

Effects of Yeast Cell Debris on Growth, Survival and Disease Resistance of Pacific White Shrimp *Litopenaeus vannamei*

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ABSTRACT

A 60-day growth trial was conducted with Pacific white shrimp (*Litopenaeus vannamei*) post-larvae (PL12) to study the use of yeast cell debris with three different formulations. Seven treatment diets were designed with yeast cell debris containing 45, 38 and 56% crude protein as formula A, B and C, respectively. Each formulation had two concentrations of yeast cell debris (1 and 5%). Commercial shrimp feed was used as the control. After 60 days of dietary administration, shrimp fed with 5% yeast cell debris of formula C had the highest average body weight, which was significantly different among all treatments. The survival rate of shrimp fed with yeast cell debris in all three formulations was higher than that of the control group. Disease resistance after challenge with a virulent strain of *Vibrio harveyi* (LD₅₀ in 48 h) altered the survival rate in shrimp fed with 5% yeast cell debris of formula C to a significantly higher level than that of the control group. The results from this study suggest that the dietary administration of 5% yeast cell debris of formula C effectively enhanced growth and survival of *L. vannamei* postlarvae.

Keywords: Yeast cell debris, Non-specific immune, Pacific white shrimp, Challenged, *Vibrio harveyi*

INTRODUCTION

Currently, Pacific white shrimp, *Litopenaeus vannamei*, native to the Pacific coast of Central and South America, is the major shrimp species cultured in China, Taiwan and Thailand (Limsuwan and Chanratchakool, 2004). Since 2001, shrimp farmers have experienced disease problems

associated with production declines in farmed *L. vannamei* (Chen *et al.*, 2005). Many scientists have attempted to solve the disease problems by enhancing non-specific immune response, the main internal defense mechanism in shrimp. Use of immunostimulants is another approach used to increase shrimp immunity against diseases (Purivirojkul *et al.*, 2006). Immunostimulants have been

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reported to increase resistance to some infectious diseases in teleost fish and shellfish (Raa, 1996; Sakai, 1999) by enhancing the non-specific immune system, the set of defenses directed against all potentially invasive, disease-causing organisms. Two types of immunostimulants have received the most attention in shrimp aquaculture: (1) fragments of bacterial cell walls, such as lipopolysaccharide (LPS) and (2) beta-glucans from one of several fungal or algal species (Raa, 1996). In recent years, the biotechnology industry has developed a number of microbial preparations to improve pond water quality, to enhance shrimp growth and survival, or to decrease shrimp disease (Aguirre-Guzma'n, 1994; Gatesoupe, 1999). Microbial preparations can compete for intestinal space and modify the microbial flora, decrease the presence of non-beneficial bacteria, and increase enzyme production, nutrient assimilation, or digestion (Conway, 1990; Roques and Dussert, 1991). The growth rate, survival, and feed conversion ratio can therefore improve by inclusion of these probiotic agents (Gatesoupe, 1991a,b; Douillet and Langdon, 1994; Garriques, 1995). Intensive shrimp farm feeding management is becoming more and more important because feed cost is about 40-50% of the total cost for the intensive culture.

Therefore, yeast cell debris is an alternative to decrease costs of production and has high protein at a low price with the benefits of increasing growth, survival rate and immune characteristics of white shrimp. We hypothesized that yeast cell debris might be useful as a growth promoter and immunostimulant in shrimp culture. The aim of the present study was to examine the optimum concentration of three different

formulae of yeast cell debris on growth, survival and tolerance of Pacific white shrimp to *Vibrio harveyi* in laboratory conditions.

MATERIALS AND METHODS

Experimental animals

Litopenaeus vannamei postlarvae (PL9) were obtained from a commercial hatchery in Chachoengsao province, Thailand. A total of 1,200-1,500 shrimp from the hatchery were transported and acclimated in fiberglass tanks at the Aquaculture Business Research Center laboratory, Faculty of Fisheries, Kasetsart University. After 3 days of acclimatization, the shrimp postlarvae (now PL12) were stocked in 21 experimental tanks at a density of 100 PL/m² or 50 PL/tank. Salinity during the acclimation and experimental periods was maintained at 20-25 ppt.

Experimental diets

Three diets were formulated for the present study. To compare efficiency, three different yeast cell debris were used. All three yeast cell debris were different in regard to the components of moisture, solid, ash, fat, total nitrogen and protein component. Among the three yeast cell debris, cell debris A and cell debris B were extracted by the enzymatic digestion of *Saccharomyces cerevisiae*, e.g., Baker's yeast and torula yeast, respectively. The C cell debris was also derived from *Saccharomyces cerevisiae*, but no enzymatic digestion was involved for extraction. The performance of these diets was compared to a commercial diet (control) (Table 1).

Table 1. Composition of yeast cell debris with all three formulations used in the present study (unit is percentage)

Diet component	Formula A	Formula B	Formula C
Moisture	5.98	5.94	7.99
Solid	94.02	94.06	92.01
Ash	3.72	5.18	4.45
Salt (Direct Mohr)	-	-	2.06
Salt (Ash Mohr)	0.96	0.82	1.66
Fat	7.60	0.60	0.17
T-N	7.33	6.22	9.03
Protein (F=6.25)	45.81	38.88	56.44

To determine the potential growth and survival effects of yeast cell debris administrated in the diet, seven treatments were used as shown in Table 2. All treatments diets except the control were mixed with commercial feed.

Table 2. Treatments used to determine effects of yeast cell debris.

Treatment no.	Treatment Name	% Yeast cell debris	Formula
1	A1%	1	A
2	A5%	5	A
3	B1%	1	B
4	B5%	5	B
5	C1%	1	C
6	C5%	5	C
7	Control	none	none

Growth and survival study

Shrimp from the seven treatment groups were fed four times daily to satiation level. Feeding rate was adjusted according to shrimp weight throughout the 60 day experimental period. Water quality parameters such as pH, dissolved oxygen (DO), ammonia, and

nitrite were maintained at suitable levels for shrimp culture throughout the experiment. The growth of all treatment groups was recorded after 30, 40, 50 and 60 days of the experiment. The survival rates of all treatment groups were recorded after 60 days of the feeding trial.

Bacterial disease resistance study

After 60 days of dietary administration, shrimp from the growth and survival study were challenged with a virulent strain of *Vibrio harveyi* using the methods as follows:

1. Shrimp from the seven treatments were acclimated in the aquarium for 3 days. Each experiment group had three replicates. A total of 21 aquaria were used in this experiment. Salinity during the acclimation period and experiment was maintained at 20-25 ppt. Shrimp were stocked at a density of 10 shrimp (size 5-6 g) per aquarium.

2. The acclimated shrimp were challenged with a virulent strain of *V. harveyi*, which had been cultured in Tryptic Soy Agar (TSA), with 1.5% NaCl (w/v). All shrimp were injected with *V. harveyi* suspension at 1.38×10^7 CFU/ml. (or an appropriate concentration depending on LC_{50} at 48 hrs). Shrimp from the seven experiment groups were injected intramuscularly by 0.1 ml *V. harveyi* whereas shrimp in the control group were injected intramuscularly with 0.1 ml 0.85% NaCl. The number of dead shrimp was recorded for 48 hrs.

3. Bacteria was then isolated from the hepatopancreas of moribund shrimp and cultured on the TCBS agar.

4. Shrimp samples which exhibited clinical signs of disease were collected for histopathological study. The Davison's fixative solution was injected into the hepatopancreas and muscle, then fixed in Davidson's solution for 24 hrs. The solution was changed to 80% alcohol before processing for the standard histological method, according to Bell and Lightner (1984).

Statistical analysis

The data were analyzed using the software SPSS 13.0. One-way ANOVA and the Duncan's New Multiple Range test were used to compare data among the treatments. Differences were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Determination of yeast cell debris on growth and survival of Pacific white shrimp in laboratory conditions

After 30 days of administering the diet, shrimp fed with 5% yeast cell debris of formula C had an average body weight of 1.37 ± 0.18 g. This was significantly higher ($P < 0.05$) than that of shrimp fed with 1% and 5% yeast cell debris of formula A and the control group. After 40 days of feeding, shrimp fed with 5% yeast cell debris of formula C had an average body weight of 2.64 ± 0.10 g, which was significantly higher ($P < 0.05$) than that of shrimp fed with 1% yeast cell debris of formula A and the control group. After 60 days, shrimp fed with 5% yeast cell debris of formula C had a significantly higher ($P < 0.05$) average body weight, 4.64 ± 0.77 g than shrimp fed with 1% yeast cell debris of formula B. At day 60, shrimp fed with 5% yeast cell debris of formula C had an average body weight 6.77 ± 0.31 g that was significantly higher ($P < 0.05$) than shrimp fed with 1% and 5% yeast cell debris of formula A, 1% and 5% yeast cell debris of formula B, 1% yeast cell debris of formula C and the control group (Figure 1).

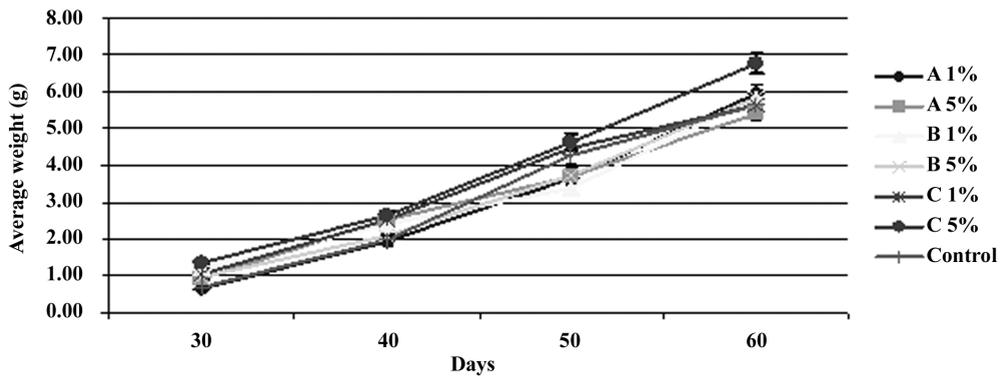


Figure 1. Average body weight of *L. vannamei* after 30, 40, 50 and 60 of feeding with yeast cell debris

Shrimp fed with 5% yeast cell debris of formula B had the highest percentage survival of 95.76 ± 4.58 . This was not significantly higher ($P > 0.05$) than shrimp fed with 1% and 5% yeast cell debris of

formula C, but was significantly higher ($P < 0.05$) than shrimp fed with 1% and 5% yeast cell debris of formula A, 1% yeast cell debris of formula B and the control group (Figure 2).

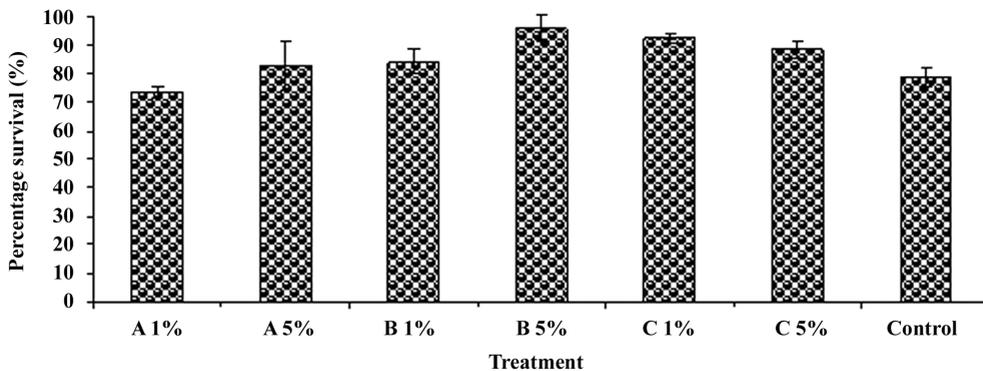


Figure 2. Survival percentage *L. vannamei* after 60 days of feeding with yeast cell debris.

Determination of the effect of yeast cell debris on the survival of white shrimp experimentally infected by Vibrio harveyi

After 48 hours of intramuscular injection with *V. harveyi* at a concentration of 1.38×10^7 CFU/ml., the shrimp were fed with three different feed formulations: A, B and C. Each formula consisted of two concentrations of yeast cell debris: 1 and

5%, with the commercial feed without yeast cell debris as the control. Shrimp fed with 5% yeast cell debris of formula C had the highest survival at $79.17\% \pm 7.21$, which was significantly higher ($P < 0.05$) than that of shrimp fed with 1 and 5% yeast cell debris of formula A, 1% yeast cell debris of formula B and 1% yeast cell debris of formula C and the control group (Figure 3).

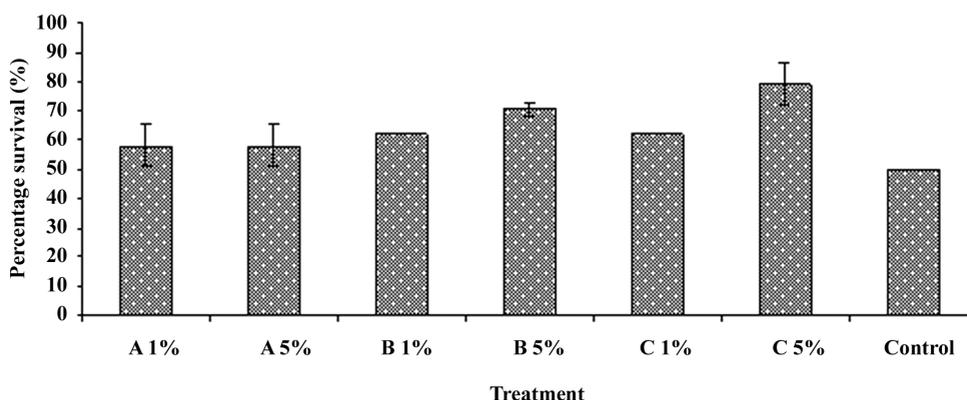


Figure 3. Survival of *L. vannamei* after being challenged with *V. harveyi* at 1.38×10^7 CFU/ml for 48 hrs.

In the present study, continuous oral administration of 5% yeast cell debris of formula C had a growth-promoting effect on the postlarvae of *L. vannamei*, evaluated by measuring the increases in body weight. This growth-promoting effect was due to yeast cell debris. The cell wall of *Saccharomyces cerevisiae* contains β -glucans, mannan-oligosaccharides and other materials, such as vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids and some living cells (Burgent *et al.*, 2004). The growth-promoting effects of yeast cell debris consisted of 56% protein. The dietary protein requirement of penaeid shrimp is an important nutritional consideration because it is a major limiting nutrient for growth. In several studies, the recommended protein concentration in feed for penaeids ranges between 30 and 45% (Andrews and Sick, 1972; Balaz, 1973; New, 1976; Neal, 1980; Piedad-Pascual, 1990; Akiyama *et al.*, 1991).

Considering the effect of yeast cell debris on bacterial disease resistance, the

results from this study suggested that the application of 5% yeast cell debris of formula C effectively enhanced tolerance to *V. harveyi*. This study exhibited that yeast cell debris is one of the immunostimulants which can be used to boost the immune system and improve resistance of shrimp to infections, which was similarly reported by Smith *et al.* (1985).

CONCLUSION

The results from this study suggest that the diet of 5% yeast cell debris formula C, during the post larval stage, effectively enhanced the growth and survival of Pacific white shrimp, *L. vannamei*.

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