Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 11 (1): 67-76, 2016
ISSN 1816-4927 / DOI: 10.3923/jfas.2016.67.76

Administration of Microencapsulated Probiotic *Bacillus* sp. NP5 and Prebiotic Mannan Oligosaccharide for Prevention of *Aeromonas hydrophila* Infection on *Pangasianodon hypophthalmus*

[1]Rifqi Tamamdusturi, [2]Widanarni and [2]Munti Yuhana
[1]Study Program of Aquaculture, Graduate School, Bogor Agricultural University, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia

Corresponding Author: Widanarni, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia Tel: 622518628755 Fax: 622518622941

ABSTRACT

A feeding trial was conducted to investigate the effects of administration of microencapsulated probiotic *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide (MOS) for the prevention of *Aeromonas hydrophila* infection on *Pangasianodon hypophthalmus*. Pangasius with initial body weight of 6.54±0.17 g were stocked into 40 L aquariums with a stocking density of 10 fish per aquarium. Control (C) diet (no addition of *Bacillus* sp. NP5 and MOS), probiotic *Bacillus* sp. NP5 1% (10 g kg^{-1}), prebiotic MOS 0.2% (2 g kg^{-1}) and synbiotic (probiotic *Bacillus* sp. NP5 1%+prebiotic MOS 0.2%). Then 10 fish of each aquarium were challenged by *Aeromonas hydrophila*. The results showed that fish with synbiotic supplementation resulted the highest Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and those were significantly different ($p<0.05$) from the other treatment. The immune responses showed that fish fed with the control diet resulted the lowest hematocrit, hemoglobin and red blood cell count and those were significantly different from the probiotic and prebiotic group ($p<0.05$). White blood cell count, phagocytic activity and respiratory burst activity of the fish fed with probiotic 1% and MOS 0.2% were not significantly different from control. Moreover, fish fed with diet supplemented with probiotic, prebiotic and synbiotic had notably lower mortality after 10 days infected with *A. hydrophila* ($p<0.05$). Dietary *Bacillus* sp. NP5 and MOS had a significant interaction on enhancing immune responses and growth performances of *Pangasianodon hypophthalmus* ($p<0.05$).

Key words: *Aeromonas hydrophila*, *Pangasianodon hypophthalmus*, probiotic, prebiotic

INTRODUCTION

Pangasius is an indigenous fish from Southeast Asia, it has been cultivated widely in freshwater aquaculture systems and being economically valuable species (Hedayati and Tarkhani, 2014). Intensification of pangasius production nowadays is also accompanied with the emergence of some disease agents such as bacteria and parasites (Phan *et al*., 2009). One of diseases in pangasius cultivation is Motile Aeromonad Septicaemia (MAS) caused by *Aeromonas hydrophila*.

This bacterial disease commonly attacks freshwater fish both farmed and wild fish around the world (Zhang *et al*., 2014b). The use of antibiotics in aquaculture practices have been limited, because their long-term use can lead to bacterial pathogen resistance and also become residues in

the aquatic environment and organisms. Exploration of environmentally friendly methods is needed for the prevention of MAS. One alternative that can be used to solve this problem is the oral application of probiotics, prebiotics and synbiotic (Talpur *et al*., 2014).

Probiotic is additional microbe which can provide beneficial effects for the host (Nayak, 2010). Prebiotic is food materials which can not be digested by the host but it gives beneficial effects by stimulating the growth and activity of beneficial bacteria (Zhang *et al*., 2012). Synbiotic is supplement which made by combining probiotic and prebiotic, so it is expected to enhance the beneficial effects for the host (Cerezuela *et al*., 2011).

According to Weinbreck *et al*. (2010), the effectiveness of probiotics depend on the ability of probiotics to survive during storage, to reach the target and to work in the gastrointestinal tract of the host. Microencapsulation is an approach as a way to provide probiotic cells with a physical barrier to protect probiotics during digestion, processing and storage. Microencapsulation becomes a profitable technology for the delivery and storage of probiotics (Mortazavian *et al*., 2007; Manojlovic *et al*., 2010). Microencapsulation using spray drying technique has low operating costs, it can be an excellent alternative for large scale production of powder microbial cultures with low humidity (Corcoran *et al*., 2004).

This study aimed to evaluate the effectiveness of administration of microencapsulated probiotic *Bacillus* sp. NP5, prebiotic mannan oligosaccharide and the combination of those materials on growth performances, immune responses and resistance to *A. hydrophila* infection on pangasius.

MATERIALS AND METHODS

Duration and time: This study was conducted for 3 months started from March-May 2015.

Microencapsulated probiotic and mannan oligosaccharide: Probiotic *Bacillus* NP5 used in this study were isolated from the digestive tract of tilapia (Putra and Widanarni, 2015). *Bacillus* NP5 have been given the rifampicin antibiotic resistant marker (*Bacillus* sp. NP5 Rf^R). Probiotic cells then were cultured in Trypticase Soy Broth (TSB) medium and incubated in a water bath shaker (140 rpm, 29°C) for 18 h. Fresh culture then harvested by centrifugation (6000-7000 rpm) for 20 min to get the probiotic biomass. Probiotic biomass homogenized with sterile 10% maltodextrin solution as the coating material and then dried in a mini BUCHI spray dryer at inlet temperature of 120°C and outlet temperature of 70°C (Utami *et al*., 2015). Probiotic viability after drying was observed to determine probiotic count in the product. Viability test was done by using spread plate technique using Trypticase Soy Agar (TSA) supplemented with 50 μg mL^{-1} rifampicin (TSA+Rif). Fresh culture of probiotic *Bacillus* sp. NP5 Rf^R at a density of 10^{10} CFU g^{-1} resulted viability as much as 10^8 CFU g^{-1} after microencapsulation process. Mannan oligosaccharide (Alltech) used in this study is derived from the cell wall of *Saccharomyces cerevisiae* (Sang *et al*., 2011).

Feed preparation: This study consisted of 5 treatments with 5 replicates, feed without any supplementation included negative control (K-), positive control (K+) and feed with the addition of 1% microencapsulated probiotic (Pro), prebiotic MOS 0.2% (Pre), microencapsulated probiotic 1% and prebiotic MOS 0.2% (Syn). Microencapsulated probiotic and prebiotic MOS was added to the feed and mixed with 2% egg white as a binder.

Rearing condition: Pangasius used in this study were pangasius with a body weight of 6.54±0.17 g which have acclimatized for 2 weeks and then reared at 50×30×25 cm^3 sized aquariums with a density of 10 fish per aquarium. The fish were fed 3 times a day by at satiation for 30 days.

Water quality was maintained by siphoning rearing media every 3 days and water replacement as much as 65% of total volume of rearing media. Water quality was also maintained stable at normal conditions for freshwater fish according to Boyd (1990), dissolved oxygen>5 mg L^{-1}, temperature at 24-30°C, pH at 6.5-9.5 and Total Ammonia Nitrogen (TAN) <0.52 ppm.

Challenge test: On day 2 after 30 days of feeding trial with probiotic, prebiotic and synbiotic, 10 experimental fish from each treatment except negative control were challenged by *A. hydrophila* by injecting bacteria suspension with a density of 10^7 CFU mL^{-1} as much as 0.1 mL per individual using a sterile syringe via intramuscular (IM) route and then they were observed for 9 days. This *A. hydrophila* is a collection of Freshwater Culture Center (FCC), Sukabumi, Indonesia. *Aeromonas hydrophila* was given chloramphenicol antibiotic resistant marker before being used for the LD_{50} assays or challenge test.

Growth performances: After 30 days of feeding trial, Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) was calculated using the following equation:

$$SGR = 100 \times \frac{\ln Wt - \ln Wo}{t}$$

$$FCR = \frac{F}{B_t - B_0}$$

where, SGR is specific growth rate (%), Wt is average weight of fish at the end of feeding trial, Wo is average initial weight of fish and t is feeding trial duration. FCR is feed conversion ratio, F is the amount of feed consumed, B_t is fish biomass at the end of feeding trial and B_0 is initial fish biomass.

Total bacteria and *Bacillus* sp. NP5 count in the pangasius intestines: Pangasius intestine (0.1 g) was taken from each aquarium then homogenized in 0.9 mL sterile Phosphate Buffer Saline (PBS), continued with plating on Trypticase Soy Agar (TSA) medium for Total Bacteria (TB) and TSA+Rif for total *Bacillus* sp. NP5 (TBNP5). The plates were incubated at a temperature of 27-29°C for 24 h, the number of bacterial colonies were counted and expressed in Colony Forming Unit (CFU g^{-1}).

Immune responses: Hematocrit level was measured by comparing blood cells volume with total blood volume in microhematocrit tube (Anderson and Siwicki, 1995). Hemoglobin level was measured using Sahlinometer according to Wedemeyer and Yasutake (1977). Total erythrocyte and leukocyte followed procedure by Blaxhall and Daisley (1973). Phagocytic activity was observed from blood slides according to Anderson and Siwicki (1995). Respiratory burst was observed using fresh blood following procedure by Liu and Chen (2004).

Total *Aeromonas hydrophila* in pangasius target organs: This enumeration was performed with spread plate count technique; the target organs such as liver and kidneys (0.1 g) were taken and homogenized with 0.9 mL sterile PBS. Serial dilutions were carried out and 0.05 mL suspension from each dilution tube was spread on TSA+chloramphenicol. Enumerations of total *A. hydrophila* in the target organs were performed on day 34, 37 and 41 of the challenge test.

Statistical analysis: The data obtained were tested their normality and homogeneity. SPSS (version 16) was used as a software for statistical analysis. One-way analysis of variance (One-way ANOVA) was used to determine whether there were significant differences between treatments, if there was a significant effect, the difference between treatments was tested by Duncan test at 95% confidence interval.

RESULTS

Growth performances: The survival rate after 30 days of feeding trial in controls, probiotic, prebiotic and synbiotic showed the same values (100±0.00%). In the specific growth rate, synbiotic treatment showed a value of 2.51±0.16% and it was significantly different ($p<0.05$, Table 1) from prebiotic, probiotic and controls. Prebiotic and probiotic also showed the higher values which were significantly different ($p<0.05$, Table 1) from controls. Feed conversion ratio of synbiotic showed the lowest value (1.34±0.13) which was significantly different ($p<0.05$, Table 1) from prebiotic, probiotic and controls. Prebiotic and probiotic treatment also showed significantly different results ($p<0.05$, Table 1) compared to controls. Administration of probiotic, prebiotic and synbiotic for 30 days of feeding trial could increase Specific Growth Rate (SGR) and decrease Feed Conversion Ratio (FCR) compared to controls.

Total bacteria and *Bacillus* sp. NP5 count in pangasius intestines: After 30 days of feeding trial, synbiotic showed TBC (9.27±0.13 log CFU g^{-1}) that was significantly different ($p<0.05$, Table 1) from prebiotic, probiotic and controls but prebiotic and probiotic treatment also showed better results and significantly different ($p<0.05$; Table 1) from controls. Total *Bacillus* sp. NP5 in pangasius intestines (TBNP5) of synbiotic treatment (7.26±0.04 log CFU g^{-1}) showed a higher value and significantly different ($p<0.05$, Table 1) from probiotic treatment (6.41±0.03 log CFU g^{-1}), while in the controls and prebiotic were not found *Bacillus* sp. NP5 Rf^R colony.

Immune responses: Administration of probiotic, prebiotic and synbiotic gave effects to hematological parameters, especially after the challenge test in which there were fluctuations in hematocrit (Hc), hemoglobin (Hb), Erythrocyte Count (EC), Leukocyte Count (LC), Phagocytic Activity (PA) and Respiratory Burst (RB) (Fig. 1). Changes in fish hematology describe fish health status.

Hematocrit (Table 2) of experimental fish treated with probiotic, prebiotic and synbiotic after feeding trial showed significantly different result ($p<0.05$) from controls. Post-challenge test hematocrit levels of synbiotic treatment showed a better value and significantly different ($p<0.05$) from positive control.

Table 1: SR, SGR, FCR, TBC and TBCNP5

	Treatments				
Parameters	K-	K+	Pro	Pre	Syn
SR (%)	100.00±0.00^a	100.00±0.00^a	100.00±0.00^a	100.00±0.00^a	100.00±0.00^a
SGR (%)	1.79±0.05^c	1.85±0.04^c	2.31±0.05^b	2.18±0.06^b	2.51±0.16^a
FCR	2.11±0.08^a	2.02±0.06^a	1.49±0.05^b	1.62±0.06^b	1.34±0.13^c
TBC (log CFU g^{-1})	7.35±0.12^c	7.14±0.19^c	8.52±0.09^b	8.17±0.15^b	9.27±0.13^a
TBCNP5 (log CFU g^{-1})	0^c	0^c	6.41±0.03^b	0^c	7.26±0.04^a

Different superscript letters on the same row indicate significantly different results (Duncan, $p<0.05$). The values shown were means and standard deviations, k-: Negative control, k+: Positive control, Pro: Probiotic, Pre: Prebiotic, Syn: Synbiotic, SR: Survival rate, SGR: Specific growth rate, FCR: Feed conversion ratio, TBC: Total bacterial count and TBCNP5: Total *Bacillus* sp. NP5 RfR count

Table 2: Hc, Hb, EC, LC, PA and RB

Parameters and day	Treatments				
	K-	K+	Pro	Pre	Syn
Hematocrit (%)					
30	25.60±0.62^{d}	26.10±0.50^{d}	29.80±0.62^{c}	31.70±0.55^{b}	33.80±0.71^{a}
34	28.20±0.45^{a}	21.80±0.57^{d}	24.30±0.46^{c}	25.00±0.25^{bc}	25.90±0.76^{b}
37	28.60±1.01^{a}	18.70±1.73^{b}	20.90±2.18^{b}	21.00±0.62^{b}	21.30±0.90^{b}
41	29.10±0.71^{a}	19.50±1.11^{d}	21.00±0.55^{c}	22.30±0.15^{bc}	22.70±0.90^{b}
Hemoglobin (gr %)					
30	6.40±0.10^{c}	6.60±0.12^{c}	7.70±0.21^{b}	8.10±0.30^{ab}	8.40±0.35^{a}
34	6.90±0.35^{a}	5.70±0.70^{b}	6.70±0.42^{a}	7.00±0.10^{a}	7.40±0.35^{a}
37	7.00±0.25^{a}	4.30±0.14^{d}	5.20±0.15^{c}	5.80±0.31^{b}	6.10±0.10^{b}
41	7.80±0.46^{a}	4.80±0.25^{d}	5.90±0.15^{c}	6.30±0.21^{bc}	6.80±0.30^{b}
Erythrocyte count (×10^{6} cells mm^{-3})					
30	1.90±0.06^{c}	1.80±0.08^{c}	2.00±0.09^{b}	2.10±0.03^{ab}	2.30±0.12^{a}
34	2.00±0.20^{a}	1.40±0.05^{c}	1.60±0.07^{bc}	1.70±0.14^{b}	1.80±0.09^{b}
37	2.10±0.04^{a}	1.10±0.06^{c}	1.30±0.06^{b}	1.40±0.13^{b}	1.40±0.08^{b}
41	2.20±0.04^{a}	1.20±0.07^{c}	1.50±0.04^{b}	1.50±0.03^{b}	1.60±0.03^{b}
Leukocyte count (×10^{6} cells mm^{-3})					
30	0.84±0.08^{a}	0.87±0.07^{a}	0.87±0.21^{a}	0.91±0.07^{a}	0.88±0.05^{a}
34	0.86±0.04^{d}	1.00±0.09^{c}	1.21±0.03^{b}	1.29±0.02^{ab}	1.34±0.07^{a}
37	0.77±0.06^{c}	1.07±0.06^{b}	1.30±0.07^{a}	1.34±0.11^{a}	1.41±0.19^{a}
41	0.93±0.10^{c}	0.88±0.08^{c}	1.07±0.05^{b}	1.12±0.07^{ab}	1.23±0.03^{a}
Phagocytic activity (%)					
30	30.30±2.08^{a}	30.60±1.53^{a}	30.60±3.21^{a}	31.60±2.52^{a}	34.30±2.08^{a}
34	34.30±3.06^{c}	40.30±1.53^{b}	43.60±3.06^{b}	44.60±4.51^{b}	51.60±3.06^{a}
37	35.60±3.51^{c}	43.30±4.04^{b}	49.60±3.51^{a}	52.00±2.65^{a}	55.30±3.21^{a}
41	38.60±4.04^{c}	40.30±1.53^{c}	46.00±2.65^{b}	48.60±2.08^{ab}	52.30±2.52^{a}
Respiratory burst (O.D 630 nm)					
30	0.18±0.01^{a}	0.18±0.03^{a}	0.20±0.01^{a}	0.20±0.001^{a}	0.21±0.001^{a}
34	0.19±0.005^{c}	0.18±0.01^{c}	0.21±0.005^{bc}	0.23±0.02^{ab}	0.26±0.03^{a}
37	0.17±0.03^{c}	0.18±0.005^{c}	0.22±0.01^{b}	0.25±0.02^{ab}	0.27±0.02^{a}
41	0.17±0.01^{cd}	0.18±0.02^{d}	0.20±0.02^{bc}	0.22±0.01^{b}	0.24±0.01^{a}

Different superscript letters on the same row indicate significantly different results (Duncan, $p<0.05$). The values shown were means and standard deviations, k-: Negative control, k+: Positive control, Pro: Probiotic, Pre: Prebiotic, Syn: Synbiotic, Hc: Hematocrit level, Hb: Hemoglobin level, EC: Erythrocyte count, LC: Leukocyte count, PA: Phagocytic activity, RB: Respiratory burst

Hemoglobin level of synbiotic treatment (Table 2) also showed the highest value followed by prebiotic and probiotic that were significantly different ($p<0.05$) from controls. At post-challenge test observation, hemoglobin levels of synbiotic treatment showed the high hemoglobin values but not significantly different ($p>0.05$) from prebiotic and probiotic treatment, but they were significantly different ($p<0.05$) from positive control.

The same condition also happened to erythrocyte count, synbiotic treatment showed the highest EC value (Table 2) that was significantly different ($p<0.05$) from probiotic and controls, but it was not significantly different ($p>0.05$) from prebiotic treatment. The EC decreased after the challenge test on day 34 and 37 and then increased on day 41. The highest EC value of synbiotic treatment was significantly different ($p<0.05$) from positive control.

Leukocyte count, phagocytic activity and respiratory burst activity in all treatments during the feeding trial did not showed a significantly different effect ($p>0.05$) to controls (Table 2). The differences of LC occurred after the challenge test; there was an increase in all treatments except K-treatment and began to decline on day 41. The peak of LC occurred on day 37, in which LC of synbiotic, prebiotic and probiotic were significantly different ($p<0.05$) to positive control.

Phagocytic activity value at the end of post-challenge test observation in which synbiotic, prebiotic and probiotic treatment showed higher values and significantly different ($p>0.05$) from positive control.

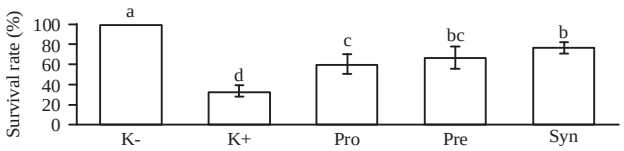

Fig. 1: Survival rate of pangasius after the challenge test. Different superscript letters on each bar indicate significantly different results (Duncan; $p<0.05$). Negative (K-), positive (K+) control, microencapsulated probiotic 1% (pro), mannan oligosaccharide 0.2% (pre) and microencapsulated probiotic 1%+mannan oligosaccharide 0.2% (Syn)

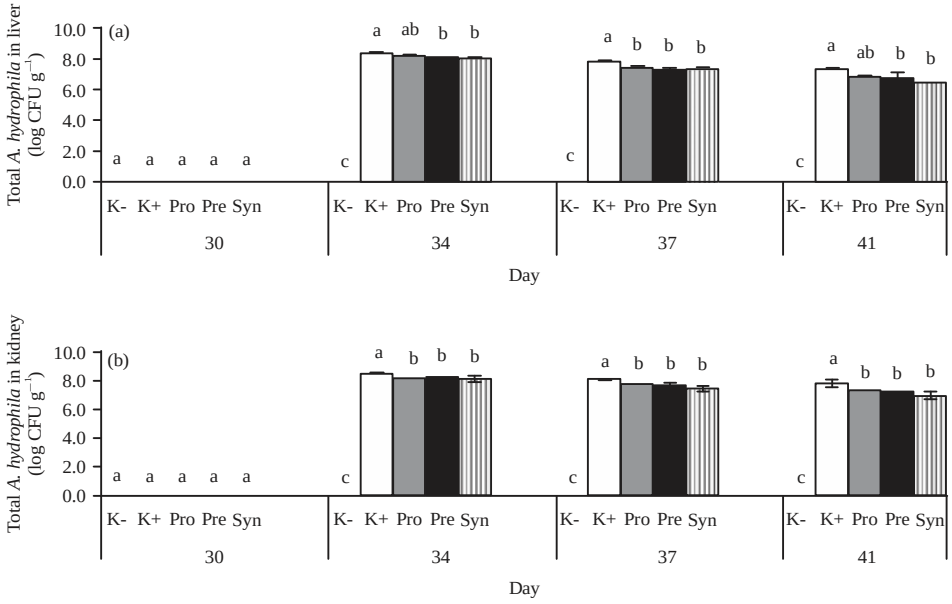

Fig. 2(a-b): Total *Aeromonas hydrophila* in the (a) Liver and (b) Kidneys of pangasius during the challenge test. Different superscript letters on each bar on the same day indicate significantly different results (Duncan; $p<0.05$). Negative (K-), positive (K+) control, microencapsulated probiotic 1% (pro), mannan oligosaccharide 0.2% (pre) and microencapsulated probiotic 1%+mannan oligosaccharide 0.2% (Syn)

Differences in respiratory burst activity during the end of the challenge test also showed that probiotic, prebiotic and synbiotic resulted higher value and significantly different ($p<0.05$) from positive control.

Total *Aeromonas hydrophila* in pangasius target organs: Observations of total *A. hydrophila* in pangasius target organs (liver and kidneys) after the challenge test (Fig. 2a-b) showed that there was no significantly differences between probiotic, prebiotic and synbiotic ($p>0.05$) but significantly different ($p<0.05$) from positive control.

DISCUSSION

The high value of specific growth rate and the low value of feed conversion ratio in synbiotic treatment were influenced by administration of combination between microencapsulated probiotic *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide. Putra *et al*. (2015) stated that

administration of amylolytic bacteria probiotic *Bacillus* sp. NP5 derived from the digestive tract of tilapia could increase digestive enzymes activity, improve nutrients digestibility and growth performance of tilapia. Dimitroglou *et al*. (2009) stated that the use of MOS on the fish is able to increase the length and density of intestinal microvilli of rainbow trout (*Onchorhynchus mykiss*), so intestinal absorption surface area will be wider, then the ability to absorb nutrients will increase and it also modulates intestinal microbes. Results of study by Daniels *et al*. (2010) showed that the feed which was given combination of *Bacillus* spp. and MOS increased specific growth rate, feed conversion ratio and survival rate. Supplementation of *Bacillus* or MOS singly also improved growth parameters, survival and post-larvae conditions significantly compared to the control group with lower values than the use of combination of those materials in European lobster larvae (*Hommarus gammarus* L.).

Good growth performance of pangasius was suspected to be influenced by the presence of microbes in the intestine as showed in the increase in total intestinal bacteria and total *Bacillus* sp. NP5 Rf^R count. Optimal improvement in synbiotic treatment was thought to be caused by the influence of MOS which was used well by intestinal microbes like *Bacillus* sp. NP5. Results of the study by Daniels *et al*. (2010) suggested a combination of the use of *Bacillus* and MOS as cost effective in increasing survival and providing additional benefit in improving growth performances compared to single administration.

Blaxhall and Daisley (1973) stated that hematological parameters such as hematocrit, hemoglobin and erythrocyte count are parameters that describe fish health status. According to Kumar *et al*. (2013) red blood cells count is the largest count and it varies ranged from 1.05-3.0×10^6 cells mm^{-3}. The high count of erythrocyte in synbiotic treatment was caused by the role of probiotic *Bacillus* sp. NP5 and MOS. In the study conducted by Andrews *et al*. (2009), there was a significant increase in red blood cells and hemoglobin of rohu (*Labeo rohita*) in group with feed given MOS supplementation compared to control diet group. The same study results demonstrated by Rodriguez-Estrada *et al*. (2013), administration of *Enterococcus faecalis* and MOS in single use or combination showed a high hematocrit value than control.

The decline occurred in Hc, Hb and EC after the challenge test with *A. hydrophila* indicated that the presence of *A. hydrophila* which produced exotoxin or endotoxin causing the red blood cells becoming lysis (Hardi *et al*., 2014). In the study by Harikrishanan *et al*. (2010), red blood cells significantly decreased after the challenge test with *A. hydrophila*. The decrease of EC, Hb and Hc in infected-fish showed that red blood cells was destroyed and it caused anemia. Hillman *et al*. (2005) stated that hemoglobin plays a role in fish resistance because it works to bind oxygen in the blood, hemoglobin level is related to erythrocyte count.

Observation of leukocyte count, phagocytic activity and respiratory burst activity in all treatments during feeding trial that were not significantly different between the treatments signified that the experimental fish was not under stress condition. Fluctuations in leukocyte count after the challenge test showed that the fish defended itself from *A. hydrophila* infection. Leukocyte according to Uribe *et al*. (2011) is one part of non-specific immune system. Leukocytes which produced in high number will occur when there is an infection in the body related to immune system working against the infection. On post-challenge test period, there was the effort of the fish to defend itself from infection by phagocytic process. Phagocytosis is the first defense of the cellular responses made by monocytes (macrophages) and granulocytes (neutrophils). Supplementation of combination of *Enterococcus faecalis* and MOS showed the highest phagocytic activity compared to control (Rodriguez-Estrada *et al*., 2013).

Respiratory burst is the basic form of antibacterial system in the fish body. Respiratory burst value obtained indicated that feeding treatment with synbiotic resulted the highest score. Increase in respiratory burst value could be associated with increase in phagocytic cells activity (Rawling *et al*., 2012). Rieger and Barreda (2011) stated that respiratory burst will increase oxygen consumption resulting in formation of superoxide anion and the process is accelerated by NADPH-oxidase and multi-component enzyme contained inside the plasma membrane after activation of phagocytic cells.

The high total *A. hydrophila* in the target organs after injection in all treatment related to the experimental fish mortality pattern. Based on this study results, the deaths began to occur in 24-48 h after injection. This was in line with the statement by Janda and Abbott (2010) which *A. hydrophila* incubation period is 1-2 days after bacterial exposure. This disease commonly goes acutely. Clinical conditions associated with systemic infection mortality result within 24-48 h, the mortality occurred is between 10 and 70% among farmed fish. This bacteria can lead to increase respiratory rate, loss of balance and bleeding in the abdominal body part.

The results obtained on the use of probiotic, prebiotic and synbiotic on *in vivo* assays could improve pangasius survival rate infected by *A. hydrophila*. This result showed the difference between probiotic, prebiotic and synbiotic compared to positive control. Based on results of the study by Widanarni and Tanbiyaskur (2015), probiotic *Bacillus* sp. NP5 and prebiotic oligosaccharide had a better survival rate. Results of the study by Zhang *et al*. (2014a), the feed supplemented with *Bacillus subtilis* and FOS with different doses in single supplementation showed cumulative mortality percentage between 40.74-62.96% on ovate pompano juveniles (*Trachinotus ovatus*) which were challenged by *Vibrio vulnificus*.

CONCLUSION

Results of this study showed that microencapsulated probiotic *Bacillus* sp. NP5 and prebiotic mannan oligosaccharide could significantly improve growth performances and immune responses of pangasius.

REFERENCES

Anderson, D.P. and A.K. Siwicki, 1995. Basic hematology and serology for fish health programs. Proceedings of the 2nd Symposium on Diseases in Asian Aquaculture, October 25-29, 1993, Phuket, Thailand, pp: 17.

Andrews, S.R., N.P. Sahu, A.K. Pal and S. Kumar, 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.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.

Boyd, C.E., 1990. Water Quality in Ponds for Aquaculture. 2nd Edn., Alabama Agricultural Experiment Station, Auburn University, Auburn, AL., USA., Pages: 482.

Cerezuela, R., J. Meseguer and M.A. Esteban, 2011. Current knowledge in synbiotic use for fish aquaculture: A review. J. Aquacult. Res. Dev. 10.4172/2155-9546.S1-008

Corcoran, B.M., R.P. Ross, G.F. Fitzgerald and C. Stanton, 2004. Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. J. Applied Microbiol., 96: 1024-1039.

Daniels, C.L., D.L. Merrifield, D.P. Boothroyd, S.J. Davies, J.R. Factor and K.E. Arnold, 2010. 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.

Dimitroglou, A., D.L. Merrifield, R. Moate, S.J. Davies, P. Spring, J. Sweetman and G. Bradley, 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Anim. Sci., 87: 3226-3234.

Hardi, E.H., C.A. Pebrianto, T. Hidayanti and R.T. Handayani, 2014. [Phatogenicity of *Aeromonas hydrophila* via some port entry in cultured nila tilapia (*Oreochromis niloticus*) from Loa Kulu Kutai Kartanegara East Kalimantan]. Jurnal Kedokteran Hewan, 8: 130-133, (In Indonesian).

Harikrishanan, R., C. Balasundaram and M.S. Heo, 2010. Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. Fish Shellfish Immunol., 28: 354-361.

Hedayati, A. and R. Tarkhani, 2014. Hematological and gill histopathological changes in iridescent shark, *Pangasius hypophthalmus* (Sauvage, 1878) exposed to sublethal diazinon and deltamethrin concentrations. Fish Physiol. Biochem., 40: 715-720.

Hillman, R.S., K.A. Ault and H. Rinder, 2005. Hematology in Clinical Practice. 4th Edn., McGraw-Hill Profesional, New York, USA., ISBN-13: 9780071440356, Pages: 471.

Janda, J.M. and S.L. Abbott, 2010. The genus *Aeromonas*: Taxonomy, pathogenicity and infection. Clin. Microbiol. Rev., 23: 35-73.

Kumar, S., R.P. Raman, P.K. Pandey, S. Mohanty, A. Kumar and K. Kumar, 2013. Effect of orally administered azadirachtin on non-specific immune parameters of goldfish *Carassius auratus* (Linn. 1758) and resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol., 34: 564-573.

Liu, C.H. and J.C. Chen, 2004. Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. Fish Shellfish Immunol., 16: 321-334.

Manojlovic, V., V.A. Nedovic, K. Kailasapathy and N.J. Zuidam, 2010. Encapsulation of Probiotics for Use in Food Products. In: Encapsulation Technologies for Active Food Ingredients and Food Processing, Zuidam, N.J. and V. Nedovic (Eds.). Springer, Berlin, Germany, ISBN-13: 978-1-4419-1007-3, pp: 269-302.

Mortazavian, A., S.H. Razavi, M.R. Ehsani and S. Sohrabvandi, 2007. Principles and methods of microencapsulation of probiotic microorganisms. Iran. J. Biotechnol., 5: 1-18.

Nayak, S.K., 2010. Probiotics and immunity: A fish perspective. Fish Shellfish Immunol., 29: 2-14.

Phan, L.T., T.M. Bui, T.T.T. Nguyen, G.J. Gooley and B.A. Ingram *et al*., 2009. Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong delta, Vietnam. Aquaculture, 296: 227-236.

Putra, A.N. and Widanarni, 2015. Screening of amylolytic bacteria as candidates of probiotics in tilapia (*Oreochromis* sp.). Res. J. Mycrobiol., 10: 1-13.

Putra, A.N., N.B.P. Utomo and Widanarni, 2015. Growth performance of tilapia (*Oreochromis niloticus*) fed with probiotic, prebiotic and synbiotic in diet. Pak. J. Nutr., 14: 263-268.

Rawling, M.D., D.L. Merrifield, D.L. Snellgrove, H. Kuhlwein, A. Adams and S.J. Davies, 2012. Haemato-immunological and growth response of mirror carp (*Cyprinus carpio*) fed a tropical earthworm meal in experimental diets. Fish Shellfish Immunol., 32: 1002-1007.

Rieger, A.M. and D.R. Barreda, 2011. Antimicrobial mechanisms of fish leukocytes. Dev. Comp. Immunol., 35: 1238-1245.

Rodriguez-Estrada, U., S. Satoh, Y. Haga, H. Fushimi and J. Sweetman, 2013. Effects of inactivated *Enterococcus faecalis* and mannan oligosaccharide and their combination on growth, immunity and disease protection in rainbow trout. North Am. J. Aquacult., 75: 416-428.

Sang, H.M., R. Fotedar and K. Filer, 2011. Effects of dietary mannan oligosaccharide on the survival, growth, immunity and digestive enzyme activity of freshwater crayfish, *Cherax destructor* Clark (1936). Aquacult. Nutr., 17: e629-e635.

Talpur, A.D., M.B. Munir, A. Mary and R. Hashim, 2014. Dietary probiotics and prebiotics improved food acceptability, growth performance, haematology and immunological parameters and disease resistance against *Aeromonas hydrophila* in snakehead (*Channa striata*) fingerlings. Aquaculture, 426-427: 14-20.

Uribe, C., H. Folch, R. Enriquez and G. Moran, 2011. Innate and adaptive immunity in teleost fish: A review. Vet. Med., 56: 486-503.

Utami, D.A.S., Widanarni and M.A. Suprayudi, 2015. Quality of dried *Bacillus* NP5 and its effect on growth performance of tilapia (*Oreochromis niloticus*). Pak. J. Biol. Sci., 18: 88-93.

Wedemeyer, G. and W.T. Yasutake, 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. Technical Paper, Vol. 89, U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC., USA.

Weinbreck, F., I. Bodnar and M.L. Marco, 2010. Can encapsulation lengthen the shelf-life of probiotic bacteria in dry products? Int. J. Food Microbiol., 136: 364-367.

Widanarni and Tanbiyaskur, 2015. Application of probiotic, prebiotic and synbiotic for the control of streptococcosis in tilapia *Oreochromis niloticus*. Pak. J. Biol. Sci., 18: 59-66.

Zhang, J., Y. Liu, L. Tian, H. Yang, G. Liang and D. Xu, 2012. 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.

Zhang, Q., H. Yu, T. Tong, W. Tong, L. Dong, M. Xu and Z. Wang, 2014a. Dietary supplementation of *Bacillus subtilis* and fructooligosaccharide enhance the growth, non-specific immunity of juvenile ovate pompano, *Trachinotus ovatus* and its disease resistance against *Vibrio vulnificus*. Fish Shellfish Immunol., 38: 7-14.

Zhang, X., W. Yang, H. Wu, X. Gong and A. Li, 2014b. Multilocus sequence typing revealed a clonal lineage of *Aeromonas hydrophila* caused motile *Aeromonas septicemia* outbreaks in pond-cultured cyprinid fish in an epidemic area in central China. Aquaculture, 432: 1-6.