Hindawi
Aquaculture Nutrition
Volume 2022, Article ID 6314978, 12 pages
https://doi.org/10.1155/2022/6314978

Research Article

Effects of Different Marine-Based Lipid Sources on Growth Performance, Activities of Digestive Enzyme, Antioxidant Responses, and Lipid Metabolism of Large Yellow Croaker (*Larimichthys crocea*) Larvae

Chuanwei Yao,[1] Wenxing Huang,[1] Yongtao Liu,[1] Youqing Miao,[1] Zhaoyang Yin,[1] Kangsen Mai,[1,2] and Qinghui Ai[1,2]

[1]*Key Laboratory of Aquaculture Nutrition and Feed (Ministry of Agriculture and Rural Affairs), Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, China*
[2]*Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao, Shandong 266237, China*

Correspondence should be addressed to Qinghui Ai; qhai@ouc.edu.cn

Received 23 March 2022; Revised 2 June 2022; Accepted 19 June 2022; Published 5 July 2022

Academic Editor: Shouqi Xie

The study of lipid nutrition is an important guarantee for the development of high-efficiency artificial microdiet for fish larvae. Existing studies on lipid nutrition of larvae mainly focus on nutrient requirements and metabolism through a single lipid source. However, there are few reports on the effects of different marine-based lipid sources on fish larvae. In this study, a 30-day feeding experiment was conducted to evaluate the effects of different dietary marine-based lipid sources on survival, growth performance, appetite gene expression, activities of digestive enzyme, antioxidant responses, and lipid metabolism of large yellow croaker (*Larimichthys crocea*) larvae (initial weight 4.71 ± 0.21 mg). Four isonitrogenous (520 g/kg crude protein) and isolipidic (190 g/kg crude lipid) diets were formulated to contain fish oil (FO), krill oil (KO), squid viscera oil (SVO), and *Schizochytrium* sp. oil (SSO), respectively. Results showed that larvae fed with the SSO diet had significantly higher survival rate (SR) than those fed with dietary FO and SVO ($P < 0.05$). However, larvae fed with the SSO diet had significantly lower final body weight (FBW) and specific growth rate (SGR) than those fed with other diets ($P < 0.05$). Furthermore, larvae fed with the SSO diet had significantly higher percentage of total n-3 long-chain polyunsaturated fatty acids (LC-PUFA) than those fed with other diets, followed by KO and SVO diets ($P < 0.05$). Larvae fed with the SSO diet had significantly lower mRNA expression of orexigenic genes (*npy* and *ghrelin*) and significantly higher mRNA expression of anorexigenic gene (*cart*) than those fed with dietary FO ($P < 0.05$). Meanwhile, larvae fed with the SSO diet had significantly higher activity of alkaline phosphatase (AKP) in intestinal segments (IS) and brush border membrane (BBM) than those fed with dietary FO and SVO ($P < 0.05$). Larvae fed with the SSO diet had significantly higher activity of total antioxidant capacity (T-AOC) than those fed with dietary FO ($P < 0.05$). The TG content and mRNA expression of lipogenesis genes (*srebp-1c*, *fas*, and *scd1*) were markedly lower in larvae fed with SSO, KO, and SVO diets than in those fed with dietary FO ($P < 0.05$). Meanwhile, larvae fed with KO and SSO diets had significantly higher mRNA expression of the lipolysis gene (*cpt-1*) than those fed with other diets ($P < 0.05$). In conclusion, results of the present study showed that FO can be completely replaced in large yellow croaker larvae feed with KO and SVO without negative effects. Moreover, the complete replacement of FO by SSO can improve survival, activities of digestive enzyme, antioxidant responses, and lipid metabolism, despite inhibiting the appetite and growth of large yellow croaker larvae.

1. Introduction

Lipid as a source of metabolic energy and essential fatty acids plays an important role in fish nutrition [1]. Traditionally, fish oil (FO) is a common lipid source in the most commercial aquatic feeds due to its effective supply of energy and essential fatty acids, such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) [2, 3]. The global aquatic feed industry is the largest consumer of FO, accounting for about 75% of the global supply [4]. However, the production of FO has continued to decline mainly due to reducing market availability from capture fisheries [5, 6]. Therefore, for the sustainable development of aquaculture, other lipid sources must be exploited.

Since the use of FO in aquaculture is regarded as unsustainable practice, based on economic and environmental considerations, most aquatic feed industries use terrestrial-based lipid sources, which mainly consist of animal fat and vegetable oil [7]. Previous studies have shown that the substitution of a terrestrial-based lipid source for fish oil will reduce the palatability and digestibility of the feed [8], which will lead to decreased growth, reduced immunity, and metabolic disorders in certain fish species [9–11].

Given the limitations of the common terrestrial-based lipid sources reported, marine-based lipid sources, including krill oil (KO), squid viscera oil (SVO), and *Schizochytrium* sp. oil (SSO), have attracted increasing attention in aquatic feeds. KO is one of the most important products extracted from Antarctic krill. It is rich in various active substances and has no side effects. Study on swimming crab found that juvenile crab fed with the KO diet had significantly higher growth performance and antioxidant response than that feed with dietary FO [12]. SVO is extracted from fresh squid viscera and other waste materials. It is a by-product of squid processing and has economic and environmental value. Study on giant freshwater prawn found that prawn fed with the squid liver oil diet had significantly higher growth performance than that fed with dietary FO [13]. SSO is the most important product extracted from *Schizochytrium*. In addition, *Schizophyllum* is a kind of marine microalgae which can be cultured by aerobic fermentation and belongs to renewable resources. Study on Atlantic salmon found that SSO can completely replace FO in feed without negatively affecting growth [14]. Given all that, KO, SVO, and SSO could be some promising alternative lipid sources to replace FO in aquatic feeds. Nevertheless, researches on marine-based lipid sources have focused on crustacea and juvenile fish which are almost scarcely reported in fish larvae.

Large yellow croaker (*Larimichthys crocea*) is an important marine fish in China due to its delicious taste and huge economic value [15–17]. Previous studies have assessed the effect of terrestrial-based lipid sources for larval and juvenile stages of large yellow croaker [18–20]. Up to now, no studies have been reported in large yellow croaker larvae to assess marine-based lipid sources as a potential replacement for FO in microdiets. Therefore, the present study intended to evaluate the effects of different dietary marine-based lipid sources on survival, growth performance, appetite gene expression, activities of digestive enzyme, antioxidant responses, and lipid metabolism of large yellow croaker larvae.

2. Materials and Methods

The present study was carried out in accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised 1 March 2017).

2.1. Feed Ingredients and Diet Formulation. Four isonitrogenous (520 g/kg crude protein) and isolipidic (190 g/kg crude lipid) diets were formulated to contain FO, KO, SVO, and SSO, respectively (Table 1).

Microdiets were manufactured by microbonding technology. All ingredients were first ground to a fine powder through a 150 μm nylon mesh. Step by step mix according to the feed formulation, and then, pour all ingredients into an electric blender and mix for 20 minutes. In the mixing process, FO, KO, SVO, and SSO were added into feed ingredients of each group, respectively. Finally, add the sodium alginate liquid until sticky mass was formed. The sticky mass was extruded into slender strip with an axial single screw spherical extruder, and then, the slender strip was made into pellets with particle size up to 1 mm by rounding machine. All pellets were dried in a constant temperature oven for 8 hours at 50°C. All pellets were stored at -20°C until use. The size of pellets ranged from 150 to 250 μm for the larvae from 15 to 25 days after hatch (DAH) and 250 to 380 μm for the larvae from 26 to 45 DAH.

2.2. Experimental Procedure. Large yellow croaker larvae used in this study were obtained from the Xiangshan Harbor Aquatic Seeds Company, Ningbo, China. The feeding experiment was conducted in Marine and Fishery Science and Technology Innovation Base, Ningbo, China. All larvae were fed with rotifers, *Brachionus plicatilis* ($0.5–1.5 \times 10^4$ individual L^{-1}) from 3 to 8 DAH and *Artemia nauplii* ($1.0–1.5 \times 10^3$ individual L^{-1}) from 6 to 11 DAH, and live copepods, *Calanus sinicus* from 10 to 14 DAH. After 14 DAH, larvae were fed with the experimental diets. Larvae (15 DAH, average body weight 4.71 ± 0.21 mg) were distributed in 12 white plastic tanks (water volume 220 L, 3500 larvae per tank). Four experimental diets were assigned to triplicate groups of larvae in a random combination. During the rearing period, water temperature ranged from 21 to 23°C, pH from 7.8 to 8.2, and salinity from 21 to 24‰. About 100–200% of the water volume was renewed daily. The experimental period was 30 days. All larvae were fed with the experimental diets seven times daily (6:30, 9:30, 12:00, 14:30, 17:00, 19:30, and 22:30) to apparent satiation during the rearing period (from 15 to 45 DAH).

2.3. Sampling and Dissection. At the beginning of the feeding experiment, initial body length and initial body weight (IBW) were measured by 50 larvae of 15 DAH collected randomly from the tank. At the end of the feeding experiment, larvae were fasted for 24 h and then anesthetized (eugenol, 1 : 10,000). The number of larvae in each tank was counted to determine survival (SR). Twenty larvae were sampled from each tank and immediately frozen in liquid nitrogen

Table 1: Formulation and proximate analysis of the experimental diets (g/kg dry matter).

Ingredients	Dietary lipid sources			
	FO	KO	SVO	SSO
White fish meal[1]	360	360	360	360
Krill meal[1]	220	220	220	220
Squid meal[2]	105	105	105	105
Yeast extract[3]	30	30	30	30
α-Starch	100	100	100	100
Sodium alginate	20	20	20	20
Vitamin premix[7]	15	15	15	15
Mineral premix[8]	10	10	10	10
Ascorbyl polyphosphate	2	2	2	2
Attractant[1]	10	10	10	10
Mould inhibitor	0.5	0.5	0.5	0.5
Antioxidant	0.5	0.5	0.5	0.5
Choline chloride	2	2	2	2
Fish oil[1]	75	0	0	0
Krill oil[4]		75		
Squid viscera oil[5]			75	
Schizochytrium sp. oil[6]				75
Soybean lecithin	50	50	50	50
Analyzed nutrient composition (dry matter basis)				
Crude protein	516.6	521.7	515.5	519.0
Crude lipid	192.9	191.5	186.9	189.5

[1]Commercially available from Great Seven Biotechnology Co., Ltd. in Qingdao, China; elementary composition (dry matter): white fish meal, a mixture of *Laemonema longipes* and *Theragra chalcogramma* meal—crude protein (71.73%) and crude lipid (4.76%); krill meal: crude protein (64.86%) and crude lipid (8.0%). [2]Commercially available from market squid and then drying and crushing; elementary composition (dry matter): squid meal—crude protein (81.81%) and crude lipid (5.16%). [3]Commercially available from Zhejiang Dongcheng Biotechnology Co., Ltd. [4]Krill oil was purchased from Kangjing Marine Biotechnology Co., Ltd. in Qingdao, China. [5]Squid viscera oil was provided by Qingdao Shenghejing Biotechnology Co., Ltd. [6]*Schizochytrium* sp. oil was purchased from Qingdao Keliyuan Biotechnology Co., Ltd. [7]Composition of vitamin premix (IU or g/kg): vitamin A palmitate, 3000000 IU; vitamin D_3, 1200000 IU; DL-α-vitamin E, 40.0 g/kg; menadione, 8.0 g/kg; thiamine-HCl, 5.0 g/kg; riboflavin, g/kg; D-calcium pantothenate, 16.0 mg/kg; pyridoxine-HCl, 4.0 mg/kg; inositol, 200.0 mg/kg; biotin, 8.0 mg/kg; folic acid, 1.5 mg/kg; 4-aminobenzoic acid, 5.0 mg/kg; niacin, 20.0 mg/kg; vitamin B_{12}, 0.01 mg/kg; and L-ascorgyl-2-monophosphate-Na (35%), 2000.0 mg/kg. [8]Composition of mineral premix (g kg^{-1} premix): $Ca(H_2PO_4)\cdot H_2O$, 675.0; $C_OSO_4\cdot H_2O$, 0.15; $CuSO_4\cdot H_2O$, 5.0; $FeSO_4\cdot 7H_2O$, 50.0; KCl, 0.1; $MgSO_4\cdot 2H_2O$, 101.7; $MnSO_4\cdot 2H_2O$, 18.0; NaCl, 80.0; $NaSeO_3\cdot H_2O$, 0.05; and $ZnSO_4\cdot 7H_2O$, 20.0.

for an antioxidant activity assay. Forty larvae were dissected from each tank to obtain visceral mass containing crude mixture of the liver, intestine, heart, pancreas, and spleen. The visceral mass was quickly put into 2.0 mL RNase-free Cryogenic Vials and then immediately frozen in liquid nitrogen for gene expression assays. Fifty larvae from each tank were separated under a dissecting microscope on a glass plate maintained at 0°C to obtain intestinal segments (IS) and pancreatic (PS) for the digestive enzyme activity assay. The remaining larvae were collected form each tank and stored at −20°C for a body composition assay.

Table 2: Fatty acid composition of experimental diets (% total fatty acids)[1].

Fatty acid (% total fatty acids)	Dietary lipid sources			
	FO	KO	SVO	SSO
C14:0	11.45	11.27	11.04	7.21
C16:0	39.62	40.05	36.20	36.14
C18:0	5.56	4.74	5.04	4.51
C20:0	0.31	0.15	0.18	0.12
Total SFA	56.94	56.21	52.47	47.98
C16:1n-7	2.36	2.00	1.85	1.86
C18:1n-9	5.81	5.47	6.73	5.10
Total MUFA	8.17	7.46	8.58	6.96
C18:3n-3	2.03	2.07	1.77	1.58
C20:5n-3	10.38	12.87	11.69	4.97
C22:6n-3	8.64	9.05	9.44	26.06
Total n-3 PUFA	21.05	23.98	22.89	32.60
C18:2n-6	9.15	8.19	9.53	9.54
C20:4n-6	0.40	0.37	0.30	0.23
Total n-6 PUFA	9.55	8.56	9.83	9.77
n-3/n-6 PUFA	2.20	2.80	2.33	3.34
Total n-3 LC-PUFA	19.03	21.92	21.12	31.03

[1]The contents of some fatty acids are minor and not detected, such as C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C18:3n-6, C20:3n-6, C18:4n-3, and C22:5n-3. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids.

2.4. Body Composition and Fatty Acid Profile Analyses. Samples of diets and larvae were dried to a constant weight in a ventilated drying oven at 105°C to determine the dry matter content. Crude protein and crude lipid of diets and larvae were measured following standard procedures [21]. Samples of diets and larvae were placed in a lyophilized chamber to freeze-dry (Alpha 1-4 LD plus; Christ, Germany). Based on Zuo et al. [22], fatty acid profiles of freeze-dried samples were determined with wispy modification (Tables 2 and 3). The fatty acid profile was separated and measured using a gas chromatograph mass spectrometer (GSMC) (Shimadzu GSMC-QP2010 Ultra, Japan). Triglyceride (TG) content and total cholesterol (TCHO) content were assayed with a triglyceride assay kit and total cholesterol assay kit (Nanjing Jiancheng Bio-Engineering Institute, China), respectively.

2.5. Detection of Enzyme Activities

2.5.1. Digestive Enzyme Analysis. Samples (45 DAH larvae) of 0.15–0.2 g IS and PS were homogenized in 0°C saline, respectively, and centrifuged at 3000 g for 10 min; then, the supernatant was collected for following assays. According to published paper [23], purified brush border membranes (BBM) were obtained from the homogenate of the intestinal segment. The activity of trypsin was assayed according to published paper [24]. The activity of leucine-aminopeptidase (LAP) was assayed in both the IS and BBM according to published paper [25]. Total protein content, amylase activity, lipase activity, and alkaline

Table 3: Effects of different dietary marine-based lipid sources on fatty acid composition of large yellow croaker larvae (% total fatty acids) (means ± SEM, $n = 3$)[1].

Fatty acid (% total fatty acids)	Dietary lipid sources			
	FO	KO	SVO	SSO
C14:0	6.96 ± 0.67^{a}	5.79 ± 0.48^{ab}	6.39 ± 0.21^{a}	3.98 ± 0.19^{b}
C16:0	43.50 ± 2.61	47.21 ± 2.53	41.99 ± 1.72	50.51 ± 3.96
C18:0	11.22 ± 0.39^{a}	10.04 ± 0.09^{ab}	9.51 ± 0.38^{b}	9.60 ± 0.46^{ab}
C20:0	0.42 ± 0.05^{a}	0.24 ± 0.01^{b}	0.23 ± 0.04^{b}	0.26 ± 0.01^{b}
Total SFA	62.09 ± 3.21	63.28 ± 2.8	58.13 ± 2.33	61.96 ± 1.59
C16:1n-7	3.38 ± 0.19^{a}	2.17 ± 0.17^{b}	3.33 ± 0.08^{a}	1.10 ± 0.09^{c}
C18:1n-9	8.50 ± 0.30^{a}	6.77 ± 0.31^{b}	8.28 ± 0.17^{a}	3.96 ± 0.38^{c}
Total MUFA	11.88 ± 0.37^{a}	8.94 ± 0.46^{b}	11.61 ± 0.24^{a}	5.06 ± 0.47^{c}
C18:3n-3	0.76 ± 0.01^{a}	0.8 ± 0.03^{a}	0.90 ± 0.03^{a}	0.49 ± 0.04^{b}
C20:5n-3	3.58 ± 0.12^{b}	6.28 ± 0.21^{a}	5.84 ± 0.16^{a}	2.18 ± 0.26^{c}
C22:6n-3	5.25 ± 0.45^{b}	7.18 ± 0.05^{b}	7.13 ± 0.24^{b}	15.47 ± 1.22^{a}
Total n-3 PUFA	9.59 ± 0.58^{c}	14.26 ± 0.29^{b}	13.87 ± 0.33^{b}	18.13 ± 1.51^{a}
C18:2n-6	9.01 ± 0.26^{a}	7.3 ± 0.34^{b}	8.54 ± 0.28^{a}	5.82 ± 0.52^{b}
C20:4n-6	0.37 ± 0.03	0.38 ± 0.01	0.4 ± 0.03	0.41 ± 0.04
Total n-6 PUFA	9.38 ± 0.29^{a}	7.68 ± 0.35^{bc}	8.94 ± 0.31^{ab}	6.23 ± 0.56^{c}
n-3/n-6 PUFA	1.02 ± 0.04^{d}	1.86 ± 0.06^{b}	1.55 ± 0.02^{c}	2.91 ± 0.04^{a}
Total n-3LC-PUFA	8.82 ± 0.57^{c}	13.46 ± 0.26^{b}	12.97 ± 0.31^{b}	17.65 ± 1.47^{a}

[1]The contents of some fatty acids are minor and not detected, such as C22:0, C24:0, C14:1, C20:1n-9, C22:1n-11, C20:2n-6, C18:3n-6, C20:3n-6, C18:4n-3, and C22:5n-3. Data in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; LC-PUFA: long-chain polyunsaturated fatty acids.

phosphatase (AKP) activity were measured according to a commercial test kit (Nanjing Jiancheng Bio-Engineering Institute, China).

2.5.2. Antioxidant Enzyme and Malondialdehyde Analysis. Samples (45 DAH larvae) were homogenized in 0°C saline and centrifuged at 3000 g for 10 min; then, the supernatant was collected for further assay. The content of malondialdehyde (MDA), catalase activity (CAT), total antioxidant capacity (T-AOC), and superoxide dismutase activity (SOD) was measured according to a commercial test kit (Nanjing Jiancheng Bio-Engineering Institute, China).

2.6. RNA Extraction, cDNA Synthesis, and Real-Time Quantitative PCR. The total RNA was extracted from head and visceral mass by using the Trizol reagent (Takara, Japan). The integrity and quality of RNA were detected using 1.2% denatured agarose gel electrophoresis and then assessed to the concentration in the NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific, USA). The total RNA was reversely transcribed to cDNA using the Prime Script-RT reagent Kit (Takara, Japan). The real-time quantitative PCR was carried out using a quantitative thermal cycler (CFX96TM Real-Time System, BIO-RAD, USA). Primer sequences for neuropeptide Y (*npy*), cocaine- and amphetamine-regulated transcript (*cart*), proopiomelanocortin (*pomc*), *ghrelin*, and stearoyl-CoA desaturase 1 (*scd1*) were designed and synthesized according to published sequences in GenBank (Table 4). The primers for *β-actin*, fatty acid synthetase (*fas*), sterol regulatory element-binding protein 1 (*srebp-1*), carnitine palmitoyl transferase 1 (*cpt-1*), peroxisome proliferator-activated receptor (*pparα*), adipose triglyceride lipase (*atgl*), diacylglycerol acyltransferase 2 (*dgat2*), acetyl CoA carboxylase 1 (*acc1*), and acyl-CoA oxidase (*aco*) were directly synthesized according to corresponding sequences in published papers [22, 26–29] (Table 4). The real-time quantitative PCR program was 95°C for 2 min, followed by 40 cycles of 95°C for 10 s, 58°C for 10 s, and 72°C for 20 s. The level of gene expression was calculated using the $2^{-\Delta\Delta CT}$ method as described according to published paper [30].

2.7. Calculations and Statistical Analysis.

$$\text{Specific growth rate (SGR, \%day}^{-1}) = (\text{Ln } W_t - Ln\ W_0) \times \frac{100}{d},$$
$$\text{Survival rate (SR, \%)} = N_t \times \frac{100}{N_0}, \quad (1)$$

where W_t is the final wet body weight, W_0 is the initial wet body weight, d is the experimental duration, and N_t and N_0 are the number of final and initial fish, respectively.

All data were subjected to statistical analysis with SPSS 17.0 (IBM, America). The data were firstly analyzed using one-way analysis of variance (ANOVA) and followed using Tukey's test. Statistics with $P < 0.05$ were considered significant, and results were presented as means ± SEM (standard error of the mean).

Table 4: Primers used for quantitative PCR.

Gene	Forward (5′-3′)	Reverse (5′-3′)	Accession number
npy	AAAGAGGTCCAGTCCTGAGATT	GTGGCGGCTCATAGTGGTAA	XM019275410
cart	GCCAACTGCAACTCCTACCT	TGCTACAGTAAATAAGCGGAAC	XM_010741284
ghrelin	TGACCTTGTGGTGCAAGTCGAC	CCGATTTCAAAGGGGGCACT	KC899122
pomc	AGCCTGATTATCTGCCTCCC	CTTGTACGTGCCGTCCTTCT	XM_019270382
srebp-1c	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	Cai et al. [26]
fas	CAGCCACAGTGAGGTCATCC	TGAGGACATTGAGCCAGACAC	Yan et al. [29]
scd1	AAAGGACGCAAGCTGGAACT	CTGGGACGAAGTACGACACC	XM_027289647
dgat2	TTCGGTGCTTTCTGCAACTTCG	AAGGATGGGGAAGCGGAAGT	Pang et al. [27]
acc1	GACTTGGCGGAATACCTACTGG	GCTTGCTGGATGATCTTTGCTT	Pang et al. [27]
cpt-1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	Yan et al. [29]
aco	AGTGCCCAGATGATCTTGAAGC	CTGCCAGAGGTAACCATTTCCT	Yan et al. [29]
pparα	GTCAAGCAGATCCACGAAGCC	TGGTCTTTCCAGTGAGTATGAGCC	Zuo et al. [22]
atgl	CCATGCATCCGTCCTTCAACC	GAGATCCCTAACCGCCCACT	Yan et al. [29]
β-Actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	Tan et al. [28]

npy: neuropeptide Y; *cart*: cocaine- and amphetamine-regulated transcript; *pomc*: proopiomelanocortin; *srebp-1c*: sterol-regulatory element-binding protein-1c; *fas*: fatty acid synthase; *scd1*: stearoyl-CoA desaturase 1; *dgat2*: diacylglycerol acyltransferase 2; *acc1*: acetyl CoA carboxylase 1; *cpt-1*: carnitine palmitoyl transferase-1; *aco*: acyl-CoA oxidase; *pparα*: peroxisome proliferator-activated receptor *α*; *atgl*: adipose triglyceride lipase.

Table 5: Effects of different dietary marine-based lipid sources on growth performance of large yellow croaker larvae (means ± SEM, $n = 3$)[1].

Parameters	Dietary lipid sources			
	FO	KO	SVO	SSO
Initial body length (mm)	6.28 ± 0.46	6.28 ± 0.46	6.28 ± 0.46	6.28 ± 0.46
Final body length (mm)	17.17 ± 0.09^{a}	17.73 ± 0.19^{a}	16.80 ± 0.40^{a}	15.76 ± 0.29^{b}
IBW (mg)	4.71 ± 0.21	4.71 ± 0.21	4.71 ± 0.21	4.71 ± 0.21
FBW (mg)	83.39 ± 0.54^{a}	87.35 ± 1.80^{a}	80.98 ± 3.44^{a}	60.72 ± 2.65^{b}
SGR (%/day)	9.58 ± 0.02^{a}	9.73 ± 0.07^{a}	9.48 ± 0.14^{a}	8.52 ± 0.14^{b}
Survival (%)	18.70 ± 1.06^{bc}	20.20 ± 0.74^{ab}	14.03 ± 1.25^{c}	24.20 ± 1.18^{a}

[1]Data in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$). IBW: initial body weight; FBW: final body weight; SGR: specific growth rate.

Table 6: Effects of different dietary marine-based lipid sources on body composition in large yellow croaker larvae (means ± SEM, $n = 3$)[1].

Parameters	Dietary lipid sources			
	FO	KO	SVO	SSO
Crude protein (g/kg)	585.6 ± 7.1	597.6 ± 3.6	579.1 ± 9.5	599.0 ± 16.1
Crude lipid (g/kg)	228.2 ± 5.9^{a}	189.6 ± 4.1^{b}	206.4 ± 14.0^{ab}	186.8 ± 3.1^{b}
Moisture (g/kg)	826.8 ± 4.6	828.8 ± 1.4	824.8 ± 2.7	834.6 ± 6.4

[1]Data in the same row sharing the same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$).

3. Results

3.1. Survival, Growth Performance, and Body Composition. Larvae fed with the SSO diet had significantly higher survival rate (SR) than those fed with dietary FO and SVO ($P < 0.05$) (Table 5). However, larvae fed with the SSO diet had significantly lower specific growth rate (SGR) and final body weight (FBW) than those fed with other diets ($P < 0.05$) (Table 5). Neither crude protein nor moisture showed any statistical differences among dietary treatments ($P > 0.05$) (Table 6). Meanwhile, larvae fed with KO and SSO diets had significantly lower crude lipid than larvae fed with the FO diet ($P < 0.05$) (Table 6).

3.2. Fatty Acid Composition. No significant differences were observed in the percentages of C16:0, total SFA, and C20:4n-6 among the dietary treatments ($P > 0.05$) (Table 3). Larvae fed with the SSO diet had significantly lower percentage of C14:0 than those fed with dietary FO and SVO ($P < 0.05$) (Table 3). Larvae fed with the FO diet had significantly

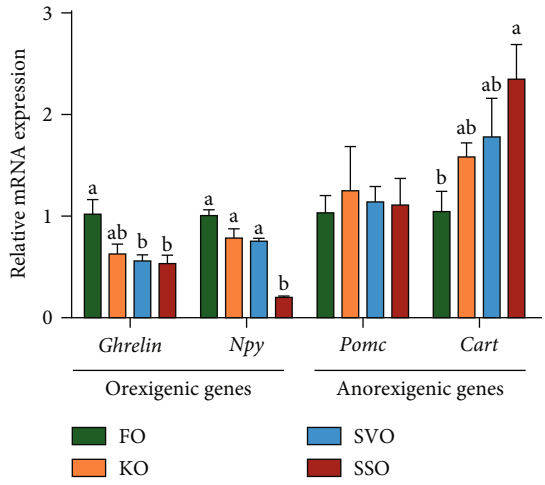

Figure 1: Effects of different dietary marine-based lipid sources on relative mRNA expression of genes involved in appetite in head and visceral mass of large yellow croaker larvae. Values are means ($n = 3$), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test). *npy*: neuropeptide Y; *cart*: cocaine- and amphetamine-regulated transcript; *pomc*: proopiomelanocortin.

higher percentage of C18:0 than larvae fed with the SVO diet ($P < 0.05$) (Table 3). Similarly, larvae fed with the FO diet had significantly higher percentage of C20:0 than those fed with other diets ($P < 0.05$) (Table 3). Larvae fed with FO and SVO diets had significantly higher percentages of C16:1n-7, C18:1n-9, and total MUFA than those fed with other diets, followed by the KO diet ($P < 0.05$) (Table 3). Meanwhile, larvae fed with the SSO diet had significantly lower percentage of C18:3n-3 than those fed with other diets ($P < 0.05$) (Table 3). Larvae fed with the KO and SVO diets had significantly higher percentage of C20:5n-3 than those fed with other diets, followed by the FO diet ($P < 0.05$) (Table 3). It is interesting to note that the SSO diet in larvae significantly increased the percentage of C22:6n-3 while it significantly decreased the percentage of C20:5n-3 compared with other diets ($P < 0.05$) (Table 3). Larvae fed with the SSO diet had significantly higher percentages of total n-3 PUFA and total n-3 LC-PUFA than those fed with other diets, followed by KO and SVO diets ($P < 0.05$) (Table 3). Moreover, larvae fed with the FO diet had significantly higher percentage of total n-6 PUFA than those fed with KO and SSO diets. The ratio n-3 to n-6 PUFA was significantly affected by different dietary marine-based lipid sources ($P < 0.05$) (Table 3). Larvae fed with the SSO diet had the highest ratio n-3 to n-6 PUFA among dietary treatments ($P < 0.05$) (Table 3).

3.3. The mRNA Levels of Appetite-Related Genes. The mRNA expression of orexigenic genes (*npy* and *ghrelin*) and anorexigenic genes (*pomc* and *cart*) was examined. Ghrelin is a key hormone of food intake regulation that acts directly on the brain in an endocrine way or triggers neural signals through afferent nerves. Moreover, ghrelin can activate NPY/AgRP neurons and inhibit POMC/CART neurons in the arcuate nucleus to increase the food intake of animal. Larvae fed with the SSO diet had significantly lower mRNA expression of *npy* in the head than those fed with other diets ($P < 0.05$) (Figure 1). Meanwhile, larvae fed with SVO and SSO diets had significantly lower mRNA expression of *ghrelin* in visceral mass than larvae fed with the FO diet ($P < 0.05$) (Figure 1). Moreover, larvae fed with the SSO diet had significantly higher mRNA expression of *cart* in the head than larvae fed with the FO diet ($P < 0.05$) (Figure 1). No significant difference was observed in the mRNA expression of *pomc* in the head among dietary treatments ($P > 0.05$) (Figure 1).

3.4. Digestive Enzyme Activities. No significant differences were observed in activities of trypsin and amylase in IS and PS among dietary treatments ($P > 0.05$) (Figures 2(a), 2(c), 2(d), and 2(f)). Similarly, there was no significant difference activity of lipase in IS among dietary treatments ($P > 0.05$) (Figure 2(e)). Larvae fed with the KO diet had significantly higher activity of lipase in PS than larvae fed with the SSO diet ($P < 0.05$) (Figure 2(b)). Moreover, larvae fed with the SSO diet had significantly higher activity of AKP in IS and BBM than those fed with dietary FO and SVO ($P < 0.05$) (Figures 2(g) and 2(i)). Larvae fed with the KO diet had significantly higher activity of LAP in IS than larvae fed with the SVO diet ($P < 0.05$) (Figure 2(h)). However, no significant difference was observed in activity of LAP in BBM among dietary treatments ($P > 0.05$) (Figure 2(j)).

3.5. Antioxidant Parameters. Larvae fed with KO and SSO diets had significantly higher activity of SOD than those fed with other diets ($P < 0.05$) (Figure 3(a)). Meanwhile, larvae fed with the SSO diet had significantly higher activity of T-AOC than larvae fed with the FO diet ($P < 0.05$) (Figure 3(c)). However, no significant differences were observed in activity of CAT and MDA content among dietary treatments ($P > 0.05$) (Figures 3(b) and 3(d)).

3.6. Triglyceride (TG) Content, Total Cholesterol (TCHO) Content, and the mRNA Levels of Lipid Metabolism-Related Genes in Visceral Mass. TG content, TCHO content, and the mRNA expression of lipogenesis genes (*srebp1*, *acc1*, *fas*, *scd1*, and *dgat2*) and lipolysis genes (*atgl*, *pparα*, *cpt1*, and *aco*) were examined. The *srebp-1*, as a transcription factor, can activate fatty acid synthesis-related gene expression including *fas* and *scd1*. The *pparα*, as a transcription factor, can activate fatty acid oxidation-related gene expression, such as *cpt-1*. Larvae fed with SSO, KO, and SVO diets had significantly lower TG content in visceral mass than those fed with dietary FO ($P < 0.05$), while no difference in THCO content was observed among dietary treatments (Figures 4(a) and 4(b)). Similarly, SSO, KO, and SVO diets in larvae significantly decreased mRNA expression of *srebp-1*, *fas*, and *scd1* in visceral mass compared to dietary FO ($P < 0.05$) (Figure 4(c)). Moreover, larvae fed with the SVO diet had significantly lower mRNA expression of *dgat2* in visceral mass than those fed with dietary FO ($P < 0.05$) (Figure 4(c)). Larvae fed with KO and SSO diets had significantly higher mRNA expression of *cpt-1* in visceral mass than those fed with other diets ($P < 0.05$) (Figure 4(d)).

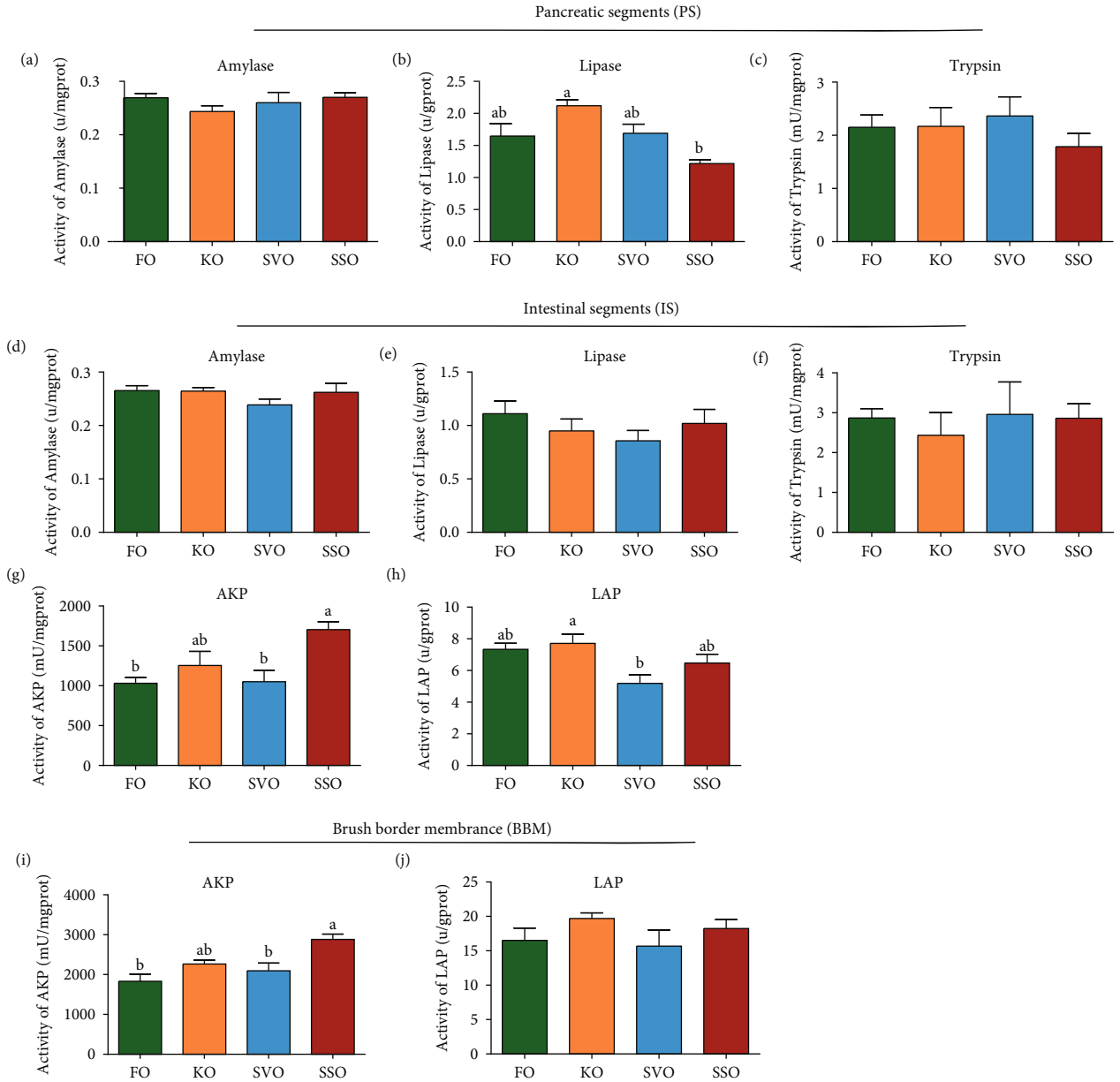

FIGURE 2: Effects of different dietary marine-based lipid sources on activities of the main digestive enzymes of large yellow croaker larvae. AKP: alkaline-phosphatase; LAP: leucine-aminopeptidase. Values are means ($n = 3$), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test).

Meanwhile, larvae fed with the KO diet had significantly higher mRNA expression of *pparα* in visceral mass than those fed with other diets ($P < 0.05$) (Figure 4(d)). However, no significant differences were observed in mRNA expressions of *aco*, *acc1*, and *atgl* among dietary treatments ($P > 0.05$) (Figures 4(c) and 4(d)).

4. Discussion

Results of the present study demonstrated that dietary marine-based lipid sources with different fatty acid compositions had specific influences on survival and growth performance in large yellow croaker larvae. It is interesting to note that larvae fed with the SSO diet had significantly improved SR while it significantly reduced FBW and SGR compared with dietary FO. Consistent with the present study, 11% *Schizochytrium* sp. meal in Atlantic salmon had significantly decreased the growth performance compared to the FO diet [6]. Appetite is an important factor in different dietary marine-based lipid sources, because appetite directly controls food intake, which plays a central role in determining the growth and body composition of cultured animals [31]. Fish food intake is regulated by neuropeptide Y (NPY), cocaine and amphetamine regulatory transcripts (CART), anorexia hormone (POMC), and ghrelin [32, 33]. Ghrelin is the key hormone of food intake regulation that acts directly on the brain in an endocrine way or triggers neural signals through afferent nerves [33]. Moreover, ghrelin can

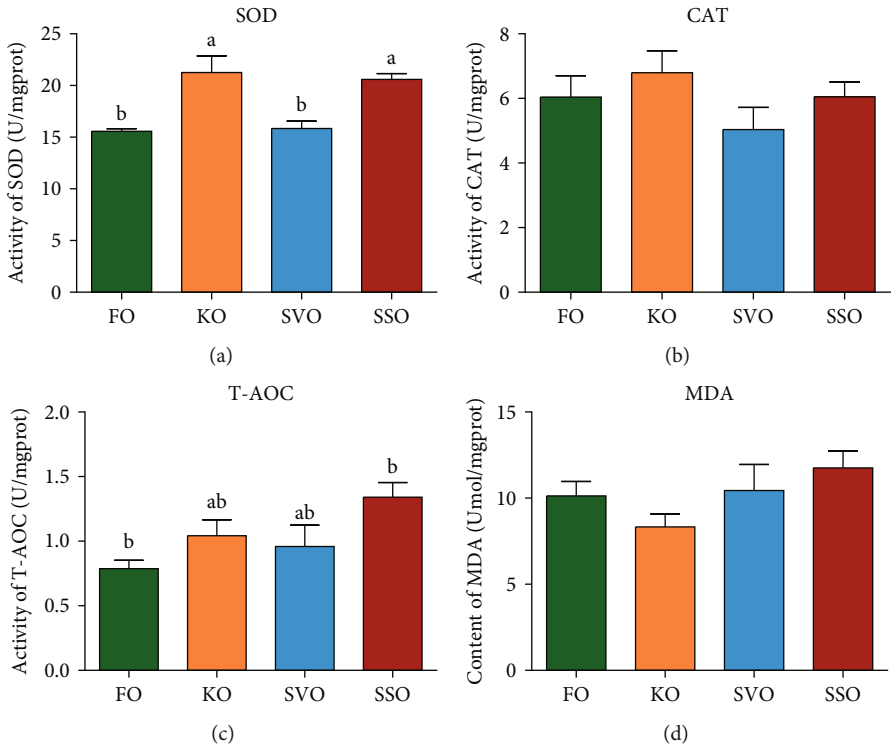

Figure 3: Effects of different dietary marine-based lipid sources on antioxidant capability in visceral mass of large yellow croaker larvae. (a) SOD: superoxide dismutase; (b) CAT: catalase; (c) T-AOC: total antioxidant capacity; (d) MDA: malondialdehyde. Values are means ($n = 3$), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test).

activate NPY/AgRP neurons and inhibit POMC/CART neurons in the arcuate nucleus to increase the food intake of animal [34]. In the present study, larvae fed with the SSO diet had significantly lower mRNA expression of *npy* in the head than those fed with other diets. Meanwhile, larvae fed with SVO and SSO diets had significantly lower mRNA expression of *ghrelin* in visceral mass than larvae fed with the FO diet. Moreover, larvae fed with the SSO diet had significantly higher mRNA expression of *cart* in the head than larvae fed with the FO diet. This result was similar to study in *Senegalese sole* larvae, which found that dietary vegetable oil significantly increased mRNA expressions of *cart1a* and *cart1b*, while it significantly decreased mRNA expression of *npy* compared to dietary cod liver oil [31]. Based on the results of this experiment, we speculate that the growth performance of larvae fed with SSO diet may be decreased due to the decreased appetite of larvae.

The fatty acid profile of larvae was a reflection of the fatty acid profile of the experimental diet. In the present study, percentages of MUFA, n-3 PUFA, and n-6 PUFA in larvae were significantly affected by different marine-based lipid sources. Similar results have been found in many studies of aquatic animals, such as swimming crab [35], Atlantic salmon [7], and golden pompano [36]. In the present study, larvae fed with FO and SVO diets had significantly higher percentages of C16:1n-7, C18:1n-9, and total MUFA than those fed with other diets, followed by the KO diet. Moreover, it is interesting to note that the SSO diet in larvae had significantly increased the percentage of DHA while it significantly decreased the percentage of EPA compared with other diets. Similarly, the negative effect of dietary FO totally replaced with SSO on EPA content in fillet was observed in seabream [37], and Atlantic salmon [6], which may be due to a lack of this fatty acid in *Schizochytrium*. Larvae fed with the SSO diets had significantly higher percentage of total n-3 PUFA and total n-3 LC-PUFA than those fed with other diets, followed by KO and SVO diets. Studies on Atlantic salmon [14, 38], gilthead seabream [39], and channel catfish [40] also pointed out that complete replacement of FO by SSO significantly increased the percentage of DHA in fish. These results may indicate that dietary SSO, KO, and SVO can improve larval quality.

Changes in digestive enzyme activities had been used as indicators for studying the nutritional status of fish larvae [41, 42]. In the present study, activities of amylase and trypsin in PS and IS had no significant differences among dietary treatments. This result was similar to study in red claw crayfish, which found that different dietary lipid sources had no significant effects on the activities of trypsin and amylase in hepatopancreas of juvenile red crayfish [43]. The onset of intestinal BBM enzymes has been successfully used to an indicator for the maturation process of the digestive function of larvae [44]. LAP and AKP are considered to be sensitive indicators of well-differentiated intestinal BBM [45, 46]. In the present study, larvae fed with the SSO diet had significantly higher activity of AKP in IS and BBM than larvae fed with dietary FO. These results confirmed that replacement of FO with SSO has beneficial effects on the maturation of larval intestinal development.

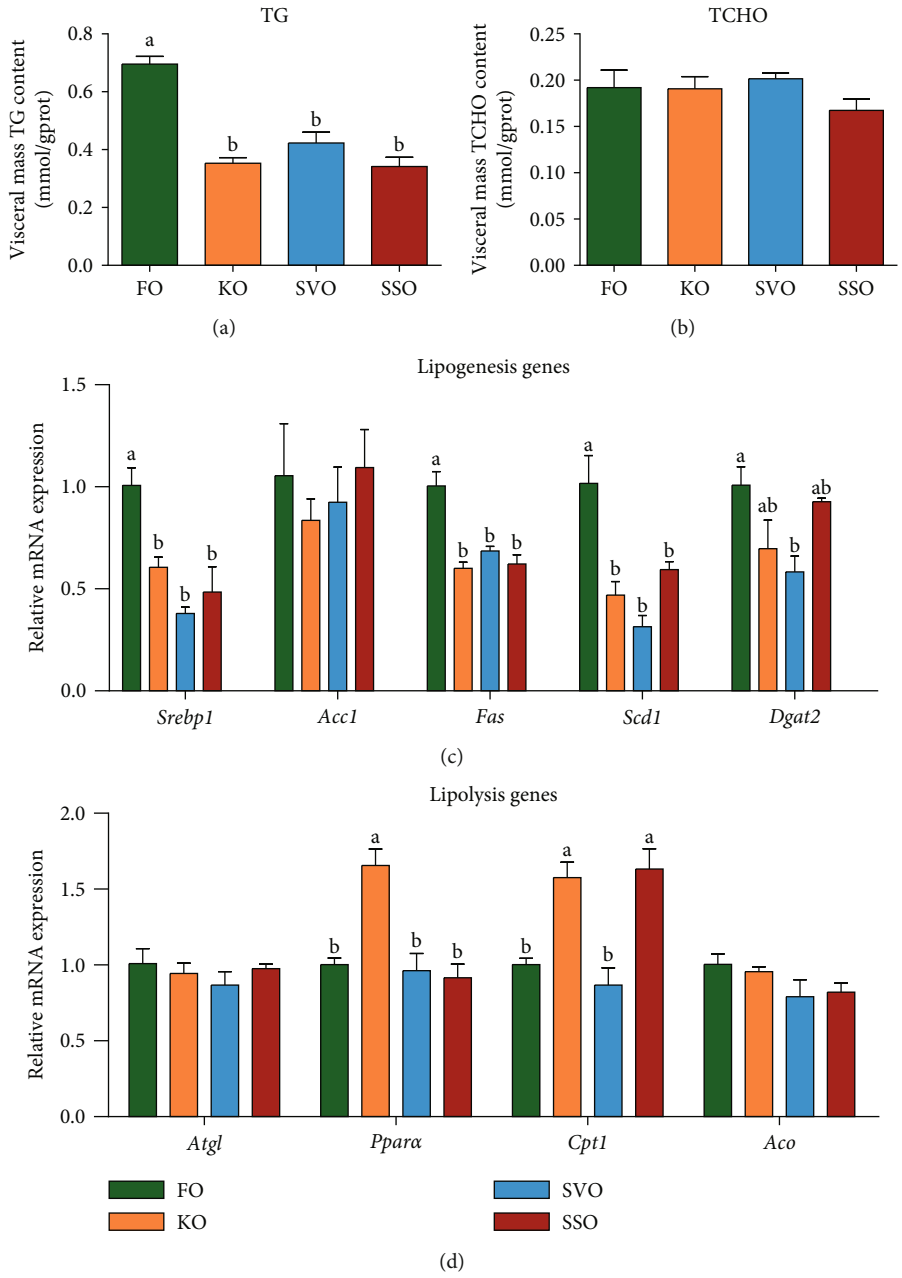

Figure 4: Effects of different dietary marine-based lipid sources on triglyceride (TG) content, total cholesterol content (TCHO), and relative mRNA expression of genes involved in lipid metabolism in visceral mass of large yellow croaker larvae. Values are means ($n = 3$), with their standard errors represented by vertical bars. Bars bearing the same letters were not significantly different ($P > 0.05$, Tukey's test). TG: triglyceride content; *srebp-1*: sterol-regulatory element-binding protein-1; *fas*: fatty acid synthase; *cpt-1*: carnitine palmitoyl transferase-1; *pparα*: peroxisome proliferator-activated receptor *α*; *aco*: acyl-CoA oxidase.

Marine fish larvae are susceptible to oxidative stress due to their high energy demand for rapid growth [47]. Oxidative stress can cause excessive production of oxygen free radicals, leading to oxidative injury such as DNA damage and lipid peroxidation [48]. SOD and CAT are two essential free radical scavenging enzymes in the cellular antioxidase system, which could sensitively reflect the antioxidant status of animals [49, 50]. In the present study, larvae fed with KO and SSO diets had significantly higher activity of SOD than those fed with other diets. However, no significant difference was observed in activity of CAT among dietary treatments. The activity of SOD response appears to be more sensitive than CAT to different dietary marine-based lipid sources. This result was similar to study in swimming crab, which found that diet KO significantly increased SOD activity, but there is no significant difference in CAT activity [12]. Moreover, larvae fed with the SSO diet had significantly higher T-AOC level than those fed with dietary FO, suggesting relatively higher antioxidant capacity. To sum up, it could be speculated that complete replacement of FO with SSO and KO could enhance the antioxidant capacity of fish larvae according to the increase in the antioxidant enzyme activities.

Previous studies have demonstrated that different dietary lipid sources have significant effects on body lipid contents of marine fish [51]. Similarly, larvae fed with KO and SSO diets in the present study had the lowest levels of body crude lipid. To find out why KO and SSO diets in larvae reduce body lipid contents, we detected TG content and mRNA expressions of key genes associated with lipid metabolism. In the present study, the TG content in visceral mass was significantly reduced in larvae fed with diets with SSO, KO, and SVO compared to those fed with dietary FO. Meanwhile, SSO, KO, and SVO diets in larvae significantly decreased mRNA expression of *srebp-1* in visceral mass compared with dietary FO. The *srebp-1* as a transcription factor plays a crucial role in lipogenesis, which can activate fatty acid synthesis-related gene expression including *fas* and *scd1* [52, 53]. Along with the inhibition of *srebp-1* expression by larvae fed with SSO, KO, and SVO diets, mRNA levels of *fas and scd1* also markedly decreased. In addition, the KO diet in larvae markedly increased the mRNA expression of *pparα* compared to other diets. The *pparα*, as a transcription factor, can activate fatty acid oxidation-related gene expression, such as *cpt-1* [54, 55]. Accordingly, SSO and KO diets in larvae markedly increased the mRNA expression of *cpt-1* compared to dietary FO and SVO. Similar results were also observed that dietary n-3 LC-PUFA increased fatty acid oxidation of mitochondria according to stimulating the activity of CPT I [56]. Overall, diets with SSO and KO in larvae might reduce lipid accumulation according to suppressing lipid synthesis and elevating lipid oxidation, while dietary SVO might reduce lipid accumulation according to suppressing lipid synthesis.

In conclusion, results of the present study demonstrated that FO can be completely replaced in large yellow croaker larvae fed with KO and SVO without negative effects on survival, growth performance, activities of digestive enzyme, and antioxidant responses. Moreover, the complete replacement of FO by SSO can improve survival, activities of digestive enzymes, antioxidant responses, and lipid metabolism, despite inhibiting the appetite and growth of large yellow croaker larvae. Further research is needed to determine the optimum level of SSO to replace FO based on survival and growth performance.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

No conflict of interest exits in the submission of this manuscript.

Authors' Contributions

The manuscript was approved by all authors for publication.

Acknowledgments

This research was financially supported by the China Agriculture Research System of MOF and MARA (CARS-47), the Key Program of National Natural Science Foundation of China (31830103), and the Leading Talent of Technological Innovation of Ten-Thousands Talents Program (CS31117200001). We thank Xueshan Li, Xiukun Zheng, Yuliang He, and Wenxuan Xu for their help during the experiment.

References

[1] S. Morais, M. J. Caballero, L. E. Conceicao, M. S. Izquierdo, and M. T. Dinis, "Dietary neutral lipid level and source in Senegalese sole (*Solea senegalensis*) larvae: effect on growth, lipid metabolism and digestive capacity," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 144, no. 1, pp. 57–69, 2006.

[2] M. Sprague, J. R. Dick, and D. R. Tocher, "Impact of sustainable feeds on omega-3 long-chain fatty acid levels in farmed Atlantic salmon, 2006-2015," *Scientific Reports*, vol. 6, no. 1, article 21892, 2016.

[3] D. R. Tocher, "Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective," *Aquaculture*, vol. 449, pp. 94–107, 2015.

[4] K. R. Thompson, T. J. Bailey, L. S. Metts, Y. J. Brady, and C. D. Webster, "Growth response and fatty acid composition of juvenile red claw crayfish (*Cherax quadricarinatus*) fed different sources of dietary lipid," *Aquaculture Nutrition*, vol. 16, no. 6, pp. 604–615, 2010.

[5] D. F. Deng, Z. Y. Ju, W. G. Dominy, L. Conquest, S. Smiley, and P. J. Bechtel, "Effect of replacing dietary menhaden oil with pollock or soybean oil on muscle fatty acid composition and growth performance of juvenile Pacific threadfin (*Polydactylus sexfilis*)," *Aquaculture*, vol. 422-423, pp. 91–97, 2014.

[6] M. Sprague, J. Walton, P. J. Campbell, F. Strachan, J. R. Dick, and J. G. Bell, "Replacement of fish oil with a DHA-rich algal meal derived from Schizochytrium sp. on the fatty acid and persistent organic pollutant levels in diets and flesh of Atlantic salmon (*Salmo salar*, L.) post-smolts," *Food chemistry*, vol. 185, pp. 413–421, 2015.

[7] S. M. Hixson, C. C. Parrish, X. Xue et al., "Growth performance, tissue composition, and gene expression responses in Atlantic salmon (*Salmo salar*) fed varying levels of different lipid sources," *Aquaculture*, vol. 467, pp. 76–88, 2017.

[8] M. Caballero, A. Obach, G. Rosenlund, D. Montero, M. Gisvold, and M. Izquierdo, "Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, Oncorhynchus mykiss," *Aquaculture*, vol. 214, no. 1-4, pp. 253–271, 2002.

[9] 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.

[10] M. Peng, W. Xu, K. Mai et al., "Growth performance, lipid deposition and hepatic lipid metabolism related gene expression in juvenile turbot (*Scophthalmus maximus* L.) fed diets with various fish oil substitution levels by soybean oil," *Aquaculture*, vol. 433, pp. 442–449, 2014.

[11] P. Tan, X. Dong, H. Xu, K. Mai, and Q. Ai, "Dietary vegetable oil suppressed non-specific immunity and liver antioxidant capacity but induced inflammatory response in Japanese sea bass (*Lateolabrax japonicus*)," *Fish and Shellfish Immunology*, vol. 63, pp. 139–146, 2017.

[12] Y. Yuan, X. Wang, M. Jin, P. Sun, and Q. Zhou, "Influence of different lipid sources on growth performance, oxidation resistance and fatty acid profiles of juvenile swimming crab, Portunus trituberculatus," *Aquaculture*, vol. 508, pp. 147–158, 2019.

[13] Y.-C. Kim, N. Romano, K.-S. Lee, C.-Y. Teoh, and W.-K. Ng, "Effects of replacing dietary fish oil and squid liver oil with vegetable oils on the growth, tissue fatty acid profile and total carotenoids of the giant freshwater prawn, *Macrobrachium rosenbergii*," *Aquaculture Research*, vol. 44, pp. 1731–1740, 2012.

[14] M. R. Miller, P. D. Nichols, and C. G. Carter, "Replacement of fish oil with thraustochytrid Schizochytrium sp. L oil in Atlantic salmon parr (*Salmo salar* L) diets," *Comparative biochemistry and physiology part A: Molecular & integrative physiology*, vol. 148, no. 2, pp. 382–392, 2007.

[15] J. Du, H. Xu, S. Li, Z. Cai, K. Mai, and Q. Ai, "Effects of dietary chenodeoxycholic acid on growth performance, body composition and related gene expression in large yellow croaker (Larimichthys crocea) fed diets with high replacement of fish oil with soybean oil," *Aquaculture*, vol. 479, pp. 584–590, 2017.

[16] R. Ji, Y. Li, X. Li et al., "Effects of dietary tea polyphenols on growth, biochemical and antioxidant responses, fatty acid composition and expression of lipid metabolism related genes of large yellow croaker (*Larimichthys crocea*)," *Aquaculture Research*, vol. 49, no. 3, pp. 1210–1218, 2018.

[17] C. Yao, W. Huang, Y. Liu et al., "Effects of dietary silymarin (SM) supplementation on growth performance, digestive enzyme activities, antioxidant capacity and lipid metabolism gene expression in large yellow croaker (*Larimichthys crocea*) larvae," *Aquaculture Nutrition*, vol. 26, no. 6, pp. 2225–2234, 2020.

[18] T. Ding, N. Xu, Y. Liu et al., "Optimal amounts of coconut oil in diets improve the growth, antioxidant capacity and lipid metabolism of large yellow croaker (*Larimichthys crocea*)," *Marine Life Science & Technology*, vol. 2, no. 4, pp. 376–385, 2020.

[19] X. Li, Q. Chen, Q. Li et al., "Effects of high levels of dietary linseed oil on the growth performance, antioxidant capacity, hepatic lipid metabolism, and expression of inflammatory genes in large yellow croaker (Larimichthys crocea)," *Frontiers in Physiology*, vol. 12, article 631850, 2021.

[20] F. Xie, *Studies on Nutritional Physiology of Amino Acid and Fatty Acid for Large Yellow Croaker (Larimichthys crocea) Larvae [PhD thesis]*, Ocean University of China, 2011.

[21] AOAC, "Official Methods of Analysis of AOAC International," 1995.

[22] R. Zuo, Q. Ai, K. Mai, and W. Xu, "Effects of conjugated linoleic acid on growth, non-specific immunity, antioxidant capacity, lipid deposition and related gene expression in juvenile large yellow croaker (*Larmichthys crocea*) fed soyabean oil-based diets," *The British journal of nutrition*, vol. 110, no. 7, pp. 1220–1232, 2013.

[23] R. K. Crane, G. Boge, and A. Rigal, "Isolation of brush border membranes in vesicular form from the intestinal spiral valve of the small dogfish (*Scyliorhinus canicula*)," *Biomembranes*, vol. 554, no. 1, pp. 264–267, 1979.

[24] H. Holm, L. E. Hanssen, Å. Krogdahl, and J. Florholmen, "High and low inhibitor soybean meals affect human duodenal proteinase activity differently: in vivo comparison with bovine serum albumin," *The Journal of Nutrition*, vol. 118, no. 4, pp. 515–520, 1988.

[25] S. Maroux, D. Louvard, and J. Barath, "The aminopeptidase from hog intestinal brush border," *Biochimica et Biophysica Acta (BBA)-Enzymology*, vol. 321, no. 1, pp. 282–295, 1973.

[26] Z. Cai, S. Feng, X. Xiang, K. Mai, and Q. Ai, "Effects of dietary phospholipid on lipase activity, antioxidant capacity and lipid metabolism-related gene expression in large yellow croaker larvae (*Larimichthys crocea*)," *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 201, pp. 46–52, 2016.

[27] Y. Pang, X. Xu, X. Xiang et al., "High fat activates O-GlcNAcylation and affects AMPK/ACC pathway to regulate lipid metabolism," *Nutrients*, vol. 13, no. 6, p. 1740, 2021.

[28] P. Tan, X. Dong, K. Mai, W. Xu, and Q. Ai, "Vegetable oil induced inflammatory response by altering TLR-NF-κB signalling, macrophages infiltration and polarization in adipose tissue of large yellow croaker (*Larimichthys crocea*)," *Fish and Shellfish Immunology*, vol. 59, pp. 398–405, 2016.

[29] J. Yan, K. Liao, T. Wang, K. Mai, W. Xu, and Q. Ai, "Dietary lipid levels influence lipid deposition in the liver of large yellow croaker (*Larimichthys crocea*) by regulating lipoprotein receptors, fatty acid uptake and triacylglycerol synthesis and catabolism at the transcriptional level," *PLoS One*, vol. 10, no. 6, article e0129937, 2015.

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

[31] K. Bonacic, A. Martínez, E. Gisbert, A. Estévez, and S. Morais, "Effect of alternative oil sources at different dietary inclusion levels on food intake and appetite regulation via enteroendocrine and central factors in juvenile Solea senegalensis (Kaup, 1858)," *Aquaculture*, vol. 470, pp. 169–181, 2017.

[32] I. Rønnestad, A. S. Gomes, K. Murashita, R. Angotzi, E. Jönsson, and H. Volkoff, "Appetite-controlling endocrine systems in teleosts," *Frontiers in endocrinology*, vol. 8, p. 73, 2017.

[33] H. Volkoff, "Fish as models for understanding the vertebrate endocrine regulation of feeding and weight," *Molecular and cellular endocrinology*, vol. 497, article 110437, 2019.

[34] M. Nakazato, N. Murakami, Y. Date et al., "A role for ghrelin in the central regulation of feeding," *Nature*, vol. 409, no. 6817, pp. 194–198, 2001.

[35] T. Han, J. Wang, S. Hu, X. Li, Y. Jiang, and C. Wang, "Effects of different dietary lipid sources on growth performance and tissue fatty acid composition of juvenile swimming crab Portunus trituberculatus," *Chinese Journal of Oceanology and Limnology*, vol. 33, no. 4, pp. 957–965, 2015.

[36] K. Liu, H. Liu, S. Chi, X. Dong, Q. Yang, and B. Tan, "Effects of different dietary lipid sources on growth performance, body composition and lipid metabolism-related enzymes and genes of juvenile golden pompano, *Trachinotus ovatus*," *Aquaculture Research*, vol. 49, no. 2, pp. 717–725, 2018.

[37] E. Ganuza, T. Benítez-Santana, E. Atalah, O. Vega-Orellana, R. Ganga, and M. S. Izquierdo, "Crypthecodinium cohnii and Schizochytrium sp. as potential substitutes to fisheries-derived oils from seabream (Sparus aurata) microdiets," *Aquaculture*, vol. 277, no. 1–2, pp. 109–116, 2008.

[38] C. G. Carter, M. P. Bransden, T. E. Lewis, and P. D. Nichols, "Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds," *Marine Biotechnology*, vol. 5, no. 5, pp. 480–492, 2003.

[39] E. Atalah, C. M. H. Cruz, M. S. Izquierdo et al., "Two microalgae Crypthecodinium cohnii and Phaeodactylum tricornutum as alternative source of essential fatty acids in starter feeds for seabream (Sparus aurata)," *Aquaculture*, vol. 270, no. 1-4, pp. 178–185, 2007.

[40] M. H. Li, E. H. Robinson, C. S. Tucker, B. B. Manning, and L. Khoo, "Effects of dried algae Schizochytrium sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish Ictalurus punctatus," *Aquaculture*, vol. 292, no. 3-4, pp. 232–236, 2009.

[41] E. Gisbert, G. Giménez, I. Fernández, Y. Kotzamanis, and A. Estévez, "Development of digestive enzymes in common dentex Dentex dentex during early ontogeny," *Aquaculture*, vol. 287, no. 3-4, pp. 381–387, 2009.

[42] E. Kjørsvik, T. F. Galloway, A. Estevez, Ø. Sæle, and M. Moren, "Effects of larval nutrition on development," *Larval Fish Nutrition*, vol. 181, pp. 219–248, 2011.

[43] Y. Lu, L. Wang, and X. Zhang, "Effects of different dietary lipid sources on growth, activities of digestive enzyme and immune ability of juvenile red claw crayfish (*Cherax quadricarinatus*)," *Journal of Southern Agriculture*, vol. 50, pp. 2095–2101, 2019.

[44] C. L. Cahu and J. L. Zambonino-Infante, "Maturation of the pancreatic and intestinal digestive functions in sea bass (Dicentrarchus labrax): effect of weaning with different protein sources," *Fish Physiology and Biochemistry*, vol. 14, no. 6, pp. 431–437, 1995.

[45] H. Ma, C. Cahu, J. Zambonino et al., "Activities of selected digestive enzymes during larval development of large yellow croaker (*Pseudosciaena crocea*)," *Aquaculture*, vol. 245, no. 1-4, pp. 239–248, 2005.

[46] J. L. Zambonino Infante and C. L. Cahu, "Ontogeny of the gastrointestinal tract of marine fish larvae," *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, vol. 130, no. 4, pp. 477–487, 2001.

[47] J. Liu, K. Mai, W. Xu, Y. Zhang, H. Zhou, and Q. Ai, "Effects of dietary glutamine on survival, growth performance, activities of digestive enzyme, antioxidant status and hypoxia stress resistance of half-smooth tongue sole (*Cynoglossus semilaevis* G *unther*) post larvae," *Aquaculture*, vol. 446, pp. 48–56, 2015.

[48] D. Tovar-Ramírez, D. Mazurais, J. F. Gatesoupe, P. Quazuguel, C. L. Cahu, and J. L. Zambonino-Infante, "Dietary probiotic live yeast modulates antioxidant enzyme activities and gene expression of sea bass (*Dicentrarchus labrax*) larvae," *Aquaculture*, vol. 300, no. 1-4, pp. 142–147, 2010.

[49] G. Mourente, D. R. Tocher, E. Diaz, A. Grau, and E. Pastor, "Relationships between antioxidants, antioxidant enzyme activities and lipid peroxidation products during early development in Dentex dentex eggs and larvae," *Aquaculture*, vol. 179, no. 1-4, pp. 309–324, 1999.

[50] M. Wan, K. Mai, H. Ma, and Z. G. Liufu, "Effects of dietary selenium and vitamin E on antioxidant enzyme activities in abalone, Haliotis discus hannai Ino," *Acta Hydrobiologica Sinica*, vol. 28, no. 5, pp. 496–503, 2004.

[51] H. Guo, C. Chen, X. Yan et al., "Effects of different dietary oil sources on growth performance, antioxidant capacity and lipid deposition of juvenile golden pompano Trachinotus ovatus," *Aquaculture*, vol. 530, article 735923, 2021.

[52] R. S. Kuipers, M. F. Luxwolda, W. S. Sango et al., "Postpartum changes in maternal and infant erythrocyte fatty acids are likely to be driven by restoring insulin sensitivity and DHA status," *Medical hypotheses*, vol. 76, no. 6, pp. 794–801, 2011.

[53] Y. Liu, D. Shi, Y. Liu et al., "Eicosapentaenoic acid-containing phosphatidylcholine alleviated lipid accumulation in orotic acid-induced non-alcoholic fatty liver," *Journal of Functional Foods*, vol. 23, pp. 294–305, 2016.

[54] R. Baillie, R. Takada, M. Nakamura, and S. Clarke, "Coordinate induction of peroxisomal acyl-CoA oxidase and UCP-3 by dietary fish oil: a mechanism for decreased body fat deposition," *Prostaglandins, leukotrienes and essential fatty acids*, vol. 60, no. 5-6, pp. 351–356, 1999.

[55] H. Poudyal, S. K. Panchal, V. Diwan, and L. Brown, "Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action," *Progress in lipid research*, vol. 50, no. 4, pp. 372–387, 2011.

[56] L. Madsen, A. C. Rustan, H. Vaagenes, K. Berge, E. Dyrøy, and R. K. Berge, "Eicosapentaenoic and docosahexaenoic acid affect mitochondrial and peroxisomal fatty acid oxidation in relation to substrate preference," *Lipids*, vol. 34, no. 9, pp. 951–963, 1999.