Effects of DVAQUA® on the Growth, Survival and Immune Characteristics of Pacific White Shrimp (*Litopenaeus vannamei*)

Chayaporn Tipsemongkol[1,2], Watchariya Purivirojkul[3], Niti Chuchird[1,2] and Chalor Limsuwan[1,2]

ABSTRACT

A study of the effects of DVAQUA® on the growth, survival and immune response of Pacific white shrimp (*Litopenaeus vannamei*) was conducted under laboratory and outdoor (ponds) conditions. For the laboratory experiments to determine the growth-promoting and immunostimulant effects of DVAQUA® administration in the diet, tests were carried out with three treatments (six replicates/treatment). Each replicate consisted of 25 shrimps (8-10 g) in 500-liter tanks. The shrimp were fed four times daily at 3% body weight per day for 50 days with pelleted feed containing graded levels of DVAQUA® (0%, 0.125% and 0.25% of the feed). After 50 days of dietary administration, shrimp fed with 0.25% DVAQUA® had a significantly higher ($P<0.05$) average body weight (16.11±2.14 g) than that of the control group (14.98±2.20 g). There was no significant difference in body weights between the control group and the group fed with 0.125% DVAQUA® (15.56±2.64 g). The survival rates of shrimp from the DV Aqua groups ranged from 88.67-95.33%, which were significantly higher ($P<0.05$) than that in the control group (75.33%). The immune characteristics of shrimp fed on diets containing 0.25% DVAQUA® had significantly higher ($P<0.05$) total hemocyte count (THC), percentage phagocytosis and superoxide dismutase activity (SOD) than the 0.125% DVAQUA® and control groups. Shrimp fed with 0.125 and 0.25% DVAQUA® had bactericidal activity at the serum dilution of 1:16 while the control group had the same at 1:8. Outdoor trials to determine the effect of DVAQUA® on growth, survival and immune response in pond-reared *L. vannamei* were carried out in eight earthen ponds (around 1 ha or 6 rais/pond). Postlarvae 10 (PL10) were stocked at a density of 1,000,000 PLs/pond. After 30 days, shrimp in four treatment ponds were fed with pelleted feed with 0.25% DVAQUA® while another four ponds were fed with regular white shrimp pelleted feed as the control group. After 90 days of culture, shrimp from both the treatment and control groups were sampled for immune parameters studies. Results showed that shrimp fed with 0.25% DVAQUA® had significantly higher THC, percentage phagocytosis, phenoloxidase, bactericidal activity and SOD ($P<0.05$) than those of the control group. After the shrimp were harvested, the average production and survival rate of the 0.25% DVAQUA® group were 2,375 kg/rai and 82%, while the control

[1] Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University, Bangkok 10900 Thailand
[2] Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok 10900 Thailand
[3] Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900 Thailand

group had 2,202 kg/rai and 73%, respectively. In conclusion, the present study indicated that oral administration of 0.25% DVAQUA® for at least 30 days could increase the growth, survival and immune response of *L. vannamei*.

Keywords: Immunostimulant, DVAQUA®, *Vibrio harveyi*, *Litopenaeus vannamei*

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 resulting in production declines in farmed *L. vannamei* (Cheng *et al*., 2005). Many scientists have attempted to solve the disease problems by enhancing the non-specific immune response, the main internal defense mechanism in shrimp. Use of immunostimulants is another approach to increase shrimp immunity against diseases (Purivirojkul *et al*., 2006). Beta-glucan, from the cell wall of yeast, has been reported to be used as an immunostimulant in aquaculture. It was found to increase survival rate and enhance protection against pathogens in shrimp (Sung *et al*., 1996; Vargas-Albores *et al*., 1998; 2000; Suphantharika *et al*., 2003). DVAQUA® is from the cell wall of *Saccharomyces cerevisiae*, which contains about 30-35% beta-glucan. The use of DVAQUA® in turbot *Scophthatmus maximus* was successful in enhancing its growth rate (Mai *et al*., 2008). However, there have been no reports on the use of DVAQUA® in shrimp culture. The objective of the present study was to determine the optimum concentration of DVAQUA® to increase growth, survival and immune response in laboratory conditions, and in intensive pond-reared *L. vannamei*, which could be recommended for use in shrimp culture.

MATERIALS AND METHODS

Laboratory Experiments

Experimental animals

Litopenaeus vannamei (8-10 g) were obtained from a commercial shrimp farm in Chantaburi province, Thailand. A total of 1,500 farm-reared shrimp were transported and acclimated in fiberglass tanks at the Aquaculture Business Research Center Laboratory, Faculty of Fisheries, Kasetsart University. After 14 days of acclimatization, the shrimp were used for the experiment. Salinity during the acclimation period and experiment was maintained at 25 ppt.

Experiment 1. Determination of the DVAQUA® effect on the growth and survival of white shrimp

To determine the growth-promoting and survival effects of DVAQUA® administration in the diet, tests were carried out with three treatments consisting of 0%, 0.125% and 0.25% DVAQUA® feed with six replications per treatment. Each replicate consisted of 25 shrimp individuals (8-10 g) in 500-liter tanks. Shrimp were fed four times daily at 3% body weight per day for 50 days with pelleted feed containing graded levels of

DVAQUA®. Feeding rate was adjusted according to shrimp weight throughout the 50-day experiment period. Water quality parameters such as pH, dissolved oxygen (DO), ammonia, nitrite were measured weekly throughout the experiment. The growth and survival rates of all treatment groups were recorded at 20, 35 and 50 days.

Experiment 2. Determination of the DVAQUA® effect on the immune characteristics of white shrimp

Three treatments (0%, 0.125% and 0.25% DVAQUA® feed) were used in experiment 2. A total of 150 shrimp individuals (8-10 g) for each treatment (at six replications per treatment) were used. The immune parameters measured every 10 days for 50 days were total hemocytes count (THC), percentage phagocytosis, phenoloxidase, superoxide dismutase and bactericidal activity.

Experiment 3. Determination of the DVAQUA® effect on survival of white shrimp experimentally infected by Vibrio harveyi

Shrimp from experiment 1 (60 shrimp per treatment) were challenged with a virulent strain of *Vibrio harveyi* which had been cultured in Tryptic Soy Agar (TSA) with 1.5% NaCl (w/v). All shrimp were injected by *V. harveyi* suspension at 8.2 x 10^6 CFU $.ml^{-1}$. (or appropriate concentration depends on LD_{50} at 96 hrs.). The number of dead shrimp was recorded for 96 hrs.

Farm Trials Experiments

Experimental ponds

The on-farm trial was conducted in a white shrimp farm located in Chantaburi province, Thailand. Eight earthen ponds with an area of around one hectare (6 rais) each were used. Postlarvae 10 (PL10) were stocked into each growout pond at a density of 1,000,000 PLs per pond. Shrimp were fed with commercial pelleted feed during the first 30 days. Salinity during the experimental period ranged from 15 to 18 ppt.

Experiment 4. Determination of the DVAQUA® effect on growth and survival of pond-reared white shrimp

To determine the growth-promoting and survival effects of DVAQUA® in the diet, tests were carried out with two treatments, i.e. 0.25% DVAQUA® and the control (four ponds/treatment) from day 30 until shrimp were harvested. Experimental pelleted feed was prepared by Charoenpokapand (CP) feed mill. Shrimp were fed four times daily and feeding rate was adjusted according to shrimp weight. Water quality parameters such as pH, DO, ammonia, and nitrite were measured biweekly throughout the experiment. The production, growth and survival rates were recorded after shrimp were harvested.

Experiment 5. Determination of the DVAQUA® effect on the immune characteristics of pond-reared white shrimp

After 90 days of culture, a total of 50 shrimp from each treatment (four ponds per treatment) were selected for the measurement of the following immune parameters: THC, percentage phagocytosis, phenoloxidase, SOD and bactericidal activity.

Immune parameters

Preparation of hemolymph samples

A blood sample of 0.5 ml from each shrimp were drawn from the base of the third walking leg by a syringe containing 1.5 ml anticoagulant (K-199 + 5% L-cysteine).

Total hemocytes

After collecting the hemolymph, hemocytes were counted using a hemocytometer and calculated as the number of blood cells (total hemocytes per cubic millimeter).

Phagocytosis

This method was modified from Itami *et al*. (1994).

Two hundred microliters of hemolymph were collected from the base of the third walking leg of the shrimp and mixed with 800 µl of sterile anticoagulant. The collected hemocytes were rinsed with shrimp saline and the viable cell number was adjusted to 1×10^6 cells. ml^{-1}. The cell suspension (200 µl) was inoculated into a cover slip. After 20 minutes, the cell suspension was removed and rinsed three times with shrimp saline. Heat-killed yeast (2) was added and the suspension was incubated for 2 hours. After incubation, heat-killed yeast was removed. The suspension was rinsed five times with shrimp saline, fixed with 100% methanol and then the cover slip was stained with Giemsa stain and mounted with permount.

Two hundred hemocytes were counted. Phagocytic activity, defined as percentage phagocytosis, was expressed as:

$$\text{percentage phagocytosis} = \frac{\text{phagocytic hemocytes}}{\text{total hemocytes}} \times 100$$

Phenoloxidase activity assay

The method was modified from Supamattaya *et al*. (2000). After the blood was withdrawn, the hemocytes were washed three times with shrimp saline (1,000 rpm, 4°C for 10 min). Haemocyte lysate (HLS) was prepared from hemocytes in a cacodylate buffer pH 7.4 using the sonicator at 30 amplitute for 5 seconds. The suspension was then centrifuged at 10,000 rpm, 4°C for 20 min. The supernatant was collected as HLS. Then 200 µl of 0.25% trypsin in cacodylate buffer was mixed to the 200 ml HLS followed by 200 µl of L-dihydroxyphenylalanine (L-DOPA) at 4 mg. ml^{-1} as the substrate. Enzyme activity was measured as the absorbance of dopachrome at 490 nm. wavelength. The amount of protein in HLS was determined using the method of Lowry *et al*. (1951). The phenoloxidase activity was calculated as the increasing of optimum density (OD) per minute per mg of protein, expressed in this equation:

$$1 \text{ unit of phenoloxidase} = \Delta \text{ OD490 /min/ mg protein}$$

Superoxide dismutase activity assay

Superoxide dismutase activity was carried out with the RANSOD kit (Randox, USA). This method is based on the formation of red formazan from the reaction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5 -phenyltetrazolium chloride (INT) and superoxide radical, which is assayed in a spectrophotometer at 505 nm.

Bactericidal activity

Serum was separated from each blood sample and diluted with 2.6% NaCl at 1:2, 1:4, 1:8, 1:16 and 1:32. Then 0.5 ml of each serum dilution and 0.5 ml of NaCl as the control were used in the study. *V. harveyi* suspension of 0.5 ml (prepared according to the method in 3) was added into each serum dilution and the control. The treatments were incubated at room temperature for 3 h before enumerating the number of bacteria by spread plate technique. The dilution which could decrease *V. harveyi* by 50% compared with the control was recorded.

Statistical analysis

Data from laboratory experiments were subjected to one-way analysis of variance followed by Duncan's multiple range test. For farm trials, T-test was used to statistically compare between treatment and control groups. Differences were considered significant if $P<0.05$.

RESULTS AND DISCUSSION

Laboratory Experiments

Experiment 1. Determination of the DVAQUA® effect on the growth and survival of white shrimp

After 35 days, shrimp fed with 0.25% DV Aqua had an average body weight that was significantly higher ($P<0.05$) than the control but not with shrimp fed with DVAQUA® at 0.125% (Table 1). As for survival rates, there was no significant difference between DV Aqua-treated shrimp but they were significantly higher ($P<0.05$) than that of the control group (Table 2).

Experiment 2. Determination of the DVAQUA® effect on the immune characteristics of white shrimp

Immune responses were measured by THC, percentage phagocytosis, phenoloxidase activity and bactericidal activity. During the experiments, water quality parameters were maintained as follows: temperature at 28 ±1oC, pH at 7.8-8.0 and salinity at 25 ppt.

Table 1. Average body weight of *L. vannamei* after 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 30)

Feeding period (days)	Average body weight (g)		
	Control	0.125% DVAQUA®	0.25% DVAQUA®
0	8.97 ± 0.85^{a}	9.00 ± 0.83^{a}	8.93 ± 0.83^{a}
20	10.87 ± 1.36^{a}	11.47 ± 1.14^{a}	11.53 ± 1.01^{a}
35	12.14 ± 1.38^{b}	13.00 ± 1.34^{ab}	13.47 ± 1.28^{a}
50	14.98 ± 2.20^{b}	15.56 ± 2.64^{ab}	16.11 ± 2.14^{a}

Average values with different superscripts in the same row are significantly different ($P<0.05$).

Table 2. Percentage survival of *L. vannamei* after 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 6)

Feeding period (days)	Percentage survival (%)		
	Control	0.125% DVAQUA®	0.25% DVAQUA®
0	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]	100.00 ± 0.00 [a]
20	92.67 ± 3.00 [b]	98.67 ± 2.07 [a]	99.33 ± 1.63 [a]
35	86.67 ± 6.02 [b]	95.33 ± 3.01 [a]	97.33 ± 2.07 [a]
50	75.33 ± 5.89 [b]	88.67 ± 7.76 [a]	95.33 ± 4.68 [a]

Average values with different superscripts in the same row are significantly different (P<0.05).

Total hemocytes count

After 40 days of the feeding trials, shrimp fed with diets containing 0.25% DVAQUA® had a THC of 48.42 ± 2.81 x 10^5 cells. ml^{-1}, which was significantly higher (P<0.05) than that of shrimp fed with diets containing 0.125% DVAQUA® and the control group which had THC 33.63 ± 5.74 x 10^5 and 27.54 ± 6.24 x 10^5 cells. ml^{-1}, respectively. However, after 50 days of feeding, both DVAQUA®-treated groups had THCs which were significantly higher (P<0.05) than that of the control group (Table 3).

Table 3. Total hemocyte count (THC) of *L. vannamei* after 0, 10, 20, 30, 40 and 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 9)

Feeding period (days)	Total Hemocyte Count (x 10^5 cells. ml^{-1})		
	Control	DVAQUA®	
		0.125%	0.25%
0	15.87 ± 3.18 [a]	16.11 ± 2.73 [a]	16.23 ± 1.55 [a]
10	15.75 ± 3.95 [a]	19.75 ± 1.32 [a]	23.13 ± 2.41 [a]
20	21.73 ± 3.15 [a]	26.13 ± 3.38 [a]	38.21 ± 3.49 [a]
30	27.25 ± 2.55 [a]	38.50 ± 4.21 [a]	49.00 ± 7.04 [a]
40	27.54 ± 6.24 [b]	33.63 ± 5.74 [b]	48.42 ± 2.81 [a]
50	35.79 ± 10.25 [c]	54.71 ± 4.07 [b]	57.92 ± 6.07 [a]

Average values with different superscripts in the same row are significantly different (P<0.05).

Phagocytic activity

After 30 days of feeding, shrimp fed with diets containing 0.25% DVAQUA® had a significantly higher (P<0.05) percentage of phagocytosis than the control group but was not significantly higher than that of shrimp fed with diets containing 0.125%. After 40 days of feeding, shrimp fed with diets containing 0.25% DVAQUA® had a significantly higher (P<0.05) percentage of phagocytosis (33.33 ± 2.08) than those of the 0.125% DVAQUA® and control groups, which had percentage of phagocytosis of 28.00 ± 2.65 and 23.67 ± 3.21, respectively (Table 4).

Table 4. Percentage phagocytosis of *L. vannamei* after 0, 10, 20, 30, 40 and 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 15)

Feeding period (days)	Percentage phagocytosis		
	Control	DVAQUA®	
		0.125%	0.25%
0	21.33 ± 2.31 ^{a}	20.67 ± 1.15 ^{a}	20.33 ± 0.58 ^{a}
10	21.33 ± 7.02 ^{a}	23.33 ± 6.43 ^{a}	23.33 ± 6.43 ^{a}
20	22.67 ± 6.11 ^{a}	24.67 ± 3.06 ^{a}	25.33 ± 3.06 ^{a}
30	23.33 ± 1.15 ^{b}	26.00 ± 7.21 ^{ab}	28.67 ± 5.03 ^{a}
40	23.67 ± 3.21 ^{c}	28.00 ± 2.65 ^{b}	33.33 ± 2.08 ^{a}
50	23.67 ± 3.51 ^{c}	29.33 ± 7.02 ^{b}	36.33 ± 2.08 ^{a}

Average values with different superscripts in the same row are significantly different (P<0.05).

Phenoloxidase activity

After 20 days of feeding, shrimp fed with diets containing 0.25% DVAQUA® had a significantly higher (P<0.05) phenoloxidase activity (386.92 ± 15.35 units. min^{-1}. mg^{-1} protein) than the control group (289.33 ± 27.75 units. min^{-1}. mg^{-1} protein) but was not significantly higher than shrimp fed with diets containing 0.125% (344.26 ± 27.75 units. min^{-1}. mg^{-1} protein) (Table 5).

Superoxide dismutase activity

After 30 days of feeding, shrimp fed with diets containing 0.25% DVAQUA® had superoxide dismutase (35.80 ± 1.12 SOD units. ml^{-1}) which was significantly higher (P<0.05) than those of shrimp fed with diets containing 0.125% DVAQUA® and control groups which had 33.88 ± 4.71 and 27.62 ± 1.42 SOD units. ml^{-1}, respectively (Table 6).

Bactericidal activity

Shrimp fed with 0.125% and 0.25% DVAQUA® had bactericidal activity at the serum dilution of 1:16 while the control group had it at 1:8 after 20 days of experiment (Table 7).

Table 5. Phenoloxidase activity of *L. vannamei* after 0, 10, 20, 30, 40 and 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 9)

Feeding period (days)	Phenoloxidase activity (units. min^{-1}. mg^{-1} protein)		
	Control	DVAQUA®	
		0.125%	0.25%
0	274.20 ± 36.12 ^{a}	295.13 ± 16.61 ^{a}	283.62 ± 15.80 ^{a}
10	299.08 ± 34.50 ^{a}	325.73 ± 48.87 ^{a}	335.60 ± 40.47 ^{a}
20	289.33 ± 27.75 ^{b}	344.26 ± 27.75 ^{ab}	386.92 ± 15.35 ^{a}
30	285.85 ± 24.98 ^{b}	333.47 ± 35.39 ^{ab}	389.14 ± 24.48 ^{a}
40	293.17 ± 29.92 ^{b}	351.75 ± 32.07 ^{ab}	381.75 ± 15.87 ^{a}
50	304.50 ± 18.86 ^{b}	358.06 ± 29.90 ^{b}	384.20 ± 14.40 ^{a}

Average values with different superscripts in the same row are significantly different (P<0.05).

Table 6. Superoxide dismutase activity of *L. vannamei* after 0, 10, 20, 30, 40 and 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 9)

Feeding period (days)	Superoxide dismutase (SOD units. ml^{-1})		
	Control	DVAQUA®	
		0.125%	0.25%
0	27.30 ± 3.80 ^{a}	30.65 ± 2.99 ^{a}	28.24 ± 4.07 ^{a}
10	25.90 ± 2.08 ^{a}	32.60 ± 5.37 ^{a}	30.58 ± 3.95 ^{a}
20	28.59 ± 2.11 ^{a}	32.02 ± 2.05 ^{a}	33.65 ± 2.39 ^{a}
30	27.62 ± 1.42 ^{b}	33.88 ± 4.71 ^{b}	35.80 ± 1.12 ^{a}
40	28.12 ± 1.12 ^{c}	32.49 ± 1.55 ^{b}	38.29 ± 1.32 ^{a}
50	31.28 ±10.52 ^{c}	37.93 ± 4.65 ^{b}	42.27 ± 1.97 ^{a}

Average values with different superscripts in the same row are significantly different (P<0.05).

Table 7. Bactericidal activity of *L. vannamei* after 0, 10, 20, 30, 40 and 50 days of feeding with DVAQUA® at 0, 0.125% and 0.25% (n = 9)

Feeding period (days)	Bactericidal activity		
	Control	DVAQUA®	
		0.125%	0.25%
0	1 : 8	1 : 8	1 : 8
10	1 : 8	1 : 8	1 : 8
20	1 : 8	1 : 16	1 : 16
30	1 : 8	1 : 16	1 : 16
40	1 : 8	1 : 16	1 : 16
50	1 : 8	1 : 16	1 : 16

Experiment 3. Determination of the DVAQUA® effect on survival of white shrimp experimentally infected by Vibrio harveyi

After challenging the shrimp which were fed with DVAQUA® diets (0.125 and 0.25%) and regular pellets (control) for 4 days with *Vibrio harveyi* at 8.2×10^6 CFU. ml^{-1}, shrimp fed with diets containing 0.25% and 0.125% DVAQUA® had significantly higher ($P<0.05$) percentages of survival than the control group, i.e. 83.33 ± 5.77, 76.67 ± 5.77 and 56.67 ± 5.77%, respectively (Table 8).

Table 8. Percentage survival of *L. vannamei* after challenging with *Vibrio harveyi* 8.2×10^6 CFU. ml^{-1} at different time period (n = 30)

Time challenged with *V. harveyi* (hrs)	Percentage survival (%)		
	Control	DVAQUA®	
		0.125%	0.25%
24	76.67 ± 5.77^{b}	93.33 ± 5.77^{ab}	96.67 ± 5.77^{a}
48	63.33 ± 5.77^{b}	76.67 ± 5.77^{ab}	83.33 ± 5.77^{a}
72	56.67 ± 5.77^{b}	76.67 ± 5.77^{a}	83.33 ± 5.77^{a}
96	56.67 ± 5.77^{b}	76.67 ± 5.77^{a}	83.33 ± 5.77^{a}

Average values with different superscripts in the same row are significantly different ($P<0.05$).

Farm Trials Experiments

Experiment 4. Determination of the DVAQUA® effect on growth and survival of pond-reared white shrimp

The average production and survival rate of shrimp from the experimental ponds (0.25% DVAQUA®) were 2,375 kg. rai^{-1} and 82%, respectively, while the control group obtained 2,202 kg. rai^{-1} and 73%, respectively (Table 9). There were no significant differences because of the variation in production from growout ponds. However, the average of 9% difference in survival rates in DVAQUA® group resulted in an increase of 173 kg. rai^{-1} production.

Experiment 5. Determination of the DVAQUA® effect on the immune characteristics of pond-reared white shrimp

After 90 days of culture or 60 days of feeding a diet containing 0.25% DVAQUA®, shrimp from this treatment group had significantly higher ($P<0.05$) levels of immune parameters (THC, percentage phagocytosis, phenoloxidase, SOD and bactericidal activity) than those of the control group (Table 10).

From the study, it was found that ß-Glucan (DVAQUA®) could be used as an immuno-stimulant for shrimp to activate its immunological functions. This has led to the improvement in the resistance of shrimp against bacteria, which correlates with other studies on ß-Glucan (Itami *et al*.,1998; Sung *et al*., 1998; Chang *et al*., 2000). DVAQUA® is a natural fermented product derived from *Saccharomyces cerevisiae* of the cell wall of yeast (β-glucans and mannan-oligosaccharides). The cell soluble material by-products from fermentation (vitamins, proteins, peptides, amino acids, nucleotides, lipids, organic acids, oligosaccharides, esters and alcohols) were found to increase the growth rate of shrimp (Burgent *et al*., 2004) which is similar to a report by Barnes *et al*. (2006). Some reports also showed that ß-Glucan can enhance growth rate since shrimp can digest ß-Glucan and use it as energy source, thereby improving the use of more protein for growth (Wigglesworth and Griffith, 1994; Johansson *et al*., 2000).

CONCLUSIONS

The results from this study suggest that oral administration of 0.25% DVAQUA® for at least 30 days effectively enhanced the growth, survival and immune responses (total hemocytes, percentage phagocytosis, superoxide dismutase and phenoloxidase activity and bactericidal activity) of *L. vannamei*.

Table 9. Production, growth and survival of pond-reared *L. vannamei*

Pond	Area (rai)	Density (shrimp per pond)	Culture period (days)	Total production (kg)	Production (kg. rai^{-1})	Mean body weight (g)	Growth rate (g . day^{-1})	FCR	Survival rate (%)
DVAQUA® pond (DVAQUA® 0.25%)									
1	6	1,000,000	108	15,015.00	2502.50	17.54	0.27	1.57	85.59
2	6	1,000,000	110	11,443.00	1907.17	17.86	0.12	1.65	64.08
3	6	1,000,000	111	15,419.00	2569.83	17.54	0.18	1.67	87.89
4	6	1,000,000	109	15,126.00	2521.00	16.67	0.24	1.66	90.76
Mean	6	1,000,000	109.50	14,250.75±1,879.58 [a]	2375.13±313.26 [a]	17.40±0.51 [a]	0.2±0.07 [a]	1.64±0.04 [a]	82.08±12.18 [a]
Control (normal feed)									
1	6	1,000,000	105	12,774.32	2129.05	18.18	0.30	1.64	70.26
2	6	1,000,000	100	12,240.00	2040.00	17.54	0.28	1.72	69.77
3	6	1,000,000	110	12,818.00	2136.33	18.18	0.25	1.86	70.50
4	6	1,000,000	112	15,035.87	2505.98	18.18	0.24	1.61	82.70
Mean	6	1,000,000	106.75	13,217.05±1,240.70 [a]	2202.84±206.78 [a]	18.02±0.32 [a]	0.27±0.03 [a]	1.75±0.1 [a]	73.31±6.27 [a]

Table 10. The immune characteristics of pond-reared *L. vannamei* after 60 days of feeding with DVAQUA® at 0 and 0.25%

Immune characteristics	Treatment	
	Control	DVAQUA® 0.25%
Total hemocyte count (x 10^7 cells. ml^{-1})	24.09 ± 2.73 ^{b}	33.95 ± 3.23 ^{a}
Percentage phagocytosis	41.34 ± 4.36 ^{b}	61.63 ± 5.94 ^{a}
Phenoloxidase activity (units. min^{-1}. mg^{-1} protein)	356.28 ± 21.91 ^{b}	423.75 ± 32.67 ^{a}
Bactericidal activity	1: 8	1:16
Superoxide dismutase (SOD units. ml^{-1})	40.30 ± 9.83 ^{b}	49.62 ± 9.52 ^{a}

Average values with different superscripts in the same row are significantly different (P<0.05).

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