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Effects of a yeast-based additive on growth and immune responses of white shrimp, *Litopenaeus vannamei* (Boone, 1931), and aquaculture environment

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Abstract

A 60 days feeding experiment was conducted to determine the effects of DV AQUA (DVA) (a yeast culture feed supplement) on growth and immune responses of white shrimp, *Litopenaeus vannamei* and aquaculture environment in commercial scale farms composed of nine ponds. Three treatments (control, Diet 1 and Diet 2) were designed to contain DVA levels of 0.0, 1.0 and 1.5 g kg^{-1} respectively. At the end of the test, the mean production of Diet 1 (0.649 ± 0.030 kg m^{-2}) and Diet 2 (0.648 ± 0.033 kg m^{-2}) were significantly higher than that of the control by 29.5% and 29.3% respectively. The feed conversion ratio decreased significantly by 12.3% (Diet 1) and 8.5% (Diet 2) compared with the control group respectively. The environmental indices indicate that the quality of water and pond sediment was improved. The endotoxin concentrations, the number of *Vibrios* and heterotrophic bacteria in the shrimp intestine of the treatment groups were significantly lower than that of the control, particularly in the later stage of the farming period. The DVA extended positive effects on growth, improved sediment quality, reduced endotoxin in shrimp intestine, and enhanced activities of lysozyme and phenoloxidase.

Keywords: yeast culture, *Litopenaeus vannamei*, growth, immune responses, aquaculture environment, endotoxin

Introduction

White shrimp, *Litopenaeus vannamei* Boone, was introduced as an alternative culture species in 1987 since then it has expanded to make up more than 80% of total shrimp production in China (Zhang, Shen, Hu, Zhang, Ren, Chen, Liang & Chen 2006). After years of farming of *L. vannamei* at high stocking density in deep ponds, numerous problems have arose, such as deteriorating farming environment, and frequent outbreaks of diseases. To counter these problems, antibiotics and disinfectants have been commonly used which may contribute to depressed ecological health and environmental safety. The improper use of antibiotics and disinfectors would disturb or destroy the normal microbial populations in aquaculture environment (Lalumera, Calamari, Galli, Castiglioni, Crosa & Fanelli 2004; Balcázar, Blas, Ruiz-Zarzuela, Cunningham, Vendrell & Múzquiz 2006; Defoirdt, Boon, Sorgeloos, Verstraete & Bossier 2007), increase the risk of drug resistance of pathogenic microbes, and threaten food safety and human health (Boyd & Massaaut 1999; Grave, Hansen, Kruse, Bangen & Kristoffersen 2008).

The need for alternative methods for regulating the number of pathogenic bacteria has led researchers to develop methods of treatment, such as probiotics and immunostimulation (Scholz, Díaz, Ricque, Suárez, Albores & Latchford 1999). Immunostimu-

lant aims at enhancing the non-specific defense mechanisms in animals, including shrimp (Song & Sung 1990) and they are believed to render animals more resistant to infectious diseases and reduce the risk of disease outbreaks if administrated prior to situations known to result in stress and impaired general performance (e.g. handling, change of environmental parameters, weaning of larvae to artificial feeds) or prior to expected increase in exposure to pathogenic microorganisms and parasites (e.g. spring and autumn blooms in the marine environment, high stocking density) (Raa 1996). When introduced into the diets or water, these compounds may enhance the immune ability of aquatic animals, control diseases and in turn reduce even stop abuse of antibiotics and other drugs to ensure food security (Skjermo, Størseth, Hansen, Handå & Øie 2006). Some studies have demonstrated that yeast culture is able to improve animal health by influencing the micro-ecological environment in digestive system, improving digestion and absorption of nutrients and stimulating immune responses (Tovar, Zambonino, Cahu, Gatesoupe, Vázquez-Juárez & Lésel 2002; Suphantharika, Khunrae, Thanardkit & Verduyn 2003; Marques, Dhont, Sorgeloos & Bossier 2004; Haddad & Goussous 2005). Besides, yeast culture could inhibit growth of pathogenic microbes colonized in digestive duct, amend the structure and shape of intestine wall and attenuate the damage of fungi toxin (Patra & Mohamed 2003; Marques, Dhont, Sorgeloos & Bossier 2006).

It has been reported that yeast culture could enhance growth, feed conversion ratio (FCR) and anti-disease ability in aquaculture animals, such as shrimps, *L. vannamei, Artemia franciscana*, *Penaeus chinesis* and fish, *Oncorhynchus mykiss*, *Paralichthys olivaceus* and *Cyprinus carpio* L. (Burgents, Burnett & Burnett 2004; Mai, Yu, Ai, Wang, Liu-Fu & Zhen 2004; Marques *et al.* 2006). However, all these experiments had been carried out in indoor systems of laboratory scale and mainly focused on the growth performance and immune responses of cultured animals. The environments are remarkably different from those in actual farming ponds, so these results probably bring some deviation from the actual culture. Because of this, in this article, a yeast culture feed supplement, DV AQUA (DVA) was selected in farming of *L. vannamei* in production scale, with the supplement levels of 0.0, 1.0 and 1.5 g kg^{-1} respectively. Effects of DVA on growth performance, immune responses and aquaculture environment were evaluated.

Materials and methods

Diet preparations

DV AQUA (DVA), a yeast culture feed supplement, was purchased from Diamond V Mills, Inc. Cedar Rapids, Iowa U.S.A.. The DVA, a kind of processed yeast not containing live yeast cells, consists of primary metabolites, such as nucleotides, polysaccharides, small peptides, organic acids and lipids.

Commercial feed for shrimps, provided by Haixin Group, Longhai, China, was used. The crude protein content was 410 g kg^{-1} and crude lipid was 75 g kg^{-1}. The DVA was added along with the additives, such as vitamins and minerals during the production process of the feed.

Three treatments (control, Diet 1 and Diet 2) were designed to contain DVA levels of 0.0, 1.0 and 1.5 g kg^{-1}, respectively, in the commercial feed.

Feeding trial

Feeding trial was conducted at Weiwei Aquaculture Base of Longhai city, Fujian Province, China, from May to August 2006. Nine ponds were randomly selected from 30 ponds with same size and shape (square pond with 4 equal length sides, 256 m^2 at the pond's water surface and 200 m^2 at the pond's bottom, 1.80 m water depth). Four sides of each pond were covered with cement and the bottom consisted of muds and sands. Each diet was randomly assigned to triplicate ponds. Control was assigned to No. 3, 16 and 27 ponds; Diet 1 was assigned to No. 7, 12 and 28 ponds; Diet 2 was assigned to No. 8, 19 and 23 ponds.

Brackish water from an inlet was used, salinity around 3–5 g L^{-1}. The shrimp juveniles with similar size (average body length of 1.2 cm, weight of 20 000 individuals kg^{-1} and age of 20 days) derived from the same patch were purchased from a commercial supplier. In actual farming of China, stocking density was usually 60–120 individuals m^{-2} in common culturing model and the yield can reach 0.5–1 kg m^{-2}. So 16 000 of shrimp juveniles (80 individuals m^{-2} pond's bottom area) were stocked in each pond on 30 April 2006.

A commercial feed without DVA was applied to all the ponds during the first 30 days. Trial feeds were applied from the 31st to 92nd day after the start of the experiment. Feed was distributed evenly over the pond's surface, twice daily at

07:00 and 15:00 hours. Each pond of shrimps was fed at apparent satiation levels during the entire experimental period. The satiation feed should ensure there were still residual feed in 2 h after the feed application. One tenth of culture water (about 45 m^3) was exchanged twice a week. Other daily management was the same as normal farming practices. During the first 30 days of farming, to stimulate phytoplankton development, culture ponds were fertilized with urea and super phosphate at the rate of 4 and 1 $g^{-1}(m^2\ week^{-1})$ during the first 4 weeks of culture. Cow dung was also added to the ponds at 20 and 10 kg dry matter/pond at the onset of the 2nd and 4th week of culture. Only feed was applied to the shrimps after starting the treatment with the DVA yeast feed.

Evaluation parameters

At the conclusion of the growth trial (94th and 95th day), the ponds were drained for harvest and the shrimp removed. The total biomass of shrimps was determined for each pond. Mean body length and weight of 100 randomly selected shrimps from each pond were measured. The number of shrimp surviving in each pond was determined by dividing the final biomass by mean body weight, FCR was calculated according to the total feed inputs of each pond divided by the total shrimp weight gain.

Water samples (2 L) were collected using a horizontal water sampler from three locations of each pond (the sampling sites were located in one corner of the pond, around 0.5 m from the sides, near the midpoint of the pond bank, around 0.5 m from the side and in the pond centre.) and pooled together in polyethylene bottles between 09:00 and 10:00 hours weekly from the 38th to 84th day after the start of the experiment (six times totally). At the time of sampling, water temperature, salinity and pH were measured using a portable YSI 63 temperature/salinity/pH metre. DO was determined by Winkler titration method (Stirling 1985). NH_3-N, NO_2-N, chemical oxygen demand (COD) and S^{2-} were determined following the methodology of Clesceri *et al.* (1998). Total number of heterotrophic bacteria and *Vibrios* expressed as colony-forming units were determined by the spread plate method, using marine agar 2216 medium and thiosulphate citrate bile salts agar medium respectively (Clesceri *et al.* 1998; Downes & Ito 2001).

Sediment samples were collected from three locations in each pond using 2-cm diameter PVC pipes and pooled just before the termination of the feeding trial (the 94th–95th day after the start of the test). The 50 g mud sample was obtained from each sampling point and 150 g mud from three points in each pond was mixed. The COD, S^{2-}, total number of heterotrophic bacteria and *Vibrios* in the sediment were also assayed.

Haemolymph of shrimp was sampled once every 10 days from the 64th to 85th day after the start of the experiment (three times totally). For total haemocyte counts (THC), haemolymph (100 μL) was withdrawn from the ventral sinus of each shrimp into a 0.5 mL sterile syringe (25 gauge) containing 200 μL anticoagulant (10 mM EDTA-Na_2, 450 mM NaCl, 10 mM KCl, 10 mM HEPES, pH 7.3, 850 mosM kg^{-1}). The anticoagulant solution for haemolymph extraction was prepared according to Vargas-Albores, Guzmán and Ochoa (1992) and Pascual, Arena, Cuzon, Gaxiola, Taboada, Valenzuela and Rosas (2004). A drop of the haemolymph mixture from ten shrimp was placed on a haemocytometer, and THC was made under an optic microscope. For enzyme activities, haemolymph (200 μL) was withdrawn from each shrimp, and the haemolymph from twenty shrimp was mixed and stored in sterile centrifuge tube at 4°C overnight. Following centrifugation (825 *g*, 10 min, 4°C), the plasma was removed and used for subsequent tests. Activities of phenoloxidase (PO), superoxide dismutase (SOD) and lysozyme were measured according to Ashida and Dohke (1980), Marklund and Marklund (1974) and Ellis (1990) respectively.

Endotoxin, total number of heterotrophic bacteria and *Vibrios* in shrimp intestine were measured once every 10 days from the 64th to 85th day after the start of the experiment (three times totally). The means of each treatment was an average of three measured values. The whole digestive tracts were removed from five shrimps of each pond, weighed and mixed with pyrogen-free saline water (8.5 g kg^{-1} of NaCl). The mixture was blended on a Vortex mixer for 10 min and centrifuged (4°C, 5000 *g*, 10 min), then the supernatant was removed, diluted to the proper concentration with pyrogen-free saline water and was detected with quantitative chromogenic limulus amebocyte lysate assay for endotoxins (Goris, Boer & Waaij 1986; China Pharmacopoeia Committee 2000).

Statistical analysis

To reflect the difference of the treatments during the whole culturing, for each parameter, six determined values of water qualities were averaged and three determined values of the immune responses, microbes and endotoxin in the shrimp intestine were averaged. The mean value of each treatment was an average of values from three replicates.

Using SPSS 11.0 software, means of percentage were firstly arcsine transformed. The One-way ANOVA was used to compare the means. Tukey's multiple comparison test was used to compare the significant differences among treatments. For statistically significant differences, it was required that $P < 0.05$.

Results

Growth performance

Shrimp growth performance of the treatments at the end of experiment (31 July) was shown in Table 1. The final body length, body weight and survival rate showed significant differences ($P < 0.05$) between the control and DVA treatments. Final body length of shrimp with DVA supplementation diets was higher than that of the control by 11.7% (Diet 1) and 12.4% (Diet 2), respectively, whereas final body weight increased by 15.7% (Diet 1) and 16.9% (Diet 2) respectively. The difference of production per square metre and FCR reached significant level. Mean production of Diet 1 and Diet 2 were significantly higher than that of the control by 29.5% and 29.3% ($P < 0.05$). The mean survival of Diet 1 and Diet 2 were significantly higher than that of the control. There were no significant differences between Diet 1 and Diet 2 in growth performance and survival rate.

Water quality

During experiment, average water temperature was 31.5°C, from 28.5 to 33.5°C, average salinity 4 g L^{-1}, from 3 to 5 g L^{-1}. Water temperature and salinity of different ponds were approximately equal at the same time. The data of six tests of other indices were given as the average value during the whole experiment period (Table 2).

The levels of NH_3-N, NO_2-N, S^{2-} and DO matched the national standard of first grade water in China. There were no significant differences in NO_2-N and DO concentrations among the three treatments ($P > 0.05$). Both S^{2-} and NH_3-N contents and *Vibrios* density were significantly lower in DVA supplementation groups than those in the control ($P < 0.05$).

Pond sediment

Both COD and S^{2-} contents in pond sediment are shown in Figure 1. Supplementation of DVA significantly reduced COD and S^{2-} contents ($P < 0.05$). However, S^{2-} content was not reduced further with higher level of supplementation.

The total *Vibrios* number in the sediment of DVA supplementation groups were reduced significantly compared with that of the control ($P < 0.05$) (Fig. 2). Total number of heterotrophic bacteria declined along with supplementation of DVA, and reached a significant level in Diet 2 ($P < 0.05$).

Microbes and endotoxin in shrimp intestine

Total number of *Vibrios* and heterotrophic bacteria in shrimp intestine declined with DVA supplementation, and reached a significant level in Diet 2 ($P < 0.05$). This trend was similar with the situation in pond mud (Fig. 3).

Table 1 Effect of DVA on the growth performance of shrimp, *Litopeneaus vannamei*[*]

Test items	Control (0.0 g kg^{-1})	Diet 1 (1.0 g kg^{-1})	Diet 2 (1.5 g kg^{-1})	*P*
Initial body weight (g)	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	–
Final body length (cm)	9.15 ± 0.35^{b}	10.22 ± 0.26^{a}	10.29 ± 0.14^{a}	0.003
Final body weight (g)	9.56 ± 0.56^{b}	11.06 ± 0.29^{a}	11.18 ± 0.44^{a}	0.007
Mean production (kg m^{-2})	0.501 ± 0.012^{b}	0.649 ± 0.030^{a}	0.648 ± 0.033^{a}	0.001
Survival rate (%)	65.52 ± 3.29^{b}	73.35 ± 1.90^{a}	72.44 ± 2.68^{a}	0.007
Total feed inputs (kg)	90.62 ± 3.87 ^{b}	103.06 ± 2.96 ^{a}	107.22 ± 3.86 ^{a}	0.003
FCR	0.905 ± 0.016^{a}	0.794 ± 0.033^{b}	0.828 ± 0.013^{b}	0.003

*Values are expressed as mean (±SD). Values in the same row with same superscript are not significantly different ($P > 0.05$).

Table 2 Effect of DVA on water qualities in the ponds[*,†]

Test items	Control (0.0 g kg^{-1})	Diet 1 (1.0 g kg^{-1})	Diet 2 (1.5 g kg^{-1})
pH	8.59 ± 0.13	8.47 ± 0.06	8.40 ± 0.02
	8.44, 8.69	8.42, 8.53	8.38, 8.42
NO_2-N (μg L^{-1})	24.43 ± 1.70	21.83 ± 1.33	20.90 ± 1.80
	22.71, 26.11	20.50, 23.16	19.52, 22.93
NH_3-N (μg L^{-1})	18.02 ± 0.62^{a}	14.96 ± 1.59^{b}	13.62 ± 1.18^{b}
	17.31, 18.41	13.71, 16.75	12.89, 14.98
DO (mg L^{-1})	7.28 ± 0.36	7.33 ± 0.13	7.33 ± 0.24
	6.96, 7.67	7.23, 7.48	7.17, 7.61
COD (μg L^{-1})	5.28 ± 0.03^{a}	5.14 ± 0.06^{ab}	5.06 ± 0.10^{b}
	5.24, 5.30	5.10, 5.21	4.94, 5.12
S^{2-} (μg L^{-1})	86.40 ± 2.56^{a}	80.32 ± 0.96^{b}	80.32 ± 0.64^{b}
	84.69, 89.34	79.22, 80.96	79.58, 80.75
Total number of *Vibrios* mL^{-1}	212 ± 22 ^{a}	166 ± 29 ^{b}	153 ± 22 ^{b}
	199, 273	132,182	136, 178

*Values are expressed as mean (±SD). Values in the same row with same superscript are not significantly different ($P > 0.05$).
†The high and low values were given in the next line following each test item.

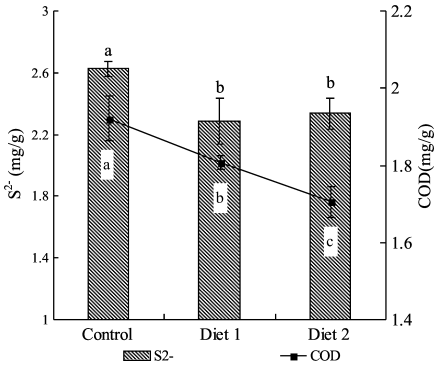

Figure 1 Effect of DVA on the S^{2-} and COD concentrations at the sediment of the ponds. Columns with the same superscript letter are not significantly different ($P > 0.05$).

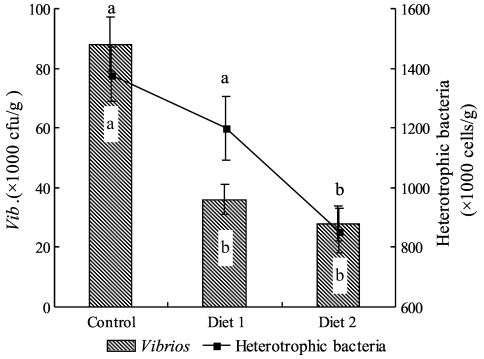

Figure 2 Effect of DVA on the numbers of V*ibrios* and heterotrophic bacteria at the sediment of the ponds. Columns with the same superscript letter are not significantly different ($P > 0.05$).

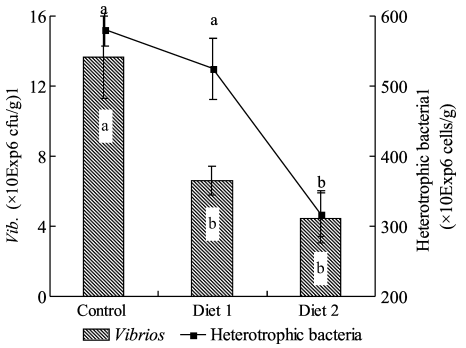

Figure 3 Effect of DVA on the numbers of V*ibrios* and heterotrophic bacteria in the shrimp intestine, *Litopeneaus vannamei*. Columns with the same superscript letter are not significantly different ($P > 0.05$).

Supplementation of DVA significantly reduced the endotoxin content in shrimp intestine ($P < 0.05$), whereas there were no significant differences between high and low level of DVA (Fig. 4).

Immune responses

During the experiment, indices for immune responses of shrimp, such as total haemocyte count, activities of PO, SOD and lysozyme were measured three times. The mean values of individual parameters are shown in Table 3.

The total haemocyte count showed a declining trend with increasing dietary DVA level, although no significant differences were found ($P > 0.05$). Lysozyme and PO activities in DVA treatments were significantly higher than those of the control

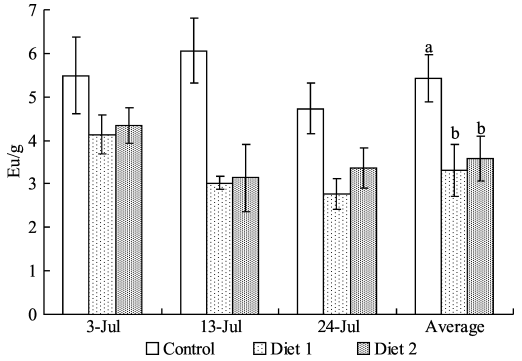

Figure 4 Effect of DVA on the endotoxin in the digestive tract of shrimp, *Litopeneaus vannamei*. Columns with the same superscript letter are not significantly different ($P > 0.05$).

($P < 0.05$), whereas the SOD activity showed no remarkable change among dietary treatments ($P > 0.05$).

Discussion

Effects of dietary DVA on growth and survival

The results revealed that DVA supplementation in feed could promote the growth performance of white shrimp cultured in commercial scale farms, and significantly decreased FCR. Similar results albeit not significant, were reported by Mai *et al.* (2004), who found that XP (a kind of yeast culture) supplementation in feed could numerically increase the body weight, and survival rate of Japanese flounder as well as decrease FCR. In the present study, supplementation of DVA demonstrated a stronger improvement of growth, production and feed efficiency.

Effects of DVA on actual culture environment

The environmental indices indicate that the quality of water and pond sediment was improved; especially contents of NH_3-N, COD and S^{2-} were reduced. However, DVA probably did not improve the water quality directly. The improvement of water quality was originated from optimization of the sediment by addition of DVA. An improved FCR would lead to a lower amount of shrimp faeces and, potentially, a lower amount of waste accumulating in the pond bottom. So the organic matter released to the water was reduced and the conditions of the pond were improved. Effects of DVA on pond mud were more pronounced than on water, which was probably due to a variety of factors. Firstly, relatively less DVA was probably released into the water because of diet supplementation. This would have been further reduced by regular water exchange. Secondly, the effect on pond mud was more pronounced presumably because DVA from shrimp faeces and uneaten feed would have directly accumulated and deposited at the pond bottom. Furthermore, the microbes probably, colonized mainly in mud rather than in water, especially those respond for the S^{2-} and NH_3 degradation. It could be concluded that DVA affected the micro-ecology and stimulate such microbe in shrimp ponds. Thus, influencing or accelerating the metabolism and recycling of organic matters. As a result, NH_3-N, COD and S^{2-} contents in pond water and mud were reduced. This is especially important in pond aquaculture because pond bottom always lacks oxygen due to weak photosynthesis and poor water exchange. The deposition of organic matter increases COD and in turn aggravates the lack of oxygen. So the oxidative degradation of organic matters slows down, and some pollutants, such as NH_3-N and H_2S are alternatively produced, which are harmful to shrimp (Chen & Chin 1988; Chen & Kou 1992). In the present study, DVA reduced NH_3-N, COD and S^{2-} contents, resulting in positive effects on culture environment, especially in the pond sediment, which was more meaningful to the zoobenthos, such as shrimp.

Table 3 Effect of DVA on the immunological parameters of shrimp, *Litopeneaus vannamei*[*]

Test items	Control (0.0 g kg^{-1})	Diet 1 (1.0 g kg^{-1})	Diet 2 (1.5 g kg^{-1})
Total haemocyte counts ($\times 10^6$ mL^{-1})	1.19 ± 0.06	1.09 ± 0.09	0.99 ± 0.06
SOD (U mL^{-1})	36.13 ± 0.73	37.17 ± 0.45	38.10 ± 0.79
Lysozyme (μg mL^{-1})	246.4 ± 23.69^b	299.77 ± 8.86^a	320.4 ± 12.16^a
PO (U mL^{-1})	10.60 ± 0.50^b	11.60 ± 0.45^{ab}	12.47 ± 0.65^a

*Values are expressed as mean (±SD). Values in the same row with same superscript are not significantly different ($P > 0.05$).

Effects of dietary DVA on microbial populations in pond and shrimp intestine

The DVA significantly reduced the total number of *Vibrios* both in pond and shrimp intestine, which are the most common pathogenic bacteria in aquaculture (Lavilla-Pitogo, Leano & Paner 1998; Sung, Hsu, Chen, Ting & Chao 2001; Chen, Wang & Chen 2005). Such inhibition on *Vibrios* might contribute to the effect of DVA on water quality and sediment. The offering of Diet 1 (1.0 g kg^{-1} of DVA) and Diet 2 (0.15 g kg^{-1} of DVA) showed no remarkable difference in inhibition of *Vibrios*, whereas shrimp and ponds receiving Diet 1 had higher levels of heterotrophic bacteria in both shrimp intestine and sediment. The supplementation level of 1.0 g kg^{-1} is considered better than 0.15 g kg^{-1} in the respects of microbe optimization. How DVA inhibits *Vibrios* remains an interesting research field. The trend in Figure 2 is identical to that of Figure 3, which might be owing to the accumulation and deposition of shrimp faeces at the pond bottom.

Environmental disturbance as well as artificial influences, like the applications of drugs and antibiotics, affects normal microorganism populations in water. High density and intensive farming always result in overload of organic matters in water, which may promote an increase in pathogenic bacteria and possible diseases outbreaks. Therefore, proper supplementation of DVA could improve water quality and inhibit *Vibrios* both in pond and shrimp intestine, then reduce diseases.

Effects of dietary DVA on endotoxin in shrimp intestine

Endotoxin refers to a heat-stable matter with bioactivity in the filtered media of Gram-negative bacteria. Studies show that the main compound of endotoxin is lipopolysaccharide (LPS), a major product secreted by Gram-negative bacteria (Raetz 1990; Holst, Ulmer, Brade, Flad & Rietschel 1996). As one of the major virulent factors, LPS is more and more widely used as an index of virulence of pathogens (Engelhardt, Otto, Mackensen, Mertelsmann & Galanos 1995; Gutsmann, Schromm & Brandenburg 2007). However, as far as we know, there is no report of studies on endotoxin in white shrimp intestine yet. The present study showed that DVA supplementation in feed significantly reduced endotoxin content in shrimp intestine, which may be related to the inhibition of DVA on *Vibrios* and other pathogenic bacteria in shrimp intestine. Comparison between Diet 1 and Diet 2 showed that the supplement dose of 1.0 g kg^{-1} was better in reduction of endotoxin.

Effects of dietary DVA on immune responses of shrimp

Activity of PO is a direct index of defense ability for animals especially in crustaceans (Ashida & Dohke 1980; Decker & Jaenicke 2004). Lysozyme hydrolyzes polysaccharides in cell wall of bacteria, thus destroys cell wall and kills bacteria, which can defense a wide spectrum of bacteria (Bulet, Hetru, Dimarcq & Hoffmann 1999; Hikima, Minagawa, Hirono & Aoki 2001; Fevolden, Røed & Fjalestad 2002). In the present study, both activities of PO and lysozyme in DVA groups were significantly higher than those of the control, and increased along with the dose of DVA. This indicates that DVA can stimulate and enhance the activities of PO and lysozyme in serum of shrimp as the cases of other probiotics (Fevolden *et al.* 2002; Fagan, O'Byrne-Ring, Ryan, Cotter, Whelan & Mac-Evilly 2003), and indicates a potential protection against diseases in shrimp. The SOD activity showed no remarkable change between the treatments, although the activity showed slight increase along with the increase in DVA. It can be related to findings in Campa-Córdova, Hernández-Saavedra, Philippis & Ascencio's study. Campa-Córdova, Hernández-Saavedra, Philippis and Ascencio (2002) reported that an elevated muscle SODA (SOD activity) of shrimp exposed to immunostimulants (sulphated polysaccharide and β-glucan), 24 h after exposure and decreased below the control group value 48 h after treatment, rising to basal values (control values) at 72 h. The increase in SOD activity of shrimps had not been found in this experiment, which may be owing to the reasons that the enzyme activities were began to be measured at the 64 th day after the start of the experiment and so the variation of SOD activity was not detected. There were no evident differences among the three measured values of SOD activity for each pond.

The total haemocyte count of shrimp showed slight decrease along with the increase in DVA. It may be explained for the stimulation of the shrimp. On the first days of stimulation there was infiltration of haemocytes in tissues which may

result in a decrease in THC. Chisholm and Smith (1995) reported that the increase in relative haemocyte count (RHC) 48 h after exposure to immunostimulants may be related to the protective effects of the shrimp immune system against potential pathogens. Campa-Córdova *et al.* (2002) considered that an increase in RHC indicated a potential protection against diseases in shrimp. In contrast, Moullac, Soyez, Saulnier, Ansquer, Avarre and Levy (1998) found a correlation between the resistance to vibriosis and the PO activity, but not to THC during the moult cycle in *Penaeus stylirostris*. In the present study, it was speculated that the low level of haemocytes in a normal range could provide much room to elevate in situations of infection.

Conclusion

The DVA extended positive effects on growth, improved sediment quality, reduced endotoxin in shrimp intestine and enhanced activities of lysozyme and PO. It was also demonstrated that the supplement dose of 1.0 g kg^{-1} was optimal on such culture conditions.

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