Animal Nutrition 24 (2026) 399–412

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

KeAi CHINESE ROOTS GLOBAL IMPACT

Original Research Article

Lipopeptides modulate lipid metabolism and immune performance in largemouth bass (*Micropterus salmoides*) across dietary lipid levels

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A R T I C L E I N F O

Article history:
Received 28 July 2025
Received in revised form
12 October 2025
Accepted 15 October 2025
Available online 8 December 2025

Keywords:
Micropterus salmoides
Lipopeptide
Lipid saving
Immunity
Energy metabolism

A B S T R A C T

Lipopeptides are versatile surface-active natural products that act as emulsifiers, enhance lipid utilization, and exhibit broad-spectrum antimicrobial properties. Whether lipopeptides have the potential to improve energy efficiency and fight bacterial infection in largemouth bass (*Micropterus salmoides*) has not been reported. In this study, a total of 300 juvenile largemouth bass (initial body weight 14.67 ± 0.01 g) were randomly divided into four groups with five replicates per group ($n = 5$), each replicate consisting of 30 fish. The fish were fed four experimental diets for 10 weeks: 10.50% lipid (low-lipid [LL]), 10.50% lipid + 0.05% lipopeptides (low-lipid with lipopeptides [LLLP]), 13.00% lipid (high-lipid [HL]), and 13.00% lipid + 0.05% lipopeptides (high-lipid with lipopeptides [HLLP]). Two-way ANOVA showed neither lipid levels nor lipopeptides had a significant effect on survival rate and weight gain ($P > 0.05$). However, the largemouth bass fed low-lipid diets exhibited significantly higher feed intake to meet energy demands, resulting in an increased feed conversion ratio ($P = 0.004$), which was then decreased after lipopeptide supplementation ($P = 0.061$). High-lipid levels led to a significant increase in plasma total cholesterol (TC) and liver lipid content ($P < 0.001$), and the addition of lipopeptides significantly improved the above conditions ($P < 0.001$). Mechanistically, lipopeptides upregulated lipid catabolism (hormonesensitive triglyceride lipase [*Hsl*], $P < 0.001$) and fatty acid oxidation genes (carnitine palmitoyltransferase 1 α [*Cpt1α*], $P = 0.004$), while suppressing lipogenesis gene (fatty acid synthase [*Fasn*], $P = 0.045$). Pathway analysis revealed that lipopeptides coordinately regulated energy metabolism by involving AMP-activated protein kinase (AMPK) energy sensing and cyclic-AMP (cAMP) response element-binding protein (Creb) signaling cascades. In an *Edwardsiella tarda* challenge, lipopeptide supplementation significantly reduced intestinal pro-inflammatory factors (interleukin [IL]-8 , $P = 0.007$ and IL-1β, $P = 0.006$) and enhanced immune function, as evidenced by elevated levels of anti-inflammatory transforming growth factor-β1 (TGF-β1; $P = 0.011$) and immunoglobulin M (IgM; $P = 0.003$). This study provides a comprehensive exploration of lipopeptide-mediated regulatory mechanisms under different dietary lipid levels, highlighting their potential for optimizing feed formulations and improving lipid metabolic health in aquaculture species.
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1. Introduction

Lipopeptides are natural surface-active compounds from various sources. The major cyclic lipopeptide families from *Bacillus* spp.—surfactins, iturins, and fengycins—are characterized by a β-hydroxy fatty acid chain conjugated to a cyclic heptapeptide (Marc and Philippe, 2008; Théatre et al., 2021). This conserved structural motif confers pronounced interfacial activity and

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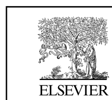
Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine



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https://doi.org/10.1016/j.aninu.2025.10.003
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underlies a wide spectrum of biological functions, including antimicrobial, antibiofilm, anti-inflammatory, and immunomodulatory activities (Dini et al., 2024; Théatre et al., 2021). The most notable of these is its emulsifying properties. For example, a class of cyclic lipopeptide compounds produced from the secondary metabolism of *Bacillus subtilis* is composed of non-polar hydrophobic fatty acid chains and polar hydrophilic cyclic peptides (Zhen et al., 2023). The ability to emulsify oil can improve the dispersion of lipids in feed, promote the absorption of lipid-soluble nutrients and enhance the growth performance of aquatic animals (Adhami et al., 2017). For example, dietary surfactin supplementation can increase intestinal lipase activity and regulate lipid metabolism in tilapia (*Oreochromis niloticus*) and orange-spotted grouper (*Epinephelus coioides*) (Samuel and Osayande, 2023; Zhai et al., 2015, 2016b, 2017b).

The potential of lipopeptides as feed additives goes far beyond their surface–active properties. They can also affect immune regulation and intestinal health. Lipopeptides can enhance the host's defense against common pathogens by disrupting cell membrane structure, inhibiting pathogen adhesion, and reducing the risk of infection, which demonstrates their potential to replace antibiotics in animal feed (Liu et al., 2019; Maget-Dana and Peypoux, 1994). For instance, dietary surfactin supplementation has been shown to elevate feed conversion efficiency and immune function in tilapia and marbled eel (*Anguilla marmaorata*) (Zhai et al., 2016a,b). Related work also shows that lipopeptide-containing diets can beneficially remodel the gut microbiome and enhance non-specific immunity in shrimp (Prathiviraj et al., 2021).

The largemouth bass (*Micropterus salmoides*) is one of the most economically significant freshwater aquaculture species globally, with a widespread distribution across Asia, North America and Europe. The production of largemouth bass in China has reached 938,509 tons in 2024. Largemouth bass have a high requirement for dietary crude protein (CP) and crude lipid (CL). The increase of dietary protein (from 46% to 50%) and lipid levels (from 8% to 16%) significantly improved the growth performance and feed utilization of largemouth bass (Chen et al., 2023a). Another study demonstrated that a high-lipid diet (18%) exhibited a significantly better protein efficiency ratio (PER) and lower feed conversion ratio (FCR) than a low-lipid diet (14%), however, excessive fat droplets increased with each increment of dietary lipid intake (Qin et al., 2025). As a representative species for studying metabolic liver diseases, largemouth bass are prone to liver lipid accumulation (Li et al., 2025; Ma et al., 2019; Zhang et al., 2020). Therefore, high-lipid diet helps improve growth and reduce FCR, but it also increases production costs and the risk of metabolic liver disease. Currently, the dietary CL level for largemouth bass aquaculture is mostly between 10% and 13%. Improving lipid utilization efficiency while reducing dietary lipid addition is crucial to the economic benefits of largemouth bass aquaculture. Simultaneously, aquaculture faces the threat of frequent diseases caused by high-density farming. Pathogens such as largemouth bass ranavirus (LMBV), *Edwardsiella* and *Aeromonas hydrophila* often cause large-scale deaths (Fu et al., 2024; Lin et al., 2025). Traditional prevention and control rely on antibiotics, but long-term use leads to an increase in drug-resistant strains, drug residues polluting the environment and food safety risks (Lulijwa et al., 2020). Lipopeptides, with their antibacterial, emulsifying, and immunomodulatory functions, can become a key technology for the green transformation of largemouth bass farming, but their application effect in this species has not been reported.

This study conducted a 10-week feeding trial with largemouth bass to systematically evaluate the effects of dietary lipid levels and lipopeptide supplementation on growth performance, lipid metabolism, and health parameters. This is the first attempt to incorporate lipopeptides into largemouth bass diets, and uniquely, the trial was designed under two distinct lipid backgrounds to examine their interactive effects. In addition to lipid utilization, further experiments with *Edwardsiella* challenge were conducted to investigate the anti-inflammatory and immune-enhancing properties of the lipopeptides. Collectively, these evaluations can provide comprehensive evidence for the application of lipopeptides in sustainable fish farming practices.

2. Materials and methods

2.1. Animal ethics statement

The Ethics Committee of the Institute of Feed Research at the Chinese Academy of Agriculture Sciences gave its approval to this study, ensuring full compliance with all pertinent ethical regulations (IFR-CAAS20240603). Throughout the experimental phase, every fish-rearing procedure was carried out in strict accordance with the Guidelines for the Welfare of Experimental Animals (China National Standard, 2016). These guidelines were issued jointly by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China and the Standardization Administration of China.

2.2. Experimental fish, feeding, and sampling

Four iso-nitrogenous experimental diets with 10.50% lipid (low-lipid [LL]) and 13.00% lipid (high-lipid [HL]) were prepared and 0.05% lipopeptides (Beijing Enhalor Biotechnology Co., Ltd., Beijing, China) were added to these two basal diets (termed low-lipid with lipopeptides [LLLP] and high-lipid with lipopeptides [HLLP], respectively). The lipopeptide product, also known as subtilis surfactant peptide, is a type of cyclic lipopeptide compound produced by secondary metabolism of *B. subtilis* through high-density liquid fermentation. The supplementation level of lipopeptides (0.05%) was determined based on previous studies (Zhai et al., 2015, 2016b). The lipopeptides used in this study is a mixture of four *Bacillus* surfactin peptides. Detailed information on the lipopeptides is provided in Table S1. Each diet was processed into 3-mm diameter floating pellets under the following extrusion conditions using a twin-screw extruder (EXT50A, Yangzhou Yanggong Machinery Co., Ltd., Yangzhou, Jiangsu, China): feeding section (90 °C for 5 s), compression section (130 °C for 5 s), and metering section (60 °C for 4 s). The feed was air-dried under natural conditions, vacuum-coated with oil, and stored in a freezer at −20 °C. Suitable feed samples were collected for nutritional composition analysis. The feed formulation and analyzed chemical composition are shown in Table 1.

This trial was conducted in an indoor recirculating aquaculture system at the National Aquatic Feed Safety Evaluation Base (Beijing, China). Juvenile largemouth bass were obtained from a commercial aquafarm (Tangshan, Hebei, China). All 600 fish were acclimated and fed the commercial diet for two weeks before the formal feeding trial. After a 24 h fasting, fish with an initial body weight of 14.67 ± 0.01 g were distributed into 20 cylindrical plastic tanks (capacity 256 L) with five replicates per treatment and 30 fish per tank, and each diet was randomly assigned to 20 tanks. Six additional fish were selected as initial samples for whole-body composition analysis. Fish were fed to apparent satiation twice daily (09:00 and 16:00) for 10 weeks. During the experiment, water temperature was maintained at 24 ± 2 °C, pH at 7.30 to 7.60, dissolved oxygen (DO) > 7.0 mg/L, and total ammonia nitrogen < 0.5 mg/L. Tanks were continuously aerated and exposed to a 12 h light:12 h dark photocycle using 40 W fluorescent lamps.

Table 1
Ingredients and composition of experimental diets (DM basis,%).

Item	Individual treatments[1]			
	LL	LLLP	HL	HLLP
Ingredients				
Fish meal (70.05% CP)	30.00	30.00	30.00	30.00
Poultry by-product	20.00	20.00	20.00	20.00
Soybean meal (47.41% CP)	18.00	18.00	18.00	18.00
Wheat gluten meal	8.00	8.00	8.00	8.00
Tapioca starch	6.00	6.00	6.00	6.00
Cottonseed concentrate protein (65.59% CP)	5.00	5.00	5.00	5.00
Micro-crystalline cellulose	4.90	4.85	2.90	2.85
Fish oil	3.00	3.00	3.00	3.00
Soybean oil	3.00	3.00	5.00	5.00
Vitamin and mineral premix[2]	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	0.60	0.60	0.60	0.60
Choline chloride	0.40	0.40	0.40	0.40
Y_2O_3	0.10	0.10	0.10	0.10
Lipopeptide		0.05		0.05
Total	100.00	100.00	100.00	100.00
Chemical composition				
Moisture	6.54	6.62	6.21	6.52
CP	48.70	48.90	48.90	49.10
CL	10.43	10.63	12.87	13.02
Gross energy, MJ/kg	19.40	19.41	20.03	19.81

DM = dry matter; CP = crude protein; CL = crude lipid; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides.
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group.
[2] Vitamin and mineral premix supplied per kg of diet: vitamin A 20 mg, vitamin D_3 10 mg, vitamin K_3 20 mg, vitamin E 400 mg, vitamin B_1 10 mg, vitamin B_2 15 mg, vitamin B_6 15 mg, vitamin B_{12} 8 mg, vitamin C 1000 mg, calcium pantothenate 40 mg, niacinaminde 100 mg, inositol 200, biotin 2 mg, folic acid 10 mg, corn gluten meal 150 mg, $CuSO_4 \cdot 5H_2O$ 10 mg, $FeSO_4 \cdot H_2O$ 300 mg, $ZnSO_4 \cdot H_2O$ 200 mg, $MnSO_4 \cdot H_2O$ 100 mg, KI, 80 mg, $CoCl_2 \cdot 6H_2O$ 5 mg, Na_2SeO_3 10 mg, $MgSO_4 \cdot 5H_2O$ 2000 mg, NaCl 100 mg, zeolite 5005 mg, tertiary butylhydroquinone 200 mg.

Growth performances were determined by batch weighing the fish at the end of the 10 weeks after starvation for 24 h. Three individuals per tank were randomly sampled to analyze whole-body composition. All the sampled fish were anesthetized with 3-aminobenzoic acid ethyl ester methanesulfonic acid salt (MS-222) at a concentration of 200 mg/L. Individual body weight, body length, viscera, liver, visceral adipose tissue, and carcass weight of four fish in each tank were recorded. Blood was rapidly drawn from the caudal vein and centrifuged (4000 × g, 10 min, 4 °C) to obtain plasma for the analysis of hematological parameters. Four liver samples from each tank were dissected and immediately frozen in liquid nitrogen, and kept at −80 °C for mRNA isolation and tissue homogenate analysis until used. Four liver samples near the bile duct in each tank were fixed in 4% paraformaldehyde (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for histology determination. The rest of the liver in each tank were pooled and then stored at −20 °C for the assay of CL.

Ten fish per group (LL, LLLP, HL, and HLLP) were challenged with *Edwardsiella tarda* (ACCC61745) via intraperitoneal injection of 200 μL bacterial suspension (5×10^8 CFU/mL) at the end of the 10 weeks. The concentration of bacteria was determined by preliminary experiments. Mortality and clinical symptoms were recorded over 7 d post-injection. After challenge with *Edwardsiella*, the survival rate (SR) of largemouth bass was 100%. Surviving fish were euthanized, and 8 samples were randomly selected from each group for blood and intestinal tissue collection for inflammatory and immune analysis.

2.3. Apparent digestibility coefficients (ADCs) determination

Following established procedures, Y_2O_3 was incorporated into all experimental diets as an inert indicator at 0.10%. The trial was conducted in a recirculating aquaculture system; feces were flushed out by the water current and intercepted by a feces screen installed at the outflow. Collection of intact fecal strands began 2 h after feeding and was repeated every 30 min; samples were immediately stored at −20 °C. Diet and fecal samples were freeze-dried and finely ground for proximate analyses. Yttrium concentrations were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) by Guobiao Testing & Certification Co., Ltd. (National Analysis and Testing Center for Nonferrous Metals and Electronic Materials, Beijing, China). ADCs were calculated using the indicator method as:

$$ADC\ (\%) = 100 \times [1 - (Y_2O_{3\ feed}/Y_2O_{3\ feces}) \times (Nutrient_{\ feces}/Nutrient_{\ feed})].$$

Methods and parameters followed Austreng et al. (2000) and Sartipiyarahmadi et al. (2023).

2.4. Chemical composition analysis of diets and fish whole body

Dry matter (DM; method 950.46), CP (method 928.08), CL (method 991.36), crude ash (method 920.153), and gross energy (method 920.87) were determined with the AOAC (2005). To analyze the DM, the samples were dried until they reached a constant weight at a temperature of 105 °C. The determination of CP was carried out using a Kjeltec 2300 Unit (FOSS Analytical A/S, Hillerød, Denmark) and the Kjeldahl method. For the analysis of CL, an acid hydrolysis process was first performed with a Soxtex System HT 1047 Hydrolyzing Unit (FOSS Analytical A/S, Hillerød, Denmark). Subsequently, a Soxhlet extraction was carried out using a Soxtex System 1043 (FOSS Analytical A/S, Hillerød, Denmark). The analysis of ash was accomplished through combustion in a CWF 1100 muffle furnace (Carbolite Gero Ltd., Hope Valley, Derbyshire, UK) at 550 °C for a duration of 3 h.

2.5. Plasma, liver, and intestinal tissues homogenate parameters

Content of plasma glucose (A154-1-1), triglycerides (TG; A110-2-1), and total cholesterol (TC; A111-2-1) were determined by

commercial assay kits (Nanjing Jiancheng Co., Nanjing, Jiangsu, China) following the manufacturer's protocols. Intestinal levels of interleukin (IL)-8 (MM-3565401), IL-10 (MM-182501), IL-1β (MM-008301), transforming growth factor-β1 (TGF-β1; MM-182901), tumor necrosis factor-α (TNF-α; MM-065501), and hepatic cyclic-AMP (cAMP; Nanjing, Jiangsu, MM-3259101) were quantified using commercial assay kits (Jiangsu Meimian Industrial Co., Ltd., Nanjing, Jiangsu, China) according to the manufacturer's protocols.

2.6. Histopathological examination

Liver histopathology of 0.5 cm^3 (bile duct-adjacent blocks) was analyzed by fixing tissues in 4% paraformaldehyde for 24 to 48 h, followed by dehydration, paraffin embedding, and sectioning into 5-μm thick slices using a Leica rotary microtome (Leica Microsystems, Wetzlar, Hesse, Germany). Sections were stained with hematoxylin and eosin (H&E) and scanned using a TissueFAXS System (TissueGnostics GmbH, Vienna, Austria) for morphological evaluation. The distal intestine was fixed in the mixture of 60% methanol and 10% acetic acid for 24 h, dehydrated and embedded in paraffin for stained with H&E and alcian blue-periodic acid-schiff (AB-PAS) and scanned using a TissueFAXS System (TissueGnostics GmbH, Vienna, Austria) for morphological evaluation.

Table 2
Primer sequences for quantitative real-time PCR.

Genes	Forward primers (5′-3′)	Reverse primers (3′-5′)
Acc1	AATCAACATCCGCCTGACTCCAAC	CCTGCTTGTCTCCGTATGCTTGG
Atgl	CTTCCTCTCCGCAACAAGTC	TGGTGCTGTCTGGAGTGTTC
Cpt1α	CATGGAAAGCCAGCCTTTAG	GAGCACCAGACACGCTAACA
Creb	GGAGTCTGTATCGCTCAGCC	GGAGTCTGTATCGCTCAGCC
Fasn	AGGCATTGTGGAGGGTGTAG	CCAGTCCACCAGTGATGATG
Hsl	TGATGTTTGCCAAGAAGCTG	CTGATGGACTGGTGCTCTGA
Mucin13	ACATCAGCAGAGTCCATTAGCAC	ACAGTCCGTATCTCCACAGTGAG
Mucin3α	CATAGGCTGACCCATTAAGTGAG	GCTGGTAATCGTTGGCTCTGT
Occludin1b	CACCACCTCCCAAGGCTTCTAT	CCCATACCCAAATCCGTTCAT
Pparα	AGGCCTGCTGAATGTGAAGC	GCTGGATGAAGTGGACGTGG
Zo1	TCTGCCCAATGACAAGCCTGA	GTTGTGCTGGTGTTTGGTGCG
Zo2	CCTTCCACCCACTACTATGATGC	TCTGACGGTCGCCACTGGTTT
Ef1α	AATCGGCGGTATTGGAACTG	TCCACGACGGATTTCCTTGA

2.7. Quantitative real-time PCR (qRT-PCR)

The extraction of total RNA and the synthesis of cDNA were carried out following the methods described in previous studies (Huang et al., 2019; Li et al., 2025). For the qRT-PCR analysis, iTaq Universal SYBR Green Supermix (1725121, Bio-Rad Laboratories Inc., Hercules, CA, USA) was employed on a CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, USA). The qRT-PCR was performed with the following conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The data were then analyzed using the $2^{-\Delta\Delta Ct}$ approach. Elongation factor 1α (Ef1α; GenBank accession number: KT827794) was used as the endogenous reference gene. The sequences of the primers are presented in Table 2. Gene abbreviations and their corresponding full names are provided in Table S2.

2.8. Western blot

Liver tissue samples were subjected to homogenization in radioimmunoprecipitation assay (RIPA) lysis buffer that had protease inhibitors incorporated. After that, the concentrations of proteins were measured using a Quick Star Bradford Protein Assay Kit 1 (5000201, Bio-Rad Laboratories Inc., Hercules, CA, USA). Subsequently, the proteins underwent a denaturation process. Next, the proteins were separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membranes. To prevent non-specific binding, the membranes were incubated with a 5% bovine serum albumin (BSA) solution at room temperature for a duration of 2 h, then incubated with primary antibodies at 4 °C overnight: glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 1:1000, Cat 2118), phospho-AMP-activated protein kinase (p-AMPK; 1:1000, Cat 2531), protein kinase B (AKT; 1:1000, Cat

Table 3
Effects of dietary lipid and lipopeptides levels on growth performance.

Item	SR[1],%	FBW,%	WG[2],%	SGR[3],%/d	FR[4],%	FCR[5]	PR[6],%	ER[7],%
Individual treatments[8]								
LL	100.00	70.66	381.80	2.30	2.33	1.21	22.62	26.43
LLLP	100.00	69.28	372.36	2.28	2.27	1.19	22.62	27.39
HL	98.67	66.77	351.18	2.23	2.18	1.17	23.02	27.46
HLLP	100.00	71.95	390.57	2.34	2.19	1.13	22.33	28.22
SEM	0.742	5.629	38.450	0.120	0.104	0.062	0.332	0.451
Main effect treatments								
Low lipid (10.50%)	100.00	69.97	377.08	2.29	2.30	1.20	22.62	26.91
High lipid (13.00%)	99.33	69.36	370.88	2.28	2.19	1.15	22.68	27.84
Lipopeptide (0)	99.33	68.72	366.49	2.27	2.26	1.19	22.82	26.76
Lipopeptide (0.05%)	100	70.62	381.47	2.31	2.23	1.16	22.47	27.80
***P*-value (two-way ANOVA)**								
Dietary lipid	0.331	0.799	0.702	0.875	0.008	0.004	0.951	0.223
Lipopeptide	0.331	0.431	0.361	0.394	0.442	0.061	0.711	0.358
Interaction	0.332	0.170	0.130	0.184	0.330	0.821	0.725	0.697

LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SR = survival rate; FBW = final body weight; WG = weight gain; SGR = specific growth rate; FR = feeding rate; FCR = feed conversion ratio; PR = protein retention; ER = energy utilization rate; SEM = standard error of the mean.

[1] SR (%) = (Number of fish at trial termination/Initial number of fish) × 100.
[2] WG (%) = [(Final weight − Initial weight)/(Initial weigh)] × 100.
[3] SGR (%/d) = [(ln Final body weight − ln Initial body weight)/Feeding days] × 100.
[4] FR (%) = [Total feed consumption/(Final fish weight + Initial fish weight)/2]/Feeding days × 100.
[5] FCR = Total feed intake/(Final fish weight + Dead fish weight − Initial fish weight).
[6] PR (%) = [(Final body protein content − Initial body protein content)/Total protein intake] × 100.
[7] ER (%) = [(Final fish energy − Initial fish energy)/(Feed intake × Feed energy)] × 100.
[8] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; *n* = 5.

Table 4
Effects of dietary lipid and lipopeptide levels on morphometric parameters (%).

Item	VSI[1]	HSI[2]	VAI[3]	CR[4]	CF[5]
Individual treatments[6]					
LL	8.28	1.85	3.24	66.92	1.75
LLLP	8.65	1.80	3.28	67.35	1.71
HL	8.47	2.04	3.41	67.39	1.76
HLLP	8.89	1.99	3.60	67.78	1.66
SEM	1.329	0.442	0.699	2.034	0.122
Main effect treatments					
Low lipid (10.50%)	8.47	1.82	3.26	67.13	1.73
High lipid (13.00%)	8.68	2.01	3.50	67.59	1.71
Lipopeptide (0)	8.38	1.94	3.32	67.14	1.76
Lipopeptide (0.05%)	8.77	1.89	3.44	67.58	1.69
***P*-value (two-way ANOVA)**					
Dietary lipid	0.538	0.153	0.181	0.407	0.443
Lipopeptide	0.263	0.735	0.506	0.442	0.034
Interaction	0.947	0.994	0.669	0.968	0.385

LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; VSI = viscera somatic index; HSI = hepatosomatic index; VAI = visceral adipose index; CR = carcass ratio; CF = condition factor; SEM = standard error of the mean.
[1] VSI (%) = (Viscera weight/Whole body weight) × 100.
[2] HSI (%) = (Liver weight/Whole body weight) × 100.
[3] VAI (%) = (Visceral fat weight/Whole body weight) × 100.
[4] CR (%) = (Carcass weight/Whole body weight) × 100.
[5] CF (%) = (Body weight/Body length^3) × 100.
[6] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 15$.

Table 5
Effects of dietary lipid and lipopeptides levels on whole-fish composition (wet weight,%).

Item	Moisture	CP	CL	Ash
Individual treatments[1]				
LL	74.09	13.62	8.28	3.17
LLLP	75.03	13.43	8.65	2.99
HL	74.15	13.53	8.47	3.13
HLLP	74.33	13.27	8.89	3.19
SEM	1.362	0.853	0.434	0.284
Main effect treatments				
Low lipid (10.50%)	74.56	13.52	8.06	3.08
High lipid (13.00%)	74.24	13.40	8.31	3.16
Lipopeptide (0)	74.12	13.57	8.36	3.15
Lipopeptide (0.05%)	74.40	13.35	8.01	3.09
***P*-value (two-way ANOVA)**				
Dietary lipid	0.619	0.772	0.188	0.531
Lipopeptide	0.387	0.588	0.081	0.677
Interaction	0.558	0.939	0.761	0.373

LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SEM = standard error of the mean; CP = crude protein; CL = crude lipid.
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 5$.

Table 6
Effects of dietary lipid and lipopeptides levels on nutrient digestibility (%).

Item	DM	CP	CL	Energy
Individual treatments[1]				
LL	76.45	87.62[a]	88.36	81.44
LLLP	75.51	86.99[ab]	89.21	80.63
HL	76.30	86.31[b]	90.32	81.60
HLLP	76.57	87.04[ab]	90.12	82.04
SEM	1.111	1.104	11.024	1.302
Main effect treatments				
Low lipid (10.50%)	75.98		88.79	81.04
High lipid (13.00%)	76.44		90.22	81.82
Lipopeptide (0)	76.38		89.34	81.52
Lipopeptide (0.05%)	76.04		89.67	81.34
***P*-value (two-way ANOVA)**				
Dietary lipid	0.196		0.011	0.127
Lipopeptide	0.336		0.503	0.707
Interaction	0.080	0.029	0.317	0.210

DM = dry matter; CP = crude protein; CL = crude lipid; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SEM = standard error of the mean.
Different lowercase superscript letters represent significant differences among groups ($P < 0.05$).
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 5$.

4691), and phospho-AKT (Ser473) (phospho-AKT, Ser473; 1:1000, Cat 4060) were obtained from Cell Signaling Technology Inc. (Danvers, MA, USA). Forkhead box O1 (FoxO1; 1:1000, ET1608–25) was purchased from Hangzhou HuaAn Biotechnology Co., Ltd. (Hangzhou, Zhejiang, China), while AMP-activated protein kinase (AMPK) and phospho-FoxO1 antibodies (1:1000, AP0176) were obtained from ABclonal Biotechnology Co., Ltd. (Wuhan, Hubei, China). After tris-buffered saline with Tween 20 (TBST) washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:10,000, ab6721, Abcam Plc., Cambridge, MA, USA) at room temperature for 1 h. Protein bands were visualized using a chemiluminescence imaging system and quantified with ImageJ software (National Institutes of Health, Bethesda, ML, USA).

2.9. Statistical analyses

Following verification of homogeneity of variance, data means were analyzed via one-way ANOVA for comparisons across dietary lipid levels. Data in the figures were expressed as mean and standard error of the mean (SEM), which reflects the standard error of the intra-group sample and is used to measure individual variation within each group. Differences among groups were evaluated using a two-way ANOVA to assess the main and interaction effects of dietary lipid level (low vs. high) and lipopeptides supplementation (absence vs. presence). The statistical model used for the analysis was as follows:

$$Y_{ijk} = \mu + S_i + G_j + (S \times G)_{ij} + e_{ijk},$$

where Y_{ijk} is the dependent variable (e.g., growth performance, lipid metabolism indicators, or immune parameters); μ is overall mean; S_i is fixed effect of dietary lipid level (i = low lipid or high lipid); G_j is fixed effect of lipopeptides supplementation ($j = 0$ or 0.05%); $(S \times G)_{ij}$ is interaction effect between lipid level and lipopeptides; e_{ijk} is random error term assumed to be normally and independently distributed with mean 0 and variance σ^2.

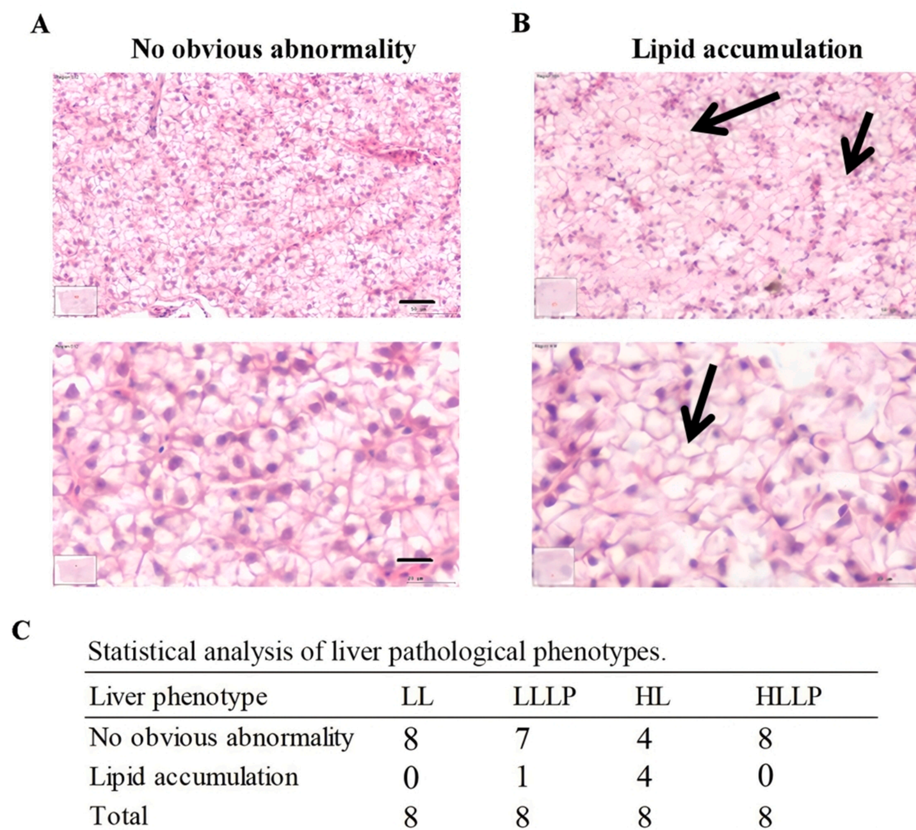
When the interaction term $(S \times G)_{ij}$ were statistically significant ($P < 0.05$), simple effect analysis was conducted and pairwise comparisons among the four dietary treatments (LL, LLLP, HL, and HLLP) were performed using Tukey's honestly significant difference (HSD) test. All parameters that showed significant main or interaction effects in the two-way ANOVA were summarized in Table 12 (Esmaeili et al., 2025). All statistical analyses were performed in R (version 3.2.5), and results were expressed as means and SEM, with statistical significance accepted at $P < 0.05$.

3. Results

3.1. Effects of dietary lipid and lipopeptide levels on growth performance and body composition

The effects of lipopeptide supplementation on growth performance and body composition of largemouth bass were shown in

Tables 3–5. SR exceeded 98.00% across all experimental groups. Two-way ANOVA revealed that neither the main effect of dietary lipid level (10.50% vs. 13.00%) nor lipopeptides supplementation (0 vs. 0.05%) significantly influenced SR, final body weight (FBW), weight gain (WG), or specific growth rate (SGR) (Table 3, $P > 0.05$), and no significant interaction was detected between lipid level and lipopeptides addition ($P > 0.05$). However, decreasing dietary lipid levels from 13.00% to 10.50% significantly increased the feeding rate (FR) and FCR (Tables 3 and 12, $P < 0.05$), while the main effect of lipopeptides supplementation decreased FCR from 1.19 to 1.16 and increased energy utilization rate (ER) from 26.76% to 27.80% (Table 3), and significantly decreased condition factor (CF) (Table 4, $P = 0.034$). No significant differences were detected in hepatosomatic index (HSI), visceral adipose index (VAI), and carcass



Statistical analysis of liver pathological phenotypes.

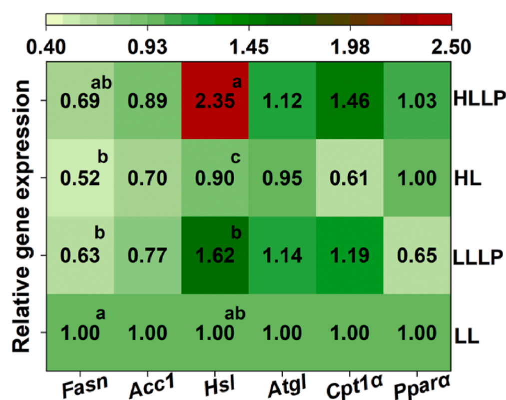
Liver phenotype	LL	LLLP	HL	HLLP
No obvious abnormality	8	7	4	8
Lipid accumulation	0	1	4	0
Total	8	8	8	8

Fig. 1. Effects of dietary lipid and lipopeptides levels on liver health of largemouth bass. (A and B) Hematoxylin and eosin (H&E) staining of two phenotypes of hepatic histopathological examination. (C) Statistical analysis of liver pathological phenotypes. Arrows point to vacuolated hepatocytes showing marked steatosis. Scale bars: 50 μm (top) and 20 μm (bottom). LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$. LL= low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides.

Table 7
Effects of dietary lipid and lipopeptides levels on lipid metabolism.

Item	Hepatic lipid,%	TC, mmol/L	TG, mmol/L	Glucose, mmol/L	Lipase, min/mg prot
Individual treatments[1]					
LL	10.57	11.52[a]	3.98	4.51	122.35
LLLP	9.97	6.62[b]	3.39	4.75	140.66
HL	13.94	12.02[a]	3.70	4.79	169.92
HLLP	12.49	13.31[a]	4.63	4.43	198.80
SEM	1.748	3.201	1.172	0.483	64.882
Main effect treatments					
Low lipid (10.50%)	10.27		3.69	4.63	131.51
High lipid (13.00%)	13.22		4.15	4.62	184.36
Lipopeptide (0)	12.26		3.84	4.65	146.14
Lipopeptide (0.05%)	11.23		4.01	4.60	169.73
***P*-value (two-way ANOVA)**					
Dietary lipid	<0.001		0.309	0.938	0.020
Lipopeptide	<0.001		0.702	0.799	0.282
Interaction	0.114	<0.001	0.086	0.137	0.808

TC = total cholesterol; TG = triglycerides; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SEM = standard error of the mean.
Different lowercase superscript letters represent significant differences among groups ($P < 0.05$).
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.



Two-way ANOVA analysis of the effects of lipid and lipopeptides levels on the gene expression of lipid metabolism.

Genes	*P*-value (two-way ANOVA)		
	Lipid level	Lipopeptide	Interaction
Fasn			0.008
Acc1			0.028
Hsl			0.027
Atgl	0.810	0.223	0.889
Cptla	0.716	0.004	0.057
Ppara	0.147	0.226	0.152

Fig. 2. Effects of dietary lipid and lipopeptides levels on the expression of lipid metabolism genes of largemouth bass. Color scale: The redder the color, the higher the gene expression, while lighter green indicates lower gene expression. LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$. LL= low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides. Different lowercase letters in the heatmap represent significant differences among groups ($P < 0.05$).

ratio (CR) across lipid levels or lipopeptides-treated groups (Table 4, $P > 0.05$). The whole-fish composition, including moisture, CP, CL, and ash, did not show significant differences among the groups (Table 5, $P > 0.05$).

3.2. Effects of dietary lipid and lipopeptide levels on digestibility

As shown in Table 6, one-way ANOVA showed the CP digestibility in the HL group was significantly lower than that in the LL group ($P = 0.019$), indicated dietary low-lipid levels promote the protein utilization. Two-way ANOVA revealed a significant main effect of dietary lipid level: CL digestibility was higher in the high-lipid background than in the low-lipid background (Tables 6 and 12, $P = 0.011$), with no significant effects observed after lipopeptide addition ($P = 0.503$).

3.3. Effects of dietary lipid and lipopeptide levels on liver health

Considering the central role of the liver in lipid metabolism, it was first examined the hepatic phenotype. Liver sections from largemouth bass were stained by H&E with eight samples observed per group. Two distinct histological phenotypes (no obvious abnormality and lipid accumulation) were identified, as illustrated in Fig. 1. Phenotype I (no obvious abnormality) exhibited intact cellular morphology, uniformly distributed cytoplasm, and minimal lipid droplets (Fig. 1A). Phenotype II (lipid accumulation) was characterized by enlarged lipid droplets, cellular vacuolation, and markedly elevated lipid content (Fig. 1B). Half of the samples in the HL group displayed lipid accumulation, whereas the HLLP group showed significant mitigation of this condition (Fig. 1C).

3.4. Effects of dietary lipid and lipopeptide levels on lipid metabolism

Subsequently, hepatic lipid metabolism was examined. The physiological and biochemical indices of largemouth bass are shown in Tables 7 and 12. Two-way ANOVA revealed a significant main effect of dietary lipid level: hepatic lipid content was higher in the high-lipid background than in the low-lipid background (Tables 7 and 12, $P < 0.001$). Lipopeptide supplementation exerted a significant main effect by reducing hepatic lipid accumulation across both lipid backgrounds (Tables 7 and 12, $P < 0.001$). Plasma TC showed a similar pattern, being significantly elevated in the high-lipid background relative to the low-lipid background (Table 7, $P < 0.001$). A significant interaction between lipid level and lipopeptides was observed for plasma TC, and lipopeptides supplementation reduced TC with the most pronounced reduction observed in the LLLP group relative to LL (Table 7, $P < 0.001$), indicating that the lipid-lowering effect of lipopeptides was dependent on dietary lipid background. No significant difference was observed on plasma TG and glucose level (Table 7, $P > 0.05$). For lipase activity, the main effect of lipid level was significant, with higher activities in the high-lipid background compared with the low-lipid background (Tables 7 and 12, $P = 0.020$). Although lipopeptides supplementation tended to increase lipase activity, the effect was not statistically significant (Table 7, $P = 0.282$).

Further analysis of the effects of different levels of lipid and lipopeptides addition on the mRNA expression of lipid metabolism genes in the liver of largemouth bass (Fig. 2). The results revealed that compared with the HL group, the lipid synthesis gene *Fasn* was significantly upregulated in the LL group ($P = 0.005$), while LLLP group significantly downregulated the mRNA levels of *Fasn* ($P = 0.037$).

3.5. Effects of dietary lipid and lipopeptide levels on energy metabolism

To elucidate the mechanisms underlying the effects of lipopeptides in largemouth bass, the relevant signaling pathways were analyzed. Western blot analysis revealed a significant main effect of lipid level: p-AKT/AKT and p-FoxO1/FoxO1 ratios were lower in the high-lipid background than in the low-lipid background (Fig. 3A–C, $P < 0.05$). The addition of lipopeptides had no significant effect on the phosphorylation levels of AKT and FoxO1 (Fig. 3A–C, $P > 0.05$). Growth performance results showed that the largemouth bass fed low-lipid diets exhibited significantly higher feed intake to meet energy demands (Table 3). Therefore, energy metabolism indicators AMPK, cAMP, and CREB signaling pathway were further analyzed. The addition of lipopeptides (HLLP) at the basis of HL group reduced the expression of p-AMPK/AMPK (Fig. 3A and D, $P = 0.035$). Both lipid and lipopeptides supplementation significantly increased the expression levels of cAMP (Tables 8 and 12, $P < 0.05$) and *Creb* (Fig. 3E and Table 12, $P < 0.05$) in largemouth bass.

3.6. Effects of dietary lipid and lipopeptide levels on intestinal health

Further analyses were conducted to evaluate the antimicrobial properties of lipopeptides. The histopathological results showed that the HL group caused inflammation in the intestinal tract of largemouth bass, manifested as lymphocyte infiltration in the lamina

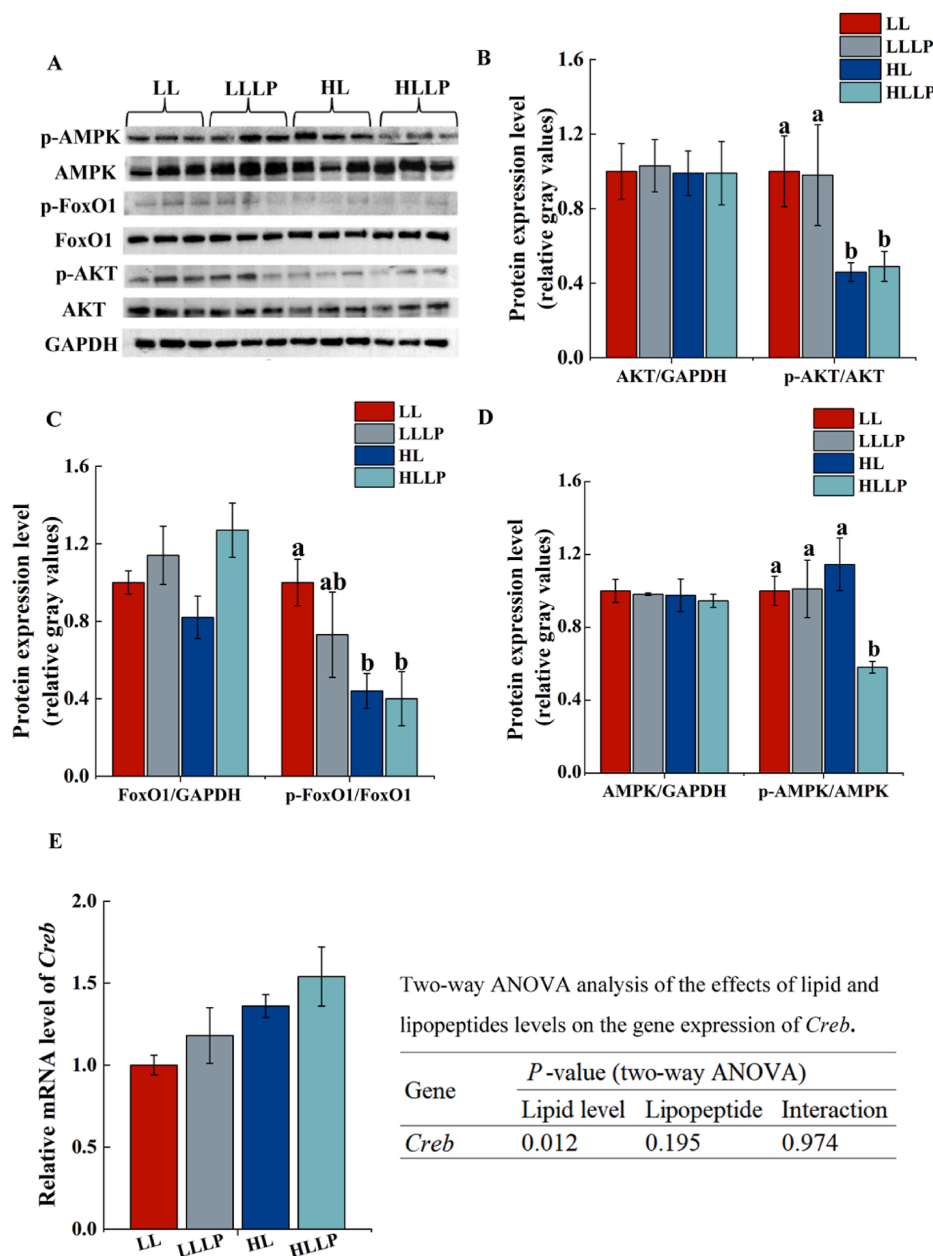


Fig. 3. Effects of dietary lipid and lipopeptides levels on energy metabolism of largemouth bass. (A–D) Hepatic AKT, p-AKT, FoxO1, p-FoxO1, AMPK, and p-AMPK protein expression, $n = 3$. (E) Hepatic *Creb* mRNA expression, $n = 8$. LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group. LL= low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides. Different lowercase letters above columns represent significant differences among treatments at $P < 0.05$.

propria and loose intestinal villi structure (Fig. 4). In the HLLP group, the intestinal tissue structure of the posterior part significantly improved, characterized by reduced inflammatory cell infiltration and recovery of intestinal epithelial integrity. Two-way ANOVA analysis revealed that the high-lipid diet significantly downregulated the expression of tight junction protein genes *Zo2* and occludin1b; lipopeptides intervention specifically upregulated the transcription levels of *Zo1* and *Zo2* (Fig. 5 and Table 12, $P < 0.05$).

3.7. Effects of dietary lipid and lipopeptide levels on the antibacterial ability

The results of intestinal inflammatory cytokines before and after pathogen challenge are shown in Tables 9 and 10. Before the challenge, ELISA analysis indicated there were no significant differences of IL-10, TGF-β1, TNF-α, and IL-8 among the different groups in the intestine (Table 9, $P > 0.05$). However, the pro-inflammatory cytokine IL-1β in the HL group was higher than the other three groups (Table 9, $P < 0.05$). Two-way ANOVA analysis indicated that IL-1β levels were significantly lower in the lipopeptides-supplemented groups compared to the non-supplemented groups (Tables 9 and 12, $P = 0.002$). After the challenge, the levels of intestinal inflammatory cytokines are presented in Table 10. The anti-inflammatory cytokine IL-10 level in the HLLP group was significantly higher than in the HL group (Table 10, $P = 0.020$), whereas the pro-inflammatory cytokine TNF-α level in the LLLP group was significantly lower than in the LL group (Table 10, $P = 0.005$). Two-way ANOVA analysis further

Table 8
Effects of dietary lipid and lipopeptides levels on hepatic cAMP content.

Item	cAMP, pmol/mg prot
Individual treatments[1]	
LL	3.36
LLLP	4.10
HL	5.40
HLLP	6.47
SEM	1.581
Main effect treatments	
Low lipid (10.50%)	3.73
High lipid (13.00%)	5.93
Lipopeptide (0)	4.38
Lipopeptide (0.05%)	5.29
***P*-value (two-way ANOVA)**	
Dietary lipid	<0.001
Lipopeptide	0.023
Interaction	0.661

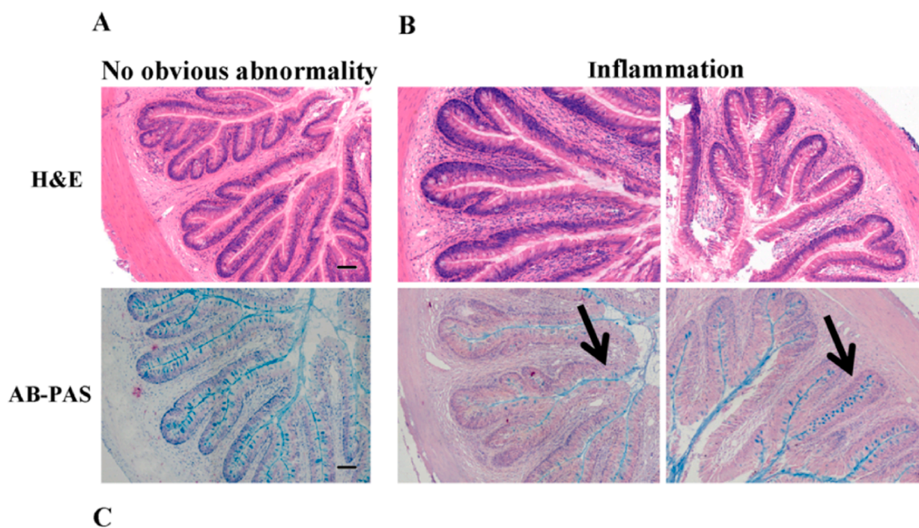
LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SEM = standard error of the mean; cAMP = cyclic-AMP; prot = protein.
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.

revealed that the main effect of lipopeptides supplementation (0 vs. 0.05%) significantly reduced the levels of TGF-β1, IL-8, and IL-1β after the pathogen challenge (Tables 10 and 12, $P < 0.05$).

The effects of pathogen challenge on IgM levels in largemouth bass are shown in Table 11. Before and after the challenge, the IgM levels in the HLLP group were significantly higher than those in the HL group (Table 11, $P < 0.05$). Additionally, two-way ANOVA analysis indicated that the main effect of lipopeptides supplementation (0 vs. 0.05%) in the diet significantly upregulated IgM levels both before and after pathogen challenge (Tables 11 and 12, $P < 0.05$), suggesting that lipopeptides enhance the humoral immune response of largemouth bass.

4. Discussion

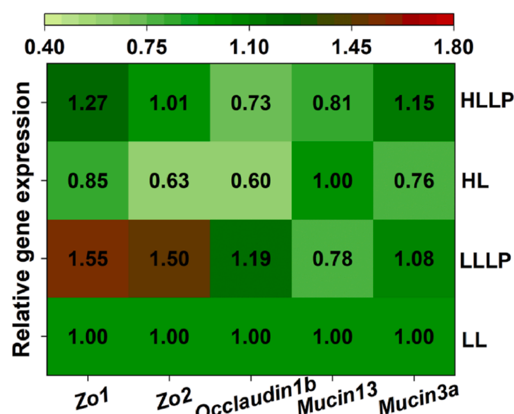
With the continuous development of global aquaculture, largemouth bass has become an important farmed species due to its excellent growth performance and high feed conversion efficiency. However, the high cost of feed, which accounts for over 60% of the cost of aquaculture, remains a significant challenge for the industry (Mustapha, 2020; Yu et al., 2024). Especially, excessive dietary CL increases feed costs and may lead to fat accumulation and metabolic issues (Zhou et al., 2020). Therefore, optimizing lipid levels to improve feed conversion efficiency while controlling costs has become a critical concern for aquaculture. Lipopeptides, as bioactive compounds, have attracted increasing attention in aquafeed applications. Studies have shown that lipopeptides effectively regulate lipid metabolism, promote fatty acid breakdown and conversion, and enhance lipid utilization. This reduces the required CL content in feed, subsequently lowering feed costs (Zhai et al., 2017a). In this experiment, two-way ANOVA indicated a significant main effect of dietary lipid level: feed intake and FCR were lower in the high-lipid background than in the low-lipid background. Similarly, energy utilization efficiency followed the same trend, with high-lipid diets showing higher energy utilization efficiency than low-lipid diets, though no significant differences were found. Otherwise, the FCR of the lipopeptides-supplemented groups slightly decreased (from 1.19 to 1.16), and the energy utilization efficiency was also improved (from 26.76% to 27.80%). The reduced feed intake observed in the high-lipid



Statistical analysis of intestinal pathological phenotypes.

Gut phenotype	LL	LLLP	HL	HLLP
No obvious abnormality	4	4	5	7
Mild inflammation	4	4	3	1
Total	8	8	8	8

Fig. 4. Effects of dietary lipid and lipopeptides levels on intestinal health of largemouth bass. (A and B) Three phenotypes of intestinal histopathological examination. Arrows point to lymphocyte infiltration into the lamina propria and disrupted villi morphology. Scale bar: 20 μm. (C) Statistical analysis of intestinal pathological phenotypes. LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$. H&E = hematoxylin and eosin; AB-PAS = alcian blue-periodic acid-schiff; LL= low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides.



Two-way ANOVA analysis of the effects of lipid and lipopeptides levels on the gene expression of tight functions in the intestine.

Genes	*P*-value (two-way ANOVA)		
	Lipid level	Lipopeptide	Interaction
Zo1	0.160	0.003	0.667
Zo2	0.042	0.039	0.755
Occlaudinlb	0.036	0.415	0.878
Muinl3	0.926	0.255	0.914
Mucin3a	0.853	0.617	0.738

Fig. 5. The effects of lipid and lipopeptides levels on the integrity of tight junctions in the intestinal tract of largemouth bass. Color scale: The redder the color, the higher the gene expression, while lighter green indicates lower gene expression. LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.

Table 9
Effects of dietary lipid and lipopeptides level on the levels of intestinal inflammatory cytokines of largemouth bass before *Edwardsiella tarda* challenge (pg/mg prot).

Item	IL-10	TGF-β1	TNF-α	IL-8	IL-1β,
Individual treatments[1]					
LL	1.50	1.74	5.54	1.30	0.81
LLLP	1.58	1.81	5.40	1.18	0.68
HL	1.88	1.67	6.48	1.31	1.00
HLLP	1.99	1.72	5.25	1.27	0.76
SEM	0.424	0.343	1.201	0.237	0.186
Main effect treatments					
Low lipid (10.50%)	1.54	1.77	5.47	1.24	0.75
High lipid (13.00%)	1.93	1.69	5.86	1.29	0.88
Lipopeptide (0)	1.69	1.70	6.01	1.30	0.90
Lipopeptide (0.05%)	1.78	1.76	5.32	1.22	0.72
***P*-value (two-way ANOVA)**					
Dietary lipid	0.070	0.539	0.335	0.546	0.019
Lipopeptide	0.494	0.641	0.102	0.336	0.002
Interaction	0.920	0.960	0.187	0.667	0.402

IL = interleukin; TGF-β1 = transforming growth factor β1; TNF-α = tumor necrosis factor α; prot = protein; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; SEM = standard error of the mean.
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.

Table 10
Effects of dietary lipid and lipopeptides level on the levels of intestinal inflammatory cytokines of largemouth bass after *Edwardsiella tarda* challenge (pg/mg prot).

Item	IL-10	TGFβ1	TNF-α	IL-8	IL-1β
Individual treatments[1]					
LL	7.60[a]	3.05	13.62[a]	4.02	2.30
LLLP	6.86[ab]	2.26	6.83[b]	3.06	1.63
HL	5.42[b]	2.95	12.22[ab]	4.03	2.31
HLLP	7.48[a]	2.46	16.02[a]	3.28	2.00
SEM	1.531	0.734	4.901	0.918	0.531
Main effect treatments					
Low lipid (10.50%)		2.66		3.54	1.97
High lipid (13.00%)		2.71		3.66	2.15
Lipopeptide (0)		3.00		4.02	2.31
Lipopeptide (0.05%)		2.36		3.17	1.81
***P*-value (two-way ANOVA)**					
Dietary lipid		0.842		0.695	0.268
Lipopeptide		0.011		0.007	0.006
Interaction	0.010	0.531	0.010	0.260	0.285

LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; prot = protein; SEM = standard error of the mean. Different lowercase superscript letters represent significant differences among groups ($P < 0.05$).
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.

background likely reflects the higher energy density of the diet. When feed energy density rises, fish may reduce their feed intake to avoid excessive energy consumption while still meeting their energy requirements (Hemre et al., 2002). This aligns with the observed decrease in feed intake under the high-lipid background in the present study. Additionally, increasing dietary lipid content can enhance feed energy utilization efficiency, leading to reduced feed intake, which is a common response in many fish species (Xie et al., 2021). Furthermore, two-way ANOVA revealed a significant main effect of dietary lipid level: fish in the high-lipid background exhibited lower FCR than those in the low-lipid background, indicating improved energy utilization efficiency. These diets typically provide more energy, allowing fish to achieve the same or better growth performance with lower feed intake (Berge et al., 2022). This has important economic implications for aquaculture, as a lower FCR translates to reduced feed costs. Although not statistically significant, lipopeptides supplementation was associated with a numerically lower FCR, suggesting potential supportive effects.

To further elucidate how high-lipid diets and lipopeptides supplementation influence feed utilization, the apparent digestibility of dietary lipids and potential adaptive responses in lipid metabolism were next examined. In this study, the apparent digestibility of CL was higher in the high-lipid background than in the low-lipid background, suggesting that when fish consume a diet with a higher lipid content, their digestive system may adapt to the high-lipid environment, thereby enhancing lipid digestion efficiency. This finding aligns with previous reports indicating that elevated dietary lipid levels can increase ADC of CL in fish, often accompanied by adaptive up-regulation of intestinal lipase or lipoprotein lipase activity and improved postprandial lipid handling (Borges et al., 2013; Chen et al., 2024; Trenzado et al., 2018; Wang et al., 2014). Related studies have indicated that an increase in dietary lipid content may facilitate lipid digestion and absorption, while lipopeptides supplementation may further enhance this process by improving lipid metabolism and increasing lipase activity (Chen et al., 2023b). Moreover, some studies have shown that although lipopeptides supplementation did not significantly improve the digestibility of CP or CL, it may indirectly enhance fish growth performance by regulating lipid metabolism, promoting digestive enzyme activity, improving lipid absorption, and increasing lipid storage (Zhai et al., 2017b; Zhu and Sun, 2021). Results in this study also showed the effects of lipopeptides

Table 11
Effects of dietary lipid and lipopeptides levels on the level of IgM in the liver of largemouth bass.

Item	IgM, pg/mg prot	
	Before *Edwardsiella tarda* challenge	After *Edwardsiella tarda* challenge
Individual treatments[1]		
LL	0.35^{b}	0.38
LLLP	0.35^{b}	0.41
HL	0.31^{b}	0.43
HLLP	0.41^{a}	0.50
SEM	0.059	0.051
Main effect treatments		
Low lipid (10.50%)		0.39
High lipid (13.00%)		0.46
Lipopeptide (0)		0.40
Lipopeptide (0.05%)		0.46
***P*-value (two-way ANOVA)**		
Dietary lipid		<0.001
Lipopeptide		0.003
Interaction	0.003	0.279

IgM = immunoglobulin M; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; prot = protein; SEM = standard error of the mean.
Different lowercase superscript letters represent significant differences among groups ($P < 0.05$).
[1] LL, basal diet with 10.50% lipid group; LLLP, basal diet with 10.50% lipid and 0.05% lipopeptides group; HL, basal diet with 13.00% lipid group; HLLP, basal diet with 13.00% lipid and 0.05% lipopeptides group; $n = 8$.

Table 12
The results of two-way ANOVA analysis for significant parameters.

Item	Main effects treatments				*P*-value		
	Low lipid (10.50%)	High lipid (13.00%)	Lipopeptide (0)	Lipopeptide (0.05%)	Dietary Lipid	Lipopeptide	Interaction
Growth performance							
FR, %	2.30^{A}	2.19^{B}			0.008	0.442	0.330
FCR	1.20^{A}	1.15^{B}			0.004	0.061	0.821
Nutrient digestibility							
CL, %	88.79^{B}	90.22^{A}			0.011	0.503	0.317
Lipid metabolism and digestion efficiency							
CF, %			1.76^{X}	1.69^{Y}	0.443	0.034	0.385
Hepatic lipid, %	10.27^{B}	13.22^{A}	12.26^{X}	11.23^{Y}	<0.001	<0.001	0.114
Lipase, min/mg prot	131.51^{B}	184.36^{A}			0.020	0.292	0.808
cAMP, pmol/mg prot	3.73^{B}	5.93^{A}	4.38^{Y}	5.29^{X}	<0.001	0.023	0.661
***Edwardsiella tarda* challenge of intestinal inflammatory cytokines, pg/mg prot**							
IL-1β (before)	0.75^{B}	0.88^{A}	0.90^{X}	0.72^{Y}	0.019	0.002	0.402
TGFβ1 (after)							3.00^{X}
IL-8 (after)							4.02^{X}
IL-1β (after)							2.31^{X}
IgM (after)					0.39^{B}	0.46^{A}	0.40^{Y}
Gene expression analysis of lipid metabolism and tight junction							
Cpt1α							0.69^{Y}
Creb					0.98^{B}	1.26^{A}	
Zo1							0.93^{Y}
Zo2					1.22^{A}	0.82^{B}	0.82^{Y}
Occlaudinlb					1.10^{A}	0.67^{B}	

FR = feeding rate; FCR = feed conversion ratio; CL = crude lipid; CF = condition factor; cAMP = cyclic-AMP; IL = interleukin; TGF-β1 = transforming growth factor β1; IgM = immunoglobulin M; LL = low-lipid; LLLP = low-lipid with lipopeptides; HL = high-lipid; HLLP = high-lipid with lipopeptides; prot = protein.
Different letters of AB, represent significant differences between dietary lipid content ($P < 0.05$) and different letters of XY, represent significant differences between dietary lipopeptide content ($P < 0.05$).

supplementation on digestibility improvement were relatively limited in both low-lipid and high-lipid diets. This finding suggests that the primary role of lipopeptides may not lie in enhancing apparent digestibility, but rather in regulating lipid metabolism and reducing lipid deposition, as evidenced by lower hepatic lipid accumulation and improved plasma lipid profiles.

Half of the sampled largemouth bass showed liver lipid accumulation in the HL group. Lipid accumulation in the liver is commonly associated with the onset of non-alcoholic fatty liver disease (NAFLD). Excessive lipid deposition in the liver may disrupt lipid metabolism (Yuan et al., 2016; Zhang et al., 2023). Lipopeptides supplementation partially alleviated the negative effects of lipid accumulation under high-lipid dietary conditions. Additionally, it was observed that lipopeptides supplementation significantly reduced the CF. Together with the observed reduction in hepatic lipid content and plasma cholesterol, the decrease in CF may be consistent with improved lipid metabolic regulation by lipopeptides. Gene expression analysis of lipid metabolism further confirmed this mechanism. In the lipid synthesis pathway, lipopeptides supplementation in the low-lipid diet suppressed the expression of *Fasn*. *Fasn* is a rate-limiting enzyme in fatty acid synthesis, and its downregulation directly reduces the de novo synthesis of long-chain fatty acids (Mir et al., 2020). Lipopeptide supplementation significantly upregulated the expression of *Hsl*, coding a key enzyme in lipid breakdown. Increased mRNA levels of *Hsl* are directly associated with enhanced triglyceride hydrolysis

capacity (Deser et al., 2016). Additionally, lipopeptide supplementation in the high-lipid diet significantly upregulated the expression of *Cpt1α*, which plays a crucial role in fatty acid oxidation. This finding aligns with the metabolic adaptation strategy of fish in high-lipid environments, where β-oxidation serves as a major energy source (Li et al., 2016). The interaction effects observed for TC, *Fasn*, *Acc1*, *Hsl*, IL-10, TNF-α, and IgM suggest that the physiological impact of lipopeptides is significantly enhanced in high-lipid diets, emphasizing the critical role of dietary lipid composition in optimizing the efficacy of functional additives.

The AKT-FoxO1 signaling pathway plays a crucial role in maintaining lipid metabolic balance by regulating lipid synthesis and breakdown. As a key molecule in the insulin signaling pathway, the decreased phosphorylation of AKT may indicate insulin resistance induced by a high-lipid diet (Alipourfard et al., 2021). FoxO1 functions as a transcriptional activator of lipid breakdown genes, such as *Atgl* and *Hsl*, and its activity depends on its dephosphorylated state (Ioannilli et al., 2020). Under normal conditions, phosphorylated FoxO1 translocated from the nucleus to the cytoplasm, thereby inhibiting its transcription activity (Tsai et al., 2016; Yu et al., 2017). The reduction in AKT activity leads to FoxO1 dephosphorylation and its retention in the nucleus, where it activates genes involved in lipid breakdown while suppressing those related to lipid synthesis. This finding is consistent with studies in mammals, which showed inhibiting AKT signaling could alleviate hepatic steatosis by restoring the nuclear localization of FoxO1 (Liang et al., 2016). The results showed that the protein expression levels of p-AKT and p-FoxO1 were significantly reduced in the HL group. However, the addition of lipopeptides had no significant effect on the phosphorylation levels of AKT and FoxO1, indicating that in this experiment, lipopeptides regulated lipid metabolism in largemouth bass rather than through the AKT-FoxO1 pathway. Given this, the focus was next placed on alternative signaling cascades that could mediate the metabolic effects of lipopeptides.

The AMPK serves as a central sensor of cellular energy status, with its phosphorylation level directly reflecting the balance between energy supply and demand (Hardie, 2014). Experimental results showed that lipopeptides supplementation in the high-lipid diet significantly reduced p-AMPK expression. This suggests that lipopeptides enhance intracellular ATP levels by promoting lipid breakdown and oxidation (e.g., activation of Hsl and Cpt1α), thereby providing sufficient cellular energy and reducing the need for AMPK activation. Meanwhile, lipopeptides supplementation significantly increased hepatic cAMP levels. As a second messenger, cAMP activates protein kinase A (PKA), which directly phosphorylates Hsl, enhancing its enzymatic activity and accelerating triglyceride hydrolysis (Dodge-Kafka et al., 2010). There is cross-regulation between the AMPK and cAMP-CREB pathways. For instance, AMPK inactivation may indirectly promote cAMP accumulation by reducing cellular energy stress signals (Sun et al., 2020). Additionally, CREB activation can provide feedback inhibition to prevent excessive AMPK activation, thereby maintaining metabolic homeostasis (Didier et al., 2018). This bidirectional regulatory network highlights the complexity of lipopeptides-mediated lipid metabolism optimization through multi-target coordination. The interplay between AMPK and the cAMP-CREB pathway forms the core framework of lipopeptides regulation of lipid metabolism. AMPK functions as an energy sensor to inhibit lipid synthesis and promote oxidation, while the cAMP-CREB cascade directly drives the transcription of genes related to lipid breakdown and oxidation (Li et al., 2018). This multilayered regulatory network not only alleviates hepatic lipid accumulation induced by high-lipid diets but also provides theoretical support for the development of functional additives in aquafeeds.

Beyond lipid metabolism, lipopeptides also exert a key antimicrobial function. High-lipid intake causes mild inflammation in the intestines of largemouth bass, which is consistent with the mechanism by which high-lipid diets induce intestinal barrier damage through the activation of pro-inflammatory pathways (such as nuclear factor kappa-light-chain-enhancer of activated B cells [NF-κB]) (He et al., 2022; Zheng et al., 2024). After adding lipopeptides, the intestinal tissue structure significantly improved, suggesting the repair effect of lipopeptides on the barrier function. Lipopeptides intervention specifically upregulated the transcription levels of *Zo1* and *Zo2*, indicating its targeted regulatory characteristics on tight junction complexes. It is noteworthy that high-lipid feed interferes with intestinal homeostasis through dual mechanisms. It not only upregulates the pro-inflammatory factor IL-1β, triggering an inflammatory cascade, but also induces an immune imbalance by causing a compensatory increase in the anti-inflammatory factor IL-10 (Liu et al., 2022; Zhang et al., 2024). However, lipopeptides intervention significantly reduced the level of IL-1β, which is consistent with previous studies on lipopeptides alleviating systemic inflammation (Kim et al., 1998; Wang et al., 2021; Zhang et al., 2015). Previous research suggests that lipopeptides can competitively bind to toll-like receptor 4 (TLR4), blocking pathogen-associated molecular pattern (PAMP) recognition and thereby inhibiting NF-κB pathway activation, which leads to a reduction in pro-inflammatory cytokine release (Buccini et al., 2022). Additionally, after the pathogen challenge, IL-10 (an anti-inflammatory cytokine) levels were significantly elevated in the intestines of the HLLP group, whereas IL-1β and IL-8 levels were significantly reduced. This suggests that lipopeptides may stimulate immune cells to secrete IL-10, reshaping the anti-inflammatory microenvironment. This mechanism aligns with the findings of Zhai et al. (2017a), who reported that lipopeptides can alleviate inflammation by modulating intestinal immune balance.

5. Conclusion

This study systematically revealed the molecular network by which lipopeptides regulate lipid metabolism in largemouth bass through multi-target synergistic interactions. Histopathological analysis revealed that a high-lipid diet led to hepatic lipid accumulation, whereas supplementation with lipopeptides significantly alleviated this condition. The molecular basis of this improvement lies in the precise regulation of lipid metabolism-related gene expression by lipopeptides. Specifically, the mRNA expression of lipid breakdown genes *Hsl* and *Atgl* was significantly upregulated, and the expression of *Cpt1α* was elevated, promoting mitochondrial β-oxidation. For the first time, this study elucidates that lipopeptides regulate lipid metabolism through a multilayered network involving AMPK energy sensing cAMP-CREB signaling cascades. These findings not only provide a molecular basis for the application of lipopeptides in aquafeeds but also highlight their potential value in the treatment of metabolic disease.

Credit Author Statement

Tong Li: Writing – original draft, Formal analysis, Data curation. **Min Li:** Writing – original draft, Formal analysis. **Min Xue:** Writing – review & editing, Supervision, Funding acquisition. **Jie Wang:** Visualization. **Hao Wang:** Software. **Lixin Wu:** Software. **Yaping Zhu:** Resources. **Wenhao Zhou:** Resources. **Xiaofang Liang:** Writing – review & editing, Supervision, Methodology, Funding acquisition.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper. The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Wenhao Zhou is currently employed by Beijing Enhalor Biotechnology Co., Ltd. Min Xue is an Editorial Board Member for Animal Nutrition and was not involved the editorial review or the decision to publish this article.

Acknowledgements

This study was supported by Beijing Natural Science Foundation (6232038); National Natural Science Foundation of China (32172981 and 32273141); The Agricultural Science and Technology Innovation Program (ASTIP) (CAAS-ZDRW202303). We thank other members in our lab for assistance in this study. We are grateful to Teng Da and Yang Na from the Active Peptide and Feed Team of the Institute of Feed Research, Chinese Academy of Agricultural Sciences for providing *Edwardsiella tarda*.

Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2025.10.003.

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