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Effects of Small Peptides on Growth, Body Composition, Non-Specific Immunity, and Disease Resistance of Juvenile Litopenaeus vannamei Postprint

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Abstract

This experiment aimed to investigate the effects of small peptides on growth, body composition, non-specific immunity, and disease resistance of juvenile Litopenaeus vannamei. Small peptides were added to the basal diet at 0 (control), 0.5%, 1.0%, 2.0%, 4.0%, and 6.0% to formulate six experimental diets designated as S0, S0.5, S1.0, S2.0, S4.0, and S6.0. Juvenile L. vannamei with an initial body weight of (0.19$±$0.01) g were randomly divided into six groups, with three replicates per group and 30 shrimp per replicate. The feeding trial lasted for 56 days. The results showed that the weight gain rate (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) of all small peptide-supplemented groups were significantly higher than those of the control group ($P<0.05$), while the feed conversion ratio (FCR) was significantly lower ($P<0.05$). No significant differences were observed in survival rate among all groups ($P>0.05$). In serum, phenoloxidase (PO) activity in S1.0 and S2.0 groups was significantly higher than in other groups ($P<0.05$); superoxide dismutase (SOD) activity in S0.5 and S1.0 groups was significantly higher than in the control group ($P<0.05$); acid phosphatase (ACP) activity in S1.0 and S2.0 groups was significantly higher than in the control group ($P<0.05$); no significant differences were observed in alkaline phosphatase (AKP) and lysozyme (LSZ) activities among all groups ($P>0.05$); malondialdehyde (MDA) content in S4.0 and S6.0 groups was significantly lower than in other groups ($P<0.05$). In hepatopancreas, LSZ activity in S0.5, S2.0, and S4.0 groups was significantly higher than in the control group ($P<0.05$), while in S6.0 group it was significantly lower than in the control group ($P<0.05$); no significant differences were observed in SOD activity among all groups ($P>0.05$); ACP activity was higher in S0.5 and S1.0 groups, but not significantly different from the control group

(P>0.05); AKP activity in S4.0 group was significantly lower than in the control group (P<0.05); MDA content in all small peptide-supplemented groups was significantly lower than in the control group (P<0.05), and S4.0 group was also significantly lower than S6.0 group (P<0.05). Through Vibrio harveyi challenge test, it was found that the cumulative mortality of juvenile L. vannamei in groups with small peptide supplementation of 0.5%-4.0% after 7 days of challenge was significantly lower than that of the control group (P<0.05), while the group with 6.0% small peptide supplementation showed no significant difference from the control group (P>0.05). In conclusion, dietary supplementation of 1.0%-2.0% small peptides could promote growth and enhance non-specific immunity and disease resistance of juvenile L. vannamei.

Full Text

Effects of Small Peptides on Growth, Body Composition, Non-Specific Immunity and Disease Resistance of Juvenile Litopenaeus vannamei

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Abstract

This study aimed to investigate the effects of small peptides on the growth, body composition, non-specific immunity, and disease resistance of juvenile *Litopenaeus vannamei*. Six experimental diets were formulated by supplementing the basal diet with 0 (control), 0.5%, 1.0%, 2.0%, 4.0%, and 6.0% small peptides, designated as S0, S0.5, S1.0, S2.0, S4.0, and S6.0, respectively. A total of 540 juvenile shrimp with an initial body weight of (0.19$±$0.01) g were randomly divided into six groups, each with three replicates of 30 individuals. The feeding trial lasted for 56 days. The results showed that the weight gain rate (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) of all small peptide-supplemented groups were significantly higher than those of the control group (P<0.05), while the feed conversion ratio (FCR) was significantly lower (P<0.05). No significant differences were observed in survival rate among all groups (P>0.05). In serum, phenoloxidase (PO) activity in the S1.0 and S2.0 groups was significantly higher than in other groups (P<0.05). Superoxide dismutase (SOD) activity in the S0.5 and S1.0 groups was significantly higher than in the control group (P<0.05). Acid phosphatase (ACP) activity in the S1.0

and S2.0 groups was significantly higher than in the control group (P<0.05). No significant differences were detected in alkaline phosphatase (AKP) or lysozyme (LSZ) activities among groups (P>0.05). Malondialdehyde (MDA) content in the S4.0 and S6.0 groups was significantly lower than in other groups (P<0.05). In hepatopancreas, LSZ activity in the S0.5, S2.0, and S4.0 groups was significantly higher than in the control group (P<0.05), whereas it was significantly lower in the S6.0 group (P<0.05). No significant differences were found in SOD activity among groups (P>0.05). ACP activity was higher in the S0.5 and S1.0 groups but did not differ significantly from the control group (P>0.05). AKP activity in the S4.0 group was significantly lower than in the control group (P<0.05). MDA content in all small peptide-supplemented groups was significantly lower than in the control group (P<0.05), with the S4.0 group also being significantly lower than the S6.0 group (P<0.05). The *Vibrio harveyi* challenge test revealed that the cumulative mortality rate after 7 days in groups supplemented with 0.5%–4.0% small peptides was significantly lower than in the control group (P<0.05), while the 6.0% supplementation group showed no significant difference from the control (P>0.05). In conclusion, dietary supplementation with 1.0%–2.0% small peptides can promote growth and enhance the non-specific immunity and disease resistance of juvenile *L. vannamei*.

Key words: small peptides; *Litopenaeus vannamei*; growth; non-specific immunity; disease resistance

Introduction

Litopenaeus vannamei, also known as the Pacific white shrimp or whiteleg shrimp, is native to Ecuador and inhabits tropical, subtropical, warm temperate, and temperate marine waters [1]. As one of the three major commercially cultured shrimp species with the highest global production, *L. vannamei* is characterized by rapid growth, strong disease resistance, significant economic benefits, high environmental adaptability, and excellent meat quality, making it the primary shrimp species cultured in South China. However, intensive aquaculture practices have led to environmental degradation and deteriorating water quality, while rising fishmeal prices have resulted in reduced fishmeal content and protein levels in feeds, severely affecting shrimp growth and causing frequent disease outbreaks. Consequently, research focused on promoting shrimp growth and enhancing immunity and disease resistance has become a critical area of interest.

Small peptides, also referred to as oligopeptides or short peptides, generally consist of two or more amino acids and can be derived from natural sources or through protein hydrolysis [2]. Research has demonstrated that the final digestive products of proteins in the gastrointestinal tract are predominantly small peptides rather than free amino acids, and these peptides can be absorbed intact and enter the bloodstream as di- and tripeptides [3]. The growth-promoting ef-

fects of small peptides as a high-quality protein source in aquatic animals have been well documented. Studies have shown that small peptides can enhance growth in *L. vannamei* [4], grass carp (*Ctenopharyngodon idellus*) [5], and juvenile Jian carp (*Cyprinus carpio* var. Jian) [6]. Building upon previous research on the growth-promoting effects of small peptides in *L. vannamei*, this study investigated the impact of dietary small peptide supplementation at varying levels on non-specific immunity and disease resistance, while simultaneously increasing dietary protein content. The findings aim to provide theoretical foundations and scientific references for the application of small peptides in aquafeeds.

Materials and Methods

1.1 Experimental Diets and Design

The basal diet was formulated using fishmeal, soybean meal, and peanut meal as protein sources, fish oil and lecithin oil as lipid sources, and supplemented with vitamin and mineral premixes. Six experimental diets were prepared by supplementing the basal diet with 0 (control), 0.5%, 1.0%, 2.0%, 4.0%, and 6.0% small peptides, designated as S0, S0.5, S1.0, S2.0, S4.0, and S6.0, respectively. The composition and nutrient levels of the experimental diets are presented in Table 1 . The small peptides used in this study were derived from microbial enzymatic hydrolysis of soybean meal protein, with a molecular weight $ $5,000 Da, provided by Shenzhen Yunong Biotechnology Co., Ltd. All dietary ingredients were ground to pass through an 80-mesh sieve, weighed accurately according to the formulation, and micro-ingredients were added using the stepwise dilution method. After thorough mixing with macro-ingredients, the diets were extruded into 1.0 mm and 1.5 mm pellets using a twin-screw pelletizer, conditioned at 60 °C for 30 min, air-dried, sealed in plastic bags, and stored at -20 °C until use.

1.2 Experimental Animals and Management

The feeding trial was conducted at the indoor aquaculture facility of the Donghai Island Marine Biology Research Base, Guangdong Ocean University. Juvenile *L. vannamei* shrimp were purchased from Zhanjiang Zhonglian Aquaculture Co., Ltd. and acclimated in outdoor cement tanks for three weeks prior to the experiment. A total of 540 healthy juvenile shrimp with uniform size and an initial body weight of $(0.19\$\pm0.01)gwererandomlyallocatedintosixgroups, eachconsistingofthreereplicatesof30individuals.Eachre$ fiberglass tank. The feeding trial lasted for 56 days. Shrimp were hand-fed to apparent satiation at 8%–10% of body weight, divided into four daily feedings at 07:00, 11:00, 17:00, and 21:00. Feed intake was monitored one hour after feeding, and rations were adjusted appropriately based on weather and water conditions. Water exchange was conducted every other day during the initial period and daily during the final two weeks of the trial, with 1/3 to 1/2 of the total water volume being replaced. Continuous aeration was maintained

throughout the experiment. Water quality parameters were monitored and maintained as follows: dissolved oxygen >6.7 mg/L, temperature 28.4–31.2 °C, salinity 26–28, pH 7.8–8.2, and ammonia nitrogen <0.03 mg/L.

1.3 Sample Collection

At the conclusion of the feeding trial, shrimp were fasted for 24 h before final counting and weighing by replicate. Five shrimp per replicate were randomly selected, blotted dry with absorbent paper, and stored at -20 °C for subsequent whole-body proximate composition analysis. An additional ten shrimp per replicate were randomly selected for hemolymph collection. Hemolymph was withdrawn from the pericardial cavity using a 1 mL sterile syringe, pooled from ten shrimp into a 1.5 mL centrifuge tube, and allowed to clot overnight at 4 °C. After centrifugation at 4,000 r/min for 10 min, the serum supernatant was collected, aliquoted, and stored at -80 °C for non-specific immune parameter analysis. Furthermore, 2–3 shrimp per replicate were randomly selected for hepatopancreas collection. The hepatopancreas was excised, blotted dry, and weighed accurately. A 1:9 (w/v) homogenate was prepared by adding nine volumes of physiological saline to the tissue, mincing, and homogenizing in an ice-water bath. The homogenate was centrifuged at 2,500–3,000 r/min for 10 min, and the supernatant was collected, aliquoted, and stored at -80 °C for hepatopancreas non-specific immune index determination.

1.4.1 Proximate Composition Analysis

Proximate composition analysis of diets and whole-body samples, including moisture, crude protein, crude lipid, crude ash, and total phosphorus, was performed according to AOAC (1995) methods [7]. Moisture content was determined by oven-drying at 105 °C to constant weight. Crude protein content was measured using the Kjeldahl method (Kjeltec TM8400, Sweden). Crude lipid content was analyzed via Soxhlet extraction using petroleum ether as the solvent. Crude ash content was determined by incineration in a muffle furnace at 550 °C. Total phosphorus content was measured using a colorimetric method.

1.4.2 Non-Specific Immune Index Determination

Serum and hepatopancreas lysozyme (LSZ) activity was measured using a turbidimetric assay. Superoxide dismutase (SOD) activity was determined using the WST-1 method. Malondialdehyde (MDA) content, acid phosphatase (ACP) activity, and alkaline phosphatase (AKP) activity were all measured using microplate methods. All these assays were performed using commercial kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s protocols. Serum phenoloxidase (PO) activity was determined according to the method described by Ashida [8].

1.5 *Vibrio harveyi* Challenge Test

The *Vibrio harveyi* strain used for the challenge test was provided by the Guangdong Provincial Key Laboratory of Aquatic Economic Animal Pathogen Biology and Epidemiology. At the end of the feeding trial, ten shrimp per replicate were randomly selected for the challenge test. A preliminary experiment determined that the 7-day median lethal dose (LD50) of *V. harveyi* for *L. vannamei* was 1.96$×10^{7}$ CFU/mL. Shrimp were injected with 30 L of this bacterial suspension into the dorsal region of the second to third abdominal segments. Mortality was recorded daily for 7 days post-challenge, and cumulative mortality rates were calculated.

1.6 Calculation Formulas

The following formulas were used for calculations: - Survival rate (SR, %) = 100 × (final number of shrimp) / (initial number of shrimp) - Weight gain rate (WGR, %) = 100 × (final mean weight - initial mean weight) / (initial mean weight) - Specific growth rate (SGR, %/d) = 100 × (ln final mean weight - ln initial mean weight) / feeding days - Protein efficiency ratio (PER) = (final body weight - initial body weight) / protein intake - Feed conversion ratio (FCR) = dry feed intake / (final body weight - initial body weight) - Cumulative mortality rate (CMR, %) = 100 × (cumulative number of dead shrimp) / (initial number of shrimp)

1.7 Data Processing and Analysis

All data are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 17.0 software. One-way analysis of variance (ANOVA) was used to evaluate differences among groups, and Duncan' s multiple range test was applied for post-hoc comparisons when significant differences were detected. Statistical significance was set at $P<0.05$.

Results

2.1 Effects of Small Peptides on Growth Performance

As shown in Table 2 , the final mean body weight of all small peptide-supplemented groups (S0.5, S1.0, S2.0, S4.0, and S6.0) was significantly higher than that of the control group (S0) ($P<0.05$). The weight gain rate, specific growth rate, and protein efficiency ratio of all supplemented groups were significantly higher than those of the control group ($P<0.05$), while the feed conversion ratio was significantly lower ($P<0.05$). No significant differences were observed in survival rate among all groups ($P>0.05$).

Table 2 Effects of small peptides on growth performance of juvenile *Litopenaeus vannamei* (n=3)

Note: Values with different letter superscripts in the same column differ significantly (P<0.05). The same applies to subsequent tables.

2.2 Effects on Body Composition

As presented in Table 3 , no significant differences were observed in whole-body moisture content among groups (P>0.05). Whole-body crude protein content in the S1.0, S2.0, S4.0, and S6.0 groups was significantly higher than in the control group (P<0.05), while the S0.5 group showed no significant difference from the control (P>0.05). Whole-body crude lipid content in the S2.0 and S4.0 groups did not differ significantly from the control group (P>0.05), but the S0.5, S1.0, and S6.0 groups were significantly higher than the control (P<0.05). Whole-body crude ash content in the S0.5 group was significantly lower than in the control group (P<0.05), whereas the S2.0, S4.0, and S6.0 groups were significantly higher (P<0.05); the S1.0 group showed no significant difference from the control (P>0.05). Whole-body total phosphorus content in the S0.5 and S4.0 groups was significantly lower than in the control group (P<0.05), with no significant differences observed in other groups (P>0.05).

Table 3 Effects of small peptides on body composition of juvenile *Litopenaeus vannamei* (DM basis) (n=3)

2.3 Effects on Serum Non-Specific Immune Indexes

As shown in Table 4 , no significant differences were observed in serum LSZ activity among groups (P>0.05). Serum PO activity exhibited a trend of initial increase followed by decrease with increasing small peptide supplementation, peaking in the S2.0 group, which along with the S1.0 group, was significantly higher than other groups (P<0.05). SOD activity in the S0.5 and S1.0 groups was significantly higher than in the control and S4.0 groups (P<0.05), with the S0.5 group also being significantly higher than the S2.0 group (P<0.05); no significant differences were detected among remaining groups (P>0.05). ACP activity in the S1.0 and S2.0 groups was significantly higher than in other groups (P<0.05). No significant differences were found in AKP activity among groups (P>0.05), although all supplemented groups showed numerically higher values than the control. MDA content in the S4.0 and S6.0 groups was significantly lower than in other groups (P<0.05), with the S4.0 group being significantly lower than the S6.0 group (P<0.05).

Table 4 Effects of small peptides on serum non-specific immune indexes of juvenile *Litopenaeus vannamei* (n=3)

2.4 Effects on Hepatopancreas Non-Specific Immune Indexes

As indicated in Table 5 , hepatopancreas LSZ activity in the S0.5, S2.0, and S4.0 groups was significantly higher than in the control group (P<0.05), while the S6.0 group showed significantly lower activity (P<0.05). No significant differences were observed in SOD activity among groups (P>0.05). ACP activity

did not differ significantly between small peptide-supplemented groups and the control group (P>0.05); however, the S0.5 and S1.0 groups were significantly higher than the S2.0 and S4.0 groups (P<0.05). AKP activity in the S4.0 group was significantly lower than in the control group (P<0.05), with no significant differences among other groups (P>0.05). MDA content in all small peptide-supplemented groups was significantly lower than in the control group (P<0.05), and the S4.0 group was also significantly lower than the S6.0 group (P<0.05).

Table 5 Effects of small peptides on hepatopancreas non-specific immune indexes of juvenile *Litopenaeus vannamei* (n=3)

2.5 Effects on Cumulative Mortality After *Vibrio harveyi* Challenge

The trend of cumulative mortality in juvenile *L. vannamei* after *Vibrio harveyi* challenge is illustrated in Figure 1 [Figure 1: see original paper]. Figure 2 [Figure 2: see original paper] demonstrates that the 7-day cumulative mortality rate after *V. harveyi* challenge was significantly lower in groups supplemented with 0.5%, 1.0%, 2.0%, and 4.0% small peptides compared to the control group (P<0.05). However, the 6.0% supplementation group showed no significant difference from the control group (P>0.05).

Discussion

3.1 Effects on Growth and Body Composition

Small peptides, composed of two or more amino acids, can be naturally occurring or produced through protein hydrolysis. As the primary digestive products of proteins, small peptides play a crucial role in amino acid digestion, absorption, and animal nutrient metabolism. In aquaculture, appropriate dietary supplementation of small peptides can enhance immunity, improve survival and weight gain rates, and increase feed utilization efficiency. The present results demonstrate that dietary small peptide supplementation promotes growth in juvenile *L. vannamei*, which is consistent with the findings of Lin et al. [4]. Additionally, Tetshima et al. [9] reported significant growth-promoting effects of small peptides in shrimp larvae. Studies on grass carp by Yu et al. [10] and Feng et al. [5] revealed that small peptides can improve feed digestibility and promote growth. Similar growth-enhancing effects have been observed in juvenile Jian carp [6] and European eel (*Anguilla anguilla*) [11].

Both small peptides and free amino acids are products of protein enzymatic hydrolysis, but their absorption involves two distinct and independent transport mechanisms [12]. Free amino acids are actively transported against a concentration gradient by intestinal cells via different sodium ion (Na^+) transport systems [13]. In contrast, small peptides are absorbed intact across the intestinal wall and transported directly into the bloodstream. The intestinal mucosa possesses

specific peptide transporters that offer advantages over free amino acid absorption, including faster transport rates, lower energy consumption, and reduced susceptibility to saturation [14]. Research indicates that after direct absorption, small peptides can participate in physiological activities and metabolic regulation, enhance protein deposition, and thereby promote growth performance.

The current results show that dietary small peptide supplementation significantly affected whole-body crude protein, crude lipid, crude ash, and total phosphorus contents in juvenile *L. vannamei*, but had no significant impact on moisture content. Previous studies on fish have reported different effects of small peptide supplementation on body composition. For instance, research on Siberian sturgeon (*Acipenser baerii* Brandt) found that replacing fishmeal with small peptides had no significant effects on whole-body moisture, crude protein, crude lipid, or crude ash contents [15]. Similar findings were reported in studies on gibel carp (*Carassius auratus gibelio*) [16]. These discrepancies with our results may be attributed to species-specific differences in the capacity of small peptides to influence nutrient deposition.

3.2 Effects on Non-Specific Immunity and Disease Resistance

Small peptides are bioactive compounds with relatively low molecular weight and flexible structures that exhibit diverse biological functions. These protein hydrolysate-derived peptides possess certain immunological properties, making them important immunostimulants that can enhance non-specific immune enzyme activities in aquatic animals.

Lysozyme (LSZ) is a crucial non-specific immune factor in shrimp that participates in various immune responses, improves and enhances macrophage phagocytic capacity and digestive function, and thereby contributes to increased growth and survival rates [17]. The present results indicate that although serum LSZ activity in small peptide-supplemented groups was not significantly different from the control group, all supplemented groups showed numerically higher values. Dietary supplementation with 0.5%–4.0% small peptides significantly increased hepatopancreas LSZ activity in *L. vannamei*. Xu et al. [17] previously reported that dietary supplementation with 1.5% small peptides could enhance LSZ activity in muscle and cephalothorax of *L. vannamei*.

Superoxide dismutase (SOD) is a critical enzyme system for scavenging oxygen free radicals [18] and plays an important role in maintaining the oxidative-antioxidant balance in organisms [19-20]. Its activity level indirectly reflects the capacity to eliminate oxygen free radicals. The current results demonstrate that dietary supplementation with 0.5%–1.0% small peptides significantly enhanced serum SOD activity in juvenile *L. vannamei*, though no significant effects were observed on hepatopancreas SOD activity.

Phenoloxidase (PO) is a defense enzyme involved in immune recognition of foreign substances and reflects the immune status of the organism. In crustaceans, PO exists as a proenzyme that is activated upon pathogen invasion or environ-

mental stress to initiate immune recognition and defense [21]. In this study, serum PO activity initially increased and then decreased with increasing small peptide supplementation, with the S1.0 and S2.0 groups showing significantly higher activity than other groups. Wang et al. [22] reported that small peptide supplementation could affect the humoral immunity of *L. vannamei* and significantly increase serum PO activity, which aligns with our findings.

Acid phosphatase (ACP) and alkaline phosphatase (AKP) are important immune-functional enzymes in *L. vannamei* that can directly kill invading pathogens and facilitate their further hydrolysis and digestion, playing a crucial role in the immune response against pathogens [22]. The present results indicate that dietary supplementation with 1.0%–2.0% small peptides enhanced serum ACP activity in juvenile *L. vannamei*.

Malondialdehyde (MDA) is the end product of lipid peroxidation induced by free radicals in organisms [23-24] and can cause cross-linking and polymerization of vital macromolecules such as proteins and nucleic acids, exhibiting cytotoxicity. Therefore, reducing MDA content decreases cellular damage. The current results show that dietary small peptide supplementation decreased MDA content in both serum and hepatopancreas of juvenile *L. vannamei*, possibly due to the protective effects of small peptides on cells, thereby improving growth performance and post-challenge survival. Previous studies have reported that dietary glutathione supplementation can reduce MDA content in the hepatopancreas of *L. vannamei* [18]. Additionally, Wang et al. [25] demonstrated that appropriate dietary small peptide supplementation effectively enhanced digestive enzyme activities in the gastrointestinal tract of juvenile starry flounder (*Platichthys stellatus*). Other research has also shown that dietary small peptides can reduce protein requirements, promote lipid metabolism, and increase digestive enzyme activities in the gastrointestinal tract of red spotted grouper (*Epinephelus akaara*) [26].

Artificial challenge tests are effective methods for evaluating non-specific immunity and disease resistance in shrimp [27]. *Vibrio harveyi* is a Gram-negative luminous bacterium widely distributed in marine environments that causes luminous disease and hepatopancreatic necrosis, ultimately leading to mortality in *L. vannamei* [28]. In this study, the *V. harveyi* challenge test revealed that supplementation with 0.5%–4.0% small peptides significantly reduced the 7-day cumulative mortality rate. These findings indicate that appropriate dietary small peptide supplementation enhances the non-specific immunity and disease resistance of *L. vannamei*, which is consistent with the results reported by Lin et al. [4].

Conclusion

Dietary supplementation with 1.0%–2.0% small peptides can promote growth and enhance the non-specific immunity and disease resistance of juvenile *Litope-*

naeus vannamei.

References

[1] Li Yuhu, Song Qinqin, Zhang Zhihuai, et al. Study on growth and development patterns and growth curve fitting of *Litopenaeus vannamei*[J]. South China Fisheries Science, 2015, 11(1): 89-95.
[2] Xiang Xiao, Zhou Xinghua, Tang Longbi. Nutrition of small peptides and their application in aquaculture[J]. Shandong Feed, 2002(8): 11-14.
[3] INFANTE J L Z, CAHU C L, CAHU A. Partial substitution of di-and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development[J]. The Journal of Nutrition, 1997, 127(4): 608-614.
[4] Lin Qicun, Fang Changfu, Zhong Guofang, et al. Effects of small peptides on growth performance and non-specific immunity of *Litopenaeus vannamei* larvae[J]. Acta Agriculturae Zhejiangensis, 2010, 22(5): 590-595.
[5] Feng Jian, Liu Donghui. Effects of small peptides in diet on growth performance of juvenile grass carp[J]. Acta Hydrobiologica Sinica, 2005, 29(1): 20-25.
[6] Gan Hui. Effects of small peptide nutrition on growth of juvenile Jian carp[J]. Feed Industry, 2005, 26(22): 30-32.
[7] AOAC. Official methods of analysis of AOAC International[S]. 16th ed. Arlington, Virginia: AOAC International, 1995.
[8] ASHIDA M. Purification and characterization of pre-phenoloxidase from the hemolymph of the silkworm *Bombyx mori*[J]. Archives of Biochemistry and Biophysics, 1971, 144(2): 749-762.
[9] TETSHIMA S I, KANAZAWA A, KOSHIO S. Recent developments in nutrition and microparticulate diets of larval prawns[J]. The Israeli Journal of Aquaculture-Bamidgh, 1993, 171(1/2): 109-119.
[10] Yu Hui, Feng Jian, Liu Donghui, et al. Effects of casein small peptides on growth and feed utilization of juvenile grass carp[J]. Acta Hydrobiologica Sinica, 2004, 28(5): 526-530.
[11] Wang Bilian, Xu Jiarui, Qian Xueqiao. Effects of small peptide products on growth characteristics of European eel[J]. Freshwater Fisheries, 2001, 31(2): 42-43.
[12] Yuan Shulin, Chen Haiyan, Wang Xiaoyan, et al. Research progress on small peptide nutrition[J]. Cereal and Feed Industry, 2002(8): 37-39.
[13] MATTHEWS D M. Intestinal absorption of peptides[J]. Physiological Reviews, 1980, 24: 734.
[14] Chen Bo, Qin Qingyu, Yang Jinbao. Current status of small peptide application in aquatic animal nutrition[J]. Aquaculture Technology Advisor, 2007(9): 111-113.
[15] Wang Chang' an, Xu Qiyou, Xu Hong, et al. Effects of replacing fishmeal with small peptides on growth and blood biochemical indices of Siberian sturgeon[J]. Journal of the Chinese Cereals and Oils Association, 2010, 25(8): 55-58, 64.

[16] Yu Yebing, Wang Kuanhua, Xu Hangfeng. Effects of replacing fishmeal with small peptides on production performance and body composition of gibel carp[J]. Journal of Anhui Agricultural Sciences, 2008, 36(36): 15925–15927.
[17] Zhang Haibo, Tan Hongxin, Wang Xingqiang, et al. Expression and activity detection of lysozyme gene from *Litopenaeus vannamei* in *Escherichia coli*[J]. Marine Sciences, 2009, 33(1): 48–53.
[18] Xu Peiyu, Zhou Hongqi. Effects of small peptide products on growth and non-specific immunity of *Penaeus vannamei*[J]. China Feed, 2004(17): 13-15.
[19] Liu Xiaohua, Cao Junming, Wu Jiankai, et al. Effects of dietary glutathione supplementation on antioxidant indices and lipid peroxide content in hepatopancreas of *Litopenaeus vannamei*[J]. Journal of Fisheries of China, 2007, 31(2): 235–240.
[20] VINCENZINI M T, IANTOMASI T, FAVILLI F. Glutathione transport across intestinal brush-border membranes: effects of ions, pH, $\Delta\Psi$, and inhibitors[J]. Biochimica Biophysica Acta: Biomembranes, 1989, 987(1): 29–37.
[21] Yang Liubing, Pan Luqing. Effects of phosphatidylserine injection on hemocyanin synthesis and phenoloxidase activity in *Litopenaeus vannamei*[J]. Journal of Fisheries of China, 2013, 37(9): 1378–1388.
[22] Wang Xiuhua, Song Xiaoling, Huang Jie. Effects of peptidoglycan preparation on humoral immune factors of *Penaeus vannamei*[J]. Journal of Fishery Sciences of China, 2004, 11(1): 26–30.
[23] Liu Shuqing, Jiang Xiaolu, Mou Haijin, et al. Effects of immunopolysaccharide on serum lysozyme, phosphatase and peroxidase in *Penaeus chinensis*[J]. Oceanologia et Limnologia Sinica, 1999, 30(3): 278–283.
[24] TRENZADO C, HIDALGO M C, GARCÍA-GALLEGO M, et al. Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss*. A comparative study[J]. Aquaculture, 2006, 254(1-4): 758–767.
[25] Wang Jiying, Jiang Kejun, Xia Bin, et al. Effects of small peptides on digestive enzyme activities, antioxidant capacity and biochemical composition of juvenile starry flounder[J]. Journal of Fishery Sciences of China, 2014, 21(6): 1154–1164.
[26] HOCHACHKA P W, SOMERO G N. Biochemical adaptation: mechanism and process in physiological evolution[M]. Oxford: Oxford University Press, 2002.
[27] Zhao Shuyan, Lin Heizhuo, Huang Zhong, et al. Effects of small peptide supplementation at different protein levels on growth, digestive enzymes, serum biochemistry and antioxidant capacity of grouper[J]. South China Fisheries Science, 2016, 12(3): 15–23.
[28] Chen Naisong, Wei Taotao, Liao Yizhao. Effects of maggot meal and β-glucan on growth and immunity of *Litopenaeus vannamei*[J]. Journal of Fisheries of China, 2007, 31(6): 771–777.
[29] Liu Wen, Qian Dong, Yang Guoliang, et al. Study on the pathogen of luminous disease during desalination of *Penaeus vannamei* postlarvae[J]. Journal of Jimei University (Natural Science Edition), 2004, 9(4): 300–304.

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