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GROWTH PERFORMANCE OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* LARVAE FED PREBIOTIC AND PROBIOTIC THROUGH ARTEMIA

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ABSTRACT

This study aimed at determining the effect of prebiotic, probiotic and synbiotic enrichment through artemia on survival, growth and stress tolerance of Pacific white shrimp larvae. The life stage of Pacific white shrimp larvae used in this study was the nauplius 6 with an average weight of 0.6±0.08 mg/larvae and the treatment was started at mysis 1. The shrimp larvae were reared in a jar with a total water volume of two liters and at a density of 100 larvae/l. The study consisted of four treatments and three replicates: control, prebiotic, probiotic and synbiotic. The stress test with 200 ppm of formalin solution was performed after the larvae reached the post-larvae 10 stage. The results showed that synbiotic enrichment through artemia had the highest survival rate (41.50±3.61%), daily growth rate (17.55 ± 0.65%) and the lowest mortality rate when tested with 200 ppm formalin solution (33.33 ± 10.41%).

Keywords: Prebiotic, probiotic, synbiotic and shrimp larvae.

INTRODUCTION

The Pacific white shrimp *Litopenaeus vannamei* is one of the primary export commodities of the fishery sector in Indonesia. As a primary commodity, a lot of the Pacific white shrimp cultivation involves the intensive cultivation system. However, in the past decade, intensive shrimp cultivation has faced many problems due to viral and bacterial infections (Zhang *et al*., 2012) which have decreased the Pacific white shrimp production. Most of the diseases occur as a result of environmental deterioration and stress associated with intensefication of shrimp farming.

Several actions have been taken to control pathogenic microbes and increase shrimp growth rate such as the use of antibiotics and disinfectants (Balcázar *et al*., 2006). However, the use of antibiotics lead to the emergence of antibiotic-resistant pathogens, environmental damage, and food-safety issues (Verschuere *et al.*, 2000).

The most common effort to improve the quality of aquatic animal larvae is to make them more resistant to disease through the application of prebiotics and probiotics. Prebiotics are non digestible food materials which stimulate microbial growth and activity and thus improve the host's health (Cerezuela *et al.*, 2011). Previous studies have shown that the use of prebiotics can improve the immune system in shrimp (Zhang *et al.*, 2012; Li *et al.*, 2009), and fish (Akrami *et al.*, 2013; Merrifield *et al.*, 2010). Probiotics are live or dead microbes or bacterial components which act upon different action models exerting beneficial effects directly on the host or its environment (Lazado and Caipang, 2014). Some probiotics that have been used in aquaculture practices are *Lactobacillus, Lactococcus*, *Leuconostoc, Enterococcus, Carnobacterium, Shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Clostridium* and *Saccharomyces* (Nayak, 2010). Some study results have proven the success of probiotics in increasing shrimp and fish resistance to disease by improving immunity or by improving water quality (Verschuere *et al.*, 2000).

The combination of a prebiotic and a probiotic is known as a synbiotic. A synbiotic gives a competitive advantage to probiotics in accessing fermented energy sources compared to the competition of endogenous populations, resulting in an effective increase in the growth of beneficial bacteria in the intestinal tract of live hosts (Schrezenmeir and Vrese, 2001). One of the important factors affecting shrimp larval quality is health status which can be tested with a stress test (Zhang *et al.*, 2012), for example using formalin solution (Samocha *et al.*, 1998). This method is widely applied in Indonesia to assess the quality of the shrimp larvae produced before they are distributed to shrimp farms.

The synbiotic used in this study is a combination between the probiotic bacteria *Vibrio alginolyticus* SKT-b and a prebiotic extracted from the sweet potato *Ipomoea batatas*. This synbiotic was used on the juvenile stage of Pacific white shrimp and the results showed that the synbiotic could improve the shrimp growth rate, immune response, and resistance (Oktaviana *et al*., 2014). Knowledge of the application of synbiotics on shrimp larvae is still very limited; therefore, this study was conducted to test the effect of the application of prebiotic, probiotic and synbiotic through artemia on Pacific white shrimp larvae's survival rate, growth and stress tolerance.

MATERIALS AND METHODS

Prebiotic preparation: The prebiotic used was extracted from sweet potato starch var. *sukuh*. The preparation of sweet potato starch was done based on the method by Marlis (2008) then the oligosaccharide was extracted according to Muchtadi (1989) using 70% ethanol. The oligosaccharide extract was measured for its total dissolved solids (TDS) according to Apriyantono *et al.* (1989).

Probiotic preparation: The probiotic used in this study was *Vibrio alginolyticus* SKT-b marked as Rifampicin resistant (Rf^R) and called SKT-b Rf^R (Widanarni *et al.*, 2003). The SKT-b Rf^R bacteria were cultured in seawater complete (SWC) broth medium (5 g bacto-peptone, 1 g yeast extract, 3 ml glycerol, 750 ml seawater and 250 ml aqua destilata) then incubated in a water bath shaker for 18 hours at 29^0C at a speed of 140 rpm.

Artemia enrichment using prebiotic and probiotic: Artemia cysts (2 g/l) were hatched in seawater with a salinity of 30 ppt then strongly aerated and harvested after 24 hours. The artemia nauplii were then enriched with prebiotic, probiotic and synbiotic. Artemia density was 100 individuals/ml seawater at a salinity of 30 ppt. Enrichment was done for 4 hours with continuous aeration (Widanarni *et al*., 2008). The enriched artemia were harvested using a plankton net and rinsed with sterile seawater. The harvested artemia were then stored in a refrigerator at 4^0C for further use on the same day.

Rearing condition: The shrimp used in this study were Pacific white shrimp larvae at nauplius 6 stage which were obtained from PT. Suri Tani Pemuka, Banten, Indonesia. The larvae were reared in two-liter-glass jars with stocking density of 100 individuals/l and the larvae weighed an average of 0.6±0.08mg. Bef o re the treatment, the larvae were reared until they reached the zoea 3 stage and were fed with *Chaetocerros* sp. (10^5 cells/ml) five times a day. Temperature of the rearing medium was between 31 and 33^0C, salinity at 32-33 ppt, pH at 7.34-8.47 and dissolved oxygen at 4.5-5.70 ppm.

Trial through artemia test administration to Pacific white shrimp: This study consisted of four treatments: control (artemia without prebiotic, probiotic or synbiotic enrichment), prebiotic treatment (artemia enriched with 2% prebiotic), probiotic treatment (artemia enriched with 10^7 cfu/ml probiotic) and synbiotic (artemia enriched with 2% prebiotic and 10^7 cfu/ml probiotic). Each treatment was performed in triplicate. Trial administration of artemia test to shrimp was begun at mysis 1 up to post-larvae (PL) 10 stage. Shrimps were fed five times a day at 06.00, 10.00, 14.00, 18.00 and 22.00 Western Indonesia Time. The number of artemia administered was 3-4 individuals/larvae during the mysis stage and 8-10 individuals/larvae during the post-larval stage (Nimrat *et al.*, 2011).

Measurement of survival rate, growth, and bacterial count: The survival rate (SR) of larval shrimp was calculated at the end of the study according to Effendie (1997) and daily growth rate (DGR) according to Huisman (1987). Bacterial count was done using the spread plate method (Madigan *et al.*, 2003) at the beginning and at the end of the rearing period. Total viable bacterial count (TBC) was assessed with SWC agar medium and total SKT-b Rf^R with thiosulphate citrate bile-salt sucrose (TCBS) agar + rifampicin 50 μg/ml.

Stress Test on Pacific White Shrimp Larvae: In this study, the stress test was done by exposing the shrimp to formalin solution based on the description by Samocha (1998). Twenty post-larval shrimp (No) from each jar were put into the rearing receptacle. Each receptacle was filled with two liters seawater and formalin solution was added to achieve a concentration of 200 ppm. Observations of the post-larvae mortality were done every 2 hours and the total mortality rate was calculated after 12 hours (Nt). The larvae mortality rate was calculated using the following formula:

$$MR = \frac{Nt}{No} \times 100\%$$

RESULTS

The best DGR was demonstrated by the synbiotic treatment (17.55±0.65%) which was significantly different (P<0.05) from the probiotic treatment (16.86±1.05%), prebiotic treatment (15.97±0.45%) and control (14.56 ±0.66%). The highest Pacific white shrimp larvae weight gain was demonstrated by the synbiotic treatment (0.65±0.07 mg/day), followed by the probiotic treatment (0.58±0.11 mg/day), prebiotic treatment (0.50±0.04 mg/ day) and control (0.39±0.04 mg/day). The highest Pacific white shrimp larvae absolute length growth was also found in the synbiotic treatment (10.05±1.18 mm) and the lowest in the control (6.85±0.27 mm). The highest survival rate was shown by the synbiotic treatment (41.50±3.61%) which was significantly different (P<0.05) from the probiotic treatment (33.00±3.33%), prebiotic treatment (28.33±3.50%) and control (27.67±0.23%) (Table 1).

Table 1. Daily growth rate (DGR), weight gain, absolute length gain, and survival rate (SR) of Pacific white shrimp (*Litopenaeus vannamei*) treated with prebiotic, probiotic and synbiotic treatments

Treatment	DGR (%)	Weight Gain (mg/day)	Absolute Length Gain (mm)	SR (%)
Control	14.56±0.66^{a}	0.39±0.04^{c}	6.85±0.27^{a}	27.67±0.23^{a}
Prebiotic	15.97±0.45^{a}	0.50±0.04^{b}	7.47±1.53^{a}	28.33±3.50^{a}
Probiotic	16.86±1.05^{a}	0.58±0.11^{ab}	7.00±0.34^{a}	33.00±3.33^{a}
Synbiotic	17.55±0.65^{b}	0.65±0.07^{a}	10.05±1.18^{b}	41.50±3.61^{b}

Notes: different letters in the same column signify significantly different results (P<0.05)

The total bacterial count in shrimp larvae at the beginning of the rearing period for all treatments was 2.08x10^{6} CFU/larvae. After the rearing period, there was an increasing total bacterial count in the shrimp larvae in all treatments. The highest total bacterial count was demonstrated by the synbiotic treatment at 1.82x10^{11} CFU/larvae, followed by the probiotic treatment at 1.36x10^{10} CFU/larvae, the prebiotic treatment at 1.22x10^{9} CFU/larvae, the lowest was the control at 9.00x10^{8} CFU/larvae. The total SKT-b Rf^{R} count at the end of the rearing period was only found in the synbiotic treatment at a value of 1.84x10^{5} CFU/larvae and the probiotic treatment at 1.52x10^{5} CFU/larvae (Table 2).

Table 2. Total bacterial count and total SKT-b Rf^{R} count in Pacific white shrimp (*Litopenaeus vannamei*) larvae

Treatment	**Total Bacterial Count (CFU/larvae)**		**Total SKT-b Rf^{R} (CFU/larvae)**	
	Initial	Final	Initial	Final
Control	2.08x10^{6}	9.00x10^{8}	0	0
Prebiotic	2.08x10^{6}	1.22x10^{9}	0	0
Probiotic	2.08x10^{6}	1.36x10^{10}	0	1.52x10^{5}
Synbiotic	2.08x10^{6}	1.82x10^{11}	0	1.84x10^{5}

No Pacific white shrimp mortality was detected at hour 2 of the stress test in any of the treatments. Initial mortality was found at hour 4 with the best value obtained in the synbiotic treatment (5.00±0.00%) which differed signify-cantly (P<0.05) from the control (20.00± 5.00%). The highest total mortality rate was found in the control (60.00±5.00%), followed by the prebiotic treatment (48.33± 2.89%), probiotic and synbiotic treatment (38.33±10.41; 38.33±10.41%). The accumulation of morta-lity rates in the synbiotic treatment was significantly different (P<0.05) from the control (Figure 1).

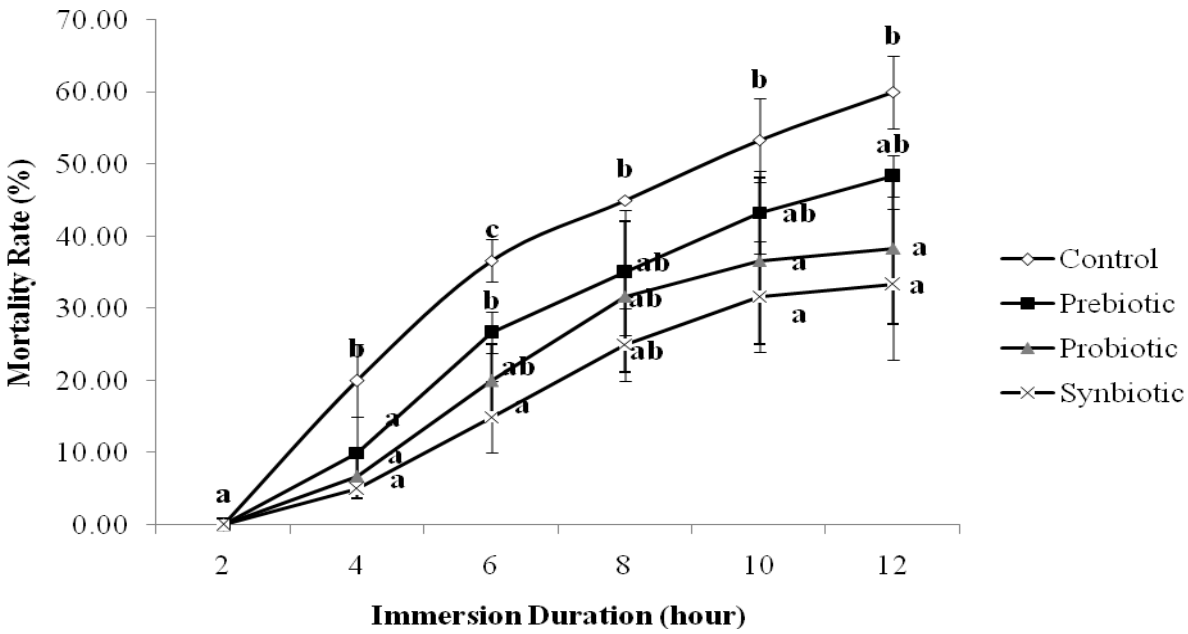

Figure 1. The Pacific white shrimp (*Litopenaeus vannamei*) post-larvae mortality rate during immersion in 200 ppm formalin solution. Different superscript letters signify significantly different results (P<0.05).

DISCUSSION

The study results showed that the administration of prebiotic and probiotic could improve the daily growth rate and absolute weight and length gain in Pacific white shrimp larvae. Some other studies have shown similar results, for example the administration of the prebiotic MOS could improve the growth of Pacific white shrimp (Zhang *et al.*, 2012) and European lobster (Daniels, 2013). Prebiotics administered to the host would be actively fermented by intestinal bacteria and would modulate bacterial activity (Ai *et al.*, 2011). Prebiotics also increase the length of intestinal microvilli (Zhang *et al.*, 2010) which help increase nutrient absorption and thus improve growth performance (Cerezuela *et al.*, 2011).

Increased growth was also observed in larvae given the probiotic. Similar results were found in several studies of other crustacean species such as *Penaeus monodon* larvae which were given the probiotic SKT-b (Widanarni *et al*., 2008; Widanarni *et al*., 2013), *L. vannamei* and *L. stylirostris* which were given the probiotic *Bacillus* spp. (Decamp *et al.*, 2008), and *L. vannamei* larvae which were given microencapsulated probiotic (Nimrat *et al.*, 2011). The probiotics administered through artemia could increase the bacterial population in the intestines and are also suspected to have improved digestive enzyme activity. This is in line with the results of the study by Widanarni *et al.* (2003) who had proven that the probiotic SKT-b could produce amylase and protease enzymes, increasing the digestibility of artemia and, as a result, improve shrimp larvae growth.

Application of the synbiotic resulted in better larval growth. This is assumed to be due to the synergistic effect of the joint administration of the prebiotic and probiotic, increasing the activity of the intestinal microflora. The increase of microflora activity was the result of the prebiotic administered through feed which could become a source of nutrients for the intestinal bacteria. This could increase feed digestibility which in turn would affect growth (Merrifield *et al*., 2010). The results of this study were in line with the study by Daniels *et al.* (2010) who showed that the administration of a synbiotic (the probiotic *Bacillus* spp. and the prebiotic MOS) through artemia could improve the growth of European lobsters larvae.

The administration of the prebiotic and probiotic SKT-b could also improve the Pacific white shrimp larvae survival rate. This is in line with the results of the study by Daniels *et al.* (2013) who showed that the administration of a synbiotic (the probiotic *Bacillus* spp. and the prebiotic MOS) through artemia could increase the survival rate of European lobster larvae. The highest survival rate in the synbiotic treatment is presumed to be because the application of the prebiotic and probiotic simultaneously would improve the benefits of the two materials.

The results of this study showed that the highest total bacterial count and highest total SKT-b Rf^R were found in larvae treated with the synbiotic. The administration of prebiotic through artemia is postulated to stimulate the growth of microflora besides the administered probiotic in the digestive tract of the Pacific white shrimp larvae, resulting in a higher total bacterial count in the synbiotic treatment compared to the other treatments. The administration of synbiotics could improve the function and increase the number of beneficial bacteria in the intestines (Delgado *et al*., 2011). Similar results were found by Daniels *et al.* (2010); the administration of MOS to the larvae of European lobster resulted in a more stable population of gastrointestinal bacteria compared to treatments without MOS.

The Pacific white shrimp used in this study for stress test were good in their health status. This has been proven by the zero mortality rate in all treatments at hour 2 which indicated good larval quality; the hatchery standard operational procedure states that the survival rate of health shrimp larvae in stress test must have a value of >95%. The application of prebiotics, probiotics and synbiotics could increase the shrimp's stress tolerance. In this study, a higher tolerance to 200 ppm formalin was found in larvae treated with the synbiotic. This is assumed due to the synbiotic ability to maintain the shrimp physiological condition to survive in environmental changes. Liu *et al.* (2010) reported that the administration of the probiotic *Bacillus subtilis* E20 in Pacific white shrimp post-larvae could highly increase tolerance to freshwater and 60 ppt seawater.

CONCLUSION

The application of the prebiotic, probiotic and synbiotic via artemia could increase the survival rate, growth rate, and tolerance to formalin in Pacific white shrimp larvae. The application of the synbiotic is more effective with the highest survival rate (41.50±3.61%) and daily growth rate (17.55±0.65%) and the lowest mortality rate (38.33±10.41%) when exposed to formalin as a stressor.

REFERENCES

Ai, Q., H. Xu, K. Mai, W. Xu, J. Wang and W. Zhang, Effect of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker, *Larimichthys crocea*. Aquaculture **317**: 155-161 (2011).

Akrami, R., Y. Iri, H.K. Rostami and M.R. Mansour, Effect of dietary supplementation of fructooligosaccharide (FOS) on growth performance, survival, lactobacillus bacterial population and hemato-immunological parameters of stellate sturgeon (*Acipenser stellatus*) juvenile. Fish Shellfish Immunol. **35**: 1235-1239 (2013).

Apriyantono, A., D. Fardiaz, N.L. Puspitasari, Sedarnawati and Budiyanti, [The Laboratory Guide for Food Analysis]. IPB Press (1989), (In Indonesian).

Balcázar, J.L., I. Blas, I. Ruiz-Zarzuela, D. Cunningham, D.Vendrell and J.L. Múzquiz, The role of probiotics in aquaculture. Veterinary Microbiology **114**: 173–186 (2006).

Cerezuela, R., J. Meseguer and M.A. Esteban, Current knowledge in synbiotic use for fish aquaculture: a review. J. Aquac. Res. Development S1-008 (2011).

Daniels, C.L., D.L. Merrifield, E. Ringø and S.J. Davies, Probiotic, prebiotic and synbiotic applications for the improvement of larval European lobster (*Homarus gammarus*) culture. Aquaculture **416–417**: 396-406 (2013).

Daniels, C.L., D.L. Merrifield, D.P. Boothroyd, S.J. Davies, J.R. Factor and K.E. Arnold, Effect of dietary *Bacillus* spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture **304**: 49–57 (2010).

Decamp, O., D.J.W. Moriarty and P. Lavens, Probiotics for shrimp larviculture: review of field data from Asia and Latin America. Aquaculture Research **39**: 334-338 (2008).

Delgado,G.T.C., W.M.S.C.Tamashiro, M.R.M. Junior, Y.M.F. Moreno and G.M. Pastero, The putative effects of prebiotics as immunomodulatory agents. Food Research International **44**: 3167-3173 (2011).

Effendie, M.I., [Fishery Biology Methods]. Yayasan Dewi Sri Bogor, In Indonesian (1997).

Huisman,E.A., The Principles of Fish Production. Departement of Aquaculture, Wageningen University (1987).

Lazado, C.C. and C.M.A. Caipang, Atlantic cod in the dynamic probiotics research in aquaculture. Aquaculture **424-425**: 53-62 (2014).

Li, J., B. Tan and K. Mai, Dietary probiotic *Bacillus* OJ and isomaltooligosaccharides influence the intestine microbial populations, immune responses and resistance to white spot syndrome virus in shrimp (*Litopenaeus vannamei*). Aquaculture **291**: 35–40 (2009).

Liu, K.F., C.H. Chiu, Y.L. Shiu, W. Cheng and C.H. Liu, Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish Shellfish Immunol. **28**: 837-844 (2010).

Madigan, M.T., J.M. Martinko and J. Parker, Brock Biology of Microorganisms. Tenth Edition. Prentice-Hall Inc. (2003).

Marlis, A., [The isolation of oligosaccharides from sweet potatoes (*Ipomea batatas* L.) and the effect of processing on its prebiotic potential]. Master Thesis submitted to Bogor Agricultural University, Indonesia, In Indonesian (2008).

Merrifield, D.L., A. Dimitroglou, A. Foey, S.J. Davies, R.T.M. Baker, J. Bøgwald, M. Castex and E. Ringø, The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture **302**: 1-18 (2010).

Muchtadi, D., [Food Nutrition Evaluation]. Depdikbud, Dirjen Dikti-PAU IPB, In Indonesian (1989),

Nayak, S.K., Probiotics and immunity: a fish perspective. Fish Shellfish Immunol. **29**: 2-14 (2010).

Nimrat,S., T.Boonthai and V.Vuthhiphandchai, Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. Animal Feed Science and Technology **169**: 244-258 (2011).

Oktaviana, A., Widanarni and M. Yuhana, The use of synbiotics to prevent IMNV and *Vibrio harveyi* co-infection in *Litopenaeus vannamei*. Hayati Journal of Biosciences **21**: 127-134 (2014).

Samocha, T.M., H. Guajardo, A.L. Lawrence, F.L. Castille, M. Speed, D.A. McKee and KI Page, A simple stress test for *Penaeus vannamei* postlarvae. Aquaculture **165**: 233–242 (1998).

Schrezenmeir, J. and M. Vrese, Probiotics, prebiotics and synbiotics-approaching a definition. Am. J. Clin. Nutr. **73**: 361-364 (2001).

Verschuere, L., G. Rombaut, P. Sorgeloos and W. Verstraete, Probiotic bacteria as biological control agents in Aquaculture. Microbiology and Molecular Biology Reviews **64**: 655-671 (2000).

Widanarni, D.T. Soelistyowati, Elly and A. Suwanto, [Administration of *Vibrio* SKT-b probiotic bacteria on tiger shrimp larvae through artemia enrichment]. Jurnal Akuakultur Indonesia (In Indonesian) **7**: 129-137 (2008) .

Widanarni, Y. Hadiroseyani and A. Sutanti, [Administration of *Vibrio* SKT-b probiotic at different dose through artemia on tiger shrimp *Penaeus monodon* post larva]. Jurnal Akuakultur Indonesia (In Indonesian) **12**: 86-93 (2013),

Widanarni, A. Suwanto, Sukenda and B.W. Lay, Potency of *Vibrio* isolates for biocontrol of vibriosis in tiger shrimp **(***Penaeus monodon*) larvae**.** Biotropia **20**: 11-23 (2003).

Zhang, J., Y. Liu, L. Tian, H. Yang, G. Liang and D. Xu, Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei.* Fish Shellfish Immunol. **33**: 1027-1032 (2012).

Zhang, Q., H. Ma, K. Mai, W. Zhang, Z. Liufu and W. Xu, Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, non-specific immunity of sea cucumber, *Apostichopus japonicus*. Fish Shellfish Immunol. **29**: 204-211 (2010).