Effects of Yeast Hydrolysate on Growth Performance, Intestinal Morphology, and Serum Non-Specific Immune Enzyme Activities in Litopenaeus vannamei (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of yeast hydrolysate on growth performance, intestinal morphology, and serum non-specific immune enzyme activities of Litopenaeus vannamei. A total of 640 juvenile L. vannamei with initial body weight of (0.41$±$0.01) g were randomly allocated into 4 groups (4 replicates per group, 40 shrimp per replicate). The supplementation levels of yeast hydrolysate were 0 (control), 0.5%, 1.0%, and 2.0%, and four isonitrogenous (42% crude protein) and isolipidic (8% crude lipid) experimental diets were formulated. An 8-week feeding trial was conducted. The results showed: 1) No significant differences were observed in survival rate, weight gain rate, or specific growth rate between any supplementation group and the control group (P>0.05). The weight gain rate and specific growth rate of the 1.0% group were significantly higher than those of the 2.0% group (P<0.05), while the feed conversion ratio was significantly lower than that of the 2.0% group (P<0.05). The hepatosomatic index of the 1.0% group was significantly higher than that of the control group (P<0.05), whereas no significant differences in condition factor were detected among all groups (P>0.05). 2) Dietary supplementation with different levels of yeast hydrolysate exerted no significant effects on the contents of dry matter, crude protein, crude lipid, or crude ash in whole shrimp or muscle (P>0.05). 3) Yeast hydrolysate had no significant effects on intestinal villus width, microvillus height, or epithelial cell height (P>0.05). However, the intestinal villus height of the 2.0% group was significantly higher than that of the 0.5% group (P<0.05). 4) Compared with the control group, supplementation with 1.0% yeast hydrolysate significantly increased serum phenoloxidase (PO) and lysozyme (LZM) activities (P<0.05), and nitric oxide synthase (NOS) activity also reached its maximum

value in the 1.0% group. In conclusion, dietary supplementation with 1.0% yeast hydrolysate can enhance serum non-specific immune enzyme activities in L. vannamei without exerting negative effects on growth performance and intestinal morphological health.

Full Text

Effects of Yeast Hydrolysate on Growth Performance, Intestinal Morphology and Serum Nonspecific Immune Enzyme Activities of Litopenaeus vannamei

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Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different levels of yeast hydrolysate on growth performance, intestinal morphology and serum nonspecific immune enzyme activities of *Litopenaeus vannamei*. A total of 640 juvenile shrimp with an initial body weight of (0.41 ± 0.01) g were randomly divided into four groups (four replicates per group, 40 shrimp per replicate). Four isonitrogenous and isolipidic experimental diets (42% crude protein and 8% crude lipid) were formulated with yeast hydrolysate supplementation levels of 0 (control), 0.5%, 1.0% and 2.0%. The feeding trial lasted for 8 weeks. The results showed: 1) No significant differences were observed in survival rate, weight gain rate or specific growth rate between the supplemented groups and the control group ($P > 0.05$). The 1.0% supplementation group exhibited significantly higher weight gain rate and specific growth rate compared with the 2.0% group ($P < 0.05$), while showing significantly lower feed conversion ratio ($P < 0.05$). Hepatosomatic index in the 1.0% group was significantly higher than that in the control group ($P < 0.05$), though no significant differences were found in condition factor among all groups ($P > 0.05$). 2) Dietary yeast hydrolysate supplementation had no significant effects on dry matter, crude protein, crude lipid or ash contents in whole body or muscle of shrimp ($P > 0.05$). 3) Fold width, microvillus height and enterocyte height of the intestine were not significantly affected by yeast hydrolysate supplementation ($P > 0.05$). However, intestinal fold height in the 2.0% group was significantly higher than that in the 0.5% group ($P < 0.05$). 4) Compared with the control group, dietary supplementation of 1.0% yeast hydrolysate significantly increased serum phenoloxidase (PO) and lysozyme (LZM) activities ($P < 0.05$), while nitric oxide synthase (NOS) activity also reached its maximum in the 1.0% group. In conclusion, dietary supplementation with 1.0% yeast hydrolysate can enhance

serum nonspecific immune enzyme activities in *L. vannamei* without negatively affecting growth performance or intestinal morphological health.

Key words: yeast hydrolysate; *Litopenaeus vannamei*; growth performance; intestinal morphology; immune enzyme

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Litopenaeus vannamei, commonly known as Pacific white shrimp, is native to warm coastal waters of the South Pacific and represents a commercially valuable aquaculture species. Valued for its nutritional quality and delicate flavor, it has gained widespread consumer acceptance. However, expanded farming operations and severe water pollution have compromised shrimp disease resistance, increasing infection rates and mortality [1]. This has led to the indiscriminate use of antibiotics and chemicals during cultivation, raising concerns about drug resistance, antibiotic residues and environmental water pollution [2]. Consequently, identifying antibiotic alternatives for *L. vannamei* aquaculture has become critically important and has attracted increasing research attention. Yeast hydrolysate, as a novel feed additive, has emerged as one of the effective antibiotic substitutes.

Yeast hydrolysate is produced from natural yeast strains through purification, autolysis or enzymatic hydrolysis, and spray drying. Its rich content of functional nutrients and immune components makes it valuable for feed applications. Research has shown that yeast cells are nutritionally rich, with a unique cell wall structure that releases immune-active substances such as β-glucans, nucleic acids, nucleotides and mannan oligosaccharides (MOS), along with unknown "growth factors" [3] when broken down. These components can improve growth performance, immune function and intestinal health in various animals including piglets [4], dairy cows [5], Japanese seabass (*Lateolabrax japonicus*) [6], common carp (*Cyprinus carpio*) [7], channel catfish (*Ictalurus punctatus*) [8], rainbow trout (*Oncorhynchus mykiss*) [9], gilthead seabream (*Sparus aurata* L) [10], giant freshwater prawn (*Macrobrachium rosenbergii*) [11], black tiger shrimp (*Penaeus monodon*) [12] and *L. vannamei* [13]. This study aimed to investigate the effects of dietary yeast hydrolysate supplementation on growth performance, intestinal morphology and serum nonspecific immune enzyme activities of *L. vannamei*, providing theoretical support for its application in formulated feeds and promoting sustainable development of *L. vannamei* aquaculture.

1.1 Feed Formulation and Preparation

Yeast hydrolysate was provided by Guangdong Hinabiotech Co., Ltd. with the following nutritional composition: crude protein 56.5%, crude lipid 0.50%, crude ash 7.10%, moisture 4.38%, amino nitrogen 2.32%, mannan oligosaccharides 6.75%, nucleic acids 14.0% and nucleotides 5.12%.

Four isonitrogenous and isolipidic experimental diets were formulated by supplementing the basal diet with 0 (control), 0.5%, 1.0% and 2.0% yeast hydrolysate.

The composition and nutrient levels of the experimental diets are presented in Table 1 . All solid ingredients were ground to pass through an 80-mesh sieve, weighed accurately according to the formula proportions, and mixed thoroughly. Vitamins and minerals were premixed using the progressive enlargement method. The mixture was processed into 1.0–1.5 mm pellets using a twin-screw extruder, cooked at 90°C for 30 minutes, air-dried, sealed and stored at -20°C until use.

Table 1 Composition and nutrient levels of experimental diets (air-dry basis) %

Items	Dietary yeast hydrolysate level/%			
Ingredients	**0**	**0.5**	**1.0**	**2.0**
Fish meal (Peru)	20.00	20.00	20.00	20.00
Local fish meal	8.00	8.00	8.00	8.00
Peanut meal	12.00	12.00	12.00	12.00
Soybean meal	22.00	22.00	22.00	22.00
Squid soluble paste	3.00	3.00	3.00	3.00
Wheat flour	22.00	22.00	22.00	22.00
Shrimp shell meal	3.00	3.00	3.00	3.00
Fish oil	2.00	2.00	2.00	2.00
Soybean lecithin oil	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50
Vitamin premix[1]	1.00	1.00	1.00	1.00
Mineral premix[2]	1.00	1.00	1.00	1.00
Cellulose	3.50	3.00	2.50	1.50
Yeast hydrolysate	0.00	0.50	1.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**
Nutrient levels[3]				
Dry matter	91.52	91.48	91.45	91.41
Crude protein	42.15	42.21	42.18	42.23
Crude lipid	8.05	8.02	8.04	8.01
Ash	10.52	10.48	10.51	10.49

[1] One kg of vitamin premix contained: VA 5,000 IU, VB_1 60 g, VB_2 50 g, VB_{12} 0.1 g, VD_3 2,000 IU, VE 100 IU, VK 60 g, inositol 200 g, nicotinic acid 100 g, biotin 6 g, folic acid 10 g, VC phosphate 0.3 g, pyridoxine 60 g.
[2] One kg of mineral premix contained: $C_{10}H_{22}N_2O_4S_2Co$ 5.0 g, $CuSO_4 \cdot 5H_2O$ 10.2041 g, KCl (99.5%) 191.62 g, $FeC_6H_5O_7 \cdot 5H_2O$ 6.8571 g, $MnSO_4 \cdot H_2O$ 6.2893 g, KI (0.99%) 1.7034 g, NaCl (99.5%) 76.69 g, $MgSO_4 \cdot 7H_2O$ (99%) 614.48 g, $C_6H_{10}CaO_6 \cdot 5H_2O$ (99.5%) 77.83 g, $ZnSO_4 \cdot 7H_2O$ 9.2754 g, Na_2SeO_3 0.5 g.
[3] Nutrient levels were measured values.

1.2 Experimental Animals and Culture Management

Juvenile *L. vannamei* shrimp were purchased from Guangdong Evergreen Group Shrimp Hatchery and cultured at the Guangdong Evergreen 863 Base for 8 weeks. Prior to the experiment, shrimp were acclimated in 1,000 L fiberglass tanks for 2 weeks and fed a commercial diet (42% crude protein, 8% crude lipid) to satiation. Feeding was stopped 24 hours before the trial began. A total of 640 healthy juvenile shrimp with uniform size and an initial body weight of (0.40 ± 0.01) g were selected and randomly distributed into 16 fiberglass tanks (300 L each) at a density of 40 shrimp per tank, with four replicates per dietary treatment. Feeding rate was adjusted according to growth stage at 8–10% of body weight, administered four times daily at 07:00, 11:00, 17:00 and 21:00. Morning and evening feedings accounted for 60–70% of total daily ration, with amounts adjusted based on daily feeding behavior and weather conditions. The experiment lasted 8 weeks. Seawater used in the trial was sand-filtered and settled. Water exchange was 100 L every two days during the early stage and 200 L daily during the middle and later stages. Continuous aeration was provided. Water temperature, salinity, dissolved oxygen and pH were recorded daily. During the experimental period, water temperature ranged 26–30°C, pH 8.0–8.2, salinity 28–32, dissolved oxygen >6 mg/L, and ammonia nitrogen $ $0.05 mg/L.

1.3 Sample Collection and Analysis

At the end of the feeding trial, shrimp were fasted for 24 hours before counting and weighing to calculate survival rate, weight gain rate and specific growth rate. Five shrimp per tank were individually measured for body length and weight, then dissected to obtain hepatopancreas weight for calculation of hepatosomatic index and condition factor. Eight shrimp per tank were randomly sampled (four whole shrimp and four muscle samples) and stored at -20°C for proximate composition analysis. Four shrimp per tank were selected for intestinal morphology analysis, with whole intestines fixed in 2 mL centrifuge tubes containing 4% paraformaldehyde. Hemolymph was collected from 10 shrimp per tank via the base of the fifth pereiopod, placed in 1.5 mL tubes, stored overnight at 4°C, then centrifuged at 5,000 r/min for 10 minutes. The supernatant was aliquoted into PCR tubes and stored at -80°C for serum nonspecific immune enzyme activity analysis.

Proximate composition of diets, whole shrimp and muscle samples was determined according to AOAC (1995) [14]: dry matter by oven drying at 105°C, crude protein using a protein analyzer (Leco, FB-528), crude lipid using a fat analyzer (OPSIS, SX-360), and ash using a muffle furnace at 550°C. Intestinal tissue sections were prepared, stained with hematoxylin-eosin (HE), mounted with neutral balsam, and observed under an optical microscope (Olympus, DP-72) to measure fold height, fold width, microvillus height and enterocyte height. Serum phenoloxidase (PO), nitric oxide synthase (NOS) and lysozyme (LZM) activities were determined using assay kits from Nanjing Jiancheng Bioengi-

neering Institute with a full-wavelength microplate reader (Thermo, Multiskan GO-1510).

1.4 Calculation Formulas

Survival rate (%) = 100 × (final shrimp number)/(initial shrimp number)
Weight gain rate (%) = 100 × (final average weight - initial average weight)/(initial average weight)
Specific growth rate (%/d) = 100 × [ln(final average weight) - ln(initial average weight)]/experimental days
Feed conversion ratio = dry weight of feed consumed/(final total weight - initial total weight)
Protein efficiency = (final body weight - initial body weight)/total protein intake
Hepatosomatic index (%) = 100 × hepatopancreas weight/shrimp body weight
Condition factor (g/cm^3) = 100 × body weight/$body\ length^3$

1.5 Statistical Analysis

Experimental data are expressed as mean ± standard error (X ± SE). Statistical analysis was performed using SPSS 17.0 software with one-way ANOVA. Differences were considered significant at $P < 0.05$. When significant differences were detected among treatments, Turkey' s multiple comparison test was applied.

2.1 Effects of Dietary Yeast Hydrolysate on Growth Performance and Feed Utilization of L. vannamei

As shown in Table 2 , no significant differences were observed in survival rate, weight gain rate or specific growth rate between supplemented groups and the control group ($P > 0.05$). Although survival rates did not differ significantly among groups ($P > 0.05$), survival showed a trend of increasing then decreasing with higher yeast hydrolysate levels, reaching a maximum of 90.00% in the 0.5% group. The 1.0% supplementation group exhibited significantly higher weight gain rate and specific growth rate compared with the 2.0% group ($P < 0.05$), while showing significantly lower feed conversion ratio ($P < 0.05$). Protein efficiency in the 1.0% group was slightly higher than other groups, though not significantly different ($P > 0.05$). Hepatosomatic index in the 1.0% group was significantly higher than that in the control group ($P < 0.05$), while condition factor was not significantly affected among all groups ($P > 0.05$).

Table 2 Effects of dietary supplementation of yeast hydrolysate on growth performance and feed utilization of *L. vannamei* (n = 4)

Items	Dietary yeast hydrolysate level/%			
	0	0.5	1.0	2.0

Items	Dietary yeast hydrolysate level/%			
Final average weight/g	7.75 ± 0.20	7.42 ± 0.10	7.83 ± 0.05	7.34 ± 0.09
Survival rate/%	86.25 ± 2.17	90.00 ± 3.65	88.28 ± 1.06	83.44 ± 1.06
Weight gain rate/%	1,786.13 ± 47.73ab	1,703.59 ± 22.93ab	1,798.19 ± 13.28b	1,690.27 ± 16.48a
Specific growth rate/(%/d)	4.93 ± 0.05ab	4.91 ± 0.04ab	4.99 ± 0.05b	4.84 ± 0.07a
Feed conversion ratio	1.20 ± 0.02ab	1.24 ± 0.01ab	1.15 ± 0.02a	1.28 ± 0.00b
Protein efficiency	1.90 ± 0.02	1.82 ± 0.02	1.93 ± 0.04	1.77 ± 0.03
Hepatosomatic index/%	5.04 ± 0.37a	5.38 ± 0.20ab	6.24 ± 0.23b	5.69 ± 0.22ab
Condition factor/(g/cm^3)	0.56 ± 0.01	0.54 ± 0.01	0.54 ± 0.01	0.56 ± 0.01

Values in the same row with different small letter superscripts indicate significant differences ($P < 0.05$). The same applies to subsequent tables.

2.2 Effects of Dietary Yeast Hydrolysate on Nutrient Composition of L. vannamei

As shown in Table 3 , dietary supplementation with different levels of yeast hydrolysate had no significant effects on dry matter, crude protein, crude lipid or ash contents in whole shrimp or muscle ($P > 0.05$).

Table 3 Effects of dietary supplementation of yeast hydrolysate on nutrient composition of whole body and muscle of *L. vannamei* (n = 4)

Items	Dietary yeast hydrolysate level/%			
	0	0.5	1.0	2.0
Whole shrimp				

Items	Dietary yeast hydrolysate level/%			
Dry matter/%	23.44 ± 0.85	22.45 ± 0.27	22.81 ± 0.72	23.83 ± 0.34
Crude protein/%	16.50 ± 0.68	15.91 ± 0.11	16.48 ± 0.44	16.74 ± 0.29
Crude lipid/%	1.76 ± 0.34	1.64 ± 0.20	1.91 ± 0.16	2.03 ± 0.09
Ash/%	3.05 ± 0.18	3.05 ± 0.11	2.92 ± 0.06	3.06 ± 0.08
Muscle				
Dry matter/%	26.33 ± 0.24	27.39 ± 0.46	26.77 ± 0.40	26.64 ± 0.19
Crude protein/%	21.94 ± 0.41	21.70 ± 0.22	22.25 ± 0.15	22.07 ± 0.16
Crude lipid/%	1.08 ± 0.07	0.99 ± 0.04	1.07 ± 0.04	0.95 ± 0.07
Ash/%	1.23 ± 0.04	1.29 ± 0.03	1.25 ± 0.02	1.28 ± 0.02

2.3 Effects of Dietary Yeast Hydrolysate on Intestinal Morphology of L. vannamei

As shown in Table 4 , the 2.0% supplementation group exhibited significantly higher intestinal fold height compared with the 0.5% group ($P < 0.05$). No significant differences were observed in fold width, microvillus height or enterocyte height among groups ($P > 0.05$). However, fold width and enterocyte height showed a trend of decreasing then increasing with higher yeast hydrolysate levels, with numerically higher values in the 2.0% group compared with the control.

Table 4 Effects of dietary supplementation of yeast hydrolysate on intestinal morphology of *L. vannamei* (n = 4)

Items	Dietary yeast hydrolysate level/%			
	0	0.5	1.0	2.0

Items	Dietary yeast hydrolysate level/%			
Fold height	34.12 ± 2.83ab	33.94 ± 6.41a	34.63 ± 4.32ab	40.67 ± 14.90b
Fold width	25.71 ± 1.40	23.02 ± 8.01	23.67 ± 4.69	26.93 ± 6.54
Microvillus height	0.92 ± 0.16	1.15 ± 0.24	0.93 ± 0.07	0.91 ± 0.05
Enterocyte height	13.74 ± 0.75	13.69 ± 3.58	10.99 ± 1.62	14.14 ± 5.41

2.4 Effects of Dietary Yeast Hydrolysate on Serum Nonspecific Immune Enzyme Activities of L. vannamei

As shown in Table 5 , the 1.0% supplementation group exhibited significantly higher serum PO and LZM activities compared with the control group ($P < 0.05$), while NOS activity was significantly higher than that in the 0.5% group ($P < 0.05$).

Table 5 Effects of dietary supplementation of yeast hydrolysate on serum immune enzyme activities of *L. vannamei* (n = 4)

Items	Dietary yeast hydrolysate level/%			
	0	0.5	1.0	2.0
PO/(U/mL)	45.73 ± 4.19a	50.57 ± 3.92ab	62.67 ± 1.22b	52.99 ± 3.38ab
NOS/(U/mL)	40.89 ± 3.15ab	35.73 ± 4.58a	47.10 ± 2.15b	38.19 ± 2.74ab
LZM/(U/mL)	77.76 ± 2.11a	81.85 ± 1.23ab	91.23 ± 6.90b	75.38 ± 2.20ab

3.1 Effects of Dietary Yeast Hydrolysate on Growth Performance and Feed Utilization of L. vannamei

Previous studies have demonstrated that yeast hydrolysate is rich in amino acids, nucleic acids and mannan oligosaccharides, which can improve nutrient digestion and absorption by maintaining intestinal health, thereby enhancing growth performance and feed utilization in aquatic animals [15]. Appropriate

dietary supplementation with yeast hydrolysate has been shown to significantly increase weight gain rate and specific growth rate while reducing feed conversion ratio in grass carp (*Ctenopharyngodon idellus*) [16], gibel carp (*Carassius auratus gibelio*) [17] and largemouth bass (*Micropterus salmoides*) [18]. In the present study, 1.0% yeast hydrolysate supplementation did not significantly affect weight gain rate, specific growth rate or feed conversion ratio in *L. vannamei*, indicating that this supplementation level does not compromise growth performance or feed utilization. These findings are consistent with Chi et al. [19], who reported that 5% yeast hydrolysate replacing 16.67% fish meal had no significant effects on weight gain rate, specific growth rate, survival rate or feed conversion ratio in *L. vannamei*. Guo et al. [20] also reported no obvious advantages of yeast hydrolysate on growth performance in early-weaned piglets. Li et al. [21] found that yeast culture had no significant effects on weight gain rate or feed conversion ratio in grass carp. Discrepancies among studies may be attributed to different supplementation levels, protein sources or protein contents in experimental diets. Another important factor could be variations in yeast strain origin, production processes and nutritional composition of yeast hydrolysate products. The current results indicate that 1.0% yeast hydrolysate does not affect shrimp growth performance, but when supplementation exceeds certain levels, weight gain and protein efficiency tend to decrease while feed conversion ratio increases, suggesting that excessive yeast hydrolysate may negatively impact growth performance. This pattern is similar to findings by He et al. [22] on yeast culture. The negative effects may be due to increased non-starch polysaccharides and antinutritional factors at high supplementation levels, which could inhibit normal digestive function and reduce nutrient absorption, thereby decreasing energy available for growth [23]. Similar results were reported by Cui et al. [24], where high replacement levels of fish meal with bio-feed yeast reduced growth performance and disrupted lipid and protein metabolism in turbot (*Scophthalmus maximus*). In this study, hepatosomatic index was significantly higher in the 1.0% group compared with the control, consistent with Chi et al. [19] who reported significantly higher hepatosomatic index in groups fed 2.5% and 5.0% yeast hydrolysate. Yeast hydrolysate supplementation had no significant effects on dry matter, crude protein, crude lipid or ash contents in whole shrimp or muscle, which aligns with Su [25] regarding yeast culture effects on *L. vannamei* body composition. These results demonstrate that dietary yeast hydrolysate does not affect body composition of *L. vannamei*. In conclusion, under the conditions of this study, 1.0% dietary yeast hydrolysate supplementation does not significantly affect growth or feed utilization in *L. vannamei*, though high supplementation levels may exert negative effects that warrant further investigation.

3.2 Effects of Dietary Yeast Hydrolysate on Intestinal Morphology of L. vannamei

The intestine is the primary site for nutrient digestion and absorption in aquatic animals, and its developmental status directly affects nutrient utilization. In-

testinal morphological structure can reflect intestinal health status to some extent [26]. Studies have shown that yeast hydrolysate affects intestinal mucosal morphology and promotes intestinal growth and health in early-weaned piglets [20] and broilers [27]. In this study, no significant differences were observed in fold width, microvillus height or enterocyte height of *L. vannamei* intestine among groups, though fold width and enterocyte height tended to increase at 2.0% supplementation. Yeast hydrolysate contains abundant nucleotides (5.12%), and previous reports indicate that dietary nucleotide supplementation can protect the intestine, improve intestinal morphology and development, and promote nutrient digestion and absorption [28-29]. Miao et al. [30] demonstrated that 150 mg/kg nucleotide supplementation increased intestinal fold height in large yellow croaker (*Pseudosciaena crocea*). Xu et al. [31] reported that 0.1–0.8 g/kg yeast nucleotides significantly increased fold thickness in shrimp. Zhu et al. [32] showed that nucleotide supplementation in fish meal-free diets significantly increased intestinal villus height and enterocyte height in common carp. In contrast, this study found no significant effects of yeast hydrolysate on fold width, microvillus height or enterocyte height in *L. vannamei*, though a positive trend was observed. This discrepancy may be related to insufficient nucleotide levels from low yeast hydrolysate supplementation. The specific mechanisms underlying yeast hydrolysate effects on intestinal morphology in *L. vannamei* require further investigation.

3.3 Effects of Dietary Yeast Hydrolysate on Nonspecific Immune Enzymes of L. vannamei

Serum PO, NOS and LZM activities are important indicators of nonspecific immunity in shrimp, reflecting immune capacity and playing crucial roles in immune defense [33]. PO exists as a proenzyme in crustaceans, and the prophenoloxidase activating system represents an important immune recognition and defense mechanism against pathogen invasion, serving as a key indicator of immune capacity [34-35]. NOS has recently gained attention for its role in immunity, catalyzing nitric oxide (NO) production. Besides neurotransmission and smooth muscle relaxation, NO exhibits antibacterial, antiparasitic and antiviral activities and modulates various immune substances, influencing immune function [36]. LZM is widely present in crustacean hemocytes and body fluid, hydrolyzing acetylaminopolysaccharides in the cell walls of Gram-positive bacteria and forming a lytic enzyme system to prevent bacterial invasion, making it an important indicator of nonspecific immunity [37].

Liu et al. [38] found that 1–2% yeast hydrolysate increased serum LZM and NOS activities in juvenile turbot. Wang et al. [39] reported that replacing 15% fish meal with yeast extract significantly increased serum LZM activity in *L. vannamei*. Chi et al. [19] demonstrated that 2.5–7.5% yeast hydrolysate enhanced nonspecific immunity in shrimp, with serum PO, NOS and LZM activities higher than the fish meal control group, indicating that appropriate yeast hydrolysate levels can maintain high immune defense status. He et al. [22] showed that

0.3%, 0.5% or 1.0% yeast culture significantly increased serum LZM and PO activities and hepatopancreas LZM activity in shrimp, suggesting that 0.3–0.5% yeast culture can significantly enhance nonspecific immunity. The present study showed similar results, with the 1.0% group exhibiting higher serum PO, NOS and LZM activities than the control group, indicating that 1.0% dietary yeast hydrolysate can enhance serum nonspecific immune enzyme activities and maintain high immune defense status.

The enhanced nonspecific immune enzyme activities may be attributed to functional components in yeast hydrolysate, including β-glucans, mannan oligosaccharides and nucleotides. Liu et al. [40] noted that yeast hydrolysate products contain 17–30% β-glucans and mannan oligosaccharides, which play important roles in improving intestinal microecology and enhancing nonspecific immunity in aquatic animals. Studies have also shown that β-glucans and mannan oligosaccharides can improve immune capacity without affecting digestion and utilization [11, 41]. Research has demonstrated that yeast nucleotides can enhance nonspecific immunity in *L. vannamei* [33], red seabream (*Pagrus major*) [42], red drum (*Sciaenops ocellatus*) [28], gibel carp [43] and tilapia (*Oreochromis niloticus*) [44], consistent with our results. Sajeevan et al. [45] indicated that nucleotides serve as important immunostimulants in aquaculture, though the specific mechanisms of nonspecific immunity in crustaceans remain unclear.

In conclusion, dietary supplementation with 1.0% yeast hydrolysate can enhance serum nonspecific immune enzyme activities in *L. vannamei* without negatively affecting growth performance or intestinal morphological health.

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