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Liver Transcriptomic Analysis of the Effects of Dietary Fish Oil Revealed a Regulated Expression Pattern of Genes in Adult Female Spotted Scat (*Scatophagus argus*)

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Despite the significance of n-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) in fish oil in promoting the maturation of female broodstocks, the detailed mechanism of the effect on the expression of hepatic reproduction-related genes is still unclear. In this study, transcriptome sequencing was used to analyze the effect of the higher dietary n-3 LC-PUFA level on gene expression in the liver of adult females spotted scat. Two-year-old female spotted scat (average weight, 242.83 ± 50.90 g) were fed with diets containing 8% fish oil (FO) or 8% soybean oil (SO) for 40 days. The fatty acid profile in the serum, liver, and ovary was analyzed, and high proportions of n-3 LC-PUFA were observed in the FO group. The final average fish body weight and gonadosomatic index were similar between the FO and SO groups. The serum vitellogenin (Vtg) and hepatosomatic index (HSI) of the FO group were significantly higher and lower than that of the SO group, respectively. Comparatively, the liver transcriptome analysis showed 497 upregulated and 267 downregulated genes in the FO group. Among them, the expression levels of three estrogen-regulated genes (i.e., *Vtga*, *Vtgb*, and *Zp4*) were significantly higher in the FO than in the SO group. This expression pattern could be related to the upregulation of *Hsd17b7* (the key gene for the synthesis of liver steroid hormone) and the downregulation of the *Hsp90* (the estrogen receptor chaperone). The expression levels of *Foxo1a* and *Lep*, which are involved in the lipid metabolism, decreased significantly in the FO group, which may be related to the lower level of HSI in the FO group. The genes related to liver LC-PUFA absorption and transport, *Fabp2* and *Mfsd2ab*, were significantly upregulated in the FO group, indicating that fish actively adapt to high-fish-oil diets. In brief, high-fish-oil diets can influence the expression of genes related to liver n-3 LC-PUFA metabolism and reproduction, inhibit the accumulation of liver fat, and promote the liver health and gonad development. This study will contribute to clarifying the mechanism of dietary n-3 LC-PUFA on promoting reproductive development in teleost fish.

Keywords: spotted scat, female broodstock, fish oil, liver, transcriptome, reproduction, lipid metabolism, vitellogenin

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INTRODUCTION

The effects of essential fatty acids in feed on the reproduction of broodstocks are among the main research areas of artificial fish breeding in recent years. Long-chain polyunsaturated fatty acids (LC-PUFA) supplied in fish oil, including docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3), and arachidonic acid (ARA, 20:4n-6), are essential nutrients for the normal growth and development of fish and can play key roles in the lipid metabolism, synthesis of sex steroid hormones, and reproductive regulation (Zhou et al., 2011; Glencross et al., 2015; Xu et al., 2017). For example, in yellowfin sea bream (*Acanthopagrus latus*) and flame angelfish (*Centropyge loriculus*), the relative fecundity, hatching rate, and larval survival rate were increased by the supplementing higher level of n-3 LC-PUFA in diet (Zakeri et al., 2011; Callan et al., 2012). When Japanese flounder (*Paralichthys olivaceus*) were fed with diets containing three different levels of n-3 LC-PUFA (i.e., 2.1, 4.8, and 6.2%), egg production increased in 6.2% n-3 LC-PUFA group. At the same time, the hatching rate and percentage of normal larvae were higher in 2.1% n-3 LC-PUFA group, indicating that excessive n-3 LC-PUFA in the diet would reduce the egg quality (Furuita et al., 2002). Consistently, in *Plectorhynchus cinctus* broodstock fed with four different levels of n-3 LC-PUFA, the moderate level of n-3 LC-PUFA improved the reproductive performance. In comparison, too high or too low n-3 LC-PUFA levels caused a reduction in reproductive performance (Li et al., 2004).

The liver is a key metabolic organ that is widely involved in the bioconversion of fatty acid and the regulation of lipid homeostasis in fish (Lazzarotto et al., 2018; Esmaeili et al., 2021). The liver is also the most important active tissue for gonadal development in oviparous vertebrates. In teleost fish, most of the yolk is synthesized and secreted by liver cells in the form of a protein precursor: the vitellogenin (Vtg) (Bemanian et al., 2004). In addition, some zona pellucida (*Zp*) genes encoding Zp glycoproteins, which constitute the protective coat surrounding oocytes, were also expressed in the teleost fish liver (Wu et al., 2018). Hence, the liver is a vital protein synthesis "factory" for the normal ovary development in fish. Previous studies have shown that the addition of fish oil to increase the dietary n-3 LC-PUFA level can promote the expression of *Vtg* in the liver of female fish, including the spotted scat (*Scatophagus argus*) (Zhang et al., 2013; Peng et al., 2015). However, the regulatory mechanism of the reproductive-related gene caused by the dietary n-3 LC-PUFA level in the liver of broodstock is still unclear, even though very pertinent in breeding programs.

In artificial breeding, the primary step is to get broodstocks with healthy oocytes and sperm in females and males, respectively. Also, the usual way to obtain mature fish with a better reproductive ability is by nutrient supplementation in the fish diet. Nutrigenomics utilizes high-throughput genomics approaches to unravel how dietary nutrients regulate gene expression at the mRNA/protein level and ultimately modulate cellular and organism metabolism. The most widely applied technique of nutrigenomics is the transcriptome analysis *via* next-generation sequencing (gene expression patterns at the mRNA level), also known as RNA-seq (Afman and Müller, 2012). Studies of the transcriptional effects of LC-PUFA on liver gene expression in fish have been carried out in postsmolts Atlantic salmon (*Salmo salar*) (Glencross et al., 2015; Xue et al., 2020) and 2-month-old golden pompano (*Trachinotus ovatus*) (Lei et al., 2019). Nonetheless, evaluating the effects of n-3 LC-PUFA on livers of female broodstocks using the transcriptional method is lacking. This study focused on the hepatic transcriptome analysis to comprehensively understand the detailed molecular mechanisms of the effects of fish oil on reproductive-related genes in the liver of adult female spotted scat.

Spotted scat is an important food fish mainly produced in the South and South-East Asian countries. Due to its good nutrient quality, high protein content, and delicious taste, the fish has gained high demand and become an important aquaculture species. It is also a popular aquaria fish due to its colorful appearance, strong adaptability, and easiness to be cultured indoors (Gupta, 2016). For these reasons, studies focusing on its reproduction biology are on the rise, including reproduction endocrinology (Jiang et al., 2017; Zhang et al., 2018; Mandal et al., 2020), sex determination and differentiation (Mustapha et al., 2018, 2021; He et al., 2019), and artificial breeding (Cai et al., 2010; Gandhi et al., 2014; Mandal et al., 2021). Its artificial breeding is successful in several countries, including China (Cai et al., 2010) and India (Mandal et al., 2021). To obtain matured spotted scat broodstock, appropriate nutritional factors should be maintained to enhance gonad development and induce reproduction (Cai et al., 2010). Although fish oil provides adequate nutrition for broodstock, the regulatory mechanism is unclear. Also, there is a delay in female gonad maturation compared with that of male (Gandhi et al., 2014). Therefore, it is important to advance studies on the effects of fish oil on the expressions of liver genes to gain insight into gene regulatory mechanisms in female spotted scat. The findings will provide essential knowledge for further functional studies on fish oil in promoting teleost fish reproduction.

MATERIALS AND METHODS

Ethical Considerations

This experiment was undertaken in accordance with the guidance, care, and use of laboratory animals in China with approval from the Committee on the Ethics of Animal Experiments of Guangdong Ocean University.

Fish Rearing

Two-year-old female spotted scats were bought from the Zhuhai HengDa Cultivation Base (Guangdong, China). The sex ratio of spotted scats is approximately 1:1 (Mustapha et al., 2021). The female spotted scats were empirically selected from the mixed-sex fish by the sexual morphological difference in the head, anal fin, body color, body weight, and genital pore (Barry and Fast, 1992). The fish were transferred to the Donghai Island experimental base, Guangdong province, China. The fish were reared in a concrete pond (12 m × 5 m × 2 m) and adapted to the rearing condition for 2 weeks. Aeration was supplied to the tank through air stones, and the average water temperature was 31.1 ± 0.8°C.

The salinity was 8‰, while pH and dissolved oxygen ranged from 7.5–8.5 and 6.0–7.0 mg/L, respectively. Fish were fed 2.5% of their body weight with commercial fish pellets consisting of 43% crude protein and 12% crude lipid. Fish were fed two times a day at 06:00 and 18:00 h throughout the adaptation period. The pond water was not recirculated. Approximately, 20% of the water in the pond was drained off and replaced with fresh water once in 3 days.

Experimental Design

After the adaptation period, 100 females (average initial weights of 242.83 ± 50.90 g, and length of 19.48 ± 1.13 cm) were randomly distributed into two treatment groups (50 fish in each group) containing different levels of fish oil (0% and 8%; ingredients are listed in **Table 1**). The diet containing 8% fish oil was marked as the FO group, while the diet of the control group containing 8% soybean oil marked the SO group. Each treatment group was duplicated and contained 25 fish in each net cage (5 m × 3.5 m × 1.8 m). Two independent concrete ponds (12 m × 5 m × 2 m) were used; one concrete pond had two net cages for both FO and SO treatments. The fish were fed with the two diets for 40 days before sampling. The culturing condition was consistent with the adaptation stage.

Growth Measurements and Sampling

At the termination of the experiment, the spotted scats were sampled after 24 h starvation. Five fish per cage were sacrificed for sampling. The final number of fish, body weight, body length, gut weight, liver weight, and gonad weight were taken individually. The survival rate (SR), condition factor (CF), viscerosomatic index (VSI), hepatosomatic index (HSI), and gonadosomatic index (GSI) were calculated using the following formulas:

SR (%) = final number of fish/initial number of fish × 100;
CF = final body weight (g)/final body length (cm) ^{3} × 100;
VSI (%) = final viscera weight (g)/final body weight (g) × 100;
HIS (%) = final liver weight (g)/final body weight (g) × 100;
GSI (%) = final gonad weight (g)/final body weight (g) × 100.

The liver and ovary samples of the fish were excised and quickly frozen in liquid nitrogen. Blood samples were collected from the caudal vein of the fish. The blood samples were stored at 4°C overnight and centrifuged (at 3,000 rpm for 10 min at 4°C), and the upper layer serums were collected. All samples were subsequently stored at −80°C for further analysis.

TABLE 1 | Formulation and proximate composition of experimental diets.

Ingredients (% dry matter)	SO	FO
Fish meal	30	30
Wheat gluten	6	6
Soybean meal	26	26
Wheat flour	25	25
Soy lecithin	2	2
Fish oil		8
Soy oil	8	
$Ca(H_2PO_4)_2$	1.5	1.5
Fish mineral and vitamin premix[a]	1	1
Choline chloride (60%)	0.5	0.5
Proximate composition (%)		
Dry matter	91.41	90.60
Crude protein	44.84	45.22
Crude lipid	12.80	12.50
Ash	10.13	10.17

[a]*Fish mineral and vitamin premix (per kg premix): 6,00,000 IU/kg axerophthol; 2,00,000 IU/kg cholecalciferol; 6,000 IU/kg a-tocopherol; 1,000 mg/kg menadione; 900 mg/kg thiamin; 900 mg/kg riboflavin; 750 mg/kg pyridoxine; 3 mg/kg cyanocobalamin; 15 mg/kg D-biotin; 3,000 mg/kg D-pantothenate; 300 mg/kg folic acid; 4,500 mg/kg nicotinamide; 9,000 mg/kg vitamin C; 8,000 mg/kg inositol; 14,000 mg/kg Fe; 350 mg/kg Cu; 1,500 mg/kg Mn; 4,000 mg/kg Zn; 10,000 mg/kg Mg; 25 mg/kg Co; 50 mg/kg I; 30 mg/kg selenium; 500 mg/kg ethoxyquinoline.*

Fatty Acid Composition of the Experimental Diets, Fish Serum, Liver, and Ovary

The total lipid from the ovary and liver (n = 6 per group) and feed were extracted using diethyl ether after acid hydrolysis, whereas lipid from the serum (n = 6 per group) was extracted with chloroform according to the procedure of the National Standards of the People's Republic of China (5009.168—2016). Extracted lipid was saponified with NaOH-methanol. Their constitutive fatty acids were methylated through the action of boron trifluoride-methanol, separated using Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, United States) on a capillary column (SPTM-2560; 100 m × 0.25 mm × 0.25 mm), and quantified using glyceryl triundecanoate as an internal standard.

RNA Extraction, Library Construction, and Sequencing

To evaluate the effects of fish oil on the genes expressed in the liver, the transcriptome analysis was carried out. First, total RNA (n = 6 per group) from the two groups was extracted from fish liver using RNeasy® Mini Kit reagents according to the instructions of the manufacturer (Qiagen GMBH, Hilden, Germany). The RNA quality was determined and quantified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, United States) and checked using the RNase-free agarose gel electrophoresis. The mRNA was enriched by oligo (dT) beads; the mRNA was interrupted. The first strand of cDNA was synthesized in M-MuLV reverse-transcriptase system, and the second strand was synthesized from dNTPs in DNA polymerase I system. Then, the cDNA fragments were ligated to Illumina sequencing adapters. The ligation products were size selected using agarose gel electrophoresis, PCR amplified, and sequenced using novaseq 6000 by Guangzhou Genedenovo Biotechnology Co. (Guangzhou, China). All clean libraries of sequencing data were submitted to the NCBI Sequence Read Archive (SRA) database (Accession No: PRJNA765892).

Data Filtering, Reads Mapping, and Differential Gene Expression Analysis

To ensure that high-quality data are achieved, the original data were filtered before analyzing. The raw reads were filtered using

fastp (version 0.18.0). The adapters read with more than 10% unknown nucleotides and over 50% low-quality nucleotides (*Q*-value $\leq$ 20) were removed. The ribosome RNA (rRNA) reads were removed with the alignment tool Bowtie2 (version 2.2.8). Then, these clean reads were mapped to the spotted scat reference genome (Huang et al., 2021) with HISAT2.2.4. Genes with unknown transcripts or in intergenic regions were defined as novel genes. The fragments per kilobase of transcript per million mapped reads (FPKM) method was used to estimate the gene expression levels. Differentially expressed genes (DEGs) in two groups were identified using the EdgeR software package. In comparison, to avoid some important genes being filtered out, genes with $|\log2$ (fold change)$| > 1$ and $P < 0.05$ were identified as significant DEGs. DEGs were annotated with the enrichment analysis of Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The KEGG pathway with $P < 0.05$ was regarded as a significant enrichment pathway.

Validation by Real-Time PCR

To validate the gene expression profiles from the RNA-seq analysis, 24 differentially expressed genes related to fat metabolism and ovarian development were selected for the real-time PCR analysis. Real-time PCR was performed using the PerfectStart^{TM} Green qPCR SuperMix (TRAN, China). The PCR reaction was carried out in a total volume of 20 μl with 10 μl of 2 × PerfectStart^{TM} Green qPCR SuperMix, 0.4 μl of each primer, and 2 μl cDNA. The thermal cycling program included an initial denaturation at 94°C for 30 s, followed by 40 cycles of 94°C for 5 s, 60°C for 15 s, and 72°C for 10 s. The reference gene, β-*actin*, was used to normalize the expression values. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of the target genes. Each group contains 10 independent samples, and each sample was detected in triplicate. Primer sequences used for real-time PCR are listed in **Supplementary Table 1**.

Vitellogenin Protein Levels of Serum

The content of serum Vtg was detected using Grouper Vitellogenin ELISA Kit according to the instructions of the manufacturer (Cusabio, Wuhan, China). The serum samples from 10 fish in each group were analyzed.

Statistical Analysis

All data are expressed as means ± standard error (SE), and an independent sample *t*-test was used with the significance level at $P < 0.05$. The statistical analysis of the data was performed using the SPSS 17.0 software (SPSS Inc., Chicago, IL, United States).

RESULTS

Fatty Acid Composition of the Feed

The DHA, EPA, and ARA contents in FO diet are higher than that of the SO diet. On the contrary, the oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) contents in FO diet are lower than that of the SO diet (**Table 2**). The differences are mainly attributed to their different oil sources.

TABLE 2 | Fatty acid composition of the experimental diets (g/100 g).

Items	SO	FO
C14:0	0.057	0.499
C15:0	0.012	0.039
C16:0	1.517	1.675
C17:0	0.019	0.036
C18:0	0.511	0.343
C20:0	0.040	0.024
C22:0	0.049	0.020
C24:0	0.024	0.012
SFA[2]	2.229	2.648
C16:1n-7	0.074	0.534
C18:1n-9	2.114	1.168
C20:1	0.189	0.184
C22:1n-9	0.039	0.041
C24:1n-9	0.045	0.058
MUFA[3]	2.461	1.985
C18:2n-6	4.783	1.240
C18:3n-6	0.032	0.018
C20:2-6	0.015	0.040
C20:3n-6	ND[1]	0.014
C20:4n-6	0.035	0.077
C22:2n-6	0.003	0.006
n-6 PUFA[4]	4.867	1.395
C18:3n-3	0.723	0.215
C20:3n-3	0.007	0.018
C20:5n-3	0.140	1.363
C22:6n-3	0.396	1.198
n-3 PUFA[5]	1.266	2.794
n-3 LC-PUFA[6]	0.543	2.579
n-3 PUFA/n-6 PUFA[7]	0.260	2.003

[1]*ND, non-detectable.*
[2]*SFA, saturated fatty acids.*
[3]*MUFA, mono-unsaturated fatty acids.*
[4]*n-6 PUFA, n-6 polyunsaturated fatty acids.*
[5]*n-3 PUFA, n-3 polyunsaturated fatty acids.*
[6]*n-3 LC-PUFA, n-3 long-chain polyunsaturated fatty acid.*
[7]*n-3 PUFA/n-6 PUFA, the ratio of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.*

Fatty Acid Composition of the Fish Serum, Ovary, and Liver

Table 3 shows fatty acid composition in different tissues of female spotted scat. In the ovary, fish oil treatment significantly increased ($P < 0.05$) the EPA concentration. Also, in the liver, fish oil treatment significantly decreased ($P < 0.05$) the C18:3n-6 concentration and increased the EPA concentration. In the serum, fish oil treatment significantly increased ($P < 0.05$) C14:0, C15:0, C16:1n-7, C17:0, C22:1n-9, EPA, C24:0, and DHA concentration, while fish oil treatment significantly decreased ($P < 0.05$) linoleic acid, C18:3n-6, C20:2-6, and C20:3n-6 concentration.

Survival Rate, Growth Performance, and Body Indices

At the end of the experiment, no fish died in both FO and SO groups. The final body weight, body length, CF, GSI, and VSI of

TABLE 3 | Fatty acid composition in different tissues of the female spotted scat fed with different oil sources.

	Ovary		Liver		Serum	
Items	**SO**	**FO**	**SO**	**FO**	**SO**	**FO**
C14:0	0.044 ± 0.011	0.105 ± 0.049	0.169 ± 0.036	0.217 ± 0.030	0.005 ± 0.001^{b}	0.014 ± 0.001^{a}
C15:0	0.011 ± 0.002	0.019 ± 0.007	0.034 ± 0.009	0.041 ± 0.006	0.002 ± 0.000^{b}	0.005 ± 0.001^{a}
C16:0	1.066 ± 0.223	1.564 ± 0.819	5.530 ± 1.577	5.612 ± 1.191	0.238 ± 0.022	0.305 ± 0.025
C17:0	0.007 ± 0.002	0.013 ± 0.006	0.038 ± 0.009	0.038 ± 0.006	0.002 ± 0.000^{b}	0.005 ± 0.000^{a}
C18:0	0.207 ± 0.038	0.265 ± 0.110	0.822 ± 0.208	0.832 ± 0.172	0.068 ± 0.007	0.087 ± 0.008
C20:0	0.002 ± 0.002	0.007 ± 0.007	0.028 ± 0.007	0.027 ± 0.007	0.001 ± 0.000	0.001 ± 0.000
C22:0	ND[1]	ND	0.013 ± 0.003	0.010 ± 0.003	ND	ND
C24:0	0.002 ± 0.002	0.007 ± 0.007	0.035 ± 0.010	0.028 ± 0.009	0.001 ± 0.000^{b}	0.004 ± 0.001^{a}
SFA[2]	1.341 ± 0.275	1.983 ± 1.007	6.669 ± 1.855	6.805 ± 1.409	0.317 ± 0.028^{b}	0.420 ± 0.035^{a}
C16:1n-7	0.171 ± 0.044	0.351 ± 0.157	0.558 ± 0.151	0.816 ± 0.153	0.014 ± 0.002^{b}	0.038 ± 0.004^{a}
C18:1n-9	1.314 ± 0.343	1.412 ± 0.645	3.857 ± 1.128	3.800 ± 0.767	0.143 ± 0.014	0.155 ± 0.015
C20:1	0.093 ± 0.022	0.191 ± 0.129	0.586 ± 0.169	0.638 ± 0.155	0.012 ± 0.002	0.014 ± 0.002
C22:1n-9	0.013 ± 0.003	0.024 ± 0.015	0.085 ± 0.022	0.094 ± 0.021	0.0020 ± 0.0005^{b}	0.0024 ± 0.0005^{a}
C24:1n-9	0.016 ± 0.004	0.021 ± 0.006	0.042 ± 0.006	0.042 ± 0.006	0.002 ± 0.000	0.002 ± 0.000
MUFA[3]	1.607 ± 0.402	2.000 ± 0.944	5.128 ± 1.464	5.390 ± 1.097	0.173 ± 0.017	0.211 ± 0.020
C18:2n-6	1.041 ± 0.318	0.624 ± 0.310	1.090 ± 0.257	0.582 ± 0.100	0.086 ± 0.011^{a}	0.043 ± 0.004^{b}
C18:3n-6	0.263 ± 0.094	0.105 ± 0.044	0.803 ± 0.181^{a}	0.329 ± 0.062^{b}	0.032 ± 0.005^{a}	0.013 ± 0.003^{b}
C20:2-6	0.079 ± 0.018	0.077 ± 0.044	0.272 ± 0.070	0.176 ± 0.031	0.015 ± 0.002^{a}	0.009 ± 0.001^{b}
C20:3n-6	0.290 ± 0.089	0.132 ± 0.052	0.572 ± 0.144	0.310 ± 0.077	0.051 ± 0.007^{a}	0.022 ± 0.003^{b}
C20:4n-6	0.088 ± 0.013	0.096 ± 0.015	0.078 ± 0.012	0.078 ± 0.011	0.020 ± 0.001	0.028 ± 0.004
C22:2n-6	0.000 ± 0.000	0.007 ± 0.006	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
n-6 PUFA[4]	1.760 ± 0.525	1.041 ± 0.463	2.815 ± 0.639	1.475 ± 0.274	0.204 ± 0.024^{a}	0.117 ± 0.011^{b}
C18:3n-3	0.074 ± 0.022	0.073 ± 0.039	0.093 ± 0.024	0.085 ± 0.018	0.005 ± 0.001	0.003 ± 0.000
C20:3n-3	0.015 ± 0.004	0.015 ± 0.009	0.032 ± 0.011	0.026 ± 0.004	0.002 ± 0.000	0.002 ± 0.000
C20:5n-3	0.028 ± 0.009^{b}	0.184 ± 0.059^{a}	0.028 ± 0.006^{b}	0.118 ± 0.018^{a}	0.006 ± 0.001^{b}	0.041 ± 0.006^{a}
C22:6n-3	0.498 ± 0.150	0.882 ± 0.279	0.549 ± 0.147	0.941 ± 0.125	0.130 ± 0.012^{b}	0.246 ± 0.023^{a}
n-3 PUFA[5]	0.614 ± 0.182	1.155 ± 0.367	0.703 ± 0.187	1.170 ± 0.162	0.143 ± 0.012^{b}	0.292 ± 0.028^{a}
n-3 LC-PUFA[6]	0.540 ± 0.162	1.082 ± 0.343	0.610 ± 0.164	1.084 ± 0.146	0.137 ± 0.012^{b}	0.289 ± 0.028^{a}
n-3 PUFA/n-6 PUFA[7]	0.345 ± 0.028^{b}	1.287 ± 0.254^{a}	0.253 ± 0.024^{b}	0.832 ± 0.076^{a}	0.730 ± 0.091^{b}	2.521 ± 0.142^{a}

Data are presented as means ± SE (n = 6). Values in the same row with different superscripts are significantly different ($P < 0.05$).
[1]ND, non-detectable.
[2]SFA, saturated fatty acids.
[3]MUFA, mono-unsaturated fatty acids.
[4]n-6 PUFA, n-6 polyunsaturated fatty acids.
[5]n-3 PUFA, n-3 polyunsaturated fatty acids.
[6]n-3 LC-PUFA, n-3 long-chain poly-unsaturated fatty acid.
[7]n-3 PUFA/n-6 PUFA, the ratio of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.

the SO and FO fish are similar ($P > 0.05$, **Table 4**). The HSI of fish in FO group was significantly lower than that of the SO group ($P < 0.05$).

Raw Sequencing Reads and Quality Statistics

Twelve cDNA libraries were constructed from livers from the SO and FO groups. In this experiment, a total of 534,586,540 (i.e., 289,243,632 in the SO group and 245,342,908 in the FO group) clean reads were obtained from the transcriptome sequencing. The percentages of Q30 bases were more than 93% for all the samples, indicating a high-quality sequence (**Table 5**). The results showed a total mapped reads of 497,792,649, and the percentage of mapped reads for each library ranged from 91.21 to 94.36% (**Table 5**). A total of 20,574 genes were detected, including 20,238 known genes and 336 novel genes, accounting for 83.44% of the reference genomes. **Supplementary Table 2** shows the number of genes detected in each sample.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichment Analysis of the Differentially Expressed Genes

In this study, a total of 764 genes were annotated to be DEGs ($P < 0.05$, $|\log2(\text{fold change})| > 1$). Compared with the SO group, 497 genes were upregulated in the FO group, while 267 genes were downregulated in the FO group (**Figure 1**).

In this study, GO and KEGG enrichment analyses were carried out to explore the biological functions of the DEGs. The DEGs

TABLE 4 | Growth performance of female spotted scat fed with different oil sources.

Item	Final weight (g)	SR (%)	body length (cm)	CF (%)	GSI (%)	HSI (%)	VSI (%)
SO	273.67 ± 10.62	100.0 ± 0.0	17.39 ± 0.15	5.19 ± 0.15	3.86 ± 0.86	3.89 ± 0.20[a]	14.26 ± 0.92
FO	282.28 ± 14.28	100.0 ± 0.0	17.41 ± 0.27	5.32 ± 0.12	5.77 ± 1.58	3.31 ± 0.18[b]	15.65 ± 0.94

Data are presented as means ± SE (n = 10). Values in the same column with different superscripts are significantly different (P < 0.05).

TABLE 5 | Summary of the sequencing data.

Sample	Raw data	Clean data (%)	After filter bases number (bp)	After filter Q20 (%)[a]	After filter Q30 (%)[b]	After filter GC (%)	Total_Mapped (%)
SO1	41,620,126	41,569,782 (99.88%)	6,201,562,361	98.38%	95.18%	51.68%	37,738,363 (91.21%)
SO2	49,145,802	49,087,598 (99.88%)	7,333,056,565	97.88%	93.75%	51.20%	45,749,559 (93.42%)
SO3	46,474,776	46,420,956 (99.88%)	6,929,984,390	97.64%	93.17%	51.61%	43,664,615 (94.23%)
SO4	57,989,442	57,917,536 (99.88%)	8,648,559,495	97.70%	93.35%	51.53%	53,425,068 (92.58%)
SO5	51,850,574	51,794,934 (99.89%)	7,737,533,380	98.00%	94.04%	50.84%	48,272,671 (93.45%)
SO6	42,494,046	42,452,826 (99.90%)	6,340,169,713	97.93%	93.85%	51.29%	39,533,702 (93.40%)
FO1	38,290,566	38,239,454 (99.87%)	5,704,221,313	98.31%	94.99%	51.03%	35,836,166 (93.91%)
FO2	37,027,228	36,982,354 (99.88%)	5,517,338,496	98.44%	95.30%	51.20%	34,573,596 (93.73%)
FO3	43,519,856	43,463,260 (99.87%)	6,488,035,759	97.88%	93.97%	51.02%	40,431,758 (93.25%)
FO4	38,476,364	38,428,274 (99.88%)	5,737,835,547	98.27%	94.89%	50.68%	36,198,089 (94.36%)
FO5	42,810,520	42,758,016 (99.88%)	6,379,194,964	97.98%	94.23%	50.82%	40,003,686 (93.77%)
FO6	45,530,902	45,471,550 (99.87%)	6,789,933,242	97.95%	94.15%	51.18%	42,365,376 (93.39%)

[a] *The percentage of bases with a phred value > 20 (error rate < 1%).*
[b] *The percentage of bases with a phred value > 30 (error rate < 0.1%).*

were annotated into GO categories of the cellular component, biological process, and molecular function (**Figure 2**). In the cellular component categories, organelle (61), cell (74), cell part (74), membrane part (45), and membrane (50) were the most enriched GO terms. In the biological process categories, cellular process (144), single-organism process (128), metabolic process (124), biological regulation (91), and response to stimulus (60) were the most enriched GO terms. In the molecular function categories, binding (140), catalytic activity (89), molecular transducer activity (22), and nucleic acid binding transcription factor activity (17) were the most enriched GO terms.

The top 20 biological pathways revealed in KEGG analysis are shown in **Figure 3**. The DEGs are mainly classified into cellular processes, environmental information processing, metabolism, organismal systems, and human diseases. Key genes for steroid biosynthesis (i.e., *Lss*, *Hsd17b7*, *Tm7sf2*, and *Nsdhl*, etc.), oocyte meiosis (i.e., *Ccnb2*, *Ppp2ca*, etc.), cell cycle (i.e., *Cdc14ab*, *Myc*, *Cdkn1a*, and *Gadd45b*, etc.), FoxO signaling pathway (i.e., *Pck1*, *Foxo1a*, *Sgk3*, *Bnip3*, and *Bcl6*, etc.), MAPK signaling pathway (i.e., *Dusp8*, *Jund*, *Hsp70*, *Epha2*, *Jun*, *Fos*, *Relb*, *Mapk8ip1*, and *Fgfr1a*, etc.), PPAR signaling pathway [i.e., *Acsbg2*, *Stearoyl-CoA desaturase 1b* (*Scd1b*), and *Fabp2*, etc.], phagosome (i.e., *Calr*, *Mr1*, etc.), as well as additional crucial genes that play roles in amino sugar and nucleotide sugar metabolism (i.e., *Cmah*, *Chia*, etc.) were identified. In addition, DEGs fell into the categories of the interaction of cytokine-cytokine receptor (i.e., *tumor necrosis factor receptor superfamily member 6B-like isoform X2*, *Ackr3*, etc.), Herpes simplex infection [i.e., *Socs3*, *aryl hydrocarbon receptor nuclear translocator-like protein 2* (*Arntl2*), etc.], and progesterone-mediated oocyte maturation (i.e., *Hsp90a.1*, etc.).

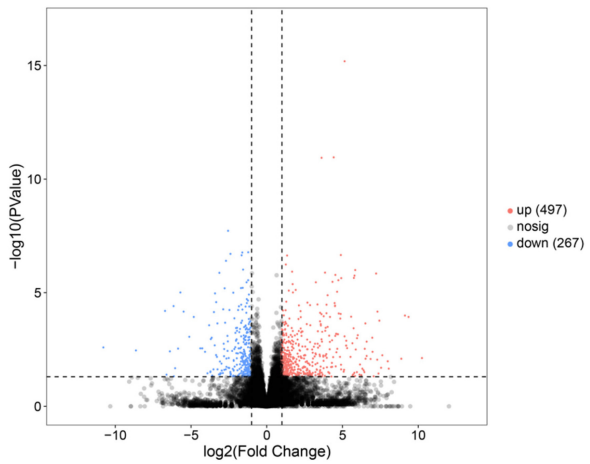

FIGURE 1 | Volcano plot of the differentially expressed genes (DEGs) in the liver of female spotted scat fed with different oil sources.

Differentially Expressed Genes Verification Using Real-Time PCR

Twenty-four genes related to ovarian development and fat metabolism from RNA-seq were verified using real-time PCR (**Figure 4**). The results showed that the expression levels of the

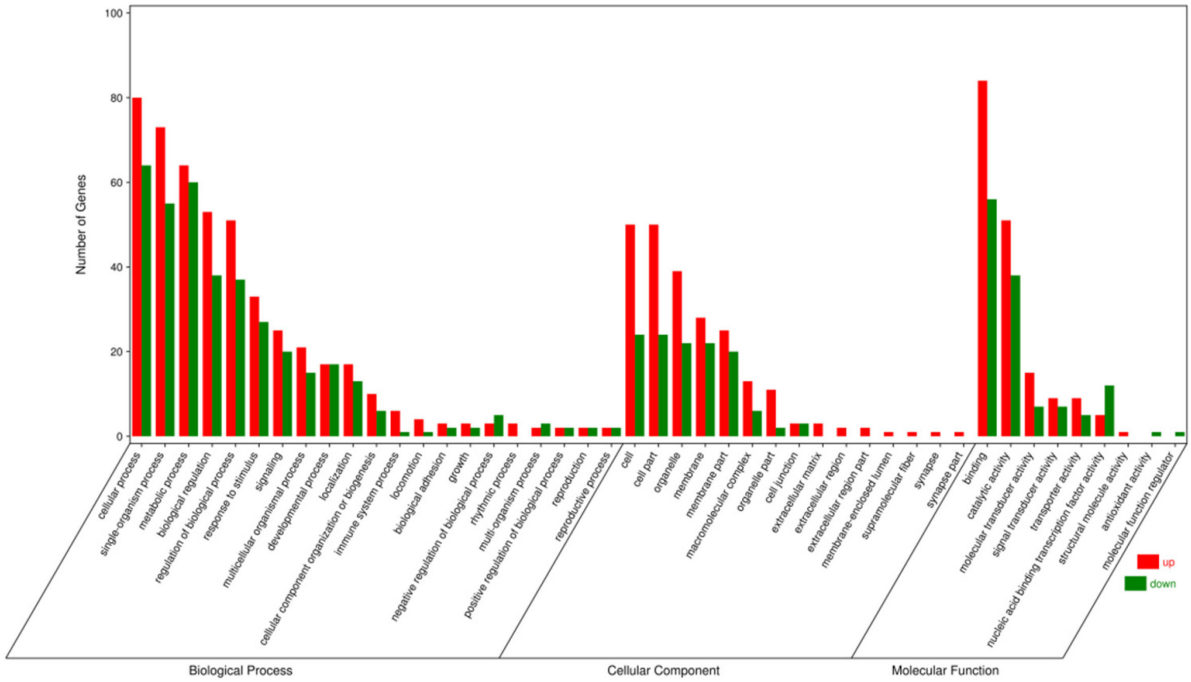

FIGURE 2 | Gene ontology annotations of the differentially expressed unigenes.

selected genes were consistent with RNA-seq, suggesting that the RNA-seq results are reliable.

Change of Serum Vitellogenin Levels

The mRNA expression levels of *Vtga* and *Vtgb* in livers of the FO group are higher than that of the SO group. The Vtg protein level in the serum was detected. The results showed that the serum Vtg level in the fish from the FO group was significantly higher than that of the SO group ($P < 0.05$; **Figure 5**). The results at the mRNA and protein levels are consistent.

DISCUSSION

Dietary n-3 Long-Chain Polyunsaturated Fatty Acids Effected Growth Performance

The growth of the fish was not affected by the oil source during the 40-day experimental stage. In sharpsnout seabream (*Diplodus puntazzo*), replacing fish oil with vegetable oil did not affect growth after 3 months of feeding (Piedecausa et al., 2007). However, in cobia (*Rachycentron canadum*), the proportional replacement of fish oil with soybean oil gradually increased (i.e., 0% SO, 33% SO, 67% SO, and 100% SO) and resulted in a gradual decrease in weight gain (Trushenski et al., 2011). The discrepancy could be related to the differences in fatty acid requirement among species (Xu et al., 2015). The current study suggests that spotted scat can be fed with a diet containing lower fish oil for normal growth. That is probably because spotted scat is omnivorous fish species. Of course, we could not conclude that the spotted scat does not need LC-PUPA for normal growth, as residual fish oil in fish meal could supply the LC-PUPA in the SO group. Decreased HSI in the FO group reveals that a high level of dietary n-3 PUFAs significantly reduced fat accumulation in the liver. It is well known that adult zebrafish suffer from hepatic adipose infiltration, which might influence reproduction and offspring (Virote et al., 2020). The liver with less fat tissue should be healthier, and the healthy liver might have a stronger ability to synthesize the proteins and lipids, such as the Vtg and Zp proteins required for gonadal development. In female spotted scat, the higher GSI values were observed in the reproduction season, indicating the maturing of the ovary (Gandhi et al., 2014). Consistently, the GSI in the FO group is relatively higher than that of SO group, although there was no statistical difference ($P = 0.306$). The possible reason for the large standard error between groups is the genetic background of the fish affected the regulation of lipid metabolism (Morais et al., 2011). These results suggest that a high level of dietary n-3 LC-PUFA might be beneficial to a healthy liver and promote ovarian development in female spotted scat.

Dietary n-3 Long-Chain Polyunsaturated Fatty Acids Affected Reproduction-Related Genes Expressions in Livers

The liver transcriptome analysis was carried out to confirm whether the high level of LC-PUPA would benefit the reproduction *via* influencing the liver genes. Vitellogenesis- and zonagenesis-related genes are induced by the estrogen in the liver

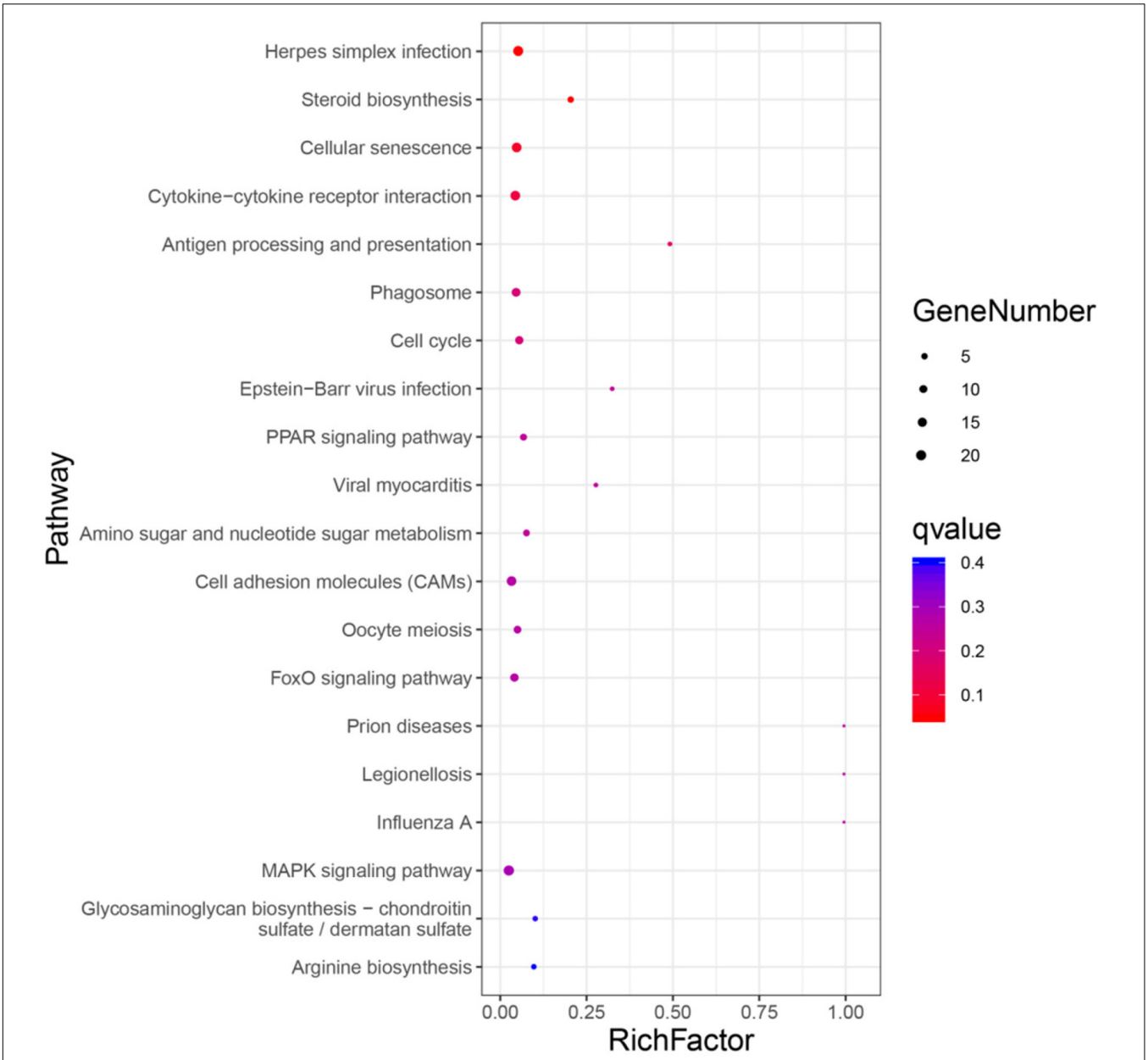

FIGURE 3 | Top 20 pathway enrichments. The *x*-axis indicates the ratio of the number of genes in the pathway of the DEGs and all genes. The *y*-axis indicates the pathway.

to synthesize Vtg and zona radiata proteins (Zrp), respectively. Vtg and Zrp are secreted and transported from blood to the ovary and are taken up by the maturing oocytes (Arukwe and Goksøyr, 2003; Levi et al., 2009). *Vtga*, *Vtgb*, and *Vtgc* are all expressed increasingly during the ovary maturation from phase II to phase IV in the female liver of spotted scat (Cui et al., 2017). In this study, both transcriptome and real-time PCR data showed that *Vtga* and *Vtgb* are highly expressed in the FO group. Consistently, the serum Vtg protein is also higher in the FO group than that of the SO group. *Zp4* is one of the genes controlling the making of Zp (in mammals) or vitelline envelope (in fish, amphibians, and birds), a glycoprotein layer surrounding the oocytes (Laine et al., 2019). In addition, *Zp4* expression is significantly upregulated in the livers of FO fish. Taken together, the fish oil might promote ovary development by upregulating genes critical for vitellogenesis (i.e., *Vtga* and *Vtgb*) and zonagenesis (i.e., *Zp4*) in spotted scat.

The estrogen signaling pathway is mediated by the nuclear receptors. Three estrogen receptors, *erα*, *erβ1*, and *erβ2*, were characterized in many fish species, including spotted scat (Cui et al., 2017). Although, the three estrogen receptors are expressed in the female livers, their expressions were not affected by the

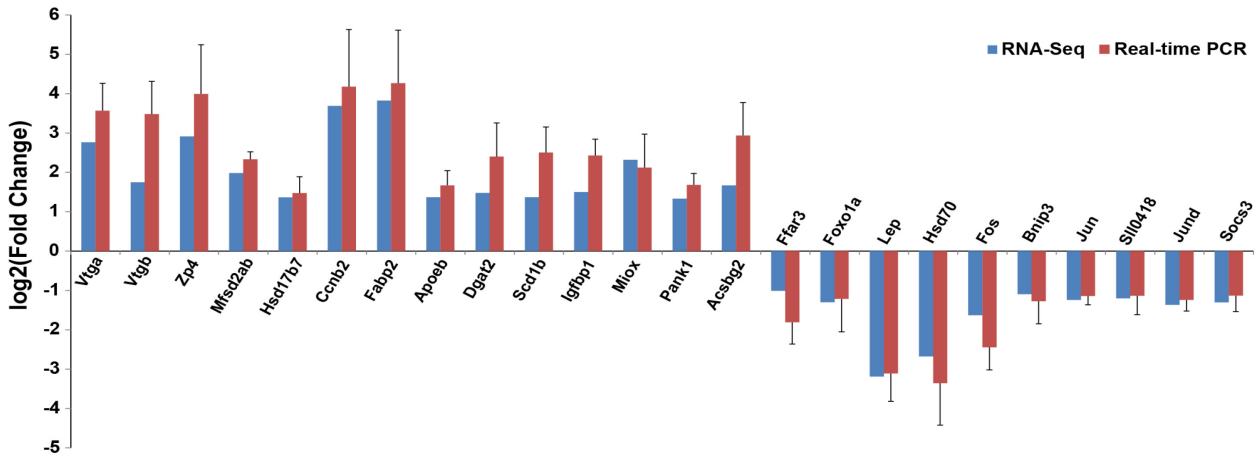

FIGURE 4 | Comparison of gene expression data between RNA-seq and real-time PCR (means ± SE).

high level of LC-PUPA in the presents study. In contrast, the liver estrogen level might be increased by the upregulation of the *Hsd17b7*. *Hsd17b7* could transform estrone to estradiol *in vitro* (Ohnesorg et al., 2006). Similarly, fish oils upregulate *17β-hsd* gene expression in the testicular tissues of rats (Mohammad et al., 2015). The regulatory mechanism of fish oil on *Hsd17b7* expression remains to be elucidated.

Hsp90 is a ubiquitously expressed molecular chaperone of estrogen receptors and is involved in the signal transduction process of estrogen receptors (Chang et al., 2014). Herein, the *Hsp90* expression was downregulated in the FO group. Whether less *Hsp90* would either promote or inhibit the estrogen signaling pathway is still unclear. Considering the upregulation of *Vtgs* and *Zp4* expressions in the FO group, the results suggested that less *Hsp90* might enhance the estrogen signaling pathway. The possible mechanism is that decreasing *Hsp90* would increase the number of free estrogen receptors. Those free receptors would combine with estrogen to be translocated into the nuclear to take action. The aryl hydrocarbon nuclear translocator (*Arnt*) has been found to be a coactivator of erα- and erβ-dependent transcription (Brunnberg et al., 2003). The *Arntl2* gene has been considered a candidate gene in the molecular regulation of reproduction seasonality (Zhao W. et al., 2020). However, its expression was decreased in the FO group. So, the n-3 LC-PUPA might have a diverse influence on the expression of the genes involved in the network of the estrogen signaling pathway. The total output of the estrogen signaling pathway in the female liver should be enhanced in the group that contains higher level n-3 LC-PUPA.

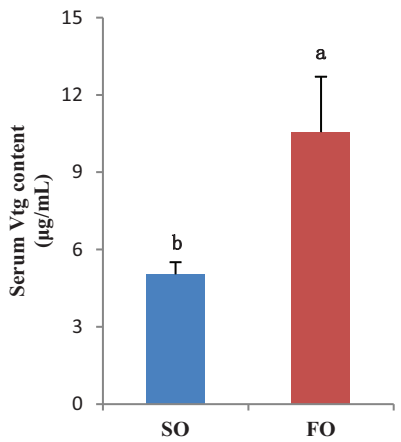

FIGURE 5 | Serum vitellogenin (Vtg) content of the female spotted scat fed with different oil sources (μg/ml, means ± SE, $n = 10$). Different letters denote statistically significant difference ($P < 0.05$).

Dietary n-3 Long-Chain Polyunsaturated Fatty Acids Affected Lipid Metabolism-Related Genes Expressions in Livers

The forkhead transcription factor *Foxo1* is a critical regulator of hepatic lipid and glucose metabolism, and it functions in hepatic lipid homeostasis (Shin et al., 2012). The DHA supplemented diets reduced the expression of *Foxo1* in the liver of pigs after 30-day feeding experiment (Chen et al., 2012). In contrast, EPA was more effective in reducing *Foxo1* expression than DHA in rats after an 8-week feeding experiment (Hong et al., 2019). DHA or EPA alone or together could reduce the expression of *Foxo1* in 3T3-L1 adipocytes *in vitro* (Martins et al., 2020). In mice, gain and loss of function proved that *Foxo1* could promote the insulin sensitivity and lipid synthesis (Matsumoto et al., 2006). *Foxo1a* was highly expressed in insulin-responsive tissues (e.g., adipose tissue, liver, and muscle) in grass carp (*Ctenopharyngodon idella*) (Sun et al., 2017). In contrast, the addition of bovine insulin *in vitro* stimulates *Vtg* expression in hepatopancreatic explants from the mud crab (*Scylla paramamosain*) (Huang et al., 2017). The decreasing of the HIS in the FO group could be explained by the DHA/EPA in the fish oil decreasing the *Foxo1a* expression and reducing the lipid synthesis. Whether the increase of

Vtg in FO group is affected by *Foxo1*-mediated insulin needs further verification.

Leptin (*Lep*) is a protein hormone that regulates body weight and energy homeostasis (Paolucci et al., 2020). In fish, the liver seems to be the primary organ for *Lep* synthesis (Frøiland et al., 2010). In large yellow crookeder (*Larimichthys crocea*), *Lep* levels in blood were significantly lower in the FO group than in palm oil (Wang et al., 2019). Similarly, the liver *Lep* mRNA in the fish of FO group was significantly lower than that of SO group in the present study. The high level of LC-PUPA might decrease the *Lep* expression level in the liver in fish. However, there was a contrary relationship between liver lipid content and liver *Lep* mRNA expression in Arctic charr (*Salvelinus alpinus*) (Frøiland et al., 2010). The upregulation of the *Lep* mRNA expression in the SO group containing high HIS might be a negative feedback mechanism to regulate the liver lipid. The E2-induced *Vtg* mRNA transcript was influenced negatively by recombinant *Lep* in the rainbow trout (*Oncorhynchus mykiss*) *in vitro* (Paolucci et al., 2020). Thereafter, the induction of the *Vtgs* expression by higher n-3 LC-PUPA level might also depend on the decreasing of *Lep*.

The Ppar-related gene expression could be regulated by dietary fat that affects hepatic lipid storage and maintenance of metabolic health (Soni et al., 2015). The fatty acid-binding protein (*Fabp*) is the DEG enriched by the KEGG analysis in the PPAR signaling pathway (ko03320). The long-chain fatty acids are assisted by some fatty acid transporters to move through the cell membrane and are then transported by *Fabps* to the corresponding metabolic target sites (Lei et al., 2019). A Fabp2$^{-/-}$ zebrafish decreased intestinal LC-PUPA and increased hepatic lipid accumulation compared with wild-type zebrafish. At the same time, transgenic overexpression *Fabp2* significantly increased intestinal LC-PUPA (Zhao Y. et al., 2020). These results suggested that *Fabp2* also promoted LC-PUPA absorption in fish. Consistently, *Fabp2* expression was upregulated in the FO group, which contains high LC-PUFA than the SO group in the present study. In golden pompano (*Trachinotus ovatus*), the liver expressions of the *Fabp -1*, *-4*, and *-6* were higher in the 2.1% LC-PUFA than that of 1.0% LC-PUFA feed group, suggesting that *Fabps* may be involved in LC-PUFA metabolism in this species (Lei et al., 2019). Taken together, *Fabp* gene family members may be essential for the LC-PUFA uptake and deposition in fish, while different subtypes of *Fabp* may be used for gene duplication and functional diversification in different species. In addition, the major facilitator superfamily domain-containing 2a (*Mfsd2a*) is confirmed as a transporter of DHA as lysophosphatidylcholine-DHA and is highly expressed in the liver (Pauter et al., 2017). Consistently, *Mfsd2ab* is also upregulated in the FO group in spotted scat. Therefore, the n-3 LC-PUFA supplementation affected the genes involved in LC-PUFA metabolism in spotted scat; hence, the regulator mechanism of these genes should be studied in the future.

CONCLUSION

The dietary supplementation of fish oil will significantly increase the deposition of n-3 LC-PUFA in spotted scat. The fish growth rates were similar between the FO and SO groups, while the HIS of the FO group was significantly lower than that of the SO group. The dietary fish oil supplementation increased the serum Vtg level, which is required for the ovary development. The liver transcription analysis showed that 764 genes were differentially expressed between the FO and SO groups. These data indicate that, the physiological response in the liver is associated with dietary oil. The expression of liver genes analyzed by RNA-seq gives us some new implications on how the dietary n-3 LC-PUFA supplied from the fish oil could promote the reproductive development of marine teleost.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA765892.

ETHICS STATEMENT

The animal study was reviewed and approved by Committee on the Ethics of Animal Experiments of Guangdong Ocean University.

AUTHOR CONTRIBUTIONS

TW and DJ designed and took part in the whole process of the experiment and wrote the draft of this manuscript. HS, SD, HC, and CZ co-conceived the experiment. ZL participated in the experiments. UM and WL revised the draft. GL supervised the experiments. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.784845/full#supplementary-material

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