Effects of Kelp Residue Supplementation Ratio and Its Enzymatic Hydrolysate on Growth, Digestion, and Non-specific Immunity of Litopenaeus vannamei (Postprint)

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Abstract

To investigate the effects of kelp residue addition ratio and its enzymatic hydrolysates on the growth, digestion, and non-specific immunity of Litopenaeus vannamei, two experiments were conducted in this study: a suitable kelp residue addition ratio experiment and an application effect experiment of kelp residue enzymatic hydrolysates. Suitable kelp residue addition ratio experiment (Experiment 1): A single-factor experimental design was adopted, and six feed formulations with kelp residue addition ratios of 0 (control group), 0.5%, 1.0%, 2.0%, 3.0%, and 5.0% were prepared (designated as groups D1–D6, respectively). After feeding Litopenaeus vannamei with an initial body weight of (1.00±0.10) g for 49 days, the growth performance indicators and the activities of gastric digestive enzymes and serum non-specific immune enzymes of the shrimp were measured. Each group had three replicates, with 35 shrimp per replicate. Application effect experiment of kelp residue enzymatic hydrolysates (Experiment 2): Based on the result from Experiment 1 that the suitable kelp residue addition ratio was 3%, the feed with 3% kelp residue addition from Experiment 1 was used as the kelp residue control group (Group A). Kelp residue hydrolyzed by -glucanase (Group B) and kelp residue hydrolyzed by protease (Group C) were then used to equally replace the kelp residue in Group A feed, respectively. Litopenaeus vannamei with an initial body weight of (1.00±0.1) g were fed for 56 days, and after the experiment, the growth performance indicators and the activities of gastric digestive enzymes and serum non-specific immune enzymes of the shrimp were measured. Each group had three replicates, with 35 shrimp per replicate. The results of Experiment 1 showed that the specific growth rate and weight gain rate of shrimp in groups D4 and D5 were significantly higher than those in the control group ($P<0.05$), and the feed coefficient of shrimp in group D5 was significantly lower than that in the control group ($P<0.05$).

With the increase of kelp residue addition ratio, the activities of pepsin in the stomach and phenoloxidase and superoxide dismutase in serum showed a trend of first increasing and then decreasing. The activities of these three enzymes were highest in group D5, and the activities of phenoloxidase and superoxide dismutase in serum of group D5 were significantly higher than those in the control group (P<0.05). The results of Experiment 2 showed that compared with untreated kelp residue, kelp residue hydrolyzed by -glucanase could significantly increase the weight gain rate and specific growth rate of shrimp (P<0.05), and significantly reduce the feed coefficient (P<0.05). Kelp residue hydrolyzed by protease could significantly increase the survival rate of shrimp (P<0.05). The cellulase activity in the stomach of shrimp in groups B and C was significantly lower than that in group A (P<0.05). The activities of acid phosphatase and phenoloxidase in serum of groups B and C, as well as the superoxide dismutase activity in serum of group C, were significantly higher than those in group A (P<0.05). It can be concluded that the suitable addition ratio of kelp residue in Litopenaeus vannamei feed is 3%. Kelp residue hydrolyzed by -glucanase can promote the growth of Litopenaeus vannamei and enhance its non-specific immunity, while kelp residue hydrolyzed by protease can enhance the non-specific immunity of Litopenaeus vannamei but has no promoting effect on its growth.

Full Text

Preamble

Effects of Kelp Meal Adding Proportion and Its Enzymatic Hydrolysates on Growth, Digestion and Non-Specific Immunity of *Litopenaeus vannamei*

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Abstract: This study investigated the effects of kelp meal adding proportion and its enzymatic hydrolysates on the growth, digestion, and non-specific immunity of *Litopenaeus vannamei* through two experiments: an optimal adding proportion trial and an enzymatic hydrolysate application trial. In Experiment 1, a single-factor design was employed to formulate six diets containing 0% (control), 0.5%, 1.0%, 2.0%, 3.0%, and 5.0% kelp meal (designated as groups D1–D6), which were fed to shrimp with an initial body weight of (1.00±0.10) g for 49 days. Growth performance metrics and activities of gastric digestive enzymes and serum non-specific immune enzymes were subsequently measured. Each group comprised three replicates of 35 shrimp. In Experiment 2, based on the optimal 3% kelp meal proportion identified in Experiment 1, a diet containing 3% untreated kelp meal served as the control (Group A). This was compared against diets where -glucanase-hydrolyzed kelp meal (Group B) or

protease-hydrolyzed kelp meal (Group C) replaced the kelp meal on an equal basis. These diets were fed to shrimp with an initial body weight of (1.00±0.10) g for 56 days, after which growth performance and enzyme activities were assessed. Each group consisted of three replicates of 35 shrimp. Experiment 1 results showed that the specific growth rate (SGR) and weight gain rate (WGR) in groups D4 and D5 were significantly higher than those in the control group ($P<0.05$), while the feed conversion ratio (FCR) in group D5 was significantly lower ($P<0.05$). With increasing kelp meal proportion, the activities of gastric pepsin and serum phenoloxidase (PO) and superoxide dismutase (SOD) initially increased then decreased, reaching maximum values in group D5, whose serum PO and SOD activities were significantly higher than the control ($P<0.05$). Experiment 2 demonstrated that -glucanase hydrolysis significantly improved WGR and SGR while reducing FCR compared to untreated kelp meal ($P<0.05$), whereas protease hydrolysis significantly increased survival rate ($P<0.05$). Both enzyme treatments significantly reduced gastric cellulase activity relative to Group A ($P<0.05$). Groups B and C exhibited significantly higher serum acid phosphatase (ACP) and PO activities than Group A ($P<0.05$), and Group C showed significantly elevated serum SOD activity ($P<0.05$). These findings indicate that the optimal dietary kelp meal proportion for *L. vannamei* is 3%. -Glucanase hydrolysis of kelp meal promotes growth and enhances non-specific immunity, while protease hydrolysis improves non-specific immunity without promoting growth.

Keywords: kelp meal; enzymatic hydrolysates; *Litopenaeus vannamei*; growth performance; digestive enzymes; non-specific immune enzymes

Litopenaeus vannamei, commonly known as Pacific white shrimp, is one of the world' s three most commercially important cultured shrimp species, characterized by rapid growth, strong disease resistance, and high environmental tolerance, offering significant economic benefits. In shrimp aquaculture, nutritionally comprehensive feed forms the foundation for healthy cultivation [1], with suitable raw materials being critical for ensuring complete nutrition. However, the variety and resources of conventional feed ingredients are limited, making the development of alternative feed materials essential.

Kelp (*Laminaria japonica* Aresch) is a major brown macroalgae species cultivated in China. Its production environment enables accumulation of various vitamins, trace elements, and growth-promoting bioactive factors, providing rich nutrition with high utilization efficiency in animals [2]. China' s kelp processing industry has developed an industrial system primarily producing alginate, mannitol, and iodine, but the utilization rate is only approximately 30%. The remaining kelp meal still contains substantial amounts of protein, cellulose, and minerals that remain underutilized. This not only wastes natural biological resources but also results in discharge of organic-rich waste, potentially contributing to eutrophication and harmful algal blooms that threaten marine fisheries and ecosystems [3]. Recent research on seaweed products has focused on poultry [4], swine [5], and shrimp [6], demonstrating that dietary seaweed

or its extracts exert positive effects on cultured animals [7-10]. Since the 1950s, kelp meal has been studied as a feed additive and continues to show promise. While direct addition of kelp meal to livestock and aquafeeds is common, its enzymatic hydrolysates are rarely used in shrimp feed production. Therefore, this study investigated varying dietary kelp meal proportions to determine the optimal level, then examined the effects of different enzymatic treatments to provide a theoretical basis for the high-value utilization of kelp meal.

1. Materials and Methods

1.1 Experimental Materials

Healthy juvenile *L. vannamei* [body weight (1.00±0.10) g] were obtained from Weifang Xindadi Aquaculture Co., Ltd. Kelp meal, a commercial byproduct from alginate extraction, was provided by Shandong Mingyue Seaweed Group Co., Ltd. Feed ingredients were purchased from Qingdao Qihao Feed Co., Ltd. Feed-grade -glucanase (powder, 50,000 U/g) and protease (powder, 20,000 U/g) were obtained from Heshibi Biotechnology Co., Ltd.

1.2 Enzymatic Hydrolysis of Kelp Meal

Based on the high protein and cellulose content in kelp meal (nutrient levels shown in Table 1), two enzyme types targeting these components were initially screened, with reducing sugar and amino acid concentrations in hydrolysates serving as evaluation indices. -glucanase and protease showing superior hydrolysis efficiency were selected. The hydrolysis procedures followed Wang et al. [11] with modifications.

For -glucanase hydrolysis (GT): -glucanase was added at 0.1% of kelp meal weight with a solid-to-liquid ratio of 1:20, followed by incubation at 50°C for 24 h with continuous stirring. The entire solid-liquid mixture was used as feed additive. The 3,5-dinitrosalicylic acid method measured reducing sugar concentration, revealing a hydrolysis efficiency of 8.98%. Nutrient levels are presented in Table 1.

For protease hydrolysis (PT): Protease was added at 0.2% of kelp meal weight with a solid-to-liquid ratio of 1:20, followed by incubation at 45°C for 24 h with continuous stirring. The complete hydrolysate was used as feed additive. Ninhydrin colorimetry determined amino acid concentration, showing a hydrolysis efficiency of 11.3%. Nutrient levels are shown in Table 1.

1.3 Experimental Design

The feeding trial was conducted at the Aquatic Laboratory of Yellow Sea Fisheries Research Institute and comprised two experiments.

1.3.1 Experiment 1: Optimal Kelp Meal Proportion Trial A basal diet (Group D1, control) was formulated using fish meal, soybean meal, high-gluten

flour, shrimp bran, vitamin premix, and mineral premix. Experimental diets were prepared by replacing high-gluten flour with 0.5% (D2), 1.0% (D3), 2.0% (D4), 3.0% (D5), and 5.0% (D6) kelp meal. Diet composition and nutrient levels are shown in Table 2 . Ingredients were ground through an 80-mesh sieve, mixed, and extruded into 2 mm pellets using a meat grinder (Hengli TJ12-H, China). Pellets were dried at 60°C to constant weight and stored at -20°C.

The 49-day trial utilized 630 healthy juvenile shrimp with an average initial weight of (1.00±0.10) g, randomly allocated into six groups with three replicates each (35 shrimp per replicate). Shrimp were cultured in 150 L white barrels. Water temperature was maintained at 21–28°C, salinity at 30–33‰, pH at 7.0–7.6, and dissolved oxygen at approximately 6.5 mg/L. After a one-week acclimation, shrimp were fed the experimental diets at 3–5% of body weight twice daily to satiation. Feed amounts were weighed before feeding, and uneaten feed was collected by siphoning after 1 h, dried, and weighed to record daily feed intake. Water was exchanged twice weekly with continuous aeration. Dead shrimp were promptly removed to prevent water quality deterioration.

1.3.2 Experiment 2: Kelp Meal Enzymatic Hydrolysate Application Trial Based on Experiment 1 results showing 3% kelp meal as optimal, the 3% kelp meal diet served as the control (Group A). Groups B and C contained 3% -glucanase-hydrolyzed or protease-hydrolyzed kelp meal, respectively, replacing untreated kelp meal on an equal basis. Diet composition and nutrient levels are shown in Table 3 . The 56-day trial used 315 healthy juvenile shrimp with an average initial weight of (1.00±0.10) g, randomly divided into three groups with three replicates each (35 shrimp per replicate). Culture conditions were identical to Experiment 1.

1.4 Sample Collection and Analysis

1.4.1 Sample Collection and Processing Shrimp in each replicate were counted and weighed at the start and end of the trial. At termination, feeding was stopped for 24 h, and five shrimp per replicate (15 per group) were randomly selected. After drying the body surface with clean gauze, hemolymph was collected from the cardiac region using anticoagulant-treated sterile syringes. Hemolymph was centrifuged at 4°C and 5000 r/min for 15 min, and the supernatant was stored at -80°C for non-specific immune enzyme analysis. The stomach was immediately dissected on ice and stored at -80°C for digestive enzyme analysis.

1.4.2 Parameter Measurement and Calculation Dietary crude protein was determined by the Kjeldahl method (VELP UDK-142 Automatic Distillation Unit, Italy), crude fat by Soxhlet extraction (SOXTEC 2050 FOSS, Sweden), crude ash by weight loss after incineration in a muffle furnace at (550±20)°C for 9 h, and moisture by direct drying at (105±2)°C for 3 h.

Growth parameters were calculated as follows: - Survival rate (SR, %) = 100 × (final shrimp number / initial shrimp number) - Weight gain rate (WGR, %) = 100 × (final mean weight –initial mean weight) / initial mean weight - Specific growth rate (SGR, %/d) = 100 × (ln final weight –ln initial weight) / culture days - Feed conversion ratio (FCR, %) = 100 × total feed intake / (final total weight –initial total weight) - Daily feeding rate (DFR, %) = 100 × total feed intake / [culture days × (final total weight + initial total weight) / 2]

Enzyme activities were measured using kits from Nanjing Jiancheng Bioengineering Institute for pepsin (PP), cellulase (CL), acid phosphatase (ACP), superoxide dismutase (SOD), and sample protein content (expressed as specific activity). Phenoloxidase (PO) activity was determined using ELISA kits from Shanghai Enzyme-Linked Biotechnology Co., Ltd.

1.5 Statistical Analysis

Data were processed using Excel 2007 and expressed as mean ± SD. One-way ANOVA was performed using SPSS 17.0 software, with Duncan's multiple range test for inter-group comparisons. Significance was set at $P<0.05$.

2. Results

2.1 Effects on Growth Performance

As shown in Table 4 , WGR and SGR in all kelp meal groups exceeded those of the control, with groups D4 and D5 showing significant differences ($P<0.05$). The lowest FCR and DFR occurred in group D5, which were significantly lower than the control and groups D2–D3 ($P<0.05$).

Table 5 reveals that group B exhibited significantly higher WGR and SGR than groups A and C ($P<0.05$), while groups B and C had significantly lower FCR and DFR than group A ($P<0.05$). Group C achieved the highest survival rate, significantly exceeding groups A and B ($P<0.05$), with no significant difference between groups A and B ($P>0.05$).

2.2 Effects on Gastric Digestive Enzyme Activities

Table 6 demonstrates that gastric cellulase activity increased with kelp meal proportion, with groups D5 and D6 significantly higher than other groups ($P<0.05$). Gastric pepsin activity initially increased then decreased, peaking in group D5 and reaching its minimum in group D6, with a significant difference between these two groups ($P<0.05$).

Table 7 shows that groups B and C had significantly lower gastric cellulase activity than group A ($P<0.05$), with group B also significantly lower than group C ($P<0.05$). Gastric pepsin activity was lowest in group B, significantly below groups A and C ($P<0.05$).

2.3 Effects on Serum Non-Specific Immune Enzyme Activities

Table 8 indicates no significant differences in serum ACP activity among groups ($P>0.05$). Serum SOD activity increased then decreased with kelp meal proportion, reaching maximum activity of 276.86 U/mL in group D5, which was significantly higher than all other groups ($P<0.05$). Serum PO activity was highest in group D5 and lowest in group D6, with groups D3–D5 significantly exceeding other groups ($P<0.05$) and group D6 significantly lower than all others ($P<0.05$).

Table 9 shows that groups B and C had significantly higher serum ACP and PO activities than group A ($P<0.05$), with no significant difference between groups B and C ($P>0.05$). Group C exhibited significantly higher serum SOD activity than groups A and B ($P<0.05$), while no significant difference existed between groups A and B ($P>0.05$).

3. Discussion

3.1 Effects of Kelp Meal Proportion on Growth, Digestion, and Immunity

The results indicate that dietary kelp meal supplementation promotes *L. vannamei* growth, with 2–3% inclusion significantly enhancing performance, consistent with Liu et al. [12]. This effect likely stems from kelp meal' s content of protein, minerals, vitamins, and bioactive growth-promoting compounds [13]. Zhou et al. [14] similarly reported that 3% dietary seaweed powder significantly improved WGR and protein efficiency while yielding the lowest FCR in *L. vannamei*.

Digestive enzyme activity reflects digestive capacity in aquatic animals. Li et al. [15] observed that increasing dietary *Enteromorpha* proportion initially enhanced then reduced intestinal cellulase activity in sea cucumbers. In this study, gastric pepsin activity followed a similar pattern, while cellulase activity continuously increased with kelp meal proportion. The rising cellulase activity likely reflects increased dietary fiber stimulating enzyme secretion. However, high fiber content accelerates gut transit [16], potentially impairing pepsin activity at excessive kelp meal levels. Additionally, 2–5% kelp meal groups showed significantly lower DFR, possibly due to reduced palatability at high inclusion levels, consequently decreasing feed intake and pepsin secretion.

Seaweed products have been shown to enhance serum SOD activity in kuruma shrimp [17] and sea cucumbers [18]. As a key antioxidant enzyme, SOD eliminates oxygen free radicals [19], and organisms' stress adaptation depends heavily on antioxidant capacity [20]. Phenoloxidase initiates melanin synthesis to inhibit pathogen proteases and chitinases, serving as an important immune indicator in crustaceans [21]. This study demonstrates that appropriate kelp meal levels elevate serum SOD and PO activities, possibly due to fucoidan release during digestion [22], which possesses immunomodulatory [23], antiviral, and antioxi-

dant properties [24-25]. The significant decline in serum SOD and PO at 5% inclusion may result from excessive polysaccharide concentrations, as high sugar levels can inhibit immune activity [26]. Although ACP, a lysosomal marker enzyme with important immune functions [27-28], showed elevated activity with kelp meal supplementation, the effect was not significant, possibly due to insufficient concentrations of digestion-derived compounds to substantially alter ACP activity.

3.2 Effects of Kelp Meal Enzymatic Hydrolysates on Growth, Digestion, and Immunity

Enzymatic treatment converts macronutrients into more absorbable small molecules. -Glucanase, a cellulase, degrades kelp cellulose into fermentable sugars. Zhang et al. [29] reported that dietary seaweed -1,3-glucan significantly improved *L. vannamei* growth. In this study, -glucanase-treated kelp meal significantly enhanced WGR and SGR while reducing FCR, likely because cellulose hydrolysis provided digestible sugars for energy, reduced gut motility, and conserved energy for growth [30-31].

The significantly lower gastric pepsin and cellulase activities in group B reflect reduced dietary fiber content and diminished stimulation of cellulase secretion. Conversely, group C' s slightly higher pepsin activity than group A may result from protease treatment generating abundant amino acids and peptides that increased substrate concentration and promoted pepsin secretion [32].

Li [33] demonstrated that seaweed polysaccharides enhance immune function, while Liu et al. [34] showed seaweed carbohydrates improve shrimp serum immune enzyme activity. This study confirmed that both enzyme treatments significantly elevated serum ACP and PO activities, with protease treatment also increasing SOD activity, indicating enhanced non-specific immunity. The lower SOD activity in group B may be attributed to high concentrations of reducing sugars inhibiting SOD activity [26,35]. Dietary amino acids are essential for maintaining normal immune function [36], and proteins are absorbed as peptides and amino acids [37] that can enhance immunity [38]. Qiu et al. [39] reported that kelp is rich in amino acids, with enzymatic hydrolysis increasing amino acid content. The 11.3% amino acid generation efficiency in this study likely increased dietary peptides and amino acids, thereby strengthening *L. vannamei* non-specific immunity [40].

Conclusions

Under the experimental conditions: (1) The optimal dietary kelp meal proportion for *L. vannamei* is 3%; (2) -Glucanase hydrolysis of kelp meal promotes growth and enhances non-specific immunity, whereas protease hydrolysis improves non-specific immunity without stimulating growth.

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