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Supplementation with *Bacillus velezensis* GY1 Improves Growth Performance, Intestine Morphology, and Intestine Microbial Diversity in Largemouth Bass (*Micropterus salmoides*)

Shilin Zhang [1], Huaping Zhu [2], Wenqing Zuo [1], Beibei Zhang [1], Tingting Lu [1], Junjin Deng [3], Jiazhou Li [1] and Huanhuan Su [1,*]

[1] Heyuan Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Heyuan 517000, China
[2] Key Laboratory of Tropical & Subtropical Fishery Resource Application & Cultivation, Ministry of Agriculture and Rural Affairs, Pearl River Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou 510380, China
[3] Agro-Biological Gene Research Center, State Key Laboratory of Livestock and Poultry Breeding, Guangdong Academy of Agricultural Sciences, No. 20 Jinying Road, Tianhe District, Guangzhou 510640, China
* Correspondence: hhsu2025@163.com; Tel.: +86-0762-2200222

Simple Summary

Largemouth bass is an important freshwater farmed species in China, valued for its good flesh quality and fast growth, which brings considerable economic benefits to the aquaculture industry. However, disease risks and feed utilization efficiency remain key issues affecting profitability during cultivation. In this study, a beneficial bacterium called Bacillus velezensis GY1 was added to the feed, and two administration strategies—continuous and intermittent supplementation—were compared to examine their effects on fish growth rate, intestinal health, digestive capacity, and immune function. The results showed that continuous supplementation significantly increased weight gain and survival rate while reducing feed wastage; intermittent supplementation also improved survival. Furthermore, fish receiving the beneficial bacterium exhibited healthier intestinal structures, enhanced digestive performance, elevated immune responses, and greater abundance and diversity of beneficial gut microbes. These findings indicate that dietary supplementation with *Bacillus velezensis* GY1 can improve growth performance and disease resistance in largemouth bass, thereby reducing farming risks and increasing production efficiency. This approach holds practical value for promoting sustainable aquaculture development and enhancing farmers' income.

Abstract

Largemouth bass (*Micropterus salmoides*) is a major freshwater farmed species in China, making substantial economic contributions owing to its desirable flesh quality, rapid growth, and strong acceptance of formulated feeds. This study assessed the effects of dietary supplementation (intermittent and continuous feeding group, $n = 3$) with the probiotic *Bacillus velezensis* (1.0×10^9 CFU/g) on growth performance, intestinal morphology, digestive enzyme activities, and gut microbial diversity in this fish. The results indicated that continuous *B. velezensis* administration significantly increased percentage weight gain, specific growth rate, and survival, while concurrently reducing feed conversion ratio relative to the basal diet ($p < 0.05$). Intermittent feeding also resulted in a markedly higher survival rate and a lower FCR. Histological examination of intestinal sections revealed elevated villus height, villus width, and muscularis thickness in the *B. velezensis* supplemented groups, with the intermittent regimen producing more pronounced effects. Compared with the control, the intermittent group exhibited substantially decreased the pepsin and

Academic Editor: Jorge Olmos-Soto

Received: 22 June 2026
Revised: 31 July 2026
Accepted: 4 August 2026
Published: 5 August 2026

lipase activities of the serum. In the continuous group, serum globulin levels rose noticeably, accompanied by significant declines in total complement and superoxide dismutase. Moreover, continuous *B. velezensis* supplementation markedly upregulated the expression levels of *TGFβ*, *myd88*, *IL34*, *IL8*, and *TNFα* in the head-kidney of the largemouth bass, whereas intermittent supplementation significantly increased *TGFβ*, *Gpx*, *Nrf2*, and *Acp* transcript levels. Dietary inclusion of *B. velezensis* was also found to enhance intestinal microbial richness and greatly increase the abundance of potentially beneficial bacteria. Collectively, these results indicate that *B. velezensis* supplementation exerts beneficial effects on intestinal digestive capacity, growth, and innate immunity, thereby lowering disease susceptibility in largemouth bass.

Keywords: *Bacillus velezensis*; *Micropterus salmoides*; growth performance; intestina microorganism

1. Introduction

Largemouth bass (*Micropterus salmoides*) has been recognized as one of the most important farmed species in China's aquaculture industry [1]. Its desirable attributes, including superior flesh quality, fast growth, good acceptability of formulated feeds, and strong tolerance to handling stress, make it a suitable candidate for intensive farming systems. These characteristics have earned it widespread favor among both culturists and consumers, with annual production continuing to rise steadily [2]. Since its introduction to mainland China in the 1980s, the species has gained a more prominent status in terms of economic and food value, and has a broad prospect in the high-end catering and seafood market [3]. In 2024, the amount of largemouth bass farmed in China reached 901,200 tonnes, compared with 702,100 tonnes in 2021, with a 28.4% increase in the total production, making it one of the major farmed species in China [4]. Largemouth bass have gradually adapted to commercial feeds, and with the continuous advancement of aquaculture technology, intensive aquaculture characterized by feed feeding and high-density culture has now become the primary farming mode for this species. However, this high-density farming model leads to the accumulation of faeces and uneaten feed residues over time, releasing harmful substances that may trigger issues such as bacterial enteritis and hepatopancreatic syndrome, impairs feed absorption efficiency and compromises fish health. Consequently, there is a significant need to investigate strategies to enhance fish immunity, thereby promoting the sustainable development of largemouth bass aquaculture.

With the rapid development of the aquaculture industry, antibiotics have become one of the commonly used drugs for the treatment of bacterial diseases in aquatic animals, significantly improving the efficiency of aquaculture. However, with the irrational use of antibiotics, it leads to elevated resistance of pathogenic bacteria, which significantly affects fish immunity and even has serious impacts on human health, even deteriorating the ecological environment [5,6]. Probiotics are widely acknowledged for their critical role in enhancing animal performance and lowering feed conversion ratio, thereby serving as effective tools in aquaculture to promote the growth performance of cultured species [7]. These beneficial microorganisms promote host health through multiple mechanisms, including maintaining intestinal microflora homeostasis, optimizing feed utilization, inhibiting pathogenic bacteria, enhancing immune responses, and strengthening disease resistance [8]. Probiotics produce antimicrobial substances, compete for adhesion receptors on the intestinal epithelium, and provide nutritional benefits to the host [9–11]. Furthermore, probiotics

reinforce the gut immune barrier and sustain immune tolerance, which attenuates pathogen invasion of the intestinal mucosa [12].

Owing to these benefits, probiotics have gained increasing attention and application in fish and shrimp culture. In these systems, they contribute to regulating body physiology, improving the rearing environment, suppressing the growth and reproduction of pathogens, enhancing host immunity, reducing malformations, and optimizing gastrointestinal morphology and microbiota [9,10]. Such effects offer new strategies for the prevention and control of aquatic animal diseases and help reduce reliance on antibiotics [11]. Currently, the most widely used probiotics in aquaculture belong to the genera *Lactobacillus* and *Bacillus* [12–14]. In particular, for largemouth bass, probiotics have been shown to improve water quality, prevent fish diseases, promote growth, and boost immunity under high-density culture conditions. *Bacillus* species regarded as the most stable of all probiotics due to their heat-resistant, easy-to-preserve, and vitality characteristics, thus making them the preferred choice of antagonistic bacteria for aquaculture diseases. *Bacillus velezensis* is recognized as a safe biological agent, playing important roles in biocontrol, pharmaceutical development, food fermentation, and industrial production, and has been extensively applied in managing plant and animal diseases [15–17]. *B. velezensis* is widely employed in agricultural settings and ranks among the most commonly used bio-control agents. However, relatively few studies have addressed the application of *B. velezensis* in aquaculture for disease prevention and immune enhancement.

B. velezensis has been observed to produce secondary metabolites with antimicrobial activity, including lipopeptide antibiotics (mainly phenoxybutyric acid, itofloxacin, and surface-active agents), polyketides, β-1,3-1,4-glucanase, and bacteriocins, which have antibacterial, antifungal, and growth-promoting effects [18–21]. Nevertheless, research on the application of *B. velezensis* in largemouth bass farming remains scarce. The current study stemmed from the isolation of a *B. velezensis* strain, with the aim of evaluating its effects on growth performance and host immunity during the rearing of this fish species. This work offers a novel microbial candidate for the bio-control of aquatic pathogens, thereby establishing a basis for future development of biological control strategies and related products for aquaculture diseases.

2. Materials and Methods

2.1. Sampling and Pretreatment

Healthy largemouth bass (*Micropterus salmoides*, 15 days after hatching) were obtained from Guangdong Liangshi Aquatic Products Breeding Co., Ltd. (Foshan, China) Following a two-week acclimation period in pond cages, the fish were randomly allocated to nine fiberglass tanks (140 L each) at a density of 20 individuals per tank. Before the start of the formal experiment, the fish were acclimatized for an additional two weeks in canvas tanks, during which they were fed a basal diet. The trial was initiated after an observation period, during which the fish (with an average initial body weight of approximately 7.0 ± 0.8 g) displayed normal swimming behavior, good appetite, and no apparent signs of injury or disease.

In this study, a potential probiotic strain, *B. velezensis* GY1, was isolated from aquaculture effluent. Subsequent in vitro antimicrobial assays revealed that this strain exhibits potent activity against a broad range of fish pathogens. To determine the optimal supplementation concentration, we conducted a preliminary gradient feeding trial with three levels: 10^7, 10^8, and 10^9 CFU/g feed. The results showed that the 10^9 CFU/g group exhibited the most favorable feeding performance; therefore, this concentration was selected for the subsequent experiments. Before the experiment, the *B. velezensis* GY1 strain was inoculated onto BHI solid medium for activation. Small-scale overnight fermentation

was conducted daily using fresh BHI liquid medium. After centrifugation and resuspension, the bacterial suspension concentration was measured and stored for later use. During the feeding period, the basal diet was weighed and mixed with the *B. velezensis* GY1 suspension to obtain a final concentration of 1.0×10^9 CFU/g, according to the methods of Srisapoome [22] and Yi et al. [23], and the mixture was freshly prepared and used immediately.

The experiment (114°14′–115°36′ E, 23°10′–24°47′ N; elevation about 110 m above sea level) was conducted using a three-group design, consisting of a control group (Group C), an intermittent feeding group (Group J), and a continuous feeding group (Group L). Each group was further divided into three replicates to ensure statistical reliability and reproducibility. The experimental period spanned a duration of 12 weeks. The control group was fed a commercial basal feed throughout the experiment. The fish in the continuous feeding group were provided with experimental feed continuously throughout the study. The intermittent feeding group received experimental feed during weeks 1–2, 5–6, and 9–10, while basal feed was provided during weeks 3–4, 7–8, and 10–12. The feed utilized in the aquaculture experiment was obtained from Guangdong Yujia Biotechnology Co., Ltd. (Guangzhou, China). The product under consideration is from Guangdong, China. Fish meal, wheat protein concentrate, and soybean protein concentrate were employed as protein sources in feed formulation, whereas soybean oil, fish oil, and soybean lecithin served as lipid sources. Proximate composition analysis of the basal diet indicated that it contained 50.92% crude protein, 7.21% crude lipid, 10.56% ash, and 10.06% moisture. The experimental feed consisted of commercial basal feed supplemented with 1.0×10^9 CFU/g *B. velezensis* GY1, which was freshly prepared and utilized throughout the entirety of the experiment.

Throughout the entire rearing period, feed was administered twice daily at 9:00 AM and 5:00 PM, with a daily feed intake of approximately 3% of the total fish weight (adjusted based on fish feeding behavior and rearing duration), with each feeding session accounting for 50% of the daily feed intake. Daily observations and records of fish feeding behavior and mortality were conducted, with deceased individuals promptly removed. All aquariums are equipped with aeration devices, which are operated continuously during the experiment. Water temperature is maintained at 28 ± 1.5 °C, pH at 7.0 ± 0.3, dissolved oxygen above 6.1 ± 0.5 mg/L, ammonia nitrogen below 0.5 mg/L, and nitrite nitrogen below 0.05 mg/L.

2.2. Determination of Growth Indicators of Largemouth Bass

Prior to sampling, fish in all treatment groups were fasted for 24 h after the conclusion of the feeding trial. The number of surviving fish in each group was recorded, and all individuals from each treatment were weighed. Growth performance parameters were calculated using the following formulas:

$$\text{Weight gain rate (WGR \%)} = (\text{Final body weight} - \text{Initial body weight})/\text{Initial body weight} \times 100\%;$$

$$\text{Survival rate (SR \%)} = \text{Number of fish at the end of the experiment}/\text{Number of fish at the beginning of the experiment} \times 100\%;$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed intake (g)}/\text{Body weight gain (g)};$$

$$\text{Specific growth rate (SGR \%)} = [(\ln \text{Final body weight} - \ln \text{Initial body weight})/\text{Number of days}] \times 100\%.$$

2.3. Serum Enzyme Activity and Biochemical Index Determination

After a 12-week feeding trial, six healthy largemouth bass (*Micropterus salmoides*) were randomly selected from each treatment group, anesthetized, and blood was collected from the caudal vein of each fish. Approximately 1 mL of blood was obtained per fish. After being left to stand at 4 °C 12 h, the blood samples were centrifuged at 5000 r/min for 5 min under refrigerated conditions to separate the serum. The serum samples were then stored at −80 °C until subsequent analysis. The serum was subsequently used to determine the levels of lysozyme (LZM), superoxide dismutase (SOD), alkaline phosphatase (AKP), albumin (ALB), and globulin (GLO). Among them, LZM and SOD were determined using the Nanjing Jiancheng kit Nanjing China (H538-1-2 and A001-3-2), and the self-control method and WST-1 method were adopted respectively. The specific operations are detailed in the kit manual. AKP, TP, ALB, and GLO were all detected on the Hitachi 7600 automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan). Nine fish were randomly selected from each treatment group and sampled after 24 h of fasting.

2.4. Intestinal and Liver Histomorphological Examination

For intestinal and liver histomorphology, three fish were randomly selected from each group. Mid-intestine and liver tissues were dissected from the abdominal region, cut into 2 cm segments, and fixed in 4% paraformaldehyde (4% PFA) solution for 18–24 h. The samples were then dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 6 μm thickness were cut using a rotary microtome and subsequently stained with hematoxylin and eosin. The liver and intestinal tissue sections were scanned under a white-light microscope, and the digital images were subjected to morphometric analysis using Saiviewer (Version 3.0.0) image analysis software. The intestinal morphological parameters measured included villus length, villus width, and the thickness of the intestinal muscularis layer.

2.5. Gene Expression Analysis

At the end of the experiment, the largemouth bass were anesthetized, and the liver and head kidney tissues were excised using sterile surgical scissors, immediately placed in liquid nitrogen, and stored at −80 °C for subsequent gene expression analysis. Total RNA was extracted from the liver and intestine tissue using TRIzol reagent (Thermo Fisher, Waltham, MA, USA), and then reverse-transcribed into cDNA. The resulting cDNA served as the template for quantitative real-time PCR, with *EF1α* used as the internal reference gene to normalize the expression levels of target genes (Table 1). The thermal cycling protocol consisted of 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, 60 °C for 15 s, and 72 °C for 10 s, with annealing temperatures optimized for each gene. All reactions were performed in triplicate for each sample. Relative gene expression levels in each tissue from different treatment groups were calculated using the $2^{-\Delta\Delta Ct}$ method, and the control group was defined as the group receiving no probiotic supplementation.

Table 1. Primers used for the quantitative real-time polymerase chain reaction analysis.

Gene	Primer Sequence Forward (5′ → 3′)	Primer Sequence Reverse (5′ → 3′)	Accession No.
EFlα [24]	TGCTGCTGGTGTTGGTGAGTT	TTCTGGCTGTAAGGGGGCTC	XM_038724777.1
Keap1 [24]	CGTACGTCCAGGCCTTACTC	TGACGGAAATAACCCCCTGC	XM_038713667.1
Nrf2 [24]	GTAATAGGGCAGCCACAGCA	AGAGCGCTTCTTACGCTTGT	XM_038720536.1
SOD	TGGCAAGAACAAGAACCACA	CCTCTGATTTCTCCTGTCACC	XM_038708943.1
gpx [25]	GAAGGTGGATGTGAATGGA	CCAACCAGGAACTTCTCAA	XM_038697220.1
Akp [25]	GATGAGTGGCAGAGCATGAA	CCATGAGGTAGTCTGTGG	XM_038692221.1
Acp1 [25]	AGGCACAGATTGAGCTTCTT	CACACTGTTCGTACACCTTCT	XM_038727000.1

Table 1. *Cont.*

Gene	Primer Sequence Forward (5′ → 3′)	Primer Sequence Reverse (5′ → 3′)	Accession No.
IL8 [26]	CCTTGAACAGACTGCGAGAGATG	AGTGGGATGGCTTCATTATCTTGT	XM_038704093.1
IL34	TTAGCAGAATGAGGTTACAGGTGG	CGTGAAGGATGTCTCAGTGGC	XM_038705745.1
TNFa [26]	CTTCGTCTACAGCCAGGCATCG	TTTGGCACACCGACCTCACC	XM_038710731
Tgf-β [26]	GCTCAAAGAGAGCGAGGATG	TCCTCTACCATTCGCAATCC	XM_038693206.1
Fas-1	GCCATCGACCACTCGATACT	GTCACCCTTGGAGAAGGTCA	XM_046069694
myd88 [25]	CTCAACCCCAAGAACACA	CGAAGATCCTCCACAATG	XM_038728827.1

2.6. Library Preparation, Sequencing, and Analysis of the Intestine Microbiota

The total intestinal contents were aseptically collected from the whole intestine, thoroughly homogenized, and used for subsequent microbial community analysis. Intestinal microbial community profiling was performed according to the procedures described below. Briefly, total genomic DNA was extracted from the intestinal contents of the fish using the E.Z.N.A. Stool DNA Kit (Omega Bio-tek Inc., Norcross, GA, USA) strictly following the manufacturer's instructions. The purity and concentration of the extracted DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The variable regions of the 16S rDNA were amplified with the primer pair 341F (CCTACGGGNGGCWGCAG) and 806R (GGACTACHVGGGTATCTAAT). Subsequently, the PCR products were purified from 2% agarose gels using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's protocol. The purified amplicons were quantified with a QuantiFluor-ST fluorometer (Promega, Madison, WI, USA), pooled in equimolar amounts, and subjected to paired-end sequencing (2 × 250 bp) on an Illumina HiSeq 2500 platform following the standard protocols.

The libraries were sequenced by Novogene Co., Ltd. (Beijing, China). Raw reads were quality-filtered to obtain high-quality reads by removing those containing >10% ambiguous nucleotides or with <80% of bases having a quality score (Q-value) >20. Paired-end clean reads were merged into tags using FLASH (v1.2.11). Subsequent analyses were performed with the QIIME pipeline (v1.9.1). Chimera detection was carried out using the UCHIME algorithm in reference-based mode against the reference database (http://drive5.com/uchime/uchime_download.html, accessed on 28 August 2025). After removing chimeric tags, the effective tags were clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the UPARSE pipeline, and the most abundant sequence in each OTU was selected as the representative sequence. Taxonomic assignment of representative sequences was performed with the RDP classifier using a naive Bayesian model against the SILVA database (https://www.arb-silva.de/, accessed on 16 September 2025). Richness estimators and diversity indices for each library were calculated using Mothur (v1.39.1). The selection of representative sequences at different taxonomic levels and multiple sequence alignment were conducted using QIIME. Alpha diversity indices, including Chao1 and ACE (for community richness) and Shannon and Simpson (for community diversity), were determined; Good's coverage was also computed to assess sequencing depth. Results were summarized at the phylum and genus levels. Beta diversity was evaluated using principal component analysis (PCA) and principal coordinate analysis (PCoA) to compare microbial community structures among groups. Additionally, a Venn diagram was constructed to illustrate unique and shared OTUs, and a heatmap was generated to display the normalized abundance of dominant taxa.

2.7. Data Analysis

Data are expressed as mean ± SD. Statistical analyses were performed with SPSS 26 (IBM, New York, NY, USA). Normality and homogeneity of variances were first checked by Shapiro–Wilk and Levene's tests, respectively. Data meeting these assumptions were analyzed by one-way ANOVA followed by Duncan's post-hoc test; when variances were unequal, natural-log or square-root transformation was applied prior to ANOVA. Growth parameters (final weight, weight gain rate, SGR, FCR, survival), intestinal morphology (villi height, crypt depth, V/C ratio), digestive enzyme activities, and gene expression were all subjected to the same ANOVA and Duncan procedure. Pearson correlation was used to assess relationships between gene expression and enzyme activities or intestinal traits. Significance was set at $p < 0.05$.

3. Results

3.1. Growth Performance, Feed Utilization, and Survival Rate

A 12-week feeding trial was conducted to evaluate the effects of *B. velezensis* supplementation. Relative to fish fed the basal diet, those receiving continuous *B. velezensis* GY1 supplementation showed significantly higher final body weight, weight gain rate (WGR), specific growth rate (SGR), and survival rate (SR), while feed conversion ratio (FCR) (Table 2) was significantly reduced ($p < 0.05$). In the intermittent supplementation group, body weight, WGR, and SGR were slightly elevated compared with the control group, but these differences were not statistically significant ($p > 0.05$). Nevertheless, intermittent feeding resulted in a significantly lower FCR and a significantly higher SR than those in the control group. The data presented herein indicate that the supplementation of diets for largemouth bass with *B. velezensis* GY1 results in a significant reduction in the feed conversion ratio and an improvement in survival rate. Furthermore, the continuous supplementation of diets with *B. velezensis* GY1 treated diet yields optimal results.

Table 2. Growth performance of juvenile largemouth bass (*Micropterus salmoides*) fed *B. velezensis* GY1.

Growth Performance	Control Group	J-Group	L-Group
Initial weight (g)	7.07 ± 0.06 ^{a}	7.12 ± 0.02 ^{a}	7.04 ± 0.01 ^{a}
Final weight (g)	42.52 ± 4.62 ^{b}	50.78 ± 4.03 ^{ab}	64.55 ± 7.41 ^{a}
Weight gain rate (WGR, %)	500.90 ± 60.43 ^{b}	613.14 ± 55.12 ^{ab}	816.80 ± 104.66 ^{a}
Specific growth rate (SGR, %/d)	2.13 ± 0.12 ^{b}	2.34 ± 0.09 ^{ab}	2.63 ± 0.13 ^{a}
Feed conversion ratio (FCR)	0.93 ± 0.02 ^{a}	0.86 ± 0.02 ^{b}	0.81 ± 0.00 ^{c}
Survival rate (SR, %)	73.33 ± 5.77% ^{a}	98.33 ± 2.89% ^{b}	96.67 ± 2.89% ^{c}

Note: J-group: intermittent feeding group; L-group: continuous feeding group. The data presented as mean ± SD. Different letters represent significant differences ($p < 0.05$).

3.2. Distal Intestinal Morphological Examination

Following the analysis of H&E staining intestinal and liver tissues, Figure 1 presents their morphological examination micrographs. The intestinal tracts across all treatment groups exhibited the typical layered structure characteristic of fish intestines, with clearly distinguishable mucosal, submucosal, muscular, and serosal layers, all displaying normal morphological features. Liver tissue sections from the control and continuous feeding groups showed tightly arranged hepatocytes with relatively uniform cell size. Hepatic cords and sinusoidal spaces exhibited relatively regular structures. No obvious cellular swelling or extensive fatty degeneration was observed; only sporadic tiny lipid vacuoles could be seen, with no apparent inflammatory cell infiltration. Based on qualitative morphological observation, no significant pathological lesions were found in the livers of these two groups. Liver tissue sections from the intermittent feeding group presented

abundant vacuolar lipid structures of variable sizes, accompanied by locally irregular hepatocyte morphology and markedly severe hepatocyte fatty degeneration (as indicated by red arrows).

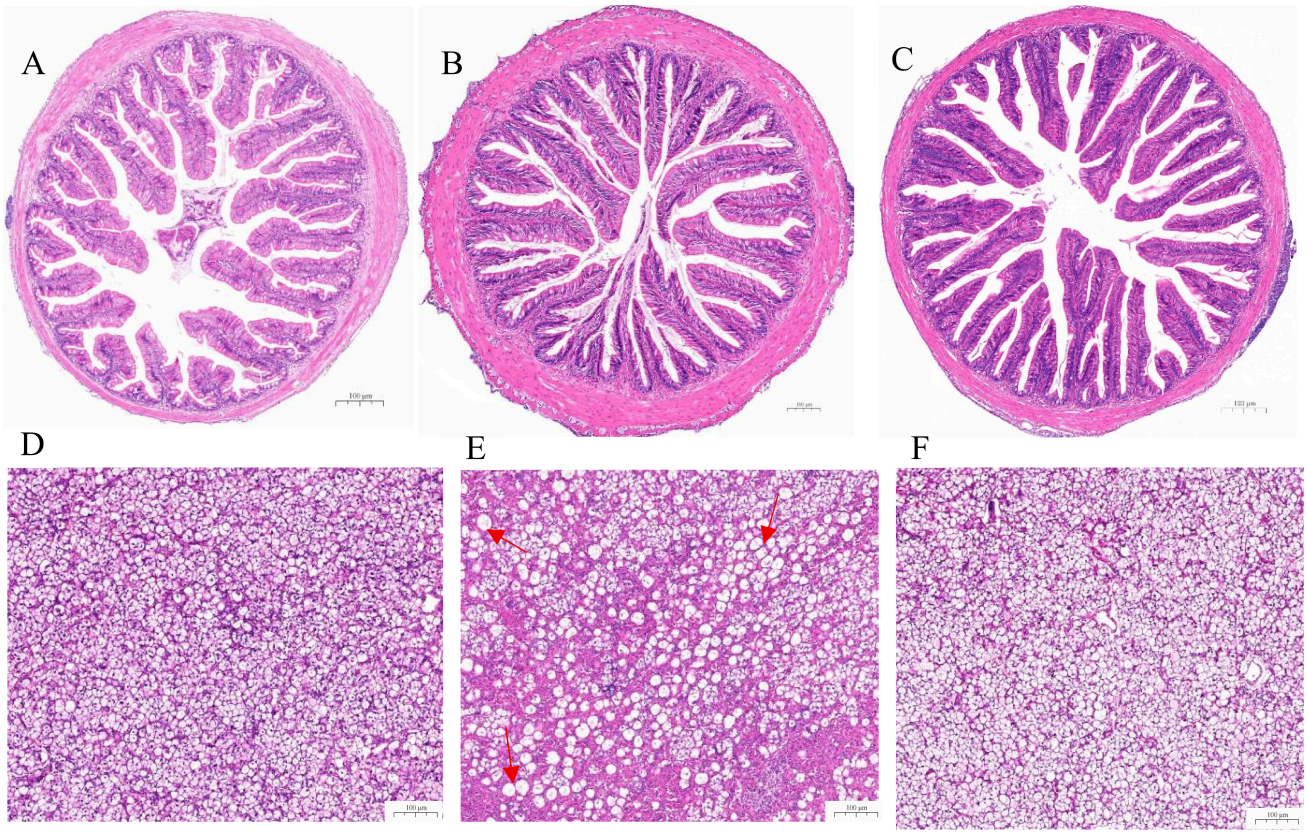

Figure 1. Effects of dietary *B. velezensis* GY1 on intestinal and hepatic morphology in largemouth bass. Note: H&E staining. (**A**) Intestine, control group; (**B**) Intestine, intermittent group (J group); (**C**) Intestine, continuous group (L group); (**D**) Liver, control group; (**E**) Liver, intermittent group; (**F**) Liver, continuous group. Red arrows indicate fatty degenerated hepatocytes.

A subsequent analysis of villus length, width, and basement membrane width revealed significant morphological changes in intestinal tissue following supplementation with host-derived probiotics. A comparison of the intestinal villi in the *B. velezensis* fed group with those in the control group revealed that the former were characterized by increased height and width, along with augmented thickness of the basal layer (Figure 2). In addition, villus height (VH), villus width (VW), and muscular layer thickness (MLT) in the intermittent group were significantly greater than those in the control and continuous groups ($p < 0.05$). Intermittent *B. velezensis* GY1 supplementation significantly decreased intestinal crypt depth relative to the control group, whereas no significant difference was noted between the continuous group and the control. Additionally, compared with the control group, the villus-to-crypt ratio in the intestines of both the intermittent and continuous groups was significantly increased after dietary supplementation with *B. velezensis* GY1, with the intermittent group showing a significantly higher ratio than both the control and continuous groups.

3.3. Serum Immune, Anti-Oxidant, and Digestive Enzyme Activities

The effects of probiotic supplementation on serum immune and antioxidant parameters were assessed, and the results are presented in Figure 3. Compared with the control group, intermittent dietary *B. velezensis* GY1 supplementation significantly elevated serum

activities of ALB, SOD, AMY, AKP, and LZM ($p < 0.05$). Globulin, lipase, pepsin, and complement activities in the intermittent group exhibited slight increases relative to the control, but these differences were not statistically significant ($p > 0.05$). After continuous dietary *B. velezensis* GY1 supplementation, activities of all measured enzymes except pepsin were significantly higher than those in the control group ($p < 0.05$). Moreover, the activities of amylase, alkaline phosphatase, and total complement were significantly greater in the continuous group than in the intermittent group ($p < 0.05$).

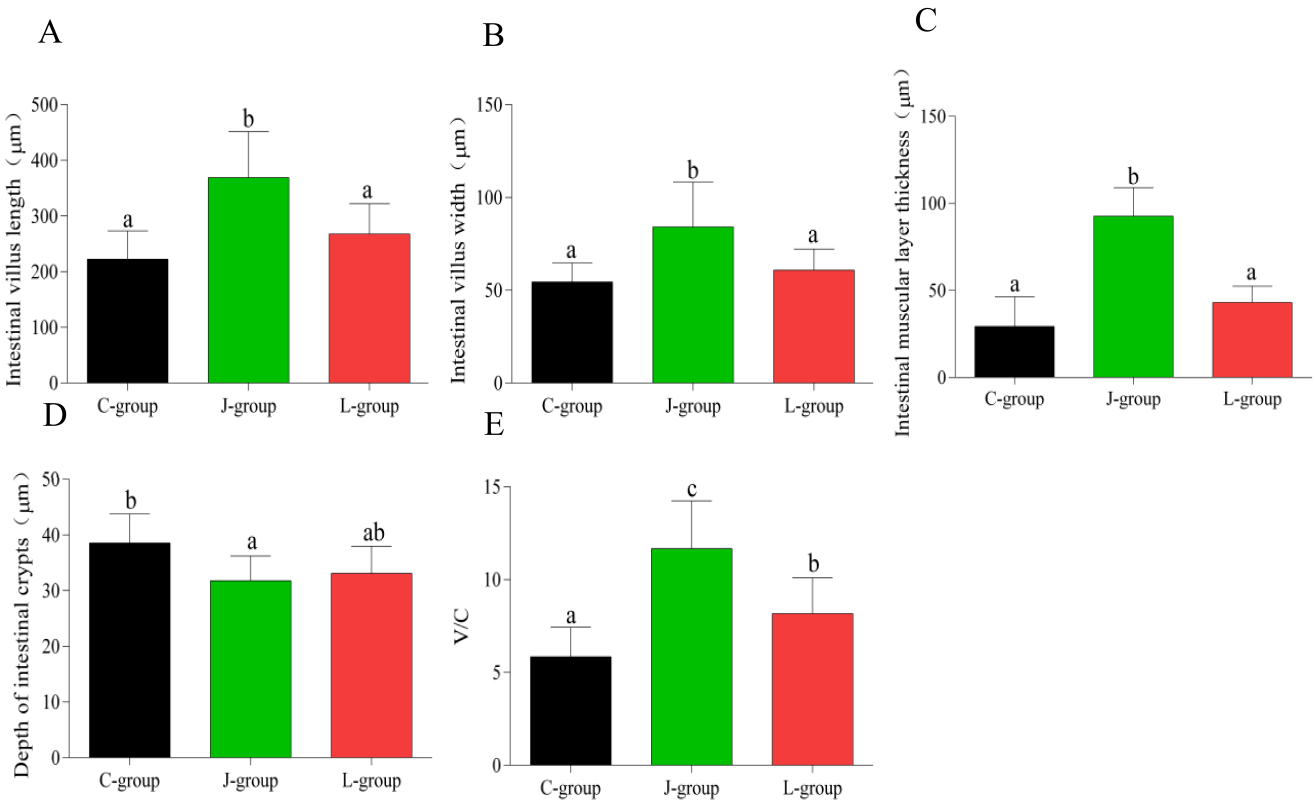

Figure 2. The villus length (VH), villus width (VW), muscular layer thickness, intestinal crypt depth (MLT) and V/C statistics of largemouth bass intestinal villi in each treatment group. Note: (**A**) intestinal villus length statistics (VH); (**B**) intestinal villus width statistics (VW); (**C**) intestinal muscular layer thickness statistics (MLT); (**D**) intestinal crypt depth statistics; (**E**) intestinal V/C statistics, V/C: Villus height/Crypt depth ratio. Note: J-group: intermittent feeding group; L-group: continuous feeding group. Note: Different letters represent significant differences ($p < 0.05$).

3.4. Gene Expression

Quantitative real-time PCR (qPCR) was employed to examine the expression levels of digestion-related genes in the liver across different treatment groups (Figure 4). Continuous *B. velezensis* GY1 supplementation significantly up-regulated *Keap1* and *Gpx* expression, while significantly down-regulating *SOD* expression compared with the control group. However, no significant changes were observed in the expression of *Nrf2*, *Akp*, or *Acp* in the continuous group relative to the control. Intermittent *B. velezensis* supplementation significantly increased the transcript levels of *Nrf2*, *Acp*, and *Gpx*, whereas no significant difference was found for *Keap1* or *Akp* compared with the control. In addition, *SOD* expression was significantly reduced in the intermittent group relative to the control.

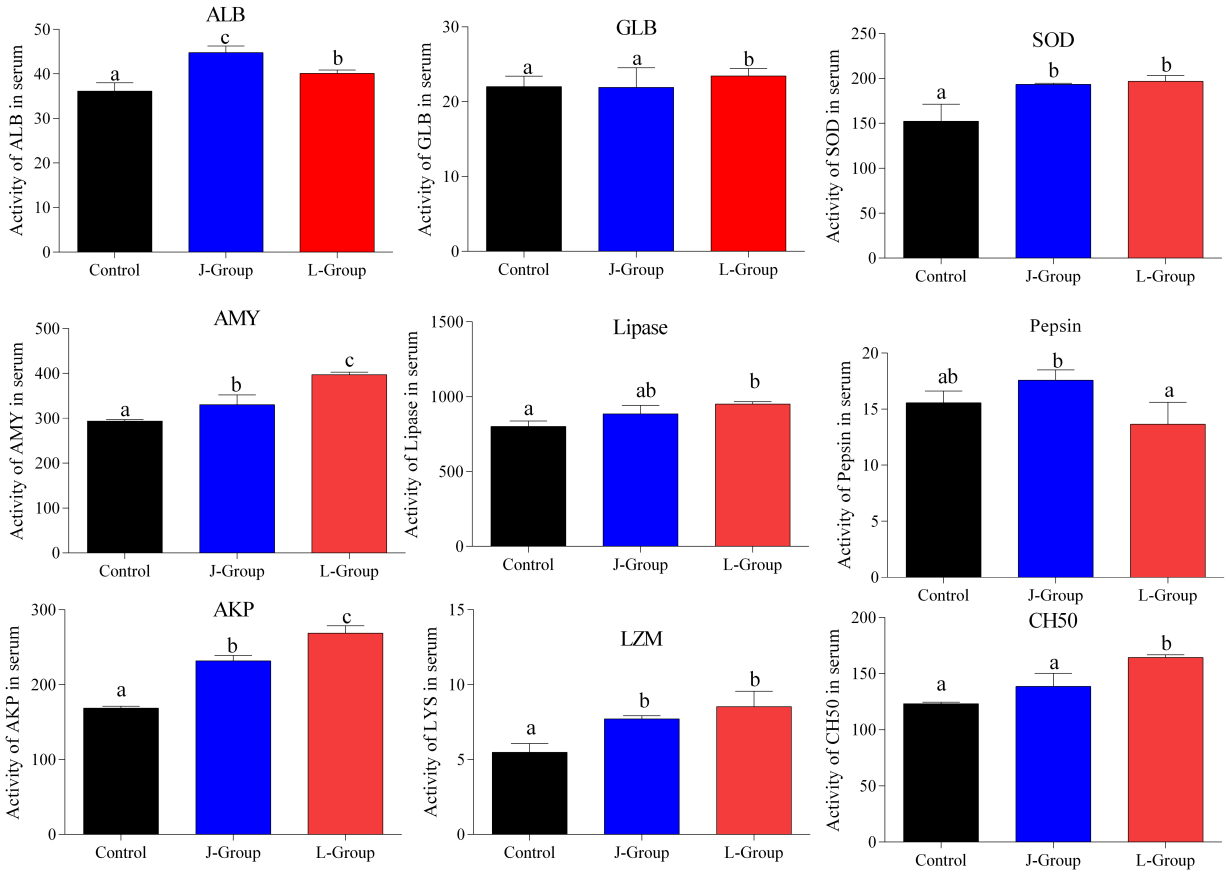

Figure 3. Activity of enzymes in serum of largemouth bass with 12 weeks of supplementation of *B. velezensis* GY1 in the diet. Note: Different letters indicate significant differences ($p < 0.05$). Note: Control: the control group; J group: the intermittent feeding group; L group: the continuous feeding group.

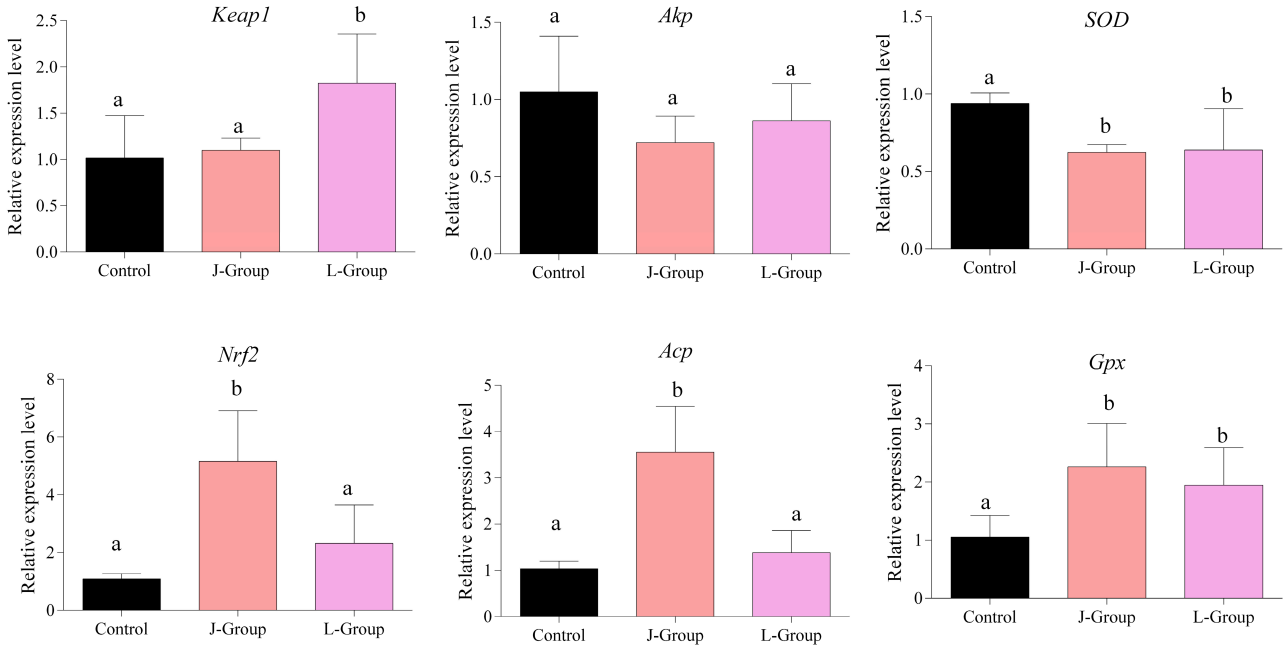

Figure 4. Gene expression levels in the liver of largemouth bass after twelve weeks of supplementation of *B. velezensis* GY1 in the diet. Note: Different letters indicate significant differences ($p < 0.05$). Note: Control: the control group; J group: the intermittent feeding group; L group: the continuous feeding group.

The expression levels of immune genes in the head kidney tissues of each treatment group were detected using qPCR technology. The results showed that compared with the control group, intermittent feeding with *B. velezensis* enhanced the expression levels of immune genes, but the difference was not statistically significant (Figure 5). Continuous feeding with *B. velezensis* significantly increased the expression levels of *TNFα*, *TGFβ*, *IL8*, *IL34* and *myd88*, while the expression levels of the genes *Fas* did not undergo significant changes.

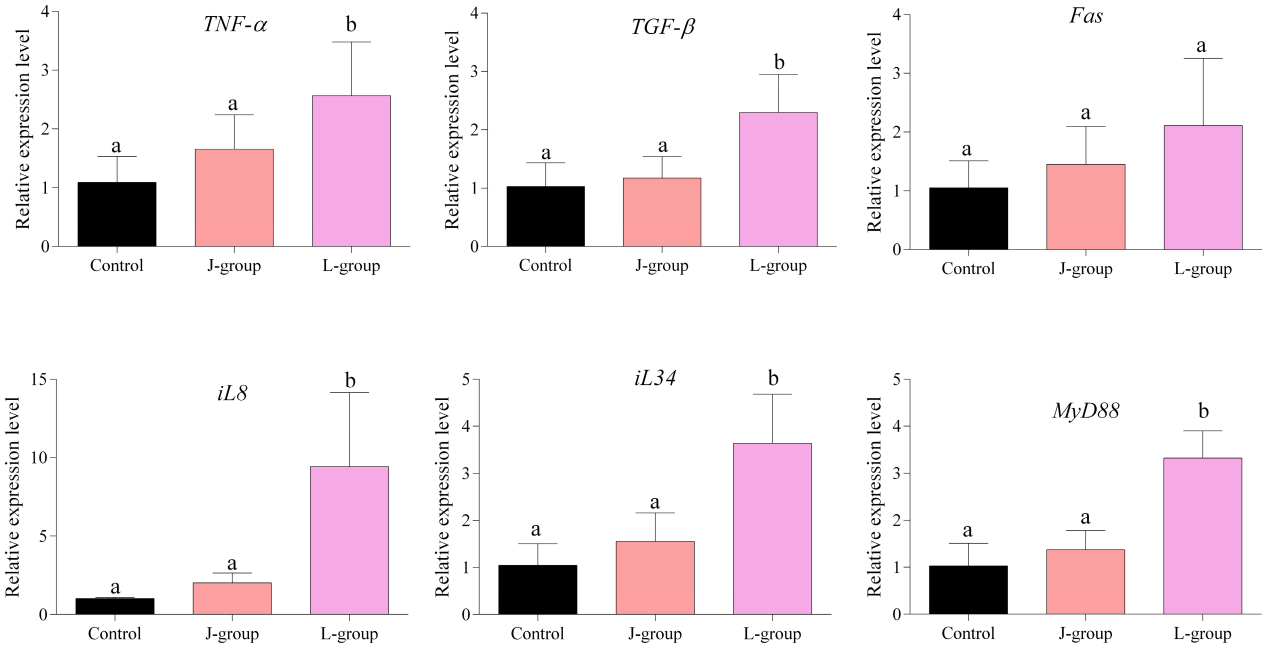

Figure 5. Gene expression levels in the head kidney tissues of largemouth bass after twelve weeks of supplementation of *B. velezensis* GY1 in the diet. Note: Different letters indicate significant differences ($p < 0.05$). Note: Control: the control group; J group: the intermittent feeding group; L group: the continuous feeding group.

3.5. High-Throughput Sequencing Analysis of 16S rRNA Gene Amplicons

3.5.1. Obtained Sequence Information of the Distal Intestinal Microbiota

A total of 682,073, 741,712 and 71,0945 raw reads were obtained from fish intestine microbiota samples of three groups (mix the intestinal contents of every three fish into one sample, with six replicates per group), with an average of 97,439, 105,959 and 101,564 sequences per group, respectively (Table S1). After data quality filtering, 676,039, 734,871 and 704,429 clean reads were acquired, averaging 96,577, 104,982 and 100,633 sequences per group. Following chimera removal, the clean reads obtained from fish intestinal samples yielded 639,233, 647,614, and 633,398 effective tags, with mean values of 91,319, 92,516, and 90,485 sequences per group, respectively. No significant differences were detected among treatment groups in terms of effective tag counts ($p > 0.05$).

3.5.2. Alpha and Beta Diversity Analysis of the Distal Intestinal Microbiota

The supplementary data (Table S1) summarized the operational taxonomic units (OTUs) and alpha diversity indices, including community richness (Chao1 and ACE) and diversity (Shannon and Simpson), for the intestinal microbiota of largemouth bass fed dietary *B. velezensis*. Overall, none of the alpha diversity indices (Chao1, ACE, Shannon, and Simpson) showed significant differences among all groups ($p > 0.05$). The Good's coverage values for the control, J, and L groups were 1 ± 0.00, 0.999 ± 0.00, and 1 ± 0.00, respectively, indicating that the sequencing depth was sufficient to capture most of the microbial

diversity within the samples. To compare overall β-diversity among groups, principal component analysis (PCA) and principal coordinate analysis (PCoA) were performed based on weighted and unweighted UniFrac distances. Weighted and unweighted UniFrac PCoA plots are shown in Figure 6A and 6B, respectively, and the two-dimensional PCA plot is presented in Figure 6C. A Venn diagram (Figure 6D) was used to illustrate the shared and unique OTUs among the three groups, revealing a total of 153 OTUs shared across all treatment groups. Furthermore, the core OTU numbers in the control, J, and L groups were 103, 223, and 65, respectively, with the J group exhibiting the highest number.

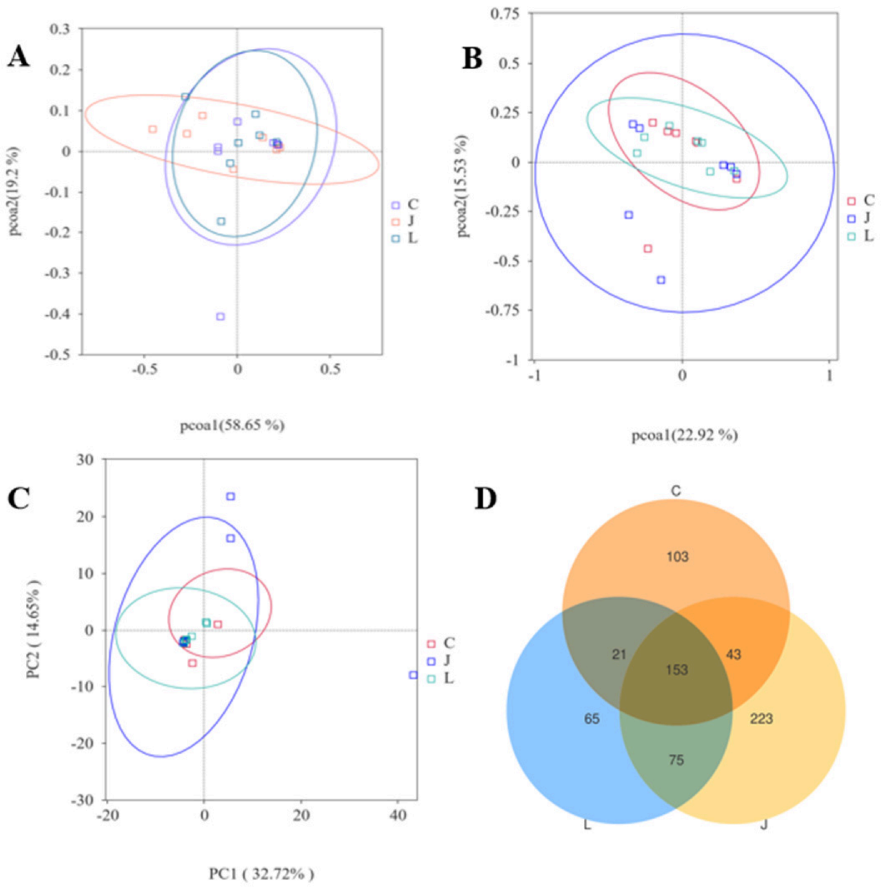

Figure 6. The Venn diagram and beta diversity of intestine microbiota based on weighted UniFrac and unweighted UniFrac distance of intestinal microbiota after dietary supplementation of *B. velezensis* GY1 in largemouth bass. Note: (**A**): PCoA of weighted UniFrac distance; (**B**): PCoA of unweighted UniFrac distance; (**C**): PCA analysis of intestinal microbiota; (**D**): Venn diagram of species diversity. C indicated the control group; J indicated intermittent feeding group; L indicated continuous feeding group.

3.5.3. Microbiota Composition, Relative Abundance Analysis, and Comparison

Microbial community composition was determined based on sequencing reads clustered at 97% similarity. A total of 25 phyla, 44 classes, 95 orders, 173 families, 323 genera, and 237 species were identified across all samples. Figure 7 presents the relative abundance of intestinal microbiota in juvenile largemouth bass after dietary *B. velezensis* supplementation. At the phylum level, the ten most dominant phyla were *Firmicutes*, *Fusobacteriota*, *Actinobacteriota*, *Proteobacteria*, *Bacteroidota*, *Cyanobacteria*, *Halobacterota*, *Chloroflexi*, *Patescibacteria*, and *Deinococcota*, with *Firmicutes* being the most abundant (Figure 7A). In comparison with the control group, the relative abundance of Fusobacteriota was significantly reduced in both the J and L groups ($p < 0.05$). However, compared with the control group, the relative abundance of *Proteobacteria*, *Actinobacteriota*, *Cyanobacteria*, *Bacteroidota*, *Deinococ-*

cota, *Halobacterota* and *Chloroflexi* were displayed a significant increase in J group ($p < 0.05$). *Proteobacteria* and *Actinobacteriota* displayed a significant increase in L group ($p < 0.05$) in comparison to the control group (Figure 7A).

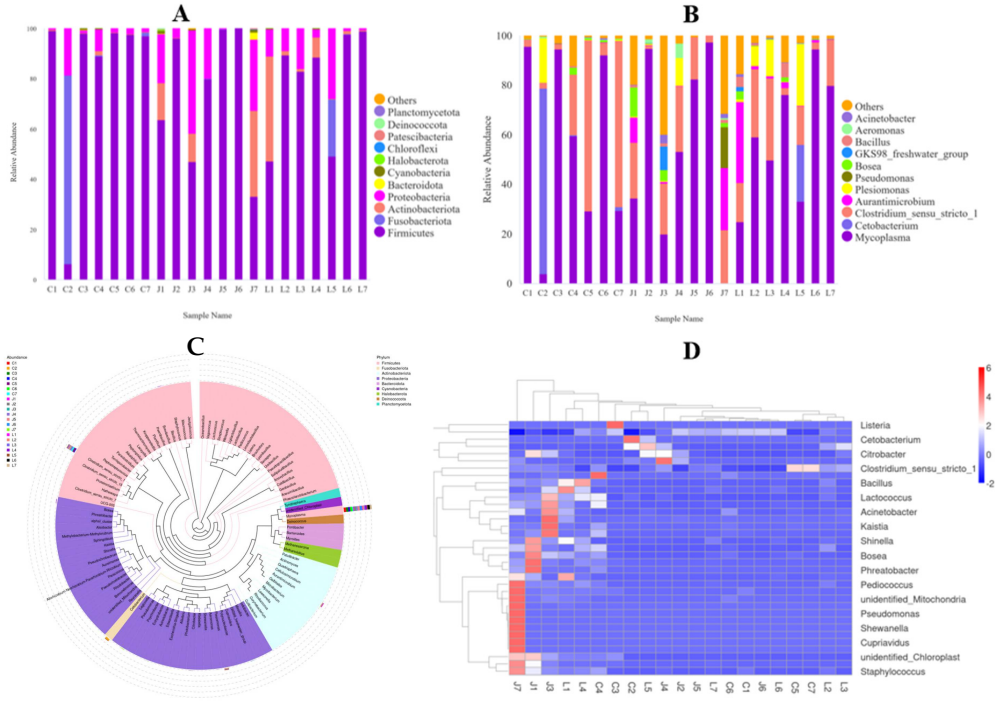

Figure 7. Comparison of bacterial composition and relative abundance of the intestinal microbiota in largemouth bass fed *Bacillus* species. (**A**) Relative abundance at the phylum level; (**B**) Relative abundance at the genus level; (**C**) Phylogenetic tree based on genus-level features (each branch represents a species, and branch length indicates the evolutionary distance between two species); (**D**) Heatmap showing the top 100 predominant genera in the distal intestinal microbiota of largemouth bass after dietary supplementation with *Bacillus* species. Note: C: the control group; J: intermittent feeding group; L: continuous feeding group.

At the genus level, the ten most abundant taxa are illustrated in Figure 7B. Compared with the control group, the J group exhibited significantly higher relative abundances of *Bosea*, *Aeromonas*, *Aurantimicrobium*, *Pseudomonas*, the GKS98 freshwater group, and Bacillus, while the abundances of *Clostridium_sensu*_stricto_1 and *Cetobacterium* were significantly lower ($p < 0.05$; Figure 7B). Similarly, the L group showed significantly increased abundances of *Aurantimicrobium*, *Plesiomonas*, the GKS98 freshwater group, and *Bacillus* relative to the control group ($p < 0.05$). In the L group, the abundances of *Cetobacterium* and *Plesiomonas* were also significantly reduced compared with the control group (Figure 7B). A phylogenetic tree based on genus-level features is displayed in Figure 7C, and a heatmap depicting the relative abundances of the top 35 genera is shown in Figure 7D.

4. Discussion

The implementation of effective disease control measures in aquaculture is of paramount importance for the attainment of high productivity in aquatic animals and the sustainability of the aquaculture industry [27]. The widespread and indiscriminate use of antibiotics has raised considerable biological and ecological issues, primarily reflected in the increasing prevalence of antibiotic-resistant bacteria [28,29]. Probiotics, broadly defined as beneficial microbes, are considered effective and environmentally sustainable alternatives to antibiotics, owing to their marked benefits in promoting host growth and health, as well as their pathogen-resistant properties [30]. Aquaculture practices

have increasingly adopted probiotics for their development, as these microorganisms are both ecologically friendly and economically advantageous, unlike antibiotics and other chemical agents, which not only cause adverse effects on animals and the environment but also are economically inefficient [31,32]. Among various known beneficial bacteria, the genus *Bacillus* has been documented as one of the most promising probiotics, due to its critical attributes, including tolerance to harsh environmental conditions, prolonged viability, enhancement of growth performance, immune responses, digestive enzyme activities, and disease resistance in aquatic animals [31]. Numerous studies have examined the effects of *Bacillus* strains isolated from various sources on the growth and development, immune responses, antioxidant activity, digestive enzyme activity, and disease resistance of different fish species. Nevertheless, the impact of *B. velezensis* on largemouth bass remains relatively understudied. To date, there is a paucity of reports on the effects of *B. velezensis* enrichment on the growth, immunity, digestion, and intestinal contents of largemouth bass. This study, based on a strain of *B. velezensis* GY1 screened from aquaculture wastewater, evaluates the effects of *B. velezensis* GY1 on the growth, immunity, and intestinal health of juvenile largemouth bass from histological and intestinal microbiological perspectives.

Probiotics are primarily introduced into the digestive tract via feed and water. In animal production, *Bacillus velezensis* is typically administered as a direct-fed microbial or as part of a multi-strain formulation [33]. Numerous studies have established that dietary *B. velezensis* supplementation enhances intestinal digestive enzyme activity, reduces feed conversion ratio (FCR), and promotes growth in aquatic species [34]. Consistent with these reports, the present study demonstrated that dietary *B. velezensis* GY1 supplementation significantly increased weight gain (WG) and specific growth rate (SGR) in largemouth bass (*Micropterus salmoides*). The growth-promoting effect of *Bacillus spp.* has been extensively documented across diverse aquaculture species. For instance, Yang et al. [34] reported that dietary supplementation with 0.3 and 0.4 g/kg of *B. velezensis* T23 fermentation products reduced FCR by 15% and 6%, and increased final body weight by 39% and 25%, respectively, in Pacific white shrimp (*Litopenaeus vannamei*). Zhao et al. [35] isolated *B. velezensis* strains MS-3 and CA-1 and found that both single and mixed supplementation significantly elevated intestinal digestive enzyme activities in largemouth bass, with the mixed group exhibiting the lowest FCR. Abdelsamad et al. [36] further confirmed that dietary *B. velezensis* significantly enhanced digestive enzyme activity, final weight, WG, and SGR in L. vannamei, while decreasing FCR by 23.6%. Beyond these, *B. velezensis* supplementation has been shown to improve growth and feed utilization in carp [37] and to significantly enhance WG and SGR in hybrid grouper (*Epinephelus fuscoguttatus* ♀× *E. lanceolatus* ♂) [38]. Yang et al. [39,40] also demonstrated that symbiotic supplementation significantly increased WG and decreased FCR in juvenile largemouth bass. Collectively, these findings corroborate our observation that *B. velezensis* GY1 supplementation exerts a consistent positive effect on growth performance in *M. salmoides*. The mechanisms underlying *B. velezensis* mediated growth promotion are multifactorial. First, *B. velezensis* can colonize the intestinal tract and produce a spectrum of extracellular digestive enzymes (e.g., proteases, amylases, and lipases), thereby enhancing the host's digestive and absorptive capacity for dietary nutrients. Second, the production of antimicrobial compounds—including lipopeptides, bacteriocins, and volatile organic compounds, suppresses pathogenic bacterial proliferation and maintains gut microbial homeostasis, indirectly supporting host health and growth. Third, *B. velezensis* competes for adhesion receptors on the intestinal epithelium and reinforces intestinal barrier function, further contributing to its probiotic efficacy. Notably, this study also compared two feeding regimens, continuous versus intermittent

supplementation, and revealed that while both effectively promoted growth, they differentially modulated intestinal microbiota composition and hepatic histology. This finding suggests that optimizing the feeding strategy may be a critical avenue for maximizing the efficacy of probiotic application, warranting further investigation. This results provide robust evidence supporting the efficacy of *B. velezensis* as a feed additive for largemouth bass and offer a practical reference for its application in aquaculture.

Intestinal morphology and digestive function are closely interrelated. Villus height and crypt depth are generally considered reliable indicators of the intestinal absorptive capacity [41]. An increase in villus height expands the epithelial surface area available for nutrient contact, thereby facilitating absorption. A higher villus height-to-crypt depth ratio is likewise associated with enhanced nutrient uptake and improved gut health [42]. Previous studies have reported that dietary *B. velezensis* significantly increases villus length in Nile tilapia [43], and that combined supplementation with *B. velezensis* and *Lactobacillus plantarum* reduces crypt depth while elevating the V/C ratio in fish [44]. Similarly, synbiotic administration has been shown to significantly increase villus height in sardines [39]. Consistently, our findings demonstrated that villus height, villus width, and muscular layer thickness were all elevated in the *B. velezensis* supplemented groups relative to the control, with the intermittent feeding regimen yielding significantly greater values than both the control and continuous feeding groups. Moreover, crypt depth was reduced and the V/C ratio was significantly increased in the probiotic treated groups compared with the control. Collectively, these observations provide circumstantial evidence that improved intestinal structural parameters may be associated with enhanced nutrient absorption, though this possibility requires direct confirmation through future digestibility or nutrient flux assays. Nevertheless, our results suggest that *B. velezensis* GY1 supplementation may benefit intestinal health and, by extension, nutrient assimilation in largemouth bass. Intermittent feeding tended to yield slightly greater intestinal improvements than continuous feeding, but this difference warrants further investigation.

The largemouth bass (*Micropterus salmoides*) is one of the most important carnivorous fish species cultured in China. This species possesses a relatively short digestive tract and inherently low amylase activity, which limits its ability to efficiently digest and utilize plant-based feed ingredients, particularly starch [45]. Carnivorous fish exhibit persistent hyperglycemia after consuming high-carbohydrate diets, and their physiological glucose intolerance renders them far less efficient at utilizing plant-derived carbohydrates than omnivorous or herbivorous species [45]. Consequently, undigested starch may reach the distal intestine, where it becomes available for fermentation by the resident intestinal microbiota. While such microbial fermentation contributes to starch breakdown, excessive production of microbial metabolites may impose an additional metabolic burden on the liver [46]. Martinez-Medina et al. [46] demonstrated that a high fat, high sugar diet induces gut dysbiosis, alters intestinal permeability, and impairs intestinal barrier function, suggesting that high starch diets may exert analogous negative effects on intestinal health and hepatic function in fish. The extracellular enzyme activity of probiotics is considered a primary mechanism by which they enhance host digestive capacity, promote growth and development, facilitate nutrient absorption, and improve growth performance [47]. Guo et al. [47] isolated an enzyme-producing strain of *Bacillus subtilis* from the intestine of grass carp and confirmed its potential as a probiotic additive in aquatic feeds. Tan et al. [48] reported that dietary supplementation with *Rummeliibacillus stabekisii*, a bacterium capable of secreting protease, cellulase, and xylanase, significantly improved weight gain rate and feed efficiency in Nile tilapia (*Oreochromis niloticus*). In rainbow trout (*Oncorhynchus mykiss*), Gao et al. [49] found that spores of *Bacillus velezensis* V4 can cross the gastric barrier, subsequently germinating and

colonizing the gastrointestinal tract, a process that has been demonstrated to influence digestive enzyme activities. Furthermore, Wang et al. [50] reported that dietary supplementation with *B. velezensis* GY65 at concentrations of 10^6 and 10^7 CFU/g significantly enhanced amylase activity in the intestine and liver of mandarin fish (*Siniperca chuatsi*), concomitantly improving weight gain rate, specific growth rate, and organ indices. Collectively, these studies indicate that enzyme-producing probiotics promote the utilization of plant-based feeds by secreting digestive enzymes such as amylase, although their efficacy may vary depending on the strain, dosage, and host species. In the present study, dietary inclusion of *B. velezensis* GY1 at 10^9 CFU/g similarly enhanced weight gain rate, specific growth rate, and feed conversion ratio in largemouth bass, with these effects being more pronounced under the continuous feeding regimen. However, it should be noted that only serum digestive enzyme activities were measured in this study; direct measurements of digestive enzyme activities in intestinal and hepatic tissues were not performed. Therefore, although previous studies have reported that *B. velezensis* possesses the capacity to secrete amylase and other digestive enzymes [50], the present study cannot directly confirm that the observed growth-promoting effects are attributable to the modulation of digestive enzyme activity by this probiotic in the host intestine. Taken together, our findings indicated that *B. velezensis* GY1 supplementation exerts a positive effect on the growth performance of largemouth bass. Nevertheless, the precise mechanisms underlying this effect—particularly whether they involve the regulation of digestive enzyme activities in the intestine and liver—warrant further investigation through direct enzymatic assays in relevant tissues.

Numerous studies have demonstrated that probiotics enhance the host's antioxidant capacity and innate immune function [51]. Superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen, is widely recognized as a reliable biomarker of antioxidant status in fish. This process has been demonstrated to mitigate the harmful effects of free radicals [52,53]. Wu et al. [54] found that feeding grass carp with *Bacillus licheniformis* B8 significantly increased SOD activity and reduced MDA content, with the 10^9 CFU/g supplementation group showing the best effect. Wu et al. [54] observed that feeding *Aeromonas veronii* a diet containing 1×10^9 CFU/g *B. velezensis* significantly elevated serum SOD and CAT activities by 34.6% and 138.1%, respectively, compared to the control group, while reducing MDA content by 47.5%. Lysozyme serves as a critical antibacterial protein within the fish non-specific immune system, constituting an important defense barrier against viral and bacterial infections [55,56]. A six-week feeding trial in Nile tilapia, as reported by Zhang et al. [57], demonstrated elevated serum lysozyme activity following probiotic administration. It has been suggested that *Bacillus* spores enhance host immunity by stimulating non-specific immune responses and interfering with pathogen cell wall components [58]. In the present study, after 12 weeks of dietary *B. velezensis* GY1 supplementation, serum superoxide dismutase and lysozyme activities in largemouth bass were significantly higher than those in the control group. Collectively, these results indicate that *B. velezensis* may protect against oxidative damage and potentiate lysozyme activity.

B. velezensis has been reported to trigger the NF-κB signaling pathway [59], enhance the transcription of immune-related genes, and regulate the production of tumor necrosis factor-α (*TNF-α*), interleukin-1β (*IL-1β*), and interleukin-6 (*IL-6*) in the liver and head kidney [60–62]. This enhances the animals' immunosuppressive capacity and self-repair capabilities, thereby reducing inflammation-induced damage to organs such as the liver [63]. *B. velezensis* has been shown to increase the activities of non-specific immune enzymes in vivo, including acid phosphatase (ACP), alkaline phosphatase (AKP), and lysozyme (LZM) [64,65], increase immunoglobulin (IgG) content, and enhance immune function [62].

Gao et al. [66] found that *Bacillus cereus* CPA1-1 exhibits potent antibacterial activity and strong antagonistic effects against *Vibrio vulnificus*, it up-regulates immune-related gene expression and increases ACP and AKP activity in the intestine. Zhang et al. [57] added *B. velezensis* LF01 to feed at 1.0×10^9 CFU/g, indicated that tilapia fed *B. velezensis* LF01 supplement exhibited significantly higher survival rates than the control group, with upregulation of immune-related gene expression in the head kidney. Li et al. [67] fed grouper with feed supplemented with 1×10^7 CFU/g *Bacillus licheniformis* K2, and observed a gradual increase in ACP and AKP activity, along with significantly elevated LZM activity and IgM and IL-8 levels. Pro-inflammatory cytokines, such as tumor necrosis factor-α (*TNFα*), have been identified as key mediators of inflammatory responses following pathogenic infections in fish [68]. IL-8, a chemotactic cytokine, has been reported to promote the recruitment of inflammatory cells through mediating immune responses associated with local inflammation [69]. Similarly, dietary supplementation with *B. velezensis* was found to upregulate the intestinal expression of tumor necrosis factor-α in grass carp [47]. In the current study, *B. velezensis* supplementation significantly increased the transcript levels of *TNFα* and *IL-8* in the head-kidney of largemouth bass, implying that probiotic administration may modulate the balance of inflammatory cytokines and thereby protect the host from excessive inflammatory responses. It is well established that probiotics can enhance the survival of fish [70].

The balance of intestine microbiota plays a crucial role in the healthy growth and development of animals [71]. *B. velezensis* exhibits strong tolerance to extreme environments [72] and can colonize the intestine [73], competing with pathogens for nutrients, occupying favourable ecological niches in the intestine, and prolonging survival time within animals. Additionally, *B. velezensis* forms biofilms on the intestinal mucosal surface [74], effectively reducing pathogen–intestine contact. *B. velezensis* produces a range of antimicrobial compounds, including lipopeptides, bacteriocins, and volatile organic compounds, which can enhance the competitive fitness of beneficial gut bacteria while simultaneously suppressing pathogenic populations. Such activity favors the establishment of a balanced intestinal microbiota in the host [75–77]. In the present 12-week feeding trial with largemouth bass, no significant difference in the relative abundance of Firmicutes was observed between the control and experimental groups. However, Actinobacteria were significantly enriched in the intestines of the probiotic-supplemented fish. Notably, intermittent feeding led to a marked reduction in Fusobacteria abundance, whereas continuous feeding did not result in a significant change in this phylum. *B. velezensis* promotes the colonization of dominant microbial groups in the animal intestine, such as the Firmicutes phylum, the genus *Bacillus*, and the *Clostridium* class, occupying favorable ecological niches while suppressing the survival space of pathogens [78]. Chen et al. [78] observed significantly increased abundances of *Clostridium* and *Bacillus* in the intestines of carp fed *B. velezensis*. Thurlow et al. [79] observed a significant decrease in Firmicutes abundance in control group spotted tilapia, whereas no notable changes were seen in the group fed *B. velezensis* AP193. This indicates that AP193 does not excessively alter the composition of the intestinal microbiota and effectively balances the intestine microbiome. Khan et al. [80] observed stable abundances of Actinobacteria and Bacillaceae in *Carassius carassius* fed *B. velezensis* FAi2, whereas the control group was dominated by *Bacillus* species. These findings indicate that appropriate supplementation with *B. velezensis* can modulate beneficial intestine microbial diversity, maintain intestinal microbial balance, repair intestinal damage, and enhance intestine function.

5. Conclusions

The results of the present study demonstrate that 12-week dietary supplementation with *Bacillus velezensis* GY1 significantly improved growth performance, enhanced digestive capacity and immune function, and maintained intestinal microbial community homeostasis in largemouth bass. Notably, the continuous and intermittent feeding regimens exhibited differential effects on several parameters: continuous feeding was more effective in promoting growth and reducing the feed conversion ratio, whereas intermittent feeding showed a greater advantage in improving intestinal morphology and modulating the gut microbiota. These findings suggest that the selection of feeding strategy should be optimized according to specific culture objectives and practical production conditions. With the advancement of precision aquaculture and green agriculture, *B. velezensis* GY1 holds promise as a key biological agent for healthy aquaculture practices. Future research should focus on the following aspects: (1) investigating the applicability of different feeding strategies under various culture cycles and stress conditions; (2) elucidating the molecular mechanisms by which *B. velezensis* modulates host physiology through multi-omics approaches; and (3) evaluating its scalability and commercial application potential under real world farming conditions, thereby driving the aquaculture industry toward an efficient, environmentally sustainable trajectory.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani16152423/s1, Table S1: The growth performance, intestinal morphology, digestive enzyme results, gene expression, and intestinal microbiota data.

Author Contributions: H.S.: Conceptualization, Investigation, Methodology, Software, Writing—original draft, Supervision, review & editing; S.Z.: Investigation, Resources, Formal analysis; H.Z.: Supervision, Resources; J.D.: Supervision, Resources; W.Z.: Investigation, Formal analysis; T.L.: Investigation, Formal analysis; B.Z.: Investigation, Resources; J.L.: Supervision, Resources, Funding Acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by grants from the Research on the application of duckweed in fish ecological cycling farming systems funds (DT20240002) and Development and demonstration of duckweed feed protein technology (2025 Provincial Science and Technology Special Project 036).

Institutional Review Board Statement: The animal study protocol was approved by Animal Care and Use Committee of Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences (LAEC-PRFRI-2025-03-54).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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