Aquaculture 306 (2010) 218–224

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of discontinuous administration of β-glucan and glycyrrhizin on the growth and immunity of white shrimp *Litopenaeus vannamei*

Nan Bai, Wenbing Zhang *, Kangsen Mai, Xiaojie Wang, Wei Xu, Hongming Ma

Key Laboratory of Mariculture (Ministry of Education), Ocean University of China, Qingdao 266003, PR China

ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 9 June 2010
Accepted 12 June 2010

Keywords:
Litopenaeus vannamei
β-glucan
Glycyrryhizin
Immunity fatigue

ABSTRACT

A 6-week growth trial was conducted to compare the effects of different feeding strategies of dietary immunostimulants on the growth and immunity of white shrimp *Litopenaeus vannamei* (4.70 ± 0.20 g). Six feeding strategies were set, including feeding immunostimulants-free diet continuously (control), feeding dietary β-glucan or glycyrrhizin continuously, feeding dietary β-glucan discontinuously, feeding dietary β-glucan and glycyrrhizin alternately. The results showed that compared with glycyrrhizin, β-glucan could maintain the immunity of shrimps at a higher level during the experimental period. However, continuously applying β-glucan or glycyrrhizin into the diet caused immunity fatigue in *L. vannamei*. On the 27th day, the total haemocyte count (THC), superoxide anion and superoxide dismutase (SOD) activity of the shrimps fed with β-glucan continuously were no longer significantly higher than those in the control group. Meanwhile, phenoloxidase (PO) activity was no longer significantly higher on the 35th day. THC, PO activity and SOD activity of the shrimps fed with glycyrrhizin were no longer significantly higher than those in the control group on the 25th, 37th, 29th day, respectively. Discontinuous administration of β-glucan or glycyrrhizin could eliminate the immunity fatigue. Shrimps fed with dietary β-glucan 2 days followed by the basal diet for 5 days showed the highest specific growth rate (SGR). It was concluded that this feeding strategy is most suitable for *L. vannamei*.

1. Introduction

In the past few years, shrimp industry has suffered from diseases due to vibrios such as *Vibrio harvey*, *V. damsela* and *V. alginolyticus* (Saulnier et al., 2000), and viruses such as monodon baculovirus virus (MBV), white spot syndrome virus (WSSV) and taura syndrome virus (TSV) (Walker and Mohan, 2009). To solve this problem, several antibiotics were applied (Roque et al., 2001). However, it arose many other problems, such as spread of drug resistant pathogens, negative impact on the environment and risk for human health (Holmström et al., 2003). Under these conditions, immunostimulants were used to boost immune system and improve resistance to infections for shrimp. Several immunostimulants, such as β-glucan, peptidoglycan, lipopolysaccharide, live bacteria and killed bacteria were widely used around the world (Smith et al., 2003). In our previous studies, it has been proved that β-glucan isolated from brewer's yeast slurry (Tan et al., 2004), soybean isoflavones (Chen et al., in press), probiotics isolated from intestine (Li et al., 2009) or culture water (Zhang et al., 2009) and glycyrrhizin (unpublished data) have immuno-enhancement effects on white shrimp *Litopenaeus vannamei*. Moreover, the optimal supplement levels of these immunostimulants in diet were also determined.

Shrimps need several months to grow from larva to adult. Therefore, long-term using of immunostimulants is needed. However, some researches have proved that continuous administration of β-glucan may cause immunity fatigue. For example, Chang et al. (2000) found that continuous administration of β-glucan at the dose of 2000 mg/kg for 40 days to *Penaeus monodon* could elevate the respiratory burst (RB) to the highest level (O.D. 630 nm 0.229) on the 24th day. After the 24th day, however, the RB decreased to the level (O.D.630 nm 0.040) as that in treatment with β-glucan-free basal diet.

Research on the immune system of drosophila revealed that different non-self particles would react with different receptors and stimulate the generation of different immune factors (Hultmark, 2003). Because of the conservation of immune system, it is suggested that alternate administration of different immunostimulants may activate different parts of immune system of shrimps and take advantage of different immunostimulants to solve the problem of immunity fatigue and enhance the immunity continuously. However, there is no report on the discontinuous administration of immunostimulants for shrimps.

In the present work, a six-week feeding trial was conducted to compare the effects of continuous and discontinuous administration of β-glucan or glycyrrhizin, and the alternate administration of these

* Corresponding author. The Key Laboratory of Mariculture, Ministry Education of China, Ocean University of China, Qingdao, Shandong, 266003, China. Tel./fax: +86 532 82032145.
E-mail address: wzhang@ouc.edu.cn (W. Zhang).

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.06.017

two immunostimulants on the growth and immunity of *L. vannamei*, furthermore, to screen the optimal administration strategy. The aim of the present study was to investigate if the discontinuous administration of immunostimulants could eliminate the potential immunity fatigue.

2. Materials and methods

2.1. Experimental design and diets

Based on the nutritional requirements of *L. vannamei* recommended previously (Shiau, 1998), a basal diet was formulated (Table 1). 0.2% β-glucan (Angel Company, Hubei, China; extracted from yeast *Saccharomyces cerevisiae*) and 0.06% glycyrrhizin (Sigma, USA; extracted from glycyrrhiza root) were added into the basal diet, respectively, to prepare the two experimental diets. The dose of β-glucan was according to concentration recommended previously (Liao et al., 1996); the dose of glycyrrhizin was according to our previous work (unpublished data). There were six treatments with different feeding strategies using the basal diet and the two experimental diets, respectively (Table 2). The six feeding strategies were as follows: (1) shrimps were fed the immunostimulant-free basal diet (Control); (2) fed with dietary β-glucan (2000 mg/kg) continuously (Treatment 1); (3) fed with dietary glycyrrhizin (600 mg/kg) continuously (Treatment 2); (4) fed with dietary β-glucan (2000 mg/kg) for 7 days, then with basal diet for 7 days, and so on (Treatment 3); (5) fed with dietary β-glucan (2000 mg/kg) for 2 days, then with basal diet for 5 days, and so on (Treatment 4); (6) fed with dietary β-glucan (2000 mg/kg) for 7 days, then with dietary glycyrrhizin (600 mg/kg) for 7 days, and so on (Treatment 5).

The diets were formulated by thoroughly mixing the dry ingredients with fish oil and then adding cold water until a stiff dough resulted. The stiff dough was then passed through an extruder with a diameter of 1.5 mm. After that, the string-like diets were broken up and sieved into proper pellet size (about 3.0 mm in length). Diets were then stored in plastic bags at −20 °C until use.

2.2. Experimental animals and culture condition

L. vannamei juveniles were bought from a commercial farm in Jiaonan, Qingdao, China and acclimated in a re-circulated seawater system for 2 weeks prior to the feeding trial.

Table 1
Composition of the basal diets (% dry weight).

Ingredients	Percentage
Fish meal[1]	25.00
Shrimp head meal	5.00
Peanut meal	14.00
Squid visceral meal	5.00
Soybean meal	18.00
Fish oil	1.00
Soy lecithin	2.00
Wheat flour	27.53
Choline chloride (50%)	0.30
Stay C[2,3]	0.05
$Ca(H_2PO_4)_2$	0.37
Vitamin premix[3]	0.50
Mineral premix[3]	1.00
Antimycin[3,4]	0.10
Molt hormone[3,5]	0.10
Ethoxyquin[3]	0.05

[1] Crude protein 67.5% (dry weight basis), crude lipid 7.8% (dry weight basis).
[2] Stay C: L-ascorbyl-2-monophosphate (35% ascorbic acid activity, Haffman La Roche, Swiss).
[3] Kindly provided by Qingdao Master Biotechnology Co. Ltd, Qingdao, China.
[4] Contained 50% calcium propionic acid and 50% fumaric acid.
[5] Contained 8% chitin and 10% gentian extract.

Table 2
Groups and feeding strategy.

Treatment abbreviations	Feeding strategy
Control	Shrimps were fed with basal diet continuously
Treatment 1	Shrimps were fed with dietary β-glucan (2000 mg/kg) continuously
Treatment 2	Shrimps were fed with dietary glycyrrhizin (600 mg/kg) continuously
Treatment 3	Shrimps were fed with dietary β-glucan (2000 mg/kg) for seven days and then with basal diet for seven days alternately
Treatment 4	Shrimps were fed with dietary β-glucan (2000 mg/kg) for two days and then with basal diet for five days alternately
Treatment 5	Shrimps were fed with dietary β-glucan (2000 mg/kg) seven days and then with dietary glycyrrhizin (600 mg/kg) for seven days alternately

Three thousand shrimps (initial mean weight 4.70 ± 0.20 g) were randomly distributed to six treatments and each treatment had ten replicates. Each 300-L cylindrical fiberglass tank with 50 shrimps was used as a replicate. The shrimps were fed to apparent satiation four times a day at 06:00, 12:00, 18:00 and 24:00. For each time, the tank was cleaned first to remove the waste matter. Then the air pump was turned off and a certain amount of feeds were put into the tank. One hour later, the air pump was turned on and the uneaten feeds were removed.

During the 6-week feeding trial, water temperature was maintained at 27–29 °C, pH 7.8–8.2, salinity 35‰.

2.3. Experimental procedure

From the 1st day of the experiment, five shrimps in the intermolt stage were randomly chosen from one tank to assay immune parameters every two days. Three tanks in one treatment were randomly chosen at each sampling time point. 100 μl haemolymph was withdrawn from the ventral sinus of each shrimp into a 1-ml sterile syringe containing 200 μl anticoagulant solution (30 mM trisodium citrate, 10 mM EDTA, 0.34 mM sodium chloride 0.12 mM glucose, adjust pH to 7.55 and osmotic pressure to 780 mOsm/kg). The haemolymph from five shrimps in one tank was pooled. The molt stage was determined by the examination of uropoda in which partial retraction of the epidermis could be distinguished (Robsertson et al., 1987). At the end of the 6-week growth experiment, the body weight of remaining shrimps was weighed and the specific growth rate (SGR) was evaluated as follows:

$$\mathrm{SGR}(\%) = [\ln(\text{final weight}) - \ln(\text{initial weight})] / \text{time(day)} \times 100$$

2.4. Immune parameters assay

A drop of the anticoagulant-haemolymph was placed on a Buker hemocytometer to measure total haemocyte count (THC) under optical microscope (XPS-BM-2GA, shanghai BM optical institution manufacture CO. LTD.). The haemocytes were counted manually in all 25 squares (= 0.1 mm^3). A 1-ml anticoagulant-haemolymph sample was centrifuged at 700 × *g* at 4 °C for 10 min, and supernatant was used to measure phenoloxidase (PO) activity and superoxide dismutase (SOD) activity. About 500 μl anticoagulant-haemolymph was used to measure the superoxide anion in haemocytes.

PO was estimated spectrophotometrically by recording the formation of dopachrome using L-3,4 dihydroxyphenylalanine (L-DOPA; Sigma, USA) as substrate according to Hernández-López et al. (1996). Briefly, 50 μl haemolymph supernatant was incubated with 50 μl trypsin (0.1% in CAC buffer: 0.45 M sodium chloride, 0.10 M trisodium citrate, 0.01 M sodium cacodylate, pH 7.0) in 96 wells micro plate at 25 °C for 10 min, and then 50 μl L-DOPA (0.3% in CAC buffer)

was added. The absorbance value was read every 2 min in microplate reader (Model Multiskan spectrum, Thermo, MA, Waltham, USA) at the wavelength of 490 nm for 20 min. Enzyme activity was expressed as the change in absorbance per minute per ml haemolymph supernatant.

Superoxide anion was determined by measuring the blue formazan reduced from nitroblue tetrazolium (NBT) (Muñoz et al., 2000). Briefly, 100 μl haemolymph with anticoagulant solution was deposited on a 96 well microplate previously coated with 100 μl 0.2% poly-L-lysine solution (sigma, USA) to improve cell adhesion. The microplate was centrifuged at 700×*g* for 20 min and the supernatant was removed. 100 μl Hank's balanced salt solution (137.93 mM NaCl, 5.33 mM KCl, 4.17 mM $NaHCO_3$, 0.441 mM KH_2PO_4, 0.338 mM Na_2HPO_4, 5.56 mM Glucose, 10 mM calcium chloride, 3 mM magnesium chloride and 5 mM magnesium sulfate) containing 0.3% NBT (Sigma, USA) was added and allowed to react for 2 h at room temperature. The NBT solution was removed and the haemocytes were fixed with 100 μl absolute methanol. After washing twice with 100 μl of 70% methanol and air-drying, formazan was dissolved in 120 μl 2 M KOH and 140 μl dimethyl sulphoxide (DMSO). The superoxide anion was expressed as the absorption value at wavelength of 630 nm (O.D. 630).

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide anion generated by xanthine and xanthine oxidase reaction system (Wan et al., 2004). One unit of SOD was defined as the amount required inhibiting the rate of xanthine reduction by 50% in the reaction system. Specific activity was expressed as SOD units per ml haemolymph supernatant.

2.5. Statistical analysis

Results are presented as mean ± S.D. (standard deviation of means). SPSS (Version 15.0) programs were used for the statistical analyses. One-way analysis of variance (One-way ANOVA) and Tukey's multiple comparison were used to determine whether significant difference existed between the treatments. All tests used a significance level of $P<0.05$.

3. Results

3.1. Effects of continuous administration of β-glucan or glycyrrhizin on the immunity of L. vannamei

Values of THC ($9.03\pm0.45\times10^6$ cells ml^{-1}, mean ± S.D.) and PO activity (0.62 ± 0.07 U) of the shrimps fed with β-glucan continuously were significantly higher ($P<0.05$) than those in the control group ($7.35\pm0.27\times10^6$ cells ml^{-1} and 0.44 ± 0.08 U, respectively) on the 5th day (Tables 3 and 4). The superoxide anion value (O.D. 630 nm 0.478 ± 0.032) and SOD activity (13.63 ± 0.83 U) were significantly higher ($P<0.05$) than those in the control group (O.D. 630 nm 0.393 ± 0.042 and 11.20 ± 0.16 U, respectively) on the 3rd day (Tables 5 and 6). After the 27th day, the THC, superoxide anion and SOD activity in haemolymph of shrimps fed with β-glucan were no longer significantly higher ($P>0.05$) than those in the control group. However, the activity of PO in β-glucan group (0.69 ± 0.04 U) was significantly higher ($P<0.05$) than that in the control group (0.55 ± 0.04 U) even on the 33rd day.

THC ($8.58\pm0.45\times10^6$ cells ml^{-1}) and PO (0.65 ± 0.07 U) of the shrimps fed with glycyrrhizin were significantly higher ($P<0.05$) than those in the control group ($7.50\pm0.24\times10^6$ cells ml^{-1} and 0.55 ± 0.07 U) on the 13th day (Tables 3 and 4). However, these two immune parameters were no longer significantly higher ($P>0.05$) after the 25th and the 37th day, respectively. Activity of SOD in the shrimps fed with glycyrrhizin was significantly higher ($P<0.05$) than those in control group from the 5th day to the 27th (Table 6). During the

Table 3
Total haemocyte count (10^6 cells per ml haemolymph) of *L. vannamei* fed with the basal diet and diets containing β-glucan or glycyrrhizin under different feeding strategies for 42 days*.

	Sampling time (day)						
Treatment	1	3	5	7	9	11	13
Control[1]	6.68 ± 0.20	6.65 ± 0.35	7.35 ± 0.27^a	7.33 ± 0.56^a	7.05 ± 0.27^a	7.68 ± 0.58^a	7.50 ± 0.24^a
Treatment 1[2]	6.53 ± 0.64	6.30 ± 0.70	9.03 ± 0.45^b	10.03 ± 0.63^b	10.18 ± 0.57^b	10.85 ± 1.31^b	10.25 ± 0.50^c
Treatment 2[3]	6.13 ± 0.27	6.10 ± 0.13	7.40 ± 0.78^a	7.43 ± 0.23^a	7.55 ± 0.86^a	7.95 ± 0.87^a	8.58 ± 0.45^b
Treatment 3[4]	6.75 ± 0.67	6.25 ± 0.54	9.08 ± 0.08^b	9.45 ± 0.34^b	9.25 ± 0.94^b	9.74 ± 0.43^{ab}	9.63 ± 0.40^c
Treatment 4[5]	6.80 ± 0.30	6.29 ± 1.02	9.65 ± 0.38^b	10.40 ± 0.31^b	10.63 ± 0.93^b	10.35 ± 1.05^b	9.98 ± 0.30^c
Treatment 5[6]	6.38 ± 0.74	6.60 ± 0.78	9.38 ± 0.60^b	9.35 ± 0.53^b	10.08 ± 0.62^b	10.60 ± 0.70^b	10.00 ± 0.56^c
	Sampling time (day)						
Treatment	15	17	19	21	23	25	27
Control[1]	7.83 ± 0.23^a	7.65 ± 0.41^a	8.13 ± 0.34^a	8.33 ± 0.43^a	8.39 ± 0.19^a	8.35 ± 0.29^a	8.50 ± 0.57^a
Treatment 1[2]	9.85 ± 0.31^b	9.68 ± 0.58^b	9.60 ± 0.22^c	10.45 ± 1.02^b	9.60 ± 0.53^b	9.55 ± 0.75^{bc}	9.50 ± 0.84^{ab}
Treatment 2[3]	9.35 ± 0.41^b	9.20 ± 0.19^b	8.93 ± 0.33^b	9.20 ± 0.31^b	9.05 ± 0.38^b	8.45 ± 0.45^{ab}	8.91 ± 0.29^a
Treatment 3[4]	9.85 ± 0.57^b	10.88 ± 0.61^{bc}	10.30 ± 0.64^d	10.03 ± 0.96^b	10.09 ± 0.69^{bc}	9.30 ± 0.54^b	10.15 ± 0.75^b
Treatment 4[5]	10.40 ± 0.28^b	11.00 ± 0.48^c	10.20 ± 0.61^{cd}	10.60 ± 0.96^b	11.17 ± 0.52^c	10.78 ± 0.73^c	10.78 ± 0.86^{bc}
Treatment 5[6]	9.93 ± 0.34^b	11.06 ± 0.86^c	11.60 ± 0.70^e	10.73 ± 0.84^b	10.90 ± 0.66^c	10.80 ± 0.61^c	11.50 ± 0.75^c
	Sampling time (day)						
Treatment	29	31	33	35	37	39	41
Control[1]	9.10 ± 0.49^a	8.58 ± 0.65^a	9.15 ± 0.53^a	9.20 ± 0.44^a	9.10 ± 0.19^a	9.38 ± 0.41^a	9.13 ± 0.68^a
Treatment 1[2]	9.20 ± 0.54^a	8.90 ± 0.55^a	8.55 ± 0.41^a	9.43 ± 0.68^{ab}	8.75 ± 0.64^a	8.78 ± 0.49^a	9.00 ± 0.60^a
Treatment 2[3]	8.23 ± 0.53^a	8.13 ± 0.64^a	8.53 ± 0.31^a	9.30 ± 0.27^a	9.20 ± 0.65^a	9.15 ± 0.47^a	8.75 ± 0.30^a
Treatment 3[4]	9.98 ± 0.67^b	9.83 ± 0.20^b	10.25 ± 0.46^b	10.58 ± 0.70^b	10.15 ± 0.56^b	10.68 ± 0.23^b	10.53 ± 0.43^b
Treatment 4[5]	10.32 ± 0.63^b	10.68 ± 0.83^b	10.88 ± 0.40^b	10.18 ± 0.35^b	9.90 ± 0.98^b	10.18 ± 0.37^b	11.28 ± 0.57^b
Treatment 5[6]	11.08 ± 0.48^b	11.13 ± 1.06^b	10.78 ± 0.62^b	10.08 ± 0.36^b	10.05 ± 0.67^b	10.10 ± 0.29^b	10.80 ± 0.49^b

[1] Shrimps were fed with basal diet continuously.
[2] Shrimps were fed with dietary β-glucan (2000 mg/kg) continuously.
[3] Shrimps were fed with dietary glycyrrhizin (600 mg/kg) continuously.
[4] Shrimps were fed with dietary β-glucan (2000 mg/kg) for seven days and then with basal diet for seven days alternately.
[5] Shrimps were fed with dietary β-glucan (2000 mg/kg) for two days and then with basal diet for five days alternately.
[6] Shrimps were fed with dietary β-glucan (2000 mg/kg) seven days and then with dietary glycyrrhizin (600 mg/kg) for seven days alternately.
* Data were expressed as mean ± S.D. Data in the same column with different letters are significantly different ($P<0.05$).

Table 4
Phenoloxidase activity (unit per ml haemolymph supernatant) of *L. vannamei* fed with the basal diet and diets containing β-glucan or glycyrrhizin under different feeding strategies for 42 days *.

	Sampling time (day)						
Treatment	1	3	5	7	9	11	13
Control[1]	0.38 ± 0.08	0.43 ± 0.08	0.44 ± 0.08[a]	0.47 ± 0.08[a]	0.48 ± 0.08[a]	0.52 ± 0.04[a]	0.55 ± 0.07[a]
Treatment 1[2]	0.39 ± 0.07	0.41 ± 0.07	0.62 ± 0.07[b]	0.67 ± 0.07[b]	0.68 ± 0.07[b]	0.63 ± 0.04[b]	0.65 ± 0.08[b]
Treatment 2[3]	0.39 ± 0.05	0.45 ± 0.05	0.47 ± 0.05[a]	0.51 ± 0.08[a]	0.43 ± 0.04[a]	0.44 ± 0.07[a]	0.65 ± 0.07[b]
Treatment 3[4]	0.45 ± 0.03	0.47 ± 0.07	0.68 ± 0.07[b]	0.73 ± 0.07[b]	0.65 ± 0.07[b]	0.70 ± 0.07[b]	0.67 ± 0.05[b]
Treatment 4[5]	0.48 ± 0.05	0.58 ± 0.08	0.57 ± 0.04[b]	0.69 ± 0.02[b]	0.68 ± 0.08[b]	0.70 ± 0.04[b]	0.62 ± 0.02[b]
Treatment 5[6]	0.42 ± 0.05	0.44 ± 0.08	0.65 ± 0.08[b]	0.70 ± 0.08[b]	0.65 ± 0.08[b]	0.67 ± 0.08[b]	0.64 ± 0.04[b]
	Sampling time (day)						
Treatment	15	17	19	21	23	25	27
Control[1]	0.48 ± 0.07[a]	0.49 ± 0.07[a]	0.47 ± 0.08[a]	0.54 ± 0.08[a]	0.49 ± 0.08[a]	0.52 ± 0.08[a]	0.51 ± 0.08[a]
Treatment 1[2]	0.61 ± 0.05[b]	0.64 ± 0.05[b]	0.67 ± 0.07[bc]	0.64 ± 0.07[ab]	0.69 ± 0.07[b]	0.72 ± 0.07[b]	0.71 ± 0.07[b]
Treatment 2[3]	0.66 ± 0.08[bc]	0.67 ± 0.08[bc]	0.58 ± 0.08[ab]	0.69 ± 0.04[b]	0.63 ± 0.04[b]	0.67 ± 0.03[b]	0.74 ± 0.08[b]
Treatment 3[4]	0.75 ± 0.06[c]	0.78 ± 0.04[c]	0.71 ± 0.04[c]	0.81 ± 0.07[c]	0.71 ± 0.07[b]	0.69 ± 0.08[b]	0.72 ± 0.08[b]
Treatment 4[5]	0.67 ± 0.05[bc]	0.64 ± 0.03[b]	0.72 ± 0.05[c]	0.67 ± 0.05[ab]	0.64 ± 0.05[b]	0.72 ± 0.07[b]	0.75 ± 0.07[b]
Treatment 5[6]	0.72 ± 0.07[bc]	0.75 ± 0.07[bc]	0.68 ± 0.07[bc]	0.78 ± 0.08[bc]	0.68 ± 0.08[b]	0.66 ± 0.04[b]	0.69 ± 0.04[b]
	Sampling time (day)						
Treatment	29	31	33	35	37	39	41
Control[1]	0.57 ± 0.08[a]	0.54 ± 0.04[a]	0.55 ± 0.04[a]	0.52 ± 0.04[a]	0.48 ± 0.08[a]	0.49 ± 0.04[a]	0.48 ± 0.02[a]
Treatment 1[2]	0.77 ± 0.07[b]	0.69 ± 0.05[bc]	0.69 ± 0.04[b]	0.61 ± 0.08[ab]	0.51 ± 0.04[a]	0.52 ± 0.07[a]	0.51 ± 0.07[ab]
Treatment 2[3]	0.73 ± 0.04[b]	0.66 ± 0.02[b]	0.68 ± 0.03[b]	0.62 ± 0.07[b]	0.45 ± 0.08[a]	0.48 ± 0.03[a]	0.53 ± 0.08[ab]
Treatment 3[4]	0.77 ± 0.03[b]	0.75 ± 0.08[bc]	0.72 ± 0.08[b]	0.71 ± 0.04[b]	0.69 ± 0.04[b]	0.62 ± 0.04[b]	0.60 ± 0.07[b]
Treatment 4[5]	0.71 ± 0.04[b]	0.78 ± 0.04[c]	0.68 ± 0.04[b]	0.66 ± 0.01[b]	0.69 ± 0.05[b]	0.74 ± 0.07[c]	0.72 ± 0.07[b]
Treatment 5[6]	0.74 ± 0.07[b]	0.72 ± 0.07[bc]	0.69 ± 0.07[b]	0.68 ± 0.03[b]	0.66 ± 0.08[b]	0.69 ± 0.08[bc]	0.57 ± 0.01[b]

[1] Shrimps were fed with basal diet continuously.
[2] Shrimps were fed with dietary β-glucan (2000 mg/kg) continuously.
[3] Shrimps were fed with dietary glycyrrhizin (600 mg/kg) continuously.
[4] Shrimps were fed with dietary β-glucan (2000 mg/kg) for seven days and then with basal diet for seven days alternately.
[5] Shrimps were fed with dietary β-glucan (2000 mg/kg) for two days and then with basal diet for five days alternately.
[6] Shrimps were fed with dietary β-glucan (2000 mg/kg) seven days and then with dietary glycyrrhizin (600 mg/kg) for seven days alternately.
* Data were expressed as mean ± S.D. Data in the same column with different letters are significantly different ($P<0.05$).

Table 5
Superoxide anion (O.D. 630) in haemocytes of *L. vannamei* fed with the basal diet and diets containing β-glucan or glycyrrhizin under different feeding strategies for 42 days *.

	Sampling time (day)						
Treatment	1	3	5	7	9	11	13
Control[1]	0.379 ± 0.059	0.393 ± 0.042[a]	0.371 ± 0.043[a]	0.351 ± 0.087[a]	0.332 ± 0.046[a]	0.364 ± 0.060[a]	0.371 ± 0.020[a]
Treatment 1[2]	0.371 ± 0.035	0.478 ± 0.032[b]	0.545 ± 0.021[b]	0.549 ± 0.065[b]	0.525 ± 0.050[b]	0.510 ± 0.031[b]	0.505 ± 0.030[b]
Treatment 2[3]	0.373 ± 0.060	0.403 ± 0.030[a]	0.402 ± 0.016[a]	0.373 ± 0.023[a]	0.364 ± 0.019[a]	0.376 ± 0.053[a]	0.395 ± 0.030[a]
Treatment 3[4]	0.384 ± 0.043	0.480 ± 0.016[b]	0.522 ± 0.010[b]	0.542 ± 0.021[b]	0.528 ± 0.083[b]	0.518 ± 0.062[b]	0.510 ± 0.028[b]
Treatment 4[5]	0.379 ± 0.061	0.488 ± 0.019[b]	0.531 ± 0.020[b]	0.538 ± 0.090[b]	0.540 ± 0.064[b]	0.565 ± 0.040[b]	0.499 ± 0.041[b]
Treatment 5[6]	0.385 ± 0.045	0.485 ± 0.036[b]	0.527 ± 0.043[b]	0.561 ± 0.073[b]	0.495 ± 0.037[b]	0.520 ± 0.039[b]	0.494 ± 0.057[b]
	Sampling time (day)						
Treatment	15	17	19	21	23	25	27
Control[1]	0.380 ± 0.031[a]	0.390 ± 0.033[a]	0.385 ± 0.042[a]	0.386 ± 0.042[a]	0.375 ± 0.024[a]	0.339 ± 0.050[a]	0.382 ± 0.042[a]
Treatment 1[2]	0.547 ± 0.057[b]	0.505 ± 0.068[b]	0.517 ± 0.042[bc]	0.487 ± 0.036[b]	0.467 ± 0.050[bc]	0.422 ± 0.023[b]	0.396 ± 0.023[a]
Treatment 2[3]	0.395 ± 0.048[a]	0.382 ± 0.040[a]	0.350 ± 0.089[a]	0.387 ± 0.053[a]	0.387 ± 0.044[ab]	0.397 ± 0.046[a]	0.362 ± 0.017[a]
Treatment 3[4]	0.496 ± 0.042[b]	0.603 ± 0.020[c]	0.592 ± 0.047[c]	0.550 ± 0.023[c]	0.551 ± 0.011[d]	0.543 ± 0.029[c]	0.572 ± 0.036[b]
Treatment 4[5]	0.494 ± 0.044[b]	0.513 ± 0.031[c]	0.510 ± 0.031[b]	0.504 ± 0.041[b]	0.521 ± 0.070[c]	0.504 ± 0.042[b]	0.528 ± 0.043[b]
Treatment 5[6]	0.524 ± 0.042[b]	0.526 ± 0.059[c]	0.484 ± 0.039[b]	0.484 ± 0.039[b]	0.515 ± 0.018[c]	0.527 ± 0.052[c]	0.553 ± 0.079[b]
	Sampling time (day)						
Treatment	29	31	33	35	37	39	41
Control[1]	0.372 ± 0.045[a]	0.411 ± 0.013[a]	0.396 ± 0.041[a]	0.408 ± 0.037[a]	0.382 ± 0.029[a]	0.390 ± 0.023[a]	0.382 ± 0.029[a]
Treatment 1[2]	0.395 ± 0.014[a]	0.431 ± 0.014[a]	0.430 ± 0.028[a]	0.419 ± 0.017[a]	0.430 ± 0.020[a]	0.410 ± 0.019[a]	0.394 ± 0.058[a]
Treatment 2[3]	0.361 ± 0.061[a]	0.405 ± 0.020[a]	0.423 ± 0.045[a]	0.426 ± 0.045[ab]	0.412 ± 0.050[a]	0.415 ± 0.077[ab]	0.401 ± 0.016[a]
Treatment 3[4]	0.521 ± 0.055[b]	0.570 ± 0.029[c]	0.559 ± 0.049[b]	0.537 ± 0.017[c]	0.560 ± 0.065[b]	0.564 ± 0.077[c]	0.550 ± 0.052[b]
Treatment 4[5]	0.518 ± 0.033[b]	0.522 ± 0.005[b]	0.515 ± 0.024[b]	0.524 ± 0.079[bc]	0.535 ± 0.057[b]	0.510 ± 0.050[bc]	0.492 ± 0.035[b]
Treatment 5[6]	0.505 ± 0.023[b]	0.526 ± 0.045[bc]	0.534 ± 0.019[b]	0.542 ± 0.016[c]	0.547 ± 0.054[b]	0.515 ± 0.034[c]	0.539 ± 0.020[b]

[1] Shrimps were fed with basal diet continuously.
[2] Shrimps were fed with dietary β-glucan (2000 mg/kg) continuously.
[3] Shrimps were fed with dietary glycyrrhizin (600 mg/kg) continuously.
[4] Shrimps were fed with dietary β-glucan (2000 mg/kg) for seven days and then with basal diet for seven days alternately.
[5] Shrimps were fed with dietary β-glucan (2000 mg/kg) for two days and then with basal diet for five days alternately.
[6] Shrimps were fed with dietary β-glucan (2000 mg/kg) seven days and then with dietary glycyrrhizin (600 mg/kg) for seven days alternately.
* Data were expressed as mean ± S.D. Data in the same column with different letters are significantly different ($P<0.05$).

Table 6
Superoxide dismutase (units per ml haemolymph supernatant) of *L. vannamei* fed with the basal diet and diets containing β-glucan or glycyrrhizin under different feeding strategies for 42 days *.

Treatment	Sampling time (day)						
	1	3	5	7	9	11	13
Control[1]	11.05 ± 0.26	11.20 ± 0.16^{a}	11.31 ± 0.33^{a}	11.24 ± 0.78^{a}	11.52 ± 0.44^{a}	11.41 ± 0.87^{a}	12.02 ± 0.41^{a}
Treatment 1[2]	11.24 ± 0.53	13.63 ± 0.83^{c}	13.53 ± 0.41^{c}	14.11 ± 0.47^{bc}	14.35 ± 0.29^{b}	13.62 ± 0.54^{b}	14.25 ± 0.52^{bc}
Treatment 2[3]	11.32 ± 0.33	11.84 ± 0.72^{ab}	12.63 ± 0.77^{bc}	13.46 ± 0.59^{b}	14.43 ± 0.52^{b}	14.11 ± 0.35^{bc}	14.32 ± 0.34^{b}
Treatment 3[4]	11.18 ± 0.41	12.87 ± 0.59^{bc}	12.66 ± 0.52^{bc}	13.57 ± 0.61^{b}	13.87 ± 0.33^{b}	14.24 ± 0.26^{bc}	15.11 ± 0.41^{c}
Treatment 4[5]	11.31 ± 0.34	13.33 ± 0.72^{c}	13.69 ± 0.77^{c}	14.45 ± 0.26^{c}	13.97 ± 0.11^{b}	14.34 ± 0.13^{c}	14.29 ± 0.34^{b}
Treatment 5[6]	11.71 ± 0.52	13.12 ± 0.35^{bc}	12.76 ± 0.34^{bc}	13.79 ± 0.72^{bc}	13.45 ± 0.77^{b}	13.23 ± 0.59^{b}	13.47 ± 0.52^{b}
	Sampling time (day)						
Treatment	15	17	19	21	23	25	27
Control[1]	11.51 ± 0.47^{a}	11.72 ± 0.29^{a}	12.03 ± 0.59^{a}	11.81 ± 0.52^{a}	11.32 ± 0.83^{a}	11.31 ± 0.44^{a}	11.24 ± 0.87^{a}
Treatment 1[2]	14.11 ± 0.61^{bc}	14.22 ± 0.33^{c}	14.44 ± 0.72^{b}	14.89 ± 0.77^{c}	13.23 ± 0.59^{bc}	12.77 ± 0.29^{b}	12.11 ± 0.54^{ab}
Treatment 2[3]	14.21 ± 0.41^{bc}	14.38 ± 0.59^{c}	14.22 ± 0.29^{b}	14.19 ± 0.59^{bc}	13.67 ± 0.52 ^{cd}	14.02 ± 0.59 ^{cd}	13.24 ± 0.29^{b}
Treatment 3[4]	14.81 ± 0.59^{c}	14.92 ± 0.52^{c}	14.82 ± 0.35^{b}	14.55 ± 0.34^{c}	14.11 ± 0.72^{d}	13.98 ± 0.33^{d}	13.82 ± 0.26^{c}
Treatment 4[5]	14.33 ± 0.72^{bc}	14.32 ± 0.77^{c}	14.39 ± 0.59^{b}	13.21 ± 0.52^{b}	12.88 ± 0.26^{b}	13.21 ± 0.34^{bc}	13.34 ± 0.72^{b}
Treatment 5[6]	13.31 ± 0.77^{b}	13.19 ± 0.59^{b}	13.41 ± 0.33^{b}	13.49 ± 0.72^{bc}	13.81 ± 0.77 ^{cd}	13.57 ± 0.59^{bcd}	13.42 ± 0.33^{bc}
	Sampling time (day)						
Treatment	29	31	33	35	37	39	41
Control[1]	11.58 ± 0.41^{a}	11.81 ± 0.47^{a}	12.29 ± 0.61^{a}	12.31 ± 0.33^{a}	11.82 ± 0.72^{a}	11.74 ± 0.77^{a}	11.63 ± 0.59^{a}
Treatment 1[2]	12.41 ± 0.52^{ab}	12.33 ± 0.61^{ab}	12.36 ± 0.59^{a}	12.22 ± 0.52^{a}	12.42 ± 0.35^{a}	12.11 ± 0.34^{a}	12.09 ± 0.72^{a}
Treatment 2[3]	12.19 ± 0.77^{ab}	12.22 ± 0.54^{ab}	12.41 ± 0.52^{a}	12.56 ± 0.61^{ab}	12.29 ± 0.33^{a}	12.33 ± 0.26^{a}	11.88 ± 0.25^{a}
Treatment 3[4]	13.95 ± 0.41^{c}	14.34 ± 0.59^{c}	14.23 ± 0.29^{c}	14.24 ± 0.59^{c}	13.25 ± 0.52^{b}	13.45 ± 0.83^{b}	13.55 ± 0.44^{b}
Treatment 4[5]	13.42 ± 0.77^{bc}	12.98 ± 0.59^{b}	13.57 ± 0.33^{b}	13.75 ± 0.72^{bc}	13.69 ± 0.77^{b}	13.26 ± 0.59^{b}	13.27 ± 0.29^{b}
Treatment 5[6]	13.46 ± 0.72^{bc}	13.36 ± 0.26^{b}	13.67 ± 0.41^{bc}	13.45 ± 0.59^{bc}	13.25 ± 0.11^{b}	13.43 ± 0.13^{b}	13.31 ± 0.21^{b}

[1] Shrimps were fed with basal diet continuously.
[2] Shrimps were fed with dietary β-glucan (2000 mg/kg) continuously.
[3] Shrimps were fed with dietary glycyrrhizin (600 mg/kg) continuously.
[4] Shrimps were fed with dietary β-glucan (2000 mg/kg) for seven days and then with basal diet for seven days alternately.
[5] Shrimps were fed with dietary β-glucan (2000 mg/kg) for two days and then with basal diet for five days alternately.
[6] Shrimps were fed with dietary β-glucan (2000 mg/kg) seven days and then with dietary glycyrrhizin (600 mg/kg) for seven days alternately.
* Data were expressed as mean ± S.D. Data in the same column with different letters are significantly different ($P<0.05$).

6 weeks, there were no significant differences ($P>0.05$) in the superoxide anion value between the shrimps fed with glycyrrhizin and those in control group (Table 5).

3.2. Effects of discontinuous administration of β-glucan on the immunity of L. vannamei

Overall, compared with those in the control group, the analyzed immune parameters of shrimps fed with β-glucan discontinuously increased to the significant level ($P<0.05$) on the same day as the shrimps fed with β-glucan continuously (Tables 3–6). After the 33rd day, the PO in shrimp fed with β-glucan continuously was no longer significantly higher ($P>0.05$) than that in the control group (Table 4). At the same time, however, the PO activity in shrimp fed with β-glucan discontinuously (Treatments 3 and 4) was still significantly higher ($P<0.05$) than that in the control group (Table 4). The same thing occurred in THC (Table 3), superoxide anion (Table 5) and SOD (Table 6) after the 25th day.

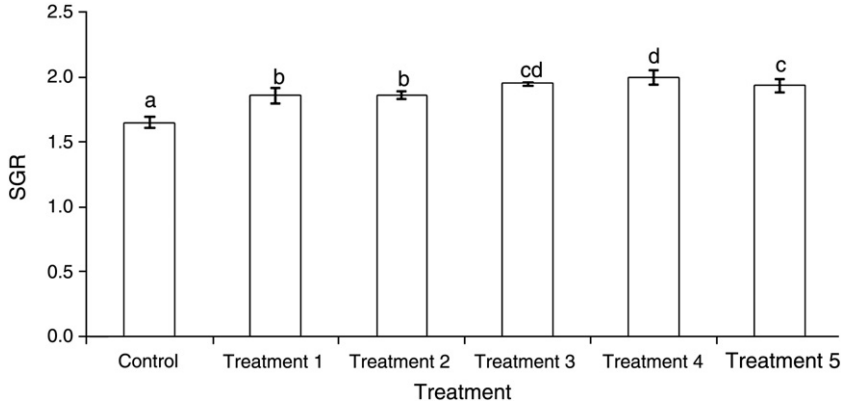

Fig. 1. Specific growth rate (SGR) of *L. vannamei* fed with basal diet (Control) and diets containing β-glucan (2000 mg/kg) or glycyrrhizin (600 mg/kg) under different feeding strategies for 42 days. Treatment 1, shrimps were fed with diet containing β-glucan continuously; Treatment 2, shrimps were fed with diet containing glycyrrhizin continuously; Treatment 3, shrimps were fed with diet containing β-glucan seven days and then with basal diet seven days alternately; Treatment 4, shrimps were fed with diet containing β-glucan two days and then with basal diet five days alternately; Treatment 5, shrimps were fed with diet containing β-glucan seven days and then with diet containing glycyrrhizin seven days alternately. Each bar represents mean value from ten replicates with standard error. Data with different letters are significantly different ($P<0.05$).

When shrimps were fed with β-glucan and glycyrrhizin alternately, the immune parameters had the same changing trend as those fed with β-glucan discontinuously (Tables 3–6).

3.3. The effects of immunostimulants on the growth of *L. vannamei*

As can be seen from Fig. 1, shrimps fed with immunostimulants regardless of continuous or discontinuous feeding showed significantly higher SGR than those in the control group ($P<0.05$). When shrimps were fed immunostimulants alternately, the SGR were significantly higher ($P<0.05$) than that of shrimps fed with β-glucan or glycyrrhizin continuously. The highest SGR (1.9973) was found in the shrimps fed with β-glucan 2 days followed by the basal diet for 5 days (Treatment 4).

4. Discussion

In the present study, continuous oral administration of β-glucan or glycyrrhizin caused the immunity fatigue in *L. vannamei*. The similar phenomenon was also seen by Chang et al. (2000), who fed dietary β-glucan continuously to *P. monodon* for 40 days. In that work, the RB of the shrimps fed with dietary β-glucan first increased to the highest level and at the end of the experiment, the RB was the same as that in shrimps fed with β-glucan-free diet. Smith et al. (2003) demonstrated that the decrease of immune parameters may be due to the consumption of the haemocytes by degranulation. After being eaten, β-glucan may act with the binding pattern-recognition proteins (PRPs) such as β-glucan binding protein (βGBP) (Romo-Figueroa et al., 2004), lipopolysaccharide and β-glucan binding protein (LGBG) (Cheng et al., 2005). Then, protein–glucan complex reacts with the haemocytes and induces degranulation (Vargas-Albores and Yepiz-Plascencia, 2000). These actions reduced the haemocyte quantity, and decreased the values of PO activity, superoxide anion and SOD activity (Smith et al., 2003). In the present study the decrease of PO activity took place later than that of THC. It is probably due to the prophenoloxidase (proPO), not phenoloxidase, released with the degranulation (Sritunyalucksana and Söderhäll, 2000). Prophenoloxidase could be cleaved to the activated phenoloxidase at sequence Arg90-Asp91 by prophenoloxidase activating enzyme (PPA) (Lai et al., 2005). It is supposed that there was still some proPO existing in the hemolymph when THC decreased. The proPO could show PO activity after being activated. These could be used to explain, in the present study, why THC, PO activity and SOD activity in hemolymph of shrimp have different changing trends at the different time intervals.

The present study proved that discontinuous feeding of β-glucan could eliminate the immunity fatigue in *L. vannamei*. The durative enhancement of immune parameters suggested that discontinuous administration of β-glucan could keep the balance of haemocytes consuming and compensating well and maintain the immunity of shrimps at a high level during a long period. When shrimps were fed with β-glucan, the haemocytes degranulated and released immune factors, and then the shrimps' haemocytes may have compensated when they were fed with β-glucan-free basal diet. When shrimps were fed with β-glucan and glycyrrhizin alternately, in the present study, the up-regulated immunity didn't show decrease during the experiment, thus indicating alternate use of two classes of immunostimulants could also eliminate the immunity fatigue.

In the present study, dietary β-glucan could improve the growth of *L. vannamei*. This is in agreement with the results from previous studies in several fish species (Sakai, 1999) and shrimps (Smith et al., 2003). However, it is still unclear that how the β-glucan improved the growth. One hypothesis is that eaten β-glucan could induce local intestinal inflammatory response and increase resistance against pathogens. The pathogen would otherwise result in decreased weight gain and maybe disease (Dalmo and Bøgwald, 2008). The beneficial effects of glycyrrhizin on growth performance of *L. vannamei* have also been found in our previous work (unpublished data). From the results of the present study, alternate feeding with β-glucan and glycyrrhizin could take advantage of these two immunostimulants to increase the growth of shrimp. However, discontinuous administration of immunostimulants (Treatments 3 and 4) showed higher SGR than continuous and alternate patterns (Fig. 1). Concerning that shrimps in treatment 4 showed no immunity fatigue and higher SGR, we concluded that feeding shrimps with dietary β-glucan for 2 days and then with immunostimulants-free diet for 5 days is most suitable for *L. vannamei*. Further study is needed to explain why and how this feeding strategy improves the growth of *L. vannamei*.

Acknowledgements

This work was financially supported by the National High-tech Research and Development Program of China (No. 2006AA100313), and the National Public Sector Funding for Special Research Project (No. 200803012). We thank Mr. Chao Liang and Lin Zhu for their help in immune parameters assay and Miss Min Gu for her assistance in experiment and revision for this manuscript.

References

Chang, C.F., Chen, H.Y., Su, M.S., Liao, I.C., 2000. Immunomodulation by dietary β-1, 3 glucan in the brooders of the black tiger shrimp *Penaeus monodon*. Fish Shellfish Immunol. 10, 505–514.

Chen, X.R., Tan, B.P., Mai, K.S., Zhang, W.B., Wang. X.J., Ai, Q.H., Liufu, Z.G., in press. Dietary administration of soybean isoflavones enhances the immunity of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Aquacult. Nutr. doi:10.1111/j.1365-2095.2009.00702.x.

Cheng, W., Liu, C.H., Tsai, C.H., Chen, J.C., 2005. Molecular cloning and characterization of a pattern recognition molecule, lipopolysaccharide- and β-1, 3-glucan binding protein (LGBP) from the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 18, 297–310.

Dalmo, R.A., Bøgwald, J., 2008. β-glucans as conductors of immune symphonies. Fish Shellfish Immunol. 25, 384–396.

Hernández-López, J., Gollas-Galván, T.S., Vargas-Albores, F., 1996. Activation of the prophenoloxidase system of the brown shrimp (*Penaeus californiensis* Holmes). Comp. Biochem. Physiol. C 113, 61–66.

Holmström, K., gräslund,, S., Wahlström, A., Poungshompoo, S., Bengtsson, B.E., Kautsky, N., 2003. Antibiotic use in shrimp farming and implications for environmental impacts and human health. Int. J. Food Sci. Technol. 38, 255–266.

Hultmark, D., 2003. *Drosophila* immunity: paths and patterns. Curr. Opin. Immunol. 15, 12–19.

Lai, C.Y., Cheng, W., Kuo, C.M., 2005. Molecular cloning and characterization of prophenoloxidase from hamocytes of the white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 18, 417–430.

Li, J.Q., Tan, B.P., Mai, K.S., 2009. Dietary probiotic *Bacillus* OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp *Litopenaeus vannamei*. Aquaculture 291, 35–40.

Liao, M.S., Chang, C.F., Her, B.Y., Kojima, T., 1996. Enhancement of the resistance of grass prawn *Penaeus monodon* against *Vibrio damsela* infection by beta-1, 3-glucan. J. Fish Soc. Taiwan 23, 109–116.

Muñoz, M., Cedeño, R., Rodríguez, J., Van der Knaap, W.P.W., Mialhe, E., Bachère, E., 2000. Measurement of reactive oxygen intermediate production in haemocytes of the penaeid shrimp, *Penaeus vannamei*. Aquaculture 191, 89–107.

Robsertson, L., Bray, W., Leung-Trujillo, J., Lawrence, A., 1987. Practical molt staging of *Penaeus setiferus* and *Penaeus stylirostris*. J. World Aquac. Soc. 18, 180–185.

Romo-Figueroa, M.G., Vargas-Requena, C., Sotelo-Mundo, R.R., Vargas-Albores, F., Higuera-Ciapara, I., Söderhäll, K., Yepiz-Plascencia, G., 2004. Molecular cloning of a β-glucan pattern-recognition lipoprotein from the white shrimp *Penaeus (Litopenaeus) vannamei*: correlations between the deduced amino acid sequence and the native protein structure. Dev. Comp. Immunol. 28, 713–726.

Roque, A., Molina-Aja, A., Bolán-Mejía, C., Gomez-Gil, B., 2001. In vitro susceptibility to 15 antibiotics of vibrios isolated from penaeid shrimps in Northwestern Mexico. Int. J. Antimicrob. Agents 17, 383–387.

Sakai, M., 1999. Current research status of fish immunostimulants. Aquaculture 172, 63–92.

Saulnier, D., Haffner, P., Goarant, C., Levy, P., Ansquer, D., 2000. Experimental infection models for shrimp vibriosis studies: a review. Aquaculture 191, 133–144.

Shiau, S.Y., 1998. Nutrient requirements of penaeid shrimps. Aquaculture 164, 77–93.

Smith, V.J., Brown, J.H., Hauton, C., 2003. Immunostimulation in crustaceans: does it really protect against infection? Fish Shellfish Immunol. 15, 71–90.

Sritunyalucksana, K., Söderhäll, K., 2000. The proPO and clotting system in crustaceans. Aquaculture 191, 53–69.

Tan, B.P., Zhou, Q.C., Zheng, S.X., Liu, L.H., 2004. Effects of dietary β-1, 3/1, 6-glucan on growth and immunity in *Litopenaeus vannamei* juveniles. High Tech. Lett. 5, 73–77 (In Chinese with English abstract).

Vargas-Albores, F., Yepiz-Plascencia, G., 2000. Beta glucan binding protein and its role in shrimp immune response. Aquaculture 191, 13–21.

Walker, P.J., Mohan, C.V., 2009. Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. Rev. Aquac. 2, 125–154.

Wan, M., Mai, K., Ma, H., Xu, W., Liufu, Z., 2004. Effects of dietary selenium and vitamin E on antioxidant enzyme activities in abalone, *Haliotis discus hannai* Ino. Acta Oceanol. Sin. 28, 51–58.

Zhang, L., Mai, K.S., Tan, B.P., Ai, Q.H., Qi, C.Z., Xu, W., Zhang, W.B., Liufu, Z.G., Ma, H.M., Wang, X.J., 2009. Effects of dietary administration of probiotic *halomonas* sp. B12 on the intestinal microflora, immunological parameters, and midgut histological structure of shrimp, *Fenneropenaeus chinensis*. J. World Aquac. Soc. 40, 58–66.