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Dietary probiotics enhance the immunity of Vietnami koi (*Anabas testudineus)* against *Pseudomonas* sp.

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Abstract. The present study evaluated the efficacy of dietary probiotic Biozyme on the immunological parameter and disease resistance against *Pseudomonas* sp. infection in Vietnam Koi (*Anabas testudineus*). Fish with an average body wt. 25±5 g were fed twice a day at 5% of their body wt.) experimental diet prepared with the supplementation of 0 g, 0.5 g, 1.0 g, 1.5 g and 2.0 g Biozyme probiotic per kg feed that enhanced the immune potentiality investigated on weeks 1, 2 and 4. The phagocytic and bactericidal activity significantly increased in *A. testudineus* fed with 2.0 g Biozyme probiotic per kg feed against *Pseudomonas* sp. on weeks 2 and 4. Further, the diet enriched with 2.0 g of Biozyme (probiotics) resulted in lowest mortality (20%) highest protection with 75% survival from *Pseudomonas* sp. infection than 0.5 g, 1.0 g and 1.5 g of addition with diet that resulted 46.67%, 40% and 33.33% mortality respectively. 2.0 g probiotic/kg of feed also showed highest growth compared to other dose. The results suggest that the dietary supplementation of 2.0 g probiotics/kg feed significantly ($p<0.05$) reduce mortality, act as immunostimulants, and promoter and showed resistance in *A. testudineus*.

Keyword: *Anabas testudineus*, dietary probiotics, *Pseudomonas* sp.

Introduction

Vietnam koi, *Anabas testudineus,* is an obligatory air-breathing fish. In addition to four pairs of gills, it bears accessory respiratory organs- one pair of labyrinthine and respiratory membranes within the suprabranchial chamber. A freshwater fish like *A. testudineus* are more prone to bacterial disease as well as *Pseudomonas* sp. *Pseudomonas* is a genus of Gram-negative, aerobic gammaproteo bacteria, belonging to the family Pseudomonadaceae containing 191 validly described species (EL-Rhman *et al*. 2009). These microorganisms are responsible for ulcer type diseases including ulcerative syndrome, Bacteria haemorrhagic septieaemia, tail and fin rot, and bacteria gill rot and dropsy (Cao *et al*. 2001). Probiotic can be defined as "A live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment" (Gomez *et al*. 2007). Probiotics is used as fish diet and contribute the benefits to increase the length and weight of organisms, bacterial control diseases, nutrients source, essential enzymes for better food digestion, elimination of organic matter and increase of immune response against pathogen organisms, reducing diseases risks and use of chemical drugs who pollute water habitat (Irianto and Austin 2002, Burr *et al*. 2005). Dietary probiotics can be used as living

cells but some studies have also shown their benefits when supplied as heat-inactivated cells (also known as heat-killed cells), formalin-killed (FKC), freeze-dried, dead cells or cell-free supernatant (CFS) (Cordero *et al*. 2011). Some commercial name of probiotics that are mainly applied into the aquaculture farm are pH Fixer , Super Biotic, Super PS, Zymetin, Mutagen, Bioprob, Alibio, Bactocell Pa10, BaoZyme-Aqua, BGY-35, BioPlus 2B, Cernivet, Ecomarine, Biostart, Liqualife, SanoCare SanoLife (Cordero *et al*. 2011).

Materials and Methods

Biozyme (dietary probiotics)

Biozyme was purchased from Syfulla Krishi and Motsho Biponi located at Chanchra, Jessore. Biozyme was manufactured by Bio-Pharmachemie VIETNAM. The useful bacteria, enzyme and nutrition of Biozyme are shown in Table I.

Table I. The useful bacteria and enzyme of biozyme[1]

Enzyme	Quantity	Useful Bacteria
Amylase	35000 units	Bacillus subtilis 7×10^6 CFU Saccharomyces cerevisiae 3×10^{12} cells
B glucanase	22500 units	
Lipase	10000 units	
Protease	137500 units	
Hemicelluloses	2500 units	

[1]Source: Bio-Pharmachemie VIETNAM, BIOZYME

Fish and husbandry

Climbing perch or Vietnam koi (*A. testudineus*) average weight 25 5 g; N=225 were collected from commercial fish farm in Jessore and taken into the laboratory in January, 2017. Continuous aeration was provided to maintain dissolved oxygen level at 7.5 ± 0.5 mg/L and one third of the aquarium water was exchanged daily by siphoning the waste materials. Fishes were provided with normal feed at the rate of 5% of their body weight twice a day at 09:00 and 17:00 hours for 3 days but at the first day of their arrival no feed was provided.

Experimental diet preparation

The experimental diet was prepared by mixing with locally available commercial feed (CP Feed) which approximately contains: 35% crude protein, 8% fat, 12% moisture, 3% fiber, (Source: C.P Bangladesh Co. Ltd) (Table II). At first commercial feed was grinded with a grinder and mixed with Biozyme probiotics powder. The crude protein was estimated by Kjeldahl method, lipid was estimated by Soxhlet extraction apparatus, moisture by moisture analyzer and ash was estimated by muffle furnace (Table III). All the ingredients were mixed thoroughly by adding water and pelletized by hand and then sundried. Five different experimental pellet diets were prepared containing five different mixture of Biozyme probiotic such as 0 g (control), 0.5 g, 1.0 g, 1.5 g and

2.0 g per kg feed. The pellet feed was stored not longer than 90 days. Feed was regularly inspected, moldy feed discarded immediately, kept in dry, cool, clean, and free of rodents and pests, in room temperature until use.

Table II. Composition of basal diet

Composition	Content (%)
Crude protein	35
Moisture	12
Fat	8
Fibre	3

Table III. Proximate composition of the experimental diet

Composition	Content (%)
Protein	15
Fat	9
Moisture	7
Ash	14

Pseudomonas sp. isolation

Pseudomonas sp. strains initially isolated from diseased *A. testudineus* with abdominal dropsy and septicemia, were collected from the fish farm in Jessore. The strains had been maintained in the laboratory since their isolation, by repeated culture in selective agar media (*Pseudomonas* agar media). Stocks were grown in *Pseudomonas* agar media for 24 hrs at 37^0C and kept in – 20^0C until use. The subculture was taken and centrifuged (5000 rpm for 12 min). After centrifugation the supernatant was discarded and the pellet was re-suspended in sterile phosphate buffer saline (PBS). The culture was adjusted to 3.0×10^{-6} colony forming units (CFU) $ml^{-1}$and incubated at 37^0C for 24 hrs (Table IV). The bacteria were confirmed by the following characterization.

Table IV. Identifying characteristics of fish pathogenic strain *Pseudomonas* sp.

Sl. No.	Character	Results
1	Colony shape	Round
2	Colony size	Medium
3	Colony color	Yellowish
4	Gram stain	Negative
5	Shape	Rod
6	Oxidase	+
7	Polar flagella	+
8	Catalase	+
9	O-F test	Oxidative
10	Motility	+
11	Methyl-Red test	-
12	Growth at37^0C	+

Reference: Foysal *et al*. 2011. + = Positive reaction; - = Negative reaction

Experimental design

The experiment was performed in a 100 L rectangular glass aquaria in the laboratory. The fishes were divided into five equal groups (T_1, T_2, T_3, T_4 and T_5) each with three replicates containing 15 fishes per replicate. Fishes were provided with adequate aeration with an air stone. All fish groups were fed on diets at the rate of 3% of the body weight during 6 weeks of the experiment. On week 2, 4 and 6, three fish were randomly collected from each group and used for blood collection for specific and nonspecific immunological assays. The experimental fish were challenged with a virulent strain of *Pseudomonas* sp. at 3.0×10^{-7} CFU ml^{-1} by injected intraperitoneally (i.p.) with 25μl PBS for analyzing cumulative mortality.

Serum collection: Blood samples were obtained from the caudal vein of chosen fish from each aquarium by using a syringe of 1 ml. The blood (2ml) was then transferred into Eppendorf containing EDTA solution as an anticoagulant to avoid blood clotting. Blood was centrifuged at 4000 rpm for 10 minute at 4°C and the serum collected.

Bactericidal activity: *Pseudomonas* sp. was used to examine the effectiveness of supplements to kill the bacterial infection to prepare stock solution of experimental bacterial strain (from previously cultured 2.4) in conical flask containing 100 ml distill water, inoculating loop was touched from single bacterial colony of fresh culture. Bacterial suspension was then diluted using diffusion method. 15μl of serum was added with 15μl of bacterial suspension and mixed properly. The serum-bacterial mixture (15μl) was plated onto the nutrient agar and BHI agar plates and incubated for 24 hours at 37^0C before the numbers of colonies were counted.

Phagocytic activity: Phosphate buffer solution (PBS) was fixed with gluteraldehyde and 6% suspension of Vietnam koi blood cells were mixed in it. 20μl of bacterial suspension (from previously cultured 2.4) was placed on a cover slip incubated for 30 min in a humid chamber. Then it was carefully washed with PBS and 20μl of blood cells was added and incubated for 40 min after air dried. After staining with giemsa the numbers of engulfed blood cell or phagocytic cell was determined by photographic microscope (Axiocam ERc 5s with Axiovixim driver, Carl Zeiss, Germany).

Challenge test: For the challenge test virulent *Pseudomonas* sp. strain were prepared from maintaining the serial dilution. Two days after the last bleeding, the fishes from each group were injected intraperitoneally (i.p.) with 1ml of 24 hours cultured *P. fluorescens* which contained 3.0×10^{-7} $CFUml^{-1}$ Challenge strain. The clinical signs and mortality was recorded up to 30 days of post challenge. Mortality percentage and Relative Percent Survival (RPS) was calculated by using the following formula:

$$\text{Percentage of mortality} = \frac{No.of\ dead\ fish\ ofier\ challange}{Total\ no.\ of\ injected\ fish} \times 100$$

$$\text{RPS} = 1\text{-}\frac{(\%\ Mortality\ in\ treated\ group)}{(\%\ Mortality\ in\ control\ group)} \times 100$$

Statistical analysis: Values for each parameter measured were expressed as the arithmetic mean ± standard error (SE) by using Tukey's statistical analysis by statistical software 2007. Effects of dietary probiotics on growth performance, hematological and immunological parameter were tested using one-way ANOVA and the mean values were compared by using Duncan's multiple range tests at 5% level of significance (Zar 1984).

Results

Disease resistance (Challenge test)

During the experimental period the mean (±SE) water temperature, pH and TDS were 22±0.8C^0, 5.94±0.21 and 434±0.29 mg/L respectively. The cumulative mortality was lowest 20% in T_5 (2 g/kg feed) compared with T_1 – control (80%) and other treatments which were 33.33%, 40% and 46.67% in case of T_4 (1.5 g/kg), T_3 (1.0 g/kg), T_2 (0.5 g/kg) The challenge with dietary probiotics (Biozyme) against *Pseudomonas* sp. of Vietnam Koi (*A. testudinesas* shown 80% survival rate and 75% RPS in T_5 for 30 days which was higher than other treatments (Table V, Fig. 1)

Table V. Challenge test of dietary probiotics against *Pseudomonas* sp. in Vietnam Koi

Treatment With probiotics	Challenge dose CFU ml^{-1}	Total fish	No of infected fish	No of death fish	Mortality %	Survival %	RPS %
T_1-control	3.0×10^{-7}	15	13	12	80.00	20.00	-
T_2(0.5 g/kg)	3.0×10^{-7}	15	11	7	46.67	53.33	41.66
T_3(1.0 g/kg)	3.0×10^{-7}	15	8	6	40.00	60.00	
T_4(1.5 g/k)	3.0×10^{-7}	15	9	5	33.33	66.67	58.33
T_5(2.0 g/kg)	3.0×10^{-7}	15	7	3	20.00	80.00	75.00

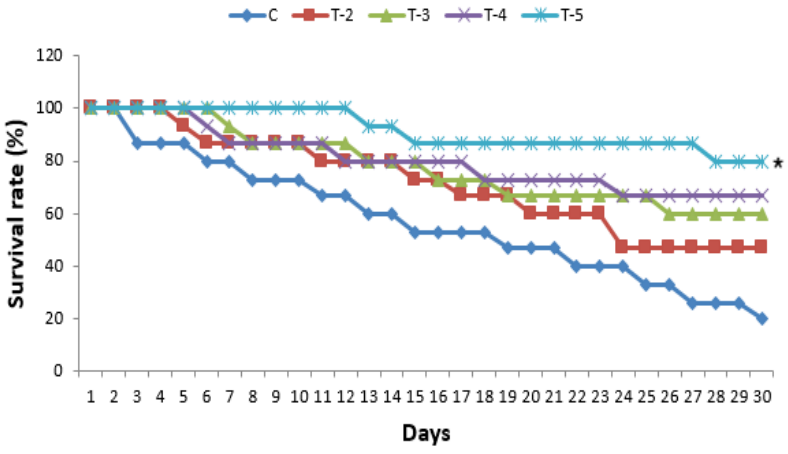

Fig. 1. Survival rates of Vietnam koi fed with different doses of dietary probiotics against *Pseudomonas* sp. [* indicates relatively significant ($p<0.05$)]

Serum bactericidal activity (Specific immunity)

Fishes feeding with different doses of dietary probiotics did not significantly enhance immune response in the first week. Immune response level significantly increased with diets in week 2 and 4. Immune response level did not significantly change in control (Fig. 2 & Fig. 3)

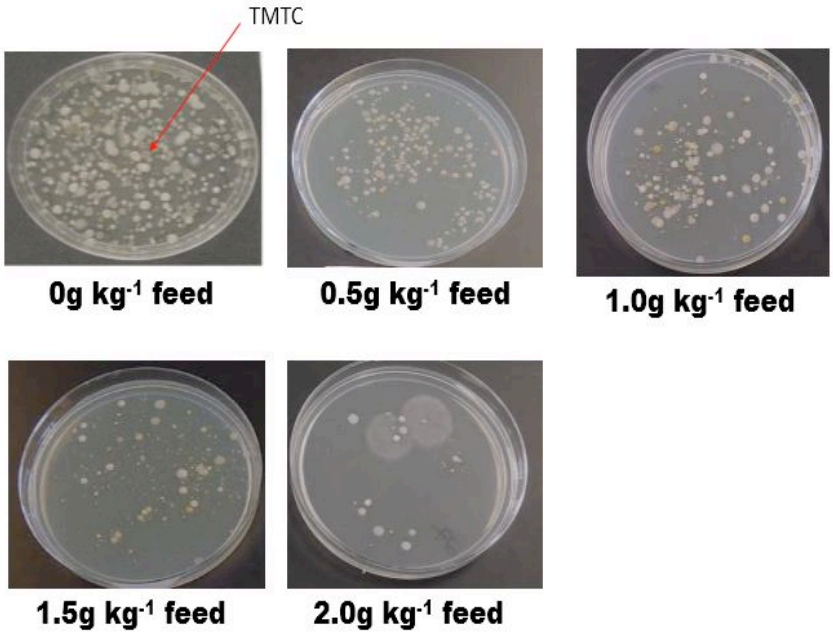

Fig. 2. Colony of *Pseudomonas* sp. grown from serum suspension of Vietnam koi.

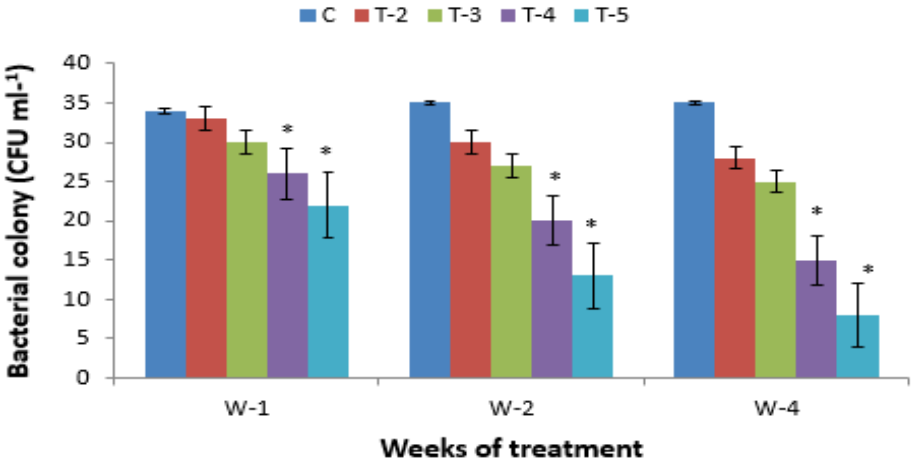

Fig. 3. Bacterial activity of serum of Vietnam koi fed dietary probiotics against *Pseudomonas* sp.[* indicates significant ($p < 0.05$)].

Phagocytic activity

Phagocytic activity did not significantly enhance with T_2 (0.5 g/kg), T_3 (1.0 g/kg), T_4 (1.5 g/kg), T_5 (2.0 g/kg) feed diet on the first week against *Pseudomonas* sp. However with T_4 (1.5 g/kg), and T_5 (2.0 g/kg feed) doses the activity significantly increased in week 2 and 4 but not with T_2 (0.5 g/kg) and T_3 (1.0 g/kg) doses as compared with T_1-control (80%) (Fig. 4 & Fig. 5).

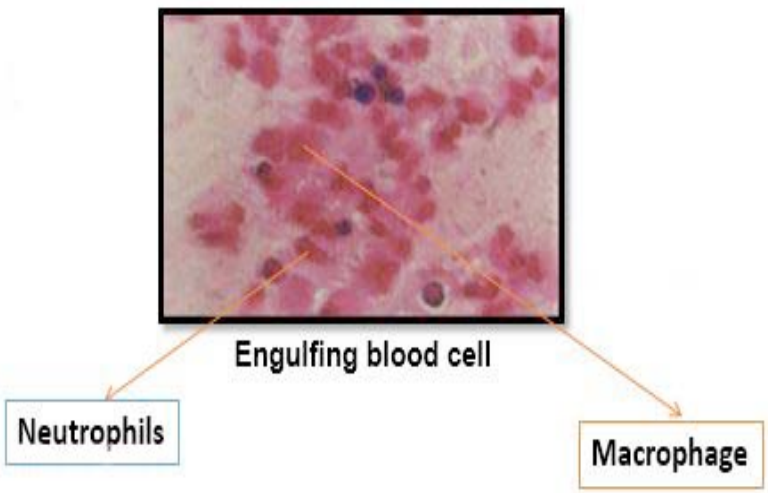

Fig. 4. Microscopic view of 2.0 g kg^{-1} experimental diet fed Vietnam koi blood cells

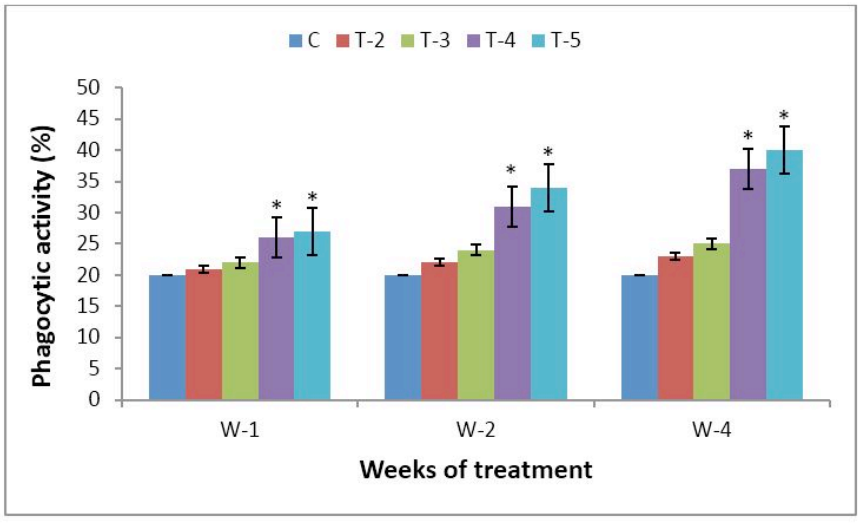

Fig. 5. Phagocytic activity (%) of Vietnam koi fed dietary probiotics against *Pseudomonas* sp. [* indicates relatively significant ($p < 0.05$)].

Discussion

The cumulative mortality was lowest (20%) in T_5 (2.0 g/kg feed) compared with T_1-control (80%) and other treatments which were 33.33%, 40% and 46.67% in case of T4 (1.5 g/kg), T_3 (1.0 g/kg), T_2 (0.5 g/kg). In this study challenge with dietary probiotics (Biozyme) against *Pseudomonas* sp. of Vietnam koi (*A. testudineus*) have showed 80% survival rate and 75% RPS at T_5 for 30 days which was higher than other treatments. The results of the present study was similar to dietary probiotics with 2.0 g kg^{-1} feed were found to enhance the disease resistance of *P. hypophthalmus* against *Pseudomonas* sp. (Balcazar *et al*. 2006). The result of the present study was also similar to the effectiveness of probiotics in terms of protection against infection has also been demonstrated to be as a result of enhanced immunity. Denev *et al*. (2009) worked on probiotic intervention in aquaculture. They showed that probiotics played an important role in developing innate immunity among the fish and hence helped them to fight against any pathogenic bacteria. Hasan *et al*. (20112) stated that probiotic use can enhance the immune response of tilapia and improve disease resistance. It is likely that the positive results reported in the present study may be due to a combination of all or some of these mechanisms. Iman *et al*. (2013) studied on *Lactobacillus plantarum* as a probiotic in *Oreocromis niloticus* on growth performance and innate immunity.

Serum bactericidal activity is a mechanism that helps to resist the growth of pathogen (Eissa and Abou-EL 2014) the lowest number of bacterial colonies cells in serum to kill pathogen. In the present study, the lowest numbers of bacterial colonies were observed in the treatment group than the control group. Fish feeding with different doses of dietary probiotics did not significantly enhance immune response in the first week. Immune response level significantly increased with diets in week 2 and 4. Immune response level did not significantly change in control. Other research showed that the use of probiotics improved the nutrition level of aquaculture and improves immunity to a pathogenic microorganism. Bandyopadhyay and Das (2009) observed that *Bacillus* sp. provided disease protection to shrimp by activating both cellular and humoral immune defenses. The implementation and application of probiotics in diets for aquatic animals are suggested as a prevention measure of diseases (Giri *et al*. 2012), and may increase the growth rate (length and weight) or increase of immune response to allow better survival when illness were shown (Essa *et al*. 2010).

Probiotics as live microorganisms can be good alternatives to chemotherapy (Cabello 2006 *et al*., Giri *et al*. 2012) since they have stimulation effect of non-specific host defense mechanisms, enhances the disease resistance and growth promotion (Irianto and Ausin 2002). Similar results were previously recorded by (Dasgupta *et al*. 2000) who studied the effect of live and dead probiotic cells on the non-specific immune system of Nile tilapia and reported that the probiotics-treatment stimulated the non-specific immune parameters, resulting in the enhancement of fish resistance against *Edwardsiella tarda* infection.

Phagocytic activity was not significantly enhanced with T_2 (0.5 g/kg), T_3 (1.0 g/kg), T_4 (1.5 g/kg), T_5 (2.0 g/kg feed) diet in the first week against *Pseudomonas* sp. However with T_4 (1.5 g/kg) and T_5 (2.0 g/kg) doses as compared with T_1-cotrol (80%) Other investigation demonstrated that betaglucans and nucleotides might be useful to increase resistance of fish. When these immunostimulants were added to the feed, a decrease in mortality ranging from 7 to 44% was observed in RTB sharks challenged with *S. iniae*. One possible explanation for the results of the first experiment is that the mechanism of action of these compounds may be through stimulation of the nonspecific immune system, which has been observed in the investigations (Kulkarni *et al*. 1986). However no specific immune function testing was done in the RT experiments to test this possibility. The efficacy of beta-glucans in increasing disease resistance has also been documented in other studies. For example, lower mortality was observed in Atlantic salmon vaccinated against *Aeromonas salmonicida* (Burrells *et al*. 2001), in turbot vaccinated against *Enterococcus* sp. (Ibrahim *et al*. 2014), in yellowtail challenged with *Enterococcus seriolicida* (Itami *et al*. 1996), and in swordtails, rosy barbs and black tetras challenged with *Aeromonas salmonicida* (Nikl *et al*. 1993) fed beta-glucans.

All Vietnam koi fed with different doses of dietary probiotics (Biozyme) added diet (T_2, T_3, T_4, T_5) showed significantly higher final body weight (FBW), weight gain (WG) and specific growth rate (SGR) compared to control group (T_1). While the feed conversion ratio (FCR) was significantly lower in fish fed diet T_5 compared to control group (T_1). This finding is supported by Tovar-Ramirez *et al*. (2004) who reported that the growth and survival of larvae of sea bass fed 1.1%live yeast was significantly higher than the control. In this study tilapia final weight, weight gain, specific growth rate, survival rate, feed intake and protein efficiency ratio were increased among *O. niloticus* fed a diet containing *L. plantarum*. These result are in agreement with Rengpipat *et al*. (1998) and Rengpipat *et al*. (2009) who report that the probiotic enhanced the growth rate of shrimps and maintain the water quality parameters. Survival of shrimps was significantly greater in the treated group compared with the control group. Rengpipat *et al*. (1998) and Rengpipat *et al*. (2009) reported that the probiotic group enhanced growth rate of Shrimps. In the present study after challenge with *P. fluorescens* (3.0*10-7CFU^{ml-1}) mortalities were significantly reduced in all groups compared to the control group. The lowest mortality 20% was observed at the dose of T5 (2.0 g/kg) of dietary probiotics (Biozyme). It can be deduced that using dietary probiotics (Biozyme) as an immunostimulants in Vietnam koi showed an immunity enhancement which supplements as immuno modulatory components in fish feed help to make an immune response in cultured fish against *Pseudomonas* sp.

In this experiment 2.0 g/kg feed probiotics enriched diet showed the highest positive response against *Pseudomonas* sp. reduce mortality and acts as immunostimulants, growth promoter and disease resistance (survival rate 80%) in Vietnam koi (*Anabas testudineus*) against *Pseudomonas* sp. infection in *A. testudineus*. It is important to develop education and demonstrative programs for commercial fish

farmers to promote and demonstrate the efficacy of preventive medicine that include also the use of vaccine and immunostimulants, instead of the mass application of drugs and antibiotics..

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Literature Cited

Balcazar J.L., I. Balsw, I.D. Ruiz-Zarzucla, Cunningham, D. Vendrell and J.L. Muzquiz, 2006. The role of probiotics in aquaculture. *Vet. Microbiol.*, 114: 173-186.

Bandyopadhyay P. and M.P.K. Das, 2009. Effect of a bacteriam *Bacillus circulans* PB7 in the formulated diets on growth, nutritional quality and immunity *of Catla catla* (Ham). *J. Fish Physiol. Biochem.*, 35(3): 467-478.

Burr, G., M. Hume, W.H. Neill and D.M. Gatlin, 2008. Effects of prebiotics on nutrient digestibility of soybean-meal-based diets by red drum (*Sciaenops ocellatus*). *Aquac. Res.,* 39: 1680-1686.

Burrells, C., P.D William and P. Forno, 2001. Dietary nucleotides: a novel supplement in fish feeds: Effects on resistance to disease in salmonids. *Aquaculture*, 199(1-2): 159-169.

Cabello F.C., 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for Human and animal health and for the environment. *Environ. Microbiol.,* 8: 1137-1114.

Cao, H., R.L. Baldini and L.G. Rahme, 2001. Common mechanisms for pathogens of plants and Animals. *Ann. Rev. Phytopathol.,* 39: 259-284.

Carver J.D. 1994. Dietary nucleotides; cellular immune, intestinal and hepatic system effect. *J. Nutri.*, 124(1): 144-148.

Cordero H. Esteban, M.A. and Cuesta A.2011. Use of Probiotic Bacteria against Bacterial and Viral Infections in Shellfish and Fish Aquaculture. *J. Invert. Pathol.*, 96; 239-266.

D. Tovar-Ramíreza, B., J. Zambonino Infante, C. Cahu, F.J. Gatesoupe and R. Vázquez-Juárez, 2004. Influence of dietary live yeast on European sea bass (*Dicentrarchus labrax*) larval development. *Aquaculture*, 234(1-4): 415-427.

Dasgupta, N., S. K. Arora and R. Ramphal, 2000. FleN, a gene that regulates flagellar number in Pseudomonas aeroginosa. J. Bacteriol., 182(2): 357-364.

Denev, S., Y. Staykov, R. Mouutafchieva and G. Beev, 2009. Microbilal ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture. *Int. Aquatic Res.,* 1: 1-29.

Eissa N. and E. Abou-ELGheit, 2014. Dietary supplementation impacts of potential non-pathogenic isolates on growth performance, hematological parameters and disease resistance in Nile tilapia (*Oreochromis niloticus*). *J. Vet. Adv.,* 4(10): 712-719.

EL-Rhman, A.M.A., Y.A. Khattab and A.M. Shalaby, 2009. *Micrococcus iuteus* and *Pseudomonas species* as probiotics for promoting the growth performance and health of Nile tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol.,* 27: 175-180.

Essa, M.A., S. EL-Serafy, M.M. EL-Ezabi, S.M. Daboor and N.A. Esmael, 2010. Effect of different dietary probiotics on growth, feed utilization and digestive enzymes activites of Nile tilapia, *Oreochromis niloticus*. *J. Arab. Aquac. Soc.*, 5: 143-161.

Foysal, M.J., M.M. Rahman and M. Alam, 2011. Antibiotic sensitivity and in vitro antimicrobial activity of plant extracts to pseudomonas fluorescensisolates collected from diseased fish. *Int. J. Nat. Sci.,* 1(4): 82-88.

Giri S. SSen S.S and V. Sukumaran, 2012. Effects of dietary supplementation of potential probiotics *Pseudomonas aeruginosa* VSG-2 on the innate immunity and disease resistance of tropical freshwater fish *Labeo rohita*. *Fish and Shellfish Immunol*. 32: 1135-1140.

Gomez, R., D. Geovanny, J.L. Balcazer and M.A. Shen, 2007. Probiotics as control agents in aquaculture. *J. Ocean Univ. China,* 16(1): 76-79.

Ibrahim B.U., J. Baba and M.S. Sheshi, 2014. Isolation and identification of bacteria associated with fresh and smoked fish (*Clarias gariepinus*) in Minna Metropolis, Niger State, Nigeria. *J. Appl. Environ. Microbiol.*, 2 (3): 81-85.

Iman M.K., W.T. Abbas, E.S. Awaad, M.M.N. Authman, G.A. El–Shafei Ibrahim, G.A Z.I. Sadek and H.S. Sayed, 2013. Evaluation of *Lactobacillus plantrum* as a probiotic in aquaculture; Emphasis on growth performance and innate immunity. *J. Appl. Sci. Res.,* 9(1): 572-582.

Irianto A. and B. Austin, 2002. Use of probiotics to Control furunclosis in rainbow trout (*Oncrhynchus mykiss*, Walbaum). *J. Fish Dis.,* 25: 333-342.

Itami, T., M. Kondo, M. Uozo, A. Suganuma, T. Abe, A. Nakagawa, N. Suzuki and Y. Takahashi, 1996. Enhancement of resistance against *Enterococcus seriolicida* infection in yellowtail, *Seriola quinqueradi-ata*, by oral administration of peptidogly can derived from *Bifidobacterium thermophilum*. *J. Fish Dis.,* 19: 185–187.

Kesarcodi-Watson, A., H. Kaspar, M.J. Lategan and L. Gibson, 2008. Probiotics in aquaculture; the need, principles and mechanisms of action and screening processes. *Aquaculture*, 274: 1-14.

Kulkarni A.D., W.C. Fanslow, F.B. Rudolph and C.T. Van Buren, 1986. Effect of dietary nucleotides on response to bacterial infections. *J. Par. Ent. Nutri.,* 10: 169-171.

Nikl, L., T.P.T. Evelyn and L.J. Albright, 1993. Trials with an orally and immersion-administered beta-1,3 glucan as an immunoprophylactic against *Aeromonas salmonicida* in juvenile chinook salmon *Oncorhynchus tshawytscha*. *Dis. Aquat. Org.,* 17: 191-196.

Rengpipat, S., N. Wongtangprasert and T. Palaga, 2009. The use of green fluorescent protein as a marker for monitoring a probiotic Bacillus S11 in the black tiger shrimp *Penaeus monodon*. *Aquac. Nutri.,* 15: 297-305.

Rengpipat, S., W. Phianphak, S. Piyatiratitivorakul and P. Menasveta, 1998. Effect of probiotics on Black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture,* 67: 301-313

Zar J.H., 1984. Production. *In*: Biostatistical Analysis (ed. By J.H. Zar), pp. 293-305. Prentice-Hall, Englewood Cliffs, NJ, USA.

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