

***Saccharomyces cerevisiae* Cell Wall Supplementation on Growth Performance and Immunity Status of White Shrimp (*Litopenaeus vannamei*)**

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Abstract: Effects of different levels of dietary *Saccharomyces cerevisiae* cell walls were evaluated on growth, survival, immune status, and disease resistance against *Vibrio harveyi* and *Vibrio parahaemolyticus* which are causative agents of Early Mortality Syndrome (EMS), in White shrimp (*Litopenaeus vannamei*). Dietary treatments were: 0, (CON) 0.25, 0.5, or 1.0% yeast cell wall product (YCW; ImmunoWall[®], ICC, Sao Paulo, Brazil) per treatment there were 8 replicate tanks (4 for growth at 14 and 28 d, and 4 for weekly immune responses to bacterial challenges, and each replicate consisted of a 1,000 L fiber tank with a stocking density of 60 shrimp/m³ in brackish water of 12-15 ppt. Individual shrimp of 6.45±0.26 g were acclimated for one week and allocated to treatment tanks. Two bacteria challenge tests were conducted. For each test, the same shrimp were continued on experimental diet to 30 d after which 30 shrimp from each treatment (3 replicate tanks of 10 shrimp each) were randomly selected and challenged 2.6×10⁶ CFU/mL of *Vibrio parahaemolyticus* or 1.0×10⁶ CFU/mL of *Vibrio harveyi* by intramuscular injection. Three hours post injection, hemolymph was collected. The YCW supplementation at 0.5 and 1.0% improved ($P < 0.05$) body weight at d 14, and feed intake at d 28. Hemolymph protein improved at d 7 and 14, and phenoloxidase activity improved at d 21 ($P < 0.05$) at all YCW feeding levels compared with control group. At 2 and 3 d after *V. harveyi* challenge, mortality rates were lower ($P < 0.05$) in groups fed YCW. Also total hemocyte count and hemolymph were higher ($P < 0.05$) in groups fed with 0.5 and 1.0% of YCW. For *V. parahaemolyticus* challenge, total hemocyte count improved for all YCW levels. The YCW supplementation at 0.5 and 1% in shrimp diets resulted in improved body weight gain and feed intake. The 1% YCW inclusion rate was shown to enhance some immunity parameters under disease challenge conditions by *Vibrio harveyi* or *Vibrio parahaemolyticus*.

Key words: early mortality syndrome, *Vibrio harveyi*, *Vibrio parahaemolyticus*, white shrimp, yeast cell wall

1. Introduction

Saccharomyces cerevisiae yeast cell wall contains β -glucans and mannan oligosaccharide which are functional carbohydrate molecules shown to stimulate the immune system (innate immunity) and prevent gut pathogen colonization, respectively, in numerous species including shrimp. Enhancing the innate immune system in shrimp is important because shrimp

do not have a specific immune system as in higher animals, thus prebiotics with high levels of β -glucans become essential. Many research trials with shrimp have shown the effectiveness of yeast cell wall, β -glucans and mannan oligosaccharide for enhancing immunity.

Thanardkit et al. (2002) [1] utilized a yeast fraction containing ~53% β -glucan at 0.2% level in the diet of Black Tiger shrimp for 3 days. Significant increases in phenol oxidase, number of hemocytes, and the bacterial killing activity against the pathogen *Vibrio*

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harveyi were observed compared to control diet. Feeding yeast beta-glucan resulted in the appearance of inducible beta-(1,3)-glucanase in the liver.

Namikoshi et al. (2004) [2] reported that yeast beta-glucan was effective against White Spot Syndrome virus injected intramuscularly into Kuruma shrimp (Japanese Tiger shrimp). Beta-glucan as a feed supplement has been shown to enhance survival of Black Tiger shrimp against White Spot Syndrome [3, 4].

Hisano et al. (2008) [5] found that a diet with yeast mannan oligosaccharide at 0.2% fed to juvenile Amazon River prawn (shrimp) for 60 days significantly increased body weight gain compared to unsupplemented control diet.

Rutchanee et al. (2008) [6] evaluated 3 inclusion rates (0, 0.1, or 0.2%) of yeast cell wall in feed of juvenile Pacific White shrimp (*Litopenaeus vannamei*) for 4 weeks. Live performance parameters were unaffected, but total hemocyte counts, granular hemocyte count, and bacterial clearance were better in shrimp fed diets supplemented with 0.1 or 0.2% yeast cell wall compared to control diet.

The research was conducted at Kasetsart University, Bangkok, Thailand. The objective of these trials was to evaluate effects of *Saccharomyces cerevisiae* cell wall dietary supplementation on growth performance, survival rate, and on immune status and disease resistance against *Vibrio harveyi* and *Vibrio parahaemolyticus*, causative agent of Early Mortality Syndrome disease (EMS) of White shrimp, *Litopenaeus vannamei*. The EMS affects shrimp that are not yet marketable size (younger than 40 days old), and since 2009 losses up to 100% have occurred with White shrimp in ponds in Asia. Surviving shrimp typically have impaired growth.

2. Materials and Methods

2.1 Phase 1

In Phase 1 the shrimp were distributed in a completely randomized design, with 4 diet treatments

of 0, (CON) 0.25, 0.5 and 1.0% yeast cell wall concentrate product (YCW; *Saccharomyces cerevisiae* cell wall product ImmunoWall[®], ICC, Sao Paulo, Brazil), with 8 replicates. These included 4 replicates for growth performance and 4 replicates for immunity (bacteria challenge). Each replicate consisted of a 1,000 liter fiber tank with a stocking density of 60 shrimp/m³ in brackish water of 12-15 ppt. Individual shrimp weighing 6.45±0.26 g were acclimated for one week and allocated to treatment tanks. They were fed a pelleted diet (38% of CP, and 7.5% of lipid) at 2.5-3% of body weight, 4 times daily at 07:00 a.m., 11:00 a.m., 15:00 p.m. and 19:00 p.m.

The measurements of growth included total feed intake (TFI, g/tank), feed intake (FI, g/shrimp), daily feed intake (DFI, g/ind/d), production (g/tank) body weight (BW, g/shrimp), body weight gain (BWG, g/shrimp), average daily gain (ADG, g/shrimp/d), specific growth rate (SGR, %/d), feed/gain ratio (FGR), and survival rate (SR, %) at 14 and 28 days.

The immunological measurements included total hemocyte count (THC, X 10⁵ cell/mL), hemolymph protein (HP, mL/dL), and phenol oxidase activity (POx, unit/mg protein) measured at 14 and 28 days.

2.2 Phase 2

For the pathogenic bacteria challenges in Phase 2, the same shrimp in Phase 1 were continued on experimental diet to 30 d after which 30 shrimp from each of the 4 treatments were randomly selected to challenge them with pathogenic bacteria by intramuscular injection. The infection doses were 1.0×10⁶ CFU/mL for *Vibrio harveyi* or 2.6×10⁶ CFU/mL for *Vibrio parahaemolyticus*. Three hours post injection, hemolymph was collected to determine immune responses and to evaluate pathogenic bacteria clearance ability, indicators of efficiency of YCW to enhance the shrimp immune defense system. The THC, HP and POx were evaluated weekly. Mortality rate (MR, %) was determined daily and reported weekly.

In Phases 1 and 2, the data were analyzed using the GLM (SAS), and means were compared by Duncan's test ($P \leq 0.05$).

3. Results and Discussion

3.1 Phase 1 (Tables 1 and 2)

The diet with YCW supplementation at 1.0% improved ($P < 0.05$) BW at d 14 compared to 0 or 0.25%

YCW, with 0.5% YCW treatment group BW being intermediate. The BW gains were not significantly different by treatments at 14 or 28 d, but at each age there was a numerical stepwise increase in gain with increasing levels of YCW product. Specific growth rate (%/d) did not differ significantly by treatment at 14 or 28 d.

Table 1 Growth performance of shrimp after fed different levels of yeast cell wall (phase 1).

Parameters	Yeast cell wall inclusion levels (%)				P value
	0.00	0.25	0.50	1.00	
Weight (g/shrimp)					
Initial	6.35 ^a ±0.37	6.32 ^a ±0.28	6.62 ^a ±0.13	6.50 ^a ±0.19	0.3542
14 days	8.31 ^b ±0.13	8.31 ^b ±0.08	8.65 ^{ab} ±0.17	8.71 ^a ±0.39	0.0466
28 days	10.11 ^a ±0.61	10.29 ^a ±0.56	10.89 ^a ±0.33	10.85 ^a ±0.28	0.0807
Weight gain (g/shrimp)					
14 days	1.97 ^a ±0.41	2.00 ^a ±0.32	2.03 ^a ±0.24	2.21 ^a ±0.29	0.7110
28 days	3.76 ^a ±0.45	3.97 ^a ±0.38	4.28 ^a ±0.27	4.35 ^a ±0.16	0.0946
Specific growth rate (%/d)					
14 days	1.94 ^a ±0.44	1.97 ^a ±0.34	1.91 ^a ±0.22	2.09 ^a ±0.23	0.8720
28 days	1.85 ^a ±0.22	1.91 ^a ±0.20	2.12 ^a ±0.11	2.10 ^a ±0.09	0.0843
Feed intake (g/shrimp)					
14 days	2.55 ^a ±0.15	2.57 ^a ±0.14	2.60 ^a ±0.18	2.72 ^a ±0.09	0.3574
28 days	5.11 ^b ±0.10	5.10 ^b ±0.29	5.44 ^a ±0.16	5.62 ^a ±0.24	0.0099
Feed/gain ratio					
14 days	1.33 ^a ±0.21	1.31 ^a ±0.17	1.29 ^a ±1.25	1.25 ^a ±0.17	0.9105
28 days	1.37 ^a ±0.15	1.29 ^a ±0.06	1.28 ^a ±0.06	1.29 ^a ±0.07	0.4681
Survival rate unchallenged (%)					
14 days	91.67 ^a ±4.30	97.50 ^a ±3.19	97.50 ^a ±3.19	95.00 ^a ±6.38	0.2561
28 days	84.17 ^a ±5.69	92.50 ^a ±5.69	91.67 ^a ±6.38	90.00 ^a ±10.54	0.4090

Each mean ± standard deviation is listed. ^{a-b} Different letters in the same row indicate significant differences between averages ($P < 0.05$).

Diets with 0.5% or 1.0% YCW increased TFI, FI and DFI at d 28 compared to 0 or 0.25% YCW diets. Feed efficiency (Feed/gain ratios) did not differ significantly by treatment at 14 or 28 d. Survival rates (%) were not significantly different by treatments at either age, but survival rates (%) were numerically higher for YCW product containing diets than for control diets.

The increased BW with 1.0% YCW confirms results of Hisano et al. (2008) [5] who reported that a diet with 0.2% yeast mannan oligosaccharide fed to

juvenile Amazon River prawn (shrimp) for 60 days significantly increased body weight gain compared to unsupplemented control diet.

Immune responses of White shrimp under normal conditions and fed YCW product are shown in Table 2.

Total hematocyte count ($\times 10^5$ cell/mL) did not differ significantly by treatment but increased numerically from 7 to 28 days with increasing levels of YCW product. Hemolymph protein (mg/dL) means

significantly increased at 7 and 14 d with YCW diets compared to control diets.

Table 2 Immune responses of white shrimp fed diets with yeast cell wall product (phase 1).

Parameters	Yeast cell wall inclusion levels (%)				P value
	0	0.25	0.5	1	
Total hemocyte count (THC; x105 cell/mL)					
7 days	6.63 ^a ±0.78	7.38 ^a ±1.04	7.78 ^a ±1.20	7.85 ^a ±1.88	0.5393
14 days	5.75 ^a ±2.55	6.18 ^a ±1.35	6.75 ^a ±0.68	7.70 ^a ±0.70	0.3420
21 days	5.60 ^a ±0.73	6.03 ^a ±1.78	6.75 ^a ±0.84	7.53 ^a ±0.71	0.1268
28 days	4.60 ^a ±1.03	5.08 ^a ±2.49	6.60 ^a ±1.63	3.8 ^a ±1.10	0.1170
Hemolymph protein (HP; mg/dL)					
7 days	2.91 ^b ±0.11	3.49 ^a ±0.15	3.52 ^a ±0.12	3.67 ^a ±0.32	0.0006
14 days	2.93 ^b ±0.34	3.42 ^a ±0.43	3.44 ^a ±0.09	3.71 ^a ±0.21	0.0213
21 days	2.76 ^a ±0.26	2.89 ^a ±0.26	3.02 ^a ±0.33	3.27 ^a ±0.39	0.1835
28 days	2.76 ^a ±0.26	2.76 ^a ±0.44	2.94 ^a ±0.51	3.09 ^a ±0.28	0.4711
Phenol oxidase activity (POx; unit/mg protein)					
7 days	153.3 ^a ±85.2	177.8 ^a ±68.3	186.4 ^a ± 144.2	207.0 ^a ±95.5	0.9017
14 days	184.3 ^a ±66.6	218.1 ^a ±103.1	224.5 ^a ±60.4	234.4 ^a ±43.0	0.7770
21 days	175.9 ^b ±42.5	248.2 ^a ±38.7	252.3 ^a ±56.2	262.3 ^a ±25.4	0.0485
28 days	203.6 ^a ±48.9	252.3 ^a ±14.7	286.3 ^a ±119.0	303.8 ^a ±63.5	0.2648

Each mean ± standard deviation is listed. ^{a-b} Different letters in the same row indicate significant differences between averages ($P < 0.05$).

At 21 d phenol oxidase activity (unit/mg protein) for control diet was significantly lower than for YCW diets.

These positive immune responses confirm those of Rutchanee et al. (2008) [6] who evaluated 3 inclusion rates (0, 0.1, or 0.2%) of yeast cell wall in feed of juvenile Pacific White shrimp (*Litopenaeus vannamei*) for 4 weeks and found that total hemocyte counts, granular hemocyte count, and bacterial clearance were better in shrimp fed diets supplemented with 0.1 or 0.2% yeast cell wall compared to control diet.

3.2 Phase 2 *Vibrio harveyi* Challenge (Tables 3 and 4)

After *V. harveyi* challenge, the d 7 SR were 6.67% and 10.00% for 0.5 and 1.00% YCW diets, respectively, compared to 0% for 0 or 0.25% YCW diets (Table 3). The THC were higher ($P < 0.05$) in the group fed 1.0% YCW diet compared to 0 or 0.25% YCW diet, with 0.50% YCW diet intermediate, at 3 hours post injection (Table 4). The HP significantly improved in the 0.5 or 1.0% YCW diet groups compared to 0 or 0.25% YCW diet groups, at 3 hours post injection. Neither POx nor

pathogen clearance ability were affected by YCW treatments in White shrimp 3 hours post injection with *Vibrio harveyi*. These results agree with those of Thanardkit et al. (2002) [1] who fed a diet with 0.2% yeast cell wall component containing ~53% beta-glucan to Black Tiger shrimp and found significant increases in phenol oxidase, number of hemocytes, and killing activity against *Vibrio harveyi*.

3.3 Phase 2 *Vibrio parahemolyticus* (Tables 5 and 6)

After *V. parahemolyticus* challenge, d 7 SR numerically increased from 30.00% in control to 53.33% in 1.0% YCW diet groups although differences were not significant (Table 5). The THC were significantly higher in White shrimp fed 0.5 or 1.0% YCW diets compared to control diet, with 0.25% YCW diet group intermediate (Table 6). No significant treatment differences were found for HP, POx, or pathogen clearance ability 3 hours post injection.

Using White Spot Syndrome virus challenges and diets with or without yeast beta-glucan, Namikoshi et al. (2004) [2] with Kuruma (Japanese Tiger) shrimp

and Citarasu et al. (2006) [3] and El-Boshy et al. (2008) [4] with Black Tiger shrimp observed that yeast beta-glucan supplemented diets were effective at enhancing survival rate.

Table 3 Cumulative mortality (%) of White shrimp fed diets with yeast cell wall product and injected with *Vibrio harveyi* (phase 2).

Day after challenge	Yeast cell wall inclusion levels (%)				P value
	0.00	0.25	0.50	1.00	
Mortality rate, %					
Day 0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-----
Day 1	63.33 ^a ±15.3	53.33 ^a ±15.3	36.67 ^a ±11.6	30.00 ^a ±10.0	0.0524
Day 2	80.00 ^c ±10.0	70.00 ^{bc} ±10.0	53.33 ^{ab} ±11.6	40.00 ^a ±10.0	0.0067
Day 3	90.00 ^c ±10.0	76.67 ^{bc} ±5.77	66.67 ^{ab} ±15.3	53.33 ^a ±5.77	0.0115
Day 4	100.0 ^a ±0.009	3.33 ^a ±11.6	80.00 ^a ±20.0	73.33 ^a ±5.77	0.0871
Day 5	100.0 ^a ±0.00	100.0 ^a ±0.00	93.33 ^a ±11.6	90.00 ^a ±10.0	0.3437
Day 6	100.0 ^a ±0.00	100.0 ^a ±0.00	93.33 ^a ±11.6	90.00 ^a ±10.0	0.3437
Day 7	100.0 ^a ±0.00	100.0 ^a ±0.00	93.33 ^a ±11.6	90.00 ^a ±10.0	0.3437
Survival rate, %					
Day 7	0.00 ^s ±0.00	0.00 ^s ±0.00	6.67 ^s ±0.83	10.00 ^s ±1.11	0.3437

Each mean ± standard deviation is listed.

Table 4 Immune responses of white shrimp fed diets with yeast cell wall product and 3 hours after injection with *Vibrio harveyi* (phase 2).

Parameters	Yeast cell wall product inclusion levels (%)				P value
	0.00	0.25	0.50	1.00	
Total hemocyte count (THC; ×10 ⁵ cell/mL)					
	1.10 ^b ±0.15	1.25 ^b ±0.10	1.50 ^{ab} ±0.38	1.74 ^a ±0.14	0.0356
Hemolymph protein (HP; mg/dL)					
	2.60 ^b ±0.10	2.95 ^b ±0.29	3.80 ^a ±0.16	3.86 ^a ±0.32	0.0004
Phenol oxidase activity (POx; unit/mg protein)					
	509.7 ^a ±19.0	526.9 ^a ±59.2	531.2 ^a ±80.8	545.2 ^a ±28.7	0.8738
Pathogen clearance ability (log cfu/mL)					
	4.72 ^a ±0.29	4.27 ^a ±0.93	4.23 ^a ±0.51	4.20 ^a ±0.38	0.6807

Each mean ± standard deviation is listed. ^{a-b} Different letters in the same row indicate significant differences between averages ($P < 0.05$).

Table 5 Cumulative mortality (%) of white shrimp fed diets with yeast cell wall product and 3 hours after injection with *Vibrio parahemolyticus* (phase 2).

Day after challenge	Yeast cell wall inclusion levels (%)				P value
	0.00	0.25	0.50	1.00	
Mortality rate, %					
Day 0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-----
Day 1	26.67±5.77	23.33±5.77	16.67±11.6	13.33±11.6	0.3300
Day 2	53.33±5.77	40.00±26.5	33.33±23.1	30.00±17.3	0.5205
Day 3	70.00±10.0	66.67±23.1	53.33±23.1	46.67±5.77	0.3659
Day 4	70.00±10.0	66.67±23.1	53.33±23.1	46.67±5.77	0.3659
Day 5	70.00±10.0	66.67±23.1	53.33±23.1	46.67±5.77	0.3659
Day 6	70.00±10.0	66.67±23.1	53.33±23.1	46.67±5.77	0.3659
Day 7	70.00±10.0	66.67±23.1	53.33±23.1	46.67±5.77	0.3659
Survival rate, %					
Day 7	30.00 ^s ±4.29	33.33 ^s ±11.5	46.67 ^s ±20.2	53.33 ^s ±6.59	0.3659

Each mean ± standard deviation is listed.

Table 6 Immune responses of white shrimp fed diets with yeast cell wall product and 3 hours after injection with *Vibrio parahemolyticus* (phase 2).

Parameters	Yeast cell wall product inclusion levels (%)				P value
	0.00	0.25	0.50	1.00	
Total hemocyte count (THC; $\times 10^5$ cell/mL)					
	2.60 ^b ± 0.44	3.73 ^{ab} ± 0.32	4.67 ^a ± 0.97	4.67 ^a ± 1.01	0.0285
Hemolymph protein (HP; mg/dL)					
	3.00 ^a ± 0.47	3.35 ^a ± 0.33	3.47 ^a ± 0.17	3.59 ^a ± 0.35	0.2732
Phenol oxidase activity (POx; unit/mg protein)					
	289.8 ^a ± 49.2	339.1 ^a ± 51.1	351.4 ^a ± 70.0	367.3 ^a ± 58.0	0.4371
Pathogen clearance ability (log cfu/mL)					
	5.73 ^a ± 0.13	5.53 ^a ± 0.07	5.49 ^a ± 0.20	5.47 ^a ± 0.18	0.2176

Each mean ± standard deviation is listed. ^{a-b} Different letters in the same row indicate significant differences between averages ($P < 0.05$).

4. Conclusions

The YCW product supplementation at 1.0% in White shrimp diets resulted in significantly improved 14 d body weight compared to 0 or 0.25% YCW diets, with 0.5% YCW diet body weight intermediate. Feed intakes by shrimp were significantly increased at 14 and 28 d with 0.50 or 1.0% YCW diets compared to control or 0.25% YCW diets. The hemolymph protein and phenol oxidase activity means were significantly increased at 7 and 14 d and at 21 d, respectively, with each YCW diet compared to control diet.

With *Vibrio harveyi* infection, survival rates were numerically increased from 0% with 0 or 0.25% YCW diets to 6.67% and 10.00%, respectively, with 0.50% and 1.0% YCW diets.

The total hemocyte count was significantly increased with 1.0% YCW diets compared to 0 or 0.25% YCW diets, with 0.50% YCW diets intermediate, at 3 hours post injection.

Hemolymph protein was significantly higher in White shrimp fed 0.50 or 1.0% YCW diets compared to control or 0.25% diets.

With *Vibrio parahemolyticus* infection, survival rates were numerically but nonsignificantly increased from 30.00% for control diet to 53.33% for 1.0% YCW diet. Total hemocyte counts were significantly increased by 0.50 or 1.0% YCW diets compared to control diet, with 0.25% YCW diet intermediate.

5. Recommendations

White shrimp fed 0% (control) or 0.25% YCW product diets were similar in many respects with regard to live performance and immune response parameters whereas 0.50% or 1.00% YCW product showed some significant benefits in live performance, immune responses, and survivability. Therefore, 0.50% or 1.00% YCW product (ImmunoWall®, ICC, Sao Paulo, Brazil) is recommended as the proper range of dietary inclusion for improving the growth, health, and survivability of White shrimp.

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