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Effects of Low Salinity on Growth, Digestive Enzyme Activity, Antioxidant and Immune Status, and the Microbial Community of *Litopenaeus vannamei* in Biofloc Technology Aquaculture Systems

Lina Long, Huang Liu * and Shimin Lu

Fishery Machinery and Instrument Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200092, China; longlina@fmiri.ac.cn (L.L.); lushimin@fmiri.ac.cn (S.L.)
* Correspondence: liuhuang@fmiri.ac.cn

Abstract: Biofloc technology (BFT) culture systems based on low salinity can meet the needs of shrimp growth and environmental protection, thus having potential application. To evaluate the effects of BFT on water quality, the microbial community, growth, digestive enzyme activity, and antioxidant and immune status of *Litopenaeus vannamei* under low salinity conditions, a four-week experiment was conducted in a BFT culture system (C/N ratio of 12: 1) with three salinity conditions: 5.0‰ (S5 group), 10.0‰ (S10 group), and 15.0‰. The results showed that water quality parameters were all within the range suitable for the culture of *L. vannamei*. There were no significant differences in growth parameters such as final weight or specific growth rate. Muscle moisture, crude protein, and crude lipid contents of *L. vannamei* did not differ among groups ($p > 0.05$). In addition, intestinal amylase and trypsin activities in the S5 group significantly increased ($p < 0.05$). In the S15 group, the superoxide dismutase activity and total antioxidant capacity in the serum and hepatopancreas of *L. vannamei*, as well as serum catalase, acid phosphatase, and alkaline phosphatase activities, markedly increased ($p < 0.05$). The microbial diversity (Shannon and Simpson indices) and richness (Chao1 and ACE indices) were higher in the S5 group than in the S15 group. Our findings indicated that although the shrimp in BFT systems with a salinity of 5‰ had lower antioxidant and immune levels, the digestive enzyme activity as well as the gut microbial diversity and richness improved compared to other groups, suggesting the possibility of culturing *L. vannamei* in low-saline areas with BFT systems.

Keywords: biofloc technology; low salinity; *Litopenaeus vannamei*; digestive activity; immune response; microbial community

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1. Introduction

Biofloc technology (BFT) is a low-cost, sustainable, and environmentally friendly aquaculture system [1,2]. By adding carbon sources to regulate the carbon/nitrogen (C/N) ratio in the water, the culture can promote the formation of biofloc that contains bacteria, fungi, protozoa, rotifers, and microalgae. Ammonia nitrogen and nitrite nitrogen can be removed through the assimilation of heterotrophic bacteria to maintain water quality [3–5]. Biofloc is rich in nutrients such as crude protein, crude lipid, and a variety of extracellular enzymes that can benefit aquatic animals. Thus, it can improve the feed utilization of cultured species [6,7]. In addition, biofloc contains bioactive compounds, including poly β-hydroxybutyric acid (PHB), polysaccharides, carotenoids, chlorophylls, phytosterols, taurine, and fat-soluble vitamins, which can significantly improve the immunity and antioxidant levels of aquatic animals [8–11]. At present, BFT is widely used in culturing *Litopenaeus vannamei* [9,12,13], *Macrobrachium rosenbergii* [14], *Oreochromis niloticus* [15–17], *Cyprinus carpio* [18], and other aquatic animals.

Litopenaeus vannamei has the advantages of wide adaptability and rapid growth. It is the world's most widely cultivated euryhaline crustacean [19]. In China, *L. vannamei* is also the most widely cultivated shrimp, producing the highest yield and accounting for 85% of the country's shrimp production [20]. The culturing of *L. vannamei* in inland areas at low-salinity conditions is a current trend, and the rearing scale will show sustained growth in the future [21]. On the one hand, such a culture mode alleviates the pollution of mariculture on the coastal marine environment, thus protecting the coastal ecosystem. On the other hand, it also promotes the development of inland aquaculture, providing fresh shrimp for inland areas that cannot otherwise obtain such commodities [22]. However, the cultivation of *L. vannamei* in inland areas may have adverse ecological and environmental impacts on the local nonsaline earth surface [23,24].

Fortunately, using BFT in inland areas to culture *L. vannamei* can necessitate little or no change in water usage during the farming process, thus reducing the discharge of salt and minimizing the impact on the local ecological environment [23]. At present, several studies successfully applied BFT to low-salinity *L. vannamei* aquaculture systems, but these studies primarily focused on growth performance [25–29], amino acid and fatty acid compositions [30], and yield [24,31] of *L. vannamei* as well as the compositions of biofloc [32]. However, there are few research reports on digestive enzyme activity, antioxidant levels, or immune status of *L. vannamei* in the BFT culture system under low-salinity conditions [30]. Therefore, this study employed *L. vannamei* as a research object to investigate the effects of BFT on water quality regulation of zero exchange aquaculture systems. Shrimp growth performance, muscle composition, digestive enzyme activity, antioxidant and immune statuses, and the composition of biofloc and the intestinal microbial community under low-salinity conditions were analyzed. Our aim was to provide references for the promotion and application of BFT in the cultivation of shrimp under low-salinity conditions in inland areas.

2. Materials and Methods

2.1. Experiment and Design

The experiment was conducted in the Pond Ecological Engineering Research Center of the Chinese Academy of Fishery Sciences. Three low-salinity groups were set up, namely, 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15). Each group had three replicates. This study used a zero-water exchange BFT system comprising the culture tanks (70 cm × 50 cm × 50 cm), sedimentation tanks, and biofilm purification tanks. Glucose was purchased from local markets and used as a supplementary carbon source to adjust the C/N ratio to 12. The C/N ratio was calculated according to the carbon–nitrogen contents of pellet feed and the carbon content contained in glucose following the protocol described by Avnimelech [3]. The culture period was four weeks.

L. vannamei used in this study were purchased from Hainan Ruyilai Aquaculture Co., Ltd. (Wenchang, Hainan Province, China). Before the experiment, *L. vannamei* were temporarily raised for two weeks at 28 ± 1 °C, pH 7.5 ± 0.7, and dissolved oxygen > 5 mg L^{-1}. The shrimp were fed with pellet feed three times a day (crude protein 43%, Jiangsu Changshou Group Nanshan Biotechnology Co., Ltd., Rugao, Jiangsu Province, China). The salinity was adjusted according to the following method: artificial sea salt powder (Shanghai Baojia Industry & Trade Co., Ltd., Shanghai, China) and marine elements supplement (Shanghai Blue Sea aquatic Products Development Co., Ltd., Shanghai, China) were added to fully aerated tap water. After being adjusted to the target salinity and stabilizing, healthy *L. vannamei* with an initial mean body length of 5.06 ± 0.29 cm and initial mean body weight of 0.65 ± 0.13 g were selected for the experiment (50 shrimp per tank). The shrimp were fed three times per day. During the experiment, water was not exchanged, and only the small amount of water lost due to sewage and evaporation was replaced irregularly.

2.2. Sampling and Measurement

2.2.1. Water Quality Parameters

During the experiment, water temperature, dissolved oxygen, pH, and salinity of the culture water were measured daily using water quality instruments (YSI-550A, Yellow Springs Instruments Inc., OH, USA; AZ8371, Hengxin Technological Co., Ltd., Guangzhou, China). The contents of total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen were measured once a week. Water samples (100 mL) were collected from all tanks and then filtered through a 0.45 μm filtration membrane for determination of total ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen according to standard methods [33].

2.2.2. Growth, Muscle Composition, Digestive Enzyme Activity, and Antioxidant and Immune Status

After the experiment, *L. vannamei* were fasted for 24 h and then were counted and weighed. Three individuals were anesthetized in an ice bath from each tank, and then hemolymph was taken using a nonheparinized tuberculin syringe. After clotting at 4 °C for one night and centrifuging at 7000 r/g at 4 °C for 15 min, serum samples were stored in a refrigerator at −80 °C for the determination of antioxidant and immune activities. The *L. vannamei* were then dissected on an ice tray immediately after hemolymph collection. The muscle, hepatopancreas, and intestinal tissues were obtained and placed in the refrigerator at −80 °C. These tissues were used for the determination of muscle composition, hepatopancreas antioxidant and immune activities, and intestinal digestive enzyme activities, respectively.

The contents of moisture, crude protein, crude lipid, and ash in shrimp muscle were determined according to standard measurement methods (GB/T 5009.3-2003, GB/T 6432-1994, GB/T 6433-2006, and GB/T 6438-2007, respectively). Serum and hepatopancreas superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), total antioxidant capacity (T-AOC), lysozyme (LZM), acid phosphatase (ACP), and alkaline phosphatase (AKP) were measured using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu Province, China) following the manufacturer's protocols. In addition, intestinal digestive enzyme activities (amylase, lipase, and trypsin) were also measured using such commercial assay kits.

2.2.3. Microbial Community Analysis

Fifty-milliliter water samples were taken from all tanks, filtered, and then stored at −80 °C for the analysis of the microbial community in the biofloc after the experiment. In addition, five shrimp were taken from each tank and disinfected with 75% alcohol on the body surface. Then, the shrimp were washed with sterile water, and the intestinal contents were removed into 2 mL sterile centrifuge tubes that were then stored at −80 °C for the analysis of the intestinal microbial community.

A Fast DNA Spin Kit for Soil (MP Biomedicals, Irvine, CA, USA) was used to extract total genomic DNA from all samples. The purity of extracted genomic DNA was determined using an ultrafine spectrophotometer (NanoDrop2000, Thermo Fisher Scientific, Waltham, MA, USA). PCR amplification of 16S rRNA (the 16S V3-V4 hypervariable region) was carried out using primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR reaction mixture as well as the amplification procedure followed those of Liu et al. [34]. Each sample had three replicates. The PCR products were extracted, purified, and quantified.

Sequencing was performed on an Illumina Miseq PE300 platform (Illumina, San Diego, CA, USA). The original sequences were controlled and spliced according to fastp and FLASH, respectively. UPARSE 7.1 software was used to carry out operational taxonomic units (OTUs) clustering on the quality-controlled spliced sequences according to 97% similarity, and chimeras were removed. The sequences were compared with the bacterial SILVA database by the RDP classifier, and OTUs species taxonomic annotation was performed.

Data analysis was performed via the Majorbio Cloud platform. Alpha diversity indices (Simpson index, Shannon index, Chao1, and ACE) were calculated by Mothur v1.30.1 software. Principal coordinate analysis (PCoA) based on the Bray–Curtis distance algorithm was used to test the similarity of microbial community structures among samples. Linear discriminant analysis (LDA) effect size (LEfSe) was used to determine the significantly abundant taxa (phylum to genus levels) of bacteria in different groups (the LDA threshold was set to 4).

2.3. Calculations and Statistics

In this study, data analysis was performed using SPSS 18.0. Before analyses, data were checked for normality and homogeneity of variances with Levine's test. One-way ANOVA followed by Duncan's multiple range test was used for significance detection. Differences were considered significant at $p < 0.05$.

Growth performance was evaluated by the following calculation formulas [16]:

$$\text{Survival rate } (\%) = 100 \times \text{ harvested number/stocking number}, \quad (1)$$

$$\text{Weight gain (WG; \%)} = 100 \times (\text{FW} - \text{IW})/\text{IW}, \quad (2)$$

$$\text{Specific growth rate } \left(\text{SGR; \% day}^{-1}\right) = 100 \times (\text{Ln FW} - \text{Ln IW})/\text{culture days}, \quad (3)$$

where FW = final weight and IW = initial weight.

3. Results

3.1. Water Quality Parameters

The water quality parameters were similar in all treatments ($p > 0.05$), including temperature at 27.75–28.36 °C, dissolved oxygen at 6.53–6.62 mg L^{-1}, and pH at 7.90–7.92. In addition, these water quality parameters were suitable for shrimp culture.

Total ammonia nitrogen showed a trend of first rising and then declining (Figure 1a). Nitrite concentration fluctuated during the first two weeks; the concentration of nitrite in the S15 group was initially higher than in other groups and then stabilized, while the concentration of nitrite in the S5 group was lower overall (Figure 1b). The concentration of nitrate nitrogen in different groups showed a rapidly rising trend during the first two weeks, then became stable from the third week (Figure 1c).

3.2. Growth and Muscle Composition

As can be seen in Table 1, no significant differences in growth performance were found among the groups ($p > 0.05$). The specific growth rate and survival rate of shrimp in different low-salinity groups were 7.25–7.41% day^{-1} and 78.34–81.22%, respectively.

Table 2 shows the muscle composition of shrimp in different low-salinity groups. Moisture, crude protein, and crude lipid contents did not differ among groups ($p > 0.05$). The S10 group had a higher ash content than the S5 and S15 groups ($p < 0.05$).

3.3. Digestive Enzyme Activity

The activities of amylase, lipase, and trypsin in the intestines of shrimp are shown in Figure 2 (colors in all the figures have no real meaning). The intestinal amylase activity of *L. vannamei* in the S5 and S10 groups was significantly higher, by 141% and 132%, respectively, than that in the S15 group ($p < 0.05$). In addition, intestinal lipase activity did not differ significantly among groups ($p > 0.05$). Trypsin activity in the intestines of *L. vannamei* was notably higher in the S5 group compared to the S10 and S15 groups ($p < 0.05$), increasing by 163% and 274%, respectively.

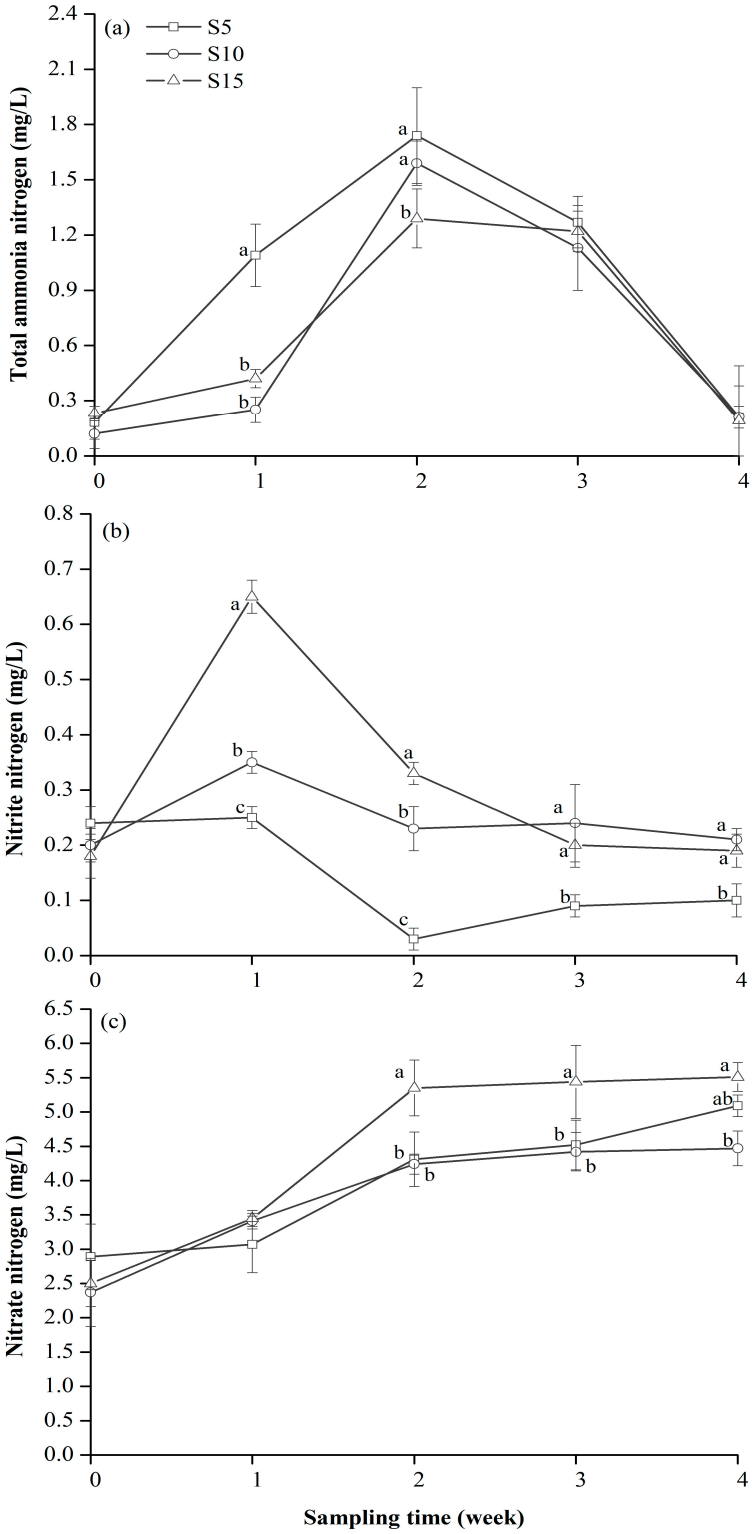


Figure 1. Weekly changes of total ammonia nitrogen (**a**), nitrite nitrogen (**b**), and nitrate nitrogen (**c**) concentrations in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15). Error bar indicates ± standard deviation (SD).

Table 1. Growth performance of *Litopenaeus vannamei* growing in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15).

Parameters	Groups		
	S5	S10	S15
Initial weight (g)	0.65 ± 0.08	0.64 ± 0.06	0.65 ± 0.04
Final weight (g)	5.21 ± 0.14	4.96 ± 0.17	5.20 ± 0.40
Specific growth rate (SGR, %day^{-1})	7.41 ± 0.16	7.25 ± 0.19	7.30 ± 0.36
Survival rate (%)	78.34 ± 1.23	80.61 ± 1.59	81.22 ± 1.74
Weight gain (%)	708.34 ± 34.80	680.43 ± 41.85	713.77 ± 43.25

Each value represents mean ± S.E. (n = 15).

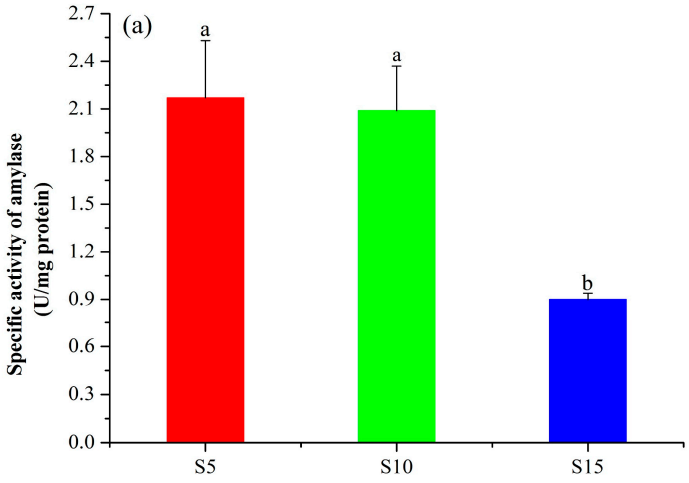
Table 2. Muscle proximate composition of *Litopenaeus vannamei* growing in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15).

Parameters	Groups		
	S5	S10	S15
Moisture (%)	79.52 ± 0.45	78.5 ± 0.52	79.06 ± 0.11
Crude protein (%)	11.42 ± 0.5	10.21 ± 0.35	11.72 ± 0.27
Crude lipid (%)	4.34 ± 0.4	4.46 ± 0.31	4.46 ± 0.41
Ash (%)	5.1 ± 0.31 ^{b}	6.73 ± 0.52 ^{a}	4.98 ± 0.57 ^{b}

Each value represents mean ± S.E. (n = 9). Means in the same row with different superscripts are significantly different at $p < 0.05$.

3.4. Antioxidant and Immune Status of Shrimp

Antioxidant capacities of the shrimp are presented in Figure 3. The serum SOD and T-AOC activities of shrimp in the S15 group were significantly higher than those in the S5 group ($p < 0.05$). In hepatopancreas, SOD activity was elevated significantly with the increase in salinity ($p < 0.05$). In the S10 and S15 groups, CAT activity was significantly greater than in the S5 group ($p < 0.05$). T-AOC in the S15 group was notably higher than in the S10 and S5 groups ($p < 0.05$). GPX activities showed no significant differences in serum or hepatopancreas among groups ($p > 0.05$).



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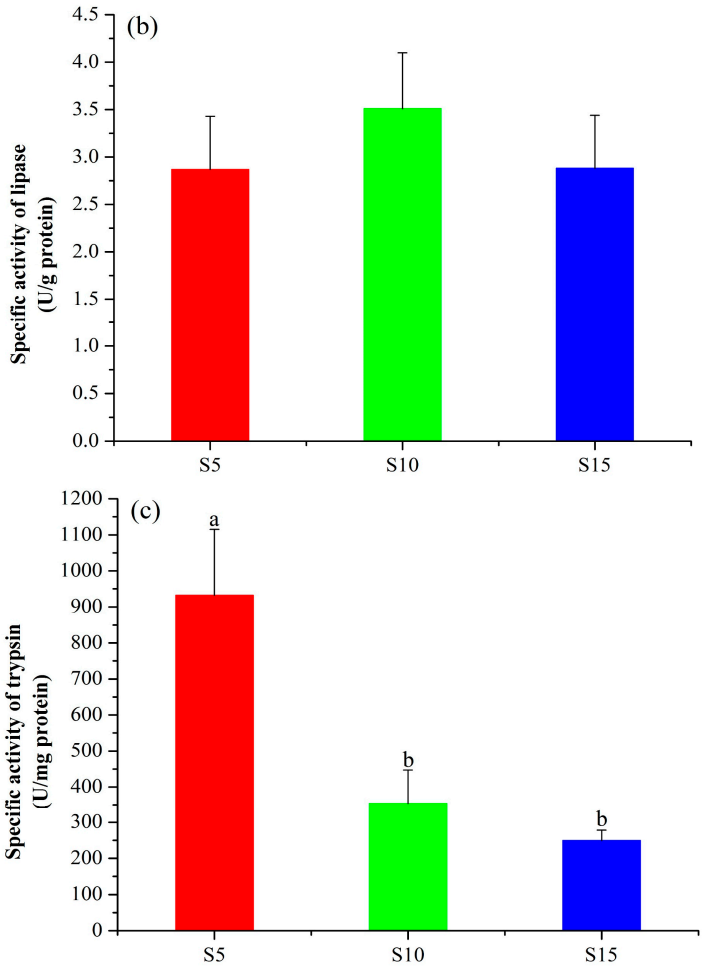

Figure 2. Specific activities of (**a**) amylase, (**b**) lipase, and (**c**) trypsin in the intestine of *Litopenaeus vannamei* reared in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment. Each value represents mean ± S.E. (n = 9). Values within the same tissue with different superscript letters are significantly different at $p < 0.05$.

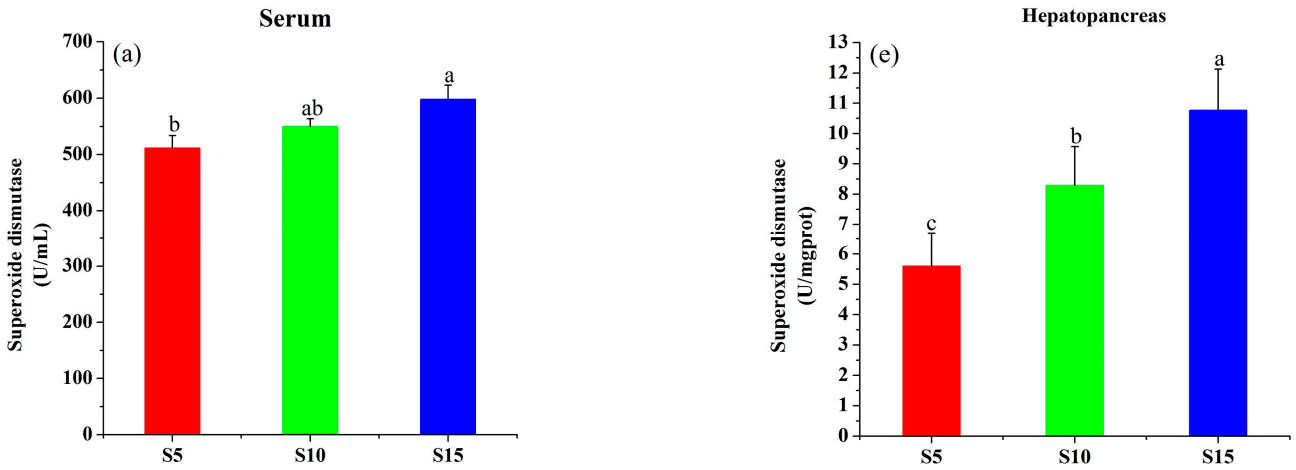

Figure 3. *Cont.*

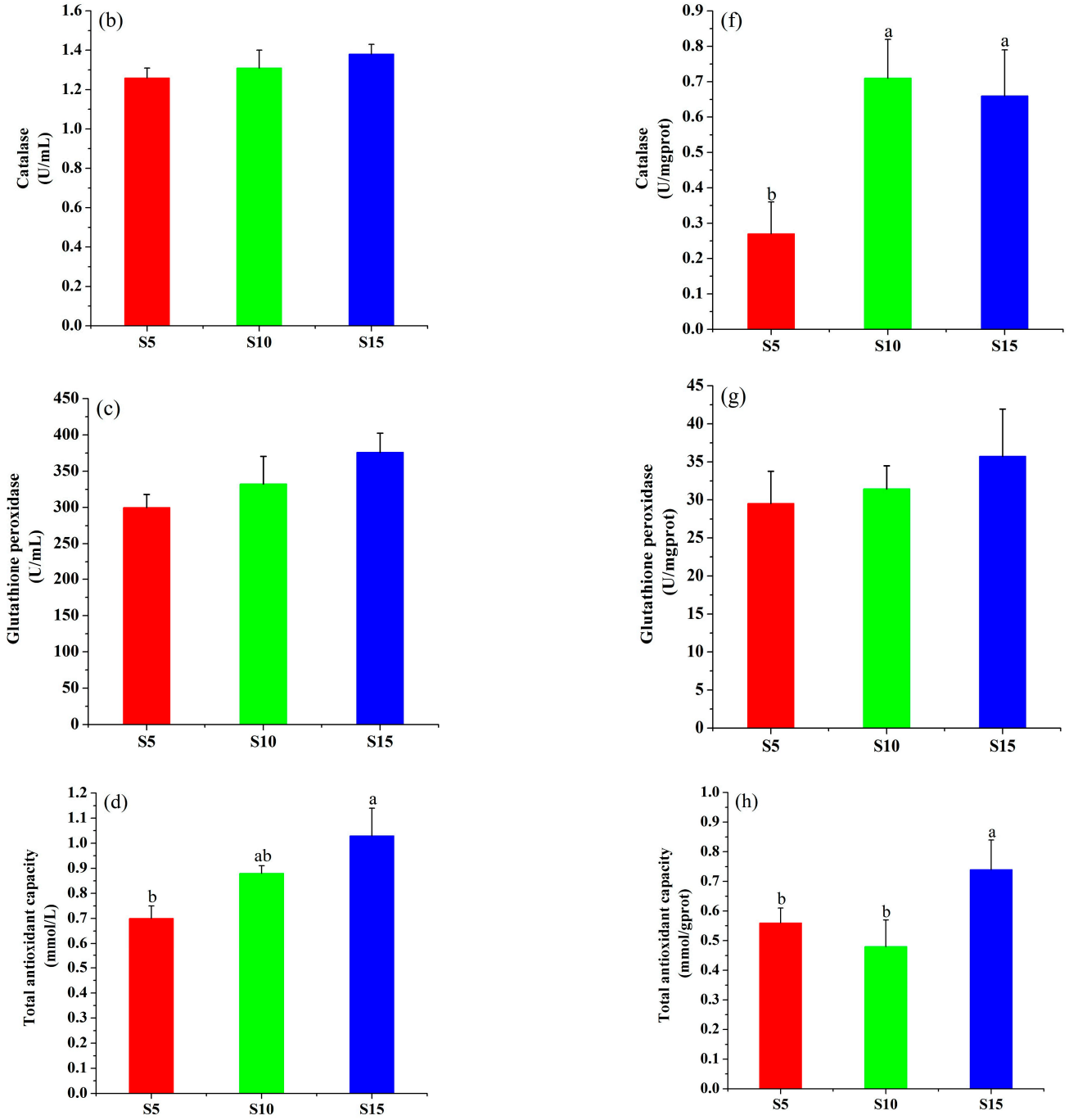

Figure 3. Antioxidants and total antioxidant capacity in serum and hepatopancreas of Litopenaeus vannamei reared in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment. (**a**) superoxide dismutase activity in serum, (**b**) catalase activity in serum, (**c**) glutathione peroxidase activity in serum, (**d**) total antioxidant capacity in serum, (**e**) superoxide dismutase activity in hepatopancreas, (**f**) catalase activity in hepatopancreas, (**g**) glutathione peroxidase activity in hepatopancreas, (**h**) total antioxidant capacity in hepatopancreas. Each value represents mean ± S.E. (n = 9). Values within the same tissue with different superscript letters are significantly different at $p < 0.05$.

Figure 4 shows the immune status of shrimp at the end of the experiment. Serum LZM activity of *L. vannamei* had no significant difference among groups, but the hepatopancreatic LZM activity of shrimp in the S10 group was significantly higher than that in the S5 group ($p < 0.05$). Serum ACP and AKP activities of *L. vannamei* in the S15 group were significantly

higher than those in the S10 and S5 groups ($p < 0.05$), but no significant difference was demonstrated in the hepatopancreas ($p > 0.05$).

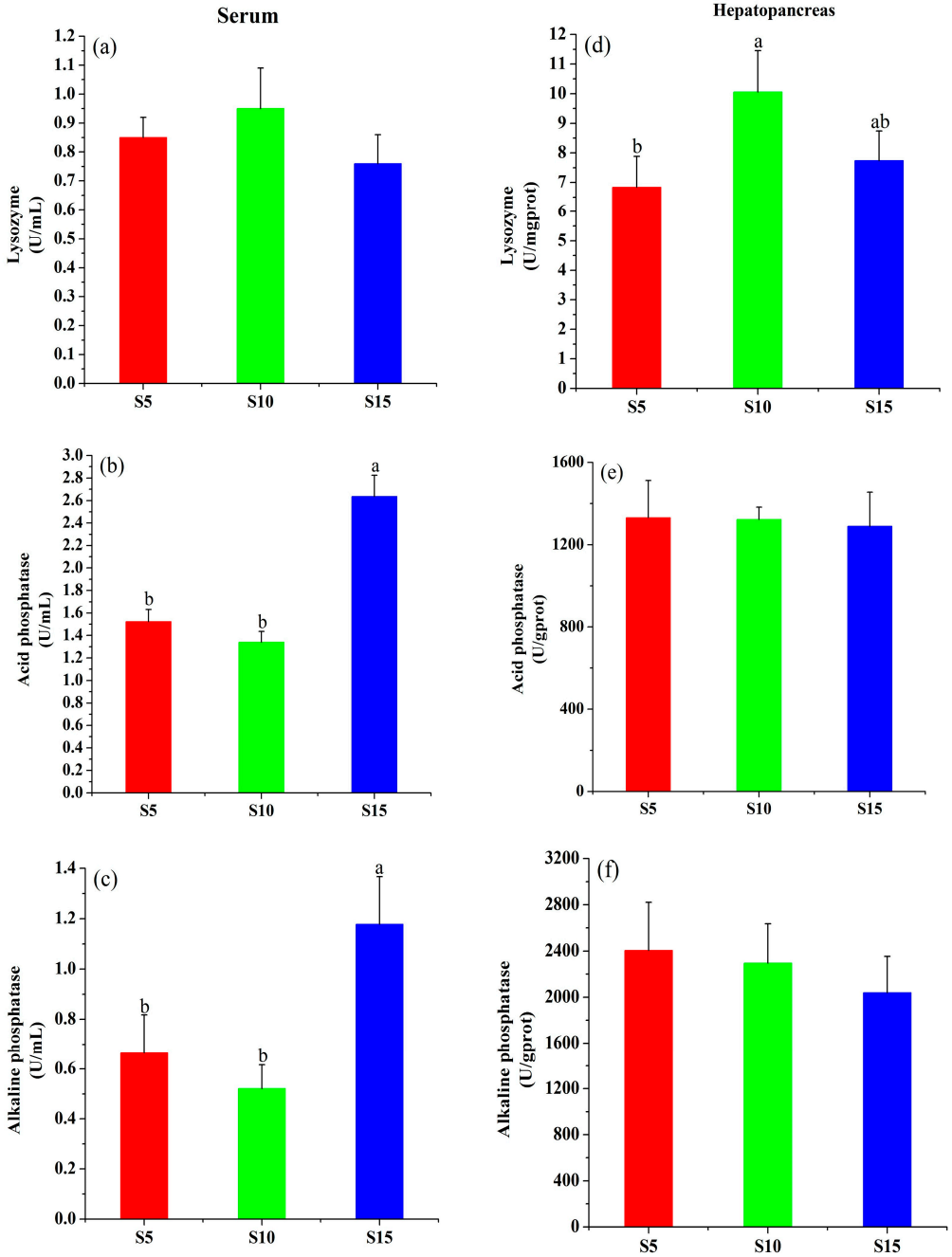

Figure 4. Immunity in serum and hepatopancreas of *Litopenaeus vannamei* reared in the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment. (**a**) lysozyme activity in serum, (**b**) acid phosphatase activity in serum, (**c**) alkaline phosphatase activity in serum, (**d**) lysozyme activity in hepatopancreas, (**e**) acid phosphatase activity in hepatopancreas, (**f**) alkaline phosphatase activity in hepatopancreas. Each value represents mean ± S.E. ($n = 9$). Values within the same tissue with different superscript letters are significantly different at $p < 0.05$.

3.5. Microbial Analysis

3.5.1. Composition of the Microbial Community

A total of 928,671 effective sequences were acquired from 18 samples after deculturization, quality control, and removal of chimeras. Each sample had an average of

51,593 sequences. The coverage rate per sample was more than 99%, indicating that the sequencing depth was good. The effective sequences were clustered into 1579 OTUs according to 97% similarity. As shown in Figure 5, 87 OTUs were shared by all groups. Except for the biofloc sample in the S10 group (WS10), all of the samples had a decreased number of OTUs with the increase in salinity. In addition, the number of OTUs in biofloc samples was higher than that in the intestine of *L. vannamei*, except for the intestine sample of shrimp in the S5 group (CS5).

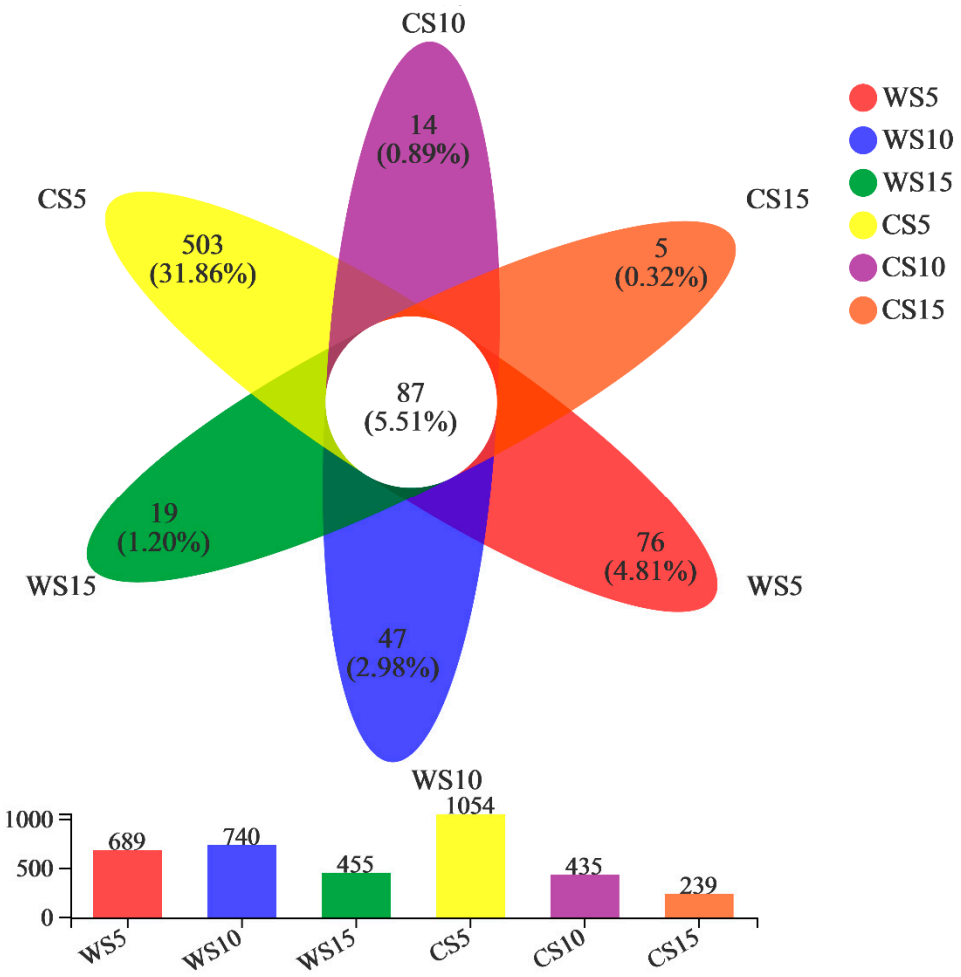

Figure 5. Venn diagram analysis of microbial communities in biofloc (WS5, WS10, and WS15) and *Litopenaeus vannamei* intestine (CS5, CS10, and CS15) among the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment.

3.5.2. Diversity of the Microbial Community

The α-diversity analysis demonstrated that the Shannon index of the biofloc sample in the S10 group was significantly higher compared to other biofloc samples ($p < 0.05$, Table 3). Furthermore, the index value of the biofloc samples was higher than that of the intestine samples. The Simpson index of the biofloc sample in the S5 group was the highest ($p < 0.05$), whereas no significant difference in diversity was found in the intestines of the shrimp. The richness indices (Chao1 and ACE) for the S5 group were significantly higher than those for the S15 group, and the values as well as variation of biofloc samples were generally higher than those of intestinal samples (Table 3). PCoA analysis of β-diversity showed differences in microbial community composition between biofloc and intestine samples under different salinity conditions (Figure 6a).

Table 3. Bacterial diversity indices (Shannon, Simpson) and estimated OTU richness indices (Chao1, ACE) for the biofloc and *Litopenaeus vannamei* intestinal bacterial diversity analysis among the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment.

Groups		Indices			
		Shannon	Simpson	Chao1	ACE
Biofloc	S5	2.39 ± 0.12 ^{b}	0.34 ± 0.04 ^{a}	650.10 ± 21.49 ^{a}	641.09 ± 18.93 ^{a}
	S10	3.29 ± 0.20 ^{a}	0.09 ± 0.02 ^{c}	676.95 ± 50.73 ^{a}	713.41 ± 36.46 ^{a}
	S15	2.34 ± 0.10 ^{b}	0.23 ± 0.01 ^{b}	436.78 ± 15.79 ^{b}	501.07 ± 52.52 ^{b}
Intestine	S5	2.19 ± 0.22	0.32 ± 0.06	547.50 ± 71.14 ^{a}	552.80 ± 70.55 ^{a}
	S10	1.90 ± 0.12	0.34 ±0.07	335.74 ± 30.48 ^{a}	379.55 ± 13.00 ^{b}
	S15	1.75± 0.29	0.31 ± 0.09	182.55 ± 29.39 ^{b}	205.19 ± 44.85 ^{c}

Each value represents mean ± S.E. (n = 3). Means in the same row with different superscripts are significantly different at $p < 0.05$.

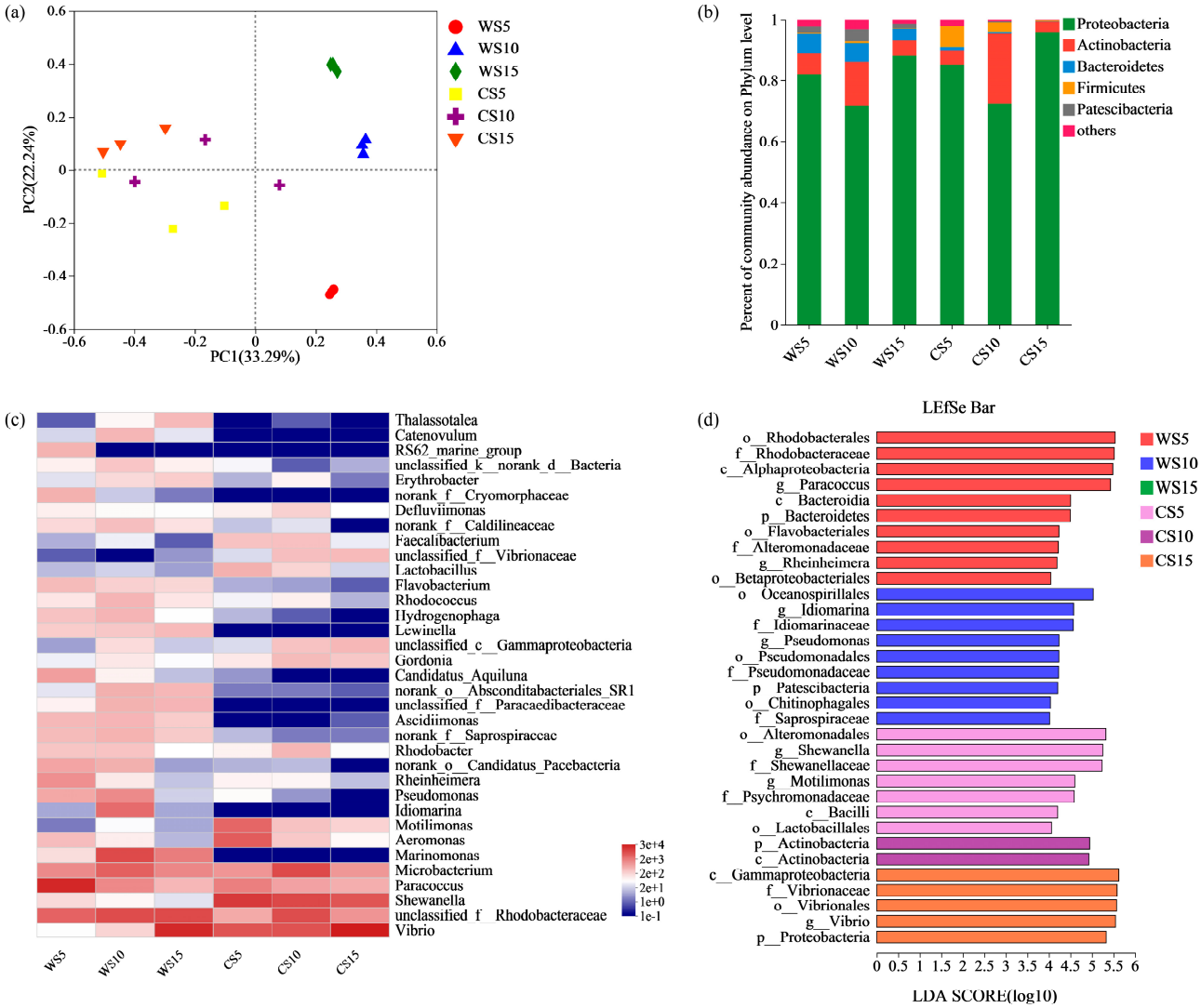

Figure 6. (**a**) Principal coordinate analysis (PCoA), (**b**) relative abundance of dominant phyla, (**c**) heatmap of the abundance of bacteria at the genus level, and (**d**) bacterial taxa identified by LEfSe using an LDA score threshold of >4 in biofloc (WS5, WS10, and WS15) and *Litopenaeus vannamei* intestine (CS5, CS10, and CS15) among the three biofloc groups at salinities of 5.0‰ (S5), 10.0‰ (S10), and 15.0‰ (S15) at the end of the 4-week experiment.

Proteobacteria was the dominant phylum in each group, accounting for 71.86–95.91% (Figure 6b). The top three predominant phyla in biofloc samples of each group were *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes*. However, in intestine samples, *Proteobacteria*, *Actinomycetes*, and *Firmicutes* were the top three phyla. A heatmap was constructed to characterize the relative abundances of the first 35 genera detected in biofloc and shrimp intestine samples of each group (Figure 6c). The abundance of *Vibrio* in biofloc and shrimp intestine samples was highest in the S15 group. In addition, LEfSe analysis showed that there were ten and nine biomarkers with significantly higher relative abundance in biofloc samples of the S5 and S10 groups, as well as seven, two, and five biomarkers in the intestine samples of the S5, S10, and S15 groups, respectively (Figure 6d). However, no biomarkers were found in biofloc samples of the S15 group. The biomarkers of biofloc samples in the S5 group included *Rhodobacterales*, *Rhodobacteraceae*, and *Paracoccus*. Vibrionaceae, Vibrionales, and *Vibrio* were the biomarkers in the intestine samples of the S15 group; these are potential pathogens of fish and shellfish.

4. Discussion

4.1. Water Quality

Total ammonia nitrogen and nitrite nitrogen are toxic to aquatic animals; they can inhibit growth and even lead to death [35]. In this study, the average concentrations of water quality parameters were all within accepted safe ranges. Biofloc was formed and gradually stabilized in about 15 days [36]. The biofloc can transform inorganic nitrogen into organic nitrogen, thereby regulating water quality in aquaculture systems. This was in accordance with our study, in which the total ammonia nitrogen concentration increased continuously during the first two weeks and then gradually decreased. The concentration of nitrate nitrogen in BFT culture systems generally shows an increasing trend [37,38]. According to the study of Ebeling et al. [39], organic nitrogen such as residual feed in culture systems and protein in the feces of farming objects will be degraded with the successive production of ammonia, nitrite, and nitrate [39]. High amounts of total ammonia nitrogen and nitrite nitrogen appear successively, followed by the continuous accumulation of nitrate. This pattern is similar to the results of the present experiment.

4.2. Growth and Muscle Composition

This work showed no significant difference in the growth of *L. vannamei* among different salinity groups. Maicá et al. [32] found that the growth of *L. vannamei* in BFT systems with 25.0‰ salinity was notably higher than those with 2.0‰ and 4.0‰ salinity. Ponce-Palafox et al. [27] demonstrated that the growth performance of *L. vannamei* was elevated with the increase in salinity (10.0‰, 20.0‰, and 30.0‰), and the values of survival rate and SGR at a salinity of 30‰ were higher compared to this experiment. However, the lower-salinity (5‰) rearing with BFT did not show significantly lower growth than that under the salinity of 30‰ in the case of *Penaeus indicus* postlarvae [40]. Such differences may have been caused by various culture conditions, including different carbon sources, the C/N ratio, the number of culture days, and shrimp growth stages [14].

As the salinity of the aquaculture water increases, moisture content in the muscle of *L. vannamei* decreases [41]. This is because *L. vannamei* belongs to the group of euryhaline shrimp that can adapt to the changes in environmental salinity through hemolymph osmosis and ion regulation: when the salinity is high, the shrimp can expel water; when salinity is low, water is absorbed to maintain isotonicity with the external environment, thus maintaining a stable state [42]. In this study, the muscle moisture contents of the three different salinity groups showed no significant difference; however, the S5 group exhibited a slightly higher content of muscle moisture than the S10 and S15 groups did. In the BFT systems, studies on *L. vannamei* [43] showed that their crude lipid content was notably higher compared to that of the control group, a result that may have been due to biofloc in these systems containing a certain amount of lipid [38]. This experiment showed no significant differences in the contents of crude protein or crude lipid in the muscle

of *L. vannamei* under three different low-salinity conditions. This indicates that salinity may not have a notable effect on the composition of the biofloc. Therefore, it may not play a role in the contents of crude protein or crude lipid of cultured aquatic animals. In addition, Li et al. [41] found that the lower the moisture content in *L. vannamei*, the higher the ash content. This study also found that the S10 group exhibited a slightly lower muscle moisture content than the S5 and S15 groups, while the ash content was relatively higher in the S10 group. The ash content is composed of inorganic substances such as mineral oxides or salts. The higher muscle ash content of the S10 group may indicate that the mineral content was relatively high. Thus, minerals could participate in the regulation of osmotic pressure.

4.3. Digestive Enzyme Activity

Digestive enzymes are closely related to growth and thus directly affect the digestive capacity of cultured animals [44]. Among the digestive enzymes, amylase and protease play essential roles in the digestion of carbohydrate and protein [45]. When aquatic animals are stressed by salinity, they need energy for osmoregulation. Researchers have shown that glucose, amino acids, and lipids can be used as energy sources for the osmotic regulation of aquatic animals [46–48]. Li et al. [49] found that the trypsin activity of shrimp was elevated during low-salinity stress. In addition, biofloc contains digestive enzymes such as amylase, lipase, and protease that can improve the digestive ability of cultured animals [9,12,50]. Biofloc can also stimulate the secretion of more endogenous digestive enzymes [51]. Therefore, we speculate that the significantly increased intestinal amylase and trypsin activities of *L. vannamei* in the S5 group may have been due to the higher digestive enzyme activity obtained by eating more biofloc in the culture system. The shrimp in this group improved their intestinal amylase and trypsin activities, thereby producing more glucose and amino acids under low-salinity pressure to provide energy and maintain osmotic pressure balance.

4.4. Antioxidant and Immune Status

Shrimp have a lipid soluble antioxidant system (LSAS) [52]. In this system, SOD has an important role in maintaining the metabolic balance of ROS [53], and T-AOC reflects the shrimp antioxidant defense capacity [54]. In addition, the antioxidant capacity of shrimp is closely related to the immune system [9]. LZM, ACP, and AKP are hydrolytic enzymes that can resist pathogens and kill or digest microorganisms during the immune response process [55,56]. In this work, SOD, CAT, and T-AOC, along with ACP and AKP, increased significantly in the S15 group, and these results were consistent with the notably enriched *Vibrio* in the biofloc and the intestines of shrimp in the S15 group (Figure 6c,d). *Vibrio* is a normal component of the flora in the gut of shrimp that is conducive to the metabolism and utilization of carbon sources. However, some *Vibrio* species are conditional pathogenic bacteria and could affect the health of the shrimp when they multiply in large numbers for some reason [57]; this usually causes considerable economic losses [58].

4.5. Microbial Analysis

Shannon and Simpson indices are good indicators of bacterial diversity, while Chao1 and ACE are good indicators of bacterial richness [59]. In this study, the overall bacterial richness and diversity indices were high in the S5 group compared to other groups (Table 3), and the richness and diversity of biofloc samples were higher than those of intestine samples, indicating that changes in salinity would alter the richness and diversity of bacteria in biofloc samples and thus affect the intestinal flora of shrimp.

The first five bacterial groups with an abundance greater than 1% were found in different salinity groups: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Patescibacteria* (Figure 6b). Most of these phyla exist widely in biofloc systems [13,60–62] and are considered to possess some ecological and functional characteristics [60]. Among all such groups, *Proteobacteria* was the dominant phylum, accounting for more than 70%. This phylum is

widely distributed in marine environments; it can be used to remove organic matter from BFT culture systems, thus regulating water quality effectively [63,64]. In this study, with respect to the genus, the abundance of *Vibrio* was significantly higher in the S15 group than in the S5 and S10 groups (Figure 6c), and this finding was similar to that of Hosain et al. [14]. It may be that *Vibrio* is more suited to surviving in brackish/marine habitats [65,66].

In addition, *Rhodobacteraceae* and *Paracoccus* were the main biomarkers in biofloc samples in the S5 group. *Rhodobacteraceae* is considered to be an excellent biofilm-forming organism with a variety of metabolic activities [67], and it can often be found in aquaculture biofilm systems [68]. It may form an antagonistic beneficial microbial community in culture systems, thus limiting the survival rate of pathogenic bacteria [69]. This may be one of the reasons why there were fewer *Vibrio* in the S5 group in this study. Furthermore, *Paracoccus*, a denitrifying bacterium, can help reduce nitrate to molecular nitrogen [13]. It is because of the existence of these microorganisms that the water quality of rearing system remains good.

5. Conclusions

L. vannamei cultured under three different salinity conditions (5.0‰, 10.0‰, and 15.0‰) in BFT was examined in this study. The water parameters measured in this study were within the acceptable ranges for *L. vannamei*. No significant differences in growth parameters were found among the three groups. *L. vannamei* cultured in BFT with a salinity of 5‰ had lower antioxidant and immune levels. However, the digestive enzyme activity, microbial diversity, and microbial richness of *L. vannamei* in this group were significantly higher than those of other groups. These findings suggest the possible application of rearing *L. vannamei* at low salinity in BFT systems.

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