Hindawi
Aquaculture Research
Volume 2023, Article ID 4869006, 9 pages
https://doi.org/10.1155/2023/4869006

Research Article

Effect of Supplemental Dietary α-linolenic Acid (18:3n-3) on the Growth Performance, Body Composition, and Fatty Acid Profile of Coho Salmon (*Oncorhynchus kisutch*) Alevins Cultured in Freshwater

Hairui Yu,[1] **Lingyao Li**,[1,2] **Leyong Yu**,[1] **Ling Zhang**,[4] **Fanghui Li**,[1,3] **Mengjie Guo**,[1,3] **Jiayi Zhang**,[1] **Jiyun Hou**,[1] **and Yijing Zhang**[1]

[1]*Key Laboratory of Biochemistry and Molecular Biology in Universities of Shandong (Weifang University), Weifang Key Laboratory of Coho Salmon Culturing Facility Engineering, Institute of Modern Facility Fisheries, College of Biology and Oceanography, Weifang University, Weifang 261061, China*
[2]*Shandong Collaborative Innovation Center of Coho Salmon Health Culture Engineering Technology, Shandong Conqueren Marine Technology Co. Ltd, Weifang 261108, China*
[3]*Guangxi Key Laboratory for Polysaccharide Materials and Modifications, Key Laboratory of Protection and Utilization of Marine Resources, Guangxi Minzu University, Nanning 530008, China*
[4]*Shenzhen Institute of Quality and Safety Inspection and Research, Shenzhen 518101, China*

Correspondence should be addressed to Hairui Yu; yhr6003@hotmail.com

Received 28 October 2022; Revised 9 December 2022; Accepted 16 December 2022; Published 7 February 2023

Academic Editor: Janice Ragaza

The present study evaluated the effects of supplemental dietary α-linolenic acid (ALA, 18:3n-3) on growth performance, body composition, hepatic fat metabolizing enzymes, and fatty acid profiles of coho salmon (*Oncorhynchus kisutch*) alevins cultured in freshwater. Six isonitrogenous and isolipidic experimental diets were formulated to attain different levels of ALA (0.09, 0.41, 0.76, 1.03, 1.32, and 1.68% dry weight) by adding linseed oil in a basal diet, respectively. Each diet was fed to triplicate groups of alevins (50 fish in each tank) with initial mean body weight of 0.352 ± 0.002 g. Fish were reared in a freshwater flow-through rearing system and fed to apparent satiation 4 times daily for 12 weeks. The specific growth rate (SGR) increased with increasing dietary ALA level, which reached the peak at 1.03% ALA group ($p < 0.05$). The final body weight (FBW) and SGR showed linear, quadratic, and cubic responses with the dietary ALA level. The whole-body crude protein increased with increasing dietary ALA level while lipid content was the opposite trend ($p < 0.05$). However, the content of crude lipid in the whole-body of fish showed quadratic and cubic responses with the dietary ALA level. In addition, except for fatty acid synthase (FAS), the hepatic enzymes activities of lipoprotein lipase (LPL), hepatic lipase (HL), and malate dehydrogenase (MDH) showed linear, quadratic, and cubic response with the dietary ALA level ($p < 0.05$) The activities of hepatic LPL and HL in 1.32% ALA group were significantly higher than other groups ($p < 0.05$). The activities of hepatic MDH and FAS in 1.32% ALA group were the lowest, which were significantly lower than those in ALA groups (form 0.09% to1.03%) ($p < 0.05$). Except for the content of MUFA, the contents of other fatty acids showed linear, quadratic, and cubic response with the dietary ALA level. The ALA and ∑n-3 PUFA content significantly increased with increasing dietary ALA levels ($p < 0.05$). These results indicated that dietary ALA actively impacted fish growth performance, hepatic lipid metabolizing enzymes, and muscle fatty acid profile. The cubic regression analysis based on SGR as evaluation indices indicated that the optimal dietary ALA content was 1.33% dry weight of diet for alevins.

1. Introduction

As the main component of fat, fatty acids (FA) are composed of a terminal carboxyl group and a long hydrocarbon chain. The FA can be divided into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) [1, 2]. For fish, the essential fatty acids (EFAs) are recognized to comprise PUFA with carbon chain lengths of 18 and highly unsaturated fatty acids (HUFA) with carbon chain lengths of 20 and 22, both the n-3 and n-6 series [1]. The EFA cannot be synthesized by fish themselves and need to be obtained from feed to maintain growth and normal physiological functions. The n-3 and n-6 series of PUFA are important EFA for fish [3]. There are certain differences in the EFA requirements of fish due to differences in fish species and growth environments. Tocher and Dick [4] found that salinity had a great impact on the EFA requirements of fish and there was a big difference between freshwater fish and marine fish. The main reason is that different living environment, diet, and access to preformed dietary DHA leads to different synthesis of long-chain PUFA. Since freshwater fish could effectively convert α-linolenic acid (ALA, 18:3n-3) to docosahexaenoic acid (DHA, 22:6n-3), marine fish lack this ability to extend. ALA is a major EFA in freshwater fish [5, 6].

PUFA plays important roles in maintaining the integrity of fish body cell structure and function, which regulates the activity of protein kinase C and the immune response, promotes the biological properties of growth factors, and also can reduce cholesterol and triglycerides [7–9]. ALA is the parent of the omega-3 series of FAs, which have 18 carbon atoms and three methylene discontinuous double bonds, starting at number 3 carbon at the omega end of the molecule (as opposed to the carboxyl group) [10]. It is important to promote animal growth, enhance immunity, improve meat quality, decrease blood lipids, and so on. Because n-3 fatty acids include ALA and DHA, which can be used to maintain the fluidity and permeability of biofilm at low temperature, it is generally believed that the requirement of n-3 fatty acids for cold-water fish is greater than that of n-6 fatty acids [11–14]. In addition, bioconversion of ALA is reported to account for 25% of total net intake in the diets of Atlantic salmon (*Salmo Salar*) that have completely replaced fish oil with camelina oil (ALA content of camelina oil is 30% total fatty acids) [13, 15].

Coho salmon (*Oncorhynchus kisutch*) is an important aquaculture Pacific salmon species [16], which belongs to cold-water fish and higher economic and nutritional value. In recent years, farming of salmon has started to be promoted in China. Previous studies have shown that the EFA demand greatly varies depending on the fish species, growth stage, and environment of the fish, but most studies focus on juvenile and adult fish stages, and studies on EFAs in the early stage have not attracted enough attention [4, 17–19]. Currently, linoleic acid (18 : 2n-6, LA) and eicosapentaenoic acid (20:5n-3, EPA) are characterized as EFAs [20, 21], there is a lack of information about the ALA requirements for this species. Therefore, this study investigated the effects of dietary ALA levels on the growth, body composition, and fatty acid profile of coho salmon alevins. To determine the optimal requirement of ALA is also helpful to improve the database of nutrition parameters of this fish species.

2. Materials and Methods

2.1. Ethics Statement. The study was performed in strict accordance with the commendations of Care and Use of Laboratory Animals in China, Animal Ethical and Welfare Committee of China Experimental Animal Society. The experimental protocol and procedures were approved by the Institutional Animal Care and Use Committee of Weifang University (approval number 202104132) (Weifang, China).

2.2. Experimental Diets. Six experimental diets were prepared by supplementing with graded levels (0.00, 0.60, 1.20, 1.80, 2.40, and 3.00%) of linseed oil (Fatty acid profile: C16:0, 5.83%; C18:0, 2.91%; C18:1n-9, 21.12%; C18:2n-6, 13.18%; and C18:3n-3, 54.32%). The final concentration of ALA in diets was 0.09, 0.41, 0.76, 1.03, 1.32, and 1.68% (Tables 1 and 2), respectively. The diets were made by using a double-screw extruder with a 2.0-mm-diameter pellet. No steam was used and the pellet temperature at diets was ranged from 90 to 100°C. And dried in a ventilated oven for 12 h at 50°C, ground and sieved into particles of 0.6–0.8 mm size, and stored in plastic bags at −20°C until used.

2.3. Experimental Procedures. Feed-trained coho salmon alevins were obtained from one base of Shandong Collaborative Innovation Center of Coho Salmon Health Culture Engineering Technology, Linyi, China. A total of 900 coho salmon alevins (initial average weight 0.352 ± 0.002 g) were randomly assigned to 18 experimental fiberglass tanks (80 cm × 60 cm × 60 cm, water volume 240-L) connected to freshwater in a re-circulating aquaculture system. Each diet treatment contained triplicate tanks (50 fish/tank). The feeding management was followed by the previous report [22]. The alevins were fed four times a day at 7 : 30, 11 : 00, 14 : 30, and 18 : 00 for 12 weeks.

2.4. Sampling Procedures. At the end of the feeding trial, fish were fasted 24 hrs, and then anaesthetized (MS 222, 20 mg·L^{-1}). Fish in each tank were netted out before being bulk weighed and counted. Nine fish were randomly from each tank stored in −20°C for chemical composition analyses. Six anesthetic fish were rapidly dissected, removed liver and muscle, and stored at −80°C until analysis of hepatic biochemical activities and muscle FA composition. Three additional anaesthetized fish were used to record fish body lengths and weights, and then dissected to excise liver and intestine for morphological indices. The liver samples were then frozen in liquid nitrogen and stored at −80°C for determining hepatic lipoprotein lipase (LPL), hepatic lipase (HL), malate dehydrogenase (MDH), and fatty acid synthase (FAS) activity.

Table 1: Formulation and proximate composition of the experimental diets for *Oncorhynchus kisutch* alevins (% in dry matter).

Ingredients	Dietary ALA level (%)					
	0.00	0.60	1.20	1.80	2.40	3.00
Degreasing fish meal[1]	40.00	40.00	40.00	40.00	40.00	40.00
Soybean protein concentrate[1]	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal[1]	20.00	20.00	20.00	20.00	20.00	20.00
Peanut meal[1]	9.80	9.80	9.80	9.80	9.80	9.80
α-Starch[1]	13.80	13.80	13.80	13.80	13.80	13.80
Sodium alginate[1]	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil[1]	2.00	2.00	2.00	2.00	2.00	2.00
Lard oil	6.00	5.40	4.80	4.20	3.60	3.00
Linseed oil[2]	0.00	0.60	1.20	1.80	2.40	3.00
Mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin C phosphate	0.05	0.50	0.50	0.50	0.50	0.50
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Proximate composition						
Moisture (%)	7.73	7.71	7.73	7.75	7.21	7.98
Crude protein (%)	46.91	46.80	46.88	46.83	46.95	46.97
Crude lipid (%)	12.30	12.89	12.87	12.79	12.82	12.45
Ash (%)	9.31	9.34	9.51	9.32	9.31	9.30
Linolenic acid (%)	0.09	0.41	0.76	1.03	1.32	1.68

[1]Provided by Shandong Conqueren Marine Technology Co., Ltd., Weifang, China. [2]Linseed oil: ALA content, 50%. [3]Composition (mg/kg mineral premix): AlK $(SO_4)_2{\cdot}12H_2O$, 123.7; $CaCl_2$, 17879.8; $CuSO_4{\cdot}5H_2O$, 31.7; $CoCl_2{\cdot}6H_2O$, 48.9; $FeSO_4{\cdot}7H_2O$, 707.4; $MgSO_4{\cdot}7H_2O$, 4316.8; $MnSO_4{\cdot}4H_2O$, 31.1; $ZnSO_4{\cdot}7H2O$, 176.7, KCl, 1191.9; KI, 5.3; NaCl, 4934.5; $Na_2SeO_3{\cdot}H_2O$, 3.4; Ca $(H_2PO_4)_2{\cdot}H_2O$, 12457.0; KH_2PO_4, 9930.2. [4]Composition (IU or g/kg vitamin premix): retinal palmitate, 10,000 IU; cholecalciferol, 4,000 IU; α-tocopherol, 75.0 IU; menadione, 22.0 g/kg; thiamine-HCl, 40.0 g/kg; riboflavin, 30.0 g/kg; D-calcium pantothenate, 150.0 g/kg; pyridoxine-HCl, 20.0 g/kg; meso-inositol, 500.0 g/kg; D-biotin, 1.0 g/kg; folic acid, 15.0 g/kg; ascorbic acid, 200.0 g/kg; niacin, 300.0 g/kg; cyanocobalamin, 0.3 g/kg.

Table 2: Fatty acids composition of the experimental diets (% total fatty acids).

Fatty acids	Dietary ALA level (%)					
	0.09	0.41	0.76	1.03	1.32	1.68
C14:0	0.36	0.34	0.32	0.30	0.27	0.26
C16:0	11.10	11.05	11.00	10.92	10.70	10.69
C18:0	16.81	12.77	9.73	6.63	3.48	0.43
∑SFA	28.27	24.16	21.05	17.85	14.45	11.38
C16:1n-7	0.34	0.36	0.40	0.42	0.41	0.44
C18:1n-9	0.38	1.74	3.23	5.22	5.60	7.13
∑MUFA	0.72	2.1	3.63	5.64	6.01	7.57
C18:3n-3	0.74	3.38	6.26	10.13	10.87	13.83
C20:5n-3	0.42	0.49	0.49	0.46	0.48	0.46
C22:6n-3	0.45	0.51	0.54	0.56	0.58	0.57
∑n-3 PUFA	1.61	4.38	7.29	11.15	11.93	14.56

2.5. Growth Performance. The calculation formulae for the indexes mentioned above are as follows:

$$\text{Survival rate (SR, \%)} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100,$$

$$\text{Specific growth rate}\left(\text{SGR}, \frac{\%}{\text{day}}\right) = \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{days}} \times 100,$$

$$\begin{aligned}\text{Condition factor}\left(\text{CF}, \frac{g}{\text{cm}^3}\right) &= 100 \times \left(\frac{\text{body weight}}{\text{body length}^3}\right),\\ \text{Hepatosomatic index (HSI, \%)} &= 100 \times \left(\frac{\text{liver weight}}{\text{body weight}}\right),\\ \text{Intestine somatic index (ISI, \%)} &= 100 \times \left(\frac{\text{intestine weight}}{\text{body weight}}\right).\end{aligned} \quad (1)$$

2.6. Assays for Chemical Composition and Fatty Acid Profile. The proximate compositions of diets and tissue samples were measured according to AOAC [23]. The whole-body sample was freeze-dried for 48 h and ground in a mortar. Then, FAs were detected with a GC-MS chromatograph (Agilent Technologies 7890-5977A, USA) according to a previous method [24].

2.7. Assays for Testing Lipid Metabolism-Related Enzymes Activities. Liver samples were homogenized in 0.1 M pH 7.4 Tris-HCl buffers at 4°C. The supernatants were used for enzyme analysis. The activities of lipase, including LPL and HL activity, were also determined using a commercial kit by the procedures described by Dong et al. [25]. LPL and HL can decompose triglycerides (TG) and hydrolyze them into glycerol and free fatty acids (FFAs). LPL and HL activity can be determined by the amount of FFA produced with a copper reagent. HL belonging glycoprotein is not needed to be activated by apolipoprotein CII and some ions and is not inhibited by high-concentration salt and protamine. 1 μmol of free fatty acid produced by per mg of tissue protein per hour at 37.0°C in the reaction system, namely, is regarded as one unit of enzyme activity (FFA μmol/mg prot. hour). The reaction mixture was used for estimation of malate dehydrogenase (MDH) with different substrate as 1 mg oxaloacetate/mL of chilled triple distilled water [26]. The activities of FA synthetase (FAS) were assayed with the commercial kits purchased from Zhuocai biology Co., Ltd. (Shanghai, China) [27].

2.8. Statistical Analysis. Results are presented as mean ± SD. The normality and homogeneity of variances among groups were tested and results were subjected to one-way analysis of variance (ANOVA), Duncan multiple comparison was used for the significantly different groups. With ALA level as independent variable, linear, quadratic, and cubic regression analyses were performed on the responses data. Difference was considered significant at $p < 0.05$. All the result data was performed using SPSS version 20.0 software (SPSS Inc., USA).

3. Results

3.1. Survival and Growth Performance. No significant differences were observed for SR among the dietary groups based on the linear analysis ($p > 0.05$), but which showed quadratic and cubic responses with the dietary ALA level (Table 3). The CF, HSI, and ISI of fish were not significantly affected by the dietary ALA level ($p > 0.05$). The final body weight (FBW) and SGR showed linear, quadratic, and cubic responses with the dietary ALA level. With the increase of the dietary ALA content, SGR increased significantly, the fish fed diet with 1.32% ALA level had the highest SGR, which was significantly higher than that of the 0.09% ALA group ($p < 0.05$), but did not differ significantly from 0.41% to 1.68% ALA groups ($p > 0.05$). Based on the cubic regression analysis, the optimal dietary ALA level for maximum SGR was 1.33% ($Y = -0.048X^3 - 0.016X^2 + 0.2962 + 2.8939$, $R^2 = 1.0000$) (Figure 1).

3.2. Proximate Composition and Fatty Acid Profile. The whole-body moisture and ash contents did not differ significantly among dietary groups ($p > 0.05$) (Table 4). The whole-body crude protein significantly increased with increasing levels of dietary ALA from 0.09% to 1.68% ($p < 0.05$). The content of crude lipid in the whole-body of fish showed quadratic and cubic responses with the dietary ALA level. Compared to the dietary 0.09% ALA level, the ALA levels of 0.41, 0.76, 1.03, 1.32, and 1.68% ALA levels decreased the content of crude lipid in the whole-body of fish; however, there was no significant difference between 0.09% and 1.32% ALA level groups ($p > 0.05$).

Except for the content of MUFA, the contents of other fatty acids showed linear, quadratic, and cubic response with the dietary ALA level. The content of C14:0, C16:0, and C18:0 fatty acids were significantly decreased, but that of C18:3n-3 was significantly increased with increasing dietary ALA levels. Compared to the fish fed 0.09% ALA level, the fish fed ALA diets supplemented resulted in a decrease of SFA and MUFA in whole-body fish ($p < 0.05$, Table 5). Fish fed diet with 1.32% ALA level had the lowest SFA and MUFA contents. The C18:3n-3 and n-3 PUFA in the whole-body were higher in fish fed ALA diets than those in fish fed 0.09% ALA level ($p < 0.05$). The contents of EPA and DHA in the whole body were significantly decreased with increasing dietary ALA, and got the lowest with increasing dietary ALA level to 1.32%.

3.3. Lipid Metabolism-Related Enzymes Activities. The hepatic enzymes activities of LPL and HL showed linear, quadratic, and cubic response with the dietary ALA level ($p < 0.05$), which significantly increased as dietary ALA level increased from 0.09% to 1.32%, and then showed a significant decrease trend when ALA level further increased to

Table 3: Survival and growth performance and feed utilization of *Oncorhynchus kisutch* alevins fed the experimental diets with different dietary ALA level for 12 weeks (means ± SD, $n = 3$).

	Dietary ALA level (%)						SEM	Regression analysis (P, R^2)		
	0.09	0.41	0.76	1.03	1.32	1.68		Linear	Quadratic	Cubic
SR	98.43	98.57	98.78	98.89	98.80	98.50	0.036	0.252, 0.059	0.000, 0.798	0.000, 0.930
IBW (g)	0.351	0.352	0.351	0.353	0.352	0.351	0.000	0.303, 0.048	0.559, 0.054	0.762, 0.055
FBW (g)	4.08^{a}	4.41^{ab}	4.70^{ab}	4.89^{b}	4.95^{b}	4.81^{b}	0.089	0.001, 0.379	0.001, 0.491	0.003, 0.496
SGR (%/day)	2.92^{a}	3.01^{ab}	3.09^{ab}	3.13^{b}	3.15^{b}	3.12^{b}	0.023	0.001, 0.409	0.000, 0.536	0.001, 0.538
CF (g/cm^3)	1.18	1.14	1.09	1.16	1.09	1.10	0.036	0.536, 0.039	0.814, 0.045	0.934, 0.050
HSI (%)	1.14	1.04	1.42	1.39	1.24	1.03	0.058	0.971, 0.000	0.117, 0.379	0.156, 0.461
ISI (%)	1.61	1.22	1.60	1.50	1.47	1.66	0.057	0.442, 0.060	0.445, 0.164	0.567, 0.213

Means in the same raw with different superscript letters are significantly different ($p < 0.05$).

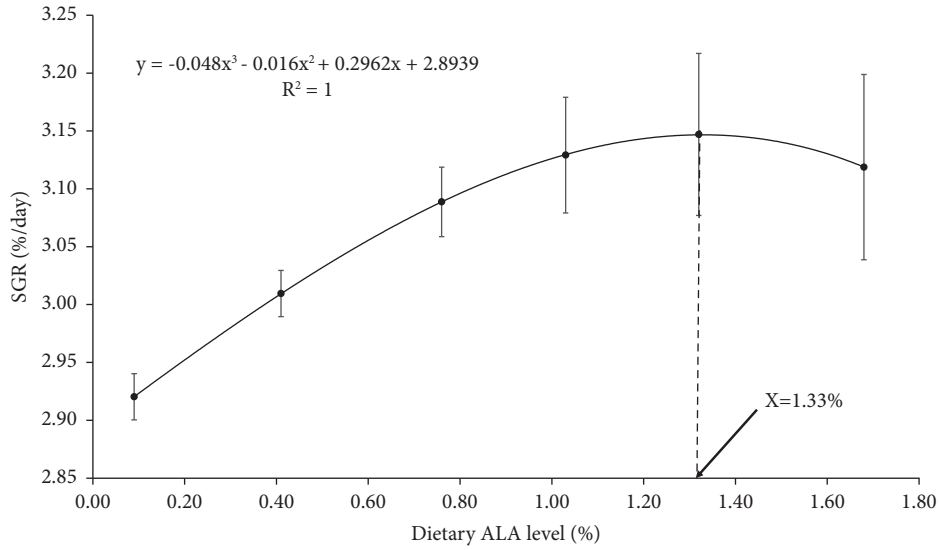

Figure 1: Relationship between specific growth rate (SGR) and dietary α-linolenic acid (ALA) levels based on the cubic regression analysis showed the predicted dietary ALA requirement was 1.33% for *Oncorhynchus kisutch* alevins.

Table 4: Whole-body composition of *Oncorhynchus kisutch* alevins fed the experimental diets with different dietary ALA level for 12 weeks (means ± SD, $n = 3$).

	Dietary ALA level (%)						SEM	Regression analysis (P, R^2)		
	0.09	0.41	0.76	1.03	1.32	1.68		Linear	Quadratic	Cubic
Moisture (%)	77.85	77.36	77.37	77.39	77.39	77.31	0.077	0.095, 0.253	0.129, 0.366	0.126, 0.493
Crude protein (%)	12.23^{a}	12.63^{a}	12.70^{a}	13.79^{b}	13.99^{b}	14.11^{b}	0.232	0.264, 0.123	0.419, 0.176	0.510, 0.239
Crude lipid (%)	6.55^{b}	6.48^{b}	6.38^{ab}	5.18^{ab}	5.07^{ab}	4.86^{a}	0.251	0.451, 0.058	0.015, 0.609	0.016, 0.708
Ash (%)	3.27	3.23	3.24	3.25	3.22	3.27	0.009	0.053, 0.324	0.170, 0.325	0.180, 0.439

Means in the same column with different superscript letters are significantly different ($p < 0.05$).

1.68% ($p < 0.05$) (Table 6, Figures 2(a) and 2(b)). However, compared to the 0.09% ALA group, the hepatic LPL and HL activities of the 1.68% ALA group were significantly increased ($p < 0.05$), the highest value were found in 1.32% group. Similarly, the hepatic enzymes activity of MDH showed linear, quadratic, and cubic response ($p < 0.05$), however, which decreased significantly with the increased of ALA levels ($p < 0.05$) (Table 6, Figure 2(c)). The hepatic enzymes activity of FAS decreased with the increase of dietary ALA level, and the lowest value was found in 1.68% group ($p < 0.05$) (Table 6, Figure 2(d)).

4. Discussion

ALA has an important physiological function, since it is an EFA for freshwater fish. As one of the best vegetable oil sources, linseed oil contains a large amount of ALA [28, 29]. The decomposition of ALA provides energy for the growth of organisms. The result showed that growth performance of alevins was greatly affected by the dietary levels of ALA and could be enhanced remarkably by the diets rich in ALA, which was in agreement with previous studies in European sea bass [30], Japanese seabass (*Lateolabrax japonicus*) [31],

Table 5: Effects of different dietary ALA levels on fatty acid composition of *Oncorhynchus kisutch* alevins (Mean ± SD).

	Dietary ALA level (%)						SEM	Regression analysis (P, R^2)		
	0.09	0.41	0.76	1.03	1.32	1.68		Linear	Quadratic	Cubic
C14:0	1.97^{c}	1.85^{b}	1.77^{b}	1.58^{a}	1.51^{a}	1.57^{a}	0.050	0.000, 0.863	0.000, 0.928	0.000, 0.976
C16:0	18.49^{c}	17.81^{bc}	17.55^{b}	15.99^{a}	15.62^{a}	15.63^{a}	0.344	0.000, 0.898	0.000, 0.912	0.000, 0.955
C18:0	5.06^{d}	4.87^{c}	4.41^{b}	3.14^{a}	3.10^{a}	3.13^{a}	0.255	0.000, 0.848	0.000, 0.868	0.000, 0.954
∑SFA	25.52^{c}	24.53^{b}	23.73^{b}	20.71^{a}	20.23^{a}	20.33^{a}	0.649	0.000, 0.884	0.000, 0.903	0.000, 0.961
C16:1n-7	6.16^{d}	5.18^{c}	5.54^{c}	4.55^{b}	4.18^{a}	4.34^{ab}	0.211	0.000, 0.787	0.000, 0.823	0.002, 0.829
C18:1n-9	13.30^{d}	13.20^{d}	12.86^{c}	10.97^{b}	9.77^{a}	10.84^{b}	0.405	0.000, 0.738	0.002, 0.745	0.000, 0.947
∑MUFA	19.46^{e}	18.38^{d}	18.4^{c}	15.52^{b}	13.95^{a}	15.18^{b}	1.222	0.035, 0.373	0.106, 0.392	0.062, 0.581
C18:3n-3	0.40^{a}	3.46^{b}	5.60^{c}	9.34^{d}	9.47^{d}	9.60^{d}	1.056	0.000, 0.887	0.000, 0.967	0.000, 0.979
C20:5n-3 (EPA)	1.14^{d}	0.98^{c}	0.90^{bc}	0.90^{bc}	0.75^{a}	0.81^{ab}	0.036	0.000, 0.797	0.000, 0.885	0.000, 0.886
C22:6n-3 (DHA)	2.80^{d}	2.46^{c}	2.45^{c}	2.25^{b}	1.92^{a}	2.22^{b}	0.082	0.001, 0.693	0.000, 0.822	0.001, 0.859
∑n-3 PUFA	4.34^{a}	6.90^{b}	8.95^{c}	12.14^{d}	12.49^{e}	12.63^{e}	0.947	0.000, 0.883	0.000, 0.960	0.000, 0.970

Means in the same column with different superscript letters are significantly different ($p < 0.05$).

Table 6: Effects of different dietary ALA levels on the activity of LPL, HL, MDH, and FAS in liver of *Oncorhynchus kisutch* alevins (Mean ± SD).

	Dietary ALA level (%)						SEM	Regression analysis (P, R^2)		
	0.09	0.41	0.76	1.03	1.32	1.68		Linear	Quadratic	Cubic
LPL (U/mgprot.)	0.95^{a}	1.01^{a}	1.14^{b}	1.41^{c}	1.69^{d}	1.16^{b}	0.054	0.000, 0.432	0.002, 0.456	0.004, 0.476
HL (U/mgprot.)	0.96^{a}	1.01^{a}	1.59^{b}	1.86^{c}	2.73^{d}	1.50^{b}	0.124	0.000, 0.579	0.000, 0.716	0.000, 0.884
MDH (U/mgprot.)	1.27^{c}	1.26^{c}	1.13^{b}	1.10^{b}	0.73^{a}	0.76^{a}	0.046	0.000, 0.723	0.000, 0.771	0.000, 0.792
FAS (U/mgprot.)	2.893^{b}	2.892^{b}	2.892^{b}	2.891^{b}	2.865^{a}	2.850^{a}	0.005	0.867, 0.003	0.060, 0.464	0.147, 0.470

Means in the same column with different superscript letters are significantly different ($p < 0.05$).

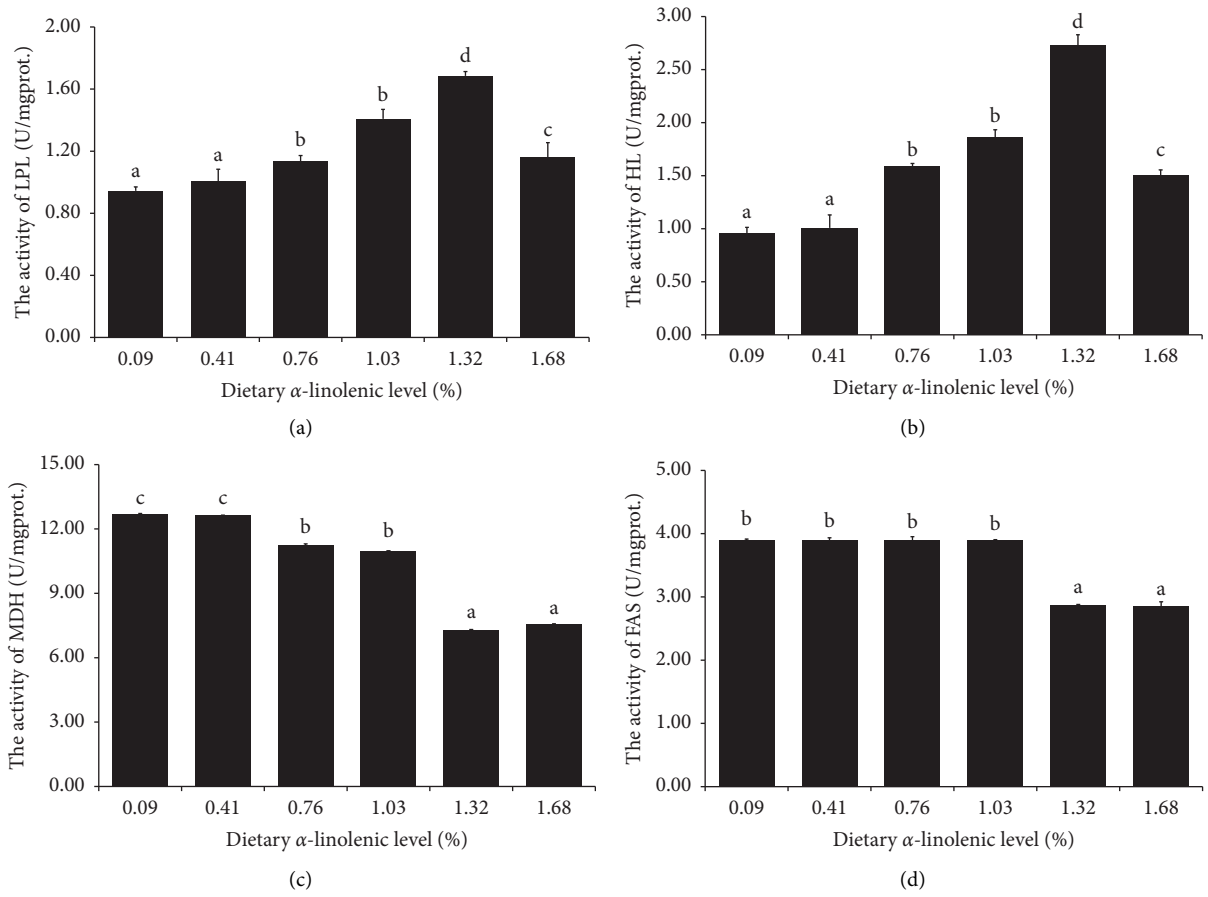

Figure 2: The activity of LPL, HL, MDH, and FAS in liver of *Oncorhynchus kisutch* alevins fed the experimental diets with graded dietary ALA levels after 12 weeks. (a) LPL; (b) HL; (c) MDH; (d) FAS.

rainbow trout [32], common carp [33], and Atlantic salmon [34, 35]. In contrast, Kanazawa et al. [36] reported that no growth improvement was found in tilapia fed a diet rich in ALA. Lacking or excess ALA in diet would impact the normal growth and development in fish. With the lacking of dietary ALA content, the feed utilization rate and SGR of yellow catfish was significantly reduced [37]. Gordon Bell et al. [38] found that 19% linseed oil supplementation in feed inhibited the growth of the juvenile turbot. It can be inferred from that adding appropriate ALA could promote the growth of fish. In addition, the differences in fish species, specifications, and breeding environment make different fish have different requirements for ALA. Fish feeding with nutritionally unbalanced diets often have abdominal swelling and liver hypertrophy, and the edible part is relatively reduced. In this experiment, the level of ALA had no significant effect on CF, HSI, and ISI. The results showed that adding ALA did not cause hepatic hypertrophy and change the form of the fish.

The studies have shown that the composition of feed has a significant effect on the chemical composition of fish [39, 40]. Different dietary ALA levels affected the whole-body protein and lipid contents of Atlantic salmon [41] and Japanese seabass [31]. In the present study, with the increase of dietary ALA content, the whole-body crude protein contents increased while the crude lipid contents decreased (quadratic and cubic responses), which indicated that the increase of dietary ALA content might decrease the whole-body lipid deposition of alevins. It is speculated that different ALA levels have effects on the expression of fatty acid synthesis and oxidation-related genes, thereby having an effect on the whole-body chemical composition. The present study showed that different ALA levels did not affect moisture and ash contents, whose result is similar to that of reported by Chen et al. [42] in Nile tilapia (*Oreochromis niloticus*).

It has also been reported that the activities of lipid metabolism-related enzymes including LPL, HL, and FAS are affected by HUFAs [43–46]. Current research indicated that low dietary ALA level significantly reduced whole-body lipid contents, which might be due to promoting the lipolysis in fish with increasing LPL and HL activities (linear, quadratic, and cubic response with the dietary ALA level). The LPL and HL activities were the highest with 1.32% ALA group, indicating that lipid utilization reached its peak at the point. However, the reduction in LPL and HL activities with dietary ALA level (>1.32% or <1.32%) might be associated with a form of liver protection, limiting lipid uptake from plasma lipoproteins. Nevertheless, Arantza-mendi [47] found that complete substitution of dietary linseed oil for fish oil did not have an effect on the LPL activity in perivisceral adipose tissue, muscle, or liver in rainbow trout. From the above, dietary ALA deficiency can affect lipid metabolism and related enzyme activities of fish. Similarly, MDH is the key enzyme for lipogenesis in the liver [48]. However, MDH activity (linear, quadratic, and cubic response) showed an opposite pattern of change compared with LPL activity in this study. Evidence suggests that MDH activity of juvenile grass carp (*Ctenopharyngodon idellus*) decreased with the increasing dietary n-3 HUFAs levels [49]. FAS enzyme is the key rate-limiting enzyme for fatty acid synthesis. This activity is of great significance for fat synthesis and fat deposition in animal body [50]. The brain is the main site for FAS transcription, followed by the kidney and liver. Studies have shown that the content of PUFA increases would inhibit FAS activity [51, 52]. In this experiment, it showed that the hepatic FAS enzyme activity decreased with the ALA content increased, which was the same as reported by Huang et al. [53] in spotted seabass (*Lateolabrax maculatus*).

The composition of dietary fatty acid directly affected the fatty acid composition of fish, which has been proved by many studies [31, 54, 55]. With the increase of dietary ALA level, muscle ALA content increased while the content of SFA and MUFA decreased. The content of ∑n-3PUFA increased in the muscle with the increased of the dietary ALA level. Similar results was also found in Nile tilapia, whereas the fish fed with flax seed oil had higher $\sum n$-3 fatty acid content than those fed with alone sunflower seed oil in Nile tilapia [56]. The muscle DHA and EPA showed downward with the increase of dietary ALA content, and the highest content obtained in 0.09% ALA content. The reason may be inferred that higher dietary ALA contents caused the increase of oxidative decomposition DHA and EPA in the muscle.

5. Conclusions

In conclusion, supplement of ALA could increase growth performance and the hepatic HL and LPL activities, and decreased the FAS and MDH activities in coho salmon alevins. Based on the results analysis of SGR, the optimal dietary ALA level for coho salmon alevins was estimated to be 1.33%.

Data Availability

The data are available upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hairui Yu designed the study, acquainted with supervised funding, project administration, and revised the article. Lingyao Li drafted the article and assisted in sampling and data analysis. LeYong Yu performed the statistical analysis and wrote the manuscript. Ling Zhang, Fanghui Li, and Mengjie Guo were responsible for sampling, assays, and data analyses. JiaYi Zhang, Ji-Yun Hou, and YiJing Zhang were responsible for the feeding experiments. All authors read and approved the final version of the manuscript.

Acknowledgments

This study was supported by the Shandong Provincial Key Research and Development Programs (Major Scientific and

Technological Innovation Projects, MSTIP), Grant/Award numbers: 2018CXGC0102 and 2019JZZY020710; Scientific and Technologic Development Program of Weifang, Grant/Award number: 2019ZJ1046; and Innovation-driven Development Special Project of Guangxi Science and Technology Major Project, Grant/Award number: AA17204044.

References

[1] D. R. Tocher, "Fatty acid requirements in ontogeny of marine and freshwater fish," *Aquaculture Research*, vol. 41, no. 5, pp. 717–732, 2010.

[2] L. Gramlich, C. Ireton-Jones, J. M. Miles, M. Morrison, and A. Pontes-Arruda, "Essential fatty acid requirements and intravenous lipid emulsions," *Journal of Parenteral and Enteral Nutrition*, vol. 43, no. 6, pp. 697–707, 2019.

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

[4] D. R. Tocher and J. R. Dick, "Essential fatty acid deficiency in freshwater fish: the effects of linoleic, alpha-linolenic, gamma-linolenic and stearidonic acids on the metabolism of [1-14C] 18:3n-3 in a carp cell culture model," *Fish Physiology and Biochemistry*, vol. 22, no. 1, pp. 67–75, 2000.

[5] R. J. Henderson, "Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids," *Archiv für Tierernaehrung*, vol. 49, no. 1, pp. 5–22, 1996.

[6] J. R. Sargent, D. R. Tocher, and J. G. Bell, "The lipids," *Fish Nutrition*, pp. 181–257, Elsevier, San Diego, CA, USA, Third edition, 2003.

[7] U. N. Das, "Long-chain polyunsaturated fatty acids in the growth and development of the brain and memory," *Nutrition*, vol. 19, no. 1, pp. 62–65, 2003.

[8] S. Yehuda, "Omega-6/omega-3 ratio and brain-related functions," *World Review of Nutrition & Dietetics*, vol. 92, pp. 37–56, 2003.

[9] C. Parolini, "Effects of fish n-3 PUFAs on intestinal microbiota and immune system," *Marine Drugs*, vol. 17, no. 6, p. 374, 2019.

[10] A. H. Stark, R. Reifen, and M. A. Crawford, "Past and present insights on alpha-linolenic acid and the omega-3 fatty acid family," *Critical Reviews in Food Science and Nutrition*, vol. 56, no. 14, pp. 2261–2267, 2016.

[11] Q. Li, H. Zhu, E. Li, J. Qin, and L. Chen, "Growth performance, lipid requirement and antioxidant capacity of juvenile Russian sturgeon *Acipenser gueldenstaedti* fed various levels of linoleic and linolenic acids," *Aquaculture Research*, vol. 48, no. 6, pp. 3216–3229, 2016.

[12] J. A. Emery, K. Hermon, N. K. A. Hamid, J. A. Donald, and G. M. Turchini, "Δ-6 Desaturase substrate competition: dietary linoleic acid (18:2n-6) has only trivial effects on α-linolenic acid (18:3n-3) bioconversion in the teleost rainbow trout," *PLoS One*, vol. 8, no. 2, Article ID e57463, 2013.

[13] S. M. Hixson, C. C. Parrish, and D. M. Anderson, "Use of camelina oil to replace fish oil in diets for farmed salmonids and Atlantic cod," *Aquaculture*, vol. 431, pp. 44–52, 2014b.

[14] G. M. Turchini and D. S. Francis, "Fatty acid metabolism (desaturation, elongation and beta-oxidation) in rainbow trout fed fish oil-or linseed oil-based diets," *British Journal of Nutrition*, vol. 102, no. 1, pp. 69–81, 2009.

[15] S. M. Hixson, C. C. Parrish, and D. M. Anderson, "Full substitution of fish oil with camelina (Camelina sativa) oil, with partial substitution of fish meal with camelina meal, in diets for farmed Atlantic salmon (*Salmo salar*) and its effect on tissue lipids and sensory quality," *Food Chemistry*, vol. 157, pp. 51–61, 2014a.

[16] J. K. Troyer, *Coho salmon (Oncorhynchus kisutch)*, pp. 15–65, USDA Forest Service-General Technical Report PNW, Fort Collins, CO, USA, 2000.

[17] M. S. Izquierdo, H. Fernández-Palacios, and A. Tacon, "Effect of broodstock nutrition on reproductive performance of fish," *Aquaculture*, vol. 197, no. 1-4, pp. 25–42, 2001.

[18] H. Quintero, E. Durland, D. A. Davis, and R. Dunham, "Effects of lipid supplementation on reproductive performance of female channel catfish *Ictalurus punctatus*, induced and strip-spawned for hybridization," *Aquaculture Nutrition*, vol. 17, 2010.

[19] E. A. Abi-Ayad, C. Melard, and P. Kestemont, "Effects of n-3 fatty acids in eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistance," *Aquaculture International*, vol. 5, pp. 161–168, 1997.

[20] H. R. Yu, L. Y. Li, C. M. Xu et al., "Effect of dietary eicosapentaenoic acid (20:5n-3) on growth performance, fatty acid profile and lipid metabolism in coho salmon (*Oncorhynchus kisutch*) alevins," *Aquaculture Reports*, vol. 23, Article ID 101084, 2022a.

[21] H. Yu, L. Li, L. Yu et al., "Effect of dietary linoleic acid (18:2n-6) supplementation on the growth performance, fatty acid profile, and lipid metabolism enzyme activities of coho salmon (*Oncorhynchus kisutch*) alevins," *Animals*, vol. 12, no. 19, p. 2631, 2022b.

[22] H. Yu, B. Chen, L. Li et al., "Dietary iron (Fe) requirement of coho salmon (*Oncorhynchus kisutch*) alevins assessed using growth, whole body and hepatic Fe concentrations and hepatic antioxidant enzymes activities," *Aquaculture Research*, vol. 52, no. 9, pp. 4489–4497, 2021a.

[23] Aoac (Association of Official Analytical Chemists), *Official Methods of Analysis*, AOAC, Arlington, VA, USA, 16 edition, 1995.

[24] H. Xu, Q. Ai, K. Mai et al., "Effects of dietary arachidonic acid on growth performance, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicus*," *Aquaculture*, vol. 307, no. 1-2, pp. 75–82, 2010.

[25] L. F. Dong, T. Tong, Q. Zhang et al., "Effects of dietary carbohydrate to lipid ratio on growth, feed utilization, body composition and digestive enzyme activities of golden pompano (*Trachinotus ovatus*)," *Aquaculture Nutrition*, vol. 24, no. 1, pp. 341–347, 2018.

[26] S. Ochoa, "Malic dehydrogenase and'malic'enzyme," in *Methods of Enzymology. I*, S. P. Coloric and N. Kaplan, Eds., pp. 735–745, Academic Press, New York, NY, USA, 1955.

[27] H. Yu, J. Wang, and D. Liu, "Effect of lipid levels on the growth performance and hepatic lipid deposition in the post-larval coho salmon (*Oncorhynchus kisutch*)," *American Journal of Biochemistry and Biotechnology*, vol. 17, no. 2, pp. 208–216, 2021b.

[28] J. G. Bell, R. J. Henderson, D. R. Tocher, and J. R. Sargent, "Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (*Salmo salar*) using a fish oil finishing diet," *Lipids*, vol. 39, no. 3, pp. 223–232, 2004.

[29] Q. Wang, G. He, and K. Mai, "Modulation of lipid metabolism, immune parameters, and hepatic transferrin expression in juvenile turbot (*Scophthalmus maximus* L.) by increasing dietary linseed oil levels," *Aquaculture*, vol. 464, no. 1, pp. 489–496, 2016.

[30] G. Mourente, J. R. Dick, J. G. Bell, and D. R. Tocher, "Effect of partial substitution of dietary fish oil by vegetable oils on desaturation and β-oxidation of [1-14C] 18:3n-3 (LNA) and [1-14C] 20:5n-3 (EPA) in hepatocytes and enterocytes of European sea bass (*Dicentrarchus labrax* L.)," *Aquaculture*, vol. 248, no. 1-4, pp. 173–186, 2005.

[31] H. Xu, Y. Zhang, J. Wang, R. Zuo, K. Mai, and Q. Ai, "Replacement of fish oil with linseed oil or soybean oil in feeds for Japanese Seabass, *Lateolabrax japonicus*: effects on growth performance, immune response, and tissue fatty acid composition," *Journal of the World Aquaculture Society*, vol. 46, no. 4, pp. 349–362, 2015.

[32] J. D. Castell, D. J. Lee, R. O. Sinnhuber, and D. J. Lee, "Essential fatty acids in the diet of rainbow trout (*Salmo gairdneri*): lipid metabolism and fatty acid composition," *Journal of Nutrition*, vol. 102, no. 1, pp. 93–99, 1972.

[33] T. Takeuchi, "Essential fatty acid requirements in carp," *Archiv für Tierernaehrung*, vol. 49, no. 1, pp. 23–32, 1996.

[34] B. Ruyter, O. Røsjø, O. Einen, and M. S. Thomassen, "Essential fatty acids in Atlantic salmon: time course of changes in fatty acid composition of liver, blood and carcass induced by a diet deficient in n-3 and n-6 fatty acids," *Aquaculture Nutrition*, vol. 6, no. 2, pp. 109–117, 2000.

[35] Nrc, *Nutrient Requirements of Fish and Shrimp*, NationalAcademic Press, Washington, DC, USA, 2011.

[36] A. Kanazawa, S. I. Teshima, M. Sakamoto, and M. A. Awal, "Requirements of *Tilapia zillii* for essential fatty acids," *Nihon Suisan Gakkai-Shi*, vol. 46, no. 11, pp. 1353–1356, 1980.

[37] M. Li, L. Chen, E. Li et al., "Growth, immune response and resistance to aeromonas hydrophila of darkbarbel catfish, Pelteobagrus vachelli (*Richardson*), fed diets with different linolenic acid levels," *Aquaculture Research*, vol. 46, no. 4, pp. 789–800, 2015.

[38] J. Gordon Bell, D. R. Tocher, F. M. Macdonald, and J. R. Sargent, "Effects of diets rich in linoleic (18:2n-6) and α-linolenic (18:3n-3) acids on the growth, lipid class and fatty acid compositions and eicosanoid production in juvenile turbot (*Scophthalmus maximus* L.)," *Fish Physiology and Biochemistry*, vol. 13, no. 2, pp. 105–118, 1994.

[39] T. Yoshikawa, H. Shimano, N. I. T. Yahagi et al., "Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements," *Journal of Biological Chemistry*, vol. 277, no. 3, pp. 1705–1711, 2002.

[40] A. C. Hansen, G. Rosenlund, Ø. Karlsen, W. Koppe, and G. I. Hemre, "Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) I-Effects on growth and protein retention," *Aquaculture*, vol. 272, no. 1-4, pp. 599–611, 2007.

[41] B. Bjerkeng, S. Refstie, K. T. Fjalestad, T. Storebakken, M. Rødbotten, and A. J. Roem, "Quality parameters of the flesh of Atlantic salmon (*Salmo salar*) as affected by dietary fat content and full-fat soybean meal as a partial substitute for fish meal in the diet," *Aquaculture*, vol. 157, no. 3-4, pp. 297–309, 1997.

[42] C. Chen, B. Sun, X. Li et al., "N-3 essential fatty acids in Nile tilapia, *Oreochromis niloticus*: quantification of optimum requirement of dietary linolenic acid in juvenile fish," *Aquaculture*, vol. 416-417, no. 2, pp. 99–104, 2013.

[43] A. Diez, D. Menoyo, S. Pérez-Benavente et al., "Conjugated linoleic acid affects lipid composition, metabolism, and gene expression in gilthead sea bream (*Sparus aurata* L)," *Journal of Nutrition*, vol. 137, no. 6, pp. 1363–1369, 2007.

[44] P. Nilsson-Ehle, A. S. Garfinkel, and M. C. Schotz, "Lipolytic enzymes and plasma lipoprotein metabolism," *Annual Review of Biochemistry*, vol. 49, no. 1, pp. 667–693, 1980.

[45] H. R. Yilmaz, A. Songur, B. Özyurt, İ. Zararsiz, M. Sarsilmaz, and M. Sarsilmaz, "The effects of n-3 polyunsaturated fatty acids by gavage on some metabolic enzymes of rat liver," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 71, no. 2, pp. 131–135, 2004.

[46] S. Santamarina-Fojo, M. Amar, C. Haudenschild, K. Dugi, and H. Brewer, "Role of hepatic lipase in lipoprotein metabolism and atherosclerosis," *Atherosclerosis*, vol. 144, no. 3, pp. 84–219, 1999.

[47] L. Arantza-mendi, *Effect of dietary lipids on production, composition and lipolytic activity in commercial fish*, PhD Thesis, University of Las Palmas de Gran Canaria, Las Palmas, Spain, 2002.

[48] J. S. Zhou, J. I. Hong, J. H. Wang, and L. X. Wang, "Influence of fish oil on growth and lipid metabolism in common carp (cyprinus carpio)," *Periodical of Ocean University of China*, vol. 38, no. 2, pp. 275–280, 2008.

[49] H. Ji, J. Li, and P. Liu, "Regulation of growth performance and lipid metabolism by dietary n-3 highly unsaturated fatty acids in juvenile grass carp, *Ctenopharyngodon idellus*," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 159, no. 1, pp. 49–56, 2011.

[50] S. Pasta, A. Witkowski, A. K. Joshi, and S. Smith, "Catalytic residues are shared between two pseudosubunits of the dehydratase domain of the animal fatty acid synthase," *Chemistry & Biology*, vol. 14, no. 12, pp. 1377–1385, 2007.

[51] L. Cruz-Garcia, J. Sánchez-Gurmaches, L. Bouraoui et al., "Changes in adipocyte cell size, gene expression of lipid metabolism markers, and lipolytic responses induced by dietary fish oil replacement in gilthead sea bream (*Sparus aurata* L)," *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 158, no. 4, pp. 391–399, 2011.

[52] S. Morais, T. Silva, O. Cordeiro et al., "Effects of genotype and dietary fish oil replacement with vegetable oil on the intestinal transcriptome and proteome of Atlantic salmon (*Salmo salar*)," *BMC Genomics*, vol. 13, no. 1, p. 448, 2012.

[53] H. Huang, Y. Zhang, M. Cao, L. Xue, and W. Shen, "Effects of fasting on the activities and mRNA expression levels of lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) and fatty acid synthetase (FAS) in spotted seabass Lateolabrax maculatus," *Fish Physiology and Biochemistry*, vol. 44, no. 1, pp. 387–400, 2018.

[54] J. Trushenski, M. Schwarz, H. Lewis et al., "Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia *Rachycentron canadum*," *Aquaculture Nutrition*, vol. 17, no. 2, pp. 437–447, 2011.

[55] R. Zuo, Q. Ai, K. Mai et al., "Effects of dietary n-3 highly unsaturated fatty acids on growth, nonspecific immunity, expression of some immune related genes and disease resistance of large yellow croaker (*Larmichthys crocea*) following natural infestation of parasites (*Cryptocaryon irritans*)," *Fish & Shellfish Immunology*, vol. 32, no. 2, pp. 249–258, 2012.

[56] K. C. Justi, C. Hayashi, J. V. Visentainer, N. de Souza, and M. Matsushita, "The influence of feed supply time on the fatty acid profile of Nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with n-3 fatty acids," *Food Chemistry*, vol. 80, no. 4, pp. 489–493, 2003.