The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

&

University of Hawai'i at Mānoa

&

AquacultureHub

http://www.aquaculturehub.org

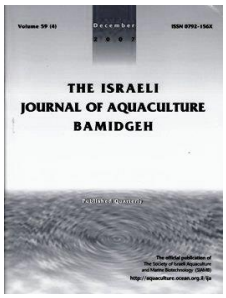
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL

Phone: + 972 52 3965809

http://siamb.org.il



Effects of Organic Selenium (OS) on Survival, Physiological and Immunological Response of Snub-Nosed Dart (*Trachinotus Blochii Lacepide*, 1801) Fingerlings When Challenged with Bacterial Infection and Copper Exposure

Huynh Minh Sang[1,*], Nguyen Thi Thanh Thuy[2] & Ho Son Lam[1]

[1] *Institute of Oceanography – Vietnam Academy of Science and Technology (VAST), 01 Cau Da – Nha Trang – Khanh Hoa - Vietnam*

[2] *Research Institute for Aquaculture No. 3. Nha Trang, Khanh Hoa, Vietnam*

Key words: snub-nosed dart fish, *Trachinotus blochii*, organic selenium, Nocardia sp, Cu toxicity

Abstract

Two trials were conducted to determine the effects of diet supplemented with organic selenium (OS, Selplex, Alltech, Kentucky USA) on survival, physiological, and immunological parameters of snub-nosed dart (*Trachinotus blochii* Lacepide, 1801) when challenged with *Nocardia sp*. bacteria or when exposed to toxic levels of environmental copper. The fish were fed two different diets containing 0% (D1,control diet) and 0.03% OS (D2) for 12 weeks before challenge. Cumulative mortality, phagocytic activity, and respiratory burst of head kidney leucocytes, and physiological parameters including moisture content and lipid accumulation in the liver, moisture content, and crude protein in the flesh were recorded. Results showed that the cumulative mortality of fish fed Se supplemented diet was lower than that of the fish fed the control diet when challenged with *Nocardia sp*. Survival rate was 100% in both treatment groups when exposed to copper only. Phagocytic activity and respiratory burst were higher in fish fed D2 (Se supplemented) compared to the D1 group when challenged with *Nocardia sp*. Phagocytic activity was higher in fish fed Se supplemented diet compared to the control group when exposed to environmental copper. No significant differences in the protein content, total lipid, and moisture in liver and flesh were recorded between the diet groups. Results indicated that supplementation of 0.3 g/Kg of OS (Sel-Plex) in the diet of snub-nosed dart is recommended to enhance fish survival and immunity when challenged with bacteria *Nocardia sp*. and environmental Cu toxicity exposure.

* Corresponding author. Tel.: 84 58 3590319, fax: 84 58 3590034, e-mail: hmsang2000@yahoo.com

Introduction

Antibiotics have been used at sub-therapeutic concentrations to improve the growth and general health of aquaculture organisms for many years. However, the use of antibiotics could enhance antibiotic-resistant pathogens and environmental deterioration (Gatlin et al., 2006). The increasing global demand for safe seafood and the need to preserve an eco-friendly environment has prompted interest in developing alternatives to antibiotics for growth and improved health of cultured organisms (Rosen, 1996). There has been a growing interest in research on the effects of dietary supplements to promote immunity as well as growth (Gatlin et al. 2006). These compounds are classified as immunonutrients and immunostimulants. The difference between the two relates to their modes of action. Selenium (Se) is an essential trace element required for normal growth and physiological function of fish (Lin and Shiau, 2007). It serves as a component of glutathione peroxidase, the enzyme which protects cell membranes against oxidative damage (Rotruck et al., 1973). Two forms of Se have been used in fish culture: the inorganic form as sodium selenite or sodium selenite, and the organic form such as selenoyeast or selenomethonine. The organic form of selenium (OS) has superior benefits to the inorganic forms (Wang, 1997). There has been an increase in the use of the organic form of Se in aquaculture (Abdel-Tawwab et al., 2007). Supplementation of selenium in many aquatic animals has improved growth performance, survival, resistance to pathogens (Nugroho and Fotedar, 2013, Sang et al., 2015, Sritunyalucksana et al., 2011) and toxicity (Abdel-Tawwab et al., 2007, Li et al., 2014).

Snub-nosed dart (*Trachinotus blochii* Lacepide, 1801) is one of main cultured fish in several Asian countries such as Hong Kong, Singapore, Taiwan, China, Malaysia and Viet Nam (Sang et al., 2015). However, *Nocardia* infection is a growing problem for this species (Hoa et al., 2012). Nocardiosis has caused increasing damage and economic losses to many marine fish industries, including cultured snub-nosed dart (Labrie et al., 2008). Copper (Cu) is a trace metal essential for cellular metabolism but it may become extremely toxic for aquatic animals, in high concentrations. Copper sulfate (CuSO4) is often used as an algaecide in commercial fishponds to control the growth of phytoplankton and filamentous algae and to control certain fish diseases (Abdel-Tawwab et al., 2007). The $CuSO_4$ used for phytoplankton control is seldom directly toxic to fish, but does kill large numbers of rotifers, cladocerans, and copepods (Boyd, 1990). Above a specific concentration, Cu is toxic to fish (Wedemeyer, 1996). The supplemented diet at OS 0.3 g/kg improved survival and higher growth rate, increased flesh protein and liver lipids, and lowered flesh moisture content of the snub-nosed dart fish. In addition, the fish fed this diet had higher hematocrit values and the highest proportions of monocytes compared to fish fed the control diet (Sang et al., 2015). However, the role of OS in enhancing the immune response to a specific pathogen and copper exposure of fish has not been investigated. The aim of this study was to determine the effects of OS supplementation on the survival, physiological, and immunological response of snub-nosed dart when challenged with *Nocardia sp.* and when exposed to copper.

Materials and Methods

Twenty four (24) composite rectangular tanks (500 x 800 x 800 mm, 360 L, capacity) were used as culture units in the experiment. Each tank was supplied with 300 L mechanically filtered seawater, aerated, and had an independent recirculating seawater system with a biological filter. Recycling rate of the water in each tank was maintained at approximately 5 L/min throughout the experiment.

The juvenile fish from a commercial hatchery (Nha Trang, Viet Nam) were shipped to the aquaculture station of the Institute of Oceanography. For a one week acclimation period prior to the commencement of the experiment, they were fed a commercial diet of Nanolis C, Guyomarc'H, Vietnam) with 52% crude protein and 8% lipid. This feed was also used as a basal diet. The fish were fed twice daily at 8:00 and 16:00 hour at the rate of 5% of total biomass of the fish. Uneaten food and feces were siphoned out prior to each feeding.

The feed was ground and supplemented with organic selenium(OS) (Selplex, Alltech, Kentucky, USA) at levels of 0 g/Kg (D1-control) and 0.3 g/Kg (D2). The mixed

ingredients were then passed through a mincer with water to obtain 2 mm diameter pellets. The pellets were dried in direct sunlight for 6 h, allowed to cool at room temperature for half an hour before being packed in plastic containers and kept in a refrigerator at 4^{o}C until used. The concentration of Selenium in each diet was set at 0.94 and 2.74 mg/Kg, respectively.

After acclimation, the fish were randomly distributed into the culture tanks at a density of 20 fish/tank. Twelve randomly selected tanks of fish were fed D1, the remaining twelve tanks were fed D2. The fish were fed twice daily at 8:00 h and 16:00 h at the rate of 5% of their body weight for 12 weeks before the commencement of the challenge tests. After 12 weeks survival in all tanks was 100% and mean weight was 48 ± 1.28 g/individual.

The bacterial challenge and Cu exposure tests are presented in Figure 1.

Bacterial challenge test:

- For D1: in 3 tanks where fish were fed with 0 selenium diet and were challenged with *Nocardia sp* bacteria. Cumulative mortality was recorded until day 13 after infection
- D2: in 3 tanks where fish were fed with 0.3 g/kg selenium diet and challenged with bacteria *Nocardia sp.* Cumulative mortality was recorded until 13 days after infection
- D1: in 3 tanks where fish were fed with 0 selenium diet and challenged with bacteria *Nocardia sp.* Immunological parameters were recorded at days 1, 3, 7 and 12 days of infection and physiological parameters at 12 days after infection.
- D2:in 3 tanks where fish were fed with 0.3 g/kg selenium diet and challenged with bacteria *Nocardia sp.* Immunological parameters were recorded at days 1, 3, 7 and 12 days after infection, and physiological parameters 12 days after infection.

Nocardia sp solution was obtained from the Institute of Veterinary Research and Development of central Vietnam, Nha Trang city, Vietnam. The concentration of the stock solution was approximately 10^3 CFU/mL. Each fish was injected intraperitoneally (IP) with 0.1 mL of bacterial solution and fed the initial basic diets.

Cu exposure test: Fish were exposed to environmental copper for 7 days at concentration of 3.5 mg Cu^{2+}/L. (Fig.1)

The experimental procedure is as follows:

- D1: in 3 tanks fish were fed with 0 selenium diet and exposed to environmental copper toxicity for 7 days. Cumulative mortality was recorded
- D2: in 3 tanks fish were fed with 0.3 g/kg selenium diet and exposed to environmental copper toxicity for 7 days. Cumulative mortality was recorded
- D1: in 3 tanks fish were fed with 0 selenium diet and exposed to environmental copper toxicity. Physiological and immunological parameters were recorded on day 1 and day 7 post exposure.
- D2: in 3 tanks fish were fed with 0.3 g/kg selenium diet and exposed to environmental copper toxicity. Physiological and immunological parameters were recorded on day 1 and day 7 post exposure.

For physiological parameters, 3 fish from each tank were collected to determine moisture content and lipid accumulation in the liver, as well as moisture content and crude protein in the flesh according to the standard methods of the Association of Official Analytical Chemists (AOAC, 1995). For immunological parameters, 2 fish from each tank were sampled for analysis of non-specific immune parameters of head kidney leucocytes (HKL).

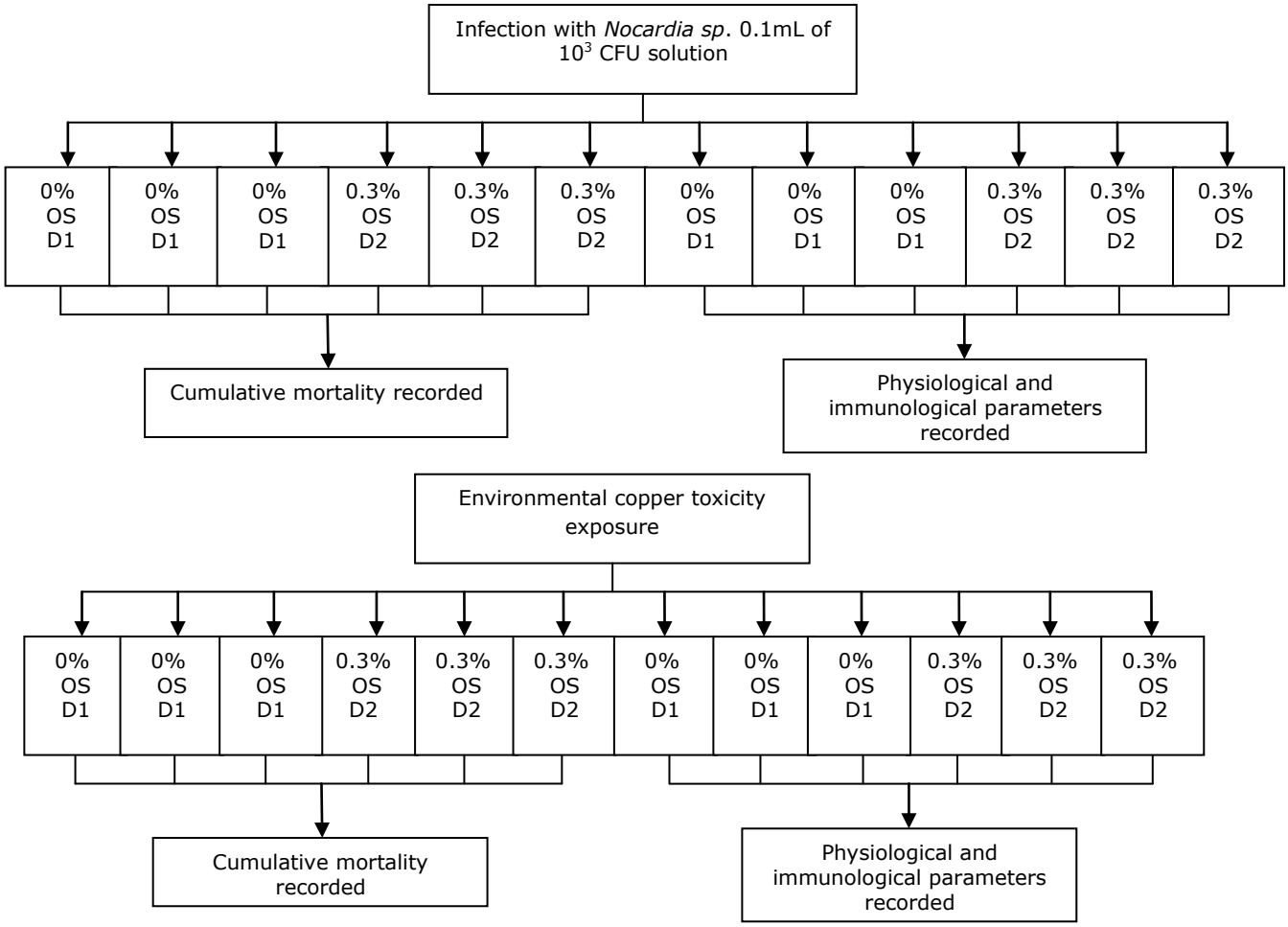


Figure 1. Experimental layout for the bacterial challenge and environmental toxicity exposure.

Isolation of head kidney leucocytes (HKL): Head kidneys were removed from the scarified fish and sieved through a 100 µm nylon mesh in L-15 medium (Leibovit). Cell suspension was placed on a 1.045/1.064 g/cm^3 Percoll density gradient and centrifuged at 1,310 rpm (400 x g) for 30 minutes at 4^{o}C. The isolated cells were harvested from the Percoll interface and washed twice with L-15 medium before microscopic examination. Cells were counted with a hematocytometer (Neubauer- Hirschmann) and cultured for non-specific immune parameters study.

Phagocytic activity assay(PA): Phagocytic activity of HKL was performed according to the method described by Yeh et al. (2008) where a suspension of HKL (500 µl of 1 x 107 cells in L-15 medium) was placed in 24 well cell culture dishes, and incubated at 28^{o}C for 2 hours. Non-adherent cells were removed and washed three times with L-15. Fluorescent latex beads (5 x 107 in L-15) were added to the leucocyte monolayer, and incubated for 2 h at 28^{o}C. The wells were washed three times with PBS to remove the uningested fluorescent latex beads, and then fixed with methanol for 5 min and washed three times with PBS. The fixed cells were then stained in Diff quick (Lucerna Chem) according to manufacturer's instructions. Phagocytic activity was determined by the percentage of phagocytes ingesting beads in 100 randomly selected phagocytes under a fluorescence microscope (Olympus BX41, Japan).

Respiratory burst activity assay (RBA): RBA produced by phagocytes in HKL was measured according to the method described by Cheng et al. (2007) where 100 µl of leucocyte suspension (1 x 10^6 cells/mL) was placed in a 96well culture dish (NuncMaxiSorpTM) and incubated for 2 h at 30^{o}C. The non-adherent cells were then

removed by washing the wells with L-15. Then, 100 µl of zymosan (Sigma) at 1 mg/mL in L-15 was added to the wells and incubated for 2 h at 30°C before adding 100 µl NBT 0.3% (Nitrobluetetrazolium) and incubated at 30°C for 30 min. The reaction was stopped by adding 100µl methanol 100%. The formazan formed in each well was then dissolved by adding 120 µl of 2 M KOH and 140 µl of dimethyl sulphoxide (DMSO). The NBT reduction was measured with a microplate reader (Bio-rad) at 630nm. Cells from each fish were placed in triplicate wells. RBA was calculated as follows:
RBA = stimulated activity (SA) - basal activity (BA) SA is the respiratory burst activity caused by stimulation with zymosan, and BA is the respiratory burst activity without stimulation by zymosan.

An independent-sample T test was used to examine the significant difference among treatments using the SPSS version 16.0. Percent data was normalized using an arcsin transformation before analysis. Difference was determined to be significant at $P < 0.05$. Results were expressed as mean ± standard error in the figures and tables.

Results

Initial mortality was observed six days post challenge with *Nocardia sp*. in both groups of fish. After day 8 following bacterial challenge, the cumulative mortality of fish fed control diet (D1) was higher ($P < 0.05$) than the cumulative mortality of fish fed OS supplemented diet (D2). Cumulative mortality of fish fed D1 reached 100 % at day 13 post challenge while in group D2 it was 74 % (Figure 2). Seven days post copper exposure, survival of fish fed both diets was 100 %.

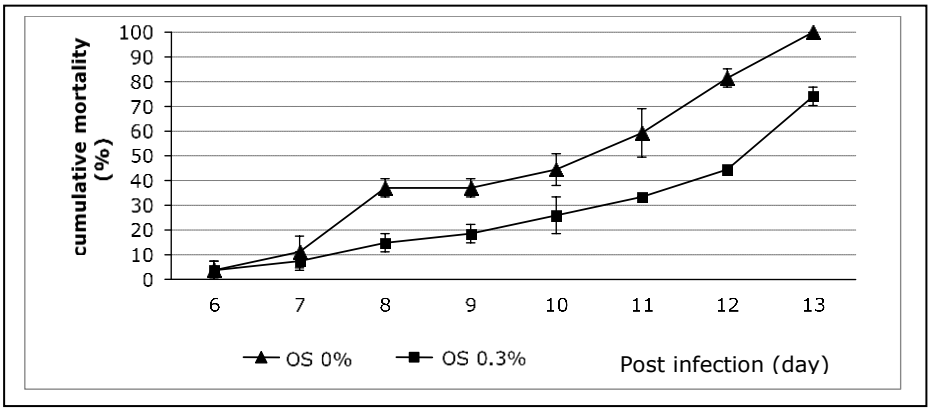


Figure 2. Mean (± S.E.) Cumulative mortality of snub-nosed dart challenged with bacteria *Nocardia sp*. Different letters indicate significantly different means ($P < 0.05$) among diets.

After 1, 3, 7 and 12 days post challenge by *Nocardia sp*., the phagocytic activity of D2 fish was significantly higher ($P<0.05$) than the D1 fish (Figure 3A). Respiratory burst activity did not differ between diets 1st and 3rd day post challenge and in D2 fish it was higher ($P<0.05$) than in D1 fish at 7th and 12th day post challenge (Figure 3B).

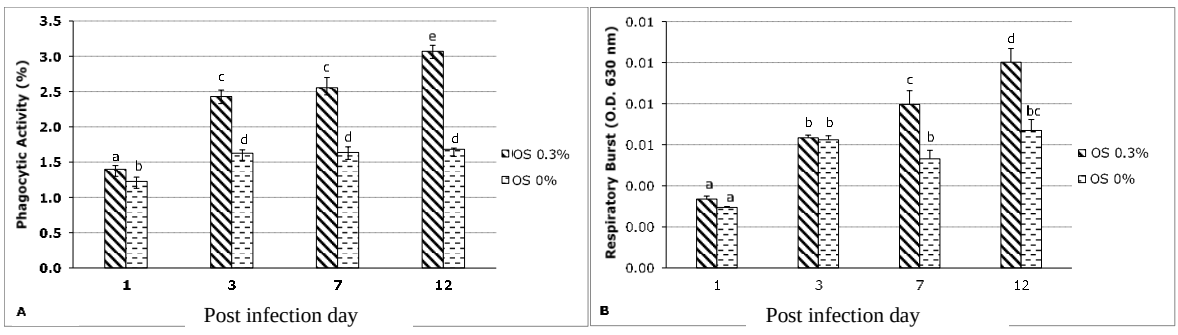


Figure 3. Mean (± SE) Phagocytic activity (A) and Respiratory Burst (B) of Snub-nosed dart challenged with bacteria *Nocardia sp*. Different letters indicate significantly different means ($P < 0.05$) among diets.

After the first day of copper exposure, no significant difference (P>0.05) in phagocytic activity was observed between the D1 and D2 fish. However, at day 7, the phagocytic activity of D2 fish was higher (P<0.05) than D1 fish (Figure 4A). Respiratory burst activity did not differ (P>0.05) between the fish fed both diets (Figure 4B).

Moisture content of liver and flesh, protein content of flesh, and total lipids in the liver were not different between fish fed OS supplemented diet and fish fed the control diet (P>0.05) and Cu toxicity exposure (Table 1).

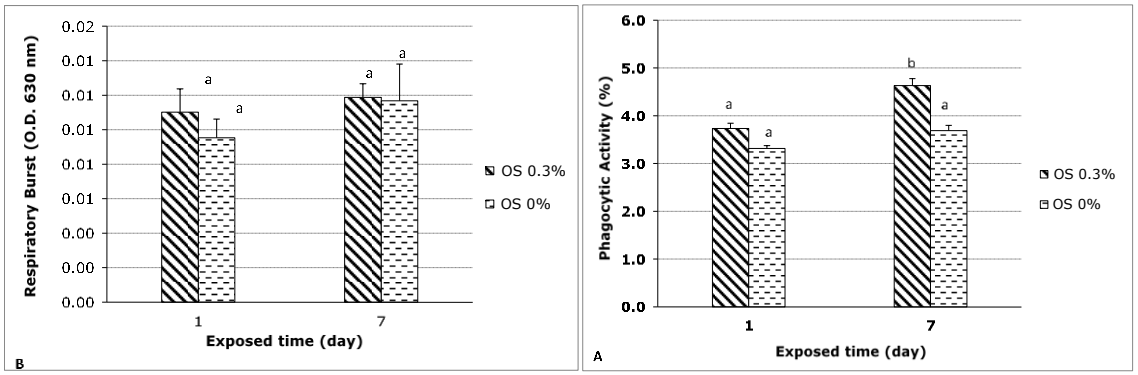


Figure 4. Mean (± SE) Phagocytic Activity (A) and Respiratory Burst (B) of Snub-nosed dart exposed to environmental copper toxicity. Different letters indicate significantly different means (P < 0.05) among diets.

Table 1. Physiological parameters of the fish fed different OS supplemented diets and challenged with *Nocardia sp.* and environmental copper exposure

	Bacterial challenge		*Copper exposure*	
	D1	*D2*	*D1*	*D2*
Liver moisture content (%)	72.39 ± 0.39	70.50 ± 1.03	67.85 ± 1.63	70.02 ± 1.00
Liver lipid (% dry weight)	4.70 ± 0.25	5.50 ± 0.60	14.04 ± 0.91[a]	16.88 ± 0.25[b]
Liver lipid (% wet weight)	1.24 ± 0.03	1.61 ± 0.19	4.64 ± 0.29	4.56 ± 0.26
Flesh moisture content (%)	73.02 ± 0.35	74.38 ± 0.78	73.48 ± 0.38	74.16 ± 0.67
Flesh protein (% wet weight)	14.33 ± 0.30	15.01 ± 0.55	16.91 ± 0.35	17.07 ± 0.34

Different superscript letters in the same row indicate significantly different means at P < 0.05

Discussion

Oral supplementation of OS for animals has been widely used and its efficacy for improving growth and pathogen resistance has been demonstrated in many species of fish, including snub-nosed dart. In our previous study, Se supplemented feed at 0.3 g/Kg OS in the diet for snub-nosed dart fish, resulted in higher survival, increased growth and highest proportion of leucocytes, particularly, monocytes and neutrophils in the blood (Sang et al., 2015). The latter play an important role in cell defense and protect animals and fish from diseases and environmental stress by strengthening their phagocytic and respiratory burst activity (Magnadóttir, 2006). Results of the present study suggest that OS supplementation of the diet improves leucocyte function of snub-nosed dart fish when challenged with bacterial infection reflected by higher phagocytic activity and respiratory burst value in fish fed OS supplemented diet. Previous research has shown that animals with better phagocytic activity and respiratory burst have higher disease resistance (Cheng et al., 2007, Solem et al., 1995). This efficacy was expressed by higher survival of fish fed Se supplemented diet than fish fed the control diet. In the current study the increase was 24 % when challenged with *Nocardia sp.* Higher phagocytic activity of fish fed OS supplemented diet when exposed to environmental copper toxicity indicates that dietary OS improved fish tolerance to environmental exposure to Cu toxicity. Survival data for fish exposed to environmental Cu toxicity were identical (100% survival) for both D1 and D2 fed fish. This may be attributable to the relatively low concentration of Cu^{2+} (3.5 mg Cu^{2+}/L) tested.

The mechanism by which dietary OS stimulates resistance of snub-nosed dart to *Nocardia sp*. and environmental toxicity has not been clearly understood. However, Selenium appeared to be an active agent in protecting cell membranes against lipid

peroxidation due to pathogen infection (Ursini and Bindoli, 1987). In addition, dietary Se can increase the ability to defend against oxidative damage via glutathione peroxides (GPx), a selenoprotein containing the 21st amino acid, selenocysteine (Sec), converted from dietary Se (Chiu et al., 2010). Lin and Shiau (2007) suggested that dietary Se supplementation prevents Cu accumulation in the tissues and prevents oxidative damage in grouper *Epinephelus malabaricus* fed diets containing high Cu level.

The results of the current research are consistent with those of previous studies on the role of Se and OS on acquired immunity of cultured fish when challenged with *Nocardia sp.* and exposed to Cu. Metal elimination from the body took place through the feces of *Cirrhinus mrigala* (James 2011) but this still needs to be studied with snub-nosed dart. In African catfish *Clarias gariepinus*, the addition of 0.3 g OS per kg diet (3.67 mg Se/Kg) improved fish growth, feed utilization, vitality against environmental copper toxicity and improved non-specific response via evaluating albumin and globulin concentration (Abdel-Tawwabet et al. 2007). However, there was no significant difference in fish survival and moisture content, while crude protein increased significantly, and total lipids decreased significantly in OS supplemented diet (Abdel-Tawwab et al., 2007). In contrast, Se had an effect on growth, muscle composition, and antioxidant acitivity in rainbow trout (Hunt et al., 2011). On the other hand, 2 mg/Kg Se supplementation had no significant effect on the survival, feed intake, hematocrit, white blood cell counts but significantly increased red blood cell count and hemoglobin concentration of yellowtail kingfish *Seriola lalandi* (Le et al., 2014). 0.5 mg/Kg Se supplementation had no effect on growth performance and disease resistance to *Edwardsiella tarda* in fingerlings of Nile tilapia *Oreochromis niloticus* L (Kim et al 2003).

In conclusion, in snub-nosed dart *Trachinotus blochii,* OS (Sel-Plex) supplemented diets enhance survival and immunity when challenged with *Nocardia sp*. and when exposed to environmental Cu toxicity. Further research is needed to evaluate the stimulation of OS on fish humoral immune response and anti-oxidant enzyme activity related to health and immunity of snub-nosed dart fish.

Acknowledgements

The authors are grateful to Mr. Hoang Duc Lu and Mrs Nguyen Kim Bich for their help in the experiment. This research was financial support by Vietnamese National Foundation for Science & Technology Development (NAFOSTED), Grant number: 106-NN.05-2013.06.

References

Abdel-Tawwab, M., Mousa, M. A. and F.E. Abbass, 2007. Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. *Aquaculture,* 272, 335-345.

Boyd, C.E., 1990. *Water Quality in Ponds for Aquaculture*. Birmingham Publishing Co., Birmingham, Alabama, USA.

Cheng, A. C., Tu, C. W., Chen, Y. Y. and H.F. Nan, 2007. The immunostimulatory effects of sodium alginate and iota-carrageenan on orange-spotted grouper *Epinephelus coioides* and its resistance against Vibrio alginolyticus. *Fish Shellfish Immunol*, 22, 197-205.

Chiu, S.-T., Hsieh, S.-L., Yeh, S.-P., Jian, S.-J., Cheng, W. and C.H. Liu, 2010. The increase of immunity and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbergii* by feeding with selenium enriched-diet. *Fish Shellfish Immun*, 29, 623-629.

Gatlin, D.M., Li, P., Wang, X., Burr, J.S., Castille, F. and A.L. Lawrence, 2006. Potential application of prebiotics in aquaculture. Symp. Int. de Nut. Acucola (Suarez, L.E.C. ed.Mexico.

Hoa, D.T., N. T. T. Giang and N. X. Nguyen, 2012. Clinical signs and histopathological characteristics of internal organ white spot disease in snub-nosed pompano *Trachinotus blochii* cultured in Nha Trang. *J Fish Sci Technol*, 1, 47-52.

Hunt A.O., Berkoz M., Ozkan F., Yalin S., Ercen Z., Erdogan E., Gunduz S.G.. 2011. Effects of Organic Selenium on Growth, Muscle Composition, and Antioxidant System in Rainbow Trout, 10 pages. *Isr. J. Aquacult. - Bamidgeh,* [IIC:63.2011.562].
James, R., 2011. Effect of Dietary Selenium Supplementation on *Cirrhinus mrigala* (Hamilton, 1822) Reared in Copper and/or Cadmium Contaminated Water, 7 pages. *Isr. J. Aquacult.- Bamidgeh,* [IIC:63.2011.573].
Kim, K.W., Wang, X., Choi, S.M., Park, G.J., Koo, J.W. and S.C. Bai, 2003. No synergistic effects by the dietary supplementation of ascorbic acid, α-tocopheryl acetate and selenium on the growth performance and challenge test of Edwardsiella tarda in fingerling Nile tilapia, *Oreochromis niloticus* L, . *Aquacult Res*, 34, 1053-1058.
Labrie, L., Tan, J. N., Z. , Komar, C., Ho, E. and L. Grisez, 2008. Nocardial Infections in Fish : An Emerging Problem in Both Freshwater and Marine Aquaculture Systems in Asia. Dis. Asian Aquac. VI, Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pp., 2008: pp. 297–312.
Le, K. T., Dao, T. T. T., Fotedar, R. and G.L. Partrigde, 2014. Effects of variation in dietary contents of selenium and vitamin E on growth and physiological and haematological responses of yellowtail kingfish, *Seriola lalandi*. *Aquacult Int*, 22, 435-446.
Li, X., Yin, D., Yin, Y., Chen, Q. and R. Wang, 2014. Dietary selenium protect against redox-mediated immune suppression induced by methylmercury exposure. *Food Chem Toxicol*, 72, 169-177.
Lin, Y.-H. and S.Y. Shiau, 2007. The effects of dietary selenium on the oxidative stress of grouper, *Epinephelus malabaricus*, fed high copper. *Aquaculture*, 267, 38-43.
Magnadóttir, B., 2006. Innate immunity of fish (overview). *Fish Shellfish Immun*, 20, 137-151.
Nugroho, R. A. and R. Fotedar, 2013. Dietary organic selenium improves growth, survival and resistance to Vibrio mimicus in cultured marron, *Cherax cainii* (Austin, 2002). *Fish Shellfish Immun*, 35, 79-85.
AOAC, 1995. Official methods of analysis, Association of Official Analytical Chemists, Washington DC.
Rosen, G.D., 1996. The nutritional effects of tetracyclines in broiler feeds In XX World's Poultry Congress, pp. 141–146 New Delhi, India (WPSA).
Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and W.G. Hoekstra, 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179: 585- 590.
Sang, H. M., Thuy, T. and D.H. Hoang, 2015. Effects of dietary organic selenium on growth, survival , physiological and hematology conditions of snub-nosed dart (*Trachinotus blochii Lacepide* , 1801). *Isr. J. Aquacult. - Bamidgeh,* 67.1181. 7 pp.
Solem, S. T., Jørgensen, J. B. and B. Robertsen, 1995. Stimulation of respiratory burst and phagocytic activity in Atlantic salmon (Salmo salar L) macrophages by lipopolysaccharide. *Fish Shellfish Immun*, 5, 475 - 491.
Sritunyalucksana, K., Intaraprasonga, A., Sa-Nguanruta, P. and K. Filer, 2011. Organic selenium supplementation promotes shrimp growth and disease resistance to Taura syndrome virus. *Science Asia,* 37, 24-30.
Wang C., Lovell T., 1997. Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (*Ictalurus punctatus*). *Aquaculture*, Volume 152, Issues 1–4, Pages 223–234.
Wedemeyer, G.A., 1996. *Physiology of Fish Intensive Culture Systems*. Chapman and Hall, New York, USA. 231 pp.
Ursini, F. and A. Bindoli, 1987. The role of Selenium peroxidases in the protection against oxidative damage of membranes. *Chem Phys Lipids*, 44, 255-276.
Yeh, S. P., Chang, C. A., Chang, C. Y., Liu, C. H. and W. Cheng, 2008. Dietary sodium alginate administration affects the fingerling growth and resistance to *Streptococcus sp. and iridovirus*, and juvenile non-specific immune responses of the orange- spotted grouper, *Epinephelus coioides*. *Fish Shellfish Immun*, 25, 19 - 27.