fishes

MDPI

Article

Dietary *Lactobacillus acidophilus* Reshapes Gut Microbiota, Alters Body Shape and Reduces Lipid Deposition in Large Yellow Croaker (*Larimichthys crocea*)

Xinghong Zhou [1,2,3,†], Subin Cui [1,2,3,†], Qin Wang [4,5,†], Kaiyue Lu [1,2,3], Zehui Su [1,2,3], Zhaowen Li [4,5,*], Kun Hu [1,2,3,*] and Yali Wang [1,2,3,*]

1 National Demonstration Center for Experimental Fisheries Science Education, Shanghai Ocean University, Shanghai 201306, China; m250100152@st.shou.edu.cn (X.Z.)
2 National Pathogen Collection Center for Aquatic Animals, Shanghai Ocean University, Shanghai 201306, China
3 Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture, Shanghai Ocean University, Shanghai 201306, China
4 National Key Laboratory of Agricultural Microbiology, Angel Yeast Co., Ltd., Yichang 443003, China
5 The Hubei Provincial Key Laboratory of Yeast Function, Angel Yeast Co., Ltd., Yichang 443003, China
* Correspondence: 13238439020@163.com (Z.L.); khu@shou.edu.cn (K.H.); yanliwang@shou.edu.cn (Y.W.)
† These authors contributed equally to this work.

Abstract

Intensive aquaculture of large yellow croaker (*Larimichthys crocea*) faces intestinal microecological imbalance and hepatic lipid deposition, requiring safe nutritional strategies. *Lactobacillus acidophilus*, a safe probiotic with strong colonization ability, produces antimicrobial metabolites and shows multiple benefits in fish. This study evaluated its effects on juvenile large yellow croaker. Fish were fed a basal diet or basal diet supplemented with *L. acidophilus* (viable counts at least 1 10^8 CFU/g) for 28 days. The results showed that, compared with the control group, supplementation did not significantly change growth performance but altered body conformation, with reduced body height and an increased body length-to-height ratio, accompanied by a decline in muscle crude lipid and lower serum lipid contents. Hepatic lipogenic gene expression (*DGAT2*, *SREBP-1*, *ACC*, *FASN*) was downregulated and *LPL* and *ATGL* were upregulated. Antioxidant capacity improved, with higher serum SOD, CAT and T-AOC and lower MDA; serum AST decreased, and *TNF-β* and *IL-8* were downregulated. Intestinal α-amylase activity, villus height and muscular layer thickness increased, and 16S profiling revealed a lower abundance of *Vibrio* together with enrichment of ABC transporter and formate dehydrogenase genes. In conclusion, dietary *L. acidophilus* altered body shape and lipid metabolism, improved antioxidant capacity, liver function and intestinal health, and reshaped the gut microbiota, providing a basis for its application in intensive large yellow croaker culture.

Keywords: *Lactobacillus acidophilus*; *Larimichthys crocea*; lipid deposition; gut microbiota; antioxidant capacity; aquaculture

Key Contribution: Dietary *L. acidophilus* reshaped the gut microbiota by suppressing *Vibrio* and altered body shape and lipid metabolism, lowering muscle lipid and producing a leaner, more elongated body conformation without affecting growth performance in large yellow croaker, providing a theoretical basis for probiotic application in healthy aquaculture.

Check for updates

Academic Editor: Seyed Hossein Hoseinifar

Received: 19 October 2026
Revised: 8 November 2026
Accepted: 9 November 2026
Published: 17 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Under the intensification of aquaculture, probiotics, as beneficial microorganisms that can enhance host health through dietary intake, have become a research focus in aquaculture because of their multiple functions in promoting growth, improving feed utilization, enhancing immunity, and increasing disease resistance [1,2]. Research has demonstrated that probiotics can greatly improve weight gain rates and feed conversion efficiency in fish, suppress pathogen growth by generating antibacterial compounds like bacteriocins, organic acids, and lysozymes, and enhance host nonspecific immune responses [3,4]. In Nile tilapia (*Oreochromis niloticus*), the inclusion of *Lactobacillus plantarum* and various *Bacillus species* in their diet promoted growth, optimized feed utilization, and elevated serum antibacterial and phagocytic activities [5,6]. *Bacillus velezensis* MVCR2 and *Latilactobacillus sakei* RDB2 demonstrated beneficial impacts on growth and *Aeromonas salmonicida* resistance in rainbow trout (*Oncorhynchus mykiss*) [7]. In marine fish, *Bacillus velezensis* NDB alleviated intestinal inflammation in *Acanthopagrus schlegelii* by boosting antioxidant enzyme activity and downregulating proinflammatory factor expression [8]. These studies indicate that probiotics have broad probiotic potential in both freshwater and marine aquaculture species, but the mechanisms of action differ among strains. Among them, *Lactobacillus acidophilus*, a representative species of the genus *Lactobacillus* with a high safety profile and strong colonization ability, has received increasing attention in aquaculture. The primary antibacterial compounds it produces are acidolin, acidophilin, lactacin B, and lactacin F, which demonstrate inhibitory effects on a range of Gram-positive and Gram-negative harmful bacteria [9]. Adding *L. acidophilus* to the diet of common carp (*Cyprinus carpio*) notably enhanced weight gain, growth rate, and feed efficiency, while also improving intestinal villus structure and antioxidant activity [10]. In rainbow trout, feeding a *L. acidophilus*-fermented diet for 60 d increased feed efficiency and intestinal lipase activity and improved intestinal conditions [11]. In *Channa striata*, supplementation with *L. acidophilus* resulted in the highest improvements in weight gain and nutrient use, while significantly enhancing the expression of immune-related genes *TGF-β1* and *NF-κB* [12]. In zebrafish (*Danio rerio*), *L. acidophilus* AC sourced from the intestine of large yellow croaker (*Larimichthys crocea*) boosted both weight gain and specific growth rates while markedly improving hepatic antioxidant and immune enzyme activity [13,14]. These observations reveal that *L. acidophilus* has probiotic effects on fish, including growth promotion, immune enhancement, and stress resistance, suggesting potential for application in aquaculture.

The fish intestine serves as the primary location for nutrient digestion and absorption while also acting as a crucial immune defense against pathogens [15]. In the past few years, research has shed light on the influence of Lactobacillus acidophilus on fish intestines. In rainbow trout, feeding L. acidophilus along with fermented red grape vinegar led to a notable increase in intestinal microvillus size, goblet cell count, lactic acid bacteria levels, and total volatile fatty acid content [16]. Dietary inclusion of *L. acidophilus* in striped catfish (*Pangasianodon hypophthalmus*) significantly boosted intestinal enzyme activities, including amylase, protease, and lipase, and led to longer villi in the anterior and posterior intestines [17]. In juvenile large yellow croaker, heat-treated *L. acidophilus* significantly upregulated the mRNA expression levels of the intestinal tight junction protein genes *zo-1*, *zo-2*, and *occludin*, while suppressing the expression of the proinflammatory gene *tnf-α*, *il-1β*, and *il-8* [18]. In Nile tilapia, fermented feed containing *L. acidophilus* led to taller intestinal villi, more goblet cells, and a decrease in intestinal pathogens [19]. In guppy (*Xiphophorus helleri*), adding L. acidophilus to their diet notably boosted the population of native intestinal lactic acid bacteria and enhanced their tolerance to salinity stress [20]. These results suggest that *L. acidophilus* may further influence fish health and physiological

metabolism by modulating intestinal digestion, the mucosal barrier, and the structure of the microbial community.

Larimichthys crocea is a key marine aquaculture species with significant economic value along China's southeastern coastline, and its production has been steadily rising in recent years [21]. As farming scales continue to expand and intensive aquaculture develops rapidly, related health issues have become more noticeable [22]. Water quality deterioration and persistent stress caused by high-density farming make the intestinal microecology of *L. crocea* prone to imbalance, with reduced microbial diversity and increased abundance of opportunistic pathogens such as *Vibrio* spp., which can further lead to digestive and metabolic disorders and immunosuppression [23]. Prolonged exposure to antimicrobial agents has accelerated the rise in antimicrobial resistance, underscoring the pressing need for safe, effective alternatives to current prevention and control strategies [24]. In practical aquaculture, dietary lipid levels in formulated feeds are generally high in order to achieve rapid growth [25]. Studies indicate that prolonged consumption of high-lipid diets significantly increases hepatic lipid deposition in *L. crocea*, raises circulating concentrations of triglyceride and low-density lipoprotein cholesterol, and induces hepatic expression of proinflammatory genes, together heightening the risk of hepatobiliary syndrome [25,26]. Therefore, exploring feasible strategies to improve hepatic lipid metabolism and reduce metabolic burden in *L. crocea* through nutritional regulation is important for maintaining aquaculture health and the sustainable development within the industry.

In response to industry issues such as intestinal microecological imbalance and increased hepatic metabolic burden in large yellow croaker under intensive aquaculture, this study used juveniles of the "Yongdai No. 1" large yellow croaker as experimental subjects. Through a 28-d feeding trial, we systematically evaluated the regulatory effects of dietary supplementation with *Lactobacillus acidophilus* on growth performance, body shape, body composition, lipid metabolism capacity, liver function, antioxidant capacity, intestinal digestive enzyme activity, inflammatory factor expression, and gut microbiota structure. Unlike existing studies that mostly focus on a single growth or immune indicator, this study conducted a correlation analysis linking gut microbiota remodeling, body condition and physiology, and growth phenotype, and explored the mechanisms by which *L. acidophilus* regulates microbiota diversity, suppresses the enrichment of harmful bacteria, and improves body condition and body shape in *L. crocea*. These findings provide a reference for the application of *L. acidophilus* in healthy aquaculture of *L. crocea* and a theoretical basis for optimizing intensive aquaculture schemes.

2. Materials and Methods

2.1. Experimental Animals

The large yellow croaker used in this experiment were juveniles of the elite strain "Yongdai No. 1", provided by the Ningbo Ocean and Fishery Research Institute. Juveniles with uniform size, healthy body condition, and no injury or disease were selected as experimental subjects, with an initial average body weight of 32.67 ± 3.30 g. Juveniles of the elite strain "Yongdai No. 1" were supplied by the Ocean and Fishery Research Institute in Ningbo and used as the experimental animals. Fish that were uniform in size, sound in body condition, and free from injury or disease were selected, with a mean initial body weight of 32.67 ± 3.30 g.

The *L. acidophilus* strain LA71 was obtained from Wuhan WECARE-Probiotics Research Institute Ltd. (Wuhan, China). Following plate cultivation, a single colony was transferred to modified MRS medium for subculturing, and its identity as *L. acidophilus* was confirmed by 16S rRNA gene sequencing and sequence comparison.

2.2. Experimental Design and Feeding Management

The experimental site was located in the Xiangshan Bay marine cage culture area of the Ningbo Ocean and Fishery Research Institute, Ningbo, Zhejiang Province. A completely randomized design was adopted. Healthy large yellow croaker were randomly distributed into six seawater cages (3.0 m × 3.0 m × 2.0 m), with 90 fish per cage. Two treatment groups were established, each consisting of three biological replicates of 30 individuals. Group C (control) received the basal diet, while group R received the same diet supplemented with *L. acidophilus* culture at 800 mL/kg feed, corresponding to viable counts of $\geq 1 \times 10^8$ CFU/g. The diet was manufactured by Zhejiang Qiangpu Biotechnology Co., Ltd. (Ningbo, China) and had a moisture content of 11%, crude protein of 46%, and crude lipid of 2%.

Water environmental conditions remained stable throughout the rearing period, with temperature at 21.0–24.5 °C, pH 8.0–8.2, dissolved oxygen 8.2–8.9 mg/L, and salinity around 27.9‰. Fish were fed at 05:00 and 16:30 each day, at a daily ration equivalent to 2.5% of body weight, and feeding continued until apparent satiation, i.e., until the fish stopped taking feed. The trial ran for 28 d. The experimental protocol and all animal procedures were approved by the Animal Ethics Committee at Shanghai Ocean University (approval no. SHOU-DW-2023-17).

2.3. Determination of Growth Performance Indices

At the end of the feeding trial, all fish were deprived of feed for 24 h. Nine healthy large yellow croakers were then randomly sampled from each group, anaesthetised with 100 mg/L tricaine methanesulfonate (MS-222, purity ≥ 99%,Sigma-Aldrich, St. Louis, MO, USA), and their body surfaces blotted dry. Body weight, body length, body height and other morphological traits were recorded for the calculation of growth parameters.

Using body weight, body length and body height recorded before and after the experiment, the core growth indices—absolute weight gain, specific growth rate, the condition factor, and the length-to-height ratio—were calculated as follows:

$$\text{Absolute weight gain (g)} = W_2 - W_1$$

$$\text{Specific growth rate (SGR, \%/d)} = (\ln W_2 - \ln W_1)/T \times 100\%$$

$$\text{Condition factor (CF, \%)} = W/L^3 \times 100\%$$

$$\text{Length-to-height ratio} = L/H$$

where W_1 denotes the mean body weight at the start of the trial (g), W_2 the mean body weight at the end (g), ln the natural logarithm, T the duration of rearing in days (d), W the body weight of a single fish (g), L body length (cm), and H body height (cm).

2.4. Determination of Body Composition

Following the feeding trial and 24 h of starvation, three fish were randomly taken from each group. Each fish was anaesthetised and the dorsal muscle dissected out. Whole fish were then collected, and all muscle was removed for the analysis of proximate composition. Moisture, crude protein, crude lipid and crude ash were determined in accordance with the National Food Safety Standards of China: moisture by the direct drying method (GB 5009.3-2016 [27]), crude protein by the Kjeldahl method (GB 5009.5-2016 [28]), crude lipid by the acid hydrolysis method (GB 5009.6-2016 [29]), and crude ash by the muffle furnace ignition method (GB 5009.4-2016 [30]).

2.5. Determination of Serum Antioxidant, Blood Biochemical, and Liver Function Indices

At the end of the trial, following 24 h of starvation, nine large yellow croakers were randomly taken from each group. Blood was drawn from the caudal vein and serum was

separated. Serum biochemical and antioxidant indices were assayed with commercial kits. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, together with the contents of triglyceride (TG), total cholesterol (T-CHO), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were measured with kits supplied by Nanjing Jiancheng Bioengineering Institute. Superoxide dismutase (SOD) and catalase (CAT) activities, total antioxidant capacity (T-AOC) and malondialdehyde (MDA) content were determined with assay kits from Shanghai Beyotime Biotechnology Co., Ltd. (Shanghai, China).

2.6. Determination of Hepatic Lipid Metabolism-Related Enzymes and Lipid Content

At the end of the trial, after 24 h of starvation, nine fish from each group were randomly chosen, anaesthetised and dissected, and liver tissue was collected without delay. Homogenates were prepared in pre-cooled normal saline(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at 1:9 (w/v), then centrifuged at 4 °C and 3000× g for 15 min, and the supernatant was retained at low temperature until assayed. Enzyme activities were quantified in the liver with commercial kits from Nanjing Jiancheng Bioengineering Institute, following the manufacturer's instructions. Assays covered hepatic lipase (HL), lipase (LPS), malate dehydrogenase (MDH), fatty acid synthase (FAS), as well as acetyl-CoA carboxylase (ACC).

2.7. Determination of Intestinal Digestive Enzyme Activities

At the end of the trial, nine fish were randomly taken from each group, anaesthetised and dissected. The intestine was excised and its contents removed. Tissue homogenates were prepared in pre-cooled normal saline at 1:9 (w/v) and centrifuged at 4 °C and 3000× g for 15 min, after which the supernatant was retained. Trypsin (Try) and α-amylase (α-AMY) activities were measured with commercial kits from Nanjing Jiancheng Bioengineering Institute, in strict accordance with the manufacturer's protocol.

2.8. Observation of Intestinal Histomorphology

At the end of the trial, after 24 h without feed, three fish per group were sampled. Following anaesthesia in 100 mg/L MS-222, the midgut was rapidly dissected, rinsed in pre-cooled 0.9% saline to clear residual contents and surface debris, and fixed in 4% paraformaldehyde(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) ahead of paraffin sectioning and histological examination. Routine dehydration, clearing and embedding then yielded serial sections, which were deparaffinised, rehydrated, stained with haematoxylin and eosin (HE, Baso Diagnostics Inc., Zhuhai, China), dehydrated, cleared and mounted in neutral balsam (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Tissue integrity and villus morphology were assessed by light microscopy (Olympus BX53, Olympus Corporation, Tokyo, Japan), and images were recorded so that histological differences could be compared.

2.9. Determination of the Expression of Inflammation- and Lipid Metabolism-Related Genes

At the end of the trial, all fish were deprived of feed for 24 h, and nine individuals per group were randomly removed for sampling. Following anaesthesia in MS-222, blood and liver were collected without delay for the analysis of immune-related gene expression in blood and of lipid-metabolism-related gene expression in liver.

For the liver, the tissue was rapidly dissected free of adherent debris, rinsed three times in pre-cooled 0.9% saline, blotted dry with filter paper(Whatman, Maidstone, UK), and 0.5 g was weighed accurately. Samples were snap-frozen in liquid nitrogen before

storage at −80 °C pending hepatic RNA extraction and quantification of lipid metabolism gene expression.

For the blood, an appropriate volume was drawn from the caudal vein with a sterile syringe into an RNase-free tube(Axygen, Union City, CA, USA) and centrifuged at 4 °C and 3000× *g* for 10 min to separate serum from the cellular fraction. The serum was then harvested, snap-frozen in liquid nitrogen, and kept at −80 °C pending RNA extraction and analysis of inflammation-related gene expression.

2.9.1. RNA Extraction and Purity Detection from Liver and Serum

Total RNA was isolated from 50 mg of liver tissue and from serum. For the liver, tissue was minced on ice, 1 mL of TRIzol(Invitrogen, Carlsbad, CA, USA) was added, and the suspension was homogenised thoroughly and held at room temperature for 5 min to allow cell lysis. For the serum, 1 mL of TRIzol was added after thawing, and the sample was pipetted repeatedly to complete lysis, then left at room temperature for 5 min. In both cases, 0.2 mL of chloroform(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was introduced, and the mixture was shaken vigorously and left to stand for phase separation. Centrifugation at 4 °C and 12,000× *g* for 15 min yielded an upper colourless aqueous phase, which was transferred to a fresh tube. RNA was precipitated by adding an equal volume of pre-chilled isopropanol(Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) together with glycogen(Thermo Fisher Scientific, Waltham, MA, USA), and the pellet was recovered by centrifugation after incubation overnight at −20 °C. This pellet was rinsed with 75% ethanol prepared with DEPC-treated water(Sangon Biotech, Shanghai, China), air-dried at room temperature and redissolved in RNase-free DEPC water, giving the liver and serum RNA preparations. RNA concentration and purity were checked spectrophotometrically(NanoDrop 2000, Thermo Fisher Scientific, Waltham, MA, USA), and samples passing quality control were aliquoted and kept at −80 °C until use.

2.9.2. cDNA Reverse Transcription Synthesis

Following the PrimeScript™ RT reagent kit(Takara Bio Inc., Kusatsu, Japan) protocol, cDNA was generated by reverse transcription with the extracted RNA as template, and the products were kept at −20 °C until use in real-time quantitative PCR (RT-qPCR).

2.9.3. Real-Time Fluorescence Quantitative PCR (qPCR) Detection

Relative transcript levels of hepatic lipid metabolism genes and serum inflammation genes in large yellow croaker were quantified by RT-qPCR, with *β-actin* as the internal reference. Lipid metabolism genes comprised *DGAT2*, *SREBP-1*, *FASN* and *ACC*, as well as *ATGL*, *CPT1*, *LPL* and *FATP1*; inflammation genes comprised *TNF-β*, *IL-6*, *IL-8* and *IL-10*. Cycling conditions began with 10 min at 95 °C for initial denaturation, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s (annealing and extension combined). Relative expression was computed by the $2^{-\Delta\Delta Ct}$ method. Primer design and synthesis were carried out by Shanghai Donghao Biotechnology Co., Ltd. (Shanghai, China), and the sequences are listed in Table 1.

Table 1. Primer sequences of the related genes.

Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Product Size/bp
TNF-β	TGGTGTTTGCTTGGT	TGTTTTCTCGGCAGTCGTCTT	95
IL-6	CAGCTGTTCTCAAGTATGTGG	GTTGTAAATAGTGGGTGTGTCG	182
IL-8	ACAACAAAAGACGCTGAACGGAC	ATGCAAACATGACATGTGAAGTTCT	144
IL-10	AGTCGGTTACTTTCTGTGGTG	TGTATGACGCAATATGGTCTG	144

Table 1. *Cont.*

Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)	Product Size/bp
DGAT2	TTCGGTGCTTTCTGCAACTTCG	AAGGATGGGGAAGCGGAAGT	111
SREBP-1	TCTCCTTGCAGTCTGAGCCAAC	TCAGCCCTTGGATATGAGCCT	94
FASN	TGGGCAATAGCAATCCAGGT	TCAAGGGGAGCATCTTTCGT	271
ACC	TGTCGGAGGAGAACTCGGAAG	GCCTTAGATTCTGCACGGGG	156
ATGL	CCATGCATCCGTCCTTCAACC	GAGATCCCTAACCGCCCACT	103
CPT1	GCTGAGCCTGGTGAAGATGTTC	TCCATTTGGTTGAATTGTTTACTGTCC	159
LPL	CCGGCAGCCCTGAAACCATTA	TGCTGGTTGGCACGAGTCAG	187
FATP1	CAACCAGCAGGACCCATTACG	CATCCATCACCAGCACATCACC	131
β-actin	ATTGCCCCACCTGAGCGTAAA	GTTGGGTGTTTGGTTGGGGAGG	183

2.10. Intestinal Microbial Diversity Analysis

At the end of the feeding trial, following 24 h of starvation, six healthy individuals were randomly taken from each group. Fish were anaesthetised with MS-222, and the intestine was dissected out, snap-frozen in liquid nitrogen and kept at −80 °C. Total genomic DNA was extracted by the CTAB method and checked on a 1% agarose gel. Barcoded, region-specific primers were synthesised for the target sequencing region, and amplification was run on an ABI GeneAmp® 9700 with TransGen AP221-02 (TransStart® FastPfu DNA Polymerase, TransGen Biotech, Beijing, China), using a reduced cycle number that was kept identical across all samples. Each sample was amplified in triplicate; the products were pooled, resolved on a 2% agarose gel, excised and recovered with an AxyPrep DNA gel extraction kit(Axygen, Union City, CA, USA), eluted in Tris-HCl, and re-checked by electrophoresis. On the basis of these gels, products were quantified on a QuantiFluor™-ST fluorometer, Promega Corporation, Madison, WI, USA and pooled in the proportions required by the sequencing depth. Illumina adapters were appended to the ends of the target region with a TruSeq™ DNA Sample Prep Kit(Illumina Inc., San Diego, CA, USA). Following recovery, elution, electrophoretic verification and denaturation into single strands with NaOH, the Illumina library was completed. Sequencing involved immobilising DNA fragments on the flow cell, generating clusters by bridge PCR, linearising them into single strands, and then reading bases through single-base extension with a modified polymerase and four fluorescently labelled dNTPs under laser scanning. After assembly, quality control filtering, and redundancy removal of the raw data, OTU clustering and taxonomic annotation were performed at 97% similarity. Alpha diversity, PCA and PCoA beta diversity with ANOSIM, and Kruskal–Wallis analysis of differential microbiota between groups were conducted. LEfSe (LDA threshold 2) was used to screen differential microbial species between groups. Based on PICRUSt2 and the KEGG database, first- and second-level functional pathways and KO genes of the intestinal microbiota were predicted and compared between groups.

2.11. Data Analysis

Data were analysed in Minitab 17.0 and are reported as mean ± standard deviation (SD). Normality was assessed with the Anderson–Darling test and homogeneity of variances with Levene's test. Differences between the control and R groups were examined by Student's *t*-test, with $p < 0.05$ taken as the threshold for significance.

3. Results

3.1. Effects of Dietary L. acidophilus on Growth-Related Phenotypes and Body Shape Traits in Large Yellow Croaker

After 28 d of feeding (Table 2), growth performance was unaffected by dietary *L. acidophilus* supplementation ($p > 0.05$); under the present experimental conditions, the strain therefore neither promoted nor restricted growth of large yellow croaker.

Table 2. Effects of dietary *L. acidophilus* on growth of large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation.

Index	C Group	R Group	t	*p*
Growth performance				
Average body weight (g)	55.16 ± 2.53	49.55 ± 4.56	1.865	0.136
Absolute weight gain (g)	22.49 ± 2.53	16.88 ± 4.56	1.865	0.136
Weight gain rate (%)	68.84 ± 7.75	51.66 ± 13.95	1.865	0.136
Specific growth rate (%/d)	1.83 ± 0.16	1.45 ± 0.32	1.854	0.137
Body shape indices				
Body length (cm)	18.02 ± 0.59	18.38 ± 0.66	−0.709	0.518
Body height (cm)	4.47 ± 0.10	4.28 ± 0.07	2.813	0.048
Length-to-height ratio	4.04 ± 0.10	4.30 ± 0.10	−3.191	0.033
Condition factor	0.95 ± 0.05	0.81 ± 0.07	3.004	0.040

For body shape, supplementation left body length unchanged ($p > 0.05$), whereas body height fell relative to the control ($p < 0.05$). The two derived indices diverged accordingly: the length-to-height ratio rose in the supplemented group ($p < 0.05$), while the condition factor declined ($p < 0.05$).

Taken together, these shifts describe a change in body conformation rather than in growth rate: fish receiving *L. acidophilus* maintained their growth trajectory yet acquired a slimmer, less plump profile, an outcome traceable to the shorter body height and the accompanying rise in the length-to-height ratio.

3.2. Effects of Dietary L. acidophilus on Whole-Body Composition in Large Yellow Croaker

As shown in Table 3, feeding diets supplemented with *L. acidophilus* significantly altered the body composition of large yellow croaker. Relative to controls, fish fed *L. acidophilus* carried less body lipid, moisture and ash ($p < 0.05$); crude protein, however, rose slightly without reaching significance ($p > 0.05$).

Table 3. Effects of *L. acidophilus* on body composition of large yellow croaker ($n = 3$, g/100 g). Data are presented as mean ± standard deviation. Within the same row, different superscript letters indicate statistically significant differences among groups ($p < 0.05$).

Item	C Group	R Group
Crude protein	17.2 ± 0.3	17.7 ± 0.4
Lipid	2.7 ± 0.2 [a]	2.2 ± 0.1 [b]
Moisture	78.2 ± 0.3 [a]	77.3 ± 0.4 [b]
Ash	2.6 ± 0.1 [a]	1.8 ± 0.1 [b]

3.3. Effects of Dietary L. acidophilus on Serum Lipid Contents in Large Yellow Croaker

Serum lipid profiles of large yellow croaker shifted markedly in fish fed a diet supplemented with *L. acidophilus* (Figure 1). All four analytes declined relative to controls: TG ($p < 0.001$), T-CHO ($p < 0.0001$), HDL-C ($p < 0.05$), and LDL-C ($p < 0.001$).

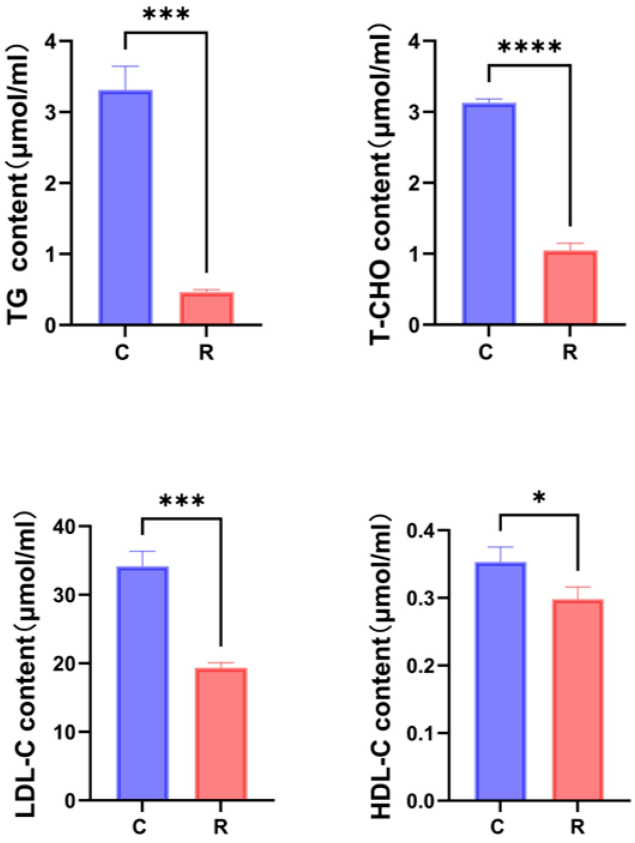


Figure 1. Serum lipid contents of TG, T-CHO, HDL-C, and LDL-C in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. TG, triglyceride; T-CHO, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. Compared with the control group, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

3.4. Effects of Dietary L. acidophilus on Serum Liver Function-Related Indices in Large Yellow Croaker

Serum liver-function markers of large yellow croaker responded to *L. acidophilus* supplementation (Figure 2). Relative to controls, both ALT ($p < 0.05$) and AST ($p < 0.0001$) activities declined.

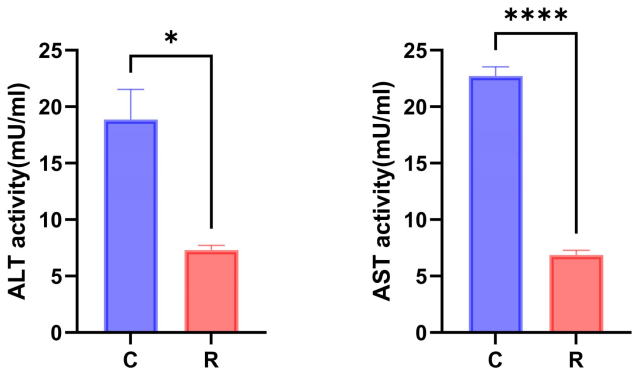


Figure 2. Serum ALT and AST activities in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. ALT, alanine aminotransferase; AST, aspartate aminotransferase. Compared with the control group, * $p < 0.05$ and **** $p < 0.0001$.

3.5. Effects of Dietary *L. acidophilus* on Serum Antioxidant Indices in Large Yellow Croaker

Antioxidant defences of large yellow croaker were reinforced by *L. acidophilus* supplementation (Figure 3). Relative to controls, SOD and CAT activities, together with T-AOC, rose ($p < 0.01$), whereas MDA dropped ($p < 0.0001$).

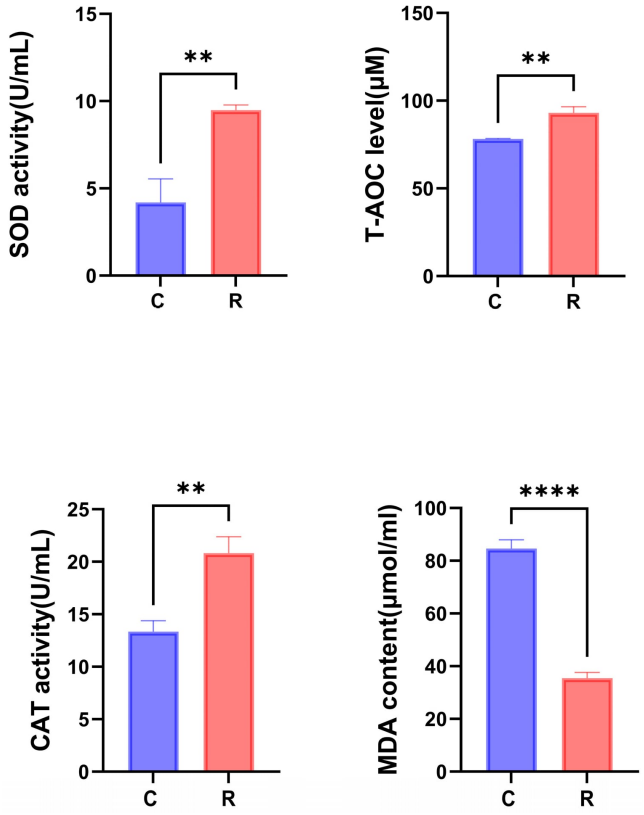


Figure 3. Serum T-AOC, SOD, and CAT activities and MDA content in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. SOD, superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity; MDA, malondialdehyde. Compared with the control group, ** $p < 0.01$ and **** $p < 0.0001$.

3.6. Effects of Dietary *L. acidophilus* on Hepatic Lipid Metabolism-Related Enzyme Activities in Large Yellow Croaker

Figure 4 shows the results of hepatic lipid metabolism-related enzyme activities in each group. Compared with the control group, hepatic ACC and FAS activities in the experimental group were significantly decreased ($p < 0.001$), MDH activity was significantly decreased ($p < 0.01$), and LPS and HL activities were significantly increased ($p < 0.01$).

3.7. Effects of Dietary *L. acidophilus* on Genes Related to Lipid Synthesis and Metabolism in Large Yellow Croaker

Hepatic mRNA levels of lipid-metabolism genes split in large yellow croaker fed *L. acidophilus* (Figure 5). Compared with the control group, the mRNA expression of *DGAT2*, *SREBP-1*, *ACC*, and *FASN* in the experimental group was significantly decreased ($p < 0.05$); the mRNA expression of *LPL* and *ATGL* was significantly increased ($p < 0.05$); *CPT1* expression was decreased, but the difference between groups was not significant ($p > 0.05$); *FATP1* expression was increased, but the difference between groups was not significant ($p > 0.05$).

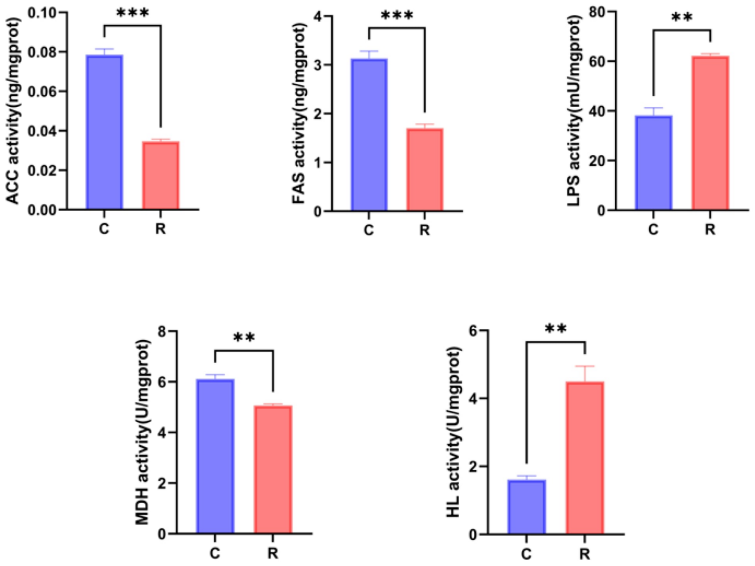


Figure 4. Hepatic ACC, FAS, LPS, MDH, and HL enzyme activities in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; LPS, lipase; MDH, malate dehydrogenase; HL, hepatic lipase. Compared with the control group, ** $p < 0.01$ and *** $p < 0.001$.

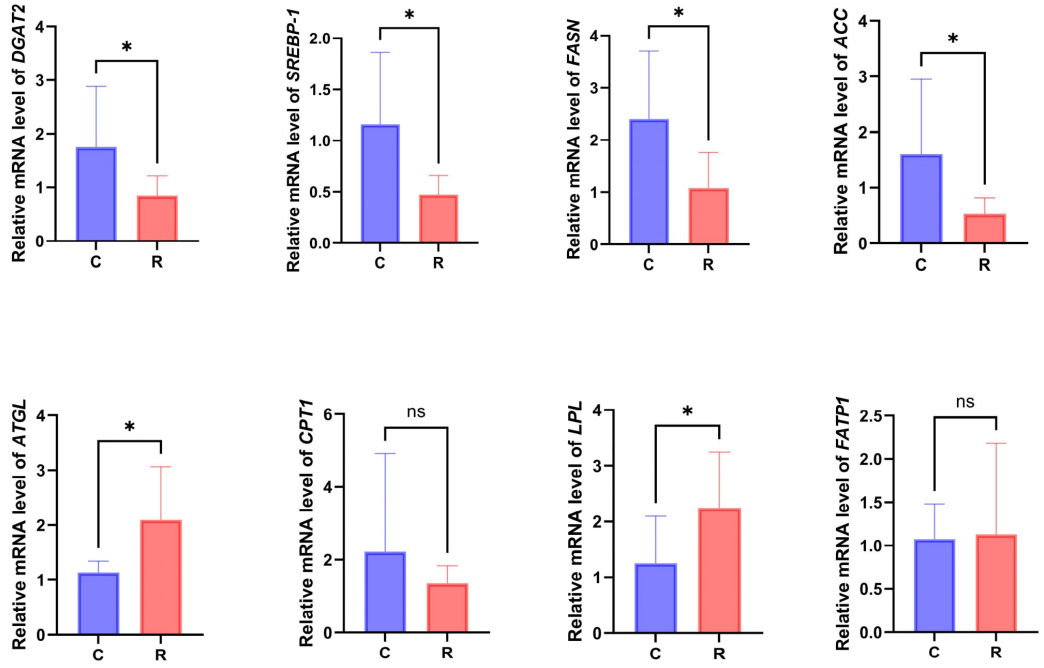


Figure 5. Hepatic mRNA expression levels of lipid metabolism-related genes in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. Compared with the control group, * $p < 0.05$; ns, not significant.

3.8. Effects of Dietary L. acidophilus on Serum Inflammation-Related Gene Expression in Large Yellow Croaker

Serum cytokine transcripts in large yellow croaker fed *L. acidophilus* showed a broadly anti-inflammatory pattern (Figure 6). Pro-inflammatory *TNF-β* and *IL-8* were downregulated ($p < 0.05$); *IL-6* also trended lower, whereas anti-inflammatory *IL-10* trended higher, though neither change was significant ($p > 0.05$).

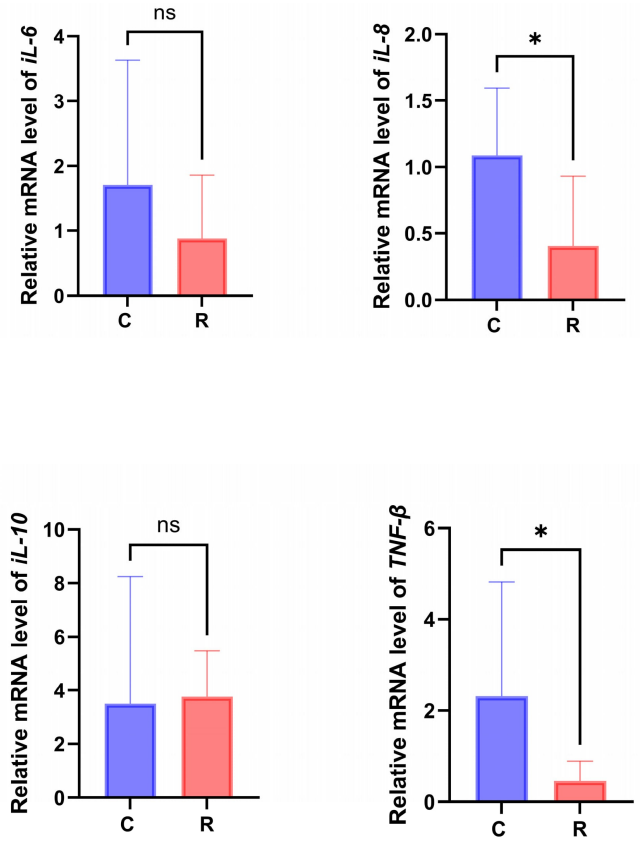


Figure 6. Serum mRNA expression levels of inflammation-related genes in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. Compared with the control group, * $p < 0.05$; ns, not significant.

3.9. Effects of Dietary L. acidophilus on Intestinal Digestive Enzyme Activities in Large Yellow Croaker

As shown in Figure 7, feeding diets supplemented with *L. acidophilus* significantly affected intestinal digestive enzyme activities in large yellow croaker. Compared with the control group, after dietary supplementation with *L. acidophilus*, Try activity was significantly decreased ($p < 0.01$), while intestinal α-AMY activity was significantly increased ($p < 0.0001$).

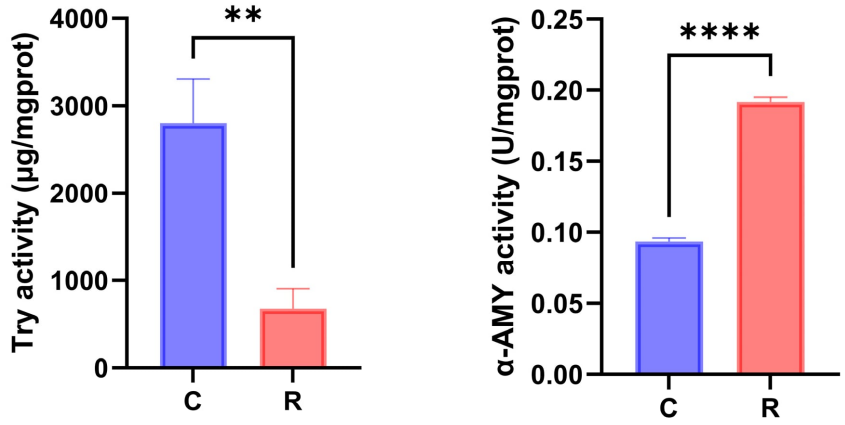


Figure 7. Intestinal Try and α-AMY activities in large yellow croaker ($n = 3$). Data are presented as mean ± standard deviation. C, control group; R, *L. acidophilus* supplementation group. Try, trypsin; α-AMY, α-amylase. Compared with the control group, ** $p < 0.01$ and **** $p < 0.0001$.

3.10. Effects of Dietary L. acidophilus on Intestinal Histomorphology in Large Yellow Croaker

Both groups displayed normal intestinal architecture with no histological lesions (Figure 8). Villi, epithelium and muscle layer remained intact across treatments, with no villus erosion, epithelial desquamation or inflammatory infiltrate. Relative to controls, large yellow croaker in experimental groups showed taller villi and a thicker muscle layer, consistent with enhanced mucosal growth.

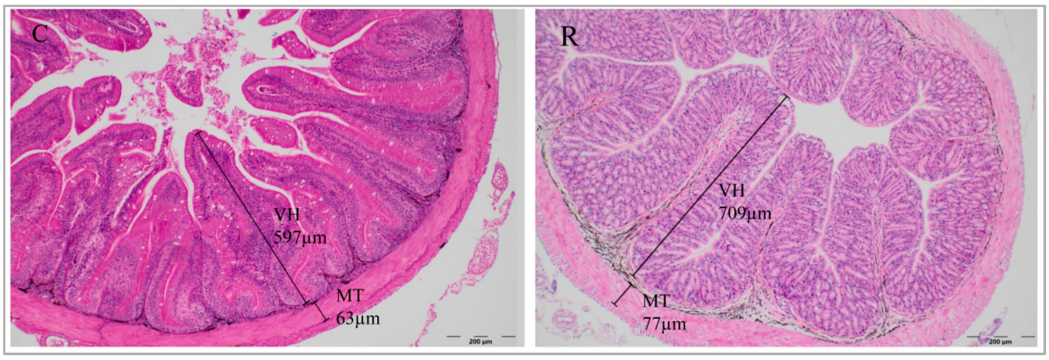


Figure 8. HE staining of intestinal tissue in large yellow croaker (10×) ($n = 3$). C, control group; R, *L. acidophilus* supplementation group. VH, intestinal villus height; MT, muscle layer thickness.

3.11. Gut Microbial Diversity Analysis

3.11.1. Sequencing Results and Alpha Diversity Analysis

Quality control retained valid sequences from 12 intestinal samples of large yellow croaker across control and L. acidophilus groups. Clustering at 97% similarity produced 1338 OTUs, assigned to 37 phyla, 100 classes, 227 orders, 378 families, 697 genera and 992 species (Table 4). Coverage exceeded 0.99 in both groups, confirming adequate sequencing depth. There were differences in the Sobs, Shannon, Simpson, ACE, and Chao indices between the control and experimental groups, although these differences did not reach statistical significance ($p > 0.05$): Compared with the control group, the experimental group showed a slight decrease in the Sobs index, increases in the Shannon, ACE, and Chao indices, and a decrease in the Simpson index.

Table 4. Alpha diversity indices of gut microbiota in large yellow croaker.

Group	Sobs	Shannon	Simpson	Ace	Chao	Coverage	Qstat
C group	184.7 ± 33.72	1.425 ± 0.3698	0.4565 ± 0.1432	218.3 ± 61.05	211.1 ± 70.54	0.9994 ± 0.0004347	63.74 ± 25.61
R group	144.9 ± 59.15	1.929 ± 0.6108	0.4161 ± 0.05761	317.9 ± 135.6	321.5 ± 137.1	0.9995 ± 0.0004188	102.5 ± 47.98

3.11.2. Beta Diversity Analysis

Beta diversity of the gut microbiota was examined by PCA, PCoA and within-group distance analysis on the genus-level abundance matrix (Figure 9). In the PCA plot, PC1 and PC2 accounted for 21.15% and 19.13% of variation; in the PCoA plot, the two axes together accounted for 94.69%. Control and experimental samples overlapped in both ordinations, with no clear separation. Within-group dispersion was broader in controls than in the *L. acidophilus* group (Figure 9C), and ANOSIM with 999 permutations confirmed no significant group difference ($p > 0.05$). The *L. acidophilus* thus shifted the gut microbiota structure of large yellow croaker, although the shift did not reach statistical significance.

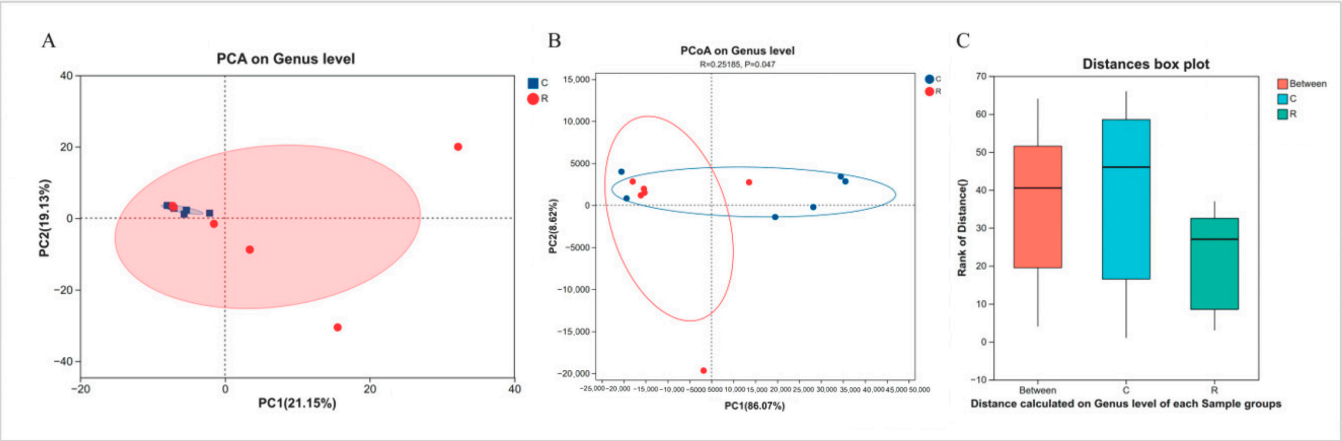


Figure 9. Analysis of genus-level differences in the gut microbiota of large yellow croaker. C, control group; R, *L. acidophilus* supplementation group. (**A**) PCA at the genus level. Blue squares represent group C, and red circles represent group R. Ellipses indicate the 95% confidence intervals for each group. PC1 and PC2 explain 21.15% and 19.13% of the total variance, respectively. (**B**) PCoA at the genus level based on Bray–Curtis distance. Blue dots represent group C, and red dots represent group R. Ellipses indicate the 95% confidence intervals for each group. PC1 and PC2 explain 86.07% and 8.62% of the total variance, respectively. (**C**) Box plot of within- and between-group distances at the genus level. The *y*-axis represents the rank of distance; Between, distances between the two groups; C, within-group distances of group C; R, within-group distances of group R.

3.11.3. Community Composition at the Phylum and Genus Levels

At the phylum level, Proteobacteria, Desulfobacterota and Firmicutes dominated the intestinal microbiota of large yellow croaker (Figure 10A). Proteobacteria was the leading phylum in both groups, rising from 75.92% in controls to 85.79% in the *L. acidophilus* group; Desulfobacterota accounted for 14.51% in controls, whereas Firmicutes reached 6.34% in the probiotic group.

At the genus level, *Achromobacter*, *Photobacterium* and *Lawsonia* were the most abundant genera (Figure 10B). In controls, *Achromobacter* (32.19%) and *Photobacterium* (30.59%) were co-dominant (Figure 10C). In the *L. acidophilus* group, *Achromobacter* reached 49.67% as the single leading genus (Figure 10D).

3.11.4. Differential Species Between Groups and LEfSe Analysis

Differential abundance analysis of the gut microbiota of large yellow croaker identified one phylum and ten genera that responded to *L. acidophilus* (Figure 11). At phylum level, Fusobacteriota declined in the *L. acidophilus* group ($p < 0.05$). At genus level, ten taxa were enriched: *norank_f__Caldilineaceae*, *Ellin6067*, *norank_f__Methyloligellaceae*, *Thioalkalivibrio*, *Hyphomicrobium*, *Mesorhizobium*, *Holdemanella*, *Variovorax*, *Pelomonas* and *Aureimonas* ($p < 0.05$).

LEfSe analysis (LDA threshold = 2) further screened the significantly differential microbial taxa across multiple taxonomic levels, and the cladogram in Figure 11C showed that the control group was enriched in Fusobacteriota and its subordinate taxa (Fusobacteriia, Fusobacteriales, Fusobacteriaceae, and *Vibrio*), while the experimental group was enriched in Deinococci, Deinococcaceae, *Deinococcus*, Xanthobacteraceae, Comamonadaceae, *Pelomonas*, *Qipengyuania*, *Variovorax*, Intrasporangiaceae, unclassified_o_Bacillales, and other taxa. Correspondingly, the LEfSe bar plot in Figure 11D further confirmed these differential taxa by LDA scores, with the control-enriched taxa showing positive LDA scores and the experimental-enriched taxa showing negative LDA scores, indicating that *L. acidophilus* supplementation mainly restructured specific microbial taxa.

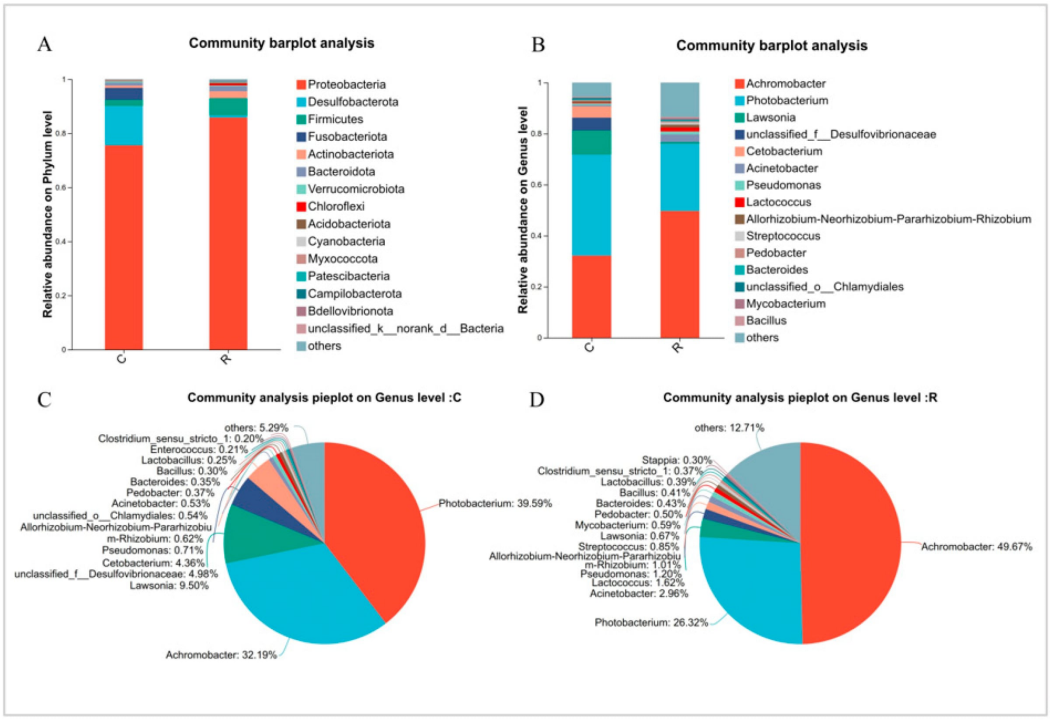


Figure 10. Community composition analysis of gut microbiota in large yellow croaker at the phylum and genus levels. C, control group; R, *L. acidophilus* supplementation group. (**A**) bar chart of phylum-level relative abundance in the two groups; (**B**) bar chart of genus-level relative abundance in the two groups; (**C**) pie chart of genus-level community composition in the control group; (**D**) pie chart of genus-level community composition in the *L. acidophilus* supplementation group. $n = 6$ per group. Data are presented as relative abundance (%).

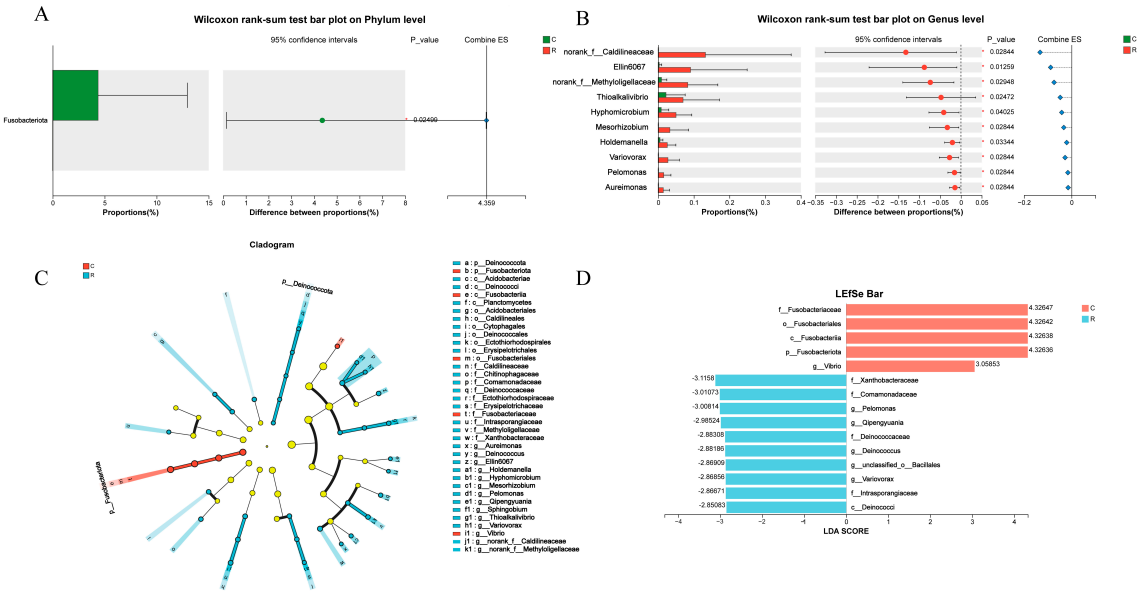


Figure 11. Differential microbial taxa and LEfSe analysis of gut microbiota in large yellow croaker. C, control group; R, *L. acidophilus* supplementation group. (**A**) Wilcoxon rank-sum test bar plot at the phylum level. (**B**) Wilcoxon rank-sum test bar plot at the genus level. (**C**) LEfSe cladogram showing differentially enriched taxa from the phylum to genus level. (**D**) LEfSe bar plot showing taxa with significant LDA scores. $n = 6$ per group.

3.11.5. PICRUSt2 Functional Prediction Analysis

PICRUSt2 against the KEGG database predicted the functional potential of the gut microbiota of large yellow croaker. At KEGG level 1, six functional categories were identified, with Metabolism dominating and the remainder ranking as Environmental Information Processing, Cellular Processes, Genetic Information Processing, Human Diseases and Organismal Systems; overall, level 1 profiles were comparable between groups (Figure 12A).

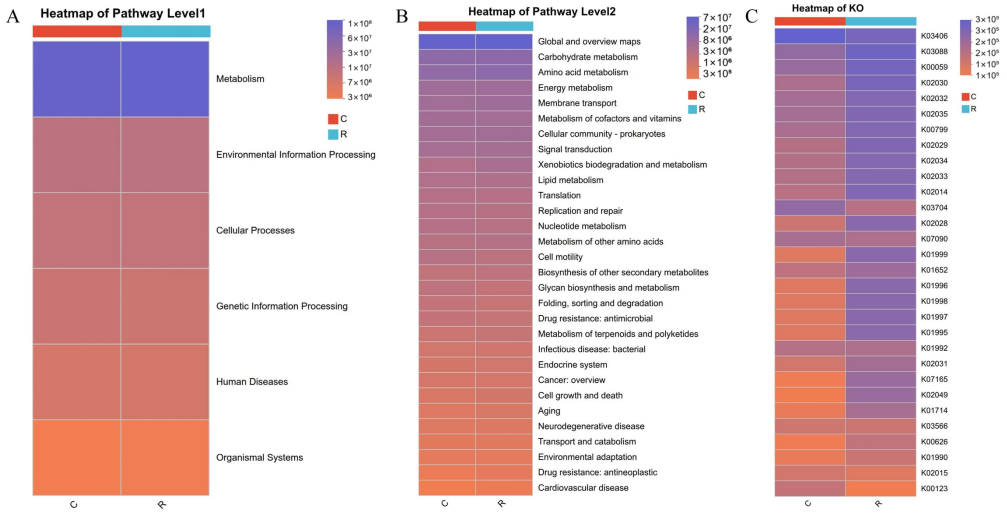


Figure 12. Functional prediction of gut microbiota in large yellow croaker based on the KEGG database. C, control group; R, *L. acidophilus* supplementation group. (**A**) Heatmap of KEGG level 1 pathway abundances in the control (C) and *L. acidophilus* supplementation (R) groups. (**B**) Heatmap of KEGG level 2 pathway abundances in the C and R groups. (**C**) Heatmap of KO abundances in the C and R groups. $n = 6$ per group. Color intensity represents the relative abundance of KEGG pathways or KO genes.

At level 2, global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, membrane transport and metabolism of cofactors and vitamins were the most abundant pathways, followed by cellular community—prokaryotes, signal transduction, xenobiotics biodegradation and metabolism, lipid metabolism, translation and replication and repair; again, profiles overlapped between control and L. acidophilus groups (Figure 12B).

At the KO level, the probiotic group enriched genes for the ABC transport system (K03088, K00059, K02030, K02032, K02035, K00799, K02029, K02034, K02033, K02014), formate dehydrogenase (K00123) and other substrate transporters, whereas K03704 and K02028 were more abundant in controls (Figure 12C).

4. Discussion

Lactobacillus acidophilus has been reported to benefit farmed fish through enhanced growth, elevated immune defence and a more robust intestinal barrier. Yet direct evidence on how these functions operate in large yellow croaker remains scarce. The present 28-day feeding trial therefore showed that dietary supplementation reoriented lipid metabolism in juveniles: hepatic lipogenesis was suppressed while lipolysis was stimulated, reducing tissue lipid accumulation. Body conformation shifted toward a leaner profile, antioxidant defenses strengthened, and the gut community was restructured—putative beneficial taxa were enriched while opportunistic/pathogenic taxa were held in check.

4.1. Effects of Dietary L. acidophilus on Growth Performance and Body Shape of Large Yellow Croaker

Probiotics are commonly credited with accelerating growth in fish [1]. However, consistent with the results, 28 d of dietary *L. acidophilus* supplementation left growth performance of large yellow croaker unaffected: final body weight, weight gain rate, absolute weight gain and specific growth rate all trended downward in the supplemented group without reaching significance, so the strain neither promoted nor restricted growth under these conditions. Similarly, in juvenile common carp, dietary supplementation with *L. acidophilus* (6×10^9 CFU/kg) for 30 d did not significantly affect growth performance or survival [31]. In sliver prussian carp (*Carassius auratus gibelio*), after feeding diets containing *L. acidophilus* (1.5×10^8–6×10^8 CFU/g) for 8 weeks, growth performance did not change significantly at any supplementation level ($p > 0.05$) [32]. And in large yellow croaker given *L. acidophilus* AC for 56 d, specific growth rate was likewise unchanged across doses, although the viscerosomatic index fell in the high-dose group and muscle lipid declined in some groups [33]. Read together, these findings indicate that growth promotion is probably not the primary probiotic function of *L. acidophilus* in all fishes. Notably, the resulting slimmer body conformation—lower condition factor coupled with a higher length-to-height ratio—aligns with the premium grading criteria for large yellow croaker (SC/T 3123—2022, Ministry of Agriculture and Rural Affairs of the People's Republic of China), under which top-grade fish require a condition factor ≤ 1.4 and a length-to-height ratio ≥ 3.8; such leaner fish also carry smaller abdominal fat depots and firmer muscle, giving this phenotype direct production value despite no measurable gain in growth.

Combined with the simultaneous decreases in muscle crude lipid content and serum lipid indices, it can be considered that *L. acidophilus* reshaped body shape by inhibiting lipid deposition. Similarly, a comparable outcome was described by Li et al. [34] in Nile tilapia: after 8 weeks of dietary *L. plantarum*, condition factor, hepatosomatic index, mesenteric fat index, hepatic lipid droplet content and both hepatic and serum triglycerides all fell significantly, alongside transcriptional suppression of lipogenic genes and induction of lipolytic and β-oxidation genes. A gnotobiotic zebrafish model further showed that mono-association with *L. plantarum* suppressed lipogenesis and stimulated lipolysis. The lipid-regulating effect of lactobacilli therefore appears strain-specific in magnitude but consistent in direction, and the same mechanism is plausibly a core function of *L. acidophilus* in large yellow croaker.

4.2. Improving Effects of Dietary L. acidophilus on Lipid Metabolism and Liver Function in Large Yellow Croaker

Regulatory roles of *lactobacilli* in lipid metabolism and hepatic health have been documented across multiple farmed fish. Wang et al. [35] reported that after adding *Lacticaseibacillus casei* K17 to largemouth bass (*Micropterus salmoides*) and observed pronounced drops in serum and hepatic total cholesterol, triglyceride and low-density lipoprotein cholesterol, alongside a rise in high-density lipoprotein cholesterol—an unequivocal hypolipidaemic response. Our data follow the same direction for TG, T-CHO and LDL-C, with serum HDL-C also reduced in the present trial. In a comparable largemouth bass trial, Yang et al. [36] reported that a fermented blend of *L. acidophilus*, *Limosilactobacillus reuteri* and *L. plantarum* lowered serum ALT, mirroring the sharp falls in both ALT and AST we observed in the *L. acidophilus* group. Since ALT and AST are established markers of hepatocellular injury [37], and their decreased activities suggest that *L. acidophilus* supplementation effectively alleviated hepatic metabolic burden and cell injury and improved liver function.

At the enzyme activity level, this study found that lipase (LPS) and hepatic lipase (HL) activities were highly significantly increased in the *L. acidophilus* group, while ACC and FAS activities were highly significantly decreased, and MDH activity was significantly decreased. Similarly, Guo et al. [38] found that *Lactococcus lactis* L19 significantly inhibited hepatic ACC and FAS activities induced by a high-carbohydrate diet in northern snakehead (*Channa argus*) and promoted CPT1 and HSL activities, suggesting that lactic acid bacteria curb hepatic lipid accumulation by suppressing lipogenesis and favouring lipolysis. LPS and HL are key enzymes that promote lipid hydrolysis [39], and their increased activities are conducive to accelerating triglyceride decomposition; ACC and FAS are rate-limiting steps in de novo fatty acid synthesis, so their suppression points to an inhibited hepatic lipogenic programme [40]. MDH, in turn, fuels the TCA cycle and supplies NADPH for fatty acid synthesis [41]. Qiao et al. [42] likewise recorded a fall in hepatic MDH in turbot (*Scophthalmus maximus*) fed the postbiotic *Lysinibacillus macroides* G117, aligning with our own decrease. Taken together, the drop in MDH likely reflects a resetting of TCA flux and a narrower NADPH supply, further constraining lipogenesis. Dietary *L. acidophilus* therefore appears to have curtailed hepatic lipogenesis, accelerated lipid breakdown and lessened lipid deposition in large yellow croaker.

Transcript profiling reinforced the enzyme-level picture: lipogenic transcripts (*DGAT2*, *SREBP-1*, *ACC*, *FASN*) were downregulated, whereas lipolytic transcripts *LPL* and *ATGL* were upregulated in the probiotic group; *CPT1* expression was slightly decreased and *FATP1* expression was slightly increased, but the differences between groups were not significant. *SREBP-1* sits at the top of the fatty-acid and triglyceride synthesis cascade [43], and its downregulation can inhibit the entire lipid synthesis pathway from upstream; *DGAT2*, the terminal enzyme of triglyceride synthesis, is itself a direct *SREBP-1* target [44]. In this study, *DGAT2* and *SREBP-1* were synchronously downregulated, suggesting that hepatic triglyceride synthesis in the experimental group was inhibited at the transcriptional level. *ACC* and *FASN* are the committed and terminal enzymes of de novo fatty-acid synthesis [45] and their synchronous downregulation further suggests that the fatty acid synthesis pathway was overall inhibited, which corroborates the decreases in ACC and FAS activities at the enzyme activity level. Increased *LPL* expression is conducive to promoting the hydrolysis of triglycerides in lipoproteins and accelerating fatty acid transport to tissues for oxidative utilization [46], aligning with the lower serum TG and LDL-C. *ATGL* is the main triglyceride lipase of cytosolic lipid droplets [47], and its upregulated expression suggests enhanced lipolysis of hepatic lipid droplets, which is conducive to reducing hepatic lipid accumulation and is consistent with the decreased crude lipid content.

Our transcript-level observations line up with prior accounts of *lactobacilli*-driven lipid control in fish. The downward trend of *SREBP-1* and *FASN* here mirrors Chen et al. [48], in which *Limosilactobacillus fermentum* E15 suppressed *SREBP-1*, *PPAR-γ*, and *Fasn* expression in a high-fat diet model of zebrafish. Similarly, Shi et al. [49] reported in hybrid grouper that *L. plantarum* 58 upregulated the expression of hepatic *ATGL*, *CPT-1*, *FXR*, and *LPL* while inhibiting the expression of *FAS* and *SCD-1*. In common carp, the *Lacticaseibacillus rhamnosus* GCC-3 fermented product likewise lowered hepatic lipid accumulation and induced the lipolytic gene *cpt1* [50].

In summary, *L. acidophilus* acts on hepatic lipid metabolism at the transcript level by repressing the lipogenic programme and inducing genes for lipid hydrolysis and transport. Combined with the results at the enzyme activity level, namely decreased ACC and FAS activities and increased LPS and HL activities, these effects jointly resulted in reduced serum lipid content and reduced body crude lipid content.

4.3. Effects of Dietary L. acidophilus on Anti-Inflammatory and Antioxidant Capacity of Large Yellow Croaker

Dietary *L. acidophilus* alleviated the inflammatory response and enhanced the antioxidant capacity of large yellow croaker. In terms of inflammation regulation, the expression of blood proinflammatory factors *TNF-β* and *IL-8* was significantly downregulated, suggesting that it effectively suppressed systemic inflammation. This direction agrees with He et al. [18], where heat-inactivated *L. acidophilus* suppressed intestinal *tnf-α*, *il-1β* and *il-8* while raising *il-10* and *tgf-β* in large yellow croaker; with Su et al. [51], who showed that *L. acidophilus*-fermented *Sargassum fusiforme* polysaccharides lowered pro-inflammatory cytokines, raised anti-inflammatory cytokines and strengthened the gut barrier in a zebrafish enteritis model; and with Yan et al. [52], who reported that *L. acidophilus* KLDS1.0901 lowered *IL-8*, *TNF-α* and *IL-1β* and repaired the gut barrier in mice. On the oxidative side, serum SOD, CAT and T-AOC all climbed in the probiotic group while MDA fell, indicating an activated antioxidant network and relieved lipid peroxidation. The same pattern has been documented in juvenile common carp [53], Nile tilapia [54] and zebrafish [14], where *L. acidophilus* raised antioxidant enzyme activities and lowered MDA. Together, these findings support a protective role of dietary *L. acidophilus* through combined anti-inflammatory and antioxidant action in large yellow croaker.

4.4. Remodeling Effects of Dietary L. acidophilus on Gut Microbiota Structure of Large Yellow Croaker

The intestine is the principal site of digestion and absorption in fish, and the resident microbiota engages in bidirectional crosstalk with the host; its stability shapes growth and health [15].

On the digestive-enzyme front, dietary *L. acidophilus* raised intestinal α-amylase while lowering trypsin in large yellow croaker. The increase in α-amylase activity helps promote carbohydrate digestion and may provide more energy substrates for the organism [53]; the decrease in trypsin activity may be related to the reduction in intestinal pH caused by organic acids secreted by *L. acidophilus*, which in turn affects the activity of trypsin [55]. This suggests that the effect of *L. acidophilus* on intestinal digestive enzymes is enzyme-selective, and its overall effect may be to optimize the intestinal digestion and absorption pattern, making the organism more inclined to use carbohydrates rather than proteins as an energy source, thereby saving protein for growth and tissue repair. Together with the taller midgut villi and thicker muscle layer seen histologically, this pattern points to a strengthened intestinal barrier and some degree of mucosal protection in *L. acidophilus*-fed large yellow croaker.

L. acidophilus supplementation restructured specific microbial taxa rather than increasing diversity on a large scale. Proteobacteria dominated the community in both groups, echoing Zhu et al. [56], who likewise identified Proteobacteria as the leading phylum in the large yellow croaker gut. LEfSe analysis further confirmed that the intestinal tract of large yellow croaker in the control group was enriched with Fusobacteriota, Fusobacteriia, Fusobacteriales, Fusobacteriaceae, and *Vibrio*, while the intestinal tract of large yellow croaker in the experimental group was enriched with Deinococci, Deinococcaceae, *Deinococcus*, Xanthobacteraceae, Comamonadaceae, *Pelomonas*, *Qipengyuania*, *Variovorax*, Intrasporangiaceae, and other microbial taxa. At the genus level, *Vibrio* is a common opportunistic pathogen in aquaculture [57], and its reduced abundance is conducive to reducing the risk of intestinal infection and maintaining intestinal health. The relative abundance of Achromobacter increased from 32.19% to 49.67%, becoming the predominant genus. Although Achromobacter is generally regarded as an opportunistic pathogen in immunocompromised humans [58], strains isolated from the fish intestine have also been reported to degrade

phthalic acid esters, suggesting possible environmental functions [59]. In largemouth bass, *L. acidophilus* also exhibited microbiota remodeling characteristics of directionally enriching potential beneficial bacteria and inhibiting opportunistic pathogens [36]. In this study, the abundances of *norank_f__Caldilineaceae*, *Ellin6067*, *norank_f__Methyloligellaceae*, *Thioalkalivibrio*, *Hyphomicrobium*, *Mesorhizobium*, *Holdemanella*, *Variovorax*, *Pelomonas*, and *Aureimonas* all rose significantly in the probiotic group. These genera are mostly involved in metabolic processes such as nitrogen cycling, organic matter degradation, and short-chain fatty acid production [60–63], and their enrichment may help enhance intestinal metabolic function and microecological stability.

PICRUSt2 predicted that dietary L. acidophilus left KEGG level-2 pathway profiles of the large yellow croaker gut community largely unchanged, yet the KO-level comparison pointed to an enriched signal in the R group: ABC transport system (K03088, K00059, K02030, K02032, K02035, K00799, K02029, K02034, K02033, K02014), formate dehydrogenase gene (K00123) and assorted substrate transporters all rose relative to controls. ABC transporters are responsible for the uptake and transport of nutrients (carbohydrates, amino acids) [64], and formate dehydrogenase generates proton motive force or regenerates NADH by oxidizing formate, and its activity level directly affects cell growth rate and energy supply [65]. Taken together, the probiotic diet appears to have strengthened the nutrient-transport and energy-metabolism capacity of the gut community, favouring nutrient uptake and utilisation by large yellow croaker.

To summarize, after *L. acidophilus* supplementation, the relative abundance of *Vibrio* in the intestine of large yellow croaker decreased, and the abundances of some genera involved in nitrogen cycling, organic matter degradation, and short-chain fatty acid production increased, indicating that the gut microbiota structure was remodeled and microecological stability and metabolic potential may be improved. In the PICRUSt2 prediction, the proportions of genes related to the ABC transport system and formate dehydrogenase were increased. Read alongside the shifted digestive-enzyme profile and the taller villi with thicker muscle layer, these results suggest that it may be beneficial to nutrient transport, energy metabolism, and intestinal barrier function.

5. Conclusions

This study showed that dietary *L. acidophilus* supplementation remodeled the gut microbiota structure of large yellow croaker, reduced the abundance of opportunistic pathogens such as *Vibrio* and enhanced the nutrient transport and energy metabolism potential of the gut microbiota. Along with the improvement of gut microecology, *L. acidophilus* optimized gut morphology and digestive enzyme activities, improved hepatic lipid metabolism and liver function, enhanced antioxidant capacity, and alleviated systemic inflammation, thereby helping to reduce the risk of hepatobiliary syndrome. The above physiological changes were ultimately reflected in an increased body length-to-body height ratio and decreased muscle crude lipid content, suggesting that *L. acidophilus* optimized the body condition and body shape of large yellow croaker while improving gut microecology. This work offers a theoretical basis for deploying *L. acidophilus* in the intensive, healthy culture of large yellow croaker.

Author Contributions: Conceptualization, X.Z., S.C., Q.W., K.L., Z.S., Z.L., K.H. and Y.W.; methodology, X.Z., S.C., Q.W., K.L., Z.S., Z.L., K.H. and Y.W.; formal analysis, X.Z. and S.C.; investigation, X.Z., S.C. and Y.W.; resources, X.Z., Z.L., K.H. and Y.W.; data curation, Q.W., K.L. and Z.S.; writing—original draft, X.Z. and S.C.; writing—review & editing, X.Z., Z.L., K.H. and Y.W.; supervision, Z.L. and K.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Scientific and Technological Project of Ningbo (2023Z121).

Institutional Review Board Statement: All animal procedures and the experimental protocol were approved by the Animal Ethics Committee of Shanghai Ocean University (approval no. SHOU-DW-2023-17 on 12 May 2023).

Data Availability Statement: The raw 16S rRNA gene sequences generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1556682 ([https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1556682] (accessed on 8 November 2026)). All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments: We would like to thank Shanghai Donghao Biotechnology Co., Ltd. (Shanghai, China) for designing and synthesizing the PCR primers.

Conflicts of Interest: Qin Wang and Zhaowen Li are employed at Angel Yeast Co., Ltd. The authors declare that their employment did not influence the study design, data collection, analysis, interpretation of results, or manuscript preparation. All experimental work, data analysis, and manuscript preparation were conducted at Shanghai Ocean University as part of a collaborative research project. The other authors declare no conflicts of interest.

References

1. Ngasotter, S.; Meitei, M.M.; Kara, T.; Meinam, M.; Sharma, S.; Rathod, S.K.; Singh, S.B.; Singh, S.K.; Bhat, R.A.H. Multifaceted Role of Probiotics in Enhancing Health and Growth of Aquatic Animals: Mechanisms, Benefits, and Applications in Sustainable Aquaculture-A Review and Bibliometric Analysis. *Aquac. Nutr.* **2025**, *2025*, 5746972. [CrossRef] [PubMed]
2. Calcagnile, M.; Tredici, S.M.; Alifano, P. A comprehensive review on probiotics and their use in aquaculture: Biological control, efficacy, and safety through the genomics and wet methods. *Heliyon* **2024**, *10*, e40892. [CrossRef] [PubMed]
3. El-Saadony, M.T.; Alagawany, M.; Patra, A.K.; Kar, I.; Tiwari, R.; Dawood, M.A.O.; Dhama, K.; Abdel-Latif, H.M.R. The functionality of probiotics in aquaculture: An overview. *Fish Shellfish Immunol.* **2021**, *117*, 36–52. [CrossRef] [PubMed]
4. Gadhiya, A.; Katariya, S.; Khapandi, K.; Chhatrodiya, D. Probiotics as a sustainable alternative to antibiotics in aquaculture: A review of the current state of knowledge. *Microbe* **2025**, *8*, 100426. [CrossRef]
5. Hagar Sedeek, D.; Nouf, A.A.; Ohaad, F.A.; Walaa, E.A.; Shahira, A.H.; Najah, M.A.; Nesreen, A.; Yasmin, M.A.E.-A.; El-Sayed Hemdan, E.; Mohammad Bodrul, M.; et al. Dietary multi-strains *Bacillus spp.* enhanced growth performance, blood metabolites, digestive tissues histology, gene expression of *Oreochromis niloticus*, and resistance to *Aspergillus flavusinfection*. *Aquac. Int.* **2024**, *32*, 7065–7086. [CrossRef]
6. Sholihuddin, T.D.; Arief, M.; Kenconojati, H. Effect of different bacterial strain in probiotics on the growth performance of Nile Tilapia *(Oreochromis niloticus)*. In *IOP Conference Series: Earth and Environmental Science*; IOP Publishing: Bristol, UK, 2020; Volume 441.
7. Dang, Y.; Sun, Y.; Zhou, Y.; Men, X.; Wang, B.; Li, B.; Ren, Y. Effects of probiotics on growth, the toll-like receptor mediated immune response and susceptibility to *Aeromonas salmonicida* infection in rainbow trout *Oncorhynchus mykiss*. *Aquaculture* **2022**, *561*, 738668. [CrossRef]
8. Zhang, Z.; Wang, Z.; Wang, Z.; Yi, H.; Su, X.; Wang, R.; Ming, T.; Xu, J. *Bacillus velezensis* NDB mitigates *Aeromonas hydrophila*-induced enteritis in black sea bream (*Acanthopagrus schlegelii*) by enhancing intestinal immunity and modulating gut microbiota. *Front. Microbiol.* **2025**, *16*, 1660494. [CrossRef] [PubMed]
9. Dinev, T.; Beev, G.; Denev, S.; Dermendzhieva, D.; Tzanova, M.; Valkova, E. Antimicrobial activity of *Lactobacillus acidophilus* against pathogenic and food spoilage microorganisms: A review. *Agric. Sci. Technol.* **2017**, *8*, 3–9. [CrossRef]
10. Adeshina, I.; Abubakar, M.I.-O.; Ajala, B.E. Dietary supplementation with *Lactobacillus acidophilus* enhanced the growth, gut morphometry, antioxidant capacity, and the immune response in juveniles of the common carp, *Cyprinus carpio*. *Fish Physiol. Biochem.* **2020**, *46*, 1375–1385. [CrossRef] [PubMed]
11. Alberto Arbelaez Rojas, G.; Neves Oliveira dos Santos, N.; Stockhausen, L.; Pacheco Kozikowski, B.; Bender, M.; Delzivo, F.R.; Cipriani, L.A.; Skoronski, E.; Toledo, P.; Araneda, G.M.; et al. Improving Rainbow Trout (*Oncorhynchus mykiss*) Juvenile Performance and Intestinal Condition Through Lactic Acid Bacteria Feed Fermentation. *Animals* **2026**, *16*, 1482. [CrossRef] [PubMed]
12. Munir, M.B.; Hashim, R.; Chai, Y.H.; Marsh, T.L.; Nor, S.A. Dietary prebiotics and probiotics influence growth performance, nutrient digestibility and the expression of immune regulatory genes in snakehead (*Channa striata*) fingerlings. *Aquaculture* **2016**, *460*, 59–68. [CrossRef]
13. Qiu, H.; Huang, L.; Wang, H.; Tao, C.; Ran, Z.; Xu, J.; Sun, H.; Wang, P. Effects of *Lactobacillus acidophilus* AC on the growth, intestinal flora and metabolism of zebrafish (*Danio rerio*). *Fish Shellfish Immunol.* **2024**, *149*, 109570. [CrossRef] [PubMed]

14. Qiu, H.; Wang, H.; Huang, L.; Tao, C.; Ran, Z.; Xu, J.; Wang, P. Effects of water supplementation with *Lactobacillus acidophilus* AC on the antioxidant, immune, intestinal flora and resistance to *Aeromonas hydrophila* of adult zebrafish (*Danio rerio*). *Aquaculture* **2025**, *594*, 741466. [CrossRef]
15. De Marco, G.; Cappello, T.; Maisano, M. Histomorphological Changes in Fish Gut in Response to Prebiotics and Probiotics Treatment to Improve Their Health Status: A Review. *Animals* **2023**, *13*, 2860. [CrossRef] [PubMed]
16. Omidi, A.H.; Adel, M.; Bahri, A.H.; Yahyavi, M.; Mohammadizadeh, F.; Impellitteri, F.; Faggio, C. Synergistic dietary influence of fermented red grape vinegar and *Lactobacillus acidophilus* on growth performance, carcass composition, and intestinal morphology of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Rep.* **2024**, *36*, 102122. [CrossRef]
17. Akter, M.N.; Hashim, R.; Sutriana, A.; Siti Azizah, M.N.; Asaduzzaman, M. Effect of *Lactobacillus acidophilus* supplementation on growth performances, digestive enzyme activities and gut histomorphology of striped catfish (*Pangasianodon hypophthalmus* Sauvage, 1878) juveniles. *Aquac. Res.* **2019**, *50*, 786–797. [CrossRef]
18. He, W.; Liu, Y.; Zhang, W.; Zhao, Z.; Bu, X.; Sui, C.; Pan, S.; Yao, C.; Tang, Y.; Mai, K.; et al. Effects of dietary supplementation with heat-killed *Lactobacillus acidophilus* on growth performance, digestive enzyme activity, antioxidant capacity, and inflammatory response of juvenile large yellow croaker (*Larimichthys crocea*). *Fish Shellfish Immunol.* **2024**, *151*, 109651. [CrossRef] [PubMed]
19. Nataly Oliveira Dos Santos, N.; Juliano De Dea, L.; Larissa, S.; Fernanda Regina, D.; Mariana, B.; Leticia, S.; Luiz Augusto, C.; Natalia, H.; Everton, S.; Enric, G.; et al. Fermentation of Plant-Based Feeds with *Lactobacillus acidophilus* Improves the Survival and Intestinal Health of Juvenile Nile Tilapia (*Oreochromis niloticus*) Reared in a Biofloc System. *Animals* **2024**, *14*, 332. [CrossRef] [PubMed]
20. Hoseinifar, S.H.; Roosta, Z.; Hajimoradloo, A.; Vakili, F. The effects of *Lactobacillus acidophilus* as feed supplement on skin mucosal immune parameters, intestinal microbiota, stress resistance and growth performance of black swordtail (*Xiphophorus helleri*). *Fish Shellfish Immunol.* **2015**, *42*, 533–538. [CrossRef] [PubMed]
21. Han, X.; Zhang, S.; Wang, Y.; Fang, H.; Peng, S.; Yang, S.; Wu, Z. Seedling Selection of the Large Yellow Croaker (*Larimichthys crocea*) for Sustainable Aquaculture: A Review. *Appl. Sci.* **2025**, *15*, 7307. [CrossRef]
22. Li, B.; Ouyang, Z.; Zhao, Z.; Miao, L.; Du, Y.; Chen, J. Pathogenic function of the Lafa gene in *Vibrio harveyi* and its regulatory role in host immune response during infection of large yellow croaker (*Larimichthys crocea*). *Aquaculture* **2026**, *612*, 743186. [CrossRef]
23. Liu, L.; Ge, M.; Zheng, X.; Tao, Z.; Zhou, S.; Wang, G. Investigation of *Vibrio alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* in large yellow croaker, *Pseudosciaena crocea* (Richardson) reared in Xiangshan Bay, China. *Aquac. Rep.* **2016**, *3*, 220–224. [CrossRef]
24. Ganguly, P.; Jones, E.A.; Giannoudis, P.V.; Tronci, G. Antibiotic-Free Strategies for Managing Antimicrobial-Resistant Infections. *Pharmaceutics* **2026**, *18*, 1136. [CrossRef] [PubMed]
25. Zhao, Z.; Wu, Z.; Ding, G.; Fang, Z.; Chen, M.; Liu, Y.; Du, J.; Li, Y.; Mai, K.; Ai, Q. The supplement of spermidine attenuates high-fat diet-induced hepatic lipid accumulation, oxidative stress, and inflammation in large yellow croaker (*Larimichthys crocea*). *Aquaculture* **2026**, *613*, 743226. [CrossRef]
26. Wang, X.; Li, Y.; Hou, C.; Gao, Y.; Wang, Y. Physiological and molecular changes in large yellow croaker (*Pseudosciaena crocea* R.) with high-fat diet-induced fatty liver disease. *Aquac. Res.* **2015**, *46*, 272–282. [CrossRef]
27. Dhanaraj, M.; Haniffa, M.A.; Singh, S.V.A.; Arockiaraj, A.J.; Ramakrishanan, C.M.; Seetharaman, S.; Arthimanju, R. Effect of Probiotics on Growth Performance of Koi Carp (*Cyprinus carpio*). *J. Appl. Aquac.* **2010**, *22*, 202–209. [CrossRef]
28. *GB 5009.3-2016*; National Food Safety Standard. Determination of Moisture in Food. China Standards Press: Beijing, China, 2016.
29. *GB 5009.5-2016*; National Food Safety Standard.Determination of Protein in Food. China Standards Press: Beijing, China, 2016.
30. *GB 5009.6-2016*; National Food Safety Standard.Determination of Fat in Food. China Standards Press: Beijing, China, 2016.
31. *GB 5009.4-2016*; National Food Safety Standard.Determination of Ash in Food. China Standards Press: Beijing, China, 2016.
32. Hosseini, M.; Miandare, H.K.; Shabani, A.; Hoseinifar, S.H.; Yarahmadi, P. Dietary *Lactobacillus acidophilus* modulated skin mucus protein profile, immune and appetite gene expression in gold fish (*Carassius auratus gibelio*). *Fish Shellfish Immunol.* **2017**, *71*, 443. [CrossRef] [PubMed]
33. Ran, Z.; Xu, J.; Wang, J.; Huang, S.; Huang, L.; Wang, S.; Wang, P. Effects of *Lactobacillus acidophilus* AC on growth, intestinal flora and metabolism of juvenile large yellow croaker (*Larimichthys crocea*). *J. Fish Biol.* **2026**, *109*, 1160–1175. [CrossRef] [PubMed]
34. Li, L.; Wang, Y.; Li, Y.; Wang, T.; Wang, Z.; Zhou, N.; Qiao, F.; Du, Z.-Y.; Zhang, M.-L. Integrated microbiota and metabolomic analysis demonstrates the growth-promoting effects of *Lactobacillus plantarum* in Nile tilapia (*Oreochromis nilotica*). *Anim. Microbiome* **2026**, *8*, 14. [CrossRef] [PubMed]
35. Wang, J.; Zhu, Z.; Tian, S.; Fu, H.; Leng, X.; Chen, L. Dietary *Lactobacillus casei* K17 Improves Lipid Metabolism, Antioxidant Response, and Fillet Quality of *Micropterus salmoides*. *Animals* **2021**, *11*, 2564. [CrossRef] [PubMed]
36. Yang, Z.; Liu, P.; Kong, Q.; Deng, Y.; Zhang, W.; Xu, G.; Tang, H. Effects of Co-Fermented Feed Using *Lactobacillus acidophilus*, *Limosilactobacillus reuteri* and *Lactiplantibacillus plantarum* on Growth, Antioxidant Capacity, Fatty Acids and Gut Microbiota of Largemouth Bass (*Micropterus salmoides*). *Fishes* **2023**, *8*, 433. [CrossRef]
37. Moreno-Torres, M.; Quintas, G.; Castell, J.V. The Potential Role of Metabolomics in Drug-Induced Liver Injury (DILI) Assessment. *Metabolites* **2022**, *12*, 564. [CrossRef] [PubMed]

38. Guo, X.; Xu, J.; Fang, Q.; Zhang, X.; Zhang, P.; Shen, Z.; Jiao, S.; Kong, Y.; Li, M.; Wang, G. Mechanism of *Lactococcus lactis* L19 in alleviating high-carbohydrate diet-induced glucose and lipid metabolism disorders in northern snakehead (*Channa argus*). *Fish Physiol. Biochem.* **2026**, *52*, 86. [CrossRef] [PubMed]
39. Li, F.; Zhang, H. Lysosomal Acid Lipase in Lipid Metabolism and Beyond. *Arterioscler. Thromb. Vasc. Biol.* **2019**, *39*, 850–856. [CrossRef] [PubMed]
40. Polakof, S.; Medale, F.; Larroquet, L.; Vachot, C.; Corraze, G.; Panserat, S. Regulation of de novo hepatic lipogenesis by insulin infusion in rainbow trout fed a high-carbohydrate diet. *J. Anim. Sci.* **2011**, *89*, 3079–3088. [CrossRef] [PubMed]
41. Ratledge, C. The role of malic enzyme as the provider of NADPH in oleaginous microorganisms: A reappraisal and unsolved problems. *Biotechnol. Lett.* **2014**, *36*, 1557–1568. [CrossRef] [PubMed]
42. Qiao, S.; Lv, C.; Zhang, X.; Lv, X.; Yang, D.; Zhao, J. Antioxidant and anti-stress properties of postbiotics produced by *Lysinibacillus macroides* G117. *Comp. Immunol. Rep.* **2024**, *6*, 200143. [CrossRef]
43. Su, F.; Koeberle, A. Regulation and targeting of SREBP-1 in hepatocellular carcinoma. *Cancer Metastasis Rev.* **2024**, *43*, 673–708. [CrossRef] [PubMed]
44. Rong, S.; Xia, M.; Vale, G.; Wang, S.; Kim, C.-W.; Li, S.; McDonald, J.G.; Radhakrishnan, A.; Horton, J.D. DGAT2 inhibition blocks SREBP-1 cleavage and improves hepatic steatosis by increasing phosphatidylethanolamine in the ER. *Cell Metab.* **2024**, *36*, 617–629.e7. [CrossRef] [PubMed]
45. Tomacha, J.; Dokduang, H.; Padthaisong, S.; Namwat, N.; Klanrit, P.; Phetcharaburanin, J.; Wangwiwatsin, A.; Khampitak, T.; Koonmee, S.; Titapun, A.; et al. Targeting Fatty Acid Synthase Modulates Metabolic Pathways and Inhibits Cholangiocarcinoma Cell Progression. *Front. Pharmacol.* **2021**, *12*, 696961. [CrossRef] [PubMed]
46. Shang, R.; Rodrigues, B. Lipoprotein Lipase and Its Delivery of Fatty Acids to the Heart. *Biomolecules* **2021**, *11*, 1016. [CrossRef] [PubMed]
47. Li, T.; Guo, W.; Zhou, Z. Adipose Triglyceride Lipase in Hepatic Physiology and Pathophysiology. *Biomolecules* **2022**, *12*, 57. [CrossRef] [PubMed]
48. Chen, Y.; Zheng, K.; Leng, Y.; Zhang, Z.; Li, X.; Li, X.; Ou, H.; Wen, M.; Qiu, F.; Yu, H. Alleviating effect of *Lactobacillus fermentum* E15 on hyperlipidemia and hepatic lipid metabolism in zebrafish fed by a high-fat diet through the production of short-chain fatty acids. *Front. Nutr.* **2025**, *12*, 1522982. [CrossRef] [PubMed]
49. Shi, H.; Jiang, L.; Cai, Y.; Chen, Z.; Hu, Y.; Zhou, Y.; Li, J.; Zhang, D.; Cao, Z.; Wang, S. Regulatory Effects of a Lipid-Lowering Strain *Lactobacillus plantarum* 58 Isolated From Dregs Vinegar on Metabolism-Related Gene Expression, Gut Microbiota, and Metabolic Biomarkers of Hybrid Grouper Under High-Fat Diets. *Aquac. Nutr.* **2026**, *2026*, 4888310. [CrossRef] [PubMed]
50. Chen, J.; Zhang, J.; Xie, M.; Hao, Q.; Liang, H.; Li, M.; Zhou, W.; Zhang, Z.; Ran, C.; Zhou, Z. The effect of dietary supplementation with *Lactobacillus rhamnosus* GCC-3 fermentation product on gut and liver health of common carp (*Cyprinus carpio*). *Aquac. Rep.* **2024**, *35*, 101983. [CrossRef]
51. Su, L.; Yang, Y.; Xu, Y.; Zhu, L.; Sun, B.; Zhou, J. In vitro digestibility of the *Sargassum fusiforme* polysaccharide fermented using *Lactobacillus acidophilus* and its improving effect on intestinal inflammation in zebrafish. *Food Funct.* **2025**, *16*, 7629–7643. [CrossRef] [PubMed]
52. Yan, F.; Li, N.; Shi, J.; Li, H.; Yue, Y.; Jiao, W.; Wang, N.; Song, Y.; Huo, G.; Li, B. *Lactobacillus acidophilus* alleviates type 2 diabetes by regulating hepatic glucose, lipid metabolism and gut microbiota in mice. *Food Funct.* **2019**, *10*, 5804–5815. [CrossRef] [PubMed]
53. Ghafarifarsani, H.; Ahani, S.; Aftabgard, M.; Ahani, S.; Yousefi, M. Efficacy of *Lactobacillus acidophilus* and yeast cell wall-derived supplements on immunity responses, growth performance, and disease resistance in *Cyprinus carpio* juveniles. *Vet. Res. Commun.* **2024**, *49*, 23. [CrossRef] [PubMed]
54. Hassaan, M.S.; El-Sayed, A.M.I.; Mohammady, E.Y.; Zaki, M.A.A.; Elkhyat, M.M.; Jarmolowicz, S.; El-Haroun, E.R. Eubiotic effect of a dietary potassium diformate (KDF) and probiotic (*Lactobacillus acidophilus*) on growth, hemato-biochemical indices, antioxidant status and intestinal functional topography of cultured Nile tilapia *Oreochromis niloticus* fed diet free fishmeal. *Aquaculture* **2021**, *533*, 736147. [CrossRef]
55. Hoseini, S.M.; Yousefi, M.; Afzali-Kordmahalleh, A.; Pagheh, E.; Mirghaed, A.T. Effects of Dietary Lactic Acid Supplementation on the Activity of Digestive and Antioxidant Enzymes, Gene Expressions, and Bacterial Communities in the Intestine of Common Carp, *Cyprinus carpio*. *Animals* **2023**, *13*, 1934. [CrossRef] [PubMed]
56. Liu, W.; Zeng, J.; Suo, N.; Ke, Q.; Zhao, J.; Wang, J.; Bai, Y.; Deng, Y.; Zhou, X.; Wang, Y.; et al. Dynamic changes in gut microbiota and production phenotypes driven by host genetic background in large yellow croaker. *Aquaculture* **2025**, *598*, 741948. [CrossRef]
57. Samsing, F.; Barnes, A.C. The rise of the opportunists: What are the drivers of the increase in infectious diseases caused by environmental and commensal bacteria? *Rev. Aquac.* **2024**, *16*, 1787–1797. [CrossRef]
58. Gabrielaite, M.; Nielsen, F.C.; Johansen, H.K.; Marvig, R.L. *Achromobacter* spp. genetic adaptation in cystic fibrosis. *Microb. Genom.* **2021**, *7*, 000582. [CrossRef] [PubMed]
59. Kolb, S.A.; O'Loughlin, E.J.; Gsell, T.C. Characterization of phthalate-degrading bacteria from Asian carp microbiomes and riverine sediments. *Int. Biodeterior. Biodegrad.* **2019**, *143*, 104727. [CrossRef]

60. Sun, J.; Wang, H.; Chen, Y.; Huang, S.; Bi, X.; Cheng, L.; Shi, X.; Zhao, W.; Zhou, X. Performance of Nitrogen Removal and Biofilm-Associated Microbial Community in a Compact Marine Shrimp Recirculating Aquaculture System with MBBR. *Microorganisms* **2026**, *14*, 841. [CrossRef] [PubMed]
61. Xian, L.; Ye, J.; Li, X.; Yuan, Y.; Li, J.; Xu, Y.; Wang, X. Effects of photoperiod on nitrogen directional conversion processes and microbial community structure in partial nitritation system. *J. Environ. Chem. Eng.* **2026**, *14*, 122640. [CrossRef]
62. Martineau, C.; Mauffrey, F.; Villemur, R. Comparative Analysis of Denitrifying Activities of *Hyphomicrobium nitrativorans*, *Hyphomicrobium denitrificans*, and *Hyphomicrobium zavarzinii*. *Appl. Environ. Microbiol.* **2015**, *81*, 5003–5014. [CrossRef] [PubMed]
63. Zagato, E.; Pozzi, C.; Bertocchi, A.; Schioppa, T.; Saccheri, F.; Guglietta, S.; Fosso, B.; Melocchi, L.; Nizzoli, G.; Troisi, J.; et al. Endogenous murine microbiota member *Faecalibaculum rodentium* and its human homologue protect from intestinal tumour growth. *Nat. Microbiol.* **2020**, *5*, 511–524. [CrossRef] [PubMed]
64. Mukherjee, S.; Thompson, L.K.; Godin, S.; Schackwitz, W.; Lipzen, A.; Martin, J.; Blanchard, J.L. Population Level Analysis of Evolved Mutations Underlying Improvements in Plant Hemicellulose and Cellulose Fermentation by *Clostridium phytofermentans*. *PLoS ONE* **2014**, *9*, e86731. [CrossRef] [PubMed]
65. Cowan, A.E.; Hillers, M.; Rainaldi, V.; Collas, F.; Choudhary, H.; Zakaria, B.S.; Bieberach, G.G.; Carruthers, D.N.; Grabovac, M.; Gin, J.W.; et al. Fast growth and high-titer bioproduction from renewable formate via metal-dependent formate dehydrogenase in *Escherichia coli*. *Nat. Commun.* **2025**, *16*, 5908. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.