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Growth, fatty acid composition, and fillet quality of largemouth bass (*Micropterus salmoides*) fed soybean oil and cottonseed oil diets

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ABSTRACT

A two-factor feeding trial evaluated the effects of soybean oil (SO) and cottonseed oil (CO), at 50 % or 100 % dietary lipid inclusion on growth, lipid metabolism, and fillet quality in largemouth bass (*Micropterus salmoides*). A total of 120 fish (51.66 ± 0.01 g) were assigned to four groups: SO50, CO50, SO100, CO100. All diets contained 30.2 % fishmeal, with approximately 0.8 % n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). SO diets had higher α-linolenic acid (ALA) level than CO. Growth performance was significantly affected by oil sources, with CO, particularly CO100, causing reduced final body weight (FBW), weight gain (WG) and specific growth rate (SGR), and higher feed conversation ratio (FCR). CO supplementation significantly reduced plasma triglycerides (TG), total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and hepatic TG, corresponding with elevated adipose triglyceride lipase (*atgl*) expression and phosphorylated AMP-activated protein kinase (p-AMPK) levels. Fillet quality parameters also changed, with decreased water holding capacity (WHC), increased pH at 100 % vegetable oil inclusion, and improved textural properties (cohesiveness, gumminess, and chewiness) in CO diets. Although dietary ALA and desaturase gene expressions were higher in the SO groups, the n-3 LC-PUFA content in muscle and liver, as well as hepatic fatty acid desaturase (FADS) activities, did not differ significantly from those in the CO groups. In conclusion, the lack of ALA in CO, combined with limited n-3 LC-PUFA conversion capacity and the elevated lipid catabolism, particularly in the CO100 group, contributed to the inferior growth performance in largemouth bass.

1. Introduction

Aquaculture has emerged as a critical component in food production, significantly bolstering global food security and nutritional supply (Zhang et al., 2022). In 2022, capture fisheries reached 91 million tons, a slight 1.22 % increase from 2020. In contrast, aquaculture production experienced a 7.64 % increase in 2020, reaching 94.4 million tons (FAO, 2024). Notably, China remains the largest producer and exporter of aquaculture products, contributing over 60 % to global production (Zheng et al., 2022). The rapid growth of aquaculture has led to a growing demand for fish feed, particularly for lipids. Fish oil (FO) is valued in fish feed for its unique fatty acid (FA) profile, which includes eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Sargent et al., 2003; Tocher et al., 2019). These FAs play a significant physiological role in aquaculture species (Cheng et al., 2018; Glencross et al., 2024). However, the rising costs and limited supply of FO necessitate alternative lipid sources to support sustainable aquaculture development (Qin et al., 2022; Tocher et al., 2019; Zorlu and Gümüş, 2022). Vegetable oils (VO), which are more cost-effective and widely available, may serve as viable alternatives to FO if they meet basic FA requirements (Sargent et al., 1999). In 2020, the worldwide production of edible VO amounted to 208.34 million tons, with soybean oil (SO) accounting for 28 % and cottonseed oil (CO) 3 %, ranking as the second and seventh most produced oils, respectively (USDA).

Throughout the preceding twenty years, numerous studies have assessed the impacts of substituting FO with VO or animal-derived oils, either partially or entirely, in various fish species (FAO, 2024; Zhang et al., 2019). Despite challenges, including imbalanced FA compositions (Sargent et al., 2003), reduced palatability, and difficulties in digestibility (Caballero et al., 2002; Guillou et al., 1995), increasing evidence suggests that VO, particularly those characterized by C18 polyunsaturated FAs (PUFA) could serve as viable alternative oil sources

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for certain species.

SO contains a substantial amount of PUFA with 51-64 % linoleic acid (LA, C18:2n-6) and 5 -8 % α-linolenic acid (ALA, C18:3n-3). The incorporation of SO into fish diets, either partially or fully, has been shown to be effective across various species. Examples include the gilthead sea bream (*Sparus aurata L.*, 48 %), black seabream (*Acanthopagrus schlegeli*, 60–80 %), sharp snout seabream (*Diplodus puntazzo*, 100 %), European sea bass (*Dicentrarchus labrax*, 50 %), grass carp (*Ctenopharyngodon idella*, 100 %), and largemouth bass (*Micropterus salmoides*, 100 %) (Figueiredo Silva et al., 2005; Gong et al., 2024; Liu et al., 2022b; Martínez-Llorens et al., 2007; Peng et al., 2008; Piedecausa et al., 2007; Yun et al., 2012; Zhang et al., 2019). However, SO is also subject to significant price fluctuations and unstable supply. Consequently, it is essential to develop alternative VO sources to diversify lipid sources in aquafeed and promote sustainable aquaculture development.

CO, a more affordable VO with stable yields, closely resembles SO and corn oil in terms of nutritional and FA composition (Eroldoğan et al., 2012), containing a high level of LA (44–55 %) (Hou et al., 2024), has gained increased attention for its high C18 PUFA content, strong antioxidant and anti-inflammatory properties (Park et al., 2019; Riaz et al., 2021). Various studies have documented successful instances that adding CO to fish diets, including gilthead seabream (*Sparus aurata,* 60 %), European seabass (*Dicentrarchus labrax*) (contained fish meal, 100 %), rainbow trout (*Oncorhynchus mykiss*, 50 %), black bream (*Acanthopagrus schlegelii*, 60 %) (Eroldoğan et al., 2012; Wassef et al., 2015; Wu et al., 2022; Yıldız et al., 2018). Additionally, the growth of spotted seabass (*Lateolabrax japonicus*) was enhanced when provided with a diet consisting of 50 % FO and 50 % CO compared to a diet containing 50 % FO and 50 % SO (Hou et al., 2024).

Largemouth bass, a significant freshwater economic fish, holds extensive potential for development within the Chinese aquaculture sector. According to FAO data, global largemouth bass production surged from 0.2 kilotons in 2000–804 kilotons in 2022, establishing it as a prevalent aquaculture species worldwide (FAO, 2024). Currently, a blend of FO and SO (50 % FO and 50 % SO, w/w) is widely used in aquaculture practices and experimental diets for largemouth bass to balance the FA composition and ensure growth (Chen et al., 2024a; Liu et al., 2022a; Sun et al., 2025; Xie et al., 2022c; Xue et al., 2022). Although extensive research has evaluated the impact of lipid sources on largemouth bass growth (Gong et al., 2024; Liang et al., 2022; Yun et al., 2012), there remains further investigation into the potential use of CO in largemouth bass diets. This study thoroughly explored the influences of SO and CO on largemouth bass, including growth efficiency, FA profile, fillet characteristics, and lipid metabolic processes, aiming to offer a theoretical basis for the utilization of CO in compound aquafeeds.

2. Materials and methods

2.1. Diet preparation

A blend of 50 % FO and 50 % SO, commonly used in commercial largemouth bass diets, was selected as the reference lipid source in this study. Based on this, a two-factor experiment was designed to comprehensively explore the impact of different oil sources and their inclusion levels on largemouth bass. The two factors were VO types (SO, CO) and VO inclusion levels (50 %, 100 %). Specifically, the experimental groups were set as follows: SO50 (50 % SO and 50 % FO, as dietary lipid source, w/w), CO50 (50 % CO and 50 % FO, w/w), SO100 (100 % SO), CO100 (100 % CO). Soybean oil was obtained from Yihai Kerry Group (China), and cottonseed oil was sourced from Xinjiang Jinlan Plant Protein Co., Ltd. (China).

Four experimental diets were formulated according to Table 1, with 30.2 % fish meal as the major protein source. The preparation and preservation of diets for future use following previous methods (Liu et al., 2022a; Xie et al., 2022b). Table 2 demonstrates the FA composition of the lipid sources and experiment diets.

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

Ingredients (%)	SO50	CO50	SO100	CO100
Fish meal	30.20	30.20	30.20	30.20
Cottonseed protein concentrate	25.00	25.00	25.00	25.00
Soybean protein concentrate	8.00	8.00	8.00	8.00
Soybean meal	14.00	14.00	14.00	14.00
Wheat flour	4.20	4.20	4.20	4.20
Cassava tuber meal	5.00	5.00	5.00	5.00
Microcrystalline cellulose	0.10	0.10	0.10	0.10
Kelp powder	1.50	1.50	1.50	1.50
Permix[1]	1.00	1.00	1.00	1.00
DL-*Met*	0.04	0.04	0.04	0.04
$Ca(H_2PO_4)_2H_2O$	0.36	0.36	0.36	0.36
Choline chloride	0.40	0.40	0.40	0.40
Fish oil	5.10	5.10	-	-
Soybean oil	5.10	-	10.20	-
Cottonseed oil[2]	-	5.10	-	10.20
Proximate Composition (%, dry matter)				
Crude protein	52.02	51.39	51.33	52.69
Crude lipid	12.07	12.23	11.33	11.82
Dry matter	96.50	96.41	97.36	95.22
Ash	10.33	10.51	10.48	10.46
Energy, MJ/Kg	21.92	21.13	21.13	21.57

1. 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, $CoCl_2{\cdot}6\ H_2O$ 5 mg, KI 80 mg, zeolite powder 705 mg; mould inhibitor 3400 mg.
2. According to the examining report provided by the manufacturer, the free gossypol content in cottonseed protein concentrate is 392 mg/kg (≤400 mg/kg), and in cottonseed oil is 12 mg/kg (≤200 mg/kg). After calculation, the content of gossypol in the SO50, CO50, SO100, CO100 diet is 98.00, 98.61, 98.00, 99.22 mg/kg, respectively, which are far lower than the requirement of the limit of China National Standard (≤150 mg/kg) (GB13078–2017).

2.2. Fish rearing conditions

The National Fisheries Technology Extension Center supplied the juvenile fish, which were subsequently transported to the National Aquafeed Safety Assessment Station (Nankou, Beijing), for reaing in a circulating water system. Before starting, the fish underwent a two-week adaptation period. After a 24-hour fasting period, fish (51.66 ± 0.01 g) were randomly distributed into 16 cylindrical plastic tanks with a volume of 0.26 m^3 each, with 4 replicates per treatment and 30 fish per tank. The fish were fed experimental diets to satiation twice daily (8:00 am and 4:00 pm). Throughout the experimental period, the water temperature was between 24 and 26°C, pH ranged from 7.4 – 7.7, dissolved oxygen was not less than 6.0 mg/L, and ammonia-N was lower than 0.2 mg/L. The fish management adhered to the China National Standard (GB/T35892–2018).

2.3. Sampling

Before the experiment, 6 fish were anesthetized using MS-222 (60 mg/L), and preserved at −20°C for initial proximate composition testing. After a 10-week feeding period, the fish in each tank were fasted for 24 h, then individually counted and weighted to determine final body weight (FBW), survival rate (SR), weight gain (WG), specific growth rate (SGR), feeding rate (FR) and feed conversion ratio (FCR). Subsequently, 3 fish from each tank were anesthetized with MS-222 (100 mg/L) and preserved at −20°C for proximate composition evaluation.

Another 3 fish from each tank were sampled and anesthetized for further analysis. Blood was extracted from the tail vein of the fish and then centrifuged (4000 ×g, 10 min, 4°C) to obtain plasma for further assessments. Body length, body weight, carcass weight (after removing

Table 2
Fatty acid composition of oil sources and diets (mg/g).

Items	Oil sources			Diets			
	FO	SO	CO	SO50	CO50	SO100	CO100
C12:0	0.89	-	-	-	-	-	-
C13:0	0.65	-	-	-	-	-	-
C14:0	56.30	0.72	6.09	4.71	4.83	2.31	2.60
C15:0	6.46	-	-	0.40	0.39	-	-
C16:0	148.00	94.40	181.00	17.56	20.88	15.56	24.53
C17:0	5.03	0.61	0.48	0.53	0.51	0.34	0.30
C18:0	32.80	35.00	17.60	4.51	3.71	4.92	3.30
C20:0	7.62	3.68	2.10	0.49	0.45	0.33	0.27
C21:0	-	-	-	1.33	1.20	0.46	0.41
C22:0	0.92	4.9	0.981	0.12	-	0.33	-
C23:0	0.38	0.36	-	-	-	-	-
C24:0	-	3.69	1.62	-	-	-	-
C16:1n−7	59.20	0.75	4.94	4.52	4.24	1.81	2.08
C17:1n−7	-	-	-	0.68	0.67	0.30	0.37
C18:1n−9	123.00	246.00	145.00	15.77	14.05	18.74	16.95
C20:1n−9	27.90	4.64	-	1.25	1.20	0.32	0.13
C22:1n−9	4.20	-	-	-	-	-	-
C24:1n−9	5.98	-	-	0.45	0.44	-	-
C18:3n−3(ALA)	8.98	53.70	1.38	5.15	0.89	8.22	0.32
C20:3n−3	1.58	-	-	2.42	2.40	-	-
C20:5n−3(EPA)	49.90	-	-	9.26	8.62	4.12	3.85
C22:6n−3(DHA)	141.00	-	-	11.76	10.28	4.37	4.20
C18:2n−6(LA)	31.50	496.00	591.00	27.73	29.23	50.18	60.84
C18:3n−6	1.04	-	-	-	-	-	-
C20:2n−6	2.35	-	-	-	-	-	-
C20:4n−6(ARA)	8.88	-	-	0.92	0.77	0.15	0.13
∑SFA	258.67	143.00	209.87	30.04	32.33	24.25	31.41
∑MUFA	220.28	251.39	149.94	22.67	20.60	21.18	19.53
∑n−3PUFA	201.46	53.70	1.38	28.60	22.19	16.71	8.37
∑n−6PUFA	43.77	496.00	591.00	28.65	30.01	50.32	60.97
n−3/n−6PUFA	4.60	0.11	0.00	1.00	0.74	0.33	0.14
PUFA	245.23	549.70	592.38	57.24	52.20	67.03	69.34
EPA+DHA	190.90	-	-	21.03	18.90	8.48	8.06

Note: some fatty acids (including C12:0, C13:0, C22:0, C23:0, C17:1n-7 etc.) content were not detected).
SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs.

the head, fins, tail, and viscera), liver weight, viscera weight, and visceral adipose tissue weight were recorded to complete the calculation of the condition factor (CF), hepatosomatic index (HSI), viscerosomatic index (VSI), and visceral adipose index (VAI). After the removal of fish scales and skin, the dorsal muscles were collected. Three cubes (3 cm×3 cm×2 cm) were immediately taken for fillet quality analysis, and the remaining portion was preserved at −20°C for FA composition analysis. The sampled liver tissues, underwent histological evaluation on sections near the bile ducts. The remaining tissues were divided into three portions: the first was snap-frozen in liquid nitrogen and stored at −80°C for subsequent gene and protein expression analyses; the second were maintained at −20°C for tissue biochemical analysis; and the third was also stored at −20°C for FA composition analysis.

2.4. Growth performance calculations

Survival (SR, %) = final number of fish (per tank)/initial number of fish (per tank) × 100.

Weight gain (WG, %) = $(Wf - Wi + Wd)/Wi \times 100$.

Specific growth rate (SGR, %d^{-1}) = [ln (Wf) – ln (Wi)]/days × 100.

Feeding rate (FR, %) = feed consumption/[$(Wf + Wi + Wd)/2$]/days × 100.

Feed conversion ratio (FCR) = feed intake/$(Wf + Wd - Wi)$.

Protein efficiency ratio (PER, %) = $(Wf - Wi)$/(protein intake) × 100.

Protein productive value (PPV, %) = obtained protein /protein intake × 100.

Lipid productive value (LPV, %) = obtained lipid (g)/lipid intake (g) × 100.

Carcass ratio (CR, %) = carcass weight/fish weight × 100.

Condition factor (CF, g/cm^3) = (fish weight/body length^3) × 100.

Viscera somatic index (VSI, %) = viscera weight (g)/fish weight (g) × 100.

Hepatopancreas somatic index (HSI, %) = hepatopancreas weight (g)/fish weight (g) × 100.

Visceral adipose index (VAI, %) = 100 × visceral adipose weight/fish weight.

Wi represents the initial weight of all fish (per tank), Wf denotes the final weight of all fish (per tank), and Wd indicates the weight of dead fish (per tank) in cultivation.

2.5. Indicator analysis

2.5.1. Proximate and FA composition

The chemical compositions of both diets and fish whole body were determined using the Official Methods of Analysis (AOAC, 2006). The gross energy content of 4 diets was analyzed using an IKAC2000 Calorimeter (IKA, Germany) (Ge et al., 2022).

Muscle and liver specimens were freeze-dried under vacuum at low temperature and then ground into powder for the determination of FA content. FAs from diets, muscle, and liver were extracted from the prepared samples using the hydrolysis procedure specified in the China

National Standard (GB5009.168–2016). The procedure was as follows: (1) approximately 1 g of freeze-dried sample was weighed and transferred into a 10 mL volumetric flask; (2) 2.5 mL each of petroleum ether and benzene was added, the mixture was shaken thoroughly, and then left to stand for 2 h; (3) 0.5 mL of 2 mol/L potassium hydroxide methanol solution was added and the mixture was allowed to stand for an additional 30 min; (4) saturated sodium chloride solution was added until the level reached 1 cm below the frosted neck of the flask, and the mixture was left to stand until the supernatant became clear; (5) the solution was filtered through a 0.22 μm organic membrane filter; (6) fatty acid composition was analyzed using a gas chromatograph (Agilent GC7890B, Agilent Technologies, USA) with the absolute internal standard method.

2.5.2. Biochemical indicators of plasma and liver, and the activity of liver enzymes

The levels of plasma glucose (GLU), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AKP), total bile acid (TBA) and hepatic total protein (TP), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) were assayed using commercial kits (Nanjing Jiancheng Co., China).

Additionally, the activities of cyclic adenosine monophosphate (cAMP), AMP-activated protein kinase (AMPK), phosphorylated AMPK (p-AMPK), Δ5 fatty acid desaturase (Δ5 FADS), and Δ6 FADS in liver tissue were assessed by ELISA kits following the instruction manual (Jiangsu Meimian, China). Similar to Western blot, ELISA kits use specific antibodies to recognize target proteins and, in a sandwich format, capture and detect them with an enzyme-linked secondary antibody that catalyzes a colorimetric reaction, where the color intensity is proportional to protein concentration for quantitative analysis (Nguyen et al., 2025; Zhang et al., 2021).

The liver samples were homogenized with a solution consisting of 9 times the volume of ice-cold phosphate buffer (pH 7.4), then centrifuged at 4, 000 × g for 10 min at 4°C. The resulting supernatant was used for the measurement of the above indicators.

2.5.3. Hepatic histopathological examination

Liver specimens were preserved in 4 % paraformaldehyde for 24 h, then dehydrated using the Slee MTM I/II tissue processing machine (Slee, Germany) and embedded in paraffin with the Leica EG1150 embedding station (Leica, Germany). Subsequently, tissue sections approximately 5–6μm thick were prepared using the Leica RM 2255 paraffin slicer (Leica, Germany). After HE staining, all tissue sections were scanned and analyzed using Tissue FAXS (Tissue Gnostics, Austria).

2.5.4. Fillet quality

The pH of the muscle sample was obtained using an HM Digital pH-80 device (HM Digital, Korea). The glass electrode was submerged into the fish muscle for a duration of 1 min at 0 h, 24 h, 48 h, and 72 h.

The experiment evaluated the water holding capacity (WHC) by measuring drip and cooking losses. Drip loss (%) was evaluated as moisture loss over holding time. Fillet samples were immediately weighed and placed in aerated plastic bags, hung vertically with iron wires (to prevent contact with bag walls), sealed, and stored at 4°C for 24 h. Subsequently, the samples were removed, gently dried with filter paper, and weighed again. Drip loss was calculated using the formula: Drip loss (%) = [initial sample weight (g) - sample weight after holding (g)] / initial sample weight (g) × 100.

The evaluation of cooking loss was based on the moisture variation that occurred during heating. The fresh fillets were first weighed, placed in a sealed plastic bag, and then subjected to heating in a water bath maintained at 70°C for 10 min. After heating, the samples'surface were wiped dry, and they were reweighed to calculate the moisture loss. Cooking loss was calculated using the formula: Cooking loss (%) = [initial sample weight (g) - sample weight after cooking (g)] / initial sample weight (g) × 100.

A TMS-Pro texture analyzer (FTC, USA) was used to conduct the texture profile analysis (TPA), which included parameters such as hardness, adhesiveness, cohesiveness, springiness, gumminess, and chewiness (Chen et al., 2024b). The fresh fillet samples (3 cm × 3 cm × 2 cm) were used for the TPA, following methods reported in previous studies (Alak et al., 2024; Yuan et al., 2022a).

2.5.5. Gene expression analysis

The expression of genes in the liver involved in FA synthesis (*acc1*, *fasn*), triglyceride hydrolysis (*atgl*, *hsl*), triglyceride β-oxidation (*cpt1*, *pparα*), triglyceride synthesis (*dgat1*, *lpin1*), and FA biosynthesis (*fads2*, *delta4 fads*, *fads6*, *elovl4a*, *elovl5*, *elovl6*, *elovl8a*) were analyzed using the qRT-PCR method with the Bio-Rad CFX96 system (Bio-Rad, USA) (Wei et al., 2019; Xie et al., 2022b). The primer sequences for *fads6* and *elovl5* were designed according to the research on largemouth bass (Liang et al., 2023), and the sequences of other genes were retrieved from the RNA-seq database. Table 3 presents the primer sequences.

2.5.6. Western blot

Liver samples underwent protein extraction and concentration determination, following a previously reported method (Huang et al., 2019; Yu et al., 2019). The primary antibody used was Δ5 FADS (ab126706, abcam), and secondary antibody was anti-rabbit IgG-HRP (CWBIO, China). Target protein bands were developed using enhanced Clarity™ Western ECL (Bio-Rad, USA). Protein expression was quantified using the total protein normalization method (Hagner-McWhirter et al., 2015; Maloy et al., 2022) and assessed with Image Lab software (Bio-Rad, USA).

2.6. Statistical methods

All findings were reported as mean ± standard error of the mean (Mean ± SEM). Statistical analysis and graphical representations were processed using GraphPad Prism 8. Differences were considered statistically significant at $P < 0.05$. A two-way ANOVA was conducted to evaluate the effects of oil type (SO and CO), inclusion level (50 % and 100 %), and their interaction on the measured variables. When a significant interaction was detected ($P < 0.05$), simple main effects were examined using one-way ANOVA followed by Tukey's test, and interaction plots were used as a visualization tool to assist in explaining significant interactions.

3. Results

3.1. Growth and proximate composition

The survival rate for each group exceeded 99 %, with no significant differences observed among the treatments (Table 4). Results from the two-way ANOVA indicated that, compared to largemouth bass fed diets with CO, those fed diets with SO exhibited significantly better performance in terms of FBW, WG, SGR, PER, PPV, LPV, CW, VAI and VSI ($P < 0.05$). A 50 % inclusion level of VO resulted in an increase in PER, PPV, and LPV, while causing a decrease in HSI and VSI ($P < 0.05$) based on. However, no significant differences were observed in FR, CR, and CF across VO sources and inclusion levels ($P > 0.05$). There was a significant interaction of oil source and inclusion level on FCR ($P < 0.05$). According to the one-way ANOVA, no significant difference in FCR was observed between SO50 and CO50 ($P > 0.05$); however, FCR was significantly higher in CO100 than in SO100 ($P < 0.05$) (Figure S1). To clarify the effects of SO and CO on largemouth bass growth, a one-way ANOVA was conducted for FBW, SGR, FR, PER, and PPV. In the CO100 group, FBW and WG were significantly reduced compared to those in the SO50 group ($P < 0.05$); SGR was significantly decreased than in the

Table 3
Real-time quantitative PCR primers.

Genes	Primers	Sequence 5′-3′	Target Size (bp)	TM (°C)	E-Value (%)
ef−1α	F	TGCTGCTGGTGTTGGTGAGTT	147	60.4	102.8
	R	TTCTGGCTGTAAGGGGGCTC			
acc1	F	ATCCCTCTTTGCCACTGTTG	121	57.5	102.1
	R	GAGGTGATGTTGCTCGCATA			
fasn	F	GGGCATCGTTAACCTGGACA	164	57	99
	R	CATCGTAGTGGCGCTTTTCG			
atgl	F	CCATGATGCTCCCCTACACT	176	58	99.1
	R	GGCAGATACACTTCGGGAAA			
hsl	F	ATCAGAGCTGGAGCACCCTA	122	60	99.3
	R	GCAGAGGAGAGCAGAAAGGA			
cpt1	F	CATGGAAAGCCAGCCTTTAG	128	60	98.8
	R	GAGCACCAGACACGCTAACA			
pparα	F	CCACCGCAATGGTCGATATG	144	59	104.3
	R	TGCTGTTGATGGACTGGGAAA			
dgat1	F	CACGCCTCTTCTTGGAGAAC	176	58.5	105.3
	R	AATGGTACCCACAGCCAGAC			
lpin1	F	TCCTACGTTCCCGAGAGAAA	136	58.5	98.8
	R	TACGAGGGAACCACTTCCTG			
fads2	F	ATCATTGCCCTACAATCACCAAC	133	62	98.9
	R	AAACCAAGCCAGATCCACCC			
delta4 fads	F	ACTTGGAACCACTCAGCCAG	162	56	97
	R	ACCCACAAGCGGTGAGAAAT			
fads6	F	TTGGAGAGCCACTGGTTTGT	134	(Liang et al., 2023)	
	R	TCGTTGAGGAGGGACTGCT			
elovl4a	F	GGCCATTCGTACTTTGGTGC	165	56	96.4
	R	CATCGTCTGGGACACGGTTA			
elovl5	F	GGGGACTTCTGCTGCTTGA	159	(Liang et al., 2023)	
	R	AGGACAAGAGTGTGACGCCC			
elovl6	F	GTCACGCAAATTCGCCATGT	227	57	93.6
	R	CAGCCGAGGCAGTTGATTTG			
elovl8a	F	TGGGGACCAAATCTAGGGGT	226	57	93.6
	R	ATCAGGAAAGACTGGCCACC			

Abbreviation: *ef−1α*, elongation factor 1α; *acc1*: acetyl-CoA carboxylase 1; *fasn*: fatty acid synthase; *atgl*: adipose triglyceride lipase; *hsl*: hormone-sensitive lipase; *cpt1*: carnitine palmitoyltransferase 1; *pparα*: peroxisome proliferator activated; *dgat1*: diacylglycerol acyltransferase 1; *lpin1*: Phosphatidic acid phosphohydrolase1; *fads2*: fatty acid desaturase 2; *elovl5*: fatty acid elongase 5; *elovl8a*: fatty acid elongase 8 a. F: forward primer; R: reverse primer.

SO50 and SO100 group ($P < 0.05$); additionally, PER and PPV were the lowest among all four groups ($P < 0.05$).

Before the experiment commenced, the initial fish contained levels of crude protein, crude lipid, dry matter, and ash at 17.07 %, 7.84 %, 29.98 % and 4.35 % (on a wet basis) respectively. At the end of experiment, it was determined that neither the various VO sources and their inclusion levels had a significant effect on the crude protein, ash, and dry matter in fish whole body ($P > 0.05$) (Table 5). However, when the VO inclusion level was increased to 100 %, there was a significant decrease in the crude lipid content of the whole fish ($P < 0.05$).

3.2. FA composition of tissues

The FA profiles of fish muscle are presented in Table 6. The levels of C18:1n-9 and C20:1n-9 were notably higher in the SO compared to the CO ($P < 0.05$). When the VO inclusion level reached 100 %, the concentrations of EPA and DHA dropped significantly, while those of LA, C20:2n-6, and total n-6 PUFA rose notably ($P < 0.05$). Besides, ALA, MUFA, and total n-3 PUFA showed significant interactions between oil source and inclusion level ($P < 0.05$). No significant differences in ALA and MUFA were found between SO50 and CO50 ($P > 0.05$); however, the levels in SO100 were significantly higher than those in CO100 ($P < 0.05$). For total n-3 PUFA, although no significant difference was observed between SO and CO at either inclusion level ($P > 0.05$), the total n-3 PUFA content significantly decreased with higher CO inclusion ($P < 0.05$) (Figures S2 - S5).

The FA profiles of fish liver are also illustrated in Table 6. In the CO groups, the levels of C16:0, LA, total saturated FAs (SFA), and total n-6 PUFA were significantly higher ($P < 0.05$). Conversely, total monounsaturated FAs (MUFA), and total n-3 PUFA levels were markedly reduced ($P < 0.05$). Specifically, no considerable difference was observed in the levels of EPA, DHA, and total n-3 PUFA between the two oil treatments ($P > 0.05$). In the 50 % VO groups, the contents of C14:0, C17:0, C16:1n-7, EPA, DHA, and total n-3 PUFA were considerably increased ($P < 0.05$). In contrast, within the 100 % VO groups, the levels of LA, total SFA, total MUFA, and total n-6 PUFA exhibited an upward trend ($P < 0.05$). In addition, significant interactions were observed for in the level of C18:0, ALA and C20:2n-6 ($P < 0.05$). Although no differences between SO and CO were found at either the 50 % or 100 % inclusion level for C18:0 and C20:2n-6 ($P > 0.05$), C18:0 increased with higher SO inclusion, while C20:2n-6 increased with higher CO inclusion ($P < 0.05$). ALA levels was consistently higher in SO than in CO at both inclusion levels, and also increased with SO inclusion ($P < 0.05$) (Figure S5–S7).

3.3. Fillet qualities

Table 7 reveals that varying VO sources did not significantly influence the drip loss or pH of fish fillet ($P > 0.05$). However, CO significantly enhanced the cohesiveness, gumminess, and chewiness of the fresh fillets ($P < 0.05$). Compared to the 50 % VO inclusion, 100 % VO significantly reduced drip loss and increased pH (24 h, 48 h, 72 h) ($P < 0.05$). while inclusion level had no significant effect on fillet texture ($P > 0.05$). Moreover, significant interactions between oil source and inclusion level were observed for cooking loss and pH (0 h) ($P < 0.05$). No significant differences were found between SO and CO at either the 50 % or 100 % inclusion level for cooking loss and pH (0 h) ($P > 0.05$). However, cooking loss significantly decreased with higher inclusion of CO, and pH (0 h) increased at higher inclusion of SO ($P < 0.05$) (Figure S8-S9).

Table 4
Growth performance and morphometric parameters of largemouth bass after 10-week culturing with different lipid sources (Mean ± SEM).

	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
Growth performance ($n = 4$)							
FBW (g)	SO	114.86 ± 5.79^{X}	112.04 ± 5.43^{XY}	113.45 ± 3.71^{A}	0.012	0.120	0.286
	CO	105.57 ± 6.16^{XY}	91.42 ± 1.31^{Y}	98.50 ± 3.96^{B}			
	Inclusion level	110.22 ± 4.29	101.73 ± 4.68				
WG (%)	SO	122.32 ± 11.19^{X}	116.92 ± 10.48^{XY}	119.62 ± 7.17^{A}	0.011	0.126	0.297
	CO	102.57 ± 11.71^{XY}	76.94 ± 2.57^{Y}	90.28 ± 7.53^{B}			
	Inclusion level	112.97 ± 8.32	96.93 ± 9.06				
SGR (%/d)	SO	1.14 ± 0.07^{X}	1.10 ± 0.07^{X}	1.12 ± 0.05^{A}	0.010	0.105	0.243
	CO	1.01 ± 0.08^{XY}	0.81 ± 0.02^{Y}	0.92 ± 0.05^{B}			
	Inclusion level	1.08 ± 0.06	0.96 ± 0.06				
FR (%)	SO	1.26 ± 0.04	1.27 ± 0.04	1.26 ± 0.02	0.101	0.937	0.848
	CO	1.20 ± 0.04	1.19 ± 0.03	1.20 ± 0.02			
	Inclusion level	1.23 ± 0.03	1.23 ± 0.02				
FCR	SO	1.18 ± 0.05^{Y}	1.22 ± 0.05^{Y}	1.20 ± 0.03^{B}	0.002	0.010	0.046
	CO	1.26 ± 0.06^{Y}	1.51 ± 0.02^{X}	1.38 ± 0.05^{A}			
	Inclusion level	1.22 ± 0.04^{b}	1.36 ± 0.06^{a}				
PER (%)	SO	1.70 ± 0.07^{X}	1.60 ± 0.07^{X}	1.68 ± 0.05^{A}	0.004	0.022	0.085
	CO	1.65 ± 0.07^{X}	1.32 ± 0.01^{Y}	1.46 ± 0.06^{B}			
	Inclusion level	1.65 ± 0.05^{a}	1.49 ± 0.07^{b}				
PPV (%)	SO	29.47 ± 1.30^{X}	28.30 ± 1.23^{X}	28.89 ± 0.86^{A}	0.009	0.011	0.073
	CO	28.18 ± 1.28^{X}	22.57 ± 0.48^{Y}	25.38 ± 1.23^{B}			
	Inclusion level	28.83 ± 0.88^{a}	25.44 ± 1.24^{b}				
LPV (%)	SO	84.59 ± 5.35	76.53 ± 6.57	80.56 ± 4.21^{A}	0.031	0.051	0.682
	CO	75.29 ± 2.60	63.36 ± 2.53	69.33 ± 2.81^{B}			
	Inclusion level	79.94 ± 3.27	69.95 ± 4.10				
Morphometric parameters ($n = 12$)							
CW (g)	SO	89.56 ± 5.28	98.92 ± 6.91	94.24 ± 4.36^{A}	0.017	0.069	0.908
	CO	75.59 ± 2.15	86.19 ± 5.87	80.89 ± 3.25^{B}			
	Inclusion level	82.57 ± 3.14	92.56 ± 4.63				
CR (%)	SO	70.73 ± 0.31	71.04 ± 0.48	70.88 ± 0.28	0.127	0.689	0.683
	CO	70.30 ± 0.40	70.30 ± 0.29	70.30 ± 0.24			
	Inclusion level	70.51 ± 0.25	70.67 ± 0.28				
VAI (%)	SO	3.35 ± 0.18	3.53 ± 0.23	3.44 ± 0.14^{A}	0.002	0.132	0.573
	CO	2.65 ± 0.09	3.03 ± 0.19	2.84 ± 0.11^{B}			
	Inclusion level	3.00 ± 0.12	3.28 ± 0.16				
HSI (%)	SO	1.43 ± 0.07	1.61 ± 0.08	1.52 ± 0.06	0.183	0.032	0.714
	CO	1.36 ± 0.06	1.49 ± 0.05	1.43 ± 0.04			
	Inclusion level	1.40 ± 0.05^{b}	1.55 ± 0.05^{a}				
VSI (%)	SO	7.61 ± 0.26	7.92 ± 0.28	7.76 ± 0.19^{A}	0.012	0.022	0.287
	CO	6.73 ± 0.14	7.55 ± 0.25	7.14 ± 0.16^{B}			
	Inclusion level	7.17 ± 0.17^{b}	7.74 ± 0.19^{a}				
CF (g/cm^3)	SO	1.76 ± 0.10	1.77 ± 0.04	1.76 ± 0.05	0.098	0.374	0.446
	CO	1.62 ± 0.02	1.71 ± 0.03	1.67 ± 0.02			
	Inclusion level	1.69 ± 0.05	1.74 ± 0.03				

A two-way ANOVA was conducted to evaluate the effects of oil sources (SO and CO) and inclusion levels (50 % and 100 %) on the measured parameters. Differences in main effects were compared: significant differences ($P < 0.05$) between VO sources are indicated by different capital letters (A, B); significant differences ($P < 0.05$) between inclusion levels are indicated by different lowercase superscript letters (a, b).
Simple main effects (one-way ANOVA) were further performed to further examine the differences among the four treatment combinations (SO50, CO50, SO100, and CO100), in cases where a significant interaction ($P < 0.05$) between the two factors was detected: significant differences ($P < 0.05$) among these groups are indicated by different capital letters (X, Y).

3.4. Biochemical indicators of plasma and liver

CO led to a significant decrease in plasma TC and LDL-C levels ($P < 0.05$) (Table 8). Additionally, neither VO source nor inclusion level significantly affected plasma GLU, AST, ALT, or hepatic TP and LDL-C levels ($P > 0.05$). Moreover, significant interaction effects between oil source and inclusion level were observed for plasma TG, HDL-C, HDL-C/LDL-C, AKP, TBA, and hepatic TG ($P < 0.05$). Further analysis showed that the levels of plasma HDL-C did not differ significantly among groups ($P > 0.05$). For plasma TG, HDL-C/LDL-C, AKP and hepatic TG, no significant differences were observed between SO50 and CO50 ($P > 0.05$); however, they were significantly higher in SO100 than in CO100 ($P < 0.05$). TBA levels showed no significant differences between SO and CO at either inclusion level ($P > 0.05$), but increased significantly with higher inclusion of both oils ($P < 0.05$) (Figure S10–S15). Preliminary histopathological analysis indicated that there were no significant abnormalities in the hepatic phenotype of largemouth bass (Fig. 1A).

3.5. Genes expression of lipid metabolism and enzymes activity of energy metabolism in liver

The VO sources as well as VO inclusion levels did not significantly affect the expression of genes related to FA synthesis, β-oxidation, and triglyceride synthesis related genes ($P > 0.05$) (Fig. 1B). Fish in the SO groups presented a decreased expression level of *atgl* compared to fish in CO groups ($P < 0.05$). Furthermore, a higher VO inclusion level significantly reduced the expression level of *atgl* ($P < 0.05$), which is associated with triglyceride hydrolysis. Liver p-AMPK activity in the CO groups was significantly higher than that in the SO groups ($P < 0.05$) (Fig. 1C). At the 100 % VO inclusion level, the activities of cAMP, p-AMPK, and the p-AMPK/AMPK ratio were significantly increased ($P < 0.05$).

Table 5
Proximate composition of whole body (%, wet basis) of largemouth bass after 10-week culturing with different lipid sources (Mean±SEM, $n = 4$).

	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
Crude protein	SO	17.22 ± 0.07	17.09 ± 0.26	17.15 ± 0.13	0.732	0.184	0.595
	CO	17.36 ± 0.08	17.06 ± 0.12	17.21 ± 0.09			
	Inclusion level	17.29 ± 0.06	17.07 ± 0.13				
Crude lipid	SO	9.87 ± 0.24	9.13 ± 0.39	9.50 ± 0.25	0.455	0.034	0.558
	CO	9.53 ± 0.08	9.09 ± 0.17	9.31 ± 0.12			
	Inclusion level	9.70 ± 0.13[a]	9.11 ± 0.20[b]				
Dry matter	SO	31.58 ± 0.22	31.21 ± 0.29	31.40 ± 0.18	0.704	0.182	0.907
	CO	31.65 ± 0.25	31.34 ± 0.18	31.49 ± 0.15			
	Inclusion level	31.61 ± 0.16	31.27 ± 0.16				
Ash	SO	4.04 ± 0.05	4.10 ± 0.11	4.07 ± 0.06	0.063	0.768	0.768
	CO	4.25 ± 0.06	4.25 ± 0.11	4.25 ± 0.06			
	Inclusion level	4.15 ± 0.05	4.17 ± 0.08				

A two-way ANOVA was conducted to evaluate the effects of oil sources (SO and CO) and inclusion levels (50 % and 100 %) on the measured parameters. Differences in main effects were compared: significant differences ($P < 0.05$) between VO sources are indicated by different capital letters (A, B); significant differences ($P < 0.05$) between inclusion levels are indicated by different lowercase superscript letters (a, b).
Simple main effects (one-way ANOVA) were further performed to further examine the differences among the four treatment combinations (SO50, CO50, SO100, and CO100), in cases where a significant interaction ($P < 0.05$) between the two factors was detected: significant differences ($P < 0.05$) among these groups are indicated by different capital letters (X, Y).

3.6. Expression of genes and protein related to LC-PUFA biosynthesis in liver

The SO and a 100 % VO inclusion level markedly enhanced the expression levels of *fads2*, *delta4 fads*, which are related to FA desaturation ($P < 0.05$) (Fig. 2A). Moreover, a significant interaction effect between oil source and inclusion level was observed for *fads6* ($P < 0.05$). No significant difference was found between SO50 and CO50 ($P > 0.05$); however, *fads6* expression was significantly higher in SO100 compared to CO100 ($P < 0.05$) (Figure S16). The expression levels of elongase genes (*elovl5* and *elovl8a*) remained unchanged irrespective of the VO sources or their inclusion levels ($P > 0.05$) (Fig. 2B).

Western blot results revealed that both CO and a 100 % VO inclusion level enhanced the protein expression of Δ5 FADS (Fig. 2C). Besides, there were no significant differences in hepatic Δ5 FADS and Δ6 FADS activities between SO and CO at either inclusion level ($P > 0.05$) (Fig. 2D).

4. Discussion

In this study, growth performance of largemouth bass was significantly affected by the oil sources (SO and CO). A meta-analysis revealed that SO, rapeseed oil, palm oil, and flaxseed oil noticeably increased the FCR, whereas CO, sunflower seed oil, corn oil, and rapeseed oil exhibited a milder effect on the FCR of major cultured fish species (Qian et al., 2024). The findings of this study were consistent with previous research, indicating that oil sources significantly affected growth of largemouth bass (Gong et al., 2024; Liang et al., 2022; Zhang et al., 2019). In this study, the effect of CO on the FCR of largemouth bass was dependent on its inclusion level in the diet. The highest FCR was observed in the 100 % CO group, which, in combination with the lowest FBW, WG, and SGR, indicated the poorest growth performance. A study on channel catfish (*Ictalurus punctatus*) proposed that CO is unsuitable for use in aquafeed due to anti-nutritional factors and harmful substances, including free gossypol (Yin et al., 2013). However, the content of gossypol in the feed ranged from 98.00 to 99.22 mg/kg, which is below the limit of the China National Standard (≤150 mg/kg) (GB13078–2017). Therefore, this was not recognized as the main factor accounting for the growth differences in largemouth bass.

ALA is renowned for its role in maintaining the body's homeostasis (Yu et al., 2023). Studies have shown that appropriate dietary supplementation of ALA can improve the lipid metabolic function of fish, enhance the ability of digestion and absorption and therefore contribute to promoting growth performance and overall health (Castell et al., 1972; Mourente et al., 2005; Xu et al., 2015; Yu et al., 2023). From this research, SO primarily contained LA (496.00 mg/g) and ALA (53.70 mg/g), whereas CO had a higher content of LA (591.00 mg/g) but significantly less ALA (1.38 mg/g). In these two oils, LA constituted over 90 % of the C18 PUFA. Compared with the other diets, the CO100 diet contained lower levels of ALA, which may be one of the reasons for the poorest growth observed among all experimental groups.

Moreover, ALA and LA are crucial FA for freshwater fish species and serve as substrates for LC-PUFA biosynthesis (Tocher, 2010; Xie et al., 2021). Desaturase and elongase are two important enzymes in the biosynthesis of LC-PUFA. As Δ6 and Δ5 desaturases are essential for the bioconversion of C18 PUFAs to LC-PUFAs (Xie et al., 2021). In largemouth bass, the biosynthesis of n-3 LC-PUFAs from ALA involves key desaturase and elongase genes, including *fads2* (Δ6 FADS), *fads6*, and *delta 4 fads*, as well as elongases such as *elovl4a*, *elovl5*, *elovl8a*, and *elovl6*. Among them, *fads2* and *delta4 fads* play central roles in desaturation steps, while *elovl5* and *elovl8a* are mainly involved in elongating intermediate fatty acids (Li et al., 2025; Liang et al., 2023; Zhang et al., 2023). Although there is limited research on *fads6*, and its function in largemouth bass remains unknown (Zhang et al., 2023), it has been identified to possess Δ4, Δ5, and Δ8 desaturase activities toward LC-PUFAs in *Trachinotus ovatus* (Zhu et al., 2018). Moreover, *fads6* has also been investigated in relation to LC-PUFA biosynthesis in several other fish species (Wang et al., 2025; Xing et al., 2023; Zhu et al., 2021).

Studies have shown that a reduction of n-3 LC-PUFA or an elevation of the ALA/LA ratio in the fish diet regulates desaturase-related genes and enzyme activities thereby promoting the n-3 LC-PUFA biosynthesis ability (Jin et al., 2017; Mohammad Ali Jalali et al., 2021; Nasopoulou and Zabetakis, 2012; Tocher et al., 2002; Xie et al., 2022a; Xie et al., 2021; Xie et al., 2013). However, in this study, largemouth bass showed no significant differences in n-3 LC-PUFA content in the muscle and liver among diets containing SO and CO with varying levels of ALA, which is consistent with previous reports (Yadav et al., 2020; Zhang et al., 2019). Expression of *fads2 and delta4 fads* in liver were upregulated in SO groups, while *elovl5*, *elovl8a* and Δ5 FADS protein levels showed no significant differences between SO and CO. The increase in *fads6* expression at SO100 suggests its regulation may depend on the level of ALA, highlighting substrate-specific responses in LC-PUFA biosynthesis. However, unchanged Δ5 FADS and Δ6 FADS activities in the liver of SO groups and CO groups suggested that the upregulation of desaturase-related gene expression does not necessarily lead to substantial changes in enzyme activity during the conversion process (Cerone and Smith, 2022). On the other hand, the similarly high LA levels in both oils compete with ALA for the same rate-limiting enzymes,

Table 6
Fatty acid compositions (mg/g, wet basis) in the muscle and liver of largemouth bass after 10-week culturing with different lipid sources (Mean ± SEM, $n = 4$).

	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
Muscle							
C14:0	SO	0.30 ± 0.04	0.23 ± 0.04	0.27 ± 0.03	0.133	0.012	0.405
	CO	0.28 ± 0.03	0.15 ± 0.01	0.21 ± 0.03			
	Inclusion level	0.29 ± 0.02^{a}	0.19 ± 0.03^{b}				
C16:0	SO	2.48 ± 0.19	2.82 ± 0.35	2.65 ± 0.20	0.165	0.747	0.256
	CO	2.42 ± 0.17	2.23 ± 0.07	2.33 ± 0.09			
	Inclusion level	2.45 ± 0.12	2.53 ± 0.20				
C17:0	SO	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.904	0.059	0.904
	CO	0.08 ± 0.01	0.11 ± 0.00	0.10 ± 0.01			
	Inclusion level	0.09 ± 0.01	0.11 ± 0.00				
C18:0	SO	0.82 ± 0.04	1.03 ± 0.09	0.93 ± 0.06	0.122	0.173	0.054
	CO	0.85 ± 0.06	0.81 ± 0.02	0.83 ± 0.03			
	Inclusion level	0.83 ± 0.03	0.92 ± 0.06				
C16:1n−7	SO	0.41 ± 0.06	0.35 ± 0.08	0.38 ± 0.05	0.070	0.070	0.381
	CO	0.35 ± 0.03	0.20 ± 0.01	0.28 ± 0.03			
	Inclusion level	0.38 ± 0.03	0.28 ± 0.04				
C18:1n−9	SO	2.05 ± 0.28	3.11 ± 0.56	2.58 ± 0.35^{A}	0.012	0.184	0.079
	CO	1.71 ± 0.11	1.54 ± 0.07	1.63 ± 0.07^{B}			
	Inclusion level	1.88 ± 0.15	2.33 ± 0.39				
C20:1n−9	SO	0.13 ± 0.02	0.11 ± 0.02	0.12 ± 0.01^{A}	0.040	0.020	0.271
	CO	0.12 ± 0.01	0.07 ± 0.00	0.09 ± 0.01^{B}			
	Inclusion level	0.13 ± 0.01^{a}	0.09 ± 0.01^{b}				
C18:3n−3	SO	0.36 ± 0.08^{XY}	0.64 ± 0.09^{X}	0.50 ± 0.08^{A}	0.000	0.176	0.011
	CO	0.21 ± 0.03^{XY}	0.11 ± 0.01^{Y}	0.16 ± 0.02^{B}			
	Inclusion level	0.28 ± 0.05	0.38 ± 0.11				
C20:5n−3	SO	0.59 ± 0.03	0.32 ± 0.04	0.46 ± 0.05	0.764	< 0.0001	0.420
	CO	0.61 ± 0.03	0.29 ± 0.01	0.45 ± 0.06			
	Inclusion level	0.60 ± 0.02^{a}	0.31 ± 0.02^{b}				
C22:6n−3	SO	2.80 ± 0.16	2.23 ± 0.16	2.51 ± 0.15	0.226	0.000	0.465
	CO	2.73 ± 0.09	1.98 ± 0.07	2.36 ± 0.15			
	Inclusion level	2.77 ± 0.08^{a}	2.10 ± 0.09^{b}				
C18:2n−6	SO	3.07 ± 0.28	5.10 ± 0.52	3.94 ± 0.49	0.163	0.001	0.229
	CO	3.00 ± 0.28	4.18 ± 0.17	3.59 ± 0.27			
	Inclusion level	3.04 ± 0.18^{b}	4.58 ± 0.31^{a}				
C20:2n−6	SO	0.08 ± 0.00	0.15 ± 0.01	0.12 ± 0.01	0.637	< 0.0001	0.280
	CO	0.08 ± 0.01	0.17 ± 0.01	0.13 ± 0.02			
	Inclusion level	0.08 ± 0.00^{b}	0.16 ± 0.01^{a}				
C20:4n−6	SO	0.16 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	> 0.9999	< 0.0001	0.479
	CO	0.16 ± 0.00	0.11 ± 0.01	0.14 ± 0.01			
	Inclusion level	0.16 ± 0.00^{a}	0.12 ± 0.00^{b}				
ΣSFA	SO	3.69 ± 0.26	4.19 ± 0.49	3.94 ± 0.27	0.198	0.791	0.145
	CO	3.63 ± 0.24	3.30 ± 0.09	3.46 ± 0.13			
	Inclusion level	3.66 ± 0.16	3.74 ± 0.29				
ΣMUFA	SO	2.58 ± 0.36^{XY}	3.57 ± 0.65^{X}	3.08 ± 0.39	0.101	0.426	0.015
	CO	2.18 ± 0.15^{XY}	1.82 ± 0.08^{Y}	1.99 ± 0.10			
	Inclusion level	2.38 ± 0.19	2.69 ± 0.45				
Σn−3PUFA	SO	3.75 ± 0.25^{X}	3.19 ± 0.29^{XY}	3.47 ± 0.21	0.154	0.001	0.026
	CO	3.55 ± 0.10^{X}	2.38 ± 0.08^{Y}	2.96 ± 0.23			
	Inclusion level	3.65 ± 0.13^{a}	2.79 ± 0.21^{b}				
Σn−6PUFA	SO	3.32 ± 0.29	5.35 ± 0.53	4.19 ± 0.50	0.185	0.001	0.248
	CO	3.25 ± 0.29	4.46 ± 0.18	3.86 ± 0.28			
	Inclusion level	3.28 ± 0.19^{b}	4.84 ± 0.31^{a}				
n−3/n−6PUFA	SO	1.14 ± 0.04	0.56 ± 0.02	0.89 ± 0.12	0.607	< 0.0001	0.909
	CO	1.11 ± 0.07	0.53 ± 0.00	0.83 ± 0.12			
	Inclusion level	1.13 ± 0.04^{a}	0.54 ± 0.01^{b}				
ΣPUFA	SO	7.07 ± 0.53	8.32 ± 0.74	7.60 ± 0.50	0.112	0.227	0.259
	CO	6.79 ± 0.39	6.84 ± 0.26	6.82 ± 0.22			
	Inclusion level	6.93 ± 0.31	7.47 ± 0.46				
EPA+DHA	SO	3.39 ± 0.18	2.55 ± 0.19	2.97 ± 0.20	0.889	< 0.0001	0.380
	CO	3.34 ± 0.12	2.27 ± 0.07	2.80 ± 0.21			
	Inclusion level	3.36 ± 0.10^{a}	2.41 ± 0.11^{b}				
DHA/EPA	SO	4.75 ± 0.18	6.96 ± 0.31	5.85 ± 0.45	0.441	< 0.0001	0.286
	CO	4.52 ± 0.16	6.80 ± 0.15	5.66 ± 0.44			
	Inclusion level	4.64 ± 0.12^{b}	6.88 ± 0.17^{a}				
Liver							
C14:0	SO	0.72 ± 0.04	0.47 ± 0.01	0.60 ± 0.05	0.053	0.001	0.280
	CO	0.76 ± 0.06	0.62 ± 0.04	0.69 ± 0.05			
	Inclusion level	0.74 ± 0.04^{a}	0.55 ± 0.03^{b}				
C16:0	SO	5.42 ± 0.22	5.81 ± 0.12	5.62 ± 0.14^{B}	0.016	0.067	0.253
	CO	6.19 ± 0.62	7.72 ± 0.67	6.96 ± 0.51^{A}			
	Inclusion level	5.81 ± 0.34	6.77 ± 0.48				
C17:0	SO	0.20 ± 0.01	0.18 ± 0.00	0.19 ± 0.01	0.762	0.008	0.278
	CO	0.22 ± 0.02	0.17 ± 0.01	0.19 ± 0.01			

(*continued on next page*)

Table 6 (*continued*)

Muscle	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
	Inclusion level	0.21 ± 0.01^{a}	0.17 ± 0.00^{b}				
C18:0	SO	3.01 ± 0.02^{Y}	3.75 ± 0.18^{X}	3.38 ± 0.16	0.137	0.009	0.031
	CO	3.55 ± 0.17^{XY}	3.64 ± 0.11^{XY}	3.59 ± 0.09			
	Inclusion level	3.28 ± 0.13^{b}	3.69 ± 0.10^{a}				
C16:1n−7	SO	0.94 ± 0.06	0.66 ± 0.01	0.8 ± 0.06	0.633	0.006	0.216
	CO	0.89 ± 0.09	0.77 ± 0.06	0.83 ± 0.05			
	Inclusion level	0.92 ± 0.05^{a}	0.71 ± 0.04^{b}				
C18:1n−9	SO	4.56 ± 0.34	6.94 ± 0.46	5.75 ± 0.52^{A}	0.002	0.001	0.126
	CO	3.73 ± 0.34	4.88 ± 0.35	4.30 ± 0.31^{B}			
	Inclusion level	4.14 ± 0.27^{b}	5.91 ± 0.47^{a}				
C20:1n−9	SO	0.33 ± 0.02	0.31 ± 0.01	0.32 ± 0.01^{A}	0.003	0.098	0.369
	CO	0.28 ± 0.02	0.23 ± 0.01	0.25 ± 0.01^{B}			
	Inclusion level	0.30 ± 0.02	0.27 ± 0.02				
C18:3n−3	SO	0.78 ± 0.05^{Y}	1.30 ± 0.04^{X}	1.04 ± 0.10^{A}	< 0.0001	< 0.0001	0.000
	CO	0.37 ± 0.05^{Z}	0.39 ± 0.02^{Z}	0.38 ± 0.03^{B}			
	Inclusion level	0.58 ± 0.09^{b}	0.84 ± 0.17^{a}				
C20:5n−3	SO	1.73 ± 0.09	0.95 ± 0.04	1.35 ± 0.15	0.063	< 0.0001	0.693
	CO	1.98 ± 0.12	1.12 ± 0.12	1.49 ± 0.19			
	Inclusion level	1.84 ± 0.08^{a}	1.04 ± 0.07^{b}				
C22:6n−3	SO	9.23 ± 0.45	7.28 ± 0.13	8.26 ± 0.43	0.488	0.008	0.225
	CO	8.98 ± 0.22	8.15 ± 0.63	8.51 ± 0.39			
	Inclusion level	9.12 ± 0.26^{a}	7.72 ± 0.34^{b}				
C18:2n−6	SO	6.21 ± 0.34	13.14 ± 0.33	9.68 ± 1.33^{B}	0.041	< 0.0001	0.278
	CO	7.13 ± 0.61	15.90 ± 1.41	11.52 ± 1.80^{A}			
	Inclusion level	6.67 ± 0.37^{b}	14.52 ± 0.85^{a}				
C20:2n−6	SO	0.19 ± 0.01^{Y}	0.43 ± 0.01^{XY}	0.31 ± 0.05^{B}	0.001	< 0.0001	0.002
	CO	0.21 ± 0.02^{Y}	0.60 ± 0.04^{X}	0.40 ± 0.08^{A}			
	Inclusion level	0.20 ± 0.01^{b}	0.52 ± 0.04^{a}				
C20:4n−6	SO	0.70 ± 0.02	0.46 ± 0.01	0.58 ± 0.05	0.106	< 0.0001	0.924
	CO	0.66 ± 0.04	0.42 ± 0.02	0.56 ± 0.05			
	Inclusion level	0.68 ± 0.02^{a}	0.44 ± 0.01^{b}				
ΣSFA	SO	9.36 ± 0.26	10.21 ± 0.26	9.79 ± 0.23^{B}	0.013	0.067	0.627
	CO	10.72 ± 0.71	12.14 ± 0.80	11.43 ± 0.56^{A}			
	Inclusion level	10.04 ± 0.43	11.18 ± 0.53				
ΣMUFA	SO	5.82 ± 0.42	7.91 ± 0.48	6.87 ± 0.49^{A}	0.006	0.005	0.233
	CO	4.90 ± 0.45	5.87 ± 0.42	5.39 ± 0.34^{B}			
	Inclusion level	5.36 ± 0.34^{b}	6.89 ± 0.48^{a}				
Σn−3PUFA	SO	11.75 ± 0.55	9.53 ± 0.18	10.64 ± 0.50	0.760	0.005	0.591
	CO	11.28 ± 0.35	9.66 ± 0.75	10.36 ± 0.54			
	Inclusion level	11.55 ± 0.35^{a}	9.60 ± 0.36^{b}				
Σn−6PUFA	SO	7.10 ± 0.35	14.04 ± 0.33	10.57 ± 1.33^{B}	0.015	< 0.0001	0.109
	CO	8.00 ± 0.66	17.71 ± 1.49	12.16 ± 2.10^{A}			
	Inclusion level	7.55 ± 0.39^{b}	15.61 ± 1.00^{a}				
n−3/n−6PUFA	SO	1.66 ± 0.09	0.68 ± 0.01	1.17 ± 0.19^{A}	0.044	< 0.0001	0.959
	CO	1.53 ± 0.03	0.56 ± 0.03	1.05 ± 0.22^{B}			
	Inclusion level	1.61 ± 0.05^{a}	0.63 ± 0.03^{b}				
ΣPUFA	SO	18.85 ± 0.77	23.57 ± 0.48	21.21 ± 0.99	0.164	0.000	0.125
	CO	18.64 ± 0.50	27.56 ± 2.29	23.1 ± 2.33			
	Inclusion level	18.76 ± 0.47^{b}	25.28 ± 1.31^{a}				
EPA+DHA	SO	10.97 ± 0.53	8.23 ± 0.17	9.60 ± 0.58	0.349	0.001	0.343
	CO	10.96 ± 0.33	9.27 ± 0.74	9.99 ± 0.54			
	Inclusion level	10.96 ± 0.32^{a}	8.75 ± 0.4^{b}				
DHA/EPA	SO	5.32 ± 0.05	7.64 ± 0.18	6.49 ± 0.45^{A}	0.021	< 0.0001	0.263
	CO	4.57 ± 0.16	7.35 ± 0.29	6.16 ± 0.59^{B}			
	Inclusion level	5.00 ± 0.17^{b}	7.5 ± 0.16^{a}				

A two-way ANOVA was conducted to evaluate the effects of oil sources (SO and CO) and inclusion levels (50 % and 100 %) on the measured parameters. Differences in main effects were compared: significant differences ($P < 0.05$) between VO sources are indicated by different capital letters (A, B); significant differences ($P < 0.05$) between inclusion levels are indicated by different lowercase superscript letters (a, b).
Simple main effects (one-way ANOVA) were further performed to further examine the differences among the four treatment combinations (SO50, CO50, SO100, and CO100), in cases where a significant interaction ($P < 0.05$) between the two factors was detected: significant differences ($P < 0.05$) among these groups are indicated by different capital letters (X, Y).

thereby constraining the conversion efficiency to EPA and DHA (Ruyter et al., 2000; Vagner et al., 2009). Overall, these results suggested that largemouth bass have limited capacity for LC-PUFA biosynthesis from dietary ALA (Yadav et al., 2020; Zhang et al., 2019).

Besides, this study shows that VO source and inclusion level significantly alters the fatty acid composition in fish muscle and liver (Turchini et al., 2010; Yadav et al., 2020). Despite the expression of desaturase-related genes and Δ5 FADS protein being up-regulated in 100 % VO groups, the accumulation of n-3 LC-PUFA content decreased in the tissues. This suggests that the dietary availability of these fatty acids plays a more decisive role than endogenous biosynthesis (Tocher, 2003; Turchini et al., 2009; Yadav et al., 2020). Previous study has shown that freshwater fish fed FO-based diets preferentially accumulate n-3 LC-PUFA in their tissues, which is consistent with the higher whole-body crude lipid content observed in the 50 % VO groups (Henderson, 1996). In addition to the reduction in n-3 LC-PUFA, a 100 % VO inclusion led to increased n-6 PUFA levels in both muscle and liver tissues. Compared with CO, SO more effectively preserved

Table 7
Fillet quality of largemouth bass after 10-week culturing with different lipid sources (Mean ± SEM, $n = 8$).

	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
WHC							
Drip loss (%)	SO	9.78 ± 0.86	9.03 ± 0.45	9.34 ± 0.42	0.119	0.028	0.209
	CO	11.87 ± 0.81	9.26 ± 0.91	10.66 ± 0.69			
	Inclusion level	10.75 ± 0.62^{a}	9.11 ± 0.42^{b}				
Cooking loss (%)	SO	9.83 ± 1.43^{XY}	8.46 ± 0.79^{Y}	9.00 ± 0.71	0.849	0.001	0.030
	CO	12.38 ± 0.65^{X}	6.30 ± 1.18^{Y}	10.04 ± 1.03			
	Inclusion level	11.10 ± 0.79^{a}	7.82 ± 0.68^{b}				
pH							
pH (0 h)	SO	6.86 ± 0.07^{Y}	7.14 ± 0.06^{X}	7.03 ± 0.05	0.967	0.352	0.009
	CO	7.07 ± 0.08^{XY}	6.93 ± 0.11^{XY}	7.01 ± 0.06			
	Inclusion level	6.96 ± 0.06	7.07 ± 0.06				
pH (24 h)	SO	6.32 ± 0.03	6.51 ± 0.04	6.43 ± 0.03	0.307	0.001	0.335
	CO	6.40 ± 0.04	6.51 ± 0.07	6.45 ± 0.04			
	Inclusion level	6.36 ± 0.03^{b}	6.51 ± 0.03^{a}				
pH (48 h)	SO	6.39 ± 0.03	6.56 ± 0.02	6.49 ± 0.02	0.091	0.000	0.288
	CO	6.48 ± 0.03	6.58 ± 0.06	6.52 ± 0.03			
	Inclusion level	6.43 ± 0.02^{b}	6.57 ± 0.02^{a}				
pH (72 h)	SO	6.37 ± 0.03	6.54 ± 0.03	6.47 ± 0.03	0.164	0.004	0.110
	CO	6.47 ± 0.02	6.52 ± 0.06	6.50 ± 0.03			
	Inclusion level	6.42 ± 0.02^{b}	6.53 ± 0.03^{a}				
Texture							
Hardness (N)	SO	5.11 ± 0.48	5.79 ± 0.32	5.51 ± 0.27	0.241	0.164	0.877
	CO	5.69 ± 0.45	6.23 ± 0.57	5.92 ± 0.34			
	Inclusion level	5.40 ± 0.32	5.93 ± 0.27				
Adhesiveness (mJ)	SO	0.08 ± 0.01	0.06 ± 0.00	0.10 ± 0.02	0.846	0.058	0.314
	CO	0.08 ± 0.01	0.07 ± 0.00	0.07 ± 0.01			
	Inclusion level	0.08 ± 0.01	0.09 ± 0.02				
Cohesiveness (Ratio)	SO	0.27 ± 0.01	0.28 ± 0.01	0.27 ± 0.00^{B}	0.001	0.940	0.413
	CO	0.30 ± 0.01	0.30 ± 0.00	0.30 ± 0.01 ^{A}			
	Inclusion level	0.29 ± 0.01	0.28 ± 0.01				
Springiness (mm)	SO	0.76 ± 0.04	0.74 ± 0.03	0.75 ± 0.02	0.224	0.958	0.636
	CO	0.78 ± 0.04	0.81 ± 0.05	0.79 ± 0.03			
	Inclusion level	0.77 ± 0.03	0.76 ± 0.03				
Gumminess (N)	SO	1.38 ± 0.15	1.60 ± 0.10	1.51 ± 0.08^{B}	0.040	0.250	0.709
	CO	1.74 ± 0.18	1.85 ± 0.15	1.79 ± 0.12 ^{A}			
	Inclusion level	1.56 ± 0.12	1.68 ± 0.08				
Chewiness (mJ)	SO	1.08 ± 0.16	1.19 ± 0.10	1.15 ± 0.08^{B}	0.039	0.428	0.958
	CO	1.40 ± 0.20	1.54 ± 0.20	1.46 ± 0.13 ^{A}			
	Inclusion level	1.24 ± 0.13	1.31 ± 0.10				

A two-way ANOVA was conducted to evaluate the effects of oil sources (SO and CO) and inclusion levels (50 % and 100 %) on the measured parameters. Differences in main effects were compared: significant differences ($P < 0.05$) between VO sources are indicated by different capital letters (A, B); significant differences ($P < 0.05$) between inclusion levels are indicated by different lowercase superscript letters (a, b).
Simple main effects (one-way ANOVA) were further performed to further examine the differences among the four treatment combinations (SO50, CO50, SO100, and CO100), in cases where a significant interaction ($P < 0.05$) between the two factors was detected: significant differences ($P < 0.05$) among these groups are indicated by different capital letters (X, Y).

beneficial fatty acids, such as ALA and MUFA in muscle, and significantly enhanced hepatic ALA levels. In contrast, CO was associated with elevated levels of LA, SFA, and n-6 PUFAs in the liver. These findings underscore the importance of carefully selecting and regulating the VO source and inclusion level to maintain optimal tissue fatty acid profiles, preventing n-3 HUFA deficiency and excessive accumulation of n-6 PUFAs in farmed fish (Emre et al., 2016; Güler and Yildiz, 2011; Turchini et al., 2009).

Findings have indicated that certain fish species are unable to biosynthesize sufficient amount of EPA and DHA from dietary ALA, necessitating the dietary supplementation of EPA and DHA in fish diet to support rapid growth and physiological functions. Cold-water carnivorous fish like rainbow trout require 0.7-1.0 % ALA and 0.4-0.5 % n-3 LC-PUFA, while channel catfish need 1.0 -2.0 % ALA and 0.5 -0.75 % n-3 LC-PUFA to support optimal growth (Henderson, 1996). Similarly, although grass carp can biosynthesize EPA and DHA, a diet containing 0.52 % n-3 LC-PUFA can support better growth and lipid metabolism (Ji et al., 2011). Largemouth bass show optimal growth at 0.76 % n-3 LC-PUFA with 0.15 % ALA (An et al., 2023), or at 1 % n-3 LC-PUFA with 3.2 % ALA (Yadav et al., 2020). In this study, 30.2 % fish meal likely provided sufficient n-3 LC-PUFA to meet the needs of largemouth bass, which may explain the lack of growth differences between the 50 % VO (2.0 % n-3 LC-PUFA) and 100 % VO (0.8 % n-3 LC-PUFA) groups. Nonetheless, further research is needed to determine the optimal combination levels of ALA and n-3 LC-PUFAs in the diet of this species.

Research has indicated that an imbalanced n-3/n-6 PUFA ratio in fish diets is a key contributor to the negative physiological impacts of a VO-based diet on fish (Hamad et al., 2024). This imbalance can potentially lead to lipid deposition and disrupt liver lipid metabolism (Bandarra et al., 2011; Li et al., 2015; Liu et al., 2022b; Menoyo et al., 2004). The VAI and VSI served as indicators to evaluate the degree of lipid accumulation in fish. In this study, changes in the VAI and VSI generally aligned with growth performance trends, suggesting that these variations were primarily growth-related. The HSI is a key metric for assessing the impact of diet on liver health. An elevated HSI (normally 1–2 % for osteichthyes), may signal metabolic imbalances (Dernekbaşı, 2012) and a risk of fatty liver. In this study, the HSI values varied between 1.36 % and 1.61 %, which is within the normal range, indicating no significant liver abnormalities.

Additionally, the impact of VO on lipid deposition in largemouth bass is also reflected in hematological indices, including TG, TC, LDL-C etc. (Guo et al., 2019; Li et al., 2014; Ye et al., 2011), which serve as indicators of lipid levels in metabolic circulation (Gong et al., 2024; Yun et al., 2012; Zhang et al., 2022). In the present study, fish fed SO

Table 8
Biochemical indicators of plasma and liver of largemouth bass after 10-week culturing with different lipid sources (Mean ± SEM, $n = 12$).

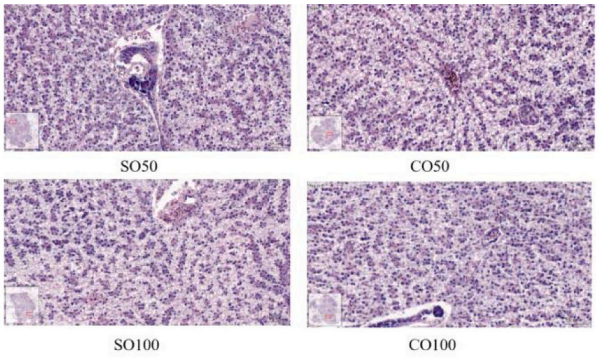
	Oil source	Inclusion level, %		Oil source	*P* Values		
		50	100		Oil source	Inclusion level	Interaction
Plasma							
GLU (mmol/L)	SO	6.08 ± 0.37	6.49 ± 0.33	6.33 ± 0.24	1.116	5.347	1.088
	CO	6.08 ± 0.64	7.35 ± 0.97	6.65 ± 0.56			
	Inclusion level	6.08 ± 0.36	6.90 ± 0.45				
TG (mmol/L)	SO	6.60 ± 0.72^{XY}	9.12 ± 0.78^{X}	7.98 ± 0.59 ^{A}	0.008	0.810	0.000
	CO	5.15 ± 0.58^{Y}	4.48 ± 0.76^{Y}	6.05 ± 0.53^{B}			
	Inclusion level	7.00 ± 0.41	7.03 ± 0.76				
TC (mmol/L)	SO	4.33 ± 0.26	4.30 ± 0.26	4.32 ± 0.18 ^{A}	0.034	0.857	0.782
	CO	3.67 ± 0.27	3.80 ± 0.26	3.73 ± 0.19^{B}			
	Inclusion level	3.99 ± 0.20	4.09 ± 0.19				
LDL-C (mmol/L)	SO	1.18 ± 0.13	1.38 ± 0.13	1.28 ± 0.09 ^{A}	0.008	0.798	0.119
	CO	1.05 ± 0.06	0.91 ± 0.07	0.99 ± 0.05^{B}			
	Inclusion level	1.11 ± 0.07	1.17 ± 0.09				
HDL-C (mmol/L)	SO	3.43 ± 0.30	2.69 ± 0.21	3.05 ± 0.19	0.773	0.751	0.014
	CO	2.70 ± 0.26	3.28 ± 0.25	2.95 ± 0.19			
	Inclusion level	3.05 ± 0.21	2.94 ± 0.17				
HDL-C/LDL-C	SO	3.46 ± 0.57^{XY}	2.22 ± 0.30^{Y}	2.82 ± 0.33	0.345	0.842	0.007
LDL-C	CO	2.70 ± 0.33^{XY}	3.77 ± 0.39^{X}	3.16 ± 0.27			
	Inclusion level	3.06 ± 0.33	2.89 ± 0.29				
AST (U/L)	SO	32.94 ± 9.61	38.69 ± 6.01	36.23 ± 5.25	0.541	0.219	0.045
	CO	43.10 ± 7.27	15.38 ± 1.64	31.51 ± 5.00			
	Inclusion level	38.29 ± 5.90	30.16 ± 4.35				
ALT (U/L)	SO	20.28 ± 7.76	19.96 ± 4.54	20.10 ± 4.10	0.420	0.076	0.085
	CO	25.74 ± 7.26	4.57 ± 0.46	16.47 ± 4.84			
	Inclusion level	23.91 ± 5.34	13.37 ± 3.07				
AKP (U/L)	SO	48.61 ± 10.62^{X}	51.26 ± 7.76^{X}	49.94 ± 6.39	0.178	0.292	0.014
	CO	37.87 ± 9.60^{XY}	16.52 ± 1.75^{Y}	26.37 ± 5.29			
	Inclusion level	44.31 ± 7.34	36.06 ± 6.20				
TBA (μmol/L)	SO	11.39 ± 0.58^{Y}	30.52 ± 0.23^{X}	20.95 ± 2.49^{B}	< 0.0001	< 0.0001	< 0.0001
	CO	19.82 ± 1.36^{Y}	31.62 ± 0.56^{X}	25.72 ± 1.68 ^{A}			
	Inclusion level	15.60 ± 1.30^{b}	31.07 ± 0.33^{a}				
Liver							
TP (gprot/L)	SO	56.57 ± 3.73	76.10 ± 5.01	61.26 ± 3.21	0.394	0.605	0.103
	CO	67.41 ± 4.71	62.50 ± 3.51	64.95 ± 2.90			
	Inclusion level	61.99 ± 3.15	64.22 ± 3.01				
TG (mmol/gprot)	SO	0.17 ± 0.02^{Y}	0.28 ± 0.02^{X}	0.22 ± 0.02 ^{A}	< 0.0001	0.001	0.014
	CO	0.13 ± 0.01^{Y}	0.15 ± 0.02^{Y}	0.14 ± 0.01^{B}			
	Inclusion level	0.15 ± 0.01^{b}	0.21 ± 0.02^{a}				
TC (mmol/gprot)	SO	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.314	0.303	0.247
	CO	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.00			
	Inclusion level	0.06 ± 0.00	0.05 ± 0.00				
LDL-C	SO	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	> 0.999	0.123	0.540
(mmol/gprot)	CO	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00			
	Inclusion level	0.02 ± 0.00	0.02 ± 0.00				

A two-way ANOVA was conducted to evaluate the effects of oil sources (SO and CO) and inclusion levels (50 % and 100 %) on the measured parameters. Differences in main effects were compared: significant differences ($P < 0.05$) between VO sources are indicated by different capital letters (A, B); significant differences ($P < 0.05$) between inclusion levels are indicated by different lowercase superscript letters (a, b).
Simple main effects (one-way ANOVA) were further performed to further examine the differences among the four treatment combinations (SO50, CO50, SO100, and CO100), in cases where a significant interaction ($P < 0.05$) between the two factors was detected: significant differences ($P < 0.05$) among these groups are indicated by different capital letters (X, Y).

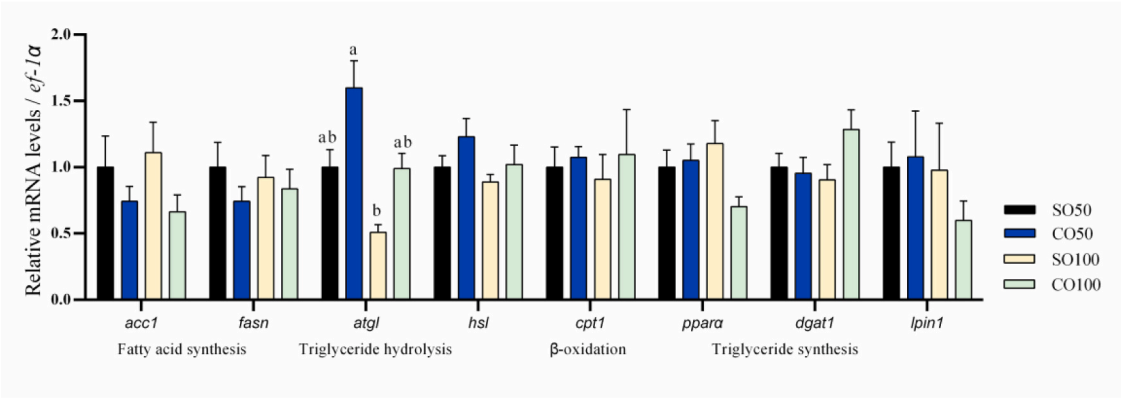
exhibited elevated plasma TC and LDL-C levels. Besides, a significant interaction effect was observed for plasma TG, HDL-C/LDL-C, AKP, and hepatic TG, suggesting that the effects of SO become pronounced only at higher inclusion levels. When considered alongside the HSI and VSI data from the SO100 group, the findings indicate that excessive SO intake may contribute to visceral lipid accumulation and disrupt the balance of lipid metabolism (Güler and Yildiz, 2011; Piedecausa et al., 2007). However, the hepatic histopathological examination revealed no significant effects in fish fed various experimental diets, suggesting that the increased liver lipid accumulation did not lead to hepatocyte vacuolization.

Several published studies have demonstrated that gene expression data can effectively reflect changes in lipid metabolism in fish (Gong et al., 2024; Tang et al., 2025; Wang et al., 2022a). We further investigated parameters of hepatic lipid metabolism and gene expression associated with several pathways, including the absorption of FA into tissues, lipid synthesis, and their subsequent breakdown (Ipsen et al., 2018; Musso et al., 2009; Saponaro et al., 2015). ATGL, primarily expressed in the liver of fish, is crucial for TG hydrolysis, and its inhibition is linked to excessive lipid accumulation (Han et al., 2021). The upregulated expression of *atgl* in the liver of fish fed CO diets or 100 % VO diets suggests a regulatory response to enhance the hydrolysis of TG, thereby influencing the cellular oxidative metabolism and energy storage (Roque-Jiménez et al., 2021). As *atgl* is functionally linked to lipid mobilization and energy balance, its activation may interact with key signaling pathways involved in energy sensing (Shi et al., 2018). AMPK is an essential protein kinase that regulates cellular energy metabolism. When an organism's energy level are insufficient, it is activated via phosphorylation (p-AMPK), which promotes lipid degradation in fish to supply energy (Nie et al., 2020; Ran et al., 2021). Furthermore, cAMP, a second messenger, regulates mitochondrial biogenesis and participates in energy metabolism (Cheng et al., 2012). High-VO diets, rich in PUFA, have been associated with increased energy metabolism (Glencross et al., 2016; Henderson, 1996; Jiang et al., 2013). In this study, the 100 % VO groups exhibited a high basal metabolic rate, characterized by upregulated cAMP and p-AMPK levels, especially with significantly

A

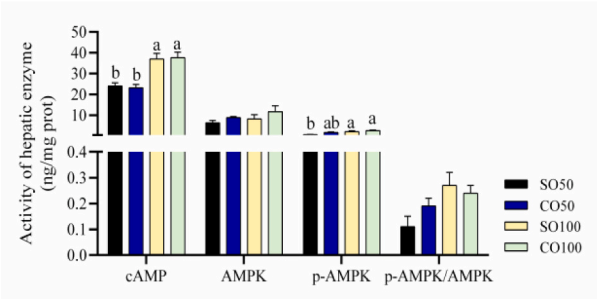


B



P-value	*acc1*	*fasn*	*atgl*	*hsl*	*cpt1*	*pparα*	*dgat1*	*lpin1*
Oil source	0.063	0.278	0.001	0.120	0.640	0.114	0.184	0.581
Inclusion level	0.933	0.957	0.001	0.165	0.704	0.523	0.341	0.364
Interaction	0.605	0.587	0.675	0.663	0.926	0.054	0.093	0.407

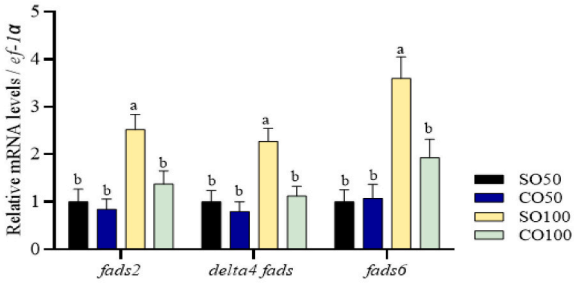
C



P-value	cAMP	AMPK	p-AMPK	p-AMPK/AMPK
Oil source	0.943	0.103	0.023	0.650
Inclusion level	<0.0001	0.185	0.002	0.030
Interaction	0.728	0.776	0.331	0.174

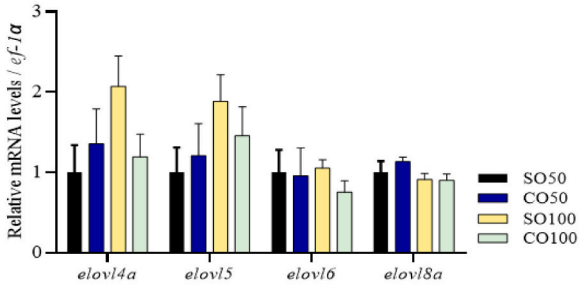
Fig. 1. Impact of different lipid sources on lipid metabolism in the liver of largemouth bass. (A) hepatic histopathological examination of largemouth bass. HE staining for histology examination, Statistical results of liver phenotypes (n = 12); (B) mRNA levels of hepatic fatty acid synthesis (*acc1 and fasn*), TG hydrolysis (*atgl* and *hsl*), β-oxidation (*cpt1* and *pparα*) and triglyceride synthesis (*dgat1* and *lpin1*) related genes (Mean±SEM, n = 8); (C) Activities of hepatic cAMP, AMPK, p-AMPK, p-AMPK/AMPK (Mean±SEM, n = 4).

A



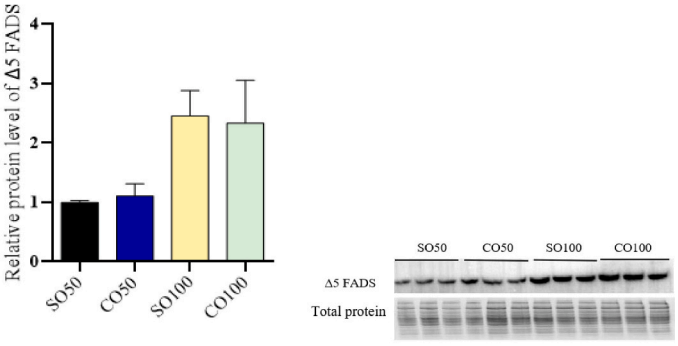
P-value	*fads2*	*delta4 fads*	*fads6*
Oil source	0.025	0.007	0.035
Inclusion level	0.001	0.002	<0.0001
Interaction	0.083	0.051	0.023

B



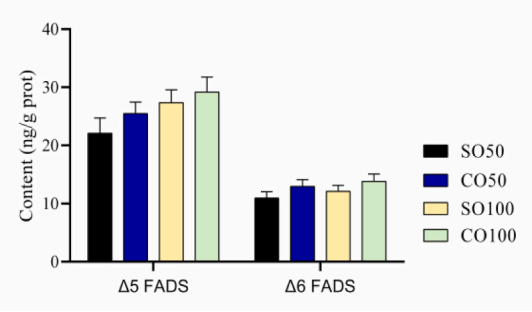
P-value	*elovl4a*	*elovl5*	*elovl6*	*elovl8a*
Oil source	0.478	0.761	0.483	0.498
Inclusion level	0.221	0.120	0.766	0.094
Interaction	0.100	0.375	0.601	0.439

C



P-value	Δ5 FADS
Oil source	0.986
Inclusion level	0.014
Interaction	0.797

D



P-value	Δ5 FADS	Δ6 FADS
Oil source	0.2743	0.1184
Inclusion level	0.0663	0.3859
Interaction	0.7443	0.9007

Fig. 2. Impact of different lipid sources on LC-PUFA biosynthesis in the liver of largemouth bass. (A) Relative mRNA levels of fatty acid desaturases (n = 8); (B) Relative mRNA levels of fatty acid elongases (Mean±SEM, n = 8); (C) Western blot of Δ5 FADS (convert C20:4n-3 to C20:5n-3) (Mean±SEM, n = 3); (D) Activities of hepatic Δ5 FADS and Δ6 FADS (Mean±SEM, n = 8).

higher p-AMPK observed in the CO100 group. Our findings suggest that largemouth bass utilize lipid catabolism as a source of energy for growth; however, excessive lipid catabolism, as noted in this study, can resulted in emaciation, which may contribute to the impaired growth observed in the fish of CO100 group (Liu et al., 2018; Tocher, 2003).

Evaluating the quality of fish fillets is essential for assessing oil sources in fish diets. Consumers typically prefer fish with firm, elastic, and juicy flesh (Yuan et al., 2022b). A gradual decline in pH is generally associated with fillet quality deterioration (Liu and Yuan, 2005). As time passed, the pH value of the fillets gradually decreased and showed a gentle change at 72 h post-sampling. Previous studies have reported that neither the type of VO source nor the content of VO significantly affects fillet pH values (Álvarez et al., 2020; Duan et al., 2014; Izquierdo et al., 2005). However, in this study, higher dietary VO inclusion was associated with increased muscle pH at 24 h, 48 h, and 72 h, and pH at 0 h was also elevated with higher SO inclusion. These findings align with observations in Atlantic salmon (*Salmo salar*), where high VO inclusion in the diet has been shown to reduce lipid oxidation (Tocher et al., 2003), potentially lowering the production of acidic metabolites such as lactic acid and contributing to a higher fillet pH.

WHC refers to the ability of muscle to retain water when exposed to external forces, which is mainly influenced by protein degradation or denaturation. In this study, the drip loss of muscle was found to be relatively high in the 100 % VO groups, whereas cooking loss significantly decreased with higher inclusion of CO. The lower crude lipid content in the whole body of 100 % VO groups may contribute to a more intact muscle structure, thereby enhancing water retention (Hocquette et al., 2010; Roth et al., 2021). In addition, a higher pH may enhance the WHC of muscle, thereby reducing water loss during processing and preservation (Andrés-Bello et al., 2013). This may also be one of the factors contributing to the higher WHC observed in the 100 % VO group.

The texture of muscle not only affects consumers' sensory experiences but also reflects the physical and chemical properties of muscle products. Therefore, texture serves as a critical criterion for assessing muscle quality, and it is influenced by the oil type and their content in the fish's diet (Szczesniak, 2002; Yang et al., 2020). In this study, the CO groups demonstrated the highest cohesiveness, gumminess, and chewiness in the fresh fillet. Although PUFA is easy to oxidize, CO contains natural antioxidants such as vitamin E, which can mitigate lipid oxidation damage to muscle proteins and thereby help maintain muscle structure stability (Riaz et al., 2023; Zia et al., 2021). Research has shown that addition of VO to the diets of grass carp and triploid rainbow trout (*Oncorhynchus mykiss*) can enhance the cohesiveness and reduce the gummy hardness of the muscle (Song et al., 2024; Wang et al., 2022b). The findings suggested that fish fed these VO in their diet may exhibit a firmer, more elastic texture upon chewing.

5. Conclusion

Under the present experimental conditions, 0.8 % n-3 LC-PUFA provided by 30.2 % fishmeal met the minimum dietary requirement, and the VO replacement level (50 % vs. 100 %) was not the primary factor limiting growth in largemouth bass. However, 50 % VO diets led to higher tissue n-3 LC-PUFA levels. The SO groups showed higher ALA content in both diets and tissues compared to the CO groups and supported better growth, whereas growth was particularly suppressed in the CO100 group. The lack of ALA in CO, combined with limited n-3 LC-PUFA conversion capacity and the elevated lipid catabolism contributed to the inferior growth performance in largemouth bass. The results showed that even if the n-3 LC-PUFA supply was sufficient, the lack of ALA in the diet of largemouth bass could still lead to lipid metabolism disorders and thus limit fish growth. Meanwhile, both 100 % VO and CO diets improved fillet quality from different aspects. Overall, 50 % CO inclusion proved to be a practical option. For 100 % CO diets, additional ALA supplementation is necessary to support fatty acid conversion and maintain optimal growth. In the future, a blended lipid strategy combining SO and CO may offer a more nutritionally balanced approach for largemouth bass aquafeeds.

Author Statement

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We have carefully considered the comments from all reviewers, and each suggested revision and comment brought forward by the reviewers was accurately incorporated and considered. For clarity and ease of review, we have also indicated the corresponding line numbers in the revised manuscript. The manuscript is submitted with tracked changes enabled to facilitate your review.

Looking forward hearing from you soon.

CRediT authorship contribution statement

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

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Declaration of Competing Interest

The authors state that they have no financial conflicts of interest or personal relationships that could potentially exert influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102938.

Data availability

Data will be made available on request.

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