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Original Article

Effect of inactive yeast cell wall on growth performance, survival rate and immune parameters in Pacific White Shrimp (*Litopenaeus vannamei*)

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Abstract

Effects of dietary inactive yeast cell wall on growth performance, survival rate, and immune parameters in pacific white shrimp (*Litopenaeus vannamei*) was investigated. Three dosages of inactive yeast cell wall (0, 1, and 2 g kg^{-1}) were tested in three replicate groups of juvenile shrimps with an average initial weight of 7.15±0.05 g for four weeks. There was no significant difference in final weight, survival rate, specific growth rate, feed conversion ratio, feed intake, protein efficiency ratio, and apparent net protein utilization of each treatments. However, different levels of inactive yeast cell wall showed an effect on certain immune parameters ($p<0.05$). Total hemocyte counts, granular hemocyte count, and bacterial clearance were better in shrimp fed diets supplemented with 1 and 2 g kg^{-1} inactive yeast cell wall as compared with the control group.

Keywords: inactive yeast cell wall, white shrimp, *Litopenaeus vannamei*, growth, survival, immune parameters

1. Introduction

The application of antibiotics in aquaculture has become limited in recent years due to its residues in products and consumer health concerns leading to a search for an alternative prevention and irradicaton methods for disease control. The use of potential natural or synthetic compounds to activate immune responses and enhance disease resistance of cultured aquatic animals has received increasing attention. Many types of immunostimulants such as synthetic chemicals, biological substances, hormones, and cytokines have been reported to increase resistance to baterial, viral, and fungal diseases in teleost fish and shellfish (Raa, 1996; Sakai, 1999). Natural immunostimulants have been used as dietary supplements in aquaculture to reduce disease occurrence via activation of organism's innate immune responses as well as improving digestibility of various dietary substances. Brewer's yeast is a natural feedstuff that contains beta glucans, chitin, mannoprotein, and nucleic acids, all of which have been found to have immunostimulatory effects in fish and shrimp (Raa, 2000; Fisher *et al*., 2001; Gatlin and Li, 2004). Recent studies in Pacific white shrimp (*L. vannamei*) showed that supplementation of yeast at 0.5% of diet improved resistance to *Vibrio penaeicida* (Burgents *et al.*, 2004), indicating a potential use to reduce common diseases such as those caused by *Vibrio* spp., which is a serious problem in all cultured species. Moreover, Hybrid striped bass (*Morone chrysops* × *M. saxatilis*) fed the diet supplemented with 2% brewers yeast were found to have significantly higher blood neutrophil oxidative radical and extracellular superoxide anion production of head kidney macrophages than that in

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the control fish (Li and Gatlin, 2003).

Inactive yeast cell wall produced commercially is a prebiotic additive for animal feed rich in mannan-oligosaccharide (MOS) and beta glucan extracted from *Saccharomyces cerevisiae*. Results in shrimp (*P. semisulcatus*) showed that MOS is an indigestible carbohydrate that stimulates growth and activity of beneficial bacteria in the intestine. With an oral application by using 3.0gkg^{-1} mannan-oligosaccharide, it enhanced growth performance and feed conversion ratio of shrimp during 48 days rearing period (Genc *et al*., 2007). Similar results have also been found in the larval stages of European lobster (*Homarus gammarus)* culture (Taylor, 2005). Moreover MOS and beta glucan can synergistically activate the innate immune responses of cultured organisms when used as a dietary supplement. When fed to black tiger shrimp (*P. monodon*) at 0.2% glucan from spent brewer's yeast preparation for 3 days, significant increases in phenol oxidase, number of haemocytes and the bacterial killing activity against *V. harveyi* were observed (Thanardkit *et al*., 2002). Furthermore, MOS supplemented at 20 ppt to lobster larval feed from hatching through to stage IV, significantly decreased mortality with a higher success rate to stage IV, V and VIII of growth (Daniel *et al*., 2006).

The objective of this study was to evaluate an effect of inactive yeast cell wall on growth performance, survival rate, and immune parameters in Pacific white shrimp (*L. vannamei*).

2. Material and Method

2.1 Experimental animals

Pacific white shrimp larvae (*L. vannamei*) were nursed in a concrete tank until attaining an average weight of approximately 6-7 g / shrimp, then sorted for use in the trial.

2.2 Expeimental diets

A practical shrimp diet (38% protein) containing fishmeal, soybean meal, wheat flour, wheat gluten, fish oil, and 1% vitamin-mineral premix as a basal ingredients was used (Table 1). Experimental diets were supplemented with three dosages of inactive yeast cell wall at 0, 1, and 2 g kg^{-1}.

2.3 Experimental system

Fifteen shrimps were stocked into each of three experimental tanks of 180 L capacity per treatment. The experimental tanks had constant aeration and 50% of the water exchanged daily. The shrimp were fed experimental

Table 1. Composition of experimental diets (% as-fed basis)

Ingredient	Inactive yeast cell wall supplemented diet		
	T1: control	T2: 1 g kg^{-1}	T3: 2 g kg^{-1}
Dehulled Soybean meal	30.00	30.00	30.00
Wheat flour	26.75	26.65	26.55
Fishmeal	29.47	29.47	29.47
Wheat gluten	3.00	3.00	3.00
Lecithin	2.03	2.03	2.03
Squid meal	2.03	2.03	2.03
Carboxy methyl celloluse	2.00	2.00	2.00
Vitamin & Mineral Premix*	1.00	1.00	1.00
Fish oil	1.70	1.70	1.70
Potassium chloride	0.71	0.71	0.71
Blood meal	0.68	0.68	0.68
Choline chloride	0.27	0.27	0.27
Monopotassium phosphate	0.24	0.24	0.24
Fylax (Antimold)	0.10	0.10	0.10
Ethoxyquin	0.02	0.02	0.02
Inactive yeast cell wall	0.00	0.10	0.20

*Vitamin & Mineral premix (values given per kg premix): vitamin A 2.5 g, vitamin D3 2.4 g, a-tocopherol acetate 2 g, menadione 2 g, thiamin HCl 2.5 g, riboflavin 2.5 g, Ca-pantothenate 5 g, pyridoxine HCl 2.0 g, niacin 10.0 g, folic acid 0.75 g, biotin 0.30 g, vitamin B_{12} 0.004 g, inositol 10.0 g, $Ca(H_2PO_4).H_2O$ 245.74 g, $NaH_2PO_4.2H_2O$ 84.06 g, $MgSO_4.7H_2O$ 88.38 g, KIO_4 0.04 g, $FeSO_4.7H_2O$ 64.53 g, $ZnSO_4.7H_2O$ 7.51 g, $CuSO_4.5H_2O$ 19.92 g, $MnSO_4.4H_2O$ 1.42 g, $CoCl.6H_2O$ 0.02 g.

diets four times a day at 8.00, 12.00, 16.00, and 20.00 h. Feed consumption for twenty-eight days was recorded and feces was removed on a daily basis.

2.4 Growth, survival rate and feed utilization

Final body weight, average daily growth (ADG), specific growth rate (SGR), feed intake (FI), and survival rate of shrimps were recorded during four-week rearing. Thirty shrimps at the beginning and five shrimps from each experimental tank at the termination were sampled for protein composition analysis according to AOAC (1990) to determine apparent net protein utilization (ANPU).

2.5 Total hemocyte count

Fifty microliters of hemolymph were withdrawn from the ventral sinus of each shrimp from five shrimps per tank into a syringe and transferred to Eppendorf tube containing 450 ìl trypan blue solution. A drop of haemolymph in trypan blue solution was placed on hemocytometer and a total haemocyte count (THC) was determined using a stereomicroscope (Supamattaya *et al*., 2000c).

2.6 Granular hemocyte count

Hemolymph (0.1ml) from the ventral sinus of the same groups of shrimp used for THC was withdrawn into a syringe and transferred to Eppendorf tube containing 0.1 ml fixative (10% formalin in 1.5% NaCl). After 10 minutes, 20 µl of the fixed hemocyte suspension was mixed with 20 µl of Rose Bengal solution (1.2% Rose Bengal in 50% ethanol) and incubated at ambient temperature (27-35^{o}C) for 20 min and smeared on microscope slides. Then the smears were completely dried before counterstaining with hematoxylin solution (50 g potassium alum, 1 g hematoxylin crystals, 0.2 g sodium iodate, 1 g citric acid, 50 g choral hydrate, and distilled water to 1 l) for 4-7 min. The slides were then rinsed with tap water for 10 min followed by dehydration with 95% ethanol and 100% ethanol. After dehydration, the slides were submerged in xylene before mounting with permount and covered with coverglass. The proportion of granula hemocyte in 200 hemocytes was recorded and this portion was calculated as percentage of hemocyte (Sritunyalucksana *et al*., 2005).

2.7 Bacterial clearance

To determine bacterial clearance, fifteen shrimps from each treatment were injected intramuscularly at the 6th abdominal segment with 10 µl of a suspension of *Vibrio harveyi* containing 10^8 cells/ml. Hemolymph was collected from the ventral sinus into a syringe after 3 hours and serially diluted 10 folds in 1.5% NaCl solution before 20 µl of hemolymph was dropped onto a plate of thiosulphate citrate bile-salt (TCBS) agar to obtain bacterial counts after incubation at 30^{o}C for 10 hours.

2.8 Statistical analysis

Data were analyzed statistically using one-way ANOVA. Differences among means were compared using Duncan's Multiple Range Test at $p < 0.05$.

3. Results

After the four-week feeding trial, there was no significant difference in final body weight and survival rate of the shrimps ($p>0.05$). The results showed that 2 g kg^{-1} inactive yeast cell wall supplemented diet had the best SGR, ADG, and FCR, but not significantly different from that of the control. Protein efficiency ratio (PER) and ANPU are shown in Table 2. A higher ANPU was noted for the shrimp given diet supplemented with 2 g kg^{-1} inactive yeast cell wall than those given diet supplemented with 1 g kg^{-1} inactive yeast cell wall and the control shrimp. PER and ANPU among the treatment groups were similar ($p>0.05$). Survival of shrimps fed with control diet, containing 1and 2 g kg^{-1} inactive yeast cell wall, was 100%.

3.1 Total hemocyte count and Granular hemocyte count

The total hemocyte counts in the 2 g kg^{-1} inactive yeast cell wall group ($13.08\times10^6 \pm 5.12\times10^6$ cell/ml) were significantly higher than those in the 1 g kg^{-1} inactive yeast cell wall group ($9.59\times10^6 \pm 3.08\times10^6$ cell/ml) and control group ($8.10\times10^6 \pm 2.57\times10^6$cell/ml) ($p<0.05$) (Figure1). Moreover, numbers of granular hemocytes in inactive yeast cell wall supplemented groups were significantly different from the control (Figure 2). These results showed that total hemocyte and granular hemocyte could be increased by feeding of inactive yeast cell wall supplemented diets.

3.2 Bacterial Clearance activity

Shrimp fed inactive yeast cell wall supplemented diets showed a significant bacterial clearance activity. The number of bacterial cells in shrimp haemolymp following pre-injection and 3 hr post-injection are shown in Figure 3.

4. Discussion

In the present study, the yeast supplemented diet fed groups showed a slightly better growth, but they were not significantly different from that of the control group. However, they showed a significant increases of total hemocyte, granular hemocyte, and bacterial clearance activity. The protective effect of the yeast supplemention might be attributed to its mannan-oligosaccharide and glucan content in inactive yeast cell wall. Many variables such as total plasma protein content, glucose concentration, alkaline phosphatase activity, clotting time, haemocyte count, prophenoloxidase

Table 2. Performance parameters and feed utilization[1] of *L. vannamei* fed control diet and diets supplemented with inactive yeast cell wall at two levels.

Parameter	Inactive yeast cell wall supplemented diet		
	T1: control	T2: 1 g kg^{-1}	T3: 2 g kg^{-1}
Initial weight (g)	7.15±0.05 ^{NS}	7.15±0.04 ^{NS}	7.16±0.03 ^{NS}
Final weight (g)	11.21±0.95 ^{NS}	11.51±1.57 ^{NS}	12.28±0.68 ^{NS}
Weight gain (%)	4.05±0.92 ^{NS}	4.37±1.58 ^{NS}	5.13±0.70 ^{NS}
FCR[2]	2.19±0.57 ^{NS}	2.23±0.74 ^{NS}	1.75±0.23 ^{NS}
SGR[3] (%)	1.59±0.30 ^{NS}	1.68±0.49 ^{NS}	1.92±0.21 ^{NS}
FI[4] (%)	3.57±0.23 ^{NS}	3.54±0.35 ^{NS}	3.37±0.20 ^{NS}
PER[5]	1.17±0.26 ^{NS}	1.26±0.44 ^{NS}	1.48±0.19 ^{NS}
ANPU[6]	15.77±5.06 ^{NS}	19.49±8.75 ^{NS}	26.54±3.93 ^{NS}

[1] Mean ± SD of three replicates and there is no difference among means under each parameter.

[2] Feed conversion ratio = total feed consumption (g) /fish weight gain (g)

[3] Specific growth rate = $\ln W_2 - \ln W_1$ / days, W_1= initial weight (g), W_2= final weight (g).

[4] Feed intake = (f*100) / [$(W_1+W_2/2)^* (N_1+N_2/2)^*t$], f = dry feed consumption, W_1 = average initial weight, W_2= average final weight, N_1= number of fish at the beginning of the experimental, N_2 = number of fish at the end of the experimental and t = days of rearing periods.

[5] Protein efficiency ratio = weight gain (g) / protein intake (g).

[6] Apparent net protein utilization = (B –Bo)/I , B = body nitrogen of fish at termination, Bo = body nitrogen of the fish at the beginning of the experiment, I = nitrogen intake.

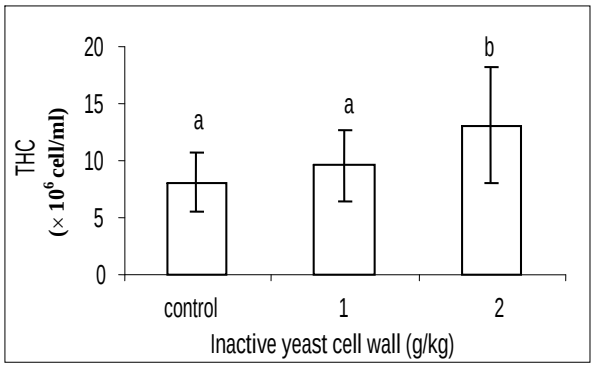

Figure 1. Total hemocyte counts of pacific white shrimp following 4 weeks feeding with control diet, diets containing 1 and 2 g kg^{-1} inactive yeast cell wall. Data are mean ± SD of total hemocyte. Alphabets indicate significance of the data compared with the control based on Duncan's multiple range test ($p<0.05$).

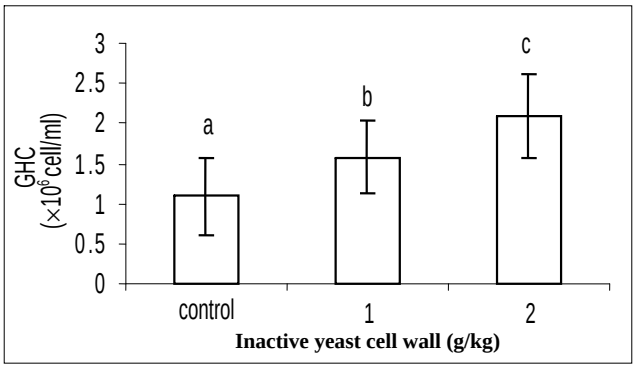

Figure 2. Granular hemocyte counts of pacific white shrimp following 4 weeks feeding with control diet or diet containing 1 or 2 g kg^{-1} inactive yeast cell wall. Data are mean ± SD of granular hemocyte. Alphabets indicate significance of the data compared with the control based on Duncan's multiple range test ($p<0.05$).

(proPO) activity, phagocytic index, release of reactive oxygen intermediates, and antibacterial activity have been considered as good health parameters in crustaceans (Rodriguez and Moullac, 2000, Supamattaya *et al.*, 2000a, Supamattaya *et al.*, 2000b, Supamattaya *et al.*, 2000c). Mannan-oligosaccharide and glucan were reported to enhance disease resistance by stimulating non specific components of the immune system or by improving processing and presentation of antigens during specific adaptive immune responses (Dobrescu, 2002). For example, the protective effects of MOS in crustaceans have been shown to be associated with the granular cells having the ability to binding to mannose and to be involved in triggering cellular and humoral defense mechanism of this animal in response to a

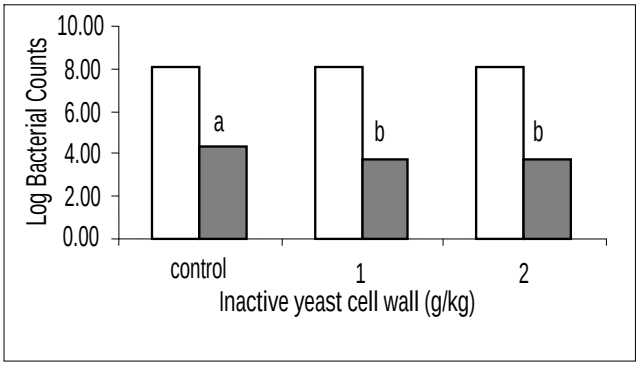

Figure 3. Bacterial clearance by shrimp fed control diet, diets supplement with 1 and 2 g kg^{-1} inactive yeast cell wall (White bars show the number of bacterial cell counts in suspension of pre-injection and black bars show the number of bacterial cell counts in shrimp haemolymph after 3 hours post-injection). Alphabets indicate significance of the data compared with the control based on Duncan's multiple range test ($p<0.05$).

pathogenic challenge. The bacterial clearance as a summative measure of humoral and cellular defense capacity and it would be useful in rapid, preliminary screening of dietary components for the potential ability to improve defense capacity. The percentage of granular cells and the rate of bacterial clearance for the yeast extract were higher than those for shrimp fed the control diet (Srithunyalucksana *et al*., 2005). The results of the present study indicated better performance and improved resistance against diseases. This may result from the effect of β-glucan of the yeast cell wall component, which has been reported to have immunostimulating activity for shrimp (Liao *et al*., 1996; Chang *et al*., 1999). Moreover Burgents *et al*. (2004) fed the dietary administration of Diamond V XP Yeast Culture® that can protect shrimps against a decline in resisitance to bacterial disease. Fisher *et al*. (2001) reported that mannan-oligosaccharide added at either 2 or 4 g kg^{-1} improved immune capacity of the white shrimp *L. vannamei*, cultivated at low or normal salinity. Similar effects have been described for fish, β-glucan has been reported to enhance the production of antibodies against pathogens (Raa *et al.*, 1992). Nile tilapia fed diets containing mannan-oligosaccharide expressed elevated respiratory burst activity (Craig and McLean, 2003) and European seabass fed mannan-oligosaccharide at 0.4 % inclusion had a significantly improved phagocytic index, NBT positive cells and were better able to resist challenge infection (Torrecillas *et al*., 2006). Data from trials with shrimp and fish clearly suggest that mannan-oligosaccharide and β-glucan supplemented in diets improved immune responses.

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