Hindawi
Aquaculture Research
Volume 2024, Article ID 2715178, 13 pages
https://doi.org/10.1155/2024/2715178

Research Article

Exogenous Fatty Acids Remodel the Muscle Fatty Acids Composition of the GIFT Tilapia (*Oreochromis niloticus*)

Zhenye Liang, Zongzheng Jiang, Sen Wu, Yujia Zhai, Shuqi You, and Chang Xu

School of Marine Biology and Fisheries, Hainan University, Haikou 570228, China

Correspondence should be addressed to Chang Xu; cxu@hainanu.edu.cn

Received 29 November 2023; Accepted 27 January 2024; Published 14 March 2024

Academic Editor: Houguo Xu

To reduce the negative impact of fish oil substitutes on the fatty acid composition of farmed fish, this experiment utilized different types of oils as dietary lipid sources for GIFT tilapia. Tilapia oil (FO) was used as the control, along with corn oil (CO), linseed oil (LO), algae oil (AO), and a mixture of linseed oil and algae oil in various ratios of 1 : 2, 1 : 1, and 2 : 1 (LA12, LA11, and LA21) to reshape the fatty acid profile of GIFT tilapia (0.205 ± 0.005 g) in the muscle. The weight gain and specific growth rate of tilapia in the LO and control groups were significantly higher than in other treatments ($P < 0.05$). Meanwhile, despite the AO group exhibited the highest docosahexaenoic acid (DHA) content ($P < 0.05$), it also exhibited the highest levels of malondialdehyde content and superoxide dismutase activity ($P < 0.05$). The mRNA expression levels of Δ6/Δ5 fatty acyl desaturase 2 (Δ6/Δ5FADs2), Δ4 fatty acyl desaturase 2 (Δ4FADs2), acetyl-CoA carboxylase α (ACCα), and elongase of very long chain fatty acids 5 (ELOVL5) in the hepatopancreas of LO group were exhibited a significant upregulation compared to the control ($P < 0.05$). The synthesis of DHA and eicosapentaenoic acid (EPA) in the muscles of LA12, LA11, and LA21 groups increased as the proportion of DHA decreased in the diets. In conclusion, the edible value of fatty acids of tilapia muscle, especially n-3 long-chain polyunsaturated fatty acids, can be significantly improved by adjusting the oil source in the diet through lipid metabolism.

1. Introduction

Lipids, as a primary nutrient in aquafeed, play a crucial role in various physiological functions of aquatic animals. Lipids in feed are carriers of fat-soluble vitamins [1], which can be used as a source of energy and provide fatty acids, phospholipids, etc. to maintain the growth, reproduction, and health of fish [2, 3]. Fatty acids are important components of lipids, among them, polyunsaturated fatty acids (PUFAs) have important effects on aquatic animals. For example, n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) are structural components of cell membranes in animals [4]. They can also alter signaling pathways which can affect the immune response [5]. N-6 long-chain polyunsaturated fatty acids (n-6 LC-PUFAs) enhance the growth performance and antioxidant capacity of aquatic animals, and participate in regulating physiological activities such as inflammatory response and lipid metabolism [6–8].

Previous research found that in aquatic animals, depending on the species, different requirements for essential fatty acids (e.g. LC-PUFAs) in developmental stages [9]. In addition, teleost fish have different synthesis capabilities of LC-PUFAs. Fatty acyl desaturase 2 (FADs2) plays an important role in the desaturation activity of LC-PUFAs synthesis. Generally, Δ5 fatty acyl desaturase 2 (Δ5FADs2) is the key enzyme for the synthesis of LC-PUFAs using C_{18} PUFAs as a precursor. Δ6/Δ5 fatty acyl desaturase 2 (Δ6/Δ5FADs2) with both Δ5FADs2 and Δ6FADs2 desaturation activities have been found in species such as zebrafish (*Danio rerio*) and Nile tilapia (*Oreochromis niloticus*) [10, 11]. Several studies have found that except for freshwater fish (e.g. Nile tilapia), salmonids (e.g. Atlantic salmon and *Salmo salar*), and a few marine fish (e.g. rabbitfish and *Siganus canaliculatus*), marine teleost fish usually lack Δ5FADs2 and the Δ6/Δ5FADs2, and thus are unable or extremely limited to use C_{18} PUFAs as a precursor to synthesize LC-PUFAs [12–14]. However, freshwater fish, salmonids, and a few marine fish can biosynthesize LC-PUFAs, but the synthesis amount is lower than that of fish fed with fish oil [15–17].

Fish oil and vegetable oil play essential roles in providing n-3 and n-6 PUFAs in aquafeed. Partially replacing fish oil with vegetable oil is feasible when the PUFAs content in the feed adequately meets the growth needs of the animals. To address the increasing prices of fish meal and fish oil, the substitution of vegetable oil for fish oil is considered the most sustainable alternative and has grown to be a typical trend in aquafeed [18]. Several previous studies in Atlantic salmon, sea bass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*) have demonstrated that substituting 50%–60% of fish oil with soybean oil is unaffected growth [19, 20]. However, further research found that while the substitution of fish oil did not impact the growth of the fish, fish muscles exhibited significant changes in the n-3/n-6 PUFAs ratio, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels, which dropped by 30%–50% [21]. This could harm consumers' health and the nutritional content of fish products, which would further damage consumers' opinions of farmed fish [22].

The amount of n-3 PUFAs in fish muscles is reduced when a substantial proportion of vegetable oil is substituted for fish oil, which impacts physical health and diminishes nutrient deposition [23, 24]. Therefore, reshaping the fatty acid composition of breeding animals and enhancing the content of PUFAs in aquatic products is essential for the growth of the aquatic sector. Previous research has demonstrated that the ratio of n-3/n-6 PUFAs is significantly altered when vegetable oil is substituted for fish oil [25]. Nevertheless, the dietary optimum content of n-3/n-6 PUFAs varies across different species. Fish exhibit better growth performance when fed diet with an optimal balance of n-3/n-6 PUFAs ratios [26]. For example, Atlantic salmon showed a great immune response when given a diet rich in n-3/n-6 PUFAs ratios [27]. Compared with the dietary n-3/n-6 PUFAs ratios of 0.93 and 2.51, gilthead seabream (*Sparus aurata*) in the 3.82 n-3/n-6 PUFAs ratios treatment exhibited better lipid composition and metabolism [28]. On the contrary, a diet n-3/n-6 PUFAs ratio of 0.66 enhanced the growth of spotted seabass (*Lateolabrax maculatus*) by altering gut microbiota and lipid metabolism [29]. Although there is extensive research on fat in aquatic animal feeds, there is still a lack of research on the efficiency of converting exogenous lipids from diet into n-3/n-6 PUFAs in aquatic animals. This area of research is crucial for reshaping the fatty acid composition of aquatic animals.

The Nile tilapia is a widely cultivated species of freshwater fish in over 120 countries worldwide. The GIFT (Genetic Improvement of Farmed Tilapia) strain was a genetically modified tilapia strain, created by crossing Nile tilapia from eight different regions [30]. The popularity of tilapia can be attributed to its muscle quality and nutritional value, which are impacted by the fatty acid composition [31]. Nevertheless, the dietary lipid sources exert a notable influence on the fatty acid profile within the muscles of the fish. Consequently, the primary objective of this study is to assess how variations in the dietary lipid sources impact the growth, antioxidant index, lipid metabolism, and fatty acid profile of tilapia.

2. Materials and Methods

2.1. Experimental Fish and Feeding Management. The GIFT tilapia utilized in the study were procured from a farm situated in Wenchang, Hainan, China. The tilapia were given 2 weeks for adaptation to the environmental conditions, during which time they were fed commercial pellet feed (Tongwei, Chengdu, China) twice times per day. Total of 315 healthy tilapia (initial body weight 0.205 ± 0.005 g) were randomly assigned to 21 glass aquariums ($80 \times 40 \times 45$ cm). The experiment consisted of seven treatments with three parallel, and 15 tilapias were placed in each parallel.

During the 105-day feeding trial, the experimental feed was provided quantitatively and regularly. The daily feeding amount was 6% of the tilapia's body weight. It was fed twice a day at 8 and 16 hr. 2 hr after feeding, uneaten feed and feces were removed through the siphon. Additionally, two-thirds of the water was replaced daily to preserve the optimal quality of water. Water quality parameters are tested every 3 days: average temperature 29.0 ± 3.0°C, average pH 7.50 ± 0.20, and dissolved oxygen 6.0–7.5 mg/L. Every experiment was carried out following Chinese mainland laws and standards regarding laboratory animals. The Hainan University Animal Ethics Committee gave its approval for this study.

2.2. Diets Preparation. The seven experimental diets were categorized based on the oil source used: tilapia oil (FO; control), corn oil (CO), linseed oil (LO), and algae oil (from *Schizochytrium* sp., AO), a 1 : 2 mixture of linseed oil and algae oil (LA12) group, a 1 : 1 mixture of linseed oil and algae oil (LA11) group, and a 2 : 1 mixture of linseed oil and algae oil (LA21). The fatty acid composition of the oil source is shown in Table 1. To prepare the experimental diets, fish meal, soybean meal, rapeseed meal, and peanut meal were ground up and crushed through a 60-mesh screen. The raw materials were then weighed according to the proportions shown in Table 2. Starting with the most minor proportion, the materials were mixed. Subsequently, the oil source was added. Following a 40-mesh screen, 120 mL/kg of distilled water was added to the mixture. The feed was extruded into particles with a particle size of 2.0 mm using a cold press double helix plodder (CD4 × 1TS, SCUT, Guangdong, China). The granules were dried in a well-ventilated room until the moisture content falls below 10%. Finally, the diets were kept in a −20°C refrigerator. A total of seven trial diets with roughly 26.08% crude protein and 7.09% crude lipid were created (Table 2).

2.3. Sample Collection. After starvation for 24 hr, tilapia were anesthetized with 30 ppm tricaine methanesulfonate (purity > 99%, AbMole) [32]. After drying the surface moisture of the tilapia, the final weight was measured on an electronic scale. Body lengths were measured with a ruler. Then, the hepatopancreas were removed by scalpel carefully and quickly frozen in liquid nitrogen, and subsequently saved at −80°C. Peel off the skin tissue of the lateral dorsal muscles, then cut the dorsal muscles and freeze them in liquid nitrogen, and finally keep them at −80°C.

Table 1: Fatty acids composition of oil sources (% of total fatty acids).

Fatty acids	Tilapia oil	Corn oil	Linseed oil	Algal oil
C14 : 0	2.35	0.55	0.72	4.27
C15 : 0	0.18	0.11	0.05	0.08
C16 : 0	15.02	11.68	7.01	9.69
C17 : 0	0.22	0.23	0.12	0.23
C18 : 0	3.85	7.22	6.78	3.65
C20 : 0	0.15	0.65	0.56	0.77
C24 : 0	0.13	0.22	0.51	0.68
C16 : 1	1.45	1.27	0.64	3.77
C18 : 1n9t	1.67	0.02	0.05	0.06
C18 : 1n9c	31.07	21.66	19.78	19.73
C20 : 1n9	0.04	0.51	0.55	0.05
C18 : 2n6c	24.57	42.37	20.66	10.67
C18 : 3n6	1.21	0.77	0.13	0.02
C20 : 4n6	0.98	0.05	3.91	0.55
C18 : 3n3	3.21	3.28	38.58	5.28
C20 : 5n3	1.31	0.52	0.20	0.44
C22 : 6n3	0.88	0.34	0.15	31.56

Note: C18 : 2n6c (Linoleic acid, LA); C18 : 3n6 (γ-linolenic acid, γ-LNA); C20 : 4n6 (Arachidonic acid, ARA); C18 : 3n3 (α-linolenic acid, α-LNA); C20 : 5n3 (Eicosapentaenoic acid, EPA); and C22 : 6n3 (Docosahexaenoic acid, DHA).

Table 2: Ingredient formulation (g/kg dry basis) and proximate composition (% dry weight) of experimental diets.

Ingredient	FO	CO	LO	AO	LA12	LA11	LA21
Fish meal	100	100	100	100	100	100	100
Soybean meal	250	250	250	250	250	250	250
Peanut meal	100	100	100	100	100	100	100
Rapeseed meal	100	100	100	100	100	100	100
Tilapia oil	60	—	—	—	—	—	—
Corn oil	—	60	—	—	—	—	—
Linseed oil	—	—	60	—	20	30	40
Algae oil	—	—	—	60	40	30	20
Wheat meal	100	100	100	100	100	100	100
Corn starch	200	200	200	200	200	200	200
Vitamin premix[1]	20	20	20	20	20	20	20
Mineral premix[2]	20	20	20	20	20	20	20
Carboxymethyl	20	20	20	20	20	20	20
Cellulose	20	20	20	20	20	20	20
$Ca(H_2PO_4)_2$	20	20	20	20	20	20	20
Total	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Proximate composition (%)			—	—	—	—	—
Crude protein	26.12	26.05	26.08	26.04	26.11	26.05	26.09
Crude lipid	7.08	7.12	7.07	7.11	7.12	7.10	7.06

[1]Vitamin premix (mg/kg): vitamin A (500,000 IU/g), 8 mg; vitamin D3 (1,000,000 IU/g), 2 mg; vitamin K, 10 mg; vitamin E, 200 mg; thiamine, 10 mg; riboflavin, 12 mg; pyridoxine, 10 mg; calcium pantothenate, 32 mg; nicotinic acid, 80 mg; folic acid, 2 mg; vitamin B12, 0.01 mg; biotin, 0.2 mg; choline chloride, 400 mg; vitamin C−2- polyphosphate (150 mg/g vitamin C activity), 400 mg. [2]Mineral premix (mg/kg): zinc ($ZnSO_4 \cdot 7H_2O$), 50.0 mg; iron ($FeSO_4 \cdot 7H_2O$), 40 mg; manganese ($MnSO_4 \cdot 7H_2O$), 15.3 mg; copper ($CuCl_2$), 3.8 mg; iodine (KI), 5 mg; cobalt ($CoCl_2 \cdot 6H_2O$), 0.05 mg; and selenium (Na_2SeO_3), 0.09 mg.

2.4. Calculations. Following a 105-day feeding trial, the performance evaluation of tilapia was as follows:

Survival (%) = 100 × final number of fish/initial number of fish;

Weight gain (WG, %) = 100 × (final body weight−initial body weight)/initial body weight;

Specific growth rate (SGR, %, day^{-1}) = (Ln(final body weight)−Ln(initial body weight)) × 105/days.

2.5. Diets Composition Assay. The detection of dietary crude lipid and crude protein contents was conducted based on the AOAC [33]. The Dumas combustion method, coupled with a protein analyzer (Elementar rapid N exceed, Germany), was employed to measure the crude protein levels in the tested diets. The determination of crude lipid contents in the diets was carried out using a Soxhlet system.

2.6. Enzymatic Assay. Hepatopancreas samples (0.1 g) were taken, and 10% tissue homogenate was prepared with 0.9 mL 0.85% normal saline. After centrifugated at 3,500 rpm at 4°C for 15 min, the activities of superoxide dismutase (SOD) and total antioxidant capacity (T-AOC), the contents of triacylglycerol (TG), total cholesterol (T-CHO), and malondialdehyde (MDA) in the hepatopancreas were detected by supernatant. The detection process was carried out in accordance with the requirements of the commercial detection kit (Nanjing Jiancheng Bioengineering Institute, China), and the optical density values were measured by a microplate reader (Epoch, BioTek, USA).

The activity of SOD was measured using the hydroxylamine method [34]. Detection of T-AOC was conducted using the Fe^{3+} reduction method [35]. The content of TG was detected using the glycerophosphate oxidase–peroxidase method [36]. The cholesterol oxidase–peroxidase method was used to detect the content of T-CHO [37]. The content of MDA was detected using thiobarbituric acid (TBA) method [38].

2.7. Gene Expression of Lipid Metabolism in the Hepatopancreas. The hepatopancreas was employed for total RNA extraction using the TRIzol reagent (Life Technologies, USA). RNA quality and concentration were evaluated with NanoDrop (Thermo Scientific, USA), and the total RNA is reverse transcribed into cDNA by using HiScript® ii Q RT SuperMix (Vazyme, China). The mRNA levels of essential genes associated with lipid metabolism were quantified by qRT-PCR.

The primers of peroxisome proliferator-activated receptor α (PPARα), carnitine palmitoyl transferase 1α (CPT-1α), acetyl-CoA carboxylase (ACCα), fatty acid synthase (FAS), Δ6/Δ5FADs2, Δ4FADs2, and elongation of very long chain fatty acid 5 (ELOVL5) are designed through Primer Premier 6.0. The primers used to amplify the lipid metabolism genes in hepatopancreas in this experiment are displayed in Table 3. The reaction system is 10 μL 2 × SYBR (Vazyme, China), 2 μL cDNA, 2 μL mixed primers (upstream and downstream primers), and 6 μL DEPC water (Sigma-Aldrich, China). The reaction procedure is 95°C/30 s, 40 cycles at 95°C/10 s, and 60°C/30 s. The $2^{-\Delta\Delta Ct}$ method was applied for the

Table 3: Primer used in this study for hepatopancreas gene expression.

Gene	5′−3′ primer sequence	GenBank accession No.
CPT-1α	F: TTTCCAGGCCTCCTTACCCA	XM_013268638.3
	R: TTGTACTGCTCATTGTCCAGCAGA	—
PPARα	F: CTGATAAAGCTTCGGGCTTCCA	XM_019346353.2
	R: CGCTCACACTTATCATACTCCAGCT	—
ACCα	F: TAGCTGAAGAGGAGGGTGCAAGA	XM_025910668.1
	R: AACCTCTGGATTGGCTTGAACA	—
FAS	F: TCATCCAGCAGTTCACTGGCATT	XM_003454056.5
	R: TGATTAGGTCCACGGCCACA	—
ELOVL5	F: GCCATACCTTTGGTGGAAGA	XM_025897214.1
	R: AGGGAGCTGTTCTGTGGATG	—
Δ6/Δ5FADs2	F: GTGGATCTGGCTTGGTTCAT	KF268464.1
	R: CCAGTCCCTGTGCTTTTCAT	—
Δ4FADs2	F: CTTACTGTGCTCGGTGATT	XM_005470635.4
	R: GGTCCTTGCTGAAGATGTT	—

calculation of relative mRNA expression levels pertaining to lipid metabolism genes in the hepatopancreas [39].

2.8. Fatty Acids Composition Analysis. Formulated diets and muscle samples were dried in a cryogenic freeze dryer for 48 hr, and the main fatty acid types in the muscle were detected by gas chromatography–mass spectrometry. Testing instrument: gas chromatograph–mass spectrometer (6890N/5975B, Agilent), chromatographic column HP-INNOWAX (30 m × 0.25 mm × 0.25 μm, Agilent). Sample processing: 50 mg of freeze-dried sample was added to 2 mL of methanol (purity ≥ 99.9%, Macklin) –chloroform (purity ≥ 99.9%, Macklin) solution, mixed thoroughly for 1 min, and placed in an 80°C water bath. Added 1 mL of n-hexane for extraction and 5 mL of clean water for washing after 30 min of methyl esterification, and took 500 μL of the supernatant. Added 25 μL of internal standard fatty acid (C19 : 0), and then used the microsampler to test on the machine after uniformity. The detection instrument parameters were set as follows: 1 μL injection volume, splitless split mode, 5 mL/min septum purge flow rate, helium carrier gas, 1.0 mL/min column flow rate, and a 50°C for 3 min column oven temperature program. Increased the temperature by 10°C per minute to 220°C and kept it for 3 min. After that, elevated it to 250°C with a 15°C gradient and held it for 10 min. Both the transfer line and the front sample inlet temperature were 250°C, and 230°C was the temperature of the ion source, the sampling mode was SIM. The raw data were calculated through the fatty acid absorption peak area and standard products to obtain the relative percentage content of crucial fatty acids.

2.9. Statistical Analysis. All the data analyses were conducted using SPSS 26.0 (IBM, Armonk, NY, USA), and all results are reported as mean ± SE (standard error). The normality and homogeneity of the data were detected before conducting a one-way analysis of variance (ANOVA). Significant differences between all treatments were determined using one-way ANOVA followed by Duncan's multiple comparison test, and significance was denoted by $P < 0.05$.

3. Results

3.1. Growth Parameter. No statistically significant differences in survival were observed among the various groups ($P > 0.05$). Tilapia in the FO and LO groups demonstrated notably elevated WG and SGR compared to the remaining groups ($P < 0.05$). Among all groups, tilapia in the AO group exhibited the lowest values for both WG and SGR ($P < 0.05$; Table 4).

3.2. Biochemical Parameters of Hepatopancreas. The AO group's tilapia showed significantly higher SOD activity and MDA content than the other groups ($P < 0.05$). The T-AOC content of tilapia was significantly increased in the AO group compared to the LO group ($P < 0.05$), but no significant distinctions were observed between the AO group and the remaining treatment groups ($P > 0.05$). The content of TG in the LO group was the lowest among all treatments. The content of T-CHO was not significant among all treatments ($P > 0.05$; Figure 1).

3.3. mRNA Expressions of Lipid Metabolism Key Genes in Hepatopancreas. The LA12 group displayed the highest mRNA expression levels of PPARα and CPT-1α among seven groups ($P < 0.05$). Among all treatments, the expression levels of Δ6/Δ5FADs2 and Δ4FADs2 were notably higher in the LO group ($P < 0.05$). ELOVL5 expression was significantly higher in CO, LO, and LA12 groups compared to others ($P < 0.05$). The relative mRNA expression of ACCα in tilapia in the AO group was the lowest ($P < 0.05$), whereas the relative mRNA expression of both ACCα and FAS in tilapia in the LO group was significantly higher than that in the AO group ($P < 0.05$; Figure 2).

Table 4: Growth performance and survival of GIFT tilapia fed diets with different oil sources for 105 days.

Items	Dietary oil sources						
	FO	CO	LO	AO	LA12	LA11	LA21
Initial weight	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
Final weight	11.78 ± 0.27^{c}	10.49 ± 0.15^{a}	11.76 ± 0.05^{c}	10.30 ± 0.09^{a}	10.69 ± 0.15^{a}	10.41 ± 0.14^{a}	11.13 ± 0.05^{b}
WG (%)	$5{,}643.90 \pm 133.98^{c}$	$5{,}015.85 \pm 72.53^{a}$	$5{,}639.02 \pm 24.99^{c}$	$4{,}923.17 \pm 45.12^{a}$	$5{,}115.85 \pm 36.90^{a}$	$4{,}975.61 \pm 33.59^{a}$	$5{,}328.05 \pm 25.53^{b}$
SGR (%/d)	3.86 ± 0.02^{d}	3.75 ± 0.01^{ab}	3.86 ± 0.01^{d}	3.73 ± 0.02^{a}	3.77 ± 0.01^{b}	3.74 ± 0.01^{ab}	3.80 ± 0.01^{c}
Survival (%)	100	100	100	100	100	100	100

Data are expressed as mean $\pm$ SE ($n \geq 8$). Mean values with different superscript letters differ significantly (a–g, $P < 0.05$).

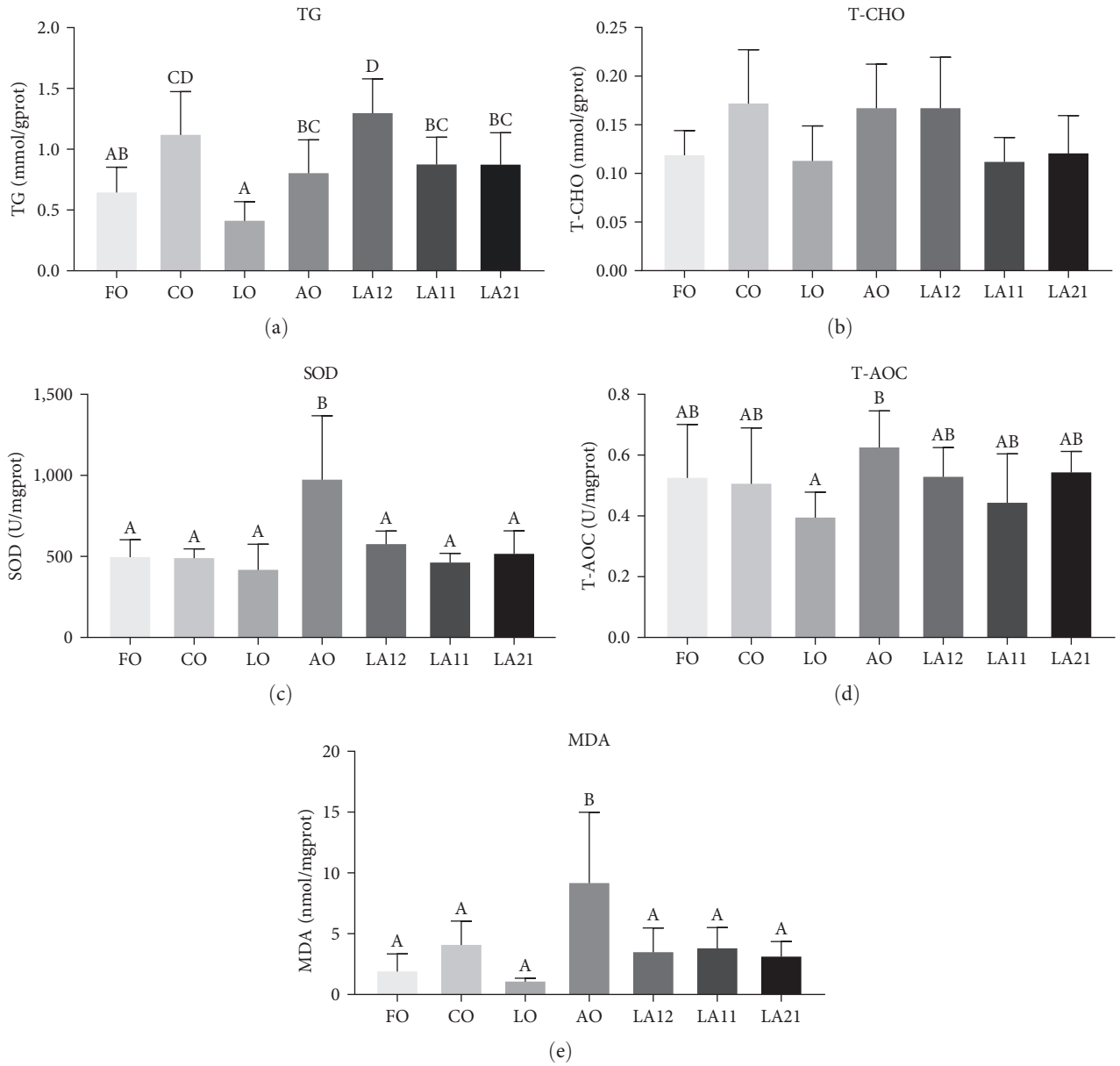

FIGURE 1: Contents of: (a) TG, (b) T-CHO, (c) SOD, (d) T-AOC, and (e) MDA in GIFT tilapia feed containing different oil sources. Data are expressed as mean ± SE ($n \geq 5$). Mean values with different superscript letters differ significantly (A–G, $P < 0.05$).

3.4. Fatty Acids Composition in the Diets and Muscle. The compositions of fatty acids in the diet and muscle are shown in Tables 5 and 6, respectively. The fatty acid deposition in the muscle of each group reflected the composition of their respective diets. The DHA proportion of muscle in the AO group was the highest ($P < 0.05$), followed by the LA21 group. Notably, the LO group had the highest proportion of EPA in muscle ($P > 0.05$), followed by the LA21 group. However, there was no detectable EPA deposition in the muscles of tilapia fed with FO and CO diets. Notably, with the decreasing proportion of DHA in the diets, the proportion of DHA in muscle decreased in the LA12, LA11, and LA21 groups, while the proportion of EPA and arachidonic acid (ARA) displayed an opposite trend. Additionally, the highest percentage of ARA in muscle was observed in the FO group ($P < 0.05$).

4. Discussion

It was found that the growth performance of tilapia in the AO group consuming diets enriched with LC-PUFAs was inferior, whereas there was no significant difference between the growth performance of tilapia in the LO group consuming diets enriched with α-linolenic acid (α-LNA) and that of the control group. Among LA12, LA11, and LA21, the LA21 group had the lowest proportion of algal oil in the feed, but the growth performance was better than the other two groups, which indicated that the addition of a high proportion of

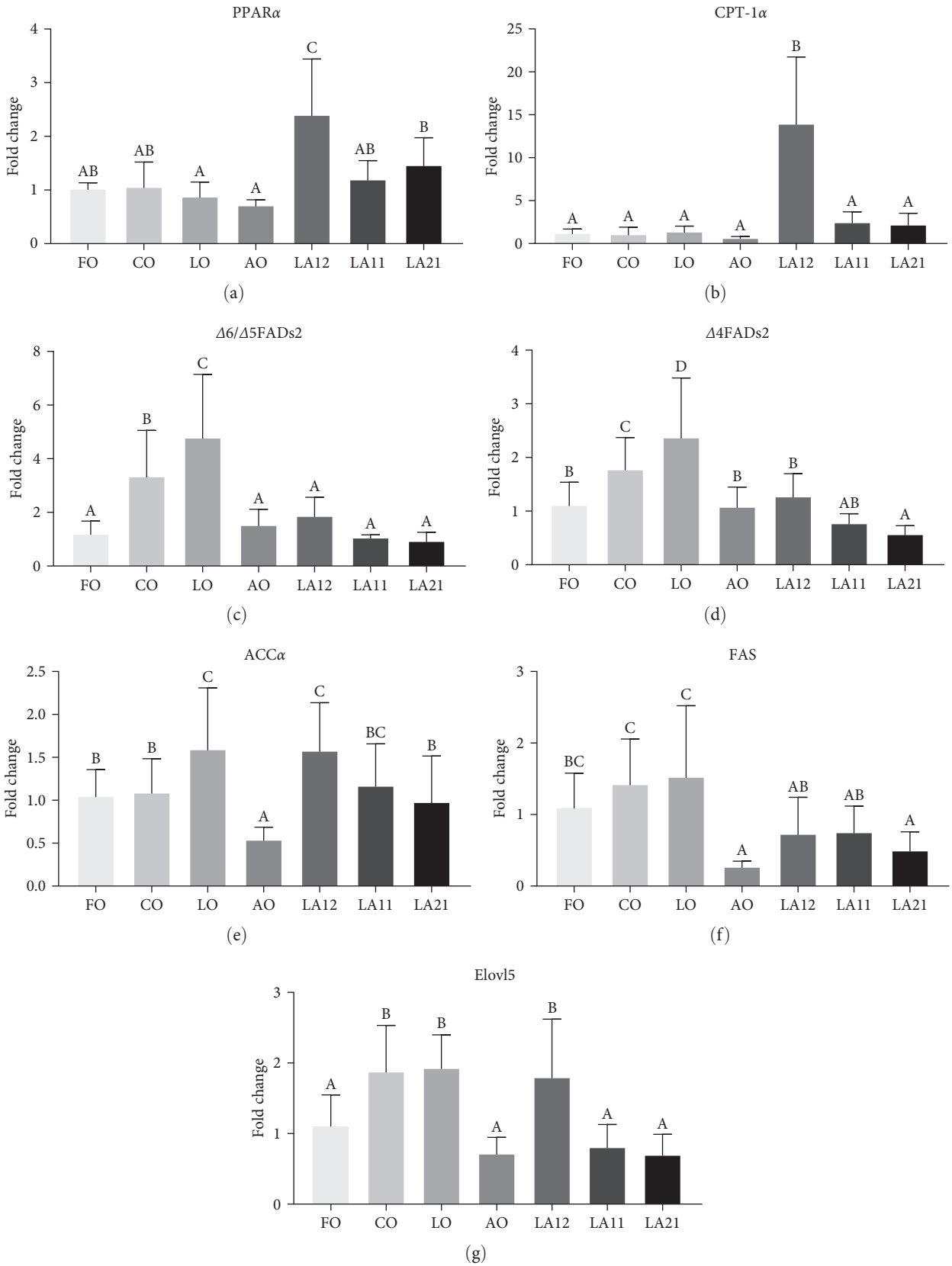

Figure 2: Expression of crucial genes in hepatopancreas lipid metabolism of GIFT tilapia feed containing different oil sources. Data are expressed as mean ± SE ($n \geq 8$). Mean values with different superscript letters differ significantly (A–G, $P < 0.05$).

Table 5: Fatty acid composition of the experimental diets (% of total fatty acids).

Fatty acid	FO	CO	LO	AO	LA12	LA11	LA21
C14:0	2.494 ± 0.005^{c}	0.798 ± 0.012^{b}	0.606 ± 0.002^{a}	10.342 ± 0.008^{g}	7.309 ± 0.005^{f}	5.875 ± 0.032^{e}	4.161 ± 0.023^{d}
C15:0	0.217 ± 0.001^{d}	0.075 ± 0.001^{c}	0.068 ± 0.001^{b}	0.064 ± 0.001^{a}	0.065 ± 0.001^{a}	0.065 ± 0.001^{a}	0.067 ± 0.001^{b}
C16:0	20.468 ± 0.018^{g}	12.921 ± 0.052^{e}	8.961 ± 0.032^{a}	13.673 ± 0.009^{f}	12.138 ± 0.003^{d}	11.359 ± 0.017^{c}	10.510 ± 0.014^{b}
C17:0	0.312 ± 0.001^{g}	0.173 ± 0.001^{f}	0.153 ± 0.001^{e}	0.133 ± 0.001^{a}	0.137 ± 0.001^{b}	0.144 ± 0.001^{c}	0.150 ± 0.001^{d}
C18:0	5.199 ± 0.008^{g}	4.238 ± 0.007^{e}	4.275 ± 0.001^{f}	2.067 ± 0.002^{a}	2.810 ± 0.001^{b}	3.123 ± 0.009^{c}	3.518 ± 0.005^{d}
C20:0	0.759 ± 0.001^{bc}	0.892 ± 0.002^{c}	0.590 ± 0.001^{ab}	0.432 ± 0.185^{a}	0.604 ± 0.001^{ab}	0.591 ± 0.001^{ab}	0.599 ± 0.001^{ab}
C24:0	0.218 ± 0.001^{a}	0.282 ± 0.001^{f}	0.231 ± 0.001^{b}	0.253 ± 0.002^{de}	0.254 ± 0.001^{e}	0.250 ± 0.001^{cd}	0.249 ± 0.001^{c}
C16:1	2.877 ± 0.002	0.730 ± 0.014	0.550 ± 0.003	2.374 ± 0.001	1.807 ± 0.001	1.528 ± 0.006	1.206 ± 0.005
C18:1n9t	0.259 ± 0.001^{a}	0.032 ± 0.008^{a}	0.084 ± 0.016^{b}	0.040 ± 0.004^{a}	0.049 ± 0.006^{a}	0.031 ± 0.004^{a}	0.027 ± 0.001^{a}
C18:1n9c	32.897 ± 0.019^{a}	28.682 ± 0.028^{e}	22.868 ± 0.048^{c}	23.073 ± 0.025^{d}	22.899 ± 0.003^{c}	22.769 ± 0.004^{b}	22.677 ± 0.003^{a}
C20:1n9	0.069 ± 0.001^{b}	0.201 ± 0.001^{d}	0.372 ± 0.001^{g}	0.039 ± 0.001^{a}	0.114 ± 0.005^{c}	0.281 ± 0.001^{e}	0.311 ± 0.001^{f}
C18:2n6c	25.662 ± 0.026^{f}	44.174 ± 0.129^{g}	21.875 ± 0.152^{e}	10.058 ± 0.007^{a}	13.080 ± 0.021^{b}	14.602 ± 0.035^{c}	16.412 ± 0.024^{d}
C18:3n6	0.925 ± 0.001^{g}	0.256 ± 0.003^{d}	0.475 ± 0.001^{f}	0.033 ± 0.001^{a}	0.181 ± 0.001^{b}	0.248 ± 0.002^{c}	0.331 ± 0.001^{e}
C20:4n6	0.698 ± 0.001	0.083 ± 0.004	—	—	—	—	—
C18:3n3	2.298 ± 0.001^{a}	4.636 ± 0.016^{c}	37.663 ± 0.243^{g}	3.657 ± 0.003^{b}	14.985 ± 0.003^{d}	20.169 ± 0.128^{e}	26.817 ± 0.083^{f}
C20:5n3	0.295 ± 0.001^{e}	0.187 ± 0.001^{b}	0.182 ± 0.001^{a}	0.192 ± 0.001^{cd}	0.193 ± 0.001^{d}	0.190 ± 0.001^{c}	0.187 ± 0.001^{b}
C22:6n3	0.589 ± 0.004^{b}	0.271 ± 0.002^{a}	0.178 ± 0.001^{a}	26.770 ± 0.037^{f}	18.560 ± 0.007^{e}	14.688 ± 0.102^{d}	9.719 ± 0.062^{c}
∑ SFA	29.667 ± 0.033^{f}	19.379 ± 0.071^{b}	14.883 ± 0.037^{a}	26.964 ± 0.173^{e}	23.317 ± 0.006^{d}	21.408 ± 0.040^{c}	19.254 ± 0.033^{b}
∑ MUFA	36.101 ± 0.016^{g}	29.645 ± 0.049^{f}	23.874 ± 0.053^{a}	25.527 ± 0.030^{e}	24.869 ± 0.010^{d}	24.609 ± 0.007^{c}	24.221 ± 0.001^{b}
∑ n-3 PUFAs	3.182 ± 0.005^{a}	5.094 ± 0.014^{b}	38.024 ± 0.242^{g}	30.619 ± 0.042^{c}	33.739 ± 0.008^{d}	35.047 ± 0.027^{e}	36.723 ± 0.022^{f}
∑ n-6 PUFAs	27.285 ± 0.026^{f}	44.513 ± 0.121^{g}	22.350 ± 0.151^{e}	10.091 ± 0.008^{a}	13.260 ± 0.021^{b}	14.850 ± 0.037^{c}	16.743 ± 0.024^{d}
n-3/n-6 PUFAs	0.117 ± 0.001	0.114 ± 0.001	1.702 ± 0.022	3.034 ± 0.006	2.544 ± 0.004	2.360 ± 0.004	2.193 ± 0.002

*Data are expressed as mean ± SE ($n = 6$). Mean values with different superscript letters differ significantly (a–g, $P < 0.05$).

Table 6: Fatty acid composition of muscle in GIFT tilapia feed diets containing different oil sources (% of total fatty acids).

Fatty acid	FO	CO	LO	AO	LA12	LA11	LA21
C14:0	1.693 ± 0.092^{a}	1.480 ± 0.108^{a}	1.448 ± 0.106^{a}	5.485 ± 0.260^{e}	3.846 ± 0.201^{d}	3.342 ± 0.161^{c}	2.706 ± 0.094^{b}
C15:0	0.274 ± 0.027^{b}	0.240 ± 0.013^{ab}	0.166 ± 0.006^{a}	0.197 ± 0.013^{ab}	0.180 ± 0.011^{ab}	0.213 ± 0.013^{ab}	0.274 ± 0.070^{b}
C16:0	22.677 ± 0.375^{e}	19.693 ± 0.618^{d}	16.686 ± 0.417^{a}	19.549 ± 0.130^{d}	18.637 ± 0.658^{cd}	17.846 ± 0.192^{bc}	17.533 ± 0.320^{ab}
C17:0	0.486 ± 0.026^{b}	0.383 ± 0.046^{a}	0.341 ± 0.050^{a}	0.313 ± 0.027^{a}	0.349 ± 0.034^{a}	0.371 ± 0.035^{a}	0.335 ± 0.016^{a}
C18:0	9.110 ± 0.460^{b}	8.163 ± 0.481^{ab}	7.142 ± 0.549^{a}	6.899 ± 0.334^{a}	7.282 ± 0.415^{a}	7.348 ± 0.416^{a}	7.488 ± 0.320^{a}
C20:0	0.525 ± 0.033^{ab}	0.574 ± 0.042^{b}	0.449 ± 0.040^{a}	0.525 ± 0.012^{ab}	0.470 ± 0.033^{a}	0.480 ± 0.023^{ab}	0.514 ± 0.022^{ab}
C24:0	0.226 ± 0.010	0.129 ± 0.003	—	—	—	—	—
C16:1	3.347 ± 0.184^{bc}	2.615 ± 0.199^{a}	2.640 ± 0.318^{a}	3.897 ± 0.116^{c}	3.332 ± 0.255^{bc}	3.219 ± 0.226^{ab}	2.948 ± 0.122^{ab}
C18:1n9t	0.322 ± 0.029^{b}	0.211 ± 0.033^{a}	0.178 ± 0.020^{a}	0.210 ± 0.013^{a}	0.197 ± 0.016^{a}	0.207 ± 0.013^{a}	0.201 ± 0.013^{a}
C18:1n9c	30.018 ± 0.638^{c}	28.496 ± 0.614^{bc}	26.553 ± 0.830^{ab}	27.170 ± 0.780^{ab}	25.930 ± 1.052^{a}	26.054 ± 0.654^{a}	25.665 ± 0.521^{a}
C20:1n9	1.489 ± 0.025^{c}	1.062 ± 0.072^{b}	0.350 ± 0.090^{a}	0.882 ± 0.055^{b}	0.526 ± 0.127^{a}	0.618 ± 0.098^{a}	0.422 ± 0.116^{a}
C18:2n6c	16.114 ± 0.424^{e}	24.177 ± 0.543^{f}	15.448 ± 0.364^{e}	8.676 ± 0.115^{a}	10.254 ± 0.218^{b}	11.239 ± 0.215^{c}	12.494 ± 0.227^{d}
C18:3n6	0.875 ± 0.022^{e}	1.072 ± 0.043^{f}	0.753 ± 0.040^{d}	0.228 ± 0.012^{a}	0.349 ± 0.020^{b}	0.382 ± 0.020^{bc}	0.442 ± 0.018^{c}
C20:4n6	4.534 ± 0.244^{e}	3.998 ± 0.241^{d}	1.757 ± 0.186^{c}	0.804 ± 0.063^{a}	1.071 ± 0.126^{ab}	1.184 ± 0.071^{ab}	1.377 ± 0.108^{bc}
C18:3n3	0.921 ± 0.032^{a}	1.567 ± 0.043^{a}	15.092 ± 0.499^{e}	1.516 ± 0.057^{a}	5.691 ± 0.338^{b}	7.600 ± 0.414^{c}	9.884 ± 0.479^{d}
C20:5n3	—	—	0.505 ± 0.034^{c}	0.274 ± 0.024^{a}	0.377 ± 0.050^{b}	0.394 ± 0.028^{b}	0.436 ± 0.023^{bc}
C22:6n3	2.320 ± 0.092^{a}	1.778 ± 0.117^{a}	5.425 ± 0.428^{b}	19.760 ± 0.753^{f}	17.210 ± 0.825^{e}	15.274 ± 0.561^{d}	12.288 ± 0.329^{c}
$\sum$ SFA	34.991 ± 0.710^{e}	30.662 ± 0.556^{c}	26.231 ± 0.509^{a}	32.968 ± 0.281^{d}	30.763 ± 0.555^{c}	29.600 ± 0.447^{bc}	28.850 ± 0.596^{b}
$\sum$ MUFA	35.175 ± 0.816^{c}	32.384 ± 0.761^{b}	29.721 ± 1.039^{ab}	32.159 ± 0.948^{b}	29.985 ± 1.257^{ab}	30.098 ± 0.765^{ab}	29.236 ± 0.431^{a}
$\sum$ n-3 PUFAs	3.241 ± 0.081^{a}	3.345 ± 0.110^{a}	21.023 ± 0.530^{b}	21.551 ± 0.755^{bc}	23.278 ± 0.709^{d}	23.268 ± 0.484^{d}	22.609 ± 0.363^{cd}
$\sum$ n-6 PUFAs	21.523 ± 0.398^{f}	29.247 ± 0.586^{g}	17.958 ± 0.439^{e}	9.707 ± 0.114^{a}	11.674 ± 0.239^{b}	12.805 ± 0.207^{c}	14.312 ± 0.150^{d}
n-3/n-6 PUFAs	0.151 ± 0.004^{a}	0.114 ± 0.003^{a}	1.171 ± 0.011^{b}	2.219 ± 0.069^{f}	1.993 ± 0.030^{e}	1.818 ± 0.034^{d}	1.580 ± 0.023^{c}

Data are expressed as mean ± SE ($n = 3$). Mean values with different superscript letters differ significantly (a–g, $P < 0.05$).

algal oil in the feed was not suitable for the growth of tilapia. In previous studies, it was found that α-LNA in feed was actively oxidized to produce energy after tilapia ingested it [40]. Under conditions of higher feed α-LNA ratios, tilapia were net producers of LC-PUFAs, but biosynthesis was low, and as the dietary α-LNA intake level increased, the LC-PUFAs ratio of tilapia gradually decreased [15, 41]. It indicated that tilapia had a limited ability to synthesize LC-PUFAs using α-LNA and consumed a small amount of α-LNA. Therefore, α-LNA in the feed was mainly oxidized after ingestion by tilapia to provide energy to maintain growth.

PUFAs, including n-3 and n-6 PUFAs, have gained significant attention for their physiological effects and preventive and healthcare functions [42]. However, it is worth noting that LC-PUFAs (e.g. DHA) are susceptible to peroxidation, leading to damage to biofilm functions and physiological health [43]. The highest content of DHA in diet and muscle was observed in the AO group. DHA is a structurally vital element of cell membranes [44]. It is susceptible to oxidative damage from oxygen free radicals, and SOD can convert superoxide anion radicals into oxygen and hydrogen peroxide. SOD plays a vital regulatory role in oxidative damage and antioxidant balance of the organism [45]. In the AO group with the highest DHA content, there was a significant increase in SOD activity, and a significant increase in MDA content as an end product of oxidative stress, and a poorer growth performance. Although the AO group had the highest T-AOC, it was not significantly different from the five groups except the LO group. The current study reveals that the excessive incorporation of DHA-rich oil into the diet results in a significant deposition of DHA in the muscle. However, high levels of DHA in the diet caused oxidative stress in the hepatopancreas of tilapia. Another aspect, both FAS and ACCα are critical enzymes in fatty acid synthesis. FAS catalyzes de novo fatty acid synthesis [46], and ACCα controls the synthesis of malonyl-CoA and is essential for the biosynthesis of long-chain fatty acids [47]. The AO group exhibited a significant decrease in the levels of gene expression for FAS and ACCα, indicating that algae oil inhibited fatty acid synthesis in tilapia. Therefore, excessive use of DHA-rich materials in aquatic feeds should be avoided.

There are two ways for vertebrates to obtain LC-PUFAs: direct intake from food, and by synthesizing n-6 PUFAs and n-3 PUFAs from linoleic acid (LA) and α-LNA, respectively, using FADs and ELOVL enzymes [48]. Generally, marine fish rely on dietary intake for LC-PUFAs, constrained by their limited synthetic capacity [49]. Freshwater fish, salmonids, and a few marine fish can convert LA and α-LNA into LC-PUFAs, including EPA and DHA [50]. FADs2 and ELOVL5 are rate-limiting enzymes in LC-PUFAs synthesis and play essential functions in fatty acid dehydrogenation and elongation. FADs2 are critical enzymes for the synthesis of unsaturated fatty acids. It can increase the double bonds of fatty acids to achieve desaturation and highly unsaturated fatty acid synthesis is directly impacted by expression and activity [51]. ELOVL controls for carbon chain elongation in PUFAs synthesis and serves as the initial regulatory procedure in the fatty acid elongation pathway [52], extending the fatty acids synthesized endogenously or provided by the diet. ELOVL5 was the first enzyme identified in zebrafish with fatty acid elongation ability and exhibits elongation activity for C_{18} PUFAs and C_{20} PUFAs in fish [53], although it is less active on C_{22} PUFAs [54]. The mRNA expression of Δ6/Δ5FADs2 and Δ4FADs2 was the highest in the hepatopancreas of the LO group, and the mRNA expression of ELOVL5 was also higher. In addition, the proportions of EPA and DHA in tilapia from the LO group far exceeded the proportions of the two fatty acids in the ingested feed. This suggests that when fed diets high in α-LNA, tilapia can convert LC-PUFAs via FADs2 and ELOVL5 using α-LNA as a synthetic precursor [55]. The conversion and deposition of DHA in the CO group muscles were not significant, and the presence of EPA was not detected, indicating that tilapia had a selective preference for the substrate fatty acids for LC-PUFAs synthesis, preferring α-LNA over LA. This finding is consistent with grass carp (*Ctenopharyngodon idellus*) [56] but contrasts with Murray cod (*Maccullochella peelii peelii*) [57]. Therefore, it can be inferred that tilapia has a preference for utilizing α-LNA for the synthesis of n-3 LC-PUFAs.

The same conversion capacity can also be found when linseed oil and algae oil are mixed in feed. Among LA12, LA11, and LA21, as the proportion of linseed oil increased, the proportion of EPA in muscle gradually increased but was not significant, and the difference in DHA ratio between muscle and feed was reduced. In particular, the proportion of DHA in muscle of the LA21 group was significantly increased, while the corresponding proportion of linolenic acid was relatively reduced. This suggested that the conversion efficiency of tilapia from α-LNA to LC-PUFAs was reduced or even inhibited in the presence of DHA as a product in substantial amounts. PPARα and CPT-1α are crucial enzymes in fatty acid β-oxidation and play a pivotal role in lipid metabolism [58, 59]. The hypolipidemic effect of PPARα activation has been widely reported [60]. PPARα can increase the demand for LC-PUFAs by accelerating fatty acid β-oxidation. CPT-1α is a crucial component of β-oxidation [61]. Decreased expression levels and activity of CPT-1α will lead to the accumulation of lipid deposits [62, 63]. In comparison to the other treatments, the tilapia fed with the LA12 diet demonstrated notably increased PPARα and CPT-1α mRNA expression in the hepatopancreas. The expression of PPARα and CPT-1α regulates the fatty acid oxidation pathway [64]. The elevated expression of PPARα and CPT-1α in the hepatopancreas of tilapia in the LA12 group suggests that a dietary oil mixture of linseed oil and algae oil in a 1 : 2 ratio can increase the breakdown and utilization efficiency of fatty acids and increase the oxidation energy ratio of fatty acids.

The DHA content in muscle corresponds to the DHA content in dietary intake, but the level of fatty acid biosynthesis is different. Previous investigations have elucidated that the accumulation of DHA in the muscles of farmed fish, such as gilthead seabream, turbot (*Scophthalmus maximus*), and Atlantic salmon is consistent with the relative amount of DHA in the diet [65–67]. In this experiment, there was no significant difference in the proportion of total n-3 PUFAs (total of α-LNA, EPA, and DHA) in tilapia muscles

among the LA12, LA11, and LA21 groups, and the total n-3 PUFAs was not significantly different between LO and AO groups, but the proportion of DHA corresponded to the amount of DHA in the ingested feed. This suggests that exogenous fatty acids can modulate the fatty acid profile of tilapia muscle, n-3 LC-PUFAs in the diet can be effectively deposited in tilapia muscle, but the amount of n-3 LC-PUFAs deposited will be affected by the conversion ability. Therefore, the n-3 LC-PUFAs content in tilapia muscle can be adjusted through feed, but to effectively increase the n-3 LC-PUFAs ratio, the proportions of α-LNA, EPA, and DHA in the feed need to be appropriately adjusted.

5. Conclusion

Exogenous fatty acids rich in DHA can accumulate in tilapia muscle in significant amounts. However, excessive intake of DHA oil sources may cause oxidative damage to the hepatopancreas of tilapia, posing a potential threat to health. Tilapia exhibits a selective preference for α-LNA as a substrate for PUFAs synthesis. The relative proportion of n-3 fatty acids in tilapia muscle can be significantly increased while ensuring body health by adjusting the intake ratio of α-LNA, EPA, and DHA.

Data Availability

The data obtain in the present study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was supported by grants from the Hainan Provincial Natural Science Foundation of China (320QN209) and the Open project of Key Laboratory of Aquatic Animal Nutrition, Jiangsu (KJS2107).

References

[1] T. Watanabe, "Lipid nutrition in fish," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 73, no. 1, pp. 3–15, 1982.

[2] G. Pang, Y. Li, Z. Zhang et al., "Evaluation of the effects of diets containing different lipid levels for juvenile yellowfin seabream *Acanthopagrus latus*," *Aquaculture Reports*, vol. 34, Article ID 101898, 2024.

[3] S. Chatzifotis, M. Panagiotidou, N. Papaioannou, M. Pavlidis, I. Nengas, and C. C. Mylonas, "Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles," *Aquaculture*, vol. 307, no. 1-2, pp. 65–70, 2010.

[4] B. D. Glencross, "Exploring the nutritional demand for essential fatty acids by aquaculture species," *Reviews in Aquaculture*, vol. 1, pp. 71–124, 2009.

[5] A. Balić, D. Vlašić, K. Žužul, B. Marinović, and Z. B. Mokos, "Omega-3 versus omega-6 polyunsaturated fatty acids in the prevention and treatment of inflammatory skin diseases," *International Journal of Molecular Sciences*, vol. 21, no. 3, Article ID 741, 2020.

[6] W. Zhu, R. Dong, L. Ge et al., "Effects of dietary n-6 polyunsaturated fatty acids (PUFA) composition on growth performances and non-specific immunity in pacific white shrimp (*Litopenaeus vannamei*)," *Aquaculture Reports*, vol. 28, Article ID 101436, 2023.

[7] C. Wei, C. Liu, X. Wang, H. Zhou, K. Mai, and G. He, "Dietary arachidonic acid supplementation improves the growth performance and alleviates plant protein-based diet-induced inflammation in juvenile turbot (*Scophthalmus maximus* L.)," *Aquaculture Nutrition*, vol. 27, no. 2, pp. 533–543, 2021.

[8] M. V. Bell, R. J. Henderson, and J. R. Sargent, "The role of polyunsaturated fatty acids in fish," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 83, no. 4, pp. 711–719, 1986.

[9] J.-L. Guo, Y.-L. Zhou, H. Zhao, W.-Y. Chen, Y.-J. Chen, and S.-M. Lin, "Effect of dietary lipid level on growth, lipid metabolism and oxidative status of largemouth bass, *Micropterus salmoides*," *Aquaculture*, vol. 506, pp. 394–400, 2019.

[10] N. K. A. Hamid, G. Carmona-Antoñanzas, Ó. Monroig, D. R. Tocher, G. M. Turchini, and J. A. Donald, "Isolation and functional characterisation of a *fads2* in rainbow trout (*Oncorhynchus mykiss*) with Δ5 desaturase activity," *PLOS ONE*, vol. 11, no. 3, Article ID e0150770, 2016.

[11] S. Tanomman, M. Ketudat-Cairns, A. Jangprai, and S. Boonanuntanasarn, "Characterization of fatty acid delta-6 desaturase gene in Nile tilapia and heterogenous expression in *Saccharomyces cerevisiae*," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 166, no. 2, pp. 148–156, 2013.

[12] D. Xie, C. Chen, Y. Dong et al., "Regulation of long-chain polyunsaturated fatty acid biosynthesis in teleost fish," *Progress in Lipid Research*, vol. 82, Article ID 101095, 2021.

[13] O. Monroig, D. R. Tocher, and L. F. C. Castro, "Polyunsaturated fatty acid biosynthesis and metabolism in fish," in *Polyunsaturated Fatty Acid Metabolism. Elsevier*, pp. 31–60, Elsevier, 2018.

[14] D. R. Tocher, "Fatty acid requirements in ontogeny of marine and freshwater fish," *Aquaculture Research*, vol. 41, pp. 717–732, 2010.

[15] C.-Y. Teoh, G. M. Turchini, and W.-K. Ng, "Erratum to, "Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend"," *Aquaculture*, vol. 316, pp. 144–154, 2011.

[16] N. Hastings, M. K. Agaba, D. R. Tocher et al., "Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs involved in the production of eicosapentaenoic and docosahexaenoic acids from α-linolenic acid in Atlantic salmon (*Salmo salar*)," *Marine Biotechnology*, vol. 6, no. 5, pp. 463–474, 2004.

[17] N. Hastings, M. Agaba, D. R. Tocher et al., "A vertebrate fatty acid desaturase with Δ5 and Δ6 activities," *Proceedings of the National Academy of Sciences*, vol. 98, no. 25, pp. 14304–14309, 2001.

[18] C. Nasopoulou and I. Zabetakis, "Benefits of fish oil replacement by plant originated oils in compounded fish feeds. A review," *LWT*, vol. 47, pp. 217–224, 2012.

[19] A. Figueiredo-Silva, E. Rocha, J. Dias et al., "Partial replacement of fish oil by soybean oil on lipid distribution and liver histology in European sea bass (*Dicentrarchus labrax*) and rainbow trout

(*Oncorhynchus mykiss*) juveniles," *Aquaculture Nutrition*, vol. 11, no. 2, pp. 147–155, 2005.

[20] G. Rosenlund, A. Obach, M. G. Sandberg, H. Standal, and K. Tveit, "Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L.)," *Aquaculture Research*, vol. 32, pp. 323–328, 2001.

[21] P. D. Nichols, B. Glencross, J. R. Petrie, and S. P. Singh, "Readily available sources of long-chain omega-3 oils: is farmed Australian seafood a better source of the good oil than wild-caught seafood?" *Nutrients*, vol. 6, no. 3, pp. 1063–1079, 2014.

[22] B. Ruyter, N. H. Sissener, T.-K. Østbye et al., "*n*-3 canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon," *The British Journal of Nutrition*, vol. 122, no. 12, pp. 1329–1345, 2019.

[23] C. Chen, J. Chen, S. Wang, C. You, and Y. Li, "Effects of different dietary ratios of linolenic to linoleic acids or docosahexaenoic to eicosapentaenoic acids on the growth and immune indices in grouper, *Epinephelus coioides*," *Aquaculture*, vol. 473, pp. 153–160, 2017.

[24] Y. Li, X. Liang, Y. Zhang, and J. Gao, "Effects of different dietary soybean oil levels on growth, lipid deposition, tissues fatty acid composition and hepatic lipid metabolism related gene expressions in blunt snout bream (*Megalobrama amblycephala*) juvenile," *Aquaculture*, vol. 451, pp. 16–23, 2016.

[25] G. M. Turchini, B. E. Torstensen, and W.-K. Ng, "Fish oil replacement in finfish nutrition," *Reviews in Aquaculture*, vol. 1, pp. 10–57, 2009.

[26] S. Glencross, "Optimizing the essential fatty acids, eicosapentaenoic and docosahexaenoic acid, in the diet of the prawn, *Penaeus monodon*," *Aquaculture Nutrition*, vol. 7, pp. 101–112, 2001.

[27] K. D. Thompson, M. F. Tatner, and R. J. Henderson, "Effects of dietary (n-3) and (n-6) polyunsaturated fatty acid ratio on the immune response of Atlantic salmon, *Salmo salar* L." *Aquaculture Nutrition*, vol. 2, pp. 21–31, 1996.

[28] N. M. Bandarra, P. Rema, I. Batista et al., "Effects of dietary n−3/n−6 ratio on lipid metabolism of gilthead seabream (*Sparus aurata*)," *European Journal of Lipid Science and Technology*, vol. 113, pp. 1332–1341, 2011.

[29] Y. Dong, Y. Wei, L. Wang et al., "Dietary n-3/n-6 polyunsaturated fatty acid ratio modulates growth performance in spotted seabass (*Lateolabrax maculatus*) through regulating lipid metabolism, hepatic antioxidant capacity and intestinal health," *Animal Nutrition*, vol. 14, pp. 20–31, 2023.

[30] T. O. Magbanua and J. A. Ragaza, "Genetic development of Nile Tilapia strains in the philippines for sustainable and efficient fish farming," *World Aquaculture*, vol. 51, no. 4, pp. 60–65, 2020.

[31] V. R. Barriviera, K. M. Tsujii, L. D. dos Santos et al., "Substitution of soybean oil with linseed oil on growth performance, fatty acid profile and texture attributes in large Nile tilapia, *Oreochromis niloticus* reared under cold suboptimal temperature," *Aquaculture Research*, vol. 52, pp. 5136–5145, 2021.

[32] U. Charoendat, N. Areechon, P. Srisapoome, and D. Chantasart, "Efficacy of synthetic eugenol as an anesthetic for Nile Tilapia (Oreochromis niloticus Linn.)," *Agriculture and Natural Resources*, vol. 43, no. 5, pp. 132–140, 2009.

[33] AOAC, *Official methods of analysis of AOAC international*, Association of Official Analysis Chemists International, 2005.

[34] J. M. McCord and I. Fridovich, "Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein)," *The Journal of Biological Chemistry*, vol. 244, no. 22, pp. 6049–6055, 1969.

[35] I. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[36] G. Bucolo and H. David, "Quantitative determination of serum triglycerides by the use of enzymes," *Clinical Chemistry*, vol. 19, no. 5, pp. 476–482, 1973.

[37] C. C. Allain, L. S. Poon, C. S. Chan, W. Richmond, and P. C. Fu, "Enzymatic determination of total serum cholesterol," *Clinical Chemistry*, vol. 20, no. 4, pp. 470–475, 1974.

[38] J. A. Buege and S. D. Aust, "Microsomal lipid peroxidation," in *Methods in Enzymology*, pp. 302–310, Elsevier, 1978.

[39] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[40] C. Chen, W. Guan, Q. Xie et al., "n-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: bioconverting LNA to DHA is relatively efficient and the LC-PUFA biosynthetic pathway is substrate limited in juvenile fish," *Aquaculture*, vol. 495, pp. 513–522, 2018.

[41] T. Thanuthong, D. S. Francis, S. P. S. D. Senadheera, P. L. Jones, and G. M. Turchini, "LC-PUFA biosynthesis in rainbow trout is substrate limited: use of the whole body fatty acid balance method and different 18:3n-3/18:2n-6 ratios," *Lipids*, vol. 46, no. 12, pp. 1111–1127, 2011.

[42] F. Pelliccia, G. Marazzi, C. Greco, F. Franzoni, G. Speziale, and C. Gaudio, "Current evidence and future perspectives on n-3 PUFAs," *International Journal of Cardiology*, vol. 170, no. 2 Suppl 1, pp. S3–S7, 2013.

[43] M. Csala, T. Kardon, B. Legeza et al., "On the role of 4-hydroxynonenal in health and disease," *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, vol. 1852, no. 5, pp. 826–838, 2015.

[44] R. Gorjão, A. K. Azevedo-Martins, H. G. Rodrigues et al., "Comparative effects of DHA and EPA on cell function," *Pharmacology & Therapeutics*, vol. 122, pp. 56–64, 2009.

[45] M. A. O. Dawood, F. I. Magouz, M. Mansour et al., "Evaluation of yeast fermented poultry by-product meal in Nile Tilapia (*Oreochromis niloticus*) feed: effects on growth performance, digestive enzymes activity, innate immunity, and antioxidant capacity," *Frontiers in Veterinary Science*, vol. 6, Article ID 516, 2020.

[46] C. B. Cowey, "Intermediary metabolism," in *Fish Nutrition*, pp. 259–329, Academic, 1989.

[47] H.-L. Cheng, N.-J. Ji, Y.-X. Peng et al., "Molecular characterization and tissue-specific expression of the acetyl-CoA carboxylase α gene from Grass carp, *Ctenopharyngodon idella*," *Gene*, vol. 487, no. 1, pp. 46–51, 2011.

[48] P. C. Calder, "Immunomodulation by omega-3 fatty acids," *Prostaglandins, Leukotrienes, and Essential fatty Acids*, vol. 77, no. 5-6, pp. 327–335, 2007.

[49] S. Sun, T. Ren, X. Li, X. Cao, and J. Gao, "Polyunsaturated fatty acids synthesized by freshwater fish: a new insight to the roles of *elovl2* and *elovl5* in vivo," *Biochemical and Biophysical Research Communications*, vol. 532, pp. 414–419, 2020.

[50] C. You, F. Lu, S. Wang, C. Chen, and Y. Li, "Comparison of the growth performance and long-chain PUFA biosynthetic ability of the genetically improved farmed tilapia (*Oreochromis niloticus*) reared in different salinities." *The British Journal of Nutrition*, vol. 121, no. 4, pp. 374–383, 2019.

[51] D. R. Tocher, X. Zheng, C. Schlechtriem, N. Hastings, J. R. Dick, and A. J. Teale, "Highly unsaturated fatty acid synthesis in marine fish: cloning, functional characterization, and nutritional regulation of fatty acyl delta 6 desaturase of Atlantic cod (*Gadus morhua* L.)." *Lipids*, vol. 41, no. 11, pp. 1003–1016, 2006.

[52] Y. Li, D. R. Tocher, Y. Pang et al., "Environmental adaptation in fish induced changes in the regulatory region of fatty acid elongase gene, *elovl5*, involved in long-chain polyunsaturated fatty acid biosynthesis," *International Journal of Biological Macromolecules*, vol. 204, pp. 144–153, 2022.

[53] M. Agaba, D. R. Tocher, C. A. Dickson, J. R. Dick, and A. J. Teale, "Zebrafish cDNA encoding multifunctional fatty acid elongase involved in production of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acids," *Marine Biotechnology*, vol. 6, no. 3, pp. 251–261, 2004.

[54] L. F. C. Castro, D. R. Tocher, and O. Monroig, "Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of fads and elovl gene repertoire," *Progress in Lipid Research*, vol. 62, pp. 25–40, 2016.

[55] D. R. Tocher, "Metabolism and functions of lipids and fatty acids in teleost fish," *Reviews in Fisheries Science*, vol. 11, pp. 107–184, 2003.

[56] Y. Luo, J. G. Jiao, A. H. Jin et al., "Metabolism of linoleic and linolenic acids in muscle cells of two freshwater fish with n-3 or n-6 fatty acid requirements," *Aquaculture*, vol. 563, Part 2, Article ID 738994, 2023.

[57] S. D. Senadheera, G. M. Turchini, T. Thanuthong, and D. S. Francis, "Effects of dietary α-linolenic acid (18: 3n-3)/ linoleic acid (18: 2n-6) ratio on fatty acid metabolism in murray cod (*Maccullochella peelii peelii*)," *Journal of Agricultural and Food Chemistry*, vol. 59, pp. 1020–1030, 2011.

[58] U. L. J. de Sousa, M. D. Koss, M. Fillies et al., "CPT1α over-expression increases long-chain fatty acid oxidation and reduces cell viability with incremental palmitic acid concentration in 293T cells," *Biochemical and Biophysical Research Communications*, vol. 338, pp. 757–761, 2005.

[59] X. Zhou, W. Wu, J. Chen, X. Wang, and Y. Wang, "AMP-activated protein kinase is required for the anti-adipogenic effects of alpha-linolenic acid," *Nutrition & Metabolism*, vol. 12, pp. 1–8, 2015.

[60] J. M. Hawkins, W. E. Jones, F. W. Bonner, and G. G. Gibson, "The effect of peroxisome proliferators on microsomal, peroxisomal, and mitochondrial enzyme activities in the liver and kidney," *Drug Metabolism Reviews*, vol. 18, no. 4, pp. 441–515, 1987.

[61] A. J. Morash, M. Kajimura, and G. B. McClelland, "Intertissue regulation of carnitine palmitoyltransferase I (CPTI): mitochondrial membrane properties and gene expression in rainbow trout (*Oncorhynchus mykiss*)," *Biochimica et Biophysica Acta (BBA)-Biomembranes*, vol. 1778, pp. 1382–1389, 2008.

[62] W. Ng and N. Romano, "A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle," *Reviews in Aquaculture*, vol. 5, pp. 220–254, 2013.

[63] Z. Sun, X. Tan, Q. Liu et al., "Physiological, immune responses and liver lipid metabolism of orange-spotted grouper (*Epinephelus coioides*) under cold stress," *Aquaculture*, vol. 498, pp. 545–555, 2019.

[64] G. Carmona-Antoñanzas, D. R. Tocher, L. Martinez-Rubio, and M. J. Leaver, "Conservation of lipid metabolic gene transcriptional regulatory networks in fish and mammals," *Gene*, vol. 534, no. 1, pp. 1–9, 2014.

[65] B. Hatlen, T. Larsson, T.-K. Østbye, O. H. Romarheim, L. M. Rubio, and B. Ruyter, "Improved fillet quality in harvest-size Atlantic salmon fed high n-3 canola oil as a DHA-source," *Aquaculture*, vol. 560, Article ID 738555, 2022.

[66] M. S. Izquierdo, D. Montero, L. Robaina, M. J. Caballero, G. Rosenlund, and R. Ginés, "Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding," *Aquaculture*, vol. 250, no. 1-2, pp. 431–444, 2005.

[67] M. Monteiro, A. Marques, R. S. Costa et al., "Beyond fish oil: assessing the implications of alternative dietary lipid sources for turbot (*Scophthalmus maximus*) on growth, nutrient utilization and muscle quality," *Aquaculture*, vol. 578, Article ID 740073, 2024.