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The Effect of *Schizochytrium* sp. on Growth, Fatty Acid Profile and Gut Microbiota of Silver Pomfret (*Pampus argenteus*)

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Abstract: *Schizochytrium* sp. is a kind of heterotrophic protist, rich in docosahexaenoic acid (DHA). To study the application value of *Schizochytrium* sp. in fish diet, a 20-day feeding experiment was initiated to evaluate its effect on growth, fatty acid contents, and the gut microbiota of the important economic fish, silver pomfret. In this study, the diets of the treatment group were added with 2% *Schizochytrium* sp. The fish in the treatment group gained more weight and had a higher growth rate than the control group. The levels of DHA, eicosapentaenoic acid, and polyunsaturated fatty acids in the edible tissue of fish were highly increased in the treatment group after 20 days. After feeding *Schizochytrium* sp., high-throughput 16S rRNA sequencing showed that the Proteobacteria, Firmicutes and Bacteroidetes were the phyla with the highest abundance, and at the genus and species level, we observed an increased abundance of probiotics. The results indicated that ingestion of *Schizochytrium* sp. could change the dominant microbiota population, which might lead to accelerated growth and improved unsaturated fatty acid content and fish health. This study provides a reference for *Schizochytrium* sp. supplementation in fish diets and could aid in the industrialization of silver pomfret.

Keywords: *Pampus argenteus*; *Schizochytrium* sp.; growth performance; fatty acid; gut microbiota

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1. Introduction

The incorporation of microalgal biomass into feeds might increase the content of vitamins, essential fatty acids, polysaccharides, monounsaturated fatty acids (MUFAs) and n-3 and n-6 polyunsaturated fatty acids (PUFAs) in the diet, which could be utilized as good feed supplements. Among these microalgae, *Schizochytrium* sp. is a kind of unicellular and heterotrophic protist that is rich in docosahexaenoic acid (DHA), whose content among total fatty acids is close to 35% [1–4]. However, there are few reports about its effect on commercial fish feed. As an important ω-3-unsaturated fatty acid (UFA), DHA has many important physiological functions, such as promoting brain cell growth and development [5,6], lowering blood fat and blood sugar, protecting eyesight, preventing cancer, and enhancing immunity [7,8]. Consequently, juvenile fish have a high dietary requirement for DHA to maintain their development, and the appropriate amount dietary DHA could improve the growth and survival of larvae [9]. Therefore, exogenous input of DHA-rich algae is beneficial to fish. Meanwhile, DHA supplementation could also significantly increase the UFA levels in tissues [10,11]. Thus, supplementation of *Schizochytrium* sp. in marine fish daily diet might improve the growth performance and UFA levels, and enhance the health state of fish.

There are particularly large and diverse populations of bacteria in fish intestines. Studies have reported that the structure and composition of the fish intestinal microbial community are affected by many factors, including diet type, aquatic environment and host health, among which diet type is the main driving factor for the formation of the intestinal bacterial community, commonly referred to as the microbiota [12–15]. The microbiota are believed to affect host development, immunity, digestion and nutrition to a certain extent [16,17]. The gut microbiota of aquatic animals can not only inhibit the growth of potential pathogens, but also promotes the digestion of algae cells and the production of amino acids and short-chain fatty acids [18]. Therefore, feeding *Schizochytrium* sp. might change the abundance and structure of intestinal microorganisms in fish.

Silver pomfret (*Pampus argenteus*) is an important aquaculture species in the western Pacific Ocean and the Indian Ocean [19,20]. Silver pomfret tastes delicious, and the DHA content in its muscle is as high as 8% [21], conferring a high nutritional value. In recent years, however, the population of wild silver pomfret has decreased sharply because of overfishing and environmental pollution. Therefore, the artificial breeding of silver pomfret is urgently required, and a breakthrough in artificial breeding technology is very important. In the past ten years, our research team has preliminarily realized mass breeding and has mastered key breeding technologies. At the same time, our team had also made certain achievements in the basic research of ovarian development, immunity, olfactory-related receptors and other aspects of silver pomfret [22–25].

To study the effects of *Schizochytrium* sp. on the growth, fatty acid composition and intestinal microbes of silver pomfret, we conducted a feeding experiment using *Schizochytrium* sp. The results will provide novel insights into the effects of adding *Schizochytrium* sp. to the daily diet of silver pomfret and will contribute to the industrialization of silver pomfret.

2. Materials and Methods

2.1. Experimental Diet and Sample

Cultured silver pomfret (initial mean length = 8.64 ± 1.0 cm, mean weight = 8.56 ± 0.67 g) were obtained from the Xiangshan Harbor Aquaculture and Larva Company Limited, Ningbo, China. The fish were fed a ration of approximately 6% (the total amount of feed provided was 2% fish body weight for 1 h 3 times per day) [26] of their body weight three times daily with a commercial feed (larve love 6#, Hayashikane Sangyo Co., Ltd., Y amaguchi-ken, Japan), keeping the ambient water at 25 °C~27 °C salinity, at 31‰~32‰ and dissolved oxygen > 6 mg/L. They were cultured in 6 farming tanks (1500 L) to acclimate for one week and fasted for 24 h before the experiment. During the experiment, 240 fish in the same growth stage were selected randomly and split into two groups (n = 3), including control (without *Schizochytrium* sp. Supplement diet) and treatment group (2% *Schizochytrium* sp. supplement diet) [27]; 40 fish were placed in each tank. The culturing water was changed totally every day.

We measured the length and weight of the fish from two groups at 0 d, 5 d, 10 d, 15 d, 20 d after anaesthetizing them by 75 mg L-1 MS-222 (Canton, Shanghai, China). Then we sampled the muscle and gut tissues of the fish which were isolated and washed with a sterile 0.85% (*w/v*) NaCl solution. Either type of tissue was pooled into 1.5 mL RNase-free tubes (n = 3, Jet, China) and stored in liquid nitrogen. One independent biological replication of gut microbiota analysis was pooled from three fish guts in either group of each sampling time (n = 3), and one independent biological replication of fatty acid analysis was pooled from three fish muscles in either group of each sampling time (n = 3). C1: control group of 0 d, C2: control group of 5 d, C3: control group of 10 d, C4: control group of 15 d and C5: control group of 20 d; S1: treatment group of 0 d, S2: treatment group of 5 d, S3: treatment group of 10 d, S4: treatment group of 15 d and S5: treatment group of 20 d.

2.2. Determination of Fish Growth Performance and Fatty Acid Composition

2.2.1. Growth Performance

The length and weight of fish at the end of feeding has recorded to measure growth performance. Initial Weight (IW), Final Weight (FW), Initial Length (IL), Final Length (FL)

Weight gain rate (WG) = final body weight − initial body weight;

Body weight gain (WG%) = 100 × (final body weight − initial body weight)/initial body weight;

Specific growth rate (SGR% day^{-1}) = 100 × (final individual weight − initial individual weight)/number of days [28].

2.2.2. Fatty Acid Composition

Total Lipid (TL) Extraction

The samples were lyophilized with a Freezone Freeze Dry System (Labconco, Kansas City, MO, USA). Lipids were extracted from 20 mg sliver pomfret muscle by a modified chloroform-methanol method (Bligh and Dyer method [29]), using chloroform/methanol (1:1, *v/v*) containing 0.05% butylated hydroxytoluene (BHT). The combined lipid extracts were evaporated on a rotary evaporator (IKA, Staufen, Germany), and the residue was stored at −20 °C. After a series of liquid/liquid phase separations, centrifugation, and evaporation under nitrogen, the lipid fraction was methylated [30] with boron trifluoromethanol.

Fatty Acid Analysis

The fatty acid composition was determined using Gas chromatography (QP2010 gas chromatograph–mass spectrometer, Shimadzu Corporation, Japan) coupled with mass spectrometry (SPB-50 capillary column (30 m × 0.25 mm, 0.25 μm, Supelo, USA), with 120, an inlet temperature of 250 °C and a column pressure of 73.0 kPa; the column temperature was maintained at 150 °C for 3.5 min, and then the temperature was set to 200 °C with a rate of 20 °C/min. Then, the temperature was raised to 280 at a rate of 5 °C/min.

The sample was dissolved in n-hexane, the injection volume was 1 μL and the split ratio was 50:1. The mass spectrometry was performed with electron impact (EI) source, the energy was set to 70 eV, the ion source temperature and interface temperature were set to 200 °C and 250 °C, respectively, and the range was m/z 40~600. All extraction procedures were performed in triplicate.

2.3. Gut Microbiota Analysis

2.3.1. DNA Extraction

The bacterial DNA kit (OMEGA, Norcross, GA, USA) was used to extract the total genome DNA. DNA was diluted to 1 ng/μL using sterile water.

2.3.2. 16S rRNA PCR and Pyrosequencing

We took 50 ng DNA from each sample as the template for bacterial 16S rRNA gene amplification and used specific primers to amplify genes in different Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Rowley, MA, USA). Electrophoretic detection was carried out on 2% (*w/v*) agarose gel, and samples with a length of 400–450 bp and bright main bands were selected for further experiments. Qiagen Gel Extraction Kit (Qiagen, Duesseldorf, Germany) was used to purify the PCR products. Sequencing libraries were generated using TruSeq. DNA PCR-Free Sample Preparation Kit (llumina, San Diego, CA, USA).

2.3.3. Operational Taxonomic Units (OTUs) Cluster

Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the QIIME (V1.7.0, http://qiime.org/index.html (accessed on 2 July 2018)) quality controlled process. The tags were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html (accessed on 2 July 2018)) using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html (accessed on 2 July 2018)) to detect chimera sequences, and then the chimera sequences were removed. Then the Effective Tags finally obtained.

Sequences analysis were performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/ (accessed on 2 July 2018)). Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs) [31].

2.3.4. Alpha and Beta Diversity

Alpha diversity is mainly fitted by six indexes (calculated with QIIME (Version 1.7.0)) and displayed with R software (Version 2.15.3), including Observed-species, Chao1, Shannon, Simpson, ACE, Good coverage.

Beta diversity on both weighted and unweighted unifrac was calculated via QIIME software (Version 1.7.0) [32].

2.4. Statistical Analysis

All data were presented as mean ± SD and subjected to independent sample t-test to test the effects of experimental diets using the 22.0 SPSS software [33]. The within-group differences were expressed in English letters and values with different letters were significant different ($p < 0.05$). The between-group differences were expressed in *, values were mean ± SEM of three replicates, and values with * were significant different ($p < 0.05$), values with ** were significant different ($p < 0.01$). One-way analysis of variance was used for within-group difference analysis, and Tukey test was used for between-group differences analysis.

3. Results

3.1. Growth Performance

The growth performance of silver pomfret fed different diets are shown in Figure 1. The results showed that the final mean weight (±SD) was 16.43 ± 0.61 g and the mean length (±SD) was 12.23 ± 0.89 cm for the treatment group, whereas for the control group these values were 14.77 ± 0.31 g and 10.65 ± 1.05 cm, respectively. The results showed that growth performance, including WG, WGR, SGR, and final weight, increased significantly in the treatment group supplemented with *Schizochytrium sp*. compared with that in the control group ($p < 0.05$). The WG, WGR, and final weight of fish in treatment group are shown in Table 1.

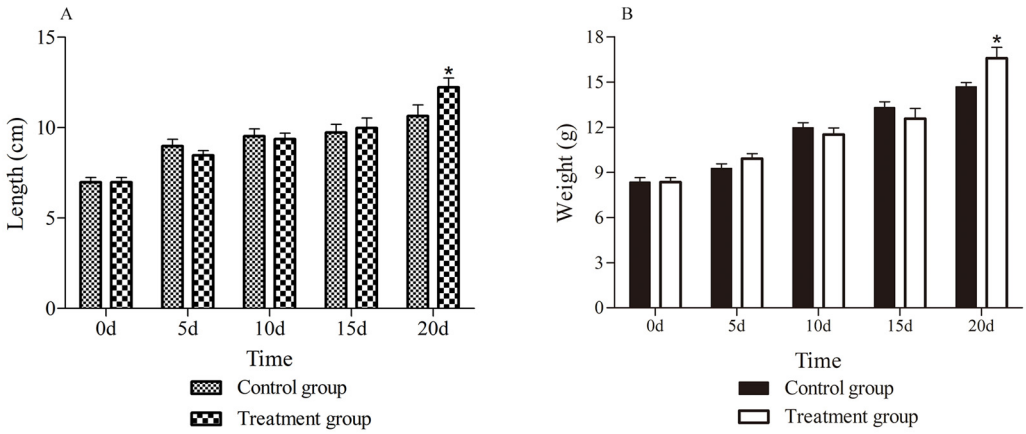

Figure 1. Length and weight of *Pampus argenteus* in the two groups. (**A**) The changes in body weight in the treatment group and control group after feeding for different times. (**B**) Body length changes of the treatment group and control group after feeding for different times. * indicates the difference between the two groups is significant ($p < 0.05$).

However, compared with other time periods, the treatment group fed for 15–20 days had the fastest growth in weight and length ($p < 0.05$). Meanwhile, the fish in the treatment group had a higher growth rate in terms of weight and body length than the fish in the control group.

Table 1. Growth performance of silver pomfret fed diets supplemented with 2% *Schizochytrium* sp. for 20 days (treatment group) and without *Schizochytrium* sp. (Control group).

Growth Parameters	Control Group	Treat Group (*Schi* 2%)
WG	6.42 ± 0.21^{a}	8.08 ± 0.66^{b}
WGR (%)	77.18 ± 7.04^{a}	99.12 ± 8.79^{b}
SGR (%)	2.86 ± 0.20^{a}	3.39 ± 0.30^{a}

Note: Values were the mean ± SEM of five replicates, and values in the same row with different letters were significantly different ($p < 0.05$). *Schi represents Schizochytrium* sp.

3.2. FA Composition

The SFAs of pomfret account for about 40.36% of the total FA (Table 2), which was the main component of fatty acids. Among the SFAs, the content of palmitic acid (16:0) was the highest, accounting for about 29.54%, followed by myristic acid (14:0) and stearic acid (18:0). MUFAs and PUFAs accounted for about 26.63% and 32.28% respectively. The main MUFA was oleic acid (18:1), accounting for 22.19%, followed by palmitoleic acid (2.75%). The dominant PUFA was DHA (22:6n-3), accounting for 21.96% of the total fatty acids, followed by eicosapentaenoic acid (EPA) (20:5n-3), AA (20:4n-6).

FA Composition at Different Times

The FA composition of the treatment group changed significantly ($p < 0.05$) as the feeding time increased: the MUFA content gradually decreased ($p < 0.05$) and the PUFA content increased ($p < 0.05$). However, there was no significant difference in total SFA or PUFA in the muscles in the control group ($p > 0.05$). At 5 d (C2, S2) and 10 d (C3, S3), compared with that in the control group, the content of MUFA increased in the treatment group. At 15 d (C4, S4), there was no significant difference in SFA ($p > 0.05$), but the content of SFA had increased compared with that in the control group. The content of MUFA in the treatment group was significantly lower than that in the control group ($p < 0.05$), while the PUFA content was significantly higher than in the control group ($p < 0.05$). At 20 d (C5, S5), fish fed the diet containing *Schizochytrium sp.* had a significantly higher total PUFA content than those fed with the commercial diet ($p < 0.05$).

In the control group, there was no significant difference in DHA and EPA as time in-creased. However, in the treatment group, the DHA content increased with time. At 5 d and 10 d, there was no significant difference in DHA content between the control and treatment groups. There was a significant difference in the DHA content between the con-trol and treatment groups at 15 d and 20 d ($p < 0.05$). The highest DHA content in muscle was observed in S5, the fish fed the diet containing *Schizochytrium sp.* for 20 days.

3.3. Effects of Schizochytrium sp. on the Gut Microbiota in Pampus argenteus

3.3.1. 16S rRNA Sequencing Results and Diversity Analysis

A total of 1,974,251 sequences remained for analysis, which were grouped into 4896 operational taxonomic units (OTUs). The diversity indices (Table 3) showed that the final number of OTUs per sample was not caused by uneven sequencing depth, and was reflected in the Simpson and Chao1 diversity indices: the two richest samples belonging to the control group (C3-2 and C5-1) and treatment group (S3-2 and S3-3). The Shannon indices indicated that the greatest microbiota diversity of the control group were C3-2 and C5-1, while S2-1 and S3-2 in the treatment group showed diverse communities. All Good's coverage values were above 99%, indicating that the vast majority of the microbiota phylotypes present were sampled. However, the overall microbiota diversity had no significant differences between the control and treatment groups at the same period ($p > 0.01$).

Table 2. Fatty acid composition of silver pomfret fed diets supplemented with 2% *Schizochytrium* sp. for 20 days (treatment group) and without *Schizochytrium* sp. (Control group).

Fatty Acid	C1	C2	C3	C4	C5	S1	S2	S3	S4	S5
C14	$3.93 \pm 0.68^{a/-}$	$2.61 \pm 0.37^{b/-}$	$3.05 \pm 1.12^{a/-}$	$3.83 \pm 0.35^{a/-}$	$1.92 \pm 0.94^{c/-}$	$3.93 \pm 0.68^{a/-}$	$4.01 \pm 0.63^{a/*}$	$3.89 \pm 0.28^{a/-}$	$2.65 \pm 1.57^{b/*}$	$1.15 \pm 0.32^{c/*}$
C15	$0.31 \pm 0.08^{b/-}$	$0.59 \pm 0.51^{a/-}$	$0.28 \pm 0.11^{b/-}$	$0.27 \pm 0.03^{b/-}$	$0.15 \pm 0.04^{a/-}$	$0.31 \pm 0.08^{a/-}$	$0.28 \pm 0.03^{a/*}$	$0.31 \pm 0.01^{a/-}$	$0.43 \pm 0.03^{a/*}$	$0.2 \pm 0.05^{b/-}$
C16	$29.81 \pm 0.59^{a/-}$	$30.42 \pm 1.95^{a/-}$	$30.08 \pm 0.43^{a/-}$	$29.17 \pm 1.2^{a/-}$	$30.46 \pm 0.5^{a/-}$	$29.81 \pm 0.59^{a/-}$	$29.11 \pm 1.04^{a/-}$	$27.61 \pm 0.64^{a/-}$	$28.37 \pm 1.16^{a/-}$	$30.52 \pm 0.65^{a/-}$
C16:1(n-9)	$3.53 \pm 1.57^{a/-}$	$2.12 \pm 0.97^{b/-}$	$3.26 \pm 1.11^{a/-}$	$3.47 \pm 0.37^{a/-}$	$0.41 \pm 0.22^{a/-}$	$3.53 \pm 1.57^{a/-}$	$3.5 \pm 0.65^{a/*}$	$3.79 \pm 0.33^{a/-}$	$3.34 \pm 0.33^{a/-}$	$0.55 \pm 0.19^{c/-}$
C17	$0.8 \pm 0.28^{b/-}$	$1.85 \pm 1.05^{a/-}$	$0.79 \pm 0.8^{b/-}$	$0.55 \pm 0.15^{c/-}$	$1.67 \pm 0.02^{a/-}$	$0.8 \pm 0.28^{c/-}$	$0.75 \pm 0.84^{c/**}$	$0.22 \pm 0.11^{c/**}$	$4.2 \pm 1.11^{a/**}$	$1.71 \pm 0.57^{c/-}$
C18	$6.82 \pm 1.92^{a/-}$	$7.65 \pm 0.49^{a/-}$	$6.94 \pm 0.98^{a/-}$	$5.96 \pm 0.19^{b/-}$	$9.37 \pm 0.83^{a/-}$	$6.82 \pm 1.92^{a/-}$	$6.17 \pm 1.88^{a/-}$	$4.87 \pm 0.47^{b/-}$	$6.31 \pm 2.25^{a/-}$	$9.96 \pm 1.31^{a/-}$
C18:1(n-9)	$22.8 \pm 1.12^{a/-}$	$16.85 \pm 0.92^{a/-}$	$19.31 \pm 0.58^{a/-}$	$21.7 \pm 0.75^{a/-}$	$20.36 \pm 0.92^{a/-}$	$22.8 \pm 1.12^{a/-}$	$21.46 \pm 1^{a/-}$	$20.47 \pm 0.78^{a/-}$	$13.95 \pm 0.57^{b/*}$	$10.87 \pm 1.39^{b/*}$
C18:1	$3.68 \pm 0.87^{a/-}$	$2.79 \pm 1.4^{b/-}$	$2.84 \pm 0.23^{b/-}$	$4.27 \pm 0.42^{a/-}$	$1.55 \pm 0.35^{c/-}$	$3.68 \pm 0.87^{a/-}$	$3.42 \pm 1.11^{b/-}$	$4.51 \pm 0.1^{a/*}$	$2.96 \pm 2.04^{b/*}$	$1.67 \pm 0.25^{c/-}$
C18:2(n-9)	$1.71 \pm 0.58^{a/-}$	$2.25 \pm 1.14^{a/-}$	$2.23 \pm 1.51^{a/-}$	$2.37 \pm 0.43^{a/-}$	$1.5 \pm 0.16^{b/-}$	$1.71 \pm 0.58^{b/-}$	$2.01 \pm 0.64^{b/-}$	$3.24 \pm 0.17^{a/*}$	$1.95 \pm 1.98^{b/-}$	$1.39 \pm 0.43^{c/-}$
C20:1	$0.79 \pm 0.11^{c/-}$	$1.79 \pm 1.06^{a/-}$	$1.21 \pm 1.03^{b/-}$	$1.19 \pm 0.58^{b/-}$	$1.02 \pm 1.23^{b/-}$	$0.79 \pm 0.11^{c/-}$	$1.09 \pm 0.28^{b/*}$	$1.57 \pm 0.31^{a/-}$	$1.65 \pm 0.98^{a/*}$	$1.29 \pm 0.28^{a/-}$
C20:4(n-6)	$0.5 \pm 0.04^{c/-}$	$1.57 \pm 0.55^{a/-}$	$1.06 \pm 0.96^{b/-}$	$0.54 \pm 0.12^{c/-}$	$0.95 \pm 0.98^{b/-}$	$0.5 \pm 0.04^{c/-}$	$0.44 \pm 0.07^{c/**}$	$0.41 \pm 0.05^{c/*}$	$1.15 \pm 0.86^{b/*}$	$1.62 \pm 1.24^{a/*}$
C20:5(n-3)	$4.9 \pm 0.55^{b/-}$	$9.27 \pm 1.12^{a/-}$	$7.02 \pm 0.68^{a/-}$	$5.95 \pm 0.91^{b/-}$	$10.11 \pm 1.12^{a/-}$	$4.9 \pm 0.55^{a/-}$	$6.5 \pm 1.19^{a/*}$	$7.18 \pm 0.43^{a/-}$	$8.96 \pm 1.61^{a/*}$	$9.27 \pm 1.35^{a/-}$
C22:1(n-9)	$0.48 \pm 0.21^{a/-}$	$0.34 \pm 0.12^{b/-}$	$0.66 \pm 0.48^{a/-}$	$0.45 \pm 0.26^{a/-}$	$0.36 \pm 0.15^{b/-}$	$0.48 \pm 0.21^{a/-}$	$0.34 \pm 0.17^{b/-}$	$0.51 \pm 0.21^{a/*}$	$0.61 \pm 0.34^{a/*}$	$0.26 \pm 0.08^{b/-}$
C22:6(n-3)	$19.94 \pm 0.33^{a/-}$	$20.14 \pm 0.44^{a/-}$	$21.28 \pm 0.7^{a/-}$	$20.3 \pm 0.51^{a/-}$	$21.72 \pm 0.65^{a/-}$	$19.94 \pm 0.33^{b/-}$	$21.08 \pm 0.38^{b/-}$	$21.42 \pm 0.81^{b/-}$	$24.15 \pm 0.96^{a/-}$	$29.65 \pm 1.51^{a/*}$
SFA	$41.67 \pm 0.72^{a/-}$	$43.11 \pm 0.72^{a/-}$	$41.14 \pm 0.3^{a/-}$	$39.77 \pm 0.49^{a/-}$	$43.57 \pm 0.41^{a/-}$	$41.67 \pm 0.72^{a/-}$	$40.32 \pm 0.55^{a/-}$	$36.9 \pm 0.23^{a/-}$	$41.95 \pm 0.53^{a/-}$	$43.53 \pm 0.42^{a/-}$
MUFA	$31.27 \pm 0.62^{a/-}$	$23.88 \pm 0.47^{b/-}$	$27.28 \pm 0.37^{a/-}$	$31.07 \pm 0.19^{a/-}$	$23.7 \pm 0.47^{b/-}$	$31.27 \pm 0.62^{a/-}$	$29.81 \pm 0.42^{a/-}$	$30.86 \pm 0.26^{a/-}$	$22.51 \pm 0.72^{b/*}$	$14.63 \pm 0.54^{c/*}$
PUFA	$27.05 \pm 0.25^{a/-}$	$33.23 \pm 0.37^{a/-}$	$31.59 \pm 0.39^{a/-}$	$29.16 \pm 0.33^{a/-}$	$34.28 \pm 0.42^{a/-}$	$27.05 \pm 0.25^{b/-}$	$30.02 \pm 0.47^{b/-}$	$32.24 \pm 0.34^{a/-}$	$36.21 \pm 0.54^{a/-}$	$41.92 \pm 0.48^{a/*}$
EPA + DHA	$24.84 \pm 0.16^{b/-}$	$29.42 \pm 0.48^{a/-}$	$28.3 \pm 0.02^{a/-}$	$26.25 \pm 0.29^{a/-}$	$31.83 \pm 0.33^{a/-}$	$24.84 \pm 0.16^{b/-}$	$27.58 \pm 0.57^{b/-}$	$28.6 \pm 0.27^{b/-}$	$33.11 \pm 0.46^{a/*}$	$38.92 \pm 0.12^{a/*}$

Note: C: control group, C1: Samples from day 0, C2: Samples from the fifth day, C3: Samples from the 10th day, C4: Samples from the 15th day, C5: Samples from the 20th day; S: treatment group; ∑SFAs: saturated fatty acids; ∑MUFAs: mono-unsaturated fatty acids; ∑PUFAs: poly-unsaturated fatty acids. The within-group differences are shown in front of /, which were expressed in English letters, values are the mean ± SEM of three replicates, and values with different letters were significantly different ($p < 0.05$). The between-group differences are shown behind/, which were expressed in *, values are the mean ± SEM of three replicates. Values with * were significantly different ($p < 0.05$) and values with ** were very significantly different ($p < 0.01$). The between-group differences were C1 vs. S1, C2 vs. S2, C3 vs. S3, C4 vs. S4, C5 vs. S5.

Table 3. Illumina high-throughput data, bacterial diversity richness (OTUs), and diversity indices (Shannon & Simpson) for intestinal bacterial diversity analysis of silver pomfret fed with different diets for 20 days.

Sample	OTU	Simpson	Chao1	Shannon	Coverage
C1-1	609	0.465	629.488	2.484	0.997
C1-2	675	0.566	681.573	2.842	0.997
C1-3	805	0.823	813.243	4.263	0.997
C2-1	1173	0.943	1160.275	6.001	0.997
C2-2	597	0.603	584.065	2.642	0.998
C2-3	461	0.266	453.928	1.32	0.998
C3-1	969	0.815	970.903	4.544	0.996
C3-2	1477	0.973	1507.891	6.99	0.995
C3-3	709	0.917	694.483	4.749	0.997
C4-1	513	0.412	504.319	1.987	0.998
C4-2	347	0.146	331.8	0.779	0.998
C4-3	2193	0.85	2148.671	5.414	0.993
C5-1	2504	0.98	2479.35	7.884	0.992
C5-2	2014	0.657	1943.744	3.963	0.992
C5-3	2299	0.936	2235.786	6.243	0.991
S1-1	609	0.465	629.488	2.484	0.997
S1-2	675	0.566	681.573	2.842	0.997
S1-3	805	0.823	813.243	4.263	0.997
S2-1	2292	0.898	2122.455	6.489	0.995
S2-2	2071	0.869	1992.766	5.433	0.992
S2-3	2463	0.759	2380.358	5.194	0.99
S3-1	1146	0.839	1101.783	5.117	0.996
S3-2	1358	0.969	1309.61	6.433	0.995
S3-3	1126	0.938	1135.107	5.726	0.996
S4-1	1072	0.948	1052.173	5.943	0.996
S4-2	940	0.942	891.114	5.485	0.997
S4-3	776	0.929	741.719	5.069	0.997
S5-1	207	0.031	211.125	0.213	0.999
S5-2	241	0.09	222.895	0.527	0.999
S5-3	218	0.07	211.526	0.425	0.999

Note: C: control group, C1: Samples from day 0, C2: Samples from the fifth day, C3: Samples from the 10th day, C4: Samples from the 15th day, C5: Samples from the 20th day; S: treatment group; The numbers followed the "-" represent each independent sample.

3.3.2. Difference in the Gut Microbiota between the Control and Treatment Groups

The core OTUs and unique OTUs in the control and treatment groups are shown in Figure 2. The results showed that the control samples (C1, C2, C3, C4, C5) shared 451 core OTUs, and the maximum number of unique OTUs was in sample C5 with 848 unique OTUs (Figure 2A). The treatment groups (S1, S2, S3, S4, S5) shared 267 core OTUs, which was less than that of the control samples, and the maximum number of unique OTUs was in sample S2, with 1636 unique OTUs. Only four unique OTUs were found in sample S5 (Figure 2B). Based on the results of both the treatment and control groups in the same period, we found that samples S2 and C2 shared 893 core OTUs. However, sample S2 had a much greater number of unique OTUs than C2 (Figure 2C). Samples S3 and C3 shared 1079 core OTUs, and S3 had slightly more unique OTUs than C3 (Figure 2D). By contrast, samples S4 and C4 shared 758 core OTUs; however, more unique OTUs were found in the C4 sample than in the S4 sample (Figure 2E). A similar situation was found in the next period, C5 had more unique OTUs (2649) than S5 (36 unique OTUs) although they shared 309 core OTUs (Figure 2F).

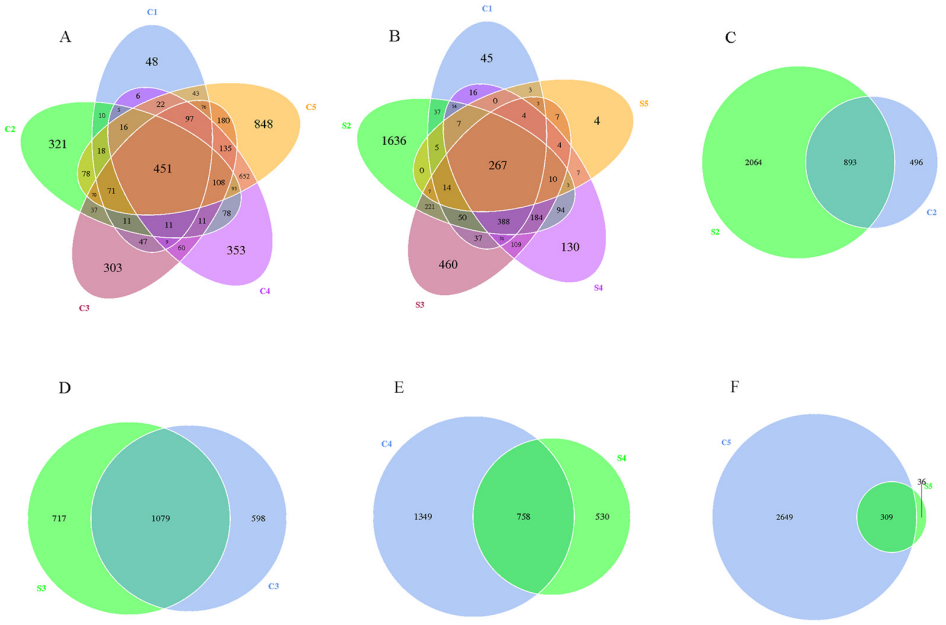

Figure 2. Venn diagram representing shared operational taxonomic units (OTUs) between samples from the control group and the treatment group of silver pomfret taken at different times. (**A**) control group, from C1 to C5; (**B**) treatment group, from S1 to S5; (**C**) C2 vs. S2; (**D**) C3 vs. S3; (**E**) C4 vs. S4; (**F**) C5 vs. S5.

The results are shown in Figure 3. Samples C1, C5, S2, S3, and S4 were closer, especially C1, S3, and S4. However, the distance between samples of C1 and S2 was large, which showed that the samples in this period had more differences, while those of sample S2 were more similar to those of S3 and S4, but were less analogous to those of S5.

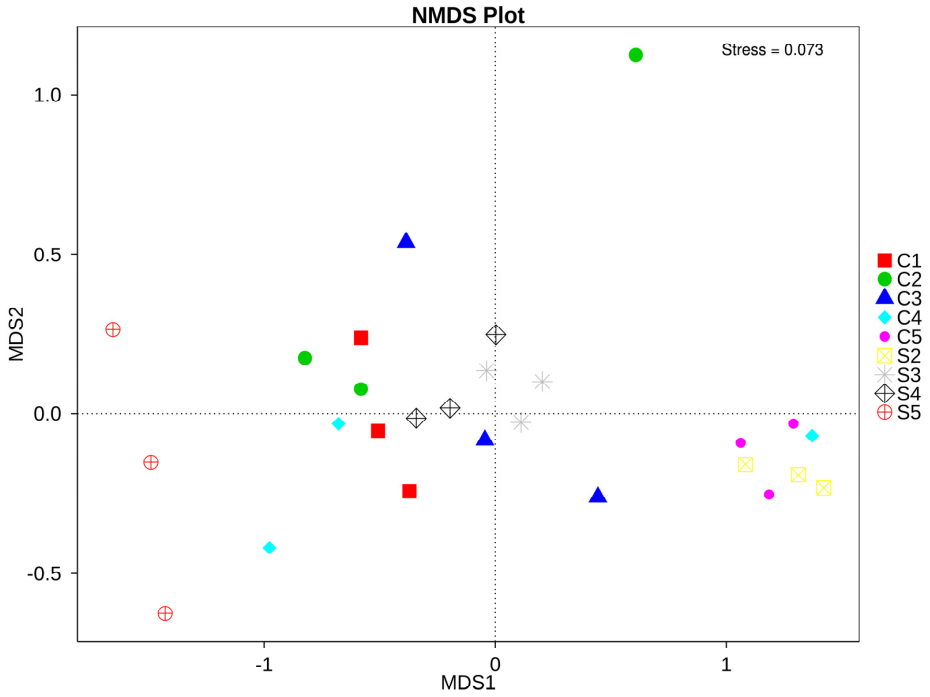

Figure 3. Nonmetric Multidimensional Scaling (NMDS) plot of the control and treatment groups. Patterns of different shapes and colors represent different samples, each point in the diagram represents one sample, and samples from the same group are represented by the same patterns.

The relative abundance of the top 10 phyla of the groups are shown in Figure 4. The top 10 dominant microbial phyla were Proteobacteria, Firmicutes, Cyanobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Spirochaetes, Chloroflexi, Gemmatimonadetes, and Verrocuomicrobia. Proteobacteria was the most predominant phylum in all samples, followed by Firmicutes. The Proteobacteria phylum included *Gammaproteobacteria*, *Alphaproteobacteria*, and *Deltaproteobacteria*.

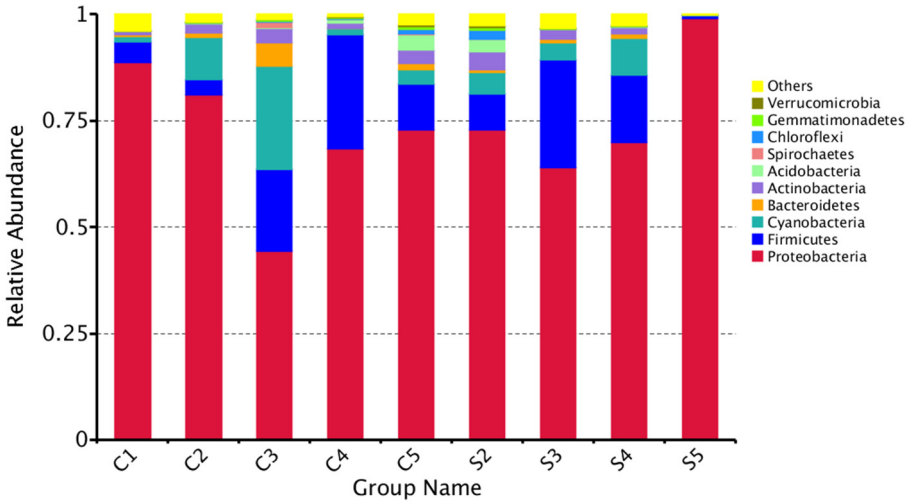

Figure 4. The relative abundance of the top 10 phyla of the control and treatment groups at the same time points.

From treatment S1 to S5, the relative abundance of Proteobacteria decreased first (S1–S3) and then increased (S3–S5), peaking at 99.10%. The control samples (C1–C5) showed the same trend. The relative abundance of Firmicutes in the treatment group samples (S1–S5) increased, with a maximum value in S3 (25.30%), and then decreased to 0.66% at S5. The tendency of control groups was similar to the treatment groups; however, the relative abundance of Firmicutes was highest in sample C4. Comparing the experimental group with the control group at the same period, we found that the relative abundances of Firmicutes, Actinobacteria and Acidobacteria of S2 were higher than those of C2, whereas the relative abundances of Proteobacteria, Cyanobacteria and Bacteroidetes were less than those in C2. The relative abundances of Proteobacteria and Firmicutes in S3 were higher than those in C3; however, the relative abundances of Cyanobacteria and Bacteroidetes in S3 were much lower than those in C3. The top three dominant microorganisms in terms of relative abundance in C4 and S4 accounted for the vast majority, except that the abundances of Firmicutes, Proteobacteria and Cyanobacteria in S4 were higher than those in C4. In S5, in addition to the Proteobacteria, the relative abundance of other microorganisms was much lower than in the C5 sample.

The microbial composition and relative abundance of the top 10 genera are shown in Figure 5, including Photobacterium, Vibrio, unidentified_Chloroplast, Lactococcus, Pseudomonas, Lactobacillus, Halomonas, Nautella, Sporolactobacillus and Enterococcus. The dominant microorganisms at the genus level were almost all different in each group. The relative abundance of Photobacterium, as the main dominant bacteria, varied greatly in each group ($p < 0.01$), ranging from 8.14% (minimum value in C3) to 96.80% (maximum value in S5). The relative abundance of Vibrio was 22.98% in C2 and was significantly higher than that in the other groups ($p < 0.01$). Interestingly, the relative abundances of Lactococcus in the C4 group, Lactobacillus in the S3 group and Halomonas in the S4 group were much higher than those in the other groups ($p < 0.01$).

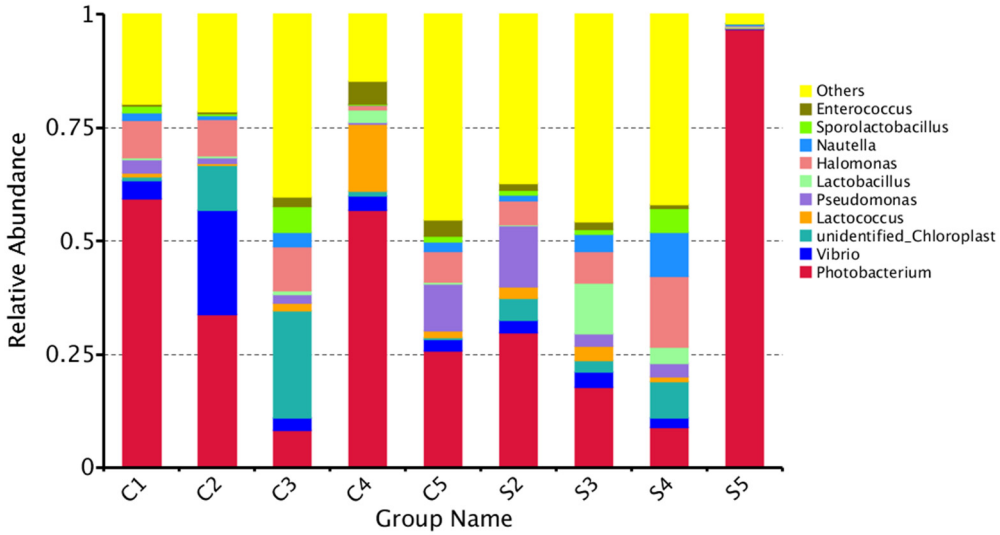

Figure 5. The relative abundance of the top 10 of the control and treatment groups at the same time points.

The structure and relative abundance of the gut microorganisms of the silver pomfret fed with *Schizochytrium* sp. had marked differences with those of the control group at the genus level. The heatmap (Figure 6A) showed that the dominant genera of S2 were *Pseudomonas*, *Acidibacter*, and *Brevundimonas.* In S3, they were mainly *Escherichia-Shigella*, *Geobacter* and *Lactobacillus.* In S4, they were *Nautella*, *Donghicola*, *Palleronia* and *Tepidimonas.* In particular, the enrichment degree of *Photobacterium* was high in S5. The genus clustering tree showed that the microorganisms in the treatment group could not be clustered as a branch. Moreover, among the top 35 relatively abundant genera, there were several genera that were present in the treatment group, but almost absent in the control group: *Bacillus*, *Escherichia-Shigella*, *Lactobacillus* and *Tepidimonas*. The abundance of these microorganisms increased after feeding *Schizochytrium* sp. for 10 days and 15 days. The heatmap in Figure 6B shows that the phyla of S2 were Chloroflexi, Fibrobacteres, JL-ETNP-Z39 and WCHB1-60. In S3, they were Chlamydiae and Fusobacteria. In S4, they were Deferribacteres and Chlorobi. Proteobacteria made up the dominant phylum in group S5. The clustering tree showed that S3 and S4 could be clustered as a branch.

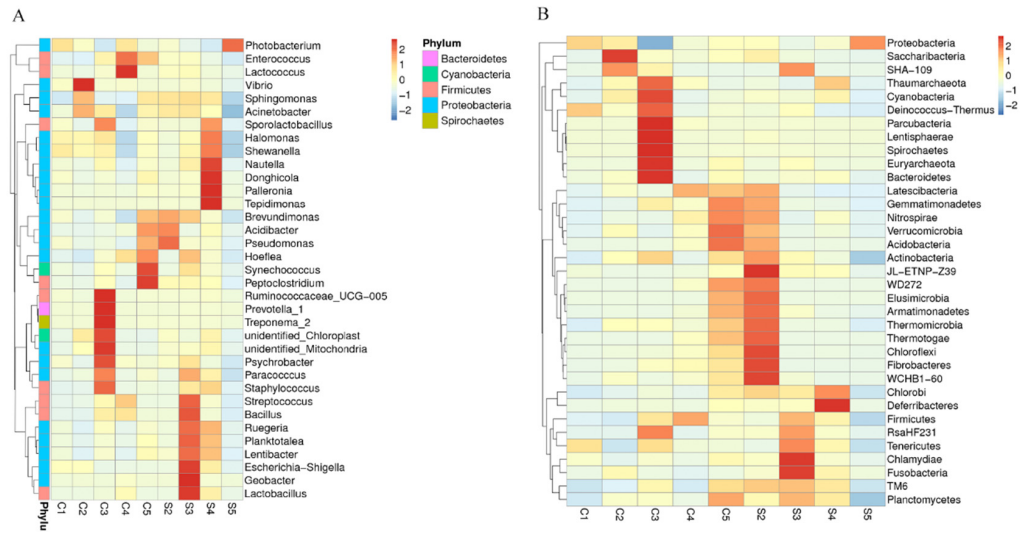

Figure 6. Heatmap at the genus (**A**) and phylum (**B**) level of the of control and treatment groups. The horizontal axis shows the sample information, and the vertical axis shows the species annotation information. The species clustering tree is on the left.

4. Discussion

4.1. Feeding Schizochytrium sp. for Better Growth Performance

The results of the present study indicated that silver pomfret fed with *Schizochytrium* sp. showed better growth performance, which is basically consistent with previous studies on zebrafish and catfish [27,34]. In addition, *Schizochytrium* sp. could be used as a lipid source in feed, which might affect the performance or condition of fish [35]. By observing the ingestive behavior of the fish, we found that the fish showed positive feeding behavior and stronger vitality. We inferred that *Schizochytrium* sp. might have a certain role in feeding attraction, because it was reported that amino acids stimulated the gustatory sense, and some specific amino acids, such as alanine, glycine, and tryptophan, could act as feeding attractants [36]. It was also found that in *Schizochytrium* sp., the content of alanine and glycine was high [36–38], which might have improved the growth rate of the experimental group by promoting food intake. At the same time, we found that the fastest growth period of fish was at 15–20 days after feeding *Schizochytrium* sp., which mean that our feeding experiment would last at least 15 days, and the specific optimal growth period needs to be determined by extending the experimental period in the later stage, which would be significant to guide the artificial culture of silver pomfret.

4.2. Fatty Acid Composition

Studies have reported that the fatty acid composition of muscle largely reflects the diets in many fish [39–43]. *Schizochytrium* sp. is rich in DHA [44,45], content which was much higher than that in the basal diet, and the inclusion of *Schizochytrium* sp. in fish feeds could significantly affect the fish muscle fatty acid content [35]. In the present study, the content of MUFAs and PUFAs was significantly different between two groups, and there was a higher total n-3 PUFA content in the muscle of the treatment group compared with that in the control, especially for DHA. Moreover, the SFA content in the treatment group was generally lower than that in the control group. DHA is an important n-3 PUFA, being the primary driver of long-chain polyunsaturated fatty acid (LC-PUFA) essentiality [46]. DHA also plays an important role in promoting brain development, physical development, vision development and immune development in young children, as well as lower blood lipids and prevention of cardiovascular disease in adults. However, excessive SFA intake might increase the risk of cardiovascular disease, harm the endothelium and increase inflammatory responses [47,48]. We could reasonably infer that feeding with *Schizochytrium* sp. could improve the quality of fish muscle to a certain extent and increase the content of PUFA, especially DHA. Thus, we improved the nutrient composition of the fish to make it more in line with the nutritional needs of the human body, enabling consumers to gain more health benefits from fish consumption.

At the same time, in response to certain external conditions, such as sudden changes in water temperature, the stress response of the treatment group was smaller than that of the control group; however, when the water temperature dropped suddenly, silver pomfret would mount a strong stress reaction and were more likely to die [49]. This point has been verified in other studies, which found that dietary DHA was effective to increase the tolerance of fish to various stress conditions, such as changes in the water temperature and salinity [50]. Therefore, the *Schizochytrium* sp. diet might also improve the stress tolerance of silver pomfret. Meanwhile, it was reported that the lipid contents, nutritional status, total lipid and phosphoglyceride DHA levels of larvae correlated directly with dietary DHA levels [51]. Therefore, feeding DHA during the development of young fish might improve their health, thereby directly improving their survival rate and growth rate. This would be of great significance for the large-scale artificial culture of silver pomfret, because marine fish larvae are vulnerable during the early life period. Thus, further *Schizochytrium* sp. feeding experiments of silver pomfret fry are required.

4.3. Changes and Functional of Dominant Phyla and Special in Different Period

The data showed that Proteobacteria were the dominant microbiota phylum in the vast majority of samples, followed by Firmicutes. In the microbiota of the mucosa, Pro-

teobacteria and Firmicutes were identified as the major bacterial phyla in the intestines of fish, such as juvenile *Larimichthys crocea*, *Salmo salar* and most other Teleosts [52–54], which was consistent with our experimental results, in which they constituted the core gut microbiota. This might mean that as marine omnivorous fish, the core gut microbiota of sliver pomfret, to a large extent, maintain a certain stability. Moreover, the composition of the diet might be an especially relevant factor in shaping the gut microbiota composition of silver pomfret.

Over the course of the feeding period, especially between feeding stages at 10 and 15 days and the earlier feeding stages, the microbiota species richness and diversity increased over time. This suggested that after feeding *Schizochytrium* sp. for a period of time, the abundance and structure of intestinal microorganisms changed and stabilized. Indeed, research has suggested that feeding microalgae could increase the abundance of intestinal microorganisms and optimize their composition [18,34,55]. Therefore, this might be related to their growth performance, because according to the growth data, the fastest growth rate after feeding *Schizochytrium* sp. was recorded at 15–20 days, which was consistent with the increased abundance of intestinal microbials.

In addition to growth conditions, we might reasonably infer that a change in intestinal microorganisms, especially some specific gut microbes, might enhance the immune status and change the fatty acid composition of silver pomfret. For example, high gut microbial richness and diversity were not conducive to the colonization of pathogens in the fish gastrointestinal tract [56]; therefore, a *Schizochytrium* sp. diet could be considered to be positive for a healthy status of the host because there might be more competition for incoming pathogens. We speculated that feeding *Schizochytrium* sp. might also partly affect the immune ability of fish. We found that the abundance of *Halomonas* in the treatment group increased gradually, peaking at 15.54% in S4, and was much higher than that in the control group at the same time point ($p < 0.01$). It was reported that *Halomonas* contained more than 70% poly-β-hydroxybutyrate (PHB) by cell dry weight. PHB can be degraded into β-hydroxybutyrate acid monomer in the intestine of aquatic animals, as a kind of SCFA, and the release of β-hydroxybutyrate protected the host from the pathogen. According to the literature, when challenged with pathogenic *Vibrio*, *Halomonas* might improve the resistance of organisms [57–60]; however, silver pomfret were susceptible to *Vibrio* infection, causing a large number of deaths in summer, which has reference significance for our breeding program. Moreover, with the increased *Halomonas* abundance, the abundance of *Photobacterium damselae* continued to decline at the species level and reached a minimum value of 8.90% in S4. As a marine Gram-negative bacterium, *Photobacterium damselae* belongs to the Vibrionaceae family [61,62], which are conditional pathogens of a variety of marine fish, including rainbow trout (*Oncorhynchus mykiss*), sea bass (*Dicentrarchus labrax*) and silver pomfret [63–65]. Therefore, it was suggested that feeding with *Schizochytrium* sp. increased the abundance of *Halomonas* in the treatment group, which could inhibit the reproduction of *Photobacterium damselae* and enhanced the resistance of fish to pathogenic bacteria, thereby improving their health. Moreover, compared with the control group, the abundance of *Lactobacillus* in the treatment group increased rapidly. At the same time, according to the change in the fatty acid content after feeding *Schizochytrium* sp., the PUFA content in the muscle of the treatment group continued to increase, and the PUFA content in each period was higher than that of the control group, except for C2 and S2. We found that while the content of *Lactobacillus* increased, the PUFA content also showed a corresponding growth trend. Thus, it was suggested that the levels of PUFAs and *Lactobacillus* were mutually reinforced in the treatment group. Interestingly, some reports confirmed that probiotics could change the fatty acid content to a certain extent. It was proven that *Lactobacillus* could incorporate exogenous free PUFAs into cellular fatty acids, thereby increasing the content of PUFAs in the body. Meanwhile, exogenous free PUFAs would increase the abundance of *Lactobacillus* [66–68]. This supported our inference that increasing specific probiotics might change the composition of fatty acids in the fish after feeding with *Schizochytrium* sp., making the fish more nutritious.

5. Conclusions

The results of the present study showed that the addition of 2% *Schizochytrium* sp. to the diet accelerated the growth of silver pomfret, facilitated the accumulation of PUFAs in muscle, and increased the relative abundance and diversity of microorganisms. This might have improved the nutritional value of muscle and the health of fish, and increased the tolerance of the fish to external stimuli and the larval survival rate. Herein, we studied the effect of feeding silver pomfret with microalgae rich in DHA for the first time, and we hope to explore the most suitable feed composition for silver pomfret in future research.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Animal Care and Use Committee of Ningbo University (ethical batch number: NBU20220079).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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