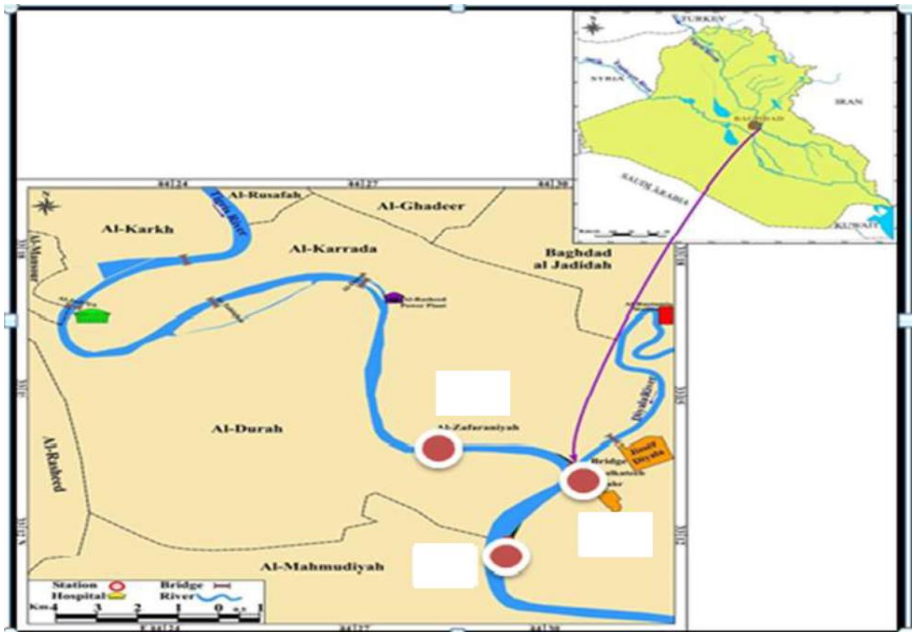

Figure 1: Study area showing fish samples collection locations at Tigris River.

Fat Content

Abdominal fat tissues deposited in the abdominal cavity and surrounding the viscera (AbFW) were weighed. The eviscerated fish (EW) were then weighed, and the percentage of AbFW to EW was calculated, which was considered Abfp. Samples from dorsal and abdominal muscles (50 g from each fish) were taken and stored at -20°C to extract total fats from dorsal muscles (MFdo) and abdominal muscles (MFab) for each fish to measure the fat content in fish muscles and compare it with gene expression results.

Fat content extraction and measurement

Fat extraction from fish muscle tissue was carried out according to Folch *et al.* (1957). The tissue samples were homogenised in a mixture of chloroform and methanol 2:1 (v/v) as an extraction solvent, containing 0.01% butylated hydroxytoluene as an antioxidant (1 g of tissue in 20 ml of solvent mixture). This step helps to break down the tissue and release the lipids into the solvent. Water was then added to the homogenate to create a biphasic system. This resulted in two distinct layers: the lower chloroform phase contained the lipids, and the upper methanol/water phase contained non-lipid contaminants. The mixture is then centrifuged to facilitate the separation of the two phases, and the lipid-containing chloroform phase is carefully collected.

Then, 1% sulfuric acid and 90% methanol were added at 70°C for 3 hours to prepare the fatty acid methyl esters (FAMEs). FAMEs were then extracted using heptane, and the samples were analysed using a gas chromatograph GC-2010 (Shimadzu, Japan) with an automatic sampler and hydrogen flame ionisation detector, and capillary columns (Varian USA) of 30 m x 0.25 mm x 0.25 µm were used. The carrier gas was nitrogen (N2), while the combustion support gases were air and hydrogen (H2). The injector and detector

temperatures were set to 250°Cm while the column temperature was initially set at 120°C for 3 minutes, followed by an increase at a rate of 10°C/min until reaching 190°C. Then, the temperature increased at a rate of 2°C/min until reaching 220°C for 15 minutes. The results were compared with commercial standard fats (Sigma, USA) and quantified using the CLASS-GC10 GC workstation (Shimadzu).

Gene Expression of Studied Genes Using Real-Time Quantitative PCR (qPCR)

Blood samples (200-500 µL) were collected from the caudal vein of each fish for RNA extraction of the ***fjx1***, ***tanc2***, and ***ankrd10a*** genes. Gene expression was measured using by Reverse Transcriptase-quantitative Polymerase Chain Reaction (RT-qPCR) using the gene -specific primers for these genes as shown in Table 1.

Table 1: The Primers and Product Size used for PCR (Zheng *et al*,2016).

Gene	Primer sequence (5'-3')	Production length(bp)
***tanc*[2]**	F: TTTTCCCCGAAGCTGACCAC R: GGCGTCACACACTACCGAAA	284
***fjx*[1]**	F: AGGAAGTGGAGAGAGCAGGT R: TTCTCCCAGCACTTGATCCG	147
Ankrd10a	F: TCCAAAAACTGCTGCCACAC R: ACCTCGACAGATTGCCAGTG	100
***18S*[2]**	F:ACGATCAGATACCGTCGTAGTTCC R: CTGTCAATCCTTTCCGTGTCCG	244

[1] The annealing temperature represents the optimal temperature during quantitative PCR.
[2] **mRNA** levels of 18s were assayed for normalisation during quantitative PCR.

The Statistical Analysis System- SAS-2012 program was used in current studyparameters. Duncan's (1955) multiple range test (ANOVA) was used to compare between means in this study. The methods Ct, ΔCt, 2-ΔCt and 2-Δ ΔCt were used to compare the levels of genes.

Results and Discussion:

Muscle fat is an important feature that determine the quality, texture, and flavour of fish meat (Yang *et al*., 2013). The present study indicated that the three genes (***fjx1***, ***tanc2*** and ***ankrd10a***), together with the internal control gene 18S, are potentially associated with the Fat content of Abdominal Muscles (MFdo) in fish. This association was inferred from the gene expression patterns observed in fish with high and low dorsal muscle fat content (Table 2). These genes also appear to play significant roles in lipid metabolism. RNA concentrations of the samples ranged from 74 to 124 ng/µL, with purities between 1.65 and 1.96. The 18S gene served as the reference or control gene.

This technique relied on the threshold cycle (CT) value, where high CT values indicate low gene expression and vice versa (Schmittgen and Livak, 2008). The reference gene used in the current study was 18s, which is an internal control gene in molecular studies

and should have stable expression in different cells and tissues under various conditions (Zheng *et al*., 2016).

The present study showed that ***tanc2*** was highly expressed in fish with elevated fat content (Table 3, Fig. 2), consistent with its association with myristic acid synthesis in muscle fat tissues (Ren *et al*., 2020) and with abdominal and overall muscle fat deposition (Ishii *et al*., 2013). One possible explanation is that ***tanc2*** may regulate lipid metabolism-related pathways, enhancing fatty acid synthesis or storage in muscle tissues. Its association with abdominal fat in poultry and cattle (Zhang *et al*., 2012; Yang *et al*., 2013) suggests that ***tanc2*** plays a conserved role in energy storage, potentially by influencing adipocyte differentiation or lipid droplet formation.

ankrd10a also showed significant expression in high-fat fish, supporting its role as a candidate gene for abdominal fat accumulation (Ankra-Badu *et al*., 2010). This gene may affect fat deposition through regulation of signaling pathways that control adipogenesis or fat cell proliferation, providing a mechanistic explanation for its effect on muscle fat content. The ***fjx1*** gene, although not directly involved in fat metabolism, was expressed in relation to body weight and lipid traits. It is involved in cellular signaling and regulates LDL receptor expression, which may indirectly influence fat deposition by affecting lipid transport and cholesterol homeostasis (May *et al*., 2007; Yao *et al*., 2010). Differences in ***fjx1*** expression could also reflect variation in metabolic efficiency or energy utilization, contributing to differences in fat content between individuals.

Other potential factors influencing gene expression include genetic polymorphisms, epigenetic modifications, and environmental factors such as diet, temperature, and growth rate, which are known to affect fat metabolism in fish. Collectively, these findings suggest that ***tanc2*** and ***ankrd10a*** may directly regulate lipid accumulation, while ***fjx1*** influences fat indirectly through metabolic and signaling pathways. These genes could serve as molecular markers for selective breeding programs aimed at enhancing fat-related production traits in aquaculture species.

Table 2: Fat content in the studied fish.

Traits (Unit)	N	Min	Max	Mean	SD	CV
Mfdo(%)	114	1.8	11.1	6.5	1.63	35.5
Mfab(%)	114	2.4	17.3	9.9	2.43	36.4
AbFW(g)	101	2.3	21.2	11.8	3.45	49.1
AbFP(%)	98	0.57	5.4	3.0	0.67	37.3

Mfdo: Dorsal muscle fat content, **Mfab**: Abdominal muscle fat content, **AbFW**: Abdominal fat weight, **AbFP**: Percentage of abdominal fat to eviscerated weight, **N**: Number of fish.

Table 3: $2^{-\Delta\Delta CT}$ values for the studied genes in fish with low and high fat content.

Gene	Mean CT values for genes with High dorsal muscle fat content	Mean CT values for genes with low dorsal muscle fat content
Tanc2	14.6	11.4
fjx1	9.6	23.6
ankrd10a	6.6	14.8
18s	25.6	25.9

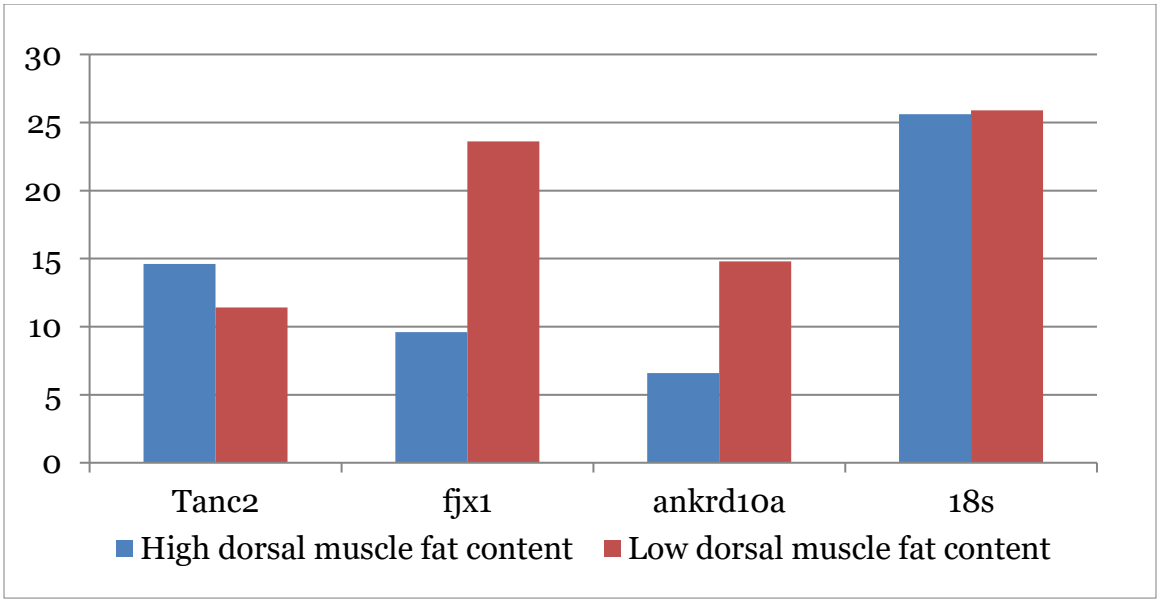

Figure 2: Gene expression of the studied genes.

Conclusions:

This is what our study concluded that the genes ***ankrd10a***, ***tanc2***, and ***fjx1*** play a significant role in regulating muscle fat content in common carp. By uncovering these genetic associations, the findings provide a strong foundation for selective breeding and genetic improvement strategies aimed at enhancing growth performance, meat quality, and nutritional value. Moreover, this work contributes to sustainable aquaculture by offering molecular targets for optimizing production efficiency and supporting the development of high-quality, genetically improved carp strains.

Recommendations:

Conduct further studies to identify the genes responsible for muscle growth in common carp.

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تعبير الجينات المرتبطة بدهون العضلات في أسماك الكارب الشائع (*Cyprinus carpio*)

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المستخلص

يعد المحتوى الدهني للعضلات من الصفات المظهرية المهمة في الأسماك، لما له من تأثير مباشر في قيمتها الغذائية وجودتها التجارية. وبناء على ذلك، يهدف البحث الحالي إلى تحديد الجينات المرتبطة بمحتوى الدهون في عضلات أسماك الكارب الشائع، وذلك باستخدام تقنية تفاعل البلمرة العكسي-Real Time Polymorphism Chain Reaction (RT-PCR) لدراسة مستوى التعبير للجينات ***fjx1*** , ***ankrd10a*** , ***tanc2*** بالاضافة الى جين السيطرة الداخلي (***18s rRNA***) لضبط مستويات التعبير. كما اشتملت الدراسة على تقدير محتوى الدهون في العضلات اعتماداً على عينات مأخوذة من منطقتي العضلات الظهرية والبطنية، وذلك بغرض مقارنتها مع مستويات التعبير للجينات المدروسة. أظهرت نتائج البحث أن التعبير للجينات الثلاثة (***ankrd10a*** , ***tanc2*** , ***fjx1***) يرتبط ارتباطاً محتملاً بالمحتوى الدهني لعضلات البطن في الأسماك(MFdo) ،والتي تلعب دورا مهما في استقلاب الدهون. تراوحت تراكيز الحمض النووي الريبي (RNA) المستخلص من العينات بين 74–124 نانوغرام/ميكرولتر، وبنقاوة ضمن المجال 1.65-1.96، مما يشير إلى ملاءمتها لإجراءات التحليل الجزيئي .اعتمد التقييم الكمي للتعبير على قيم دورة العتبة (Threshold cycle CT)، حيث اظهرت النتائج أعلى مستوى للتعبير في جين ***Tanc2*** بواقع 14.6، في حين سجّل جين ***ankrd10a*** أدنى مستوى تعبير بلغ 6.6 في الأسماك ذات المحتوى المرتفع من الدهون مقارنة بنظيراتها ذات المحتوى المنخفض. تشير نتائج البحث إلى إمكانية ارتباط الجينات المدروسة في تنظيم استقلاب الدهون وتراكمها في عضلات الأسماك، مما يوفر أساساً علمياً يمكن الاعتماد عليه في تطوير برامج الانتقاء الوراثي الهادفة إلى تحسين جودة لحوم أسماك الكارب الشائع.

الكلمات المفتاحية: التعبير الجيني، الكارب الشائع، المحتوى الدهني، العضلات، ***ankrd10a***, ***fjx1***, ***tanc2***.