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Functional characterization of fatty acid desaturases and dietary lipid sources modulation of fatty acid conversion in largemouth bass (*Micropterus salmoides*)

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ABSTRACT

To evaluate n-3 long-chain polyunsaturated fatty acids (LC-PUFA) biosynthesis capacity and the selected dietary lipid sources effects in largemouth bass (*Micropterus salmoides*), we combined functional characterization of fatty acid desaturase genes with an 8-week feeding trial. Yeast expression identified three candidate desaturase genes: *fads2* (fatty acid desaturase 2) catalyzing C18:3n-3 → C18:4n-3, and *delta4 fads* (acyl-CoA Delta4 desaturase-like) exhibiting dual Δ5/Δ4 activity (C20:4n-3 → C20:5n-3; C22:5n-3 → C22:6n-3), and *fads6* (fatty acid desaturase 6) showed no detectable desaturase activity. In a 3 × 2 factorial design, 450 juvenile largemouth bass (17.99 ± 0.02g, 25 fish per tank, triplicate) received diets containing—linseed oil (LO, 54.24% ALA), cottonseed oil (CO, 0.14% ALA) or black soldier fly oil (BSFO, 2.28% ALA), with or without DHA inclusion (3% *Schizochytrium sp.* meal). Two-way ANOVA revealed that LO and BSFO improved growth (FBW, WG, SGR) over CO, and LO raised tissue n-3 PUFAs levels aboved CO and BSFO ($P < 0.05$). CO and BSFO upregulated liver *delta4 fads* relative to LO, and CO further increased *elovl4a* and *elovl5* under DHA inclusion ($P < 0.05$), whereas desaturase proteins remained unchanged among oils ($P > 0.05$). CO lowered liver TG levels and upregulated monoacylglycerol lipase (*mgl*) against LO and BSFO, and elevated acetyl-CoA carboxylase 1 (*acc1*) and fatty acid synthase (*fasn*) expression under DHA-inclusion ($P < 0.05$). Dietary DHA improved growth and tissue DHA levels, and reduced liver crude lipid and TG levels ($P < 0.05$), while downregulating *fads2*, *delta4 fads*, *fads6* and Δ5/Δ4 Fads protein ($P < 0.05$). Overall, the results are consistent with largemouth bass possessing the enzymatic capacity to convert dietary ALA into n-3 LC-PUFA. Adequate dietary ALA supported growth and DHA biosynthesis, and severe ALA deficiency upregulated part n-3 LC-PUFA biosynthesis-related genes but still resulted in reduced tissue DHA. Dietary DHA inclusion alleviated growth constraints under ALA-deficient conditions.

1. Introduction

In 2022, global aquaculture production of aquatic animals exceeded capture fisheries for the first time, accounting for 51% of the total aquatic animal output and highlighting aquaculture's growing contribution to global food supply (FAO, 2024). Traditionally, fish oil (FO) has been the primary source of n-3 long-chain polyunsaturated fatty acids (LC-PUFA, fatty acids with ≥20 carbon atoms and ≥2 double bonds), including eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), which are critical for supporting growth, immunity, and metabolism in fish (Bou et al., 2017; Chen et al., 2018; Lei et al., 2016; Sargent et al., 2003; Tocher et al., 2019), while also contributing to human nutrition and health (Beyer et al., 2023; Tabilo et al., 2025). Thus, the increasing demand for EPA and DHA has intensified pressure on global fish oil supplies, driving up prices and limiting availability, thereby necessitating the development of sustainable alternative lipid sources (FAO, 2024). The success of this strategy, however, largely depends on the ability of farmed fish species to biosynthesize n-3 LC-PUFA from dietary C18 PUFA precursors (ALA), a capacity that varies significantly among species (Xie et al., 2021).

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Largemouth bass (*Micropterus salmoides*), a typical carnivorous economic freshwater fish species, has become one of the most important freshwater aquaculture species worldwide—especially in China (FAO, 2024). Although previous studies suggest a restricted ability of largemouth bass to convert ALA to EPA and DHA, these assessments were made under dietary conditions that included fish meal or fish oil as sources of n-3 LC-PUFAs (Li et al., 2025; Yadav et al., 2020; Zhang et al., 2019a). Nevertheless, the specific roles of three annotated *fads* genes in the largemouth bass genome—*fads2* (fatty acid desaturase 2), *delta4 fads* (acyl-CoA Delta4 desaturase-like), and *fads6* (fatty acid desaturase 6), in LC-PUFA biosynthesis, particularly in the key desaturation steps remain to be elucidated (Xie et al., 2020).

Plant-based and insect-derived oils have emerged as promising alternatives to fish oil due to their wide availability, cost-effectiveness, and distinct fatty acid profiles (Gu et al., 2025; Regost et al., 2003). Furthermore, the fatty acid composition of these oils sources directly influences the fish growth performance, tissue fatty composition, and the level or capacity of n-3 LC-PUFA biosynthesis (Liang et al., 2022a; Liu et al., 2018). Therefore, to provide insight into the endogenous LC-PUFA biosynthesis pathway of largemouth bass in a context free from dietary preformed EPA and DHA, it is necessary to select oil sources with distinctly different fatty acid compositions.

Linseed oil (LO), rich in α-linolenic acid (ALA, C18:3n-3), the biosynthesis precursor of n-3 LC-PUFA, has been evaluated as a partial replacement for FO in aquafeeds across multiple farmed fish species (Asghar et al., 2025; Li et al., 2016a; Turchini et al., 2009). Dietary LO has been shown to alter tissue fatty acid composition and modulate the expression of desaturase and elongase genes involved in LC-PUFA biosynthesis in species such as silver barb (*Puntius gonionotus*) and rainbow trout (*Oncorhynchus mykiss*)(Nayak et al., 2020; Turchini et al., 2018). In largemouth bass, LO significantly affected growth and whole-body fatty acid composition, with dietary ALA influencing tissue EPA and DHA levels (Liang et al., 2022a; Shi et al., 2019; Tidwell et al., 2007). Overall, LO represents an appropriate dietary oil source for studying LC-PUFA metabolism.

Cottonseed oil (CO), characterized by high linoleic acid (LA, C18:2n-6) content, and relatively low in n-3 PUFA, was therefore selected as an n-6-dominant dietary lipid source to contrast with n-3 precursor-rich oils. Although CO is nutritionally and compositionally comparable to widely used vegetable oils such as soybean and corn oils, it has been far less extensively evaluated in fish nutrition studies (Eroldoğan et al., 2012). Previous studies have reported variable effects on growth performance, tissue fatty acid composition, and lipid metabolism in largemouth bass (Liu et al., 2025) and other species (Hou et al., 2024; Wassef et al., 2015). Accordingly, using cottonseed oil as a high-LA background diet, in contrast to a high-ALA diet, offers a model for elucidating n-3 LC-PUFA–dependent metabolic responses in fish.

Black soldier fly oil (*Hermetia illucens*, BSFO), has recently emerged as an ecologically sustainable option for carnivorous fish (Fawole et al., 2021; Moutinho et al., 2025; Yuan et al., 2024a). Unlike conventional plant oils dominated by C18 PUFAs, BSFO is characterized by a high proportion of lauric acid (C12:0), a medium-chain saturated fatty acid (MCFA, FAs with 6-12 carbon atoms and no carbon–carbon double bonds) that is rapidly oxidized via β-oxidation, thereby supplying efficient metabolic energy and limiting lipid deposition (Dabbou et al., 2021). Therefore, BSFO can be selected as a contrasting dietary oil to C18 PUFA-rich plant oils in the evaluation of lipid metabolism and LC-PUFA-related responses in fish.

Moreover, previous studies have demonstrated that dietary inclusion of approximately 1% EPA + DHA (of total fatty acids) can enhance the growth performance and n-3 LC-PUFA deposition of largemouth bass (An et al., 2023; Yadav et al., 2020). In this study, in order to further investigate the interactive effects of dietary precursor supply and end-product feedback on LC-PUFA biosynthesis, we therefore included *Schizochytrium sp*. meal (SCM) in selected diets. The inclusion level was calculated to achieve approximately 1% dietary EPA + DHA, based on the established requirement for this species and the specific fatty acid profile of the SCM used. In addition, studies in fish species, such as rainbow trout (Bélanger et al., 2021; Santigosa et al., 2020) and Atlantic salmon (*Salmo salar*)(Zatti et al., 2023), have demonstrated that DHA-rich microbial sources, including SCM, when used at practical inclusion level, can improve both nutritional quality and sensory attributes.

Therefore, the primary objective of this study was to clarify the LC-PUFA biosynthesis capacity of largemouth bass by functionally characterizing its *fads* genes using heterologous expression. Second, to complement this molecular insight with a feeding trial evaluating the the combined effects of three contrasting oil sources (LO, CO, and BSFO), with or without dietary DHA inclusion, on growth, lipid metabolism, and LC-PUFA biosynthesis.

2. Materials and methods

2.1. Functional characterization of largemouth bass fads genes by heterologous expression

2.1.1. Materials preparation

Analytical-grade fatty acid substrates utilized for functional assays were purchased from the following suppliers, with purities ≥99% as stated by the manufacturers: ALA (C18:3n-3; Solarbio, Beijing, China), eicosatetraenoic acid (C20:4n-3; ZZstandard Co., Ltd., Zhengzhou, China), and docosapentaenoic acid (C22:5n-3; Nu-Chek Prep, USA). Stock solutions were prepared in ethanol at appropriate concentrations and stored at −20 °C until use. All substrates used in the experiments were from the same manufacturer batch to ensure consistency.

2.1.2. Molecular Cloning of largemouth bass fads genes

Three *fads* genes annotated as fatty acid desaturase–related sequences were identified from the genome of largemouth bass, including *fads2* (Gene ID: 119893991; coding sequence, CDS 1338bp), *delta4 fads* (Gene ID: 119884848; CDS 1326bp), and *fads6* (Gene ID:119915965; CDS 1220bp)(Table S1). For clarity and consistency with prior literature, gene names are presented using English form (e.g., *fads2*, *delta4 fads*, *fads6*), whereas enzyme functions are indicated with Greek symbols (e.g., Δ4 Fads activity) to denote the positions of the desaturation reaction.

Gene annotation was based on NCBI genome annotation and pairwise amino acid sequence similarity analyses. As summarized in Table S1, *delta4 fads* showed complete nucleotide query coverage (100%) and high amino acid identity (80%) relative to *fads2*, indicating that these two genes represent closely related *fads2*-like paralogs. In contrast, *fads6* exhibited low query coverage (11%) and low sequence identity (34%) compared with *fads2*, supporting its annotation as a distinct desaturase gene.

The CDS of *fads2*, *delta4 fads*, and *fads6* were amplified by PCR using 2×T8 high-fidelity DNA polymerase (Tsingke Biotechnology, Beijing, China). Following amplification, the DNA fragments were digested with the appropriate restriction endonucleases (Thermo Fisher Scientific, USA) and ligated into the pYES2 vector (Invitrogen, USA) to generate the recombinant plasmids named pYES2-*fads2*, pYES2-*delta4 fads*, and pYES2-*fads6*. Three verified recombinant vectors were individually transformed into *S. cerevisiae* strain INVSc1 through the pYES2-INVSc1 yeast expression system (Thermo Fisher Scientific, USA) (Kabeya et al., 2021; Li et al., 2010a). Yeast cells harboring the empty pYES2 vector were grown under identical conditions and used as the control. Following the transformation, plasmid DNA was extracted from recombinant yeast colonies, and Sanger sequencing covering the entire ORF was performed to verify the correct insertion and integrity of the target genes.

2.1.3. Heterologous expression of largemouth bass fads genes in yeast

Verified transformed yeast colonies carrying pYES2 (empty-vector control), pYES2-*fads2*, pYES2-*delta4 fads*, and pYES2-*fads6* (Fig. S1,

corresponding to clone numbers 3, 4, 9, and 12, respectively) were cultured overnight in 15 mL SD/-Ura medium at 30°C and 250 rpm. After being pelleted by centrifugation (1,500 × g, 5 min, 4°C), the cells were resuspended in 50 mL of SG/-Ura induction medium for subsequent induction. Gradual inoculation was performed to adjust to an optical density at 600 nm (OD_{600}) of 1.

Each of the four transformed yeast cultures was supplemented with one of the following three FA substrates. These substrates, initially dissolved in ethanol, were added from stock solutions to the culture medium, to achieve final concentrations of 0.3 mmol/L ALA, 0.3 mmol/L C20:4n-3, and 0.5 mmol/L C22:5n-3; the elevated C22:5n-3 level was set in accordance with previously published protocols (Fonseca-Madrigal et al., 2014; Geay et al., 2016; Li et al., 2010a). For each transformed yeast group, a vehicle control receiving an equivalent volume of ethanol was included. In total, 16 treatments (4 constructs × 4 substrate/control conditions) were prepared, each with two independent technical replicates.

After incubation at 30°C with agitation for 48 h, cells were harvested by centrifugation (500 × g, 2 min, 4°C), rinsed twice with 5 mL of ice-cold HBSS, and subsequently freeze-dried for fatty acid analysis (Abdul Hamid et al., 2016; Hastings et al., 2001; Li et al., 2010a; Oboh et al., 2017).

2.1.4. Fatty acid analysis of yeast

Lipids were extracted from the sample, derivatized using the DMAQ reagent, and analyzed by liquid chromatography-mass spectrometry (LC-MS), with detailed procedures and analytical principles described previously (Feng et al., 2022). Analyses were performed using an ACQUITY Ultra Performance Liquid Chromatography system (Waters, USA) interfaced with a QTRAP 6500 triple quadrupole mass spectrometer (USA). FA peaks were identified by comparing retention times and ion patterns with authentic standards analyzed under identical conditions. Chromatograms of standards peaks are shown in Fig. S2. Based on LC-MS data, extracted ion chromatograms (EICs) were generated in Origin 2021 to illustrate variations in the fatty acid profile of yeast. The relative conversion rate of FA substrates to their desaturated FA products was calculated as [peak area of FA product/(peak area of FA product + peak area of FA substrate)] × 100% (Abdul Hamid et al., 2016; Hastings et al., 2001; Li et al., 2010a; Oboh et al., 2017). The relative conversions obtained from two independent technical replicates were highly consistent (variation < 5%), confirming the reliability of the results. The data are presented as the mean values of the two replicates.

2.2. Dietary lipid source effects on fatty acid conversion in largemouth bass

2.2.1. Diet preparation

A 3 × 2 factorial design was employed to evaluate the effects of different dietary lipid sources and the inclusion of DHA (from SCM, 43.27% DHA and 2.88% EPA of total fatty acids) on fish growth performance and fatty acid biosynthesis. Three different oil sources were used as the primary oil sources: LO (Xilin Gol League Hongjingyuan Oil Co., Ltd., China), CO (Xinjiang Jinlan Plant Protein Co., Ltd., China), and BSFO (Guangzhou Konfu Biotechnology Co., Ltd., China). BSFO was extracted from larvae reared for 8–9 days on the three-phase residue from kitchen waste oil-water separation and steaming, followed by drying (75–85°C) and mechanical pressing, with a total MCFA content exceeding 30%, primarily composed of C12:0. For each oil source, two levels of DHA inclusion were applied: no SCM (D-) or 3% SCM (D+), resulting in six isonitrogenous and isoenergetic experimental diets, designated LO, LOD, CO, COD, BSFO, and BSFOD. The primary protein sources consisted of 35% cottonseed protein concentrate and 5.5% yeast extract (FA28, Guangxi, China). The six experimental diets were formulated based on the compositions detailed in Table 1 and prepared according to the procedures described in earlier studies (Liu et al., 2022; Xie et al., 2022). Detailed fatty acid composition of main dietary lipid ingredients and diets are presented in Table S2 and Table S3.

Table 1
Feed formulation and proximate composition of the experimental diets.

Ingredients (%, as-is basis)	LO	CO	BSFO	LOD	COD	BSFOD
Yeast extract[1]	5.50	5.50	5.50	5.50	5.50	5.50
Cottonseed protein concentrate	35.00	35.00	35.00	35.00	35.00	35.00
Defatted superworm meal	5.00	5.00	5.00	5.00	5.00	5.00
Wheat gluten	4.00	4.00	4.00	3.50	3.50	3.50
Clostridium autoethanogenum protein	15.00	15.00	15.00	15.00	15.00	15.00
Wheat flour	8.00	8.00	8.00	8.00	8.00	8.00
Cassava tuber meal	6.00	6.00	6.00	6.00	6.00	6.00
Linseed oil	11.60	-	-	10.00	-	-
Cottonseed oil	-	11.60	-	-	10.00	-
Black soldier fly oil	-	-	11.60	-	-	10.00
Schizochytrium sp. meal, 43.27% DHA[2]	-	-	-	3.00	3.00	3.00
Permix[3]	1.10	1.10	1.10	1.10	1.10	1.10
DL-Met	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	1.00	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2H_2O$	2.45	2.45	2.45	2.45	2.45	2.45
Taurine	0.50	0.50	0.50	0.50	0.50	0.50
Choline chloride (60% choline)	0.40	0.40	0.40	0.40	0.40	0.40
Microcrystalline cellulose	3.95	3.95	3.95	3.05	3.05	3.05
Total	100	100	100	100	100	100
Proximate composition (dry matter basis)						
Crude protein (%)	52.42	51.99	51.49	51.85	51.71	52.10
Crude lipid (%)	11.91	12.03	12.75	12.08	11.88	11.90
Dry matter (%)	94.26	94.25	94.54	94.35	94.24	94.12
Ash (%)	7.71	7.55	7.50	7.51	7.41	7.52
Gross energy (MJ/Kg)	21.93	21.98	21.98	21.92	22.09	22.07
E/P (kJ/g protein)[4]	41.83	42.29	42.69	42.27	42.72	42.36

1. Yeast extract (FA28, protein content > 68.7%), was obtained from Angel Yeast CO., Ltd.
2. *Schizochytrium sp.* meal (SCM, ALGAMAC-3050), protein content >17.6%, Oil content >56.2%, was obtained from Aquafauna Bio-Marine, Inc.
3. The premix provides the following per kg of diets: vitamin premix: VA 20 mg, VD3 10 mg, VE 400 mg, VB1 10 mg, VB2 15 mg, VB6 15 mg, niacin 100 mg, VC ester 1000 mg, calcium pantothenate 40 mg, VK3 20 mg, biotin 2 mg, VB12 8 mg, folic acid 10 mg, corn gluten powder 150 mg; inositol 200 mg; mineral premix: $MgSO_4$ 2040 mg, $FeSO_4{\cdot}H_2O$ 300 mg, $ZnSO_4{\cdot}H_2O$ 200 mg, $MnSO_4{\cdot}H_2O$ 100 mg, Na_2SeO_3 10 mg, $CoCl_2{\cdot}6H_2O$ 5 mg, KI 80 mg, zeolite powder 705 mg; mould inhibitor 3400 mg; tert-butylhydroquinone (TBHQ) 200 mg.
4. E/P(kJ/g protein), the ratio of diet gross energy(MJ/Kg) / diet crude protein (%)×100.

2.2.2. Rearing conditions

The experimental fish sourced from the National Fisheries Technology Extension Center were adapted in a recirculating aquaculture system housed at the National Aquatic Feed Safety Evaluation Base (Nankou, Beijing). Before the trial commenced, 450 fish (17.99 ± 0.02 g) were randomly assigned to 18 tanks (0.26 m^3 each, 25 fish per tank) with three replicates per treatment, and subjected to a 24-hour fasting period. During the 8-week trial, fish were fed to apparent satiation status twice daily, at 08:00 and 16:00. Daily feed intake was recorded for each tank throughout the feeding trial. The recirculating aquaculture system was kept at a stable temperature of 24.3± 0.3°C, pH 7.2-7.8, dissolved oxygen ≥ 7mg/L, and ammonia nitrogen < 0.2 mg/L, with water quality parameters regularly monitored to ensure optimal fish health. The feeding trial complied with the guidelines for animal welfare and was approved by the Animal Ethics Committee of Chinese Academy of Agricultural Sciences (Ethics Approval ID: IFR-CAAS20240611).

2.2.3. Sampling

Samples were collected according to the established protocol (Liu

et al., 2025). Before the trial, six fish were sedated with 60 mg/L MS-222 and stored at −20 °C for baseline analysis of proximate composition. Following the 8-week feeding trial, fish in each tank underwent a 24-hour fasting period and weighed to assess the growth performance. After these measurements, three fish from each tank were anesthetized using 100 mg/L MS-222 and subsequently frozen at −20 °C, and analyzed as one pooled sample for proximate composition analysis.

Four fish were collected from each tank, anesthetized and individually sampled. Measurements including body length, body weight, carcass weight (CW, after removal of the head, fins, tail, and internal organs), liver weight, total viscera weight, and visceral adipose weight were recorded. These data were used to calculate the morphometric parameters. Blood samples were drawn from the caudal vein and spun (4,000 × g, 10 minutes, 4 °C) to isolate plasma for biochemical determinations. Liver tissues were sampled and histologically evaluated on sections adjacent to the bile ducts. The residual liver tissue was divided into two distinct portions: one was rapidly frozen in liquid nitrogen and preserved at −80°C for gene expression assays; a second was stored at −20°C for biochemical determinations.

An additional four fish were collected from each tank, and their muscle and liver tissues were pooled within each tank and stored at −20°C for lipid content and fatty acid composition.

2.2.4. Analytical procedures

Growth performance parameters such as survival rate (SR), initial and final body weight (FBW), weight gain (WG), specific growth rate (SGR), feeding rate (FR), feed conversion ratio (FCR), as well as morphometric parameters, including carcass weight (CW), carcass ratio (CR), condition factor (CF), visceral adipose index (VAI), hepatopancreas somatic index (HSI), and viscerosomatic index (VSI) were calculated using methods previously reported (Liang et al., 2022b; Liu et al., 2025).

Proximate and fatty acid composition. The proximate composition of the experimental diets, whole-body samples, and selected tissues was analyzed in accordance with the Official Methods of Analysis (AOAC, 2002). The gross energy of the six experimental diets was measured using an IKA C2000 bomb calorimeter (IKA, Germany). Unlike feed and oil ingredient analyses, muscle and liver samples were freeze-dried under vacuum, ground into fine powder, and subsequently analyzed for fatty acid composition according to the Chinese National Standard (GB5009.168-2016, 2016).

Biochemical indicators. Commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) were used to determine plasma concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c), along with liver total protein (TP), TG, and TC content according to the manufacturer's protocols. Liver protein abundances of Δ6, Δ5, and Δ4 Fads were quantified using commercial ELISA kits (Jiangsu Meimian Industrial Co., Ltd., China) following the manufacturer's instructions. The assays employ a sandwich ELISA format using epitope-specific antibodies against Δ6, Δ5, and Δ4 Fads as capture and HRP-conjugated detection antibodies, forming an antibody–antigen–HRP complex that generates a chromogenic signal proportional to the protein abundance of each desaturase.

Hepatic histology examination. Histological analysis was processed according to established protocols (Yu et al., 2019a; Zhang et al., 2019b), which included fixation, dehydration, paraffin embedding, and hematoxylin–eosin (H&E) staining. Stained liver sections were scanned and quantitatively analyzed using Tissue FAXS imaging technology (Tissue Gnostics GmbH, Austria). Histological quantification was conducted using standardized procedures, with three non-overlapping regions of interest (ROIs) randomly selected from each section. All histological sections were digitally imaged using identical scanning parameters to minimize imaging-related variability across samples. Histological evaluation was conducted based on morphological criteria, including hepatocellular swelling, nuclear displacement, and severe vacuolization. Scale bars in images represent 50 μm.

Quantitative Real-Time PCR. The expression levels of genes associated with liver lipid metabolism were quantified via qRT-PCR, including those involved in lipogenesis (*acc1*, *fasn*, *pparγ*, *lpin1*, *dgat1*), lipolysis (*atgl*, *hsl*, *mgl*, *cpt1*, *pparα*), and fatty acid biosynthesis (*fads2*, *delta4 fads*, *fads6*, *elovl4a*, *elovl5*, *elovl6*, *elovl8a*), following the protocols previously published (Wei et al., 2019; Xie et al., 2022). RNA quality was verified prior to reverse transcription, with A260/280 ratios between 1.9 and 2.1. Amplification efficiencies ranged from 94% to 105% ($R^2 > 0.99$) based on melt-curve. Melt-curve analysis confirmed a single, specific peak for each primer pair, and both no-reverse-transcription (NRT) and no-template (NTC) controls showed no detectable amplification. The detailed primer information is listed in Table S4. Candidate reference genes (*ef-1α* and *β-actin*) were systematically evaluated prior to target gene analysis. Both genes exhibited satisfactory amplification efficiencies (102.1% and 102.8%, respectively) and and stability (M values < 1 in geNorm analysis). According to the geNorm results, *ef-1α* (M = 0.326) was identified as the most stable reference gene and was therefore selected for normalizing gene expression data in the main text. To confirm the robustness of our findings, normalization was also performed using *β-actin* (M = 0.709), with the results presented in Supplementay figures (Fig. S3 and S4).

2.2.5. Statistical analyses

For statistical analyses, the tank served as the experimental unit (n = 3 tanks per treatment). Individual fish measurements were averaged within each tank before analysis. Statistical analyses and figure generation were performed using GraphPad Prism 8 and Origin 2021.

Dietary oil sources, DHA inclusion and their interaction were first evaluated by two-way ANOVA. In the absence of a significant interaction, main effects are reported based on the pooled data, and Tukey's HSD test was applied to significant main effects. When a significant interaction was observed, simple effects were analysed at each level of the other factor (oil sources within each DHA level, and DHA effect within each oil source) with Sidak-adjusted pairwise comparisons.

Because preliminary ANOVA revealed that FR differed among treatments, growth parameters (FBW, WG and SGR) were re-analysed with two-way ANCOVA using FR as a covariate. Model assumptions were verified: residuals were normal (Shapiro-Wilk ≥ 0.273), variances were homogeneous (Levene ≥ 0.078), regression slopes were parallel (Oil sources × DHA inclusion × FR interaction, $P > 0.05$; both DHA inclusion × FR and Oil sources × FR interactions, $P > 0.05$) and no influential points were detected (Cook's D ≤ 0.27); adjusted $R^2 \geq 0.985$ for all three parameters.

For tissue fatty acid composition data, false discovery rate (FDR) control was applied across fatty acids within each tissue and experimental contrast using the Benjamini–Hochberg procedure.

Statistical significance was accepted at $P < 0.05$. When significant main effects were detected, differences were indicated by lowercase letters (a, b) and (x, y) for oil source and DHA inclusion, respectively. When significant interaction effects were detected, differences were indicated by uppercase letters (A, B, X, Y) or asterisks (*) in the corresponding figures and tables.

3. Results

3.1. Functional characterization of genes related to fatty acid desaturation

Based on NCBI genome annotations, three candidate desaturase genes (*fads2*, *delta4 fads*, and *fads6*), potentially involved in fatty acid desaturation, were chosen from the genome of largemouth bass. Yeast transformed with the pYES2 vector alone exhibited the main fatty acids typically found in *S. cerevisiae* specifically C16:0, C16:1n-7, C18:0, and C18:1n-9, along with the exogenously added FAs (Fig. 1A, E, I, M). Yeast expressing pYES2-*fads2* and cultured with the Δ6 substrate (ALA)

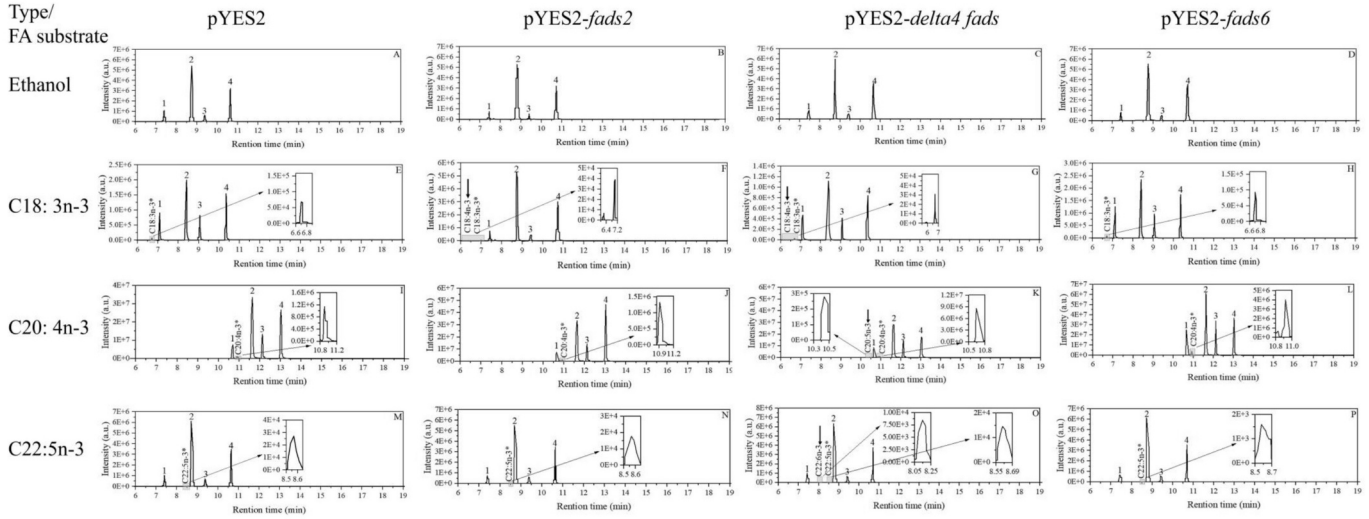


Fig. 1. Functional characterization of fatty acid desaturase genes from largemouth bass the largemouth bass expressed in yeast (*S. cerevisiae*). Extracted ion chromatograms (EICs) reconstructed from LC-MS data show retention times (min, x-axis) and signal intensities (cps, y-axis). Yeast tranformed with pYES2 (empty-vector control) (A, E, I, M), pYES2-*fads2* (B, F, J, N), pYES2-*delta 4 fads* (C, G, K, O), or pYES2-*fads6* (D, H, L, P) were cultured with ethanol (vehicle control, A-D) and fatty acid substrates C18:3n-3 (E-H), C20:4n-3 (I-L), and C22:5n-3 (M-P). Peaks 1 to 4 represent the main endogenous fatty acids in *S. cerevisiae*: C16:1n-7, C16:0, C18:1n-9, and C18:0. Arrows mark desaturation products: C18:4n-3 (F, G), C20:5n-3 (K), and C22:6n-3 (O).

exhibited new fatty acid peaks in their profiles (Fig. 1F). Similarly, yeast expressing pYES2-*delta4 fads* displayed additional fatty acid peaks when cultured with pathway precursors (ALA, C20:4n-3, C22:5n-3), reflecting Δ6, Δ5, and Δ4 desaturation steps (Fig. 1G, K, O).

The results in Table 2 showed that yeast expressing pYES2-*fads2* demonstrated Δ6 Fads activity (relative conversion rate, 13.60%). In comparison, yeast transformed with pYES2-*delta4 fads* only a very small amount of conversion was detected when a Δ6-specific substrate (4.52%) was supplied, while Δ4 and Δ5 Fads activities were detected with relative conversion rates of 40.52% and 20.49%, respectively. No desaturase activity was detected in yeast expressing pYES2-*fads6*.

3.2. Growth performance and proximate composition

No significant effects of either dietary oil source or DHA inclusion were observed on the SR of largemouth bass ($P > 0.05$, Table 3). Feeding diets based on CO significantly decreased FR ($P < 0.05$), while DHA inclusion markedly enhanced FR ($P < 0.05$). Consequently, FBW, WG, and SGR were further re-analysed by two-way ANCOVA with FR as a covariate. Adjusted means for each Oil sources × DHA inclusion combination, estimated under the common FR-slope model, are given in Table 3; the Oil sources × DHA inclusion interaction remained significant for all three traits ($P < 0.05$), while covariate slopes did not differ among treatments ($P > 0.05$). Particularly, FBW, WG, and SGR were notably higher in LO- and BSFO-fed fish than in CO-fed fish ($P < 0.05$), with no significant differences between LO and BSFO ($P > 0.05$), regardless of DHA inclusion. Moreover, DHA inclusion further enhanced growth in the COD and BSFOD groups compared with their respective basal diets ($P < 0.05$). The interaction effect was also significant for FCR ($P < 0.05$). Specifically, in the absence of DHA, FCR was significant higher in the CO group than in both the LO and BSFO groups ($P < 0.05$), and was further elevated compared with the COD group ($P < 0.05$).

Oil source had no significant effects on CR, CF, VAI, or VSI, but significantly affected HSI ($P < 0.05$, Table 4), which was higher in BSFO-fed fish than in CO-fed fish, with no difference from LO ($P > 0.05$). DHA inclusion also had no significant effect on CF, VAI, HSI, and VSI, but significantly increased CR ($P < 0.05$). A significant interaction effect was observed for CW ($P < 0.05$). Without DHA, the CO group exhibited lower CW than the LO and BSFO groups ($P < 0.05$) and was further reduced compared with the COD group ($P < 0.05$). In contrast, CW did not differ among treatments under DHA-inclusion conditions ($P > 0.05$).

Dietary oil source had no significant effect on the content of whole-body crude protein, dry matter, or ash, nor on muscle or liver lipid ($P > 0.05$, Table 5), except that BSFO-fed fish exhibited a higher whole-body crude lipid content than those fed LO or CO ($P < 0.05$). Dietary DHA inclusion did not affect the content of whole-body crude protein or ash, or muscle lipid ($P > 0.05$), but significantly increased the whole-body lipid content, decreased whole-body dry matter content, and reduced liver lipid content ($P < 0.05$).

Table 2
Relative conversions catalyzed by recombinant pYES2-*fads2*, pYES2-*delta4 fads*, and pYES2-*fads6* in transformed yeast with Δ6, Δ5, and Δ4 fatty acid substrates.

Fatty acid substrate	Fatty acid product	Desaturase activity	Recombine pYES2	Conversion (%)
C18:3n-3	C18:4n-3	Δ6 Fads	pYES2-*fads2*	13.60
			pYES2-*delta4 fads*	4.52
			pYES2-*fads6*	-
C20:4n-3	C20:5n-3	Δ5 Fads	pYES2-*fads2*	-
			pYES2-*delta4 fads*	20.49
			pYES2-*fads6*	-
C22:5n-3	C22:6n-3	Δ4 Fads	pYES2-*fads2*	-
			pYES2-*delta4 fads*	40.52
			pYES2-*fads6*	-

1. Δ6, Δ5, and Δ4 Fads indicate desaturase activities at the corresponding Δ6, Δ5, and Δ4 positions, respectively.
2. Conversion (%) was calculated using the formula: [peak area of fatty acid product/(peak area of fatty acid product + peak area of fatty acid substrate)] × 100%.

3.3. Fatty acid composition of muscle and liver tissue

3.3.1. Muscle fatty acid composition

For SFAs and Monounsaturated FAs (MUFAs, containing one carbon-carbon double bond), oil sources did not significantly affect the contents of C11:0, C16:0, and C18:0, or ΣSFA in muscle ($P > 0.05$, Table 6). In contrast, the contents of C14:0, C17:0, C16:1n-7, C17:1n-9, C18:1n-9, C20:1n-9, and ΣMUFA in fish fed the BSFO diets were significantly higher than in those fed LO or CO diets, with fatty acid-specific

Table 3
Growth performance of largemouth bass fed with different lipid sources.

Item	SR (%)	FBW (g)	WG (%)	SGR (%·d^{-1})	FR (%)	FCR
Individual treatment means						
LO	97.33±2.67	54.30±0.88^{A}	198.20±4.23^{A}	2.01±0.03^{A}	2.14±0.01	1.18±0.01^{B}
CO	100.00±0.00	38.99±1.90^{B}	116.24±9.12^{B}	1.34±0.07^{B}	2.00±0.02	1.71±0.06^{A}*
BSFO	100.00±0.00	51.32±1.09^{A}	185.47±5.24^{A}	1.91±0.04^{A}	2.20±0.02	1.23±0.01^{B}
LOD	98.67±1.33	55.26±1.11^{X}	205.76±5.35^{X}	2.04±0.04^{X}	2.21±0.03	1.17±0.00
COD	98.67±1.33	50.69±0.93^{Y}*	179.27±4.47^{Y}*	1.87±0.04^{Y}*	2.12±0.03	1.25±0.02
BSFOD	97.33±1.33	57.29±1.09^{X}*	214.25±5.24^{X}*	2.10±0.04^{X}*	2.20±0.02	1.15±0.01
Means of main effect						
LO	98.00				2.18^{a}	
CO	99.33				2.06^{b}	
BSFO	98.67				2.20^{a}	
D–	99.11				2.12^{y}	
D+	98.22				2.17^{x}	
Two-way ANOVA: *P*-value						
Oil sources	0.661				<0.001	
DHA inclusion	0.464				0.006	
Interaction	0.397	<0.001	<0.001	<0.001	0.056	<0.001

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. FBW, WG and SGR are FR-adjusted marginal means (ANCOVA); all other variables are raw means (ANOVA).
3. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively (P < 0.05). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.

Table 4
Morphometric parameters of largemouth bass fed with different lipid sources.

Item	CW (g)	CR (%)	CF (g/cm^3)	VAI (%)	HSI (%)	VSI (%)
Individual treatment means						
LO	43.31 ±3.06^{A}	62.64 ±0.64	1.75 ±0.02	3.82 ±0.16	2.08 ±0.11	9.78 ±0.23
CO	29.17 ±0.71^{B}	62.63 ±0.68	1.75 ±0.02	3.48 ±0.14	1.77 ±0.10	9.95 ±0.06
BSFO	40.49 ±0.89^{A}	63.36 ±0.41	1.78 ±0.01	3.65 ±0.08	2.22 ±0.03	10.32 ±0.11
LOD	42.39 ±1.58	63.33 ±0.25	1.71 ±0.01	3.63 ±0.23	2.03 ±0.05	9.50 ±0.24
COD	39.81 ±2.26*	63.33 ±0.39	1.74 ±0.05	3.21 ±0.13	1.92 ±0.20	9.61 ±0.37
BSFOD	43.67 ±0.88	64.62 ±0.21	1.71 ±0.02	3.49 ±0.24	2.13 ±0.08	9.68 ±0.34
Means of main effect						
LO		62.99	1.73	3.73	2.06^{ab}	9.64
CO		62.98	1.74	3.34	1.84^{b}	9.78
BSFO		63.99	1.75	3.57	2.17^{a}	10.00
D–		62.88^{y}	1.76	3.65	2.02	10.02
D+		63.76^{x}	1.72	3.44	2.03	9.60
Two-way ANOVA: *P*-value						
Oil sources		0.081	0.778	0.126	0.030	0.373
DHA inclusion		0.039	0.080	0.166	0.985	0.064
Interaction	0.021	0.790	0.579	0.946	0.531	0.751

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively ($P < 0.05$). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.

Table 5
Proximate composition of largemouth bass fed with different lipid sources (%, wet basis).

Item	Whole-body				Muscle	Liver
	Crude protein	Crude lipid	Dry matter	Ash	Crude lipid	Crude lipid
Individual treatment means						
LO	17.40 ±0.05	7.59 ±0.39	29.87 ±0.35	4.81 ±0.14	1.09 ±0.15	4.02 ±0.70
CO	17.11 ±0.25	8.56 ±0.81	28.17 ±0.17	4.83 ±0.22	0.90 ±0.12	4.56 ±0.36
BSFO	16.86 ±0.22	10.99 ±0.29	28.59 ±0.82	4.95 ±0.26	0.97 ±0.19	3.54 ±0.45
LOD	17.32 ±0.22	12.36 ±0.69	27.71 ±0.85	5.24 ±0.11	0.85 ±0.08	2.03 ±0.39
COD	17.61 ±0.25	11.18 ±0.94	28.14 ±0.61	4.83 ±0.14	0.70 ±0.14	1.95 ±0.37
BSFOD	17.43 ±0.20	12.46 ±0.93	26.84 ±0.54	5.05 ±0.30	0.77 ±0.09	2.11 ±0.19
Means of main effect						
LO	17.36	9.98^{b}	28.79	5.02	0.97	3.02
CO	17.36	9.87^{b}	28.16	4.83	0.80	3.25
BSFO	17.15	11.72^{a}	27.71	5.00	0.87	2.82
D–	17.13	9.05^{y}	28.88^{x}	4.86	0.99	4.04^{x}
D+	17.45	12.00^{x}	27.57^{y}	5.04	0.78	2.03^{y}
Two-way ANOVA: *P*-value						
Oil sources	0.507	0.043	0.246	0.607	0.486	0.629
DHA inclusion	0.077	<0.001	0.022	0.324	0.078	<0.001
Interaction	0.275	0.108	0.220	0.564	0.981	0.427

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively ($P < 0.05$). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.

differences in statistical significance ($P < 0.05$). DHA inclusion had no effect on the contents of individual SFAs and ΣSFA either, whereas it significantly reduced the contents of individual MUFAs ($P < 0.05$).

For n-6 PUFAs, oil sources significantly influenced the contents of LA, C20:4n-6, and Σn-6 PUFA, with CO-based diets resulting in markedly higher contents of LA and Σn-6 PUFA than the other oil sources ($P < 0.05$), while fish fed BSFO diets exhibited a higher content of C20:4n-6 than those fed LO diets ($P < 0.05$). Moreover, DHA inclusion significantly reduced the contents of LA and Σn-6 PUFA in muscle tissue ($P < 0.05$). Specifically, a significant interaction was observed for C20:2n-6: its content was higher in CO-fed fish than in those fed other oil sources under both non-DHA and DHA-inclusion conditions, and was also higher in the CO group than in the COD group ($P < 0.05$).

For n-3 PUFAs, oil sources significantly influenced ALA content, with LO-fed fish showing the highest values compared with CO- and BSFO-fed fish ($P < 0.05$). DHA inclusion had no effect on the ALA content ($P >$

Table 6
Muscle fatty acid composition of largemouth bass fed different lipid sources (mg/g, wet basis).

Item	C11:0	C14:0	C16:0	C17:0	C18:0	C16:1n-7	C17:1n-9	C18:1n-9	C20:1n-9	C18:2n-6 (LA)	C20:2n-6	C20:4n-6 (ARA)
Individual treatment means												
LO	2.02 ±0.06	0.09 ±0.01	2.22 ±0.19	0.05 ±0.00	1.07 ±0.06	0.13 ±0.02	0.06 ±0.00	2.85 ±0.36	0.09 ±0.01	2.61±0.36	0.08 ±0.01^{C}	0.10 ±0.01
CO	1.98 ±0.05	0.15 ±0.01	2.79 ±0.19	0.05 ±0.00	1.06 ±0.04	0.09 ±0.01	0.05 ±0.01	1.68 ±0.17	0.07 ±0.01	4.92±0.44	0.36 ±0.02^{A}*	0.15 ±0.04
BSFO	1.85 ±0.08	0.57 ±0.10	3.27 ±0.45	0.07 ±0.01	1.01 ±0.10	0.31 ±0.05	0.08 ±0.00	3.31 ±0.48	0.16 ±0.02	3.47±0.39	0.14 ±0.02^{B}	0.20 ±0.01
LOD	1.88 ±0.05	0.14 ±0.00	2.17 ±0.05	0.05 ±0.00	0.98 ±0.03	0.07 ±0.00	0.06 ±0.00	1.85 ±0.11	0.06 ±0.00	1.74±0.14	0.07 ±0.00^{Y}	0.09 ±0.00
COD	1.98 ±0.08	0.15 ±0.03	2.64 ±0.23	0.05 ±0.00	1.09 ±0.05	0.06 ±0.01	0.05 ±0.01	1.25 ±0.19	0.05 ±0.01	3.71±0.59	0.26 ±0.03^{X}	0.11 ±0.01
BSFOD	1.92 ±0.07	0.35 ±0.04	2.57 ±0.16	0.07 ±0.01	0.93 ±0.02	0.16 ±0.02	0.08 ±0.00	2.01 ±0.19	0.09 ±0.01	2.16±0.17	0.11 ±0.01^{Y}	0.15 ±0.01
Means of main effect												
LO	1.95	0.12^{b}	2.19	0.05^{b}	1.03	0.10^{b}	0.06^{b}	2.35^{a}	0.07^{b}	2.17^{b}		0.10^{b}
CO	1.98	0.15^{b}	2.71	0.05^{b}	1.08	0.07^{b}	0.04^{c}	1.46^{b}	0.06^{b}	4.32^{a}		0.13^{ab}
BSFO	1.88	0.46^{a}	2.92	0.07^{a}	0.97	0.23^{a}	0.07^{a}	2.66^{a}	0.13^{a}	2.82^{b}		0.17^{a}
D–	1.95	0.27	2.76	0.06	1.05	0.17^{x}	0.06^{x}	2.61^{x}	0.11^{x}	3.67^{x}		0.15
D+	1.93	0.21	2.46	0.05	1.00	0.09^{y}	0.05^{y}	1.70^{y}	0.07^{y}	2.54^{y}		0.12
Two-way ANOVA: FDR-adjusted *P*-value												
Oil sources	1.000	<0.001	0.173	0.032	1.000	<0.001	<0.001	0.007	0.001	0.001		0.015
DHA inclusion	1.000	0.821	0.598	1.000	1.000	0.005	0.027	0.003	0.003	0.008		0.125
Interaction	0.535	0.050	0.994	1.000	1.000	0.176	1.000	0.725	0.212	1.000	0.045	1.000

Item	C18:3n-3 (ALA)	C20:5n-3 (EPA)	C22:6n-3 (DHA)	DHA+EPA	DHA/EPA	∑SFA	∑MUFA	∑n-3PUFA	∑n-6PUFA	n-3/n-6PUFA	∑PUFA
Individual treatment means											
LO	3.48 ±0.56	0.20 ±0.01^{A}*	0.88 ±0.05^{A}	1.09 ±0.06^{A}	4.39±0.02^{B}	5.62 ±0.30	3.29 ±0.41	4.78 ±0.65^{A}	2.78 ±0.38	1.72±0.03^{A}	7.56 ±1.03
CO	0.14 ±0.02	0.09 ±0.00^{B}*	0.65 ±0.02^{B}	0.74 ±0.03^{B}	7.27±0.10^{A}	6.03 ±0.27	1.89 ±0.20	0.87 ±0.04^{B}	5.56 ±0.51	0.16±0.01^{B}	6.43 ±0.55
BSFO	0.21 ±0.03	0.10 ±0.01^{B}*	0.67 ±0.03^{B}	0.77 ±0.03^{B}	6.61±0.54^{A}	7.54 ±0.87	3.85 ±0.55	0.98 ±0.06^{B}	3.95 ±0.42	0.25±0.01^{B}	4.92 ±0.48
LOD	2.21 ±0.20	0.11±0.00^{X}	1.37 ±0.03*	1.47 ±0.03*	12.95 ±0.38^{Y}*	5.27 ±0.12	2.08 ±0.13	3.88 ±0.23^{X}	1.90 ±0.15	2.05 ±0.04^{X}*	5.78 ±0.38
COD	0.06 ±0.01	0.06±0.00^{Y}	1.45 ±0.07*	1.51 ±0.07*	23.74 ±0.40^{X}*	5.91 ±0.34	1.40 ±0.21	1.57 ±0.07^{Y}	4.16 ±0.62	0.39 ±0.04^{Z}*	5.73 ±0.69
BSFOD	0.15 ±0.01	0.07±0.00^{Y}	1.47 ±0.07*	1.53 ±0.08*	22.54 ±0.25^{X}*	6.29 ±0.22	2.32 ±0.22	1.69 ±0.08^{Y}	2.47 ±0.19	0.68 ±0.02^{Y}*	4.16 ±0.27
Means of main effect											
LO	2.85^{a}					5.45	2.68^{a}		2.34^{b}		6.67^{a}
CO	0.10^{b}					5.97	1.64^{b}		4.86^{a}		6.08^{ab}
BSFO	0.18^{b}					6.92	3.09^{a}		3.21^{b}		4.54^{b}
D–	1.28					6.40	3.01^{x}		4.09^{x}		6.30
D+	0.81					5.82	1.93^{y}		2.84^{y}		5.22
Two-way ANOVA: FDR-adjusted *P*-value											
Oil sources	<0.001					0.058	0.004		<0.001		0.043
DHA inclusion	0.082					0.417	0.003		0.005		0.152
Interaction	0.066	<0.001	0.013	0.005	<0.001	1.000	0.613	0.029	1.000	0.015	1.000

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively ($P < 0.05$). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.
3. SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs.

0.05). Moreover, the interaction for the contents of EPA, DHA, DHA + EPA, Σn-3 PUFA, the DHA/EPA and n-3/n-6 ratio was significant ($P < 0.05$). Based on simple effect analyses, under both non-DHA and DHA-inclusion conditions, LO-fed fish showed the highest contents of EPA, Σn-3 PUFA, and the n-3/n-6 ratio compared with CO- and BSFO-fed fish ($P < 0.05$), whereas the DHA/EPA ratio exhibited the opposite trend ($P < 0.05$). Notably, the contents of DHA and DHA + EPA were higher only in the LO group compared with the CO and BSFO groups ($P < 0.05$). The effects of DHA inclusion within each oil sources were variable across individual n-3PUFAs. Across all oil sources, DHA inclusion consistently reduced EPA but concurrently elevated the contents of DHA and DHA + EPA, the DHA/EPA ratio and the n-3/n-6 ratio, without materially altering total Σn-3 PUFA content ($P < 0.05$). In addition, ΣPUFA content was significantly higher in fish fed LO diets than in those fed BSFO diets ($P < 0.05$), but did not differ significantly from those fed the CO diets.

3.3.2. Liver fatty acid composition

Oil source did not significantly influence the contents of liver C11:0, C17:0, and C18:0 ($P > 0.05$, Table 7). CO inclusion significantly decreased the contents of C18:1n-9, C20:1n-9, and ΣMUFA compared to BSFO inclusion ($P < 0.05$). DHA inclusion also had no effect on C11:0 content ($P > 0.05$); however, noticeable declines in the contents of

Table 7
Liver fatty acid composition of largemouth bass fed different lipid sources (mg/g, wet basis).

Item	C11:0	C14:0	C16:0	C17:0	C18:0	C20:0	C16:1n-7	C17:1n-9	C18:1n-9
Individual treatment means									
LO	2.34±0.12	0.33±0.05^{B}	5.76±0.87^{B}	0.16±0.03	3.64±0.35	0.16±0.01^{AB}*	0.38±0.05^{B}*	0.12±0.05^{B}	9.75±1.54
CO	2.28±0.08	0.50±0.01^{B}	11.40±0.34^{A}*	0.23±0.01	4.80±0.20	0.19±0.00^{A}*	0.33±0.02^{B}*	0.22±0.00^{A}*	7.09±0.29
BSFO	2.28±0.11	1.60±0.22^{A}*	7.05±0.70^{B}	0.20±0.02	3.45±0.19	0.12±0.01^{B}*	0.94±0.08^{A}*	0.09±0.01^{B}	10.14±0.86
LOD	2.39±0.09	0.23±0.03^{Y}	3.40±0.61	0.10±0.02	2.36±0.41	0.10±0.02	0.14±0.01^{Y}	0.06±0.01	3.52±0.73
COD	2.42±0.21	0.24±0.05^{Y}	4.03±0.80	0.10±0.01	2.39±0.13	0.09±0.00	0.09±0.02^{Y}	0.06±0.01	1.97±0.47
BSFOD	2.19±0.08	0.81±0.04^{X}	4.57±0.20	0.13±0.01	2.11±0.15	0.07±0.00	0.44±0.03^{X}	0.06±0.00	4.77±0.23
Means of main effect									
LO	2.37			0.13	3.00				6.63^{ab}
CO	2.35			0.16	3.59				4.53^{b}
BSFO	2.24			0.16	2.78				7.45^{a}
D–	2.30			0.20^{x}	3.96^{x}				8.99^{x}
D+	2.33			0.11^{y}	2.29^{y}				3.42^{y}
Two-way ANOVA: FDR-adjusted *P*-value									
Oil sources	1.000			0.783	0.150				0.039
DHA inclusion	1.000			0.001	<0.001				<0.001
Interaction	1.000	0.012	0.003	0.264	0.223	0.035	0.022	0.044	1.000

Item	C20:1n-9	C18:2n-6 (LA)	C20:2n-6	C20:3n-6	C20:4n-6 (ARA)	C18:3n-3 (ALA)	C20:5n-3 (EPA)	C22:6n-3 (DHA)
Individual treatment means								
LO	0.56±0.10	6.68±1.45	0.34±0.06	0.14±0.01	0.09±0.01^{B}	7.77±1.88^{A}*	0.78±0.06^{A}*	2.46±0.17
CO	0.44±0.02	20.44±5.32	1.58±0.37	0.57±0.22	0.17±0.09^{B}	0.16±0.08^{B}	0.13±0.02^{B}	0.81±0.31
BSFO	0.79±0.03	7.63±0.89	0.45±0.01	0.66±0.01	0.83±0.03^{A}*	0.26±0.04^{B}	0.11±0.01^{B}	1.40±0.05
LOD	0.20±0.03	2.97±0.68	0.18±0.03	0.08±0.01	0.25±0.03^{Y}	3.24±0.72^{X}	0.36±0.05^{X}	3.31±0.36
COD	0.11±0.01	6.32±1.68	0.61±0.05	0.19±0.01	0.24±0.05^{Y}	0.07±0.02^{Y}	0.04±0.01^{Y}	2.80±0.22
BSFOD	0.30±0.03	4.21±0.17	0.29±0.02	0.21±0.02	0.44±0.02^{X}	0.24±0.03^{XY}	0.06±0.01^{Y}	2.96±0.30
Means of main effect								
LO	0.38^{b}	4.82^{b}	0.26^{b}	0.11^{b}				2.89^{a}
CO	0.28^{b}	13.38^{a}	1.09^{a}	0.38^{a}				1.81^{b}
BSFO	0.55^{a}	5.92^{b}	037^{b}	0.44^{a}				2.18^{ab}
D–	0.60^{x}	11.58^{x}	0.79^{x}	0.46^{x}				1.56^{y}
D+	0.21^{y}	4.50^{y}	0.36^{y}	0.16^{y}				3.02^{x}
Two-way ANOVA: FDR-adjusted *P*-value								
Oil sources	0.001	0.025	<0.001	0.018				0.008
DHA inclusion	<0.001	0.014	0.023	0.006				<0.001
Interaction	1.000	0.156	0.059	0.334	<0.001	0.040	<0.001	0.446

Item	DHA+EPA	DHA/EPA	∑SFA	∑MUFA	∑n-3PUFA	∑n-6PUFA	n-3/n-6PUFA	∑PUFA
Individual treatment means								
LO	3.24±0.22	3.18±0.18	12.88±1.51^{B}*	10.81±1.72	12.00±2.15^{A}*	7.25±1.51	1.68±0.07	19.24±3.66
CO	0.94±0.33	4.76±2.32	19.40±0.18^{A}*	8.08±0.32	1.10±0.41^{B}	22.76±5.99	0.05±0.01	23.87±6.36
BSFO	1.51±0.05	12.76±1.58	15.64±1.22^{AB}*	11.95±0.98	1.77±0.07^{B}	9.57±0.95	0.19±0.02	11.33±1.00
LOD	3.67±0.40	9.25±0.25^{Z}	8.69±1.06	3.92±0.77	7.46±1.21^{X}	3.49±0.75	2.19±0.12	10.95±1.95
COD	2.84±0.23	83.99±20.03^{X}*	9.25±1.10	2.24±0.51	2.91±0.25^{Y}	7.36±1.76	0.42±0.06	10.27±2.00
BSFOD	3.02±0.31	46.72±3.47^{Y}*	10.62±0.55	5.57±0.26	3.27±0.33^{Y}	5.15±0.15	0.63±0.06	8.42±0.42
Means of main effect								
LO	3.46^{a}			7.36^{ab}		5.37^{b}	1.94^{a}	15.10
CO	1.89^{b}			5.16^{b}		15.06^{a}	0.23^{b}	17.07
BSFO	2.27^{b}			8.76^{a}		7.36^{b}	0.41^{b}	9.88
D–	1.90^{y}			10.28^{x}		13.19^{x}	0.64^{y}	18.15^{x}
D+	3.18^{x}			3.91^{y}		5.33^{y}	1.08^{x}	9.88^{y}
Two-way ANOVA: FDR-adjusted *P*-value								
Oil sources	<0.001			0.014		0.025	<0.001	1.000
DHA inclusion	<0.001			<0.001		0.014	<0.001	0.045
Interaction	0.113	0.003	0.048	1.000	0.020	0.200	1.000	1.000

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively ($P < 0.05$). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.
3. SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs.

C17:0, C18:0, C18:1n-9, and C20:1n-9 were observed when DHA was included in the diets ($P < 0.05$). The interaction effects were significant for the contents of C14:0, C16:0, C20:0, C16:1n-7, C17:1n-9, and ΣSFA ($P < 0.05$). In particular, the BSFO-fed groups exhibited significantly higher contents of C14:0 and C16:1n-7 than LO- and CO-fed groups ($P < 0.05$) under both non-DHA and DHA-inclusion conditions. In contrast, under non-DHA conditions, CO-fed fish showed higher contents of C16:0 and C17:1n-9 than both LO- and BSFO-fed fish, higher C20:0 than BSFO-fed fish, and higher ΣSFA than LO-fed fish ($P < 0.05$). However, the effects of DHA inclusion on these fatty acids varied among oil sources: C14:0 content was significantly reduced in BSFOD compared with BSFO; the contents of C16:0 and C17:1n-9 significantly declined in the COD

group compared with CO; whereas the contents of C20:0, C16:1n-7, and ΣSFA decreased consistently across all oil sources with DHA inclusion ($P < 0.05$).

Fish fed LO and BSFO diets showed significantly lower contents of LA, C20:2n-6, and Σn-6 PUFA than those fed the CO diets ($P < 0.05$). In contrast, the content of C20:3n-6 in LO-fed fish was lower than in both BSFO- and CO-fed fish ($P < 0.05$). DHA inclusion markedly reduced the contents of LA, C20:2n-6, C20:3n-6, and Σn-6 PUFA ($P < 0.05$). Analysis revealed a significant interaction effect for C20:4n-6 content ($P < 0.05$). Fish fed BSFO diets exhibited a higher C20:4n-6 content than those fed LO and CO diets regardless of DHA inclusion ($P < 0.05$), whereas DHA inclusion resulted in a decline in the BSFOD group compared with the BSFO group ($P < 0.05$).

Fish fed LO diets exhibited a significantly higher DHA content than those fed the CO diets ($P < 0.05$), but showed no difference from the BSFO diets. Diets containing LO also markedly elevated DHA+EPA content and the n-3/n-6 PUFA ratio compared with other oil sources ($P < 0.05$). In addition, DHA inclusion significantly increased the contents of DHA and DHA+EPA as well as the n-3/n-6 PUFA ratio, whereas ΣPUFA content decreased under DHA inclusion ($P < 0.05$). The interaction effects for the contents of ALA, EPA, Σn-3 PUFA, and the DHA/EPA ratio were significant ($P < 0.05$). LO-fed groups exhibited significantly higher contents of ALA, EPA, and Σn-3 PUFA compared with the CO- and BSFO-fed groups, regardless of DHA inclusion ($P < 0.05$). Although ALA in the LOD group remained significantly higher than in COD ($P < 0.05$), no significant difference was observed compared with the BSFOD group ($P > 0.05$). Notably, all three indices declined in the LOD group compared with the LO group following DHA inclusion ($P < 0.05$). Among the three DHA inclusion groups, the COD group showed the highest DHA/EPA ratio, followed by the BSFOD group ($P < 0.05$); additionally, the DHA/EPA ratio in COD group were significantly higher than in the CO group ($P < 0.05$).

3.4. Plasma and liver lipid metabolism

Oil sources did not influence the plasma TG, TC, HDL-c, or liver TC levels; in contrast, LO inclusion led to higher liver TG levels than CO and BSFO ($P < 0.05$, Table 8). DHA inclusion significantly increased plasma HDL-c and reduced liver TG levels ($P < 0.05$). A significant interaction between dietary oil source and DHA inclusion was observed for plasma LDL-c levels and the HDL-c/LDL-c ratio ($P < 0.05$). Without DHA, the CO group exhibited the lowest LDL-c levels compared with LO and BSFO, and the higher HDL-c/LDL-c ratio than BSFO ($P < 0.05$). Under DHA-inclusion conditions, LOD group showed the highest HDL-c/LDL-c ratio compared with COD and BSFOD ($P < 0.05$). Comparing diets with and without DHA within the same oil source, DHA inclusion reduced the LDL-c levels in the LOD group and increased the HDL-c/LDL-c ratio in both LOD and BSFOD groups ($P < 0.05$). Histopathological examination of the liver revealed no obvious hepatocellular swelling, nuclear displacement, or severe vacuolization among different groups (Fig. 2A).

Fish fed CO diets exhibited significantly higher *mgl* expression than LO and BSFO ($P < 0.05$; Fig. 2B). DHA inclusion had no significant effect on the expression of lipid metabolism-related genes ($P > 0.05$). However, significant interaction effects were observed for *acc1*, *fasn*, and *pparγ* ($P < 0.05$). Without DHA, the expression of *acc1* and *fasn* did not differ significantly among the LO, CO, and BSFO groups ($P > 0.05$). Under DHA-inclusion conditions, *acc1* expression was higher in the COD group compared with LOD, while *fasn* expression was elevated in COD relative to both LOD and BSFOD ($P < 0.05$).

3.5. Liver n-3 LC-PUFA biosynthesis

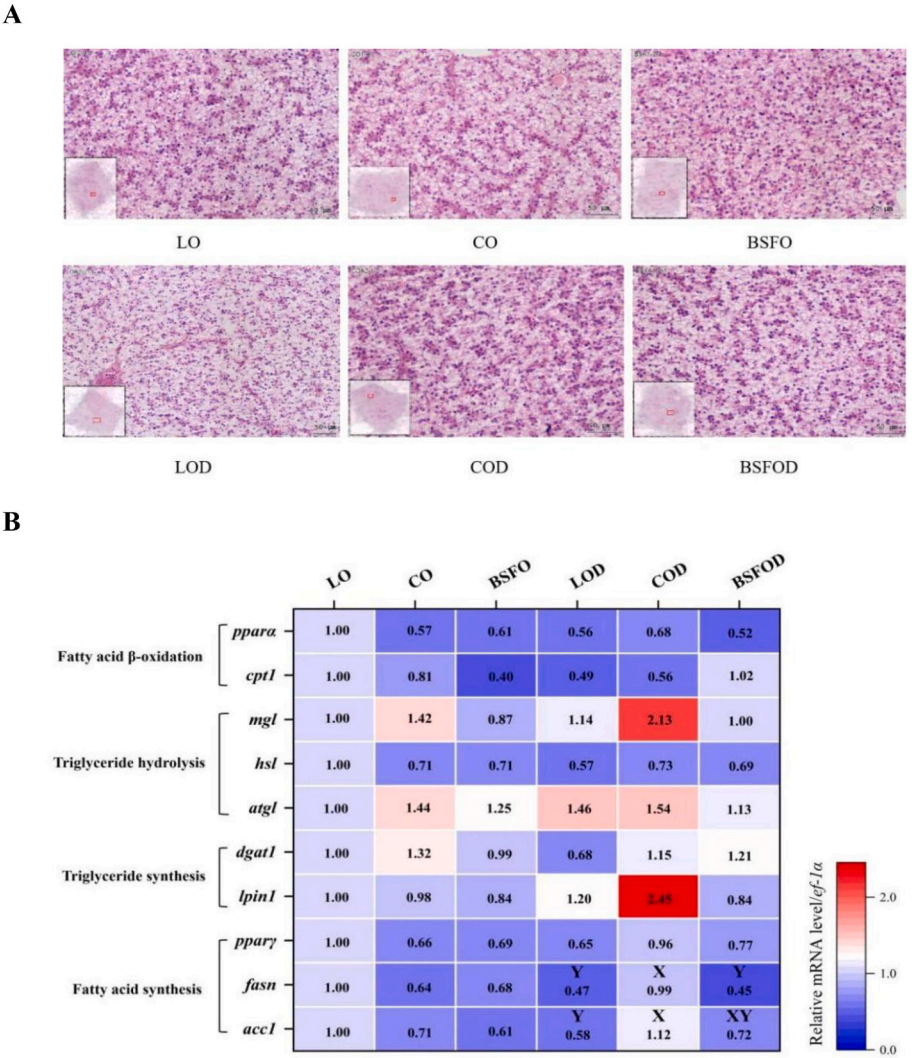
Relative to CO and BSFO, LO markedly suppressed liver *delta4 fads* expression ($P < 0.05$), while no difference between CO and BSFO (Fig. 3A). DHA inclusion further downregulated the expression of *fads2*, *delta4 fads*, and *fads6* ($P < 0.05$). In addition, significant interaction effects were observed for the expression of *elovl4a*, *elovl5*, and *elovl8a* ($P < 0.05$). Under non-DHA conditions, *elovl8a* expression was higher in the LO group than in the BSFO group ($P < 0.05$). Under DHA-inclusion conditions, *elovl4a* and *elovl5* expression were highest in COD group, reduced in LOD and BSFOD groups; *elovl8a* expression was upregulated in BSFOD compared with LOD ($P < 0.05$). Among BSFO-fed fish, DHA inclusion significantly decreased *elovl5* while it increased *elovl8a* expression ($P < 0.05$).

The liver protein abundances of Δ6, Δ5 and Δ4 Fads were not affected by dietary oil sources ($P > 0.05$, Fig. 3B). DHA inclusion did not significantly influence protein abundance of Δ6 Fads ($P > 0.05$), but markedly reduced that of Δ5 and Δ4 Fads ($P < 0.05$).

Table 8
Biochemical indicators in the plasma and liver of largemouth bass fed with different lipid sources.

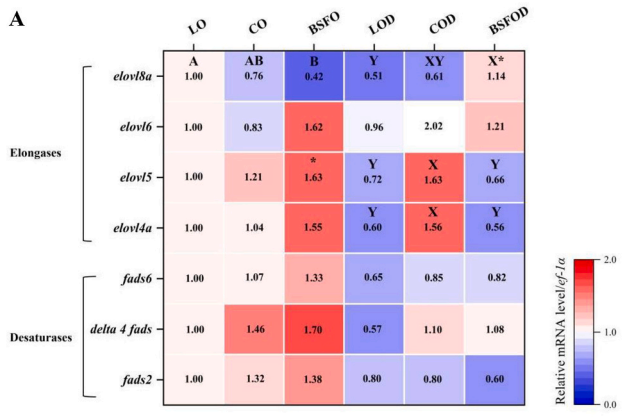
Item	Plasma					Liver	
	TG (mmol/L)	TC (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	HDL-c/LDL-c	TG (mmol/g prot)[1]	TC (mmol/g prot)
Individual treatment means							
LO	4.67±0.93	5.16±0.63	8.14±0.65	0.74±0.08^{A*}	11.89±1.34^{AB}	0.23±0.03	0.08±0.02
CO	3.32±0.47	4.74±0.39	8.29±1.77	0.59±0.04^{B}	15.18±1.56^{A}	0.18±0.01	0.03±0.01
BSFO	4.60±0.66	5.88±0.34	6.51±0.0.76	0.96±0.11^{A}	7.54±1.49^{B}	0.18±0.03	0.06±0.00
LOD	3.62±0.16	5.33±0.12	11.37±0.39	0.54±0.04	21.65±0.88^{X*}	0.17±0.02	0.05±0.01
COD	3.87±0.27	5.43±0.18	11.87±0.32	0.73±0.04	16.66±0.82^{Y}	0.13±0.02	0.04±0.01
BSFOD	6.26±1.12	6.10±0.59	10.83±0.25	0.76±0.03	14.83±0.93^{Y*}	0.11±0.01	0.04±0.01
Means of main effect							
LO	4.15	5.25	9.75			0.20^{a}	0.07
CO	3.60	5.08	10.08			0.15^{b}	0.04
BSFO	5.43	5.99	8.67			0.15^{b}	0.05
D−	4.20	5.26	7.65^{y}			0.20^{x}	0.06
D+	4.58	5.62	11.35^{x}			0.14^{y}	0.05
Two-way ANOVA: *P*-value							
Oil sources	0.056	0.112	0.267			0.030	0.084
DHA inclusion	0.500	0.317	<0.001			0.002	0.264
Interaction	0.184	0.796	0.817	0.020	0.016	0.985	0.374

1. Data are expressed as mean ± SEM. The tank was considered the experimental unit (n = 3 tanks per treatment).
2. Lowercase letters (a, b; x, y) indicate significant differences for main effects of oil source (LO, CO, BSFO) and DHA inclusion (D−, D+), respectively ($P < 0.05$). Uppercase letters (A, B; X, Y) indicate differences among treatment combinations when a significant interaction is detected, and an asterisk (*) denotes a significant DHA inclusion effect within each oil source.
3. This unit (mmol/g prot) reflects the lipid content per unit of protein rather than per tissue weight, allowing for normalization across samples with variable liver sizes.

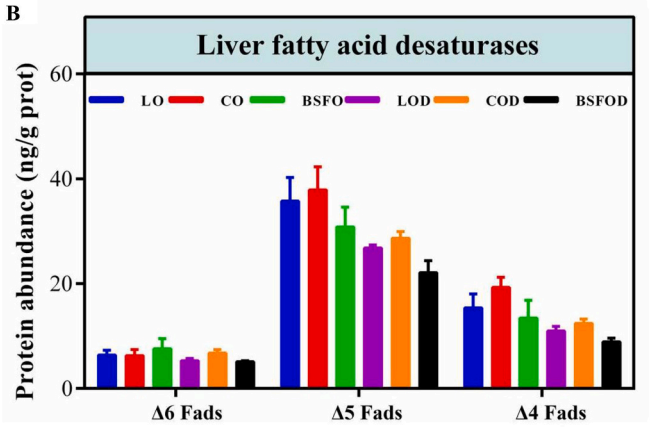


Item	*P*-value			Means of main effect				
	Oil sources	DHA inclusion	Interaction	LO	CO	BSFO	D–	D+
acc1			0.040					
fasn			0.021					
pparγ			0.026					
lpin1	0.092	0.093	0.140	1.10	1.72	0.84	0.94	1.49
dgat1	0.214	0.615	0.446	0.84	1.23	1.10	1.10	1.01
atgl	0.659	0.622	0.708	1.23	1.49	1.19	1.23	1.38
hsl	0.647	0.106	0.083	0.79	0.72	0.70	0.81	0.66
mgl	0.048	0.240	0.610	1.07[b]	1.78[a]	0.93[b]	1.10	1.43
cpt1	0.981	0.873	0.211	0.75	0.68	0.71	0.73	0.69
ppara	0.188	0.175	0.092	0.78	0.63	0.56	0.72	0.59

Fig. 2. Effect of different lipid sources on liver lipid metabolism of largemouth bass. (A) Representative H&E-stained liver sections (n = 3), no marked hepatocellular swelling, nuclear displacement, or severe vacuolization observed; (B) Relative mRNA levels of liver lipogenesis/proliferation (*acc1*, *fasn*, *pparγ*, *lpin1*, *dgat1*) and lipolysis/β-oxidation (*atgl*, *hsl*, *mgl*, *cpt1*, *pparα*) related genes (Mean±SEM, n = 3). Lowercase letters (a, b; x, y) denote significant main effects of oil source and DHA inclusion, respectively; uppercase letters (A, B; X, Y) denote differences under significant interaction, and * indicates DHA effect within each oil source ($P < 0.05$).



Item	*P*-value			Means of main effect				
	Oil sources	DHA inclusion	Interaction	LO	CO	BSFO	D−	D+
fads2	0.856	0.011	0.286	0.90	0.97	0.99	1.17^{x}	0.73^{y}
delta4 fads	0.046	0.027	0.842	0.78^{b}	1.28^{a}	1.39^{a}	1.39^{x}	0.92^{y}
fads6	0.363	0.023	0.691	0.83	0.96	1.07	1.13^{x}	0.77^{y}
eovl4a			0.040					
elovl5			0.024					
elovl6	0.310	0.354	0.062	0.98	1.42	1.41	1.15	1.40
elovl8a			0.004					



Item	*P*-value			Means of main effect				
	Oil sources	DHA inclusion	Interaction	LO	CO	BSFO	D−	D+
Δ6 Fads	0.805	0.264	0.409	5.83	6.51	6.33	6.73	5.72
Δ5 Fads	0.128	0.005	0.997	31.26	33.28	26.44	34.80^{x}	25.85^{y}
Δ4 Fads	0.106	0.008	0.788	13.16	15.83	11.14	16.01^{x}	10.74^{y}

Fig. 3. Impact of different lipid sources on liver LC-PUFA biosynthesis of largemouth bass. (A) Relative mRNA levels of liver desaturases (*fads2*, *delta4 fads*, *fads6*) and elongases (*elovl4a*, *elovl5*, *elovl6*, *elovl8a*) genes (Mean±SEM, n=3); (B) Protein abundance of liver fatty acid desaturase (Δ6, Δ5, Δ4 Fads) (Mean±SEM, n = 3). Letters and asterisks are as described in Fig. 2.

4. Discussion

4.1. Largemouth bass fads genes exhibit distinct desaturase activities

The conversion of ALA into n-3 LC-PUFA in largemouth bass is mediated by a complex biosynthesis pathway involving multiple desaturase genes (*fads2, delta 4 fads*, *fads6*) and elongase genes (*elovl4a*, *elovl5*, *elovl6*, *elovl8a*)(Li et al., 2025; Liang et al., 2023; Zhang et al., 2023). Our study provides evidence that largemouth bass possess the enzymatic basis for n-3 LC-PUFA biosynthesis by demonstrating, through heterologous expression, that *fads2* and *delta4 fads* exhibit intrinsic desaturase activity.

The yeast-based heterologous expression assay was employed to characterize the intrinsic catalytic function of desaturase genes, as previously described in zebrafish (*Danio rerio*)(Hastings et al., 2001) and *S. canaliculatus* (Li et al., 2010b). Although these results suggest potential in vivo pathway capacity, actual conversion efficiency in largemouth bass may be influenced by factors such as substrate availability, tissue specificity, and competing metabolic pathway. This interpretation is consistent with previous reports showing that desaturase and elongase activities are tissue-dependent and substrate-driven (Valenzuela et al., 2025; Valenzuela et al., 2024).

The expression of *fads2* conferred Δ6 desaturase activity, as evidenced by the conversion of ALA to its corresponding Δ6 desaturation product, with a relative conversion rate of 13.6%. This activity is consistent with the monofunctional desaturase function of Fads2 reported in most marine carnivorous species (Amara et al., 2009; Tocher et al., 2006). In contrast, *fads2* gene of largemouth bass showed neither Δ5 nor Δ4 desaturase activity, differing from the bifunctional Fads2 enzyme of the striped snakehead (*Channa striata*)(Kuah et al., 2015).

Earlier research has demonstrated that *delta4 fads* encodes a broad-specificity desaturase, capable of catalyzing multiple desaturation steps (Fonseca-Madrigal et al., 2014; Oboh et al., 2017). Consistent with previous reports, the *delta4 fads* gene in largemouth bass exhibited multifunctional desaturation activity, with Δ4 (40.52%) and Δ5 (20.49%) activities. When a Δ6-specific substrate was supplied, only 4.52% of the product was converted—a value too low to be deemed functional Δ6 Fads activity according to the previous studies (Monroig et al., 2018; Shi et al., 2018). Therefore, our results in this study support active Δ4 and Δ5 desaturase functions, whereas Δ6 activity appears negligible under experimental conditions.

Notably, *delta4 fads* exhibited their highest enzymatic activity toward the Δ4 substrate (C22:5n-3), which is consistent with a Δ4 desaturation capacity reported for this enzyme in other teleosts. This activity trend is compatible with a potential role of "Δ4-Fads2" in DHA biosynthesis via the "Δ4 pathway" (i.e., direct Δ4 desaturation of C22:5n-3 to DHA). Previous comparative studies have suggested that more recently evolved teleosts may rely less on the "Sprecher pathway", (i.e., DHA biosynthesis via elongation of C22:5n-3 to C24:5n-3 followed by Δ6 desaturation to C24:6n-3 and subsequent β-oxidation), which aligns temporally with the appearance of "Δ4-Fads2" (Oboh et al., 2017; Xie et al., 2021). Nevertheless, the present data do not distinguish between the "Δ4 pathway" and "Sprecher pathway" in vivo.

Based on the yeast transformation results, the *fads6* gene failed to exhibit any detectable desaturation activity, its role in largemouth bass remains unknown. Although *fads6* has been reported to contribute to LC-PUFA biosynthesis in other teleost species (Wang et al., 2025; Xing et al., 2023; Zhu et al., 2021), its function in largemouth bass remains unclear and warrants further investigation.

4.2. Dietary control of n-3 LC-PUFA biosynthesis in largemouth bass

Among the three oil sources, LO supplied the highest dietary ALA level but did not enhance liver LC-PUFA biosynthesis gene expression. By contrast, BSFO- and CO-fed fish received substantially lower dietary ALA inputs, while maintaining relatively higher *delta4 fads* expression compared with LO-fed fish. This combination of low precursor supply but maintained gene expression is consistent with the "substrate–limited but not gene–suppressed" pattern previously described in Atlantic salmon and rainbow trout (Gregory et al., 2016; Panserat et al., 2009). This compensatory mechanism, however, failed to offset the limited substrate availability, resulting in suboptimal DHA biosynthesis (Liu et al., 2025). Similar compensatory upregulation under limited n-3 precursor supply has been reported in Japanese seabass (*Lateolabrax*

japonicus), where SFA- and MUFA-rich diets, such as BSFO diets, enhanced desaturase-related gene expression relative to ALA-rich diets (Xu et al., 2014), suggesting that transcriptional regulation is influenced not only by substrate availability but also by broader lipid-class composition (Fawole et al., 2021).

From a pathway perspective, although desaturases catalyse rate-limiting steps early in the n-3 LC-PUFA biosynthsis pathway, elongases operate downstream and display broad, overlapping substrate specificities (e.g., C18–C24 PUFAs)(Xie et al., 2021). In this study, *elovl4a* and *elovl5* expression under DHA-inclusion conditions was highest in the COD group and lower in LOD and BSFOD groups. These gene-expression responses mirrored the dietary ALA/DHA ratios—LOD (7.01), BSFOD (0.39), and COD (0.07), suggesting that a high precursor-to-product ratio may be associated with substrate-related regulatory effects. Similar effects have been reported in teleosts, where ALA-rich diets to reduced desaturase and elongase expression, likely reflecting end-product feedback (Li et al., 2025; Tocher et al., 2002).

In this study, dietary DHA inclusion consistently downregulated the liver expression of desaturase genes (*fads2*, *delta4 fads* and *fads6*), accompanied by reduced protein abundances of Δ5 and Δ4 Fads. When dietary DHA is provided in adequate amounts, fish generally exhibit a downregulation of critical genes associated with the endogenous synthesis of LC-PUFAs, a trend consistent with the well-established negative feedback response to exogenous LC-PUFAs in teleosts (Glencross et al., 2015; Gregory et al., 2016; Panserat et al., 2009; Tocher et al., 2002). Notably, the protein abundance of Δ6 Fads remained unaffected by either oil sources or DHA inclusion. Previous studies have suggested that Δ6 Fads functions at an early step of the n-3 PUFA biosynthesis pathway, and may exhibit relatively conserved regulation across dietary conditions (Nakamura and Nara, 2004; Tocher, 2010).

4.3. Evidence for ALA conversion based on tissue fatty acid composition

In this study, LO, CO, and BSFO each contributed distinct fatty acid profiles, with LO enriching the contents of ALA and total n-3 PUFA, CO elevating the contents of LA and n-6 PUFA, and BSFO enhancing the contents of SFAs and MUFAs which were also reflected in both muscle and liver tissues (Chatelier et al., 2006; Yu et al., 2019b). As expected, fish fed LO, which contained high ALA content (54.24%), exhibited higher tissue contents of n-3 PUFAs, including EPA and DHA, compared with those fed CO or BSFO. This observation is consistent with a greater availability of n-3 precursors and may reflect a combination of dietary deposition and metabolic transformation (Li et al., 2025; Yadav et al., 2020; Zhang et al., 2019a). Although the present study reinforces this metabolic capability, it does not quantify absolute conversion flux (Yuan et al., 2024b), which will require further work integrating isotopic tracing or metabolic rate analyses.

High dietary DHA availability is known to promote its preferential retention in tissues, often reducing the relative abundance of other fatty acids, even when their absolute intake remains unchanged (Glencross, 2009; Liao et al., 2022; Tocher, 2003; Turchini et al., 2009). In this study, tissue DHA content significantly increased in the LOD group following DHA inclusion, whereas the observed decline EPA content may be attributed to their initially higher baseline concentrations in this group. Notably, although the SCM inclusion had a high DHA-to-EPA ratio (15:1), tissue EPA content did not increase significantly. Similar findings have been reported in teleosts, where DHA tends to accumulate more readily than EPA, whereas EPA often shows greater metabolic turnover or utilization across physiological pathways (Glencross, 2009; Liao et al., 2022; Sargent et al., 2003; Tocher, 2010; Turchini et al., 2009; Wu et al., 2021).

4.4. Dietary ALA and DHA benefit growth performance

ALA not only exerts intrinsic effects on growth (Glencross, 2009; Tocher, 2003; Yu et al., 2023) but also serves as a metabolic precursor for n-3 LC-PUFA, thereby influencing growth performance (Song et al., 2024; Tocher, 2010; Turchini et al., 2022; Xie et al., 2021). Consistent with previous studies and the results of the present work, largemouth bass can convert dietary ALA into n-3 LC-PUFAs to meet growth and physiological demands, supporting growth and DHA biosynthesis under the experimental conditions (Sargent et al., 2003).

In this study, fish fed diets containing LO or BSFO grew similarly, while those fed the CO diet showed significantly reduced growth, regardless of DHA inclusion. Dietary inclusion of LO has been investigated for its potential to promote body growth (Turchini and Francis, 2009), as well as stimulate endogenous n-3 LC-PUFA biosynthesis in aquatic species. However, these effects vary with species and replacing the proportion of FO (Li et al., 2016a; Menoyo et al., 2005; Shi et al., 2019). Previous studies have consistently shown that BSFO improves energy utilization and growth performance (Fawole et al., 2021; Hervé et al., 2025; Moutinho et al., 2025; Yuan et al., 2025). Moreover, in this study, the higher lipid content of whole-body in BSFO-fed fish likely provided energy to support growth (Li et al., 2016b; Xu et al., 2024b), thereby corroborating previous observation.

Whereas CO-fed fish exhibited the poorest growth, consistent with its minimal ALA content (0.14%), this limitation could not be compensated despite the marked upregulation of genes and increased protein abundance of key enzymes involved in n-3 LC-PUFA biosynthesis (Liu et al., 2025). Previous studies show that CO can maintain growth in some species but consistently alters tissue fatty acid composition (Hou et al., 2024), whereas excessive CO in the dietary of rainbow trout, gilthead seabream (*Sparus aurata*), and largemouth bass (Eroldoğan et al., 2012; Güler and Yildiz, 2011; Liu et al., 2025; Wassef et al., 2015) impair growth and influence lipid metabolic markers and liver function. In addition to insufficient n-3 PUFA, the high dietary LA in CO may also contribute to reduced growth, as suggested by similar observations in large yellow croaker (*Larmichthys crocea*) under FO-SO substitution (Duan et al., 2014). Collectively, these findings indicate that the poor growth of CO-fed fish may result from the combined effects of extremely low ALA and excessive LA, leading to an imbalanced n-6/n-3 PUFA ratio that constrains growth.

Although the COD group still exhibited markedly lower performance compared with the LOD and BSFOD groups, DHA inclusion resulted in a significant relative improvement in growth, as observed in both COD and BSFOD fish. This suggests that incorporating exogenous EPA and DHA into the diet can help mitigate the nutritional deficiencies of oils low in ALA (such as CO and BSFO) (Dernekbaşı et al., 2021; Song et al., 2024).

Moreover, in this study, supplementation with SCM, a DHA-rich microbial source, was used to increase dietary EPA and DHA levels and was associated with changes in growth performance, feed intake, and whole-body lipids in largemouth bass. This agrees with studies attributing increased palatability and energy retention to DHA-rich microbial ingredients (Santigosa et al., 2020; Shields and Lupatsch, 2012). A comparable trend was seen in juvenile black seabream (*Acanthopagrus schlegelii*), where moderate elevation of the DHA/EPA ratio (0.65 → 1.16) raised whole-body lipids, whereas a further increase reversed the effect (Jin et al., 2017b). In contrast, a study on largemouth bass reported that dietary inclusion of n-3 LC-PUFAs had no notable impact on feed intake or whole-body composition (Yadav et al., 2020). Such divergence in findings likely reflects differences in fish developmental stage, basal diet composition, or the form and level of n-3 PUFA provided, and the underlying mechanisms warrant further investigation (Norambuena et al., 2015).

4.5. Contrasting effects of CO and DHA inclusion on lipid metabolism

Circulating levels of TG, TC, HDL-c, and LDL-c reflect lipid metabolism in the bloodstream in fish (Gong et al., 2024; Yun et al., 2012; Zhang et al., 2022). Among them, HDL-c and LDL-c function oppositely and are widely applied as lipid-transport markers to evaluate lipid

metabolism (Caballero et al., 2006; Guo et al., 2019; Xu et al., 2024a). Previous studies indicate that the HDL-c/LDL-c ratio reflects the direction of cholesterol transport toward the liver in fish (Huang et al., 2022; Ma et al., 2020). Molecularly, ACC1, FASN, and MGL are key regulators involved in lipid synthesis, storage, and mobilization.

Under non-DHA conditions, the CO group exhibited lower plasma LDL-c levels and a higher HDL-c/LDL-c ratio. CO-fed fish also exhibited lower liver TG levels, accompanied by significant upregulation of liver *acc1*, *fasn*, and *mgl* expression compared with other oil sources, especially under DHA inclusion conditions. These changes indicate an altered liver lipid metabolic state associated with CO feeding. Despite the activation of genes involved in lipid mobilization, CO-fed fish still exhibited poor growth. This result may be related to the low ALA content or imbalanced fatty acid composition of CO diets, which could limit lipid availability or metabolic efficiency and be reflect in reduced liver lipid deposition (Malinska et al., 2015).

In contrast, DHA inclusion improved plasma HDL-c and reduced liver TG levels. Notably, fish in the LOD group exhibited a significantly higher HDL-c/LDL-c ratio compared with LO group. Consistent with previous findings that the HDL-c/LDL-c ratio reflects cholesterol transport direction in fish. These changes suggest that DHA inclusion may alter lipoprotein distribution and promote cholesterol transport toward liver metabolism. Generally, insufficient dietary DHA leads to lipid accumulation, particularly elevated liver TG levels, whereas DHA inclusion helps modulate lipid storage and circulation, consistent with previous observations in teleosts (Glencross et al., 2014; Jin et al., 2017a; Lei et al., 2016). Consistently, compositional analysis revealed lower liver crude lipid content in fish receiving DHA, reflecting reduced overall lipid deposition at the tissue level.

5. Conclusion

This study integrates heterologous functional characterization of desaturase genes with in vivo nutritional regulation analyses, providing new evidence for elucidating the n-3 LC-PUFA biosynthesis capacity of largemouth bass. Two desaturases (Δ6 Fads and Δ5/Δ4 Fads) exhibited catalytic activity in heterologous expression assays, supporting the potential enzymatic capacity for DHA biosynthesis in largemouth bass. Nutritional trials further demonstrated that, in largemouth bass, dietary ALA can serve as an effective precursor for DHA formation and, when provided at an adequate level in the tested oil-based diets, supported growth performance during the experimental period. Conversely, severe ALA deficiency upregulated desaturase gene expression but still resulted in reduced tissue DHA and impaired growth, suggesting that increased gene expression alone may be insufficient to compensate for limited substrate availability.

In largemouth bass, dietary DHA effectively compensated for limited precursor availability and restored growth performance, while simultaneously reducing the mRNA expression of desaturase genes and the protein abundance of key desaturases. Together, these findings indicate that dietary ALA supports DHA biosynthesis, while DHA inclusion mitigates ALA deficiency under the experimental conditions, providing a insight relevant to sustainable aquafeed strategies aquafeed design and future LC-PUFA research.

However, the link between relative gene expression and actual metabolic flux through the n-3 LC-PUFA pathway remains unresolved and should be clarified through isotopic tracing or flux measurements. In addition, although dietary DHA downregulated *fads* genes expression, the regulatory basis of this response remains unresolved and warrants further investigation using time-course expression data, enzyme-activity assays validation. Addressing these gaps will improve the understanding of LC-PUFA biosynthesis and inform more effective lipid nutrition strategies for largemouth bass.

CRediT authorship contribution statement

Yangyang Liu: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Rudy Caparros Megido:** Writing – review & editing, Validation, Conceptualization. **Frédéric Francis:** Conceptualization. **Jie Wang:** Methodology. **Hao Wang:** Methodology. **Liang Hu:** Resources. **Xiaofang Liang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Min Xue:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.743641.

Data availability

Data will be made available on request.

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