ELBA BIOFLUX
Extreme Life, Biospeology & Astrobiology
International Journal of the Bioflux Society

Improved resistance against White Spot Virus (WSV) infection in tiger shrimp, *Penaeus monodon* by combined supplementation of peptidoglycan and mannan oligosaccharide (MOS)

[1]Mary Jane S. Apines-Amar, [1]Karen Grace S. Andrino, [2]Edgar C. Amar, [1]Rowena E. Cadiz, [1]Valeriano L. Corre Jr.

[1] Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo, Philippines; [2] Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo, Philippines. Corresponding author: M. J. S. Apines-Amar, janeamar09@gmail.com

Abstract. An eight-week feeding trial was conducted to evaluate the effects of combined supplementation of peptidoglycan and mannan oligosaccharide (MOS) in tiger shrimp, *Penaeus monodon*. Tiger shrimp (0.29 ± 0.02 g) were fed diets supplemented with different levels of peptidoglycan + (MOS) as immunostimulants for six (6) and eight (8) weeks. Four (4) experimental diets were formulated to contain 0, 0.1, 0.2, and 0.4% peptidoglycan + MOS. The feeding trial was conducted in 250 L capacity concrete circular tanks (replicated four (4) times) with 20 shrimp per tank. Growth, survival, respiratory burst activity, total hemocyte count (THC), and *in vivo* resistance to WSV infection were evaluated. Weight gain of the shrimp was significantly higher in the immunostimulant-fed groups compared to the control. However, different levels of the immunostimulants did not differ in their effect on the the growth of the shrimp. On the other hand, respiratory burst activity and total haemocyte count (THC) were significantly higher in the group supplemented with 0.2% peptidoglycan + MOS than the rest of the treatments. Likewise, survival after infection with White Spot Virus (WSV) was significantly increased in the 0.2% peptidoglycan + MOS compared to the other groups. The present results demonstrated that using peptidoglycan and MOS together at 0.2% of the diet improves growth, activates immune responses such as respiratory burst activity and THC in *P. monodon* and give better protection to the shrimp against WSV infection.
Key Words: immunostimulant, shrimp, feeding, infection trial.

Introduction. Microbial diseases are among the major threats in aquaculture in recent years owing to stresses brought about by intensification of culture systems. One of the practical approaches in managing diseases is by combining good culture techniques and use of improved feed formulations containing prophylactic agents such as immunostimulants. The use of immunostimulants as a tool to improve growth, survival and overall performance in fish and shrimp is fast gaining importance in aquaculture. Its beneficial role in increasing disease resistance in farmed aquatic species is being recognized as it complements the use of chemotherapeutants and vaccines.

Dietary immunostimulants can enhance disease resistance in shrimp by modulating host innate immune functions that are necessary for protection against infectious disease agents.

Compared to teleosts and other vertebrates, shrimp have a more primitive immune system, so that they are primarily dependent on nonspecific immune processes to fight infection (Soderhall & Cerenius 1992). Shrimp postlarvae in hatcheries for instance, are highly susceptible to infections that can result in high mortalities often requiring heavy use of chemotherapeutants. Therefore, in order to reduce antimicrobial use in hatcheries, the use of immunostimulants to improve survival and growth of postlarvae is a promising alternative approach to consider.

The most commonly tested immunostimulants in fish and shrimp include glucan (Chen & Ainsworth 1992; Jorgensen & Robertsen 1995; Jeney et al 1997; Sakai 1999, Galindo-Villegas & Hosokawa 2004; Pais et al 2008; Apines-Amar et al 2012, 2013), levamisole (Siwicki 1987; Siwicki et al 1990; Jeney & Anderson 1993; Sakai 1999; Galindo-Villegas & Hosokawa 2004) and vitamin C (Liu et al 1989; Navarre & Halver 1989; Hardie et al 1991; Waagbo et al 1993; Sakai 1999; Adham et al 2000; Cuesta et al 2002; Sobhana et al 2002; Galindo-Villegas & Hosokawa 2004; Lin & Shiau, 2005; Apines-Amar et al 2012, 2013). The effects of immunostimulants, however, may vary depending on the species and size of fish, the method of administration, and the concentration of the immunostimulant being used.

In the case of feeding, length of administration also affects the efficiency of the immunostimulants (Raa 1996). Sung et al (1994) reported that immersion of postlarval shrimp in β-glucan (MacroGard) improved growth, whereas vibriosis resistance was markedly enhanced after 10 days for shrimp immersed in 0.5 and 1.0 mg mL^{-1} of MacroGard, but not at higher or lower concentrations. Still, commercial immunostimulants are currently available in the market that exhibit promising results in humans and other animals but have not been extensively used in aquatic species.

Biostim, a capsular glycoprotein extracted from *Klebsiella pneumoniae* has been reported to improve the resistance to microbial infections in warm-blooded animals including humans (Laval et al 1988; Smets et al 1988). Also, Betafectin, a β-1,3 glucan, was first shown to enhance survival against bacterial infection in mice (Nemunaitis 1993). These compounds are now being used in aquaculture as the β-1,3 side chains have been found to be immunostimulatory in fish and in shrimp as well (Engstad & Robertsen 1993, 1994; Raa 1996; Galindo-Villegas & Hosokawa 2004).

Results from studies with higher animals are good basis for the use of immunostimulatory and other related compounds in aquaculture although their efficacy in aquatic species needs to be validated. Among non-digestible carbohydrates, peptidoglycan and mannan oligosaccharide (MOS) have been studied individually in aquatic organisms but results vary in terms of growth and immune responses (Staykov et al 2007; Torrecillas et al 2007; Welker et al 2007; Yilmaz et al 2007; Peterson et al 2010; Peterson et al 2012).

The responses to peptidoglycan and MOS administration may be confounded by differences in species, source of the immunostimulants, and length of time the immunostimulants were administered. Most previously mentioned studies tested only one immunostimulant at a time. However, a few studies have combined different immunostimulants in order to enhance the immune response of aquatic animals (Ortuño et al 2001; Selvaraj et al 2006; Zhang et al 2010; Gu et al 2011).

The present study was conducted to determine the effects of combined supplementation of peptidoglycan and MOS in *Penaeus monodon* in terms of growth, immune responses and resistance to White Spot Virus (WSV) infection. The identification of potential and available immunostimulants and including them in the formulation of improved shrimp diets could result in better growth and survival that may consequently augment production of this important aquatic species.

Material and Method

Experimental shrimp, feeds, and feeding. The feeding trial was conducted on February 2012 at the Insitute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas. Four hundred *P. monodon* weighing about 0.2 g were stocked in 16 250-L capacity tanks at 25 each. Four experimental diets containing 0, 0.1, 0.2, and 0.4% peptidoglycan + (MOS) (Table 1) were prepared and fed to the shrimp for 8 weeks. Each diet was replicated 4 times. Weight of shrimp was measured at the start of the feeding trial and every 2 weeks thereafter. At the end of the 8-week feeding trial, hemolymph samples were collected to measure immunity parameters.

Table 1

Formulation and proximate composition of the experimental diets (%)

Ingredients	*0%*	*0.1%*	*0.2%*	*0.4%*
Basal diet*	93.0	93.0	93.0	93.0
Cellulose	7.0	6.9	6.8	6.6
Peptidoglycan + MOS	0.0	0.1	0.2	0.4
Proximate composition (%)				
Moisture	6.78	6.45	7.00	6.37
Crude protein	47.64	47.31	48.83	49.32
Crude fat	6.41	6.36	5.58	4.98
Crude fiber	0.91	0.94	0.78	0.63
Ash	12.43	12.69	13.33	12.74

* Fish meal 36 g, Squid meal 10 g, Soybean meal 20 g, Fish oil 5 g, Vitamin mix 2 g, Mineral mix 1 g, Butylated Hydroxy Toluene (BHT) 0.5 g, Lecithin 0.5 g, Starch 18 g.

Immunity parameters

Total Hemocyte Count (THC) - was done following the method of Le Moullac et al (1998) with modifications. Hemolymph was withdrawn using tuberculin syringe with anticoagulant, mixed gently and transferred to sterile microfuge tubes. Some amount of hemolymph was fixed with an equal volume of 10% buffered formalin. Total hemocyte count was performed using a Neubauer hemocytometer and expressed as THC per mL hemolymph.

Superoxide Anion Production - the production of superoxide anion was measured by the reduction of nitroblue tetrazolium (NBT) following the method of Muñoz et al (2000) with modification. Hemocytes were placed in a 96-well microtiter plate in triplicate and incubated for 2 h at room temperature. The supernatant was discarded and replaced with Modified Hank's Balanced Salt Solution, after which another medium with or without zymosan was added. Then, NBT working solution was immediately distributed to the wells. After incubation, the supernatant was removed, cells were fixed with absolute methanol, washed twice with 70% methanol and dried. KOH and DMSO were added to the wells to homogenize the cells and the absorbance was read at 620 nm in a microplate reader.

Challenge trial. To further evaluate the influence of the immunostimulants on disease resistance, the shrimp that had been fed with the immunostimulant-supplemented diets were challenged with WSV. The infection trial was conducted in SEAFDEC AQD, Tigbauan, Iloilo. The shrimp were immersed in WSV solution at 10^{-4} dilution for 1 hour. Thereafter, the shrimp were transferred to a clean water in duplicate 100-L capacity tank containing 10 shrimp each. Cumulative mortality was monitored daily for 10 days.

Statistical analysis. Results were analyzed by one-way ANOVA using SYSTAT version 8.0 (SPSS, Chicago, USA). Data for the cumulative mortality were arc sine transformed before performing the statistical analysis. Differences between treatments were compared by Tukey's test. Significance level was set at $p < 0.05$.

Results and Discussion. The benefits of using peptidoglycan + MOS mixture to stimulate the non-specific immunity in *P. monodon* was clearly demonstrated in the present study. The growth of tiger shrimp showed an increasing trend in response to different levels of peptidoglycan + MOS (Figure 1). However, supplementation of peptidoglycan + MOS at 0.2% exhibited the highest weight gain among the groups and significantly higher weight gain over the control. Supplementation of peptidoglycan + MOS at 0.1 and 0.4% level in the diet did not improve weight gain. Also, growth did not vary significantly among the supplemented groups. At the end of the 8-week feeding trial, survival of shrimp was similar for all diet groups (Figure 1). Effects of immunostimulation with peptidoglycan on growth have been reported previously. Boonyaratpalin et al (1995) reported that *P. monodon* fed with peptidoglycan alone at

0.01% showed better growth and feed conversion ratio than the group fed unsupplemented diet but this effect was not observed at the highest level (0.1%) administered. Likewise, *P. monodon* grew faster when immersed in glucan at 0.5, 1, and 2 mg mL^{-1} than at 0.25 mg mL^{-1} or in control solutions (Sung et al 1994). On the other hand, Matsuo & Miyazono (1993) reported that peptidoglucan did not influence the growth of rainbow trout (*Oncorhynchus mykiss*) after 60 days of oral administration. The addition of Bio-Mos® (derived from *Saccharomyces cerevisiae* outer cell wall which is rich in mannan oligosaccharides) has shown promise in promoting growth and improving feed efficiency in fish (Staykov et al 2007; Torrecillas et al 2007; Yilmaz et al 2007). Similarly, the effects of dietary MOS on growth performance have been observed in lobster (*Homarus gammarus*) and shrimp (*Penaeus semisulcatus*) (Daniels et al 2006; Genc et al 2007). In sea cucumber (*Apostichopus japonicus*), the combination of β-glucan and MOS resulted in a significantly higher growth compared to the unsupplemented control or supplementation of β-glucan or MOS alone (Gu et al 2011).

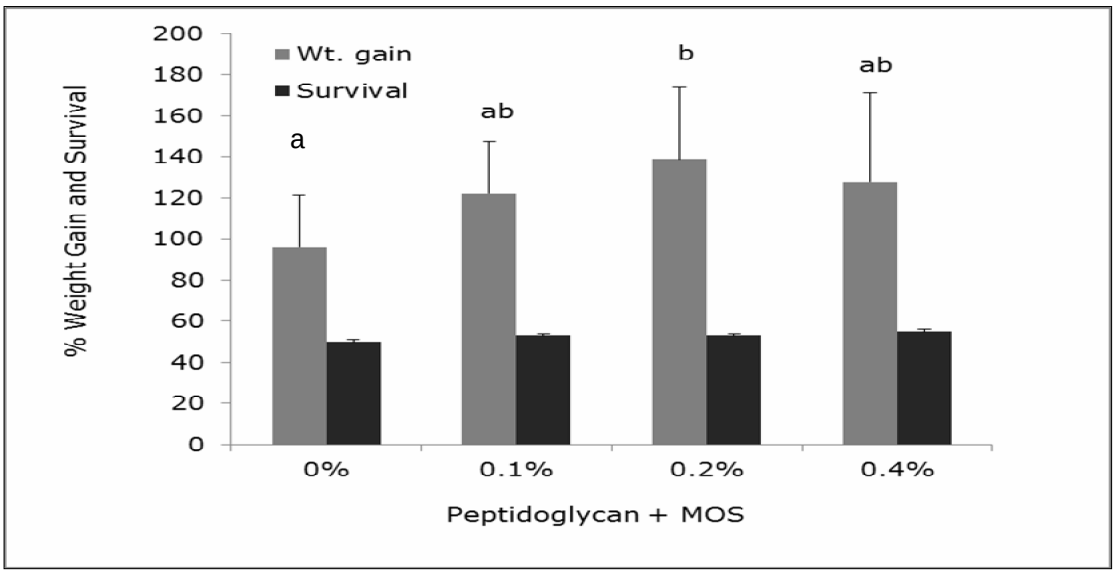

Figure 1. Growth and survival in *P. monodon* supplemented with different levels of peptidoglycan + MOS for 8 weeks.

Apart from the growth enhancement observed in the present study, immunity parameters such as respiratory burst activity and THC were also amplified. The shrimp fed with peptidoglycan + MOS at 0.2% exhibited significantly higher respiratory burst activity and total hemocyte count (THC) compared to the control and the group fed the highest concentration (0.4%) (Figures 2 and 3). In catfish (*Clarias gariepinus*), oral administration of oligosaccharide also resulted in higher nitroblue tetrazolium (NBT) reduction values (Yoshida et al 1995). Dietary MOS, β-glucan and their combinations significantly increased the superoxide anion production and total coelomocytes count (TCC) in sea cucumbers (Gu et al 2011). The combined supplementation of chitosan oligosaccharide (COS) and *Bacillus coagulans* in koi (*Cyprinus carpio*) not only enhanced growth but also significantly improved immune responses such as total leukocyte count (WBC) and respiratory activity (Lin et al 2012). Among the immunity parameters, the activation of the cellular component of the shrimp immune response such as the THC and the respiratory burst are considered sensitive indicators of immunostimulation. THC suddenly drops (due to granule exocytosis of cells containing effector substances or due to necrosis and apoptosis) in response to injury or infectious agents such as WSV (Khanobdee et al 2002) and fast recovery of THC post-infection or increase from basal level post-immunostimulation is indicative of a strong immune response and ability to maintain homeostasis (Amar & Faisan 2012). The respiratory burst is also carried out by shrimp immune cells to complement its cytotoxic ability. In addition to producing lytic enzymes, the shrimp immune cells also produce reactive oxygen intermediates (such as the superoxide anion, hydrogen peroxide, and other intermediates to degrade phagocytized agents) after which they are neutralized enzymatically to prevent damage

to the host. Poor respiratory burst response can result in inability to kill phacocytized infectious agents.

The shrimp fed at 0.2% peptidoglycan + MOS and experimentally infected by bath-immersion with WSV in the present study were observed to have significantly higher (80%) survival than the rest of the diet groups (0%, 40%, and 25% for control, 0.1% peptidoglycan + MOS and 0.4% peptidoglycan + MOS, respectively (Figure 4)). Improved survival against yellow-head baculovirus infection was also observed in shrimp fed with peptidoglucan-supplemented diets (Boonyaratpalin et al 1995). Similarly, Japanese flounder (*Paralichthys olivaceus*) supplemented with peptidoglycan exhibited higher survival than the other immunostimulant-fed groups (Galindo-Villegas et al 2006). In rainbow trout and yellowtail (*Seriola quinqueradiata*) fed peptidoglucan, survival was enhanced upon infection with *Vibrio anguillarum* and *Enterococcus seriolicida*, respectively (Matsuo & Miyazono 1993; Itami et al 1996). In addition, oral administration of Bio-Mos® reduced mortality in rainbow trout (Staykov et al 2007) and enhanced protection against *Edwardsiella ictaluri* challenge in channel catfish (Peterson et al 2010).

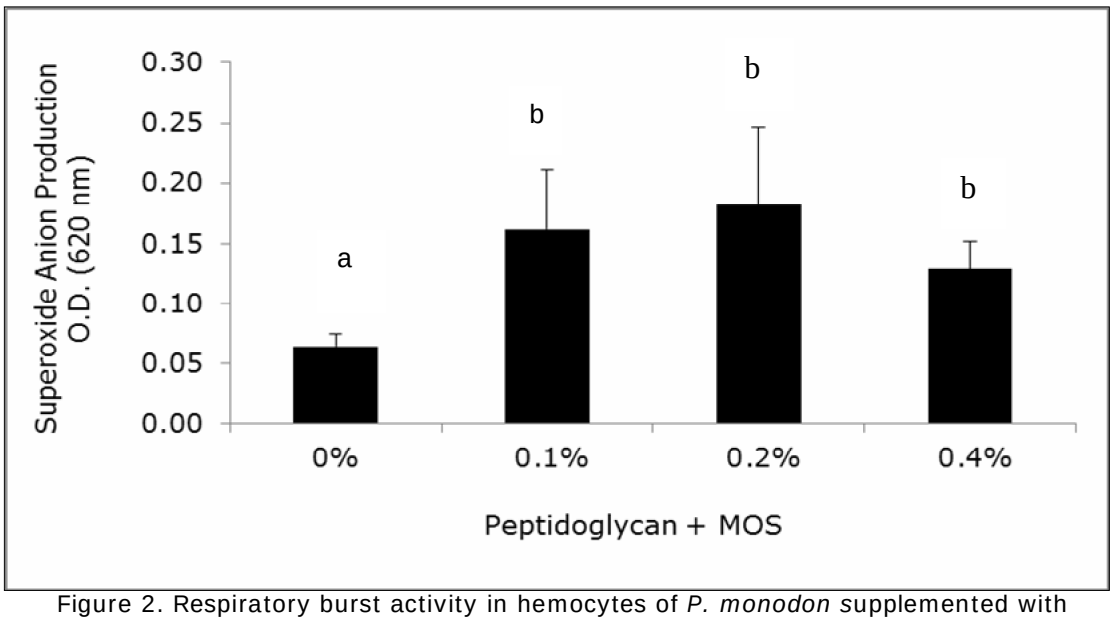

Figure 2. Respiratory burst activity in hemocytes of *P. monodon* supplemented with different levels of peptidoglycan + MOS for 8 weeks.

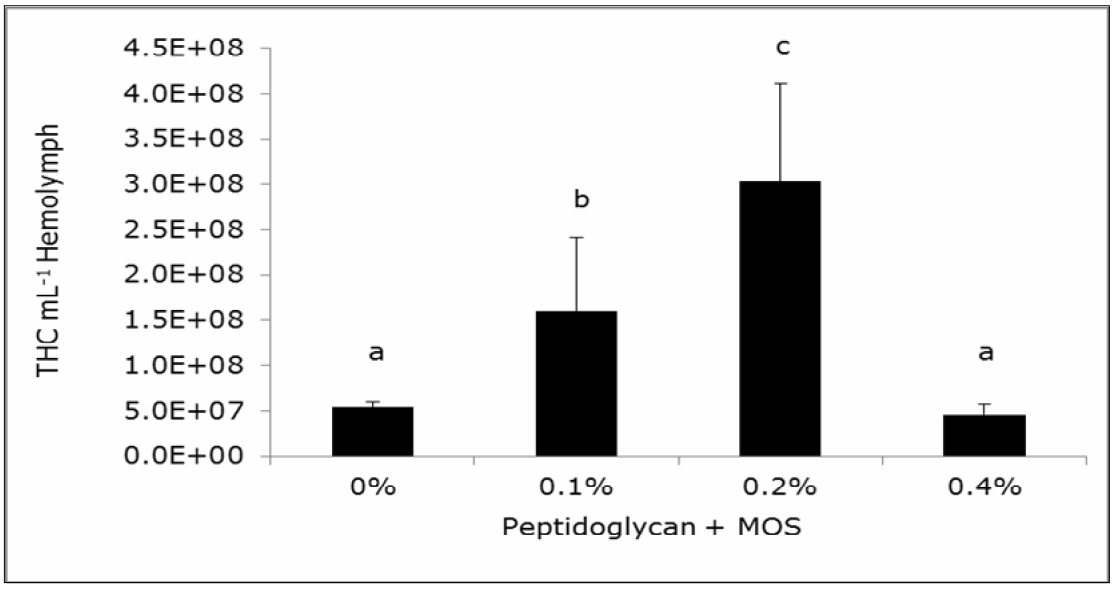

Figure 3. Total hemocyte count in *P. monodon* supplemented with different levels of peptidoglycan + MOS for 8 weeks.

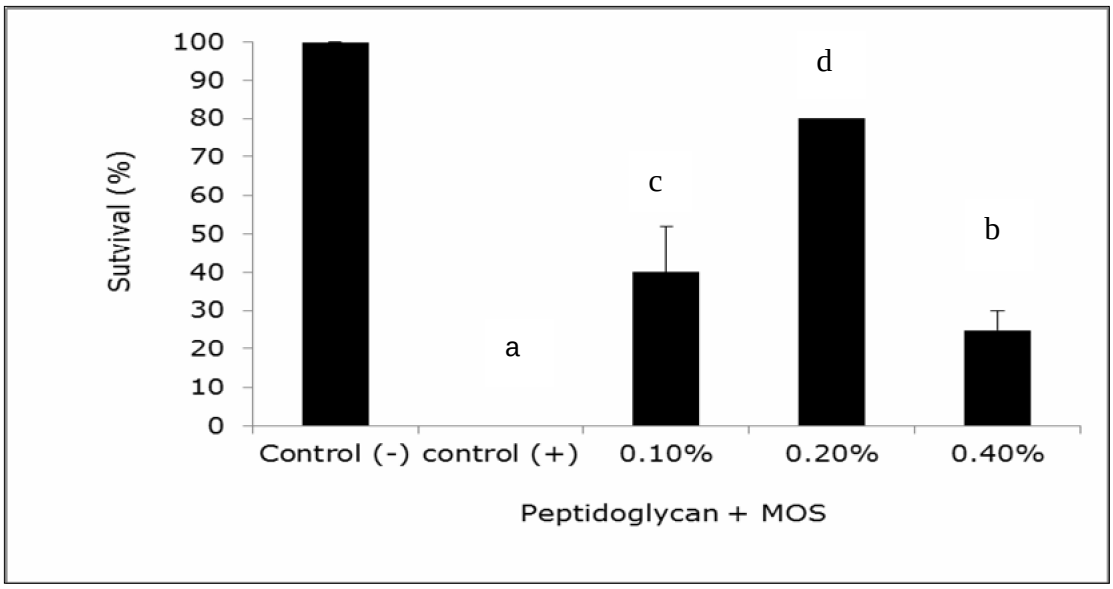

Figure 4. Disease resistance in *P. monodon* supplemented with different levels of peptidoglycan + MOS for 8 weeks, and experimentally infected with WSV.

The significant effects of peptidoglycan + MOS in this study were observed at 0.2% supplementation in the diet. Higher concentration (0.4%) was not found beneficial to the shrimp. Andrews et al (2009) reported that supplementation of MOS alone at 1% of the diets resulted in improved growth, survival and immunity in rohu (*Labeo rohita*) fingerlings. They observed that higher inclusion levels of immunostimulants led to immunosuppression in the fish. According to Sakai (1999), high doses of immunostimulants may inhibit immune responses, which may be due to receptor overload or immune fatigue due to higher energy cost of sustained immunostimulation (Moret & Schmid-Hempel 2000). On the other hand, synergistic and adjuvant effects have been found between different immunostimulants and these effects resulted in amplified immune responses and protection against pathogens (Ortuño et al 2001; Selvaraj et al 2006; Zhang et al 2010; Gu et al 2011). It is apparent from the above studies and the present results that the efficacy of peptidoglycan and MOS, as well as other polysaccharide-based immunostimulants whether administered individually or in combination is influenced by the mode and dose of administration, immunostimulant preparation, species, strain, batch, or history of the animals used in the experiment, and the prevailing experimental conditions. Nonetheless, a combination of two differently-acting immunostimulants may be better than only one immunostimulant in order to harness possible synergistic effects and lower the dose of each constituent immunostimulant. This strategy might also avoid the inhibition of immune response observed with higher doses of a single immunostimulant.

Conclusions. The present results demonstrated that using pepditoglycan and MOS together at 0.2% of the diet improves growth, activates immune responses such as respiratory burst activity and THC in *P. monodon* and give better protection to the shrimp against WSV infection.

Acknowledgements. This study was fully supported by the Department of Science and Technology (DOST) through the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD). Likewise, SEAFDEC AQD and Chemoforma Ltd. are gratefully acknowledged for the use of research facilities, and for supplying the immunostimulant, respectively.

References

Adham K. G., Hashem H. O., Abu-Shabana M. B., Kamel A. H., 2000 Vitamin C deficiency in the catfish *Clarias gariepinus*. Aquacult Nutr 6:129-139.

Amar E. C., Faisan J. P. Jr., 2012 Induction of immunity and resistance to white spot syndrome virus (WSSV) in shrimp *Penaeus monodon* (Fabricius) by synthetic oligodeoxynucleotide and bacterial DNA. Philipp Agric Scientist 95:267-277.

Andrews S. R., Sahu N. P., Pal A. K., Kumar S., 2009 Haematological modulation and growth of *Labeo rohita* fingerlings: effect of dietary mannan oligosaccharide, yeast extract, protein hydrolysate and chlorella. Aquacult Res 41:61-69.

Apines-Amar M. J. S., Amar E. C., Faisan Jr. J. P., Pakingking Jr. R. V., Satoh S., 2012 Dietary onion and ginger enhance growth, hemato-immunological responses, and disease resistance in brown-marbled grouper, *Epinephelus fuscoguttatus*. AACL Bioflux 5(4):231-239.

Apines-Amar M. J. S., Amar E. C., Faisan Jr. J. P., 2013 Growth, plasma cortisol, liver and kidney histology, and resistance to vibriosis in brown-marbled grouper, *Epinephelus fuscoguttatus* fed onion and ginger. AACL Bioflux 6(6):530-538.

Boonyaratpalin S., Boonyaratpalin M., Supamattaya K., Toride Y., 1995 Effects of peptidoglucan (PG) on growth, survival, immune responses, and tolerance to stress in black tiger shrimp, *Penaeus monodon*. In: Diseases in Asian Aquaculture. Vol. 11. Fish Health Section. Shariff M., Subasighe R. P., Arthur J. R. (eds), Asian Fisheries Society, Manila, Philippines, pp. 469–477.

Chen D., Ainsworth A. J., 1992 Glucan administration potentiates immune defence mechanisms of channel catfish, *Ictalurus punctatus* Rafinesque. J Fish Dis 15:295-304.

Cuesta A., Esteban M. A., Meseguer J., 2002 Natural cytotoxic activity in seabream (*Sparus aurata* L.) and its modulation by vitamin C. Fish Shellfish Immunol 13:97-109.

Daniels C., Boothroyd D., Davies S., Pryor R., Taylor D., Wells C., 2006 Bio-MOS improves growth and survival of cultures lobsters. Shellfish News 21:23-25.

Engstad R. E., Robertsen B., 1993 Recognition of yeast cell wall glucan by Atlantic salmon (*Salmo salar* L.) macrophages. Dev Comp Immunol 17:319-330.

Engstad R., Robertsen B., 1994 Specificity of a ß-glucan receptor on macrophages from Atlantic salmon (*Salmo salar* L.). Dev Comp Immunol 18:397-408.

Galindo-Villegas J., Hosokawa H., 2004 Immunostimulants: towards temporary prevention of diseases in marine fish. In: Avances en nutricion acuicola VII. Memorias del VII symposium internacional de nutricion acuicola. Cruz Suarez L. E., Ricque Marie D., Nieto Lopez M. G., Villarreal D., Scholz U., Gonzales M. (eds), Noviembre 16-19, 2004. Hermosillo, Sonora, Mexico.

Galindo-Villegas J., Fukada H., Masumoto T., Hosokawa H., 2006 Effect of dietary immunostimulants on some innate immune responses and disease resistance against *Edwardsiella tarda* infection in Japanese flounder (*Paralichthys olivaceus*). Aquaculture Science 54:153-162.

Genc M. A., Aktas M., Genc E., Yilmaz E., 2007 Effects of dietary mannan oligosaccharides on growth, body composition and hepatopancreas histology of *Penaeus semisulcatus* (de Haan 1844). Aquacult Nutr 13:156-161.

Gu M., Ma H., Mai K., Zhang W., Bai N., Wang X., 2011 Effects of dietary ß-glucan, mannan oligosaccharide and their combinations on growth performance, immunity and resistance against *Vibrio splendidus* of sea cucumber, *Apostichopus japonicus*. Fish Shellfish Immunol 31:303-309.

Hardie L. J., Fletcher T. C., Secombes C. J., 1991 The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar* L.). Aquaculture 95:201-214.

Itami T., Kondo M., Uozu M., Suganuma A., Abe T., Nakagawa A., Suzuki N., Takahashi Y., 1996 Enhancement of resistance against *Enterococcus seriolicida* infection in yellowtail, *Seriola quinqueradiata* (Temminck and Schlegel), by oral administration of peptidoglucan derived from *Bifidobacterium thermophilum*. J Fish Dis 19:185–187.

Jeney G., Anderson D. P., 1993 An *in vitro* technique for surveying immunostimulants in fish. Aquaculture 112:283-287.

Jeney G., Galeotti M., Volpatti D., Jeney Z., Anderson D. P., 1997 Prevention of stress in rainbow trout (*O. mykiss*) fed diets containing different doses of glucan. Aquaculture 154: 1-15.

Jorgensen J. B., Robertsen B., 1995 Yeast beta-glucan stimulates respiratory burst activity of Atlantic salmon (*Salmo salar* L.) macrophages. Dev Comp Immunol 19:43-57.
Khanobdee K., Soowannayan C., Flegel T. W., Ubol S., Withyachumnarnkul B., 2002 Evidence for apoptosis correlated with mortality in the giant black tiger shrimp *Penaeus monodon* infected with yellow-head virus. Dis Aquat Org 48:79-90.
Laval A., Rommain M., Fortier M., Zalisz R., Dahan R., Smets P., 1988 Immunomodulating effects of orally administered RU 41740 (Biostim) in the swine. Adv Biosci 68:103-109.
Le Moullac G., Soyez C., Saulnier D., Ansquer D., Avarre J. C., Levy P., 1998 Effects of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. Fish Shellfish Immunol 8:621-629.
Lin M.-F., Shiau S.-Y., 2005 Dietary L-ascorbic acid affects growth, nonspecific immune responses and disease resistance in juvenile grouper, *Epinephelus malabaricus*. Aquaculture 244:215-221.
Lin S., Mao S., Guan Y., Luo L., Luo L., Pan Y., 2012 Effects of dietary chitosan oligosaccharides and *Bacillus coagulans* on the growth, innate immunity and resistance of koi (*Cyprinus carpio koi*). Aquaculture 342-343:36–41.
Liu P. R., Plumb J. A., Guerin M., Lovell R. T., 1989 Effect of megalevels of dietary vitamin C on the immune response of channel catfish *Ictalurus punctatus* in ponds. Dis Aquat Org 7:191-194.
Matsuo K., Miyazono I., 1993 The influence of long-term administration of peptidoglucan on disease resistance and growth of juvenile rainbow trout. Nippon Suisan Gakkaishi 59:1377–1379.
Moret Y., Schmid-Hempel P., 2000 Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1166-1168.
Muñoz M., Cedeno R., Rodriguez J., van der Knaap W. P. W., Mialhe E., Bachere E., 2000 Measurement of reactive oxygen production in haemocytes of penaeid shrimp, *Penaeus vannamei*. Aquaculture 191:89-107.
Navarre O., Halver J. E., 1989 Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. Aquaculture 79:207-221.
Nemunaitis J., 1993 Macrophage function activating cytokines: potential clinical application. Crit Rev Oncol Hematol 14:153-171.
Ortuño J., Cuesta A., Angeles Esteban M., Meseguer J., 2001 Effect of oral administration of high vitamin C and E dosages on the gilthead sea bream (*Sparus aurata* L.) innate immune system. Vet Immunol Immunopathol 79:167-180.
Pais R., Khushiramani R., Karunasagar I., Karunasagar I., 2008 Effect of immunostimulants on the haemolymph haemagglutinins of tiger shrimp *Penaeus monodon*. Aquacult Res 39:1339–1345.
Peterson B. C., Bramble T. C., Manning B. B., 2010 Effects of Bio-Mos® on growth and survival of channel catfish challenged with *Edwardsiella ictaluri*. J World Aquacult Soc 41:149-155.
Peterson B. C., Booth N. J., Barrows F. T., Manning B. B., 2012 Improved survival in channel catfish fed mannanoligosaccharides in an extruded diet. Open J Anim Sci 2:57-61.
Raa J., 1996 The use of immunostimulatory substances in fish and shellfish farming. Rev Fish Sci 4:229-288.
Sakai M., 1999 Current research status of fish immunostimulants. Aquaculture 172:63-92.
Selvaraj V., Sampath K., Sekar V., 2006 Adjuvant and immunostimulatory effects of beta-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with *Aeromonas hydrophila*. Vet Immunol Immunopathol 114:15-24.
Siwicki A. K., 1987 Immunomodulating activity of levamisole in carp spawners, *Cyprinus carpio*. J Fish Biol 31:245-246.
Siwicki A. K., Anderson D. P., Dixon O. W., 1990 *In vitro* immunostimulation of rainbow trout *Oncorhynchus mykiss* spleen cells with levamisole. Dev Comp Immunol 14: 231-237.
Smets P., Zalisz R., Boisnic F., 1988 Biostim® (RU41740), immune functions and infections. Adv Biosci 68:85-102.
Sobhana K. S., Mohan C. V., Shankar K. M., 2002 Effect of dietary vitamin C on the disease susceptibility and inflammatory response of mrigal, *Cirrhinus mrigala* (Hamilton) to experimental infection of *Aeromonas hydrophila*. Aquaculture 207:225-238.
Soderhall K., Cerenius L., 1992 Crustacean immunity. Annu Rev Fish Dis 2:3-23.

Staykov Y., Spring P., Denev S., Sweetman J., 2007 Effect of a mannan oligosaccharide on the growth performance and immune status of rainbow trout (*Oncorhynchus mykiss*). Aquacult Int 15:153–161.
Sung H. H., Kou G. H., Song Y. L., 1994 Vibriosis resistance induced by glucan treatment in tiger shrimp (*Penaeus monodon*). Fish Pathol 29:11-17.
Torrecillas S., Makol A., Caballero M. J., Montero D., Robaina L., Real F., Sweetman J., Tort L., Izquierdo M. S., 2007 Immune stimulation and improved infection resistance in European sea bass (*Dicentrarchus labrax*) fed mannan oligosaccharides. Fish Shellfish Immunol 23:969–981.
Waagbo R., Glette J., Raa-Nilsen E., Sandnes K., 1993 Dietary vitamin C, immunity and disease resistance in Atlantic salmon (*Salmo salar*). Fish Physiol Biochem 12:61-73.
Welker T. L., Lim C., Yildirim-Aksoy M., Shelby R., Klesius P. H., 2007 Immune response and resistance to stress and *Edwardsiella ictaluri* challenge in channel catfish, *Ictalurus punctatus*, fed diets containing commercial whole-cell yeast or yeast subcomponents. J World Aquacult Soc 38:24-35.
Yilmaz E., Genc M. A., Genc E., 2007 Effects of dietary mannan oligosaccharides on growth, body composition, intestine and liver histology of rainbow trout, *Oncorhynchus mykiss*. Isr J Aquacult Bamid 59:182-188.
Yoshida T., Kruger R., Inglis V., 1995 Augmentation of non-specific protection in African catfish, *Clarias gariepinus* (Burchell), by the long-term oral administration of immunostimulants. J Fish Dis 18:195–198.
Zhang Q., Ma H. M., Mai K. S., Zhang W. B. Liufu Z. G., Xu W., 2010 Interaction of dietary *Bacillus subtilis* and fructooligosaccharide on the growth performance, nonspecific immunity of sea cucumber, *Apostichopus japonicus*. Fish Shellfish Immunol 29:204-211.

Recieved: 14 December 2013. Accepted: 27 December 2013. Published online: 19 January 2014.
Authors:
Mary Jane S. Apines-Amar, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023 Philippines, email: janeamar09@@gmail.com
Karen Grace S. Andrino, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023 Philippines, email: karen_perhaps21@yahoo.com
Edgar C. Amar, Aquaculture Department, Southeast Asian Fisheries Development Center, Tigbauan, Iloilo 5021, Philippines, email: eamar@seafdec.org.ph
Rowena E. Cadiz, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023 Philippines, email: weng_1990@yahoo.com
Valeriano L. Corre, Jr., Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Miagao, Iloilo 5023 Philippines, email: profcorre@yahoo.com

How to cite this article:
Apines-Amar M. J. S., Andrino K. G. S., Amar E. C., Cadiz R. E., Corre Jr. V. L., 2014 Improved resistance against White Spot Virus (WSV) infection in tiger shrimp, *Penaeus monodon* by combined supplementation of peptidoglycan and mannan oligosaccharide (MOS). ELBA Bioflux 6(1):1-9.