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Beta 1,3/1,6-glucan and vitamin C immunostimulate the non-specific immune response of white shrimp (*Litopenaeus vannamei*)

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ABSTRACT

This study mainly evaluated the effects of orally administered beta 1,3/1,6-glucan and vitamin C on the nonspecific immune responses of white shrimp (*Litopenaeus vannamei*). In this study, we found that the white shrimp oral administration with 1 g/kg of beta 1,3/1,6-glucan effectively enhanced O_2^- production and phenoloxidase and superoxide dismutase activity. Shrimp were oral administration with 0.2 g/kg of vitamin C presented beneficial nonspecific immune responses and enzyme activity and also observed in the beta 1,3/1,6-glucan treatment groups. Consequently, we compared the alterations in the immune activity between the beta 1,3/1,6-glucan and vitamin C groups and the evidence illustrated that combination of beta 1,3/1,6-glucan and vitamin C presented an additive effect on inducing the nonspecific immune responses of white shrimp.

1. Introduction

Immune responses of crustaceans are largely dependent on their nonspecific immune activity to resist pathogenic infection [1–3]. To date, no credible evidence has been presented illustrating the specific immunological memory function of crustaceans [4]. The crustacean cellular defence mechanism can be induced by foreign pathogens but only for a short time, with no specific antibody production. Three main types of crustacean haemolymph cells participate in phagocytosis, encapsulation, nodule formation, and cellular cytotoxicity. Crustaceans mainly use haemolymph agglutination, haemolytic factors, lectins (agglutinins), the original prophenoloxidase (proPO) system, and reactive oxygen species to inhibit microbial activity and decomposition [5,6]. To avoid the loss of blood and lymphatic fluid from wounds and the penetration of pathogens from wounds into the body in case of injury to the crustacean exoskeleton, rapid haemolymph coagulation is necessary [7,8]. Evidence indicates that three pathways of hyaline cells result in blood aggregation; type A enables rapid blood cell agglutination but not plasma coagulation. Type B indicated that blood clotting is accompanied by haemagglutination and some plasma coagulation, and type C presented partial cell rupture and that plasmalysis induces slight haemagglutination [9,10]. The haemolymph of horseshoe crab reportedly possesses a blood clotting protein called coagulogen that induces the formation of colloidal plasma [11,12].

According to their morphology and functions, blood cells are divided into hyaline, semi-granular, and granular cells [13]. Hyaline cells are the smallest cells among shrimp blood cells, with a high nuclear–cytoplasmic ratio and major function in phagocytosis. Following hyaline cell phagocytosis, the infection pathogen can induce a reactive oxygen reaction to synthesise reactive oxygen intermediates (ROIs) and produce transglutaminase [14,15]. The nuclear mass ratio of semi-granular cells ranged between that of granulocytes and hyaline cells. Semi-granular cells contain a small number of particles; they mainly participate in cytotoxicity, encapsulation, and activation of the phenol-oxidising enzyme to induce the proPO system [16–18]. Granulocytes contain large numbers of particles and proPO, and they are activated by lipopolysaccharide- and glucan-binding proteins [19,20].

Vitamin C had many different functions in organisms. It role as an antioxidant as a co-factor in enzyme reactions [21]. Research has identified the enhancing immune related protein by treating with the vitamin C in the shrimp [22]. In the *Aeromonas hydrophila* infected *Labeo rohita* (Ham.) experiment, it presented that treated with vitamin C exactly induce the non-specific immune response to against the bacteria infection [23]. Beta-1-3/1–6 glucan, a

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heterogeneous group of glucose polymers, comprises β-(1,3)-linked β-D-glucopyranosyl units with a β-(1,6)-linked side chain of varying distributions and lengths that mainly construct the outer cell walls of many Chinese medicinal mushrooms and fungi [24,25]. These polysaccharides have different chemical compositions, with most belonging to the group of β-1-3/1–6 glucans; these glucans have β-(1 → 3) linkages in their main chain and additional β-(1 → 6) branch points that are required for their bioactive responses [26]. Research has shown that β-1-3/1–6 glucans are pathogen-associated molecular pattern molecules and are recognized by pattern recognition receptors, such as toll-like receptors or nucleotide oligomerisation domain-like receptors, and activate the transcription of proinflammatory genes [27]. Moreover, beta 1,3/1,6-glucan have immunostimulatory activities and enhance wound healing, particularly by increasing macrophage infiltration into injury sites and stimulating tissue regeneration [28,29]. Vitamin C used in pioneering work in the synthesis of collagen species for repairing tissue injury. Evidence has indicated that the lack of vitamin C in shrimp feed results in slow growth, poor feed conversion efficiency, reduced wound healing, and high mortality [30,31].

In multivariate analysis, the Mahalanobis distance (MD) is commonly used for detecting outliers and selecting calibration samples from a large set of measurements [32]. MD considers correlations in data analysis and for calculation uses the inverse of the variance–covariance matrix of the data set of interest. A preliminary study indicated that these features follow a normal distribution, thus allowing the use of the MD as their classification criterion [33].

This study mainly evaluated the effects of orally administered beta 1,3/1,6-glucan and vitamin C on the nonspecific immune responses of white shrimp (*Litopenaeus vannamei*). We measured the immunological parameters, such as the production of superoxide anion (O_2^-) and activity of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), phenoloxidase (PO), and superoxide dismutase (SOD). These data were analysed using the MD to determine the alterations among treatments.

2. Materials and methods

2.1. Animal

White shrimp were obtained from Aquatic Animal Center, National Ocean Taiwan University. After being transferred to the laboratory, the shrimp were cultured until the body weight of each was ranging between 13 and 15 g. They were subsequently divided into 16 groups (n = 40, individuals/each group) and stocked in a 200-L fibreglass-reinforced plastic pond; the temperature was maintained at 25 °C ± 1 °C. The salt content of the cultured sea water was maintained at 32–34 ppt, and the pH was maintained at 7.5 ± 0.5.

3. Experiment design

3.1. Experimental procedure

Experimental shrimp were randomly divided into 16 groups (n = 40). After culturing for 1 week, the groups were fed various concentrations of β-1-3/1–6 glucan or vitamin C; three shrimp from each group were sampled at 0, 1, 2, 4, 8, 16, and 30 days to observe GOT, GPT, and PO activity as well as O_2^- production and SOD alteration.

In this investigation, beta 1,3/1,6-glucan [34], is a purified, water-soluble (1–3), (1–6)-β-glucan derived from the *Ganoderma lucidum* and provided by Super Beta Glucan Inc., Irvine, California, U.S.A. The beta-glucan extracted from the edible mushroom that has been generally recognized as safe (GRAS) under the US Food and Drug Administration (USFDA) regulation.

3.2. Treatment

The experimental feeds were the basic diet of the shrimp supplemented with different concentrations of beta 1,3/1,6-glucan (0, 1, 2, and 4 g/kg) or vitamin C (0, 0.2, 1.0, and 1.5 g/kg), followed by incubation at 20 °C, as shown in Table 1.

3.3. Sample preparation

Haemolymph was drawn from the shrimp abdomen close to the central location of the carapace and was immediately placed into a 1.0 mL microcentrifuge tube. Subsequently, a volume of 100 μL haemolymph was added to 900 μL of anticoagulants, and centrifuged at 14,000 rpm for 5 min. The supernatant was removed and incubated at 4 °C for analysing GOT and GPT activity. The muscle tissue was removed from the shrimp and incubated in a freezer at −20 °C. The hepatopancreas was weighed and placed in a homogeniser with an SOD buffer (1:1). The homogenised liquid was centrifuged at 14,000 rpm at 4 °C to collect the middle layer of the suspension and incubated at −20 °C for SOD analysis.

3.4. Detection of haemolymph oxaloacetate transaminase and glutamate pyruvate transaminase

Haemolymphatic GOT and GPT concentrations were measured using a biochemical analyser (COBAS MIRA Plus ANALYSIS PARAMETER; Roche).

GOT was measured at 546 nm and GPT was measured at 340 nm.

GOT: Asparate + α-ketoglutamate → Glutamate + Oxaloacetate

Table 1
Ingredient and composition of basal diet.

Ingredient	Composition (%)
Fish meal	39.8
Flour	15
Oil[a]	11.6
Corn starch	10
Cellulose	9.6
Dextrin	4.5
Mineral mix[b]	4
Vit premix[c]	4
Choline choride	0.5
Cholesterol	0.5
Vit E	0.2
Astaxanthin	0.1
Vit A	0.1
Vit D_3	0.1

[a] Cod liver oil/corn oil = 2:1.
[b] Thiamin HCl 0.5%, riboflavin 0.8%, niacinamide 2.6%, D-biotin 0.1%, Ca-pantothenate 1.5%, pyridoxin HCl 0.3%, folic acid 0.5%, inositol 18.1%, *para*-aminobenzoic acid 3%, cyanocobalamine 0.1%, BHT 0.1%, α-cellolose 60.3%, ascorbic acid 12.1%, ascorbic acid 12.1%.
[c] Calcium carbonate 2.1%, calcium phosphate dibasic 73.5%, citric acid 0.227%, cupric acid 0.046%, ferric acid (16–17% Fe) 0.558%, magnesium oxide 2.5%, magnesium citrate 0.825%, potassium iodideium sulfate 6.8%, sodium chloride 3.06%, sodium phosphate 2.14%, zinc citrate 0.133%, potassium iodine 0.001%, potassium phosphate dibasic 8.1%.