Effects of Yeast Culture on Growth Performance, Non-Specific Immunity, and Disease Resistance of Litopenaeus vannamei Postprint

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Abstract

This study aimed to investigate the effects of yeast culture on growth performance, non-specific immunity, and disease resistance of Litopenaeus vannamei. Four isonitrogenous and isolipidic experimental diets were formulated by supplementing yeast culture to the basal diet at levels of 0 (control), 0.30%, 0.50%, and 1.00%, designated as Y0, Y0.3, Y0.5, and Y1.0, respectively. A total of 800 L. vannamei with an initial body weight of (1.20$±$0.01) g were randomly allocated into 4 groups with 5 replicates per group and 40 shrimp per replicate. The feeding trial lasted for 56 days. The results showed that the weight gain rate and specific growth rate of shrimp in the Y0.3 group were significantly higher than those in the Y0.5 group (P<0.05). The Y0.3 group exhibited the highest protein efficiency and lowest feed conversion ratio, which differed significantly from the other groups (P<0.05). Yeast culture demonstrated certain feeding attractant effects, and the feeding rate of shrimp in the Y0.3 group was significantly higher than that in the Y0 group (P<0.05). The muscle crude protein content in the Y0.3, Y0.5, and Y1.0 groups was significantly higher than that in the Y0 group (P<0.05), reaching the maximum value in the Y0.3 group (91.69%). Dietary supplementation with 0.30%, 0.50%, or 1.00% yeast culture significantly enhanced the activities of lysozyme, phenoloxidase, and alkaline phosphatase in serum, as well as lysozyme, peroxidase, superoxide dismutase, and alkaline phosphatase in the hepatopancreas of shrimp (P<0.05). The malondialdehyde content in serum of shrimp in the Y0.5 group was significantly lower than that in the other groups (P<0.05). After challenge with Vibrio harveyi for 7 days, the cumulative mortality of shrimp in the Y0.3 and Y0.5 groups was significantly lower than that in the Y1.0 group (P<0.05), but showed no significant difference from the Y0 group (P>0.05). Thus, thus, dietary supplementation of 0.30% yeast culture could significantly improve the growth performance of L. vannamei, while supplementation with 0.30%–0.50% yeast culture could significantly enhance the

non-specific immunity of L. vannamei.

Full Text

Effects of Yeast Culture on Growth Performance, Nonspecific Immunity and Disease Resistance of *Litopenaeus vannamei*

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Abstract: This study investigated the effects of dietary yeast culture on growth performance, nonspecific immunity and disease resistance of *Litopenaeus vannamei*. Four isonitrogenous and isolipidic experimental diets were formulated by supplementing a basal diet with 0 (control), 0.30%, 0.50%, and 1.00% yeast culture, designated as Y0, Y0.3, Y0.5, and Y1.0, respectively. A total of 800 *L. vannamei* juveniles with an initial body weight of (1.20$±$0.01) g were randomly divided into 4 groups, each consisting of 5 replicates of 40 individuals. The feeding trial lasted for 56 days. The results showed that the weight gain rate (WGR) and specific growth rate (SGR) in the Y0.3 group were significantly higher than those in the Y0.5 group (P<0.05). The Y0.3 group exhibited the highest protein efficiency ratio (PER) and lowest feed conversion ratio (FCR), which differed significantly from all other groups (P<0.05).

Yeast culture demonstrated certain attractant effects, with the feeding rate (FR) in the Y0.3 group being significantly higher than that in the Y0 group (P<0.05). Muscle crude protein content in the Y0.3, Y0.5, and Y1.0 groups was significantly higher than in the Y0 group (P<0.05), reaching a maximum value of 91.69% in the Y0.3 group. Dietary supplementation with 0.30%, 0.50%, or 1.00% yeast culture significantly elevated the activities of lysozyme (LSZ), phenoloxidase (PO), and alkaline phosphatase (ALP) in serum, as well as LSZ, peroxidase (POD), superoxide dismutase (SOD), and ALP activities in hepatopancreas (P<0.05). Serum malondialdehyde (MDA) content in the Y0.5 group was significantly lower than in all other groups (P<0.05). Following a 7-day challenge with *Vibrio harveyi*, the cumulative mortality rates in the Y0.3 and Y0.5 groups were significantly lower than that in the Y1.0 group (P<0.05), but showed no significant difference compared with the Y0 group (P>0.05). It can be concluded that dietary supplementation with 0.30% yeast culture significantly improves the growth performance of *L. vannamei*, while supplementation with 0.30%–0.50% significantly enhances their nonspecific immunity.

Keywords: yeast culture; *Litopenaeus vannamei*; growth performance; non-specific immunity; disease resistance

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Litopenaeus vannamei is a commercially important shrimp species favored by consumers. With the continuous expansion of aquaculture scale and intensification, the risk of disease infection has increased substantially, leading to environmental deterioration and frequent outbreaks of bacterial and viral diseases [1-2]. This has necessitated extensive antibiotic use in farming practices. However, long-term antibiotic application results in drug resistance and residues, making the search for alternatives a research priority in feed industry. Yeast culture, as a microecological preparation, represents one effective substitute for antibiotics.

Yeast culture is a microecological preparation produced through anaerobic fermentation of high-performance yeast strains under specific conditions. It primarily consists of yeast extracellular metabolites, fermented culture medium, and a small number of inactive yeast cells. In addition to B vitamins, minerals, digestive enzymes, organic acids, amino acids, and oligosaccharides, it contains important "unknown growth factors" [3]. Previous studies have demonstrated that yeast culture can improve growth performance, enhance gastrointestinal digestion and absorption, and boost immunity and disease resistance in various aquatic animals, including grass carp (*Ctenopharyngodon idellus*) [4], sea cucumber (*Apostichopus japonicus* Selenka) [5], Japanese seabass (*Lateolabrax japonicus*) [6], catfish (*Pangasianodon hypophthalmus*) [7], and giant freshwater prawn (*Macrobrachium rosenbergii*) [8]. This study evaluated the appropriate use of yeast culture in *L. vannamei* feed by examining changes in growth indices, muscle nutritional composition, and nonspecific immune parameters.

1.1 Experimental Diets and Design

Four isonitrogenous and isolipidic experimental diets were formulated by adding 0 (control), 0.30%, 0.50%, and 1.00% yeast culture (product of Beijing Enhalor Biotechnology Co., Ltd.) to a basal diet. Based on the essential amino acid requirements of *L. vannamei* [9], dietary amino acid levels were balanced. The diets were designated as Y0, Y0.3, Y0.5, and Y1.0. Dietary composition and nutrient levels are presented in Table 1 . Ingredients such as brown fish meal were ground to pass through an 80-mesh sieve, accurately weighed according to formulation requirements, and mixed using a stepwise expansion method for micro-ingredients. The mixture was processed into 1.0 mm and 1.5 mm pellet diets using a twin-screw extruder, then cooked at 60 °C for 30 min. The prepared diets were air-dried, sealed in self-sealing bags, and stored at -20 °C until use.

1.2 Experimental Animals and Management

L. vannamei postlarvae were purchased from Zhanjiang Yuehai Aquatic Seedling Co., Ltd. and temporarily reared in outdoor cement tanks for

2 weeks before the experiment. After 24 h of fasting, healthy juveniles from the same genetic background with an initial body weight of $(1.20\$\pm0.01)gwererandomlyallocatedinto4groups(Y0, Y0.3, Y0.5, andY1.0)correspondingtotheexperimentalc$ fiberglass tanks. The culture period lasted 8 weeks.

The initial feeding rate was 5%–8% of body weight, adjusted appropriately based on consumption and weather conditions. Feeding occurred four times daily at 07:00, 11:00, 17:00, and 21:00, with feeding behavior observed 1 h post-feeding. During the initial phase, two-thirds of the water was exchanged every 2 days, which was increased to half the volume daily during the final 10 days. Water temperature was maintained at 28.5–30.0 °C, salinity at 26.5–28.0, with continuous aeration ensuring dissolved oxygen >6.8 mg/L, pH at 7.8–8.2, and ammonia nitrogen <0.03 mg/L.

1.3 Sample Collection and Analysis

At the end of the feeding trial, shrimp were fasted for 24 h before final weighing to calculate growth performance. From each replicate, 5 individuals were randomly selected for measurement of body length and weight, followed by muscle collection for determination of crude protein, crude lipid, crude ash, and moisture content. An additional 10 shrimp per replicate were sampled for hemolymph collection using a 1 mL sterile syringe from the base of the fifth pereiopod. Hemolymph samples from 10 shrimp were pooled as one sample, transferred to 1.5 mL centrifuge tubes, and immediately placed on ice. After sampling, samples were stored at 4 °C overnight, then centrifuged at 8,000 r/min for 10 min at 4 °C. The supernatant was collected and stored at -80 °C for serum immune parameter analysis. Following hemolymph collection, hepatopancreas was dissected, snap-frozen in liquid nitrogen, and stored at -80 °C for immune parameter detection.

Conventional nutrient analysis was performed on feed and muscle samples [10]. Moisture content was determined by oven drying at 105 °C to constant weight, crude protein by the Kjeldahl method, crude lipid by Soxhlet extraction, and crude ash by combustion at 550 °C.

Phenoloxidase (PO) activity was measured following the method of Ashida [11] with appropriate modifications. Alkaline phosphatase (ALP) activity was determined by microplate assay, lysozyme (LSZ) activity by turbidimetry, superoxide dismutase (SOD) activity by WST-1 method, peroxidase (POD) activity by colorimetry, and malondialdehyde (MDA) content by thiobarbituric acid (TBA) method. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. For hepatopancreas ALP, LSZ, SOD, and POD activity measurements, appropriate tissue amounts were weighed and homogenized with physiological saline at a 1:9 ratio (g/mL) on ice to prepare 10% tissue homogenate. The homogenate was centrifuged at 2,500 r/min for 10 min at 4 °C, and the supernatant was carefully collected. Protein concentration was determined using a bicinchoninic acid (BCA) assay kit from Nanjing Jiancheng Bioengineering

Institute.

1.4 *Vibrio harveyi* Challenge Test

At the end of the feeding trial, 10 shrimp per replicate were randomly selected for the *Vibrio harveyi* challenge test. The *V. harveyi* strain was provided by the Guangdong Provincial Key Laboratory of Aquatic Animal Pathogen Biology and Epidemiology. A pre-test determined the 7-day median lethal concentration (LD50) for *L. vannamei* as 1.89$×10^{7}$ CFU/mL. During challenge, 300 L of bacterial suspension at this concentration was injected into the dorsal muscle between the 2nd and 3rd abdominal segments. Mortality was recorded for 7 days to calculate cumulative mortality rate and relative percent survival.

1.5 Calculation Formulas

Weight gain rate (WGR, %) = 100 × (final average weight - initial average weight) / initial average weight
Specific growth rate (SGR, %/d) = 100 × (ln final average weight - ln initial average 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)
Feeding rate (FR, %) = 100 × dry feed intake / [(final body weight + initial body weight)/2 × feeding days]
Survival rate (SR, %) = 100 × final shrimp number / initial shrimp number
Cumulative mortality rate (CMR, %) = 100 × cumulative mortality / initial number
Relative percent survival (RPS, %) = 100 × (1 - mortality in treatment group / mortality in control group)

1.6 Statistical Analysis

All data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using SPSS 17.0 software. When significant differences were detected among groups, Duncan' s multiple range test was applied. The significance level was set at $P<0.05$.

2.1 Effects of Yeast Culture on Growth Performance of *L. vannamei*

As shown in Table 2 , no significant differences were observed in survival rate among all groups ($P>0.05$). The WGR and SGR in the Y0.3 group were significantly higher than those in the Y0.5 group ($P<0.05$). The Y0.3 group exhibited the highest PER and lowest FCR, which differed significantly from all other groups ($P<0.05$). Yeast culture demonstrated certain attractant properties, with the FR in the Y0.3 group being significantly higher than that in the Y0 group ($P<0.05$).

2.2 Effects of Yeast Culture on Muscle Nutrient Composition of *L. vannamei*

Table 3 shows that no significant differences were detected in muscle moisture content among groups (P>0.05). Muscle crude protein content in the Y0.3, Y0.5, and Y1.0 groups was significantly higher than in the Y0 group (P<0.05), with the maximum value (91.69%) observed in the Y0.3 group. Muscle crude lipid content in the Y0.5 group was significantly lower than in the Y0.3 group (P<0.05). With increasing yeast culture supplementation, muscle crude ash content gradually increased, with the Y1.0 group being significantly higher than the Y0 and Y0.3 groups (P<0.05).

2.3 Effects of Yeast Culture on Serum Immune Parameters of *L. vannamei*

Table 4 indicates that PO, ALP, and LSZ activities in serum were significantly higher in the Y0.3, Y0.5, and Y1.0 groups compared with the Y0 group (P<0.05). Serum SOD activity in the Y0.3 and Y0.5 groups was significantly higher than in the Y0 and Y1.0 groups (P<0.05). Serum ALP activity in the Y0.3 group was significantly higher than in all other groups (P<0.05), while serum LSZ activity in the Y0.5 group was significantly higher than in all other groups (P<0.05). Serum MDA content in the Y0.5 group was significantly lower than in all other groups (P<0.05).

2.4 Effects of Yeast Culture on Hepatopancreas Immune Parameters of *L. vannamei*

As presented in Table 5 , ALP, POD, and LSZ activities in hepatopancreas were significantly higher in the Y0.3, Y0.5, and Y1.0 groups compared with the Y0 group (P<0.05), though no significant differences were observed among these three treatment groups (P>0.05). Hepatopancreas LSZ activity peaked in the Y0.3 group. Hepatopancreas SOD activity exhibited an increasing trend with increasing yeast culture supplementation, with significant differences among all groups (P<0.05).

2.5 Effects of *Vibrio harveyi* Challenge on Cumulative Mortality and Relative Percent Survival

As shown in Figure 1 [Figure 1: see original paper], the cumulative mortality rates in the Y0.3 and Y0.5 groups were significantly lower than that in the Y1.0 group (P<0.05), but showed no significant difference compared with the Y0 group (P>0.05). Figure 2 [Figure 2: see original paper] demonstrates that the relative percent survival in the Y0.3 and Y0.5 groups was significantly higher than that in the Y1.0 group (P<0.05), with no significant difference compared with the Y0 group (P>0.05).

3.1 Effects of Yeast Culture on Growth Performance of *L. vannamei*

Yeast culture is rich in amino acids, organic acids, and oligosaccharides that improve intestinal morphology, enhance digestive enzyme activities, and promote nutrient digestion, absorption, and utilization, thereby improving growth performance in aquatic animals [12]. In this study, dietary yeast culture exhibited attractant effects in *L. vannamei*, consistent with reports in turbot (*Scophthalmus maximus*) [13]. This is attributed to flavor compounds such as glutamic acid and nucleotides in yeast culture that impart a unique aroma to the feed. Previous studies have reported that appropriate dietary yeast culture supplementation significantly improves SGR and reduces FCR in various aquatic species, including grass carp (*Ctenopharyngodon idellus*) [14], Jian carp (*Cyprinus carpio* var. Jian) [15], Japanese flounder (*Paralichthys olivaceus*) [16], gibel carp (*Carassius auratus gibelio*) [17], blunt snout bream (*Megalobrama amblycephala*) [18], and *L. vannamei* [19]. Furthermore, yeast culture can compensate for reduced SGR caused by fish meal replacement with soybean meal in turbot [13]. In the present study, the Y0.3 group showed significantly higher WGR and SGR and lower FCR compared with other groups. However, when yeast culture supplementation exceeded optimal levels, WGR, SGR, and PER decreased while FCR increased, indicating negative effects. This suggests that excessive yeast culture does not further improve growth performance, possibly due to increased non-starch polysaccharides that exert anti-nutritional effects and impair nutrient utilization [14]. The underlying mechanisms require further investigation. Under our experimental conditions, yeast culture effectively promoted *L. vannamei* growth at appropriate levels, with 0.30% supplementation showing the best results. However, Su *et al.* [19] reported that 0.075%–0.100% yeast culture (XP) significantly improved *L. vannamei* growth performance, suggesting that optimal supplementation levels may vary depending on initial body weight, yeast strain, and production process.

Previous studies have shown that dietary yeast culture supplementation significantly increases crude protein content in muscle, liver, and whole body of blunt snout bream [18]. In this study, yeast culture significantly improved muscle crude protein content in *L. vannamei*, though some studies reported no significant effects on muscle composition in aquatic animals [16,20], possibly due to species differences or variations in yeast culture production processes. The improved muscle quality may result from yeast culture components providing essential nutrients, participating in metabolism, and exerting synergistic effects through various unknown growth factors, thereby promoting growth and enhancing muscle quality.

3.2 Effects of Yeast Culture on Nonspecific Immunity and Disease Resistance of *L. vannamei*

Similar to terrestrial animals, aquatic animals possess both humoral and cellular nonspecific immune functions [21]. Superoxide dismutase (SOD) is closely associated with immune status, catalyzing the dismutation of oxygen radicals

to molecular oxygen and hydrogen peroxide while enhancing phagocytic defense capacity, representing a crucial component of the antioxidant defense system [22]. In this study, dietary yeast culture significantly increased serum SOD activity in *L. vannamei*, consistent with findings in gibel carp [17] and Chinese soft-shelled turtle (*Pelodiscus sinensis*) [23]. This effect is closely related to the abundant β-glucans and mannan oligosaccharides (MOS) in yeast culture. β-glucans can enhance hemocyte SOD activity and antioxidant capacity [24-25], while MOS, possessing certain immunogenicity, can stimulate immune responses and increase plasma SOD activity [26-27].

Lysozyme (LSZ) is an enzyme that hydrolyzes N-acetylmuramic acid and N-acetylglucosamine bonds, found in fish mucus, serum, and tissues, particularly abundant in hemocytes, and represents an important nonspecific immune factor [28]. In this study, dietary yeast culture significantly increased LSZ activities in both serum and hepatopancreas of *L. vannamei*, consistent with reports in Japanese flounder [16], gibel carp [17], and Chinese soft-shelled turtle [23]. LSZ clears residual bacterial cell walls after antimicrobial factor action, enhances sensitivity of other immune factors to bacteria, and cooperates with other immune components to resist pathogen invasion, with increased serum LSZ activity corresponding to enhanced immune capacity [29].

Phenoloxidase (PO) exists as a proenzyme in crustaceans, and the PO-activating system represents an important immune recognition and defense mechanism against pathogen invasion and environmental stress [30]. Studies have shown that dietary yeast culture increases serum PO activity in sea cucumber [5] and *L. vannamei* [19]. In this study, yeast culture significantly elevated PO activity in serum and hepatopancreas, likely attributable to β-glucans. Numerous reports have demonstrated that yeast β-glucans can increase PO activity in cultured animals. *In vitro* studies confirmed that yeast β-glucan enhances serum PO activity in black tiger shrimp (*Penaeus monodon*) [31]. Duvic and Söderhäll [32] detected β-glucan binding proteins (BGBP) in plasma of freshwater crayfish (*Astacus astacus* and *Procambarus clarkii*), which activate the PO system by enhancing prophenoloxidase-activating enzyme and PO activity upon binding to β-glucans, thereby improving immune defense. Wang [33] detected continuous expression of BGBP in hepatopancreas and prophenoloxidase in hemocytes of *L. vannamei*, suggesting the presence of BGBP in shrimp hepatopancreas. The increased serum PO activity observed in this study likely resulted from BGBP-β-glucan binding in hepatopancreas activating the PO system, though the specific mechanism requires further investigation.

Malondialdehyde (MDA), a toxic lipid peroxidation product, indicates excessive oxygen radical production when its content increases. Oxygen radicals strongly oxidize unsaturated fatty acid double bonds in cell membranes, causing lipid peroxidation and reduced membrane fluidity [34]. In this study, appropriate yeast culture supplementation decreased serum MDA content and enhanced antioxidant capacity in *L. vannamei*, consistent with findings by Xu *et al.* [17] and Zhang *et al.* [35]. Additionally, yeast culture water-soluble extracts can

improve the growth status of isolated grass carp intestinal mucosal cells damaged by MDA, reduce MDA-induced membrane permeability increase, and enhance cellular antioxidant capacity [36].

The enhanced antioxidant capacity and nonspecific immunity observed in this study may also be attributed to nucleotides in yeast culture. Research has shown that yeast nucleotides significantly improve antioxidant capacity and serum nonspecific immunity in narrow-clawed crayfish (*Astacus leptodactylus*) [37], giltthead seabream (*Sparus aurata*) [38], and Nile tilapia (*Oreochromis niloticus*) [39], highlighting their importance as immunostimulants in crustaceans [40].

Yeast culture also positively affects disease resistance in aquatic animals. Dietary yeast culture alone significantly reduced cumulative mortality and improved disease resistance in sea cucumber challenged with *Vibrio splendidus* [5]. Yeast cell wall supplementation increased survival rate after *Aeromonas* challenge in Japanese seabass [6]. In Japanese flounder naturally infected with vibriosis, dietary supplementation with 0.07% yeast culture (XP) showed a trend toward improved disease resistance. Burgents *et al.* [3] reported that dietary supplementation with 1% XP significantly improved survival after weekly *Vibrio* sp. challenges in *L. vannamei*. Su *et al.* [19] demonstrated that 0.10% yeast culture reduced cumulative mortality during the first 3 days after *Vibrio alginolyticus* challenge in *L. vannamei*. In this study, dietary supplementation with 0.30%–0.50% yeast culture reduced cumulative mortality and provided certain immune protection against *V. harveyi* challenge, though the effect was not significant compared with the control group. However, increasing supplementation to 1.00% elevated cumulative mortality and reduced relative percent survival, possibly due to excessive immune stimulation causing immunosuppression, which requires further investigation.

Under our experimental conditions, dietary yeast culture exhibited attractant effects, with 0.30% supplementation significantly improving growth performance. Compared with the control, dietary supplementation with 0.30%–0.50% yeast culture significantly enhanced the nonspecific immunity of *L. vannamei*.

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Note: Figure translations are in progress. See original paper for figures.