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Dietary supplementation with polypeptides improved growth performance, antibacterial immune and intestinal microbiota structure of *Litopenaeus vannamei*

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ABSTRACT

Antibacterial peptides (AMPs) are expected to replace some or all of the antibiotics and become a new feed additive. However, the high production cost and unclear mechanism limited the application of AMPs. In this research, the effects of a commercial polypeptide (Polypeptide S100) whose main components are AMPs on the growth, antibacterial immune and intestinal microbial of *Litopenaeus vannamei* were study. *L. vannamei* (initial weight of 0.16 ± 0.03 g) were fed for 123 days with basal diet added Polypeptide S100 at two levels each (0.5% and 1%) as experimental groups, and a basal diet as control. Dietary inclusion of Polypeptide S100 at 1% level significantly increased the weight gain (WG) and specific growth rate (SGR) of *L. vannamei*. The survival rates of *L. vannamei* in 0.5% and 1% Polypeptide S100 groups were significantly higher than the control when infected by *Vibrio harveyi* but not *Vibrio parahaemolyticus*. The activities of total superoxide dismutase (T-SOD) and lysozyme (LZM) in the two experimental groups were all significantly higher than the control. Differently, the activities of amylase (AMS) and lipase (LPS) were significantly higher in 0.5% Polypeptide S100 group but lower in 1.0% Polypeptide S100 group. Illumina MiSeq high-throughput sequencing showed that the dominant phyla in the intestine of *L. vannamei* were Proteobacteria, followed by Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Fusobacteria and Tenericutes, and the abundance of predominant phyla Cyanobacteria were upregulated significantly in the experimental groups. At the family level, significant increase was observed in Pseudomonadaceae and Xanthomonadaceae but decrease in Vibrionaceae in the 1.0% Polypeptide S100 group. The abundance of predominant genus *Photobacterium* were obviously downregulated in the two experimental groups. Unlikely, the abundance of *Pseudomonas* and *Stenotrophomonas* were distinctly increased in the 1.0% Polypeptide S100 group but not significantly different from the control in 0.5% Polypeptide S100 group. All these results suggested that Polypeptide S100 could improve the growth performance, antibacterial immune and intestinal microbiota structure of *L. vannamei*.

1. Introduction

Shrimp farming is one of the most important economic aquaculture activities worldwide. Pacific white shrimp (*Litopenaeus vannamei*), one of the most profitable species in shrimp farming, accounts for over two-third of the global shrimp production [1]. However, with the high-density, intensive and large-scale production of shrimp culture, the problems of disease overflow, ecological imbalance and environmental deterioration have become increasingly prominent, leading to severe challenges for shrimp industry [2,3]. Generally speaking, frequent outbreaks of diseases, including some bacterial diseases, such as early mortality syndrome (EMS), acute hepatopancreatic necrosis disease (AHPND) and hepatopancreas necrosis syndrome (HPNS), are the bottleneck restricting the healthy development of shrimp culture [4]. Antibiotics have long been used for promoting growth and controlling disease in aquaculture. However, their application is restricted due to their numerous adverse effects on the environment and the human healthy in recent years [5]. Thus, identification of potential alternatives

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for antibiotics is necessary for health and sustainable development of shrimp aquaculture. Polypeptide is a good choice.

Peptides are distinguished from proteins on the basis of size, and as an arbitrary benchmark can be understood to contain approximately 50 or fewer amino acids [6]. A polypeptide is a long, continuous, and unbranched peptide chain. It is a class of intermediate substances consisting of amino acids but differing in structure from proteins [7]. Proteins consist of one or more polypeptides arranged in a biologically functional way, often bound to ligands such as coenzymes and cofactors, or to another protein or other macromolecule (DNA, RNA, etc.), or to complex macromolecular assemblies [8]. Bioactive polypeptides existed widely in animals and are an significant part of the innate immune system [9]. They have broad-spectrum antimicrobial activity and multiple immunomodulatory functions, with the characteristics of strong stability, good water solubility, safety, no residue, no drug resistance and synergism with other antimicrobial substances [10]. Hence more and more attention has been paid to the physiological functions of polypeptides in organisms recently [11]. Swedish scientist G. Boman et al. discovered antibacterial peptides (AMPs) in the body of the Samia cynthia, and became the world's first discovered antimicrobial peptide, named Cecropins [12]. As a kind of small molecule polypeptide ubiquitous in animals, plants and bacteria, AMPs are the effectors molecules of innate immunity, and mainly studied in livestock, poultry, shrimp and crab farming [13,14]. The rational use of antibacterial peptides in aquaculture can effectively improve the resistance of water-producing substances to pathogenic microorganisms, and can significantly increase the weight-increasing rate of water-producing products [15]. Researches of AMPs in shrimp mainly focused on separation, purification and antimicrobial activities [16,17], but few and less in-depth in the application research during the feeding period, not to mention the functional mechanism.

Intestine is an important digestive and absorption organ, which is closely related to the healthy growth of animals [18]. Intestinal microbiota is a complex organ ecosystem with multiple functions in maintaining host health, including nutrient metabolism, pathogen defense, and antibiotic resistance [19,20]. Because of the simple structure of the digestive tract and the dual functions of nutrition and absorption, intestines play particularly important roles in aquatic animals, and intestinal microbiota were paid more and more attention as a target of immune resistance and nutritional metabolism [21,22]. In some aquatic animals, such as *Ctenopharyngodon idellus*, *Scophthalmus maximus* and *L. vannamei*, the intestinal microbial communities have been characterized, focusing on the factors that shape the intestinal microbiota, including diet, habitat environment, developmental stage, and host genotype and so on [23,24]. α-defensins, a kind of AMPs, are essential effectors that modulate immunity in Paneth cells through their ability to regulate the composition of the intestinal microbiota [25]. However, little is known about the relationship between antimicrobial peptides and intestinal microbiota in aquatic animals. In this study, polypeptide S100 which is rich in AMPs were added in the feed to observe the effect on growth performance, antibacterial immune and intestinal health of *Litopenaeus vannamei*, hoping that it could provide theoretical basis for the application of antimicrobial peptide preparations in aquaculture.

2. Materials and methods

2.1. Experimental diets and shrimp rearing

The polypeptides (Polypeptide S100) were obtained from Shandong Sci-health Biotechnology Company in Yantai, Shandong, China. Its main components are antimicrobial peptide, body defense peptide, tissue repair peptide, probiotics, etc. Three diets were produced using polypeptide S100 evenly mixed into commercial feed (Guangdong Evergreen Conglomerate Co., Ltd, China) with different levels at 0% (the control), 0.5% and 1.0%.

Juvenile *L. vannamei* were purchased from Guangdong Haimao Aquatic Seed Industry Technology Co., Ltd in Zhanjiang (Guangdong, China). They were transported into indoor ponds and fed with commercial diet to adapt to the experimental conditions for 14 days in East Island Marine Biological Research Base, Guangdong Ocean University in Zhanjiang, Guangdong, China. After the adaptation period, shrimps (approximate initial weight 0.16 ± 0.03 g) were transferred into 9 net-cages (3 m × 3 m × 1.5 m) at a density of 3000 shrimps in each cage. All cages were put in a pond. 9 net-cages were divided into 3 groups on average, fed by 3 different diets described above. Water quality parameters were measured daily (23.26 ± 2.35 ppt salinity, 7.46 ± 0.85 pH and 28.6 ± 3.6 °C temperature). The experiment was performed in triplicates and shrimps were fed to apparent satiation with experimental diets four times daily (7:00, 11:00, 18:00, 23:00) for 123 days.

2.2. Sample collection

At the end of the feeding trial, shrimps were fasted for 24 h. 15 shrimps were randomly selected from each cages to measure individual body length and body weight, and hepatopancreas were immediately placed into ice and stored at −80 °C for the analysis of the enzymatic activities. Intestine sampling was according to the previously reported methods [26]. The shrimps were washed twice with 70% alcohol before dissecting. 15 intestines were aseptically dissected and the contents of guts were mixed in a sterilized 1.5 mL autoclaved tube on dry ice, and were immediately stored −80 °C before DNA extraction.

2.3. Challenge test

After the growth trial, 50 shrimps from each cage were captured randomly for bacterial challenge test according to previously described methods [27]. *Vibrio parahaemolyticus* and *Vibrio harveyi* preserved in our laboratory were re-streaked on the thiosulfate citrate bile salts sucrose agar culture medium (TCBS) plate prior to culture of a single colony in 2 mL Luria Broth (LB) containing 1.5% NaCl at 30 °C. The bacterial inocula were subsequently transferred to 150 mL LB with vigorous shaking at 30 °C until OD600 = 0.6–0.8 which was equivalent to a bacterial density of approximately 10^8 colony forming units (cfu)/mL. The bacterial cultures were collected at 3000 rpm for 10 min prior to dilution with 0.1 M PBS (pH 7.4) to a concentration of 10^7 cfu/50 μL. The shrimp were injected intramuscularly with 50 μL bacterial suspension at the third abdominal segment. Survival rates were recorded every 6 h.

2.4. Enzymes activities

Total superoxide dismutase (T-SOD), lysozyme (LZM), amylase (AMS) and lipase (LPS) in the hepatopancreas were measured with commercial assay kits (Cat. No. A001–1, A050, C016–1 and A054, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the instructions of the manufacturer. As a result, enzyme activity units were expressed as the enzyme activity per mg of tissue protein.

2.5. DNA extraction and sequencing

Total bacterial DNA was extracted by the PowerFecal DNA Isolation Kit (MoBio, Palo Alto, CA, USA) following the manufacturer's directions. The concentration and purity of total DNA were determined by NanoVuePlus Spectrophotometer (GE Healthcare, USA) and 1% agarose gels. The primer pair 341F 5′-CCTAYGGGRBGCASCAG-3′ and 806R 5′-GGACTACNNGGGTATCTAAT-3′ were used to amplify the V3–V4 hypervariable region of 16S rRNA gene, which was modified with a barcode tag with a random 6-base oligos [28]. Sequencing libraries were generated via using TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA). In addition, the library quantity was assessed on Qubit 2.0 Fluorometer (Thermo Scientific, Waltham, MA,

USA). The libraries were sent for sequencing by Illumina Hiseq2500 platform (Illumina, San Diego, CA, USA), which was conducted by Guangzhou Sagene Biotech Co., Ltd. (Guangzhou, China). Raw data generated from Hiseq2500 platform were paired-end reads.

2.6. Analysis of the intestine microbiota

Sequences from raw data were analyzed and filtered by Quantitative Insights Into Microbial Ecology as reported previously (QIIME, http://qiime.org/index.html) [29]. Sequences were assigned to the same operational taxonomic units (OTUs) with 97% similarity by Uparse (Version 7.0.1001, http://drive5.com/uparse/).To determine the level of sequencing depth, rarefaction curves were performed by plotting the number of observed OTUs against the number of sequences. Taxonomic richness and diversity estimators were determined in Mothur. In addition, alpha diversity was determined to assess community diversity and it was analyzed using ACE and Shannon-Wiener indexes. The abundance of these OTUs was significantly different for experimental groups compared with the control using Student's t-test. A Venn diagram was generated to represent the number of unique and shared species among percentages and groups of OTUs. The normalized abundance was exhibited by heatmap.

2.7. Calculations and statistical analysis

Growth performance was calculated by the following formulas:

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

Specific growth rate (SGR, %) = 100 × (ln final body weight − ln initial body weight) / days of the experiment.

The Kaplan–Meier plot (log-rank χ^2 test) was used to identify significant differences in mortality levels between the bacteria challenged group and the control group [30]. All results were subjected to one-way analysis of variance followed by Duncan's multiple range tests to determine significant differences among treatment groups using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). The data were presented as means ± standard deviation (SD). Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Growth performance

Growth performance of *L. vannamei* was presented in Table 1. Addition of Polypeptide S100 results in better growth performance of *L. vannamei* by the amount increasing. *L. vannamei* fed the diet containing the 1.0% S100 had the highest final weight (14.08 ± 0.84 g), final length (10.58 ± 0.33 cm), SGR (3.64 ± 0.05) and WG (8701.3 ± 528.0), which were significantly higher than that in 0.5% S100 and control groups ($p < 0.05$). However, diet with 0.5% S100 did not significantly influence the growth of *L. vannamei* when compared with the control group ($p > 0.05$).

Table 1
Growth performance of *L. vannamei* fed the different experimental diets for123 days.

Parameters	Groups		
	0.0%	0.5%	1%
Initial weight (g)	0.16 ± 0.03	0.16 ± 0.03	0.16 ± 0.03
Final weight (g)	12.20 ± 0.80[a]	13.18 ± 1.01[a]	14.08 ± 0.84[b]
Initial length (cm)	2.35 ± 0.19	2.35 ± 0.19	2.35 ± 0.19
Final length (cm)	9.98 ± 0.26[a]	10.17 ± 0.16[a]	10.58 ± 0.33[b]
SGR (%)	3.52 ± 0.04[a]	3.59 ± 0.06[a]	3.64 ± 0.05[b]
WG (%)	7525.0 ± 500.4[a]	8139.0 ± 636.0[a]	8701.3 ± 528.0[b]

Data represent means ± SD from three repetitions. The experimental groups were compared with the control (0.0%) using one-way analysis of variance (ANOVA). Dissimilar letters show significant difference ($p < 0.05$).

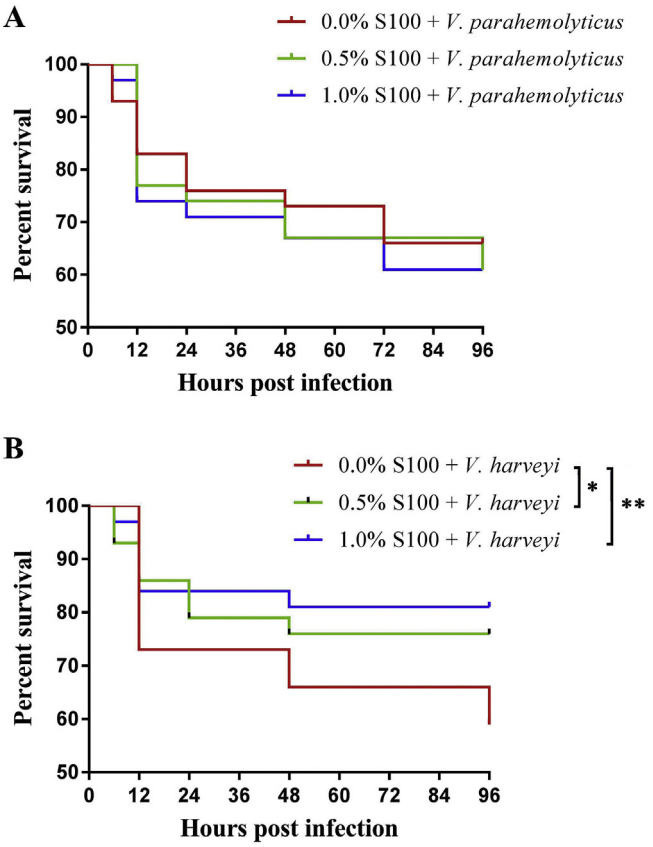

Fig. 1. Cumulative survival rates of polypeptides fed *L. vannamei* after *V. parahaemolyticus* (A) and *V. harveyi* (B) infection. At 123 d fed with diets containing 0%, 0.5% and 1.0% S100, *L. vannamei* were injected intramuscularly with *V. parahaemolyticus* or *V. harveyi*. Mortality was measured in each treatment group (n = 50) and was recorded every 6 h post-challenge. Differences in cumulative mortality levels between the experimental and control groups were analyzed by Kaplan-Meier log-rank χ^2 tests (* indicates $p < 0.05$ and ** indicates $p < 0.01$).

3.2. Cumulative survival rates of polypeptides fed L. vannamei after V. parahaemolyticus and V. harveyi infection

There was no statistically significance on the survival rates of *L. vannamei* between the test groups and the control after *V. parahemolyticus* infection (Fig. 1A). After infection by *V. harveyi*, the survival rates in 0.5% (Kaplan-Meier log-rank χ^2: 5.147, $p < 0.05$) and 1% (Kaplan-Meier log-rank χ^2: 10.194, $p < 0.01$) S100 groups were significantly higher than the control starting at 12 h post infection (hpi) (Fig. 1B). At 96 hpi, *L. vannamei* in 1% S100 group had the highest survival (80.00 ± 8.16%).

3.3. Immune and digestive enzymes activities in hepatopancreas of L. vannamei

At the end of the 123 days of the experiment, the activities of T-SOD and LZM in hepatopancreas were detected to evaluate the effect of S100 on immune enzymes of *L. vannamei* while the activities of AMS and LPS used for the digestive enzymes. The activities of T-SOD (Fig. 2A) and LZM (Fig. 2B) increased significantly in 0.5% and 1.0% S100 groups when compared with the control ($p < 0.05$), with the highest level

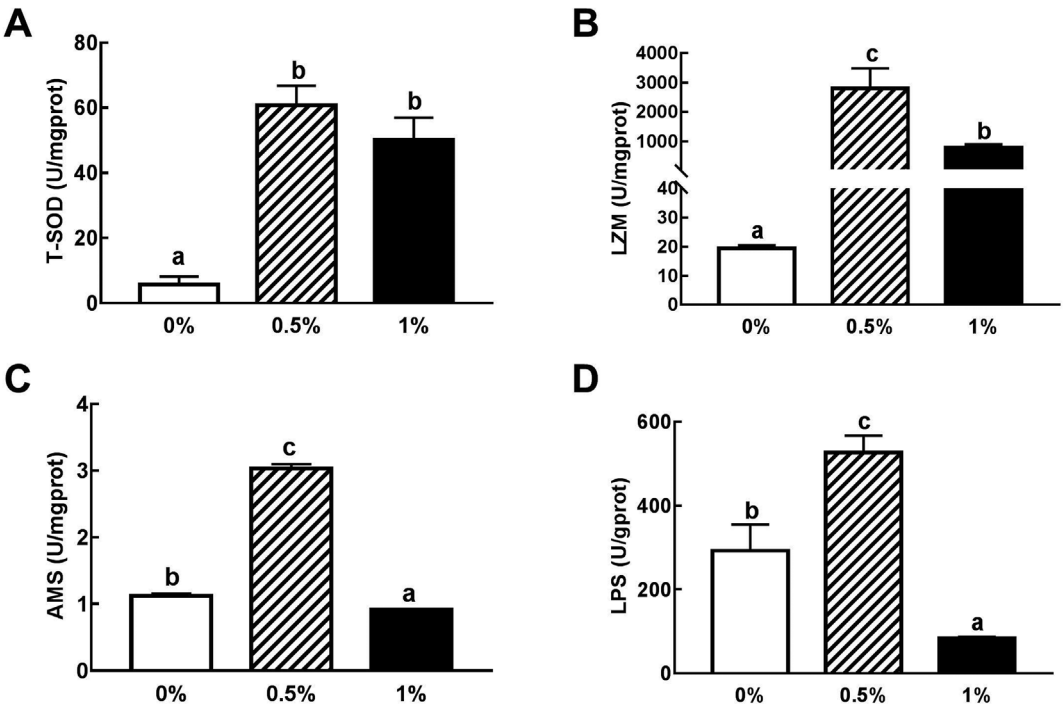

Fig. 2. (A) The activity of total superoxide dismutase (A), lysozyme (B), amylase (C) and lipase (D) in hepatopancreas of *L. vannamei* fed different diets. Dissimilar letters show significant difference ($p < 0.05$).

both in the 0.5% S100 group at 60.80 U/mgprot and 2801.12 U/mgprot, respectively. In 0.5% S100 group, the activities of AMS (Fig. 2C) and LPS (Fig. 2D) in hepatopancreas of *L. vannamei* were 3.04 U/mgprot and 526.05 U/gprot, respectively, both of which were obviously higher than the control group ($p < 0.05$). However, the activities of AMS and LPS reduced to the significantly lower level than the control group when the addition of S100 is 1.0%.

3.4. Intestinal microbiota analysis

The clean tags range from 42,109 to 54,822, with an average of 48,759 tags per sample. The total number of observed taxonomic units (OTUs) was 6879, of which 891 OTUs (34.6%) shared among the three treatments (Fig. 3). The Observed-Otus varied from 1013 to 1982. The

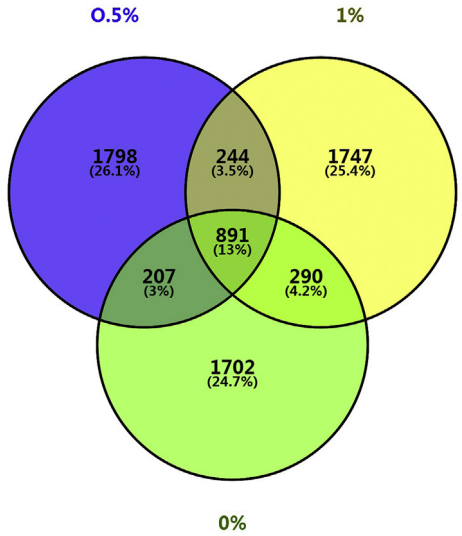

Fig. 3. Venn diagram showing the unique and shared OTUs in different level polypeptides fed shrimps.

Table 2
The summary of high-throughput sequencing read analysis, bacterial community richness (OTUs), sample coverage (Tags), Shannon index, and estimated OTU richness (Ace) for Alpha diversity analysis of *L. vannamei* fed with different polypeptide levels for 123 days.

Samples	Sequences data		Alpha diversity	
	Tags	Observed_Otus	Ace	Shannon
0 %-1	50,107	1013	2448.07	3.64
0 %-2	43,009	1200	2242.98	5.52
0 %-3	42,109	1982	5271.42	6.43
0.5 %-1	47,390	1301	2227.27	6.42
0.5 %-2	48,472	1539	4118.73	6.4
0.5 %-3	54,822	1534	3608.63	3.73
1 %-1	53,221	1760	3958.95	5.99
1 %-2	45,644	1350	2472.89	5.91
1 %-3	54,057	1454	3074.66	5.71

alpha diversity index was calculated from the OTUs of each library: the Ace ranged from 2227.27 to 5271.42 phylotypes and the Shannon diversity index varied from 3.64 to 6.43 (Table 2).

According to the abundance of taxa, OTUs were identified to 38 phyla. Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Fusobacteria, Proteobacteria and Tenericutes were the dominant phylum (relative abundance > 1%) in the *L. vannamei* intestinal microbiota. In the 0.5% S100 and 1% S100 groups, Proteobacteria (80.69% and 81.73%) was the most abundant phyla (Fig. 4A). In all detected samples, most OTUs were mapped to Proteobacteria (Fig. 4B), but there was no significant difference between the groups ($p > 0.05$). Additionally, the relative abundances of other dominant phylum in the S100-added groups were not significantly higher than that in the control group (Fig. 4C).

At the family level, a total of 178 taxa were identified. The abundance of Pseudomonadaceae and Xanthomonadaceae displayed an obvious increase following add 1% S100. In contrast, Vibrionaceae showed significant decrease in the abundance ($p < 0.05$) (Fig. 5).

At genus level, a total of 185 taxa were identified. The top 5 genera

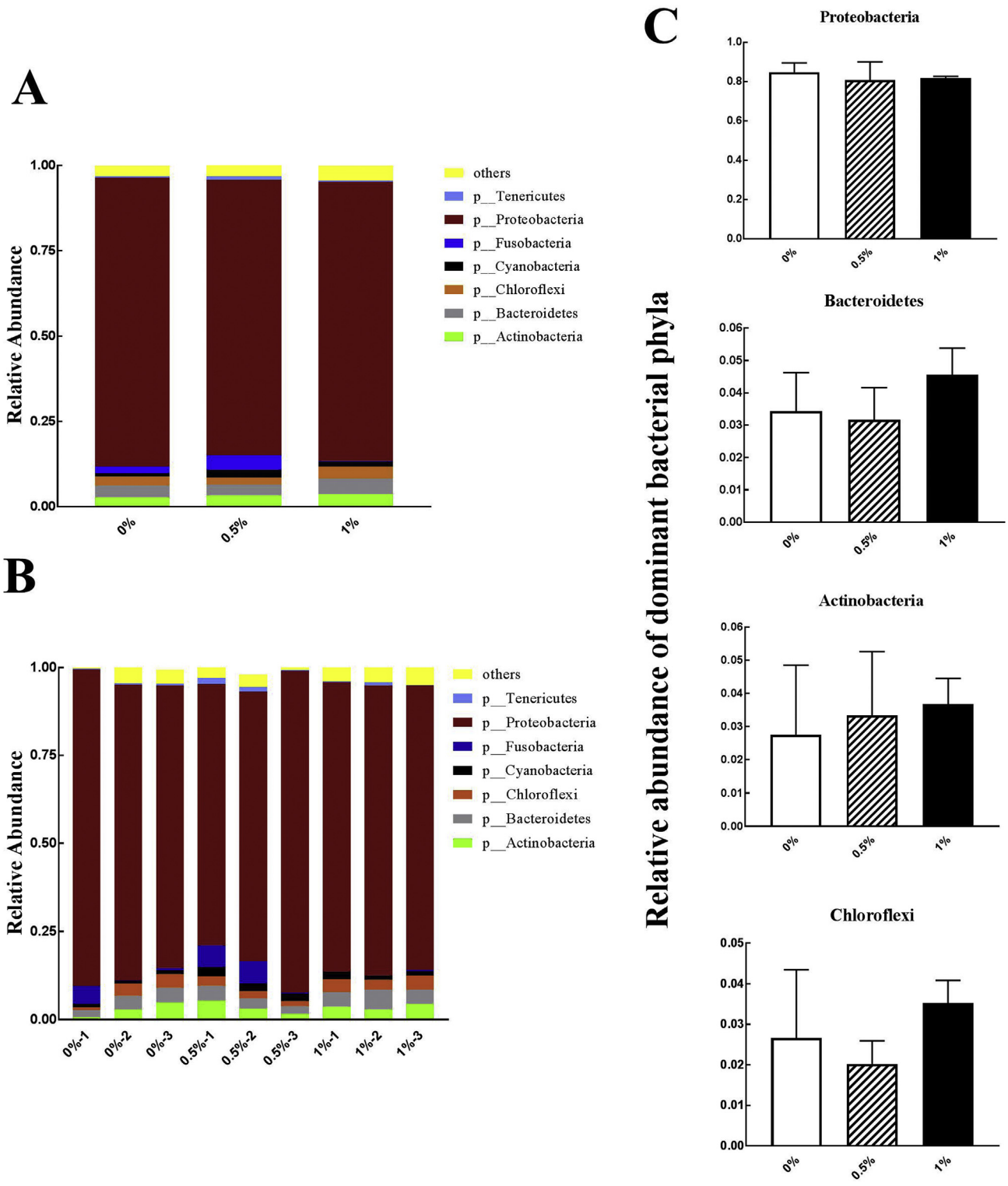

Fig. 4. Structure and composition of the intestinal bacterial communities in different level polypeptides fed shrimps on phylum level of taxonomy. (A). Means representing as three groups, (B) appearing in each sample and (C) the changes in abundance of dominant bacterial phyla. Dissimilar letters show significant difference ($p < 0.05$).

were *Pseudomonas*, *Photobacterium*, *Stenotrophomonas*, *Vibrio*, and *Propionigenium* (Fig. 6A). In all samples of experimental groups, the relative abundance of *Photobacterium* was significantly lower than that in every control sample ($p < 0.05$) (Fig. 6B). The relative abundance of *Pseudomonas* and *Stenotrophomonas* in the 1% group were significantly higher than that in the 0.5% and the control groups ($p < 0.05$) (Fig. 6C).

A PCA plot was used to show the microbial community compositions for shrimp fed with different polypeptide level diets based on the Unifrac distance (Fig. 7). Microbial communities from the different treatment groups were divided into two major clusters (0.5%, 1% groups and the 0% groups) along PCA1, which could describe 76.96% of total variation. The 0.5%, 1% and the 0% groups also showed somewhat distinct patterns in community composition along PCA2,

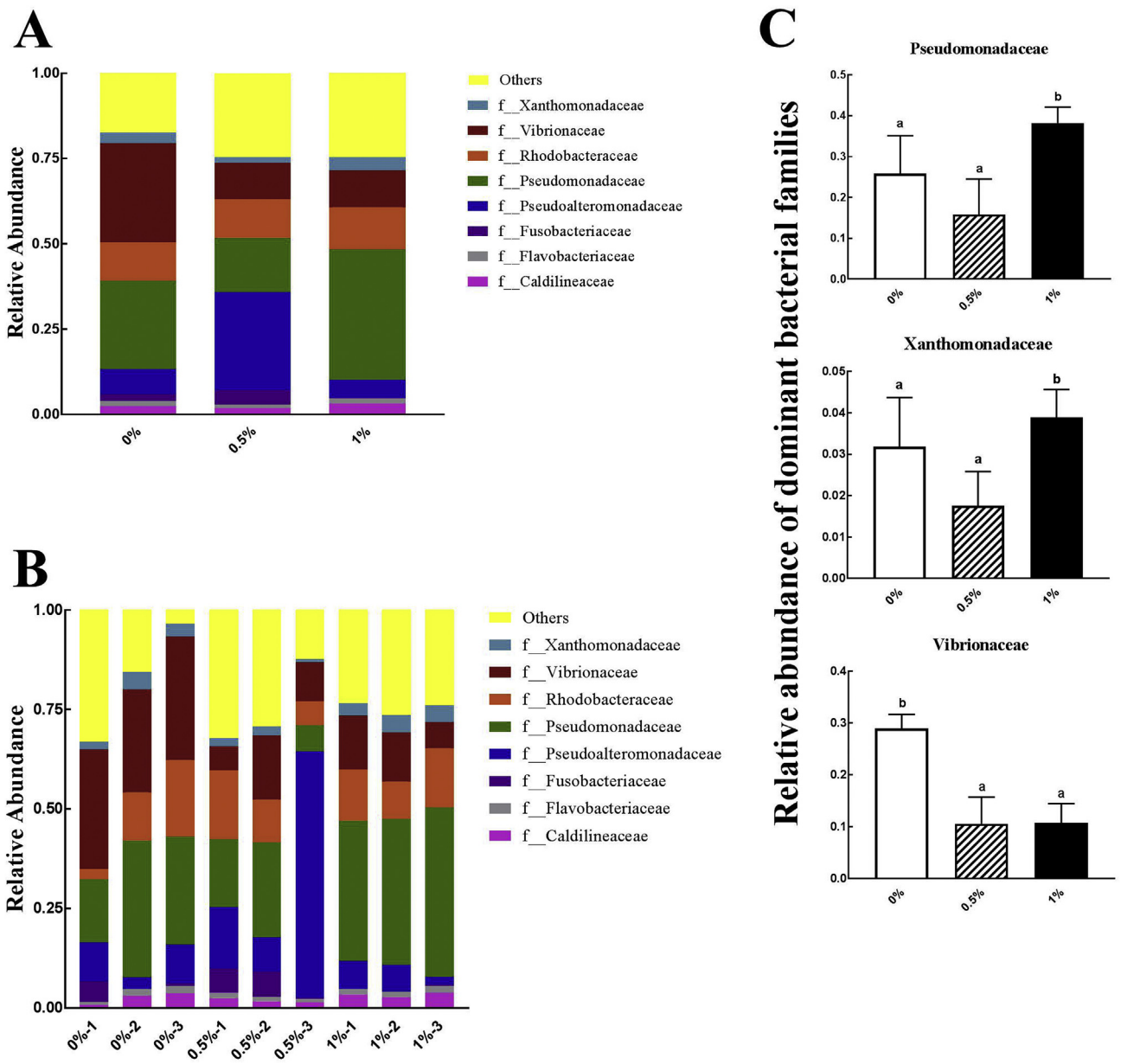

Fig. 5. Structure and composition of the intestinal bacterial communities in different level polypeptides fed shrimps on family level of taxonomy. (A). Means representing as three groups, (B) appearing in each sample and (C) the changes in abundance of dominant bacterial families. Dissimilar letters show significant difference ($p < 0.05$).

which could describe 19.28% of total variation.

4. Discussion

The extensive use of antibiotics in aquatic animals has serious consequences, such as enhancing the resistance of pathogens to antibiotics, seriously polluting the environment, being harmful to human health, and so on. AMPs, the most suitable substitute for antibiotics, have good prospects for application in aquaculture. However, there is still a long way to go. The utilization of antimicrobial peptides is few because of high production cost, and the mechanism of action of AMPs remains unclear. In this paper, the effect of Polypeptide S100 whose main components are AMPs on growth performance, antibacterial immune and intestinal microbiota structure of *Litopenaeus vannamei* were studied, aiming to explore the mechanism of action of AMPs in shrimp.

From the current research results, AMPs were considered to play an important role in promoting the growth and development and enhancing the immunity of aquatic animals. Teleost AMPs have been considered as modulators of the innate immune system. The regulation of AMP expression by exogenous factors is useful in fish to promote growth and prevent disease, particularly in aquaculture settings where crowded conditions and environmental stress pre-dispose these fish to infection [31]. For the invertebrates, non-specific immunity is the main defensive means. Thus, as one of the most important immune effectors, AMPs is an important defense line of invertebrates, including shrimp, which is a common species in aquaculture [32]. Destoumieux et al. found that the complete structure of the shrimp AMPs Penaeidins can adhere to the chitin phase, which allows Penaeidins to participate in the chitin polymerization and wound healing process, and the antibacterial activity of the AMPs can provide the body protection in the molting cycle of the shrimps [9]. Recently, the potential use of ALFPm3 in shrimp aquaculture disease control has been reported. Administration of dietary rALFPm3 as a potential immunostimulant enhanced the transcription level of immune-related genes and increased the shrimp

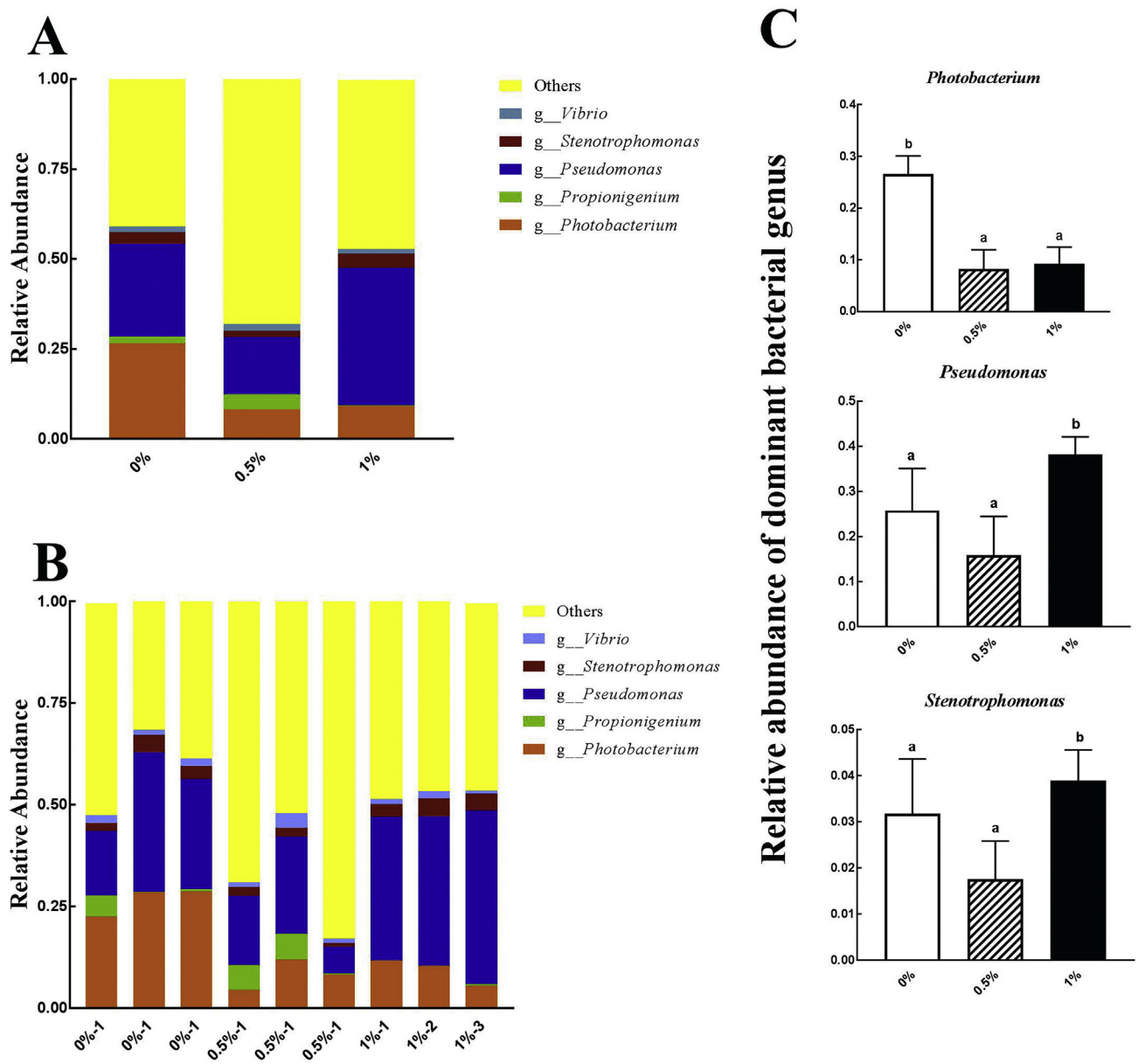

Fig. 6. Structure and composition of the intestinal bacterial communities in different level polypeptides fed shrimps on genus level of taxonomy. (A). Means representing as three groups, (B) appearing in each sample and (C) the changes in abundance of dominant bacterial genera. Dissimilar letters show significant difference ($p < 0.05$).

survival following the WSSV challenge [33]. The results of this study showed that the addition of Polypeptides S100 improved the growth of *L. vannamei*, and the effect was obvious when the added level reached to 1%. In addition, the ability of *L. vannamei* was promoted to defense *V. harveyi*. Consistently, the activities of two important immune enzymes T-SOD and LZM in shrimp were significantly higher in the Polypeptides S100-added groups than the control. However, the addition of Polypeptides S100 did not significantly impact the survival rates of *L. vannamei* when suffered *V. parahemolyticus* infection. This is probably due to the different species, sources and activities of the bacteria. In any case, all these results suggested that the addition of Polypeptides S100 had a positive effect on the growth and immunity of *L. vannamei*. As the main components of Polypeptides S100 are AMPs, it is natural to suppose AMPs plays a major role.

There are many kinds of AMPs and their antimicrobial mechanisms are different. Up to now, it is recognized that AMPs generally function through two pathways. One is that AMPs bind to specific receptors on the microbial cell membrane to change intracellular and extracellular osmotic pressure, resulting in cell death by the outflow of intracellular content. The other is that the AMPs pass through the cell membrane directly to play roles in cells, to inhibit biological processes such as DNA replication, RNA transcription and protein translation, and so on [34]. Nevertheless, little is known about the antimicrobial mechanism of AMPs. In this study, the activities of LPS and AMS, which are two common digestive enzymes, increased strikingly in the 0.5% Polypeptide S100 added group while decreased remarkably in the 1.0% Polypeptide S100 added group when compared with the control group. This indicated that the digestive capacity of *L. vannamei* could be regulated by adding appropriate amount of AMPs. Based on these results, we focus our study on the health of intestine, the vital digestive organ of shrimp which is also important for immunity [35]. Intestinal flora is the primary concern.

Intestinal flora has been considered to be an indicator of the health status of several animals, including pigs [36], chickens [37] and

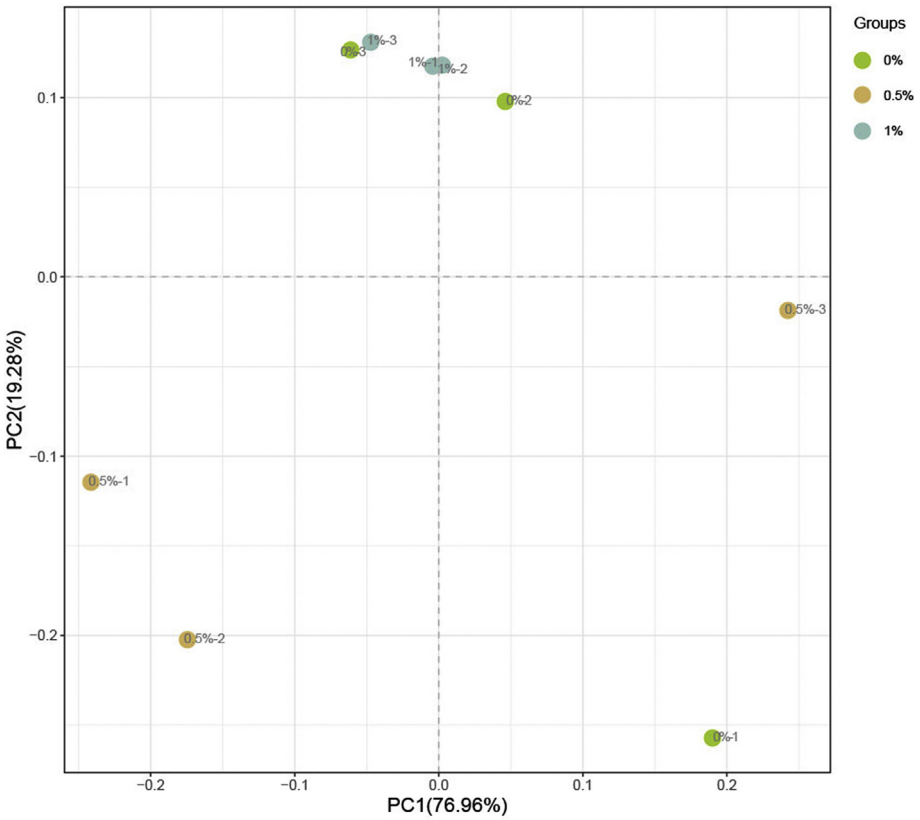

Fig. 7. Principal co-ordinate analysis (PCA), based on weighted-Unifrac distance, of the intestinal microbial communities of *L. vannamei* fed different polypeptide levels. Samples from the same group were clustered closer.

shrimps [38]. Illlumina high-throughput sequencing data revealed that the dominant phyla in *L. vannamei* was within Proteobacteria, followed by Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Fusobacteria and Tenericutes. Proteobacteria were the most prevalent members in each sample, which is consistent with previous studies in other aquatic animals, such as *Brachydanio rerio* [39], *Ctenopharyngodon idellus* [21], *L. vannamei* [29,40] and *P. monodon* [26]. After fed by Polypeptide S100, a clear shift in the microbial community structure was observed based on the PCoA plot, suggesting that AMPs may play an important role in shaping the gut microbiota of *L. vannamei*. *Vibrio* is one of the important pathogens in marine invertebrate, while *Pseudomonas* and *Stenotrophomonas* are the dominant bacteria in the intestine of healthy animals [32]. In this study, the addition of Polypeptide S100 obviously reduced *Photobacterium*, which also belongs to the Vibrionaceae family as *Vibrio*. Additionally, the relative abundance of *Pseudomonas* and *Stenotrophomonas* were significantly increased by 1.0% Polypeptide S100, which is consistent with the results of the researches by Yoon et al. [41] and Choi et al. [42]. In fact, 0.5% Polypeptide S100 reduced the amount of *Pseudomonas* and *Stenotrophomonas* in *L. vannamei*, but the difference was not significant. All these results showed that suitable addition amount of Polypeptide S100, actually AMPs, could improve the intestinal flora structure of shrimp.

In conclusion, the findings of the present study revealed that oral diet with AMPs could improve the growth performance and antibacterial immune which may via modulating the intestinal flora structure.

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