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# Application of Micro-Encapsulated Probiotic *Bacillus* NP5 and Prebiotic Mannan Oligosaccharide (MOS) to Prevent Streptococcosis on Tilapia *Oreochromis niloticus*

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## ABSTRACT

*Streptococcus agalactiae* is one of the fish pathogen which can cause mortality up to 90% in commercial tilapia farms. This study aimed to evaluate the effectiveness of supplementation of micro-encapsulated probiotic (*Bacillus* NP5), prebiotic (MOS) and combination of those materials (synbiotic) through the feed on growth performances and immune responses of tilapia infected with *S. agalactiae*. The probiotic cells were encapsulated by spray drying method. The experimental fish were reared for 40 days and fed with feed-supplemented with probiotic, prebiotic and synbiotic and without any supplementation to the feed (positive and negative control). On day 40, all fish except negative control were challenged by *S. agalactiae* via intraperitoneal route injection in amount of 0.1 mL ($10^6$ CFU $mL^{-1}$). This study showed that administration of 0.4% prebiotic to the feed resulted the best growth performance in the end of feeding trial and survival rate after the challenge test with *S. agalactiae*.

**Key words:** Micro-encapsulated probiotic, prebiotic, synbiotic, tilapia, *S. agalactiae*

## INTRODUCTION

Tilapia is one of commercially important fish species cultured around the world with 72% population produced in Asia (Li *et al*., 2014). Disease outbreak is one of the obstacles in tilapia (*Oreochromis niloticus*) cultivation. The disease that often leads to the high mortality in tilapia cultivation is streptococcosis, which is caused by *Streptococcus agalactiae*. Taukhid and Purwaningsih (2011) reported that in Indonesia, streptococcosis ever caused outbreaks and tilapia mortality. Streptococcosis-suspected-tilapia generally showed clinical symptoms such as red spots, exophthalmia, opacity, purulent, melanosis, whirling, "C" shape and internal organs hemorrhage. Mass mortality of tilapia due to *S. agalactiae* infection can occur on 10 days (Li *et al*., 2014).

Disease prevention in fish can be performed by administration of probiotics, prebiotics and synbiotics. Probiotic is additional microbe which gives beneficial effects to the host health (Nayak, 2010). Prebiotic is material which cannot be digested by the host, but it gives beneficial effects by improving the growth and activity of beneficial bacteria in the digestive tract (Reid *et al*., 2003). While, synbiotic is supplement which is made by combining probiotic and prebiotic (Cerezuela *et al*., 2011). Several studies on the supplementation of probiotics and prebiotics in tilapia has been done. The study by Aly *et al*. (2008) showed that administration of *Bacillus subtilis*

could enhance immune response and survival of tilapias which were attacked by *Streptococcus iniae*. While, Samrongpan *et al*. (2008), stated that the administration of prebiotic (MOS) in tilapia larvae improved growth and survival of tilapia which were injected by *S. agalactiae*.

The obstacle of fresh culture probiotic application is viability of probiotic which decrease after storage, so the probiotic preparation should be done every day. In addition, the ability of probiotic to survive and work in the digestive tract of tilapia greatly affects the effectiveness of the probiotic. Therefore, micro-encapsulation is needed to be carried out on the probiotics preparation. Micro-encapsulation method is a technology for coating or lining of core material with a polymer wall layer which makes it becoming micro-particles. Micro-encapsulation method is useful in stabilizing the core material, improving shelf life and protecting bacterial components against environmental conditions in the fish digestive tract (Anal and Singh, 2007).

Probiotic used in this study was *Bacillus* NP5 isolated by Putra and Widanarni (2015). In the previous study, supplementation of *Bacillus* NP5 (fresh culture) and prebiotic from sweet potato extract could increase the growth and survival of tilapias which were challenged by *S. agalactiae* (Tanbiyaskur *et al*., 2015). Utami *et al*. (2015) reported that supplementation of dried *Bacillus* NP5 $Rf^R$ to the feed could improve the growth performance of tilapia. This study aimed to evaluate the effectiveness of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and a combination of those materials (synbiotic) in improving growth, immune responses and resistance of tilapia against *S. agalactiae* infection.

## MATERIALS AND METHODS

**Preparation of probiotic, prebiotic and synbiotic:** Probiotic used in this study was *Bacillus* NP5 isolated by Putra and Widanarni (2015) which has been marked as resistant to antibiotic rifampicin at a dose of 50 μg $mL^{-1}$ (*Bacillus* NP5 $Rf^R$). Probiotic biomass production was done by subculture technique in TSB (Trypticase Soy Broth) medium. *Bacillus* NP5 $Rf^R$ cells were cultured in 50 mL of TSB medium for 18 h using thermo-shaker at a speed of 200 rpm and at a temperature of 29°C, then it was re-cultured in 500 mL of TSB medium in the same culture condition. The bacterial suspension was harvested by centrifugation (6000-7000 rpm) for 20 min to obtain probiotic biomass, then it was homogenized by stirrer in 500 mL of sterile distilled water containing 10% maltodextrin as a coating material. Spray drying process was carried out using a spray drying machine (Buchi mini) to produce micro-encapsulated probiotic. Inlet temperature used was 100-110°C and outlet temperature was 55-58°C. 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 micro-encapsulation process. The micro-encapsulated probiotic was stored in a refrigerator (-20°C) before using. Prebiotic used in this study was commercial mannan oligosaccharide BIO-MOS® (Altech company), which is derived from the cell wall of *Saccharomyces cerevisiae*. While, synbiotic used in this study was a mixture of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$ and prebiotic MOS.

**Rearing condition and experimental design:** Tilapia (*Oreochromis niloticus*) used in this study was tilapia strain nirwana with an average weight of 5.56±0.16 g derived from Fish Seed Center Cibitung, Bogor, Indonesia. The rearing of fish was conducted in the aquariums sizing 50×30×25 $cm^3$ with a density of 10 fish per aquarium. Before the treatment, tilapias were acclimatized for a week. Experimental design used in this study was a Completely Randomized Design (CRD) with five treatments and five replications. Feed used in this study (basal diet) was

a commercial feed (Hi-provite) with a protein content of 30%. Test feed used in this study was commercial feed with the addition of micro-encapsulated probiotic at a dose of 0.5% (A), prebiotic MOS at dose of 0.4% (B) and the addition of synbiotic (micro-encapsulated probiotic at a dose of 0.5 and 0.4% prebiotic MOS) (C). Feed without the addition of micro-encapsulated probiotic, prebiotic MOS and synbiotic (basal diet) was used for negative control (KN) and positive control (KP). Test feed were prepared by mixing micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and synbiotic with egg white as much as 2% as a binder, then the test feed were aired-dried and stored in a refrigerator at a temperature of 4°C before using. The feeding trial was conducted for 40 days. Tilapias were fed with the test feed three times a day (08.00, 12.00 and 16.00 Western Indonesia Time) by at satiation. On day 40, the fish were challenged by *S. agalactiae* via intraperitoneal route injection at a dose of 100 μL ($10^6$ CFU $mL^{-1}$) except for negative control. Observation of survival rate after the challenge test was carried out for 10 days. Water quality was maintained during the rearing period (temperature at 29°C, dissolved oxygen at 6.3-6.8 mg $L^{-1}$, TAN at 0.04-0.1 mg $L^{-1}$ and pH at 7.18-7.25).

**Observation of growth performance parameters:** Growth performance parameters observed were Survival Rate (SR), Specific Growth Rate (SGR) (Huisman, 1987) and Feed Conversion Ratio (FCR) (Takeuchi, 1988) using the formula as follow:

$$SR\,(\%) = \frac{Nt}{N0} \times 100$$

Where:
Nt : The number of fish in the end of study
N0 : The initial number of fish

$$SGR\,(\%) = \frac{\ln WE - \ln WS}{d} \times 100$$

Where:
WE : The final body weight of fish
WS : The initial body weight of fish
d : Rearing duration

$$FCR = \frac{\text{Fish weight gain}}{\text{Feed intake}}$$

**Enumeration of total intestinal bacteria and total *Bacillus* NP5 $Rf^R$ in the intestine:** Enumeration of total intestinal bacteria and total *Bacillus* NP5 $Rf^R$ used spread plate method. Fish intestine with a weight of 0.1 g was homogenized in 0.9 mL of sterile PBS (phosphate buffer saline) for serial dilution. Then, 50 μL from each dilution tube was spread on TSA (trypticase soy agar) medium for enumeration of total intestinal bacteria and TSA+rifampicin medium for enumeration of total *Bacillus* NP5 $Rf^R$ in the intestine. These enumerations were performed at the beginning and the end of feeding trial (day 0 and day 40).

**Observation of immune response parameters:** Immune response parameters observed in this study included Erythrocyte Count (EC) (Blaxhall and Daisley, 1973), hematocrit level (Ht) (Anderson and Siwicki, 1995), hemoglobin level (Hb) (Wedemeyer and Yasutake, 1977), leukocyte count (LC) (Blaxhall and Daisley, 1973), Phagocytic Activity (PA) (Anderson and Siwicki, 1995) and Respiratory Burst activity (RB) (Liu and Chen, 2004).

**Enumeration of total *Streptococcus agalactiae* in the target organs:** Enumeration of total *S. agalactiae* in the target organs used spread plate method. Target organs (brain, eyes, kidneys and liver) with a weight of 0.1 g were homogenized in 0.9 mL of sterile PBS for serial dilution. Then, 50 μL from each dilution tube was spread on BHIA (Brain Heart Infusion Agar) medium. These enumerations were performed on day 0, 45 and 50.

**Statistical analysis:** All data were analyzed with one-way ANOVA using SPSS (version 16). Then, the data were tested with Duncan Multiple Range Test (DMRT), if there were significant differences between the treatments.

## RESULTS

**Growth performances:** The survival rate of tilapia after administration of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$ (A), prebiotic MOS (B) and synbiotic (C) for 40 days did not show significant differences between treatments ($p>0.05$) with a range value of 96.67-100%. Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of A, B and C treatment did not show significant differences ($p>0.05$) between treatments, but significantly different with controls (KN and KP) ($p<0.05$). The highest specific growth rate was 2.79±0.14% and the lowest feed conversion ratio was 1.26±0.07 found in B treatment (Table 1).

**Total intestinal bacteria and total probiotic *Bacillus* NP5 $Rf^R$:** On day 40, there were the increases in total intestinal bacteria and total probiotic *Bacillus* NP5 $Rf^R$ in tilapia intestines (Table 2). Total intestinal bacteria after feeding trial did not show significant differences among

Table 1: Survival rate, specific growth rate and feed conversion ratio of tilapia in the end of feeding trial

| | Treatments | | | | |
|---|---|---|---|---|---|
| Parameters | KN | KP | A | B | C |
| SR (%) | 96.67±5.77$^a$ | 96.67±5.77$^a$ | 100±0.00$^a$ | 100±0.00$^a$ | 100±0.00$^a$ |
| SGR (%) | 2.54±0.05$^a$ | 2.51±0.03$^a$ | 2.69±0.05$^b$ | 2.79±0.14$^b$ | 2.77±0.09$^b$ |
| FCR | 1.52±0.06$^a$ | 1.55±0.09$^a$ | 1.33±0.05$^b$ | 1.26±0.07$^b$ | 1.31±0.08$^b$ |

SR: Survival rate, SGR: Specific growth rate, FCR: feed conversion ratio, Different superscript letters in the same row indicate significantly different results ($p<0.05$)

Table 2: Total bacterial count and total probiotic count in the tilapia intestine during feeding trial

| Parameters and treatments | Before feeding trial (log CFU $g^{-1}$) | After feeding trial (log CFU $g^{-1}$) |
|---|---|---|
| **TBC** | | |
| A | 7.68±0$^a$ | 9.52±013$^b$ |
| B | 7.68±0$^a$ | 9.63±0.07$^b$ |
| C | 7.68±0$^a$ | 9.55±0.14$^b$ |
| KP | 7.68±0$^a$ | 8.71±0.18$^a$ |
| KN | 7.68±0$^a$ | 8.75±0.22$^a$ |
| **TPC** | | |
| A | 0.00±0$^a$ | 7.90±0.01$^a$ |
| B | 0.00±0$^a$ | 0.00±0$^b$ |
| C | 0.00±0$^a$ | 7.97±0.03$^a$ |
| KP | 0.00±0$^a$ | 0.00±0$^b$ |
| KN | 0.00±0$^a$ | 0.00±0$^b$ |

TBC: Total bacterial count, TPC: Total probiotic count, A: Administration of 0.5% micro-encapsulated probiotic, B: 0.4% prebiotic, C: Synbiotic , KN: Negative control, KP: Positive control, different superscript letters in the same parameter and period indicate significantly different results ($p<0.05$)

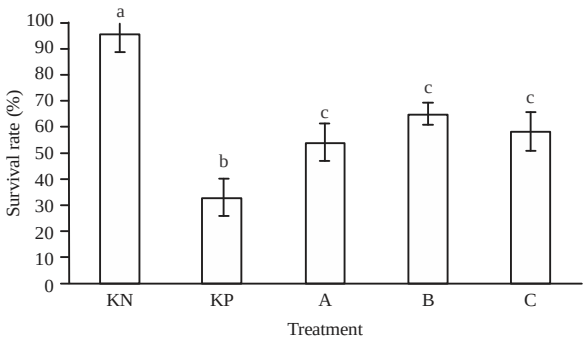


Fig. 1: Survival rate of tilapia after the challenge test with *S. agalactiae*. The different superscript letters on bars indicate significantly different results (Duncan; $p<0.05$), negative (KN), positive (KP) control, administration of 0.5% micro-encapsulated probiotic (A), 0.4% prebiotic (B) and synbiotic (C)

A, B and C treatment ($p>0.05$), but significantly different with controls (KN and KP) ($p<0.05$). The highest total intestinal bacteria was found in B treatment with a value of 9.67±0.03 log CFU $g^{-1}$. Total *Bacillus* NP5 $Rf^R$ were detected in tilapia intestines fed with feed-supplemented with micro-encapsulated probiotic (A) and synbiotic (C). While, in controls (KN and KP) and prebiotic MOS (B) treatment, there was no colony of *Bacillus* NP5 $Rf^R$. This showed that in A and C treatment, *Bacillus* NP5 $Rf^R$ were able to reach the target and to survive in tilapia intestine.

**Survival rate after the challenge test:** The survival rate of tilapia after the challenge test with *S. agalactiae* (Fig. 1) showed no significant differences between treatments, administration of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$ (A), prebiotic MOS (B) and synbiotic (C) ($p>0.05$), but showed significant differences with negative control (KN) and positive control (KP) ($p<0.05$). The highest survival rate was found in B treatment (65±4.3%) while the lowest survival rate was found in positive control (KP) which was 33.16±7.07%.

**Immune response parameters:** Health status and immune response of fish can be evaluated through hematological parameters. Hematological parameters of tilapia during this study presented in Fig. 2a-f. The hematological parameters observed in this study included Erythrocyte Count (EC), hematocrit level (Ht), hemoglobin level (Hb), Leukocyte Count (LC), phagocytic activity (PA) and Respiratory Burst activity (RB). Administration of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and synbiotic gave effects to the fish hematology; there were fluctuations in hematological parameters during this study. Administration of micro-encapsulated probiotic, prebiotic and synbiotic showed better value on hematological parameters than controls.

The EC of tilapia increased at the end of feeding trial (day 40). This was in line with hematocrit and hemoglobin level. The A, B and C treatment gave significant effect ($p<0.05$) to EC, Ht and Hb of tilapia on day 40 compared to controls (KN and KP). The B treatment showed the highest EC, Hb and Ht on day 40 ($2.91±0.11\times10^6$ cells $mm^{-3}$; 5.66±0.15 g%; 33.63±0.85%). Fluctuations of EC, Hb and Ht occurred on day 43, 46 and 50 (3, 6 and 10 days after the challenge test); there were declines in these parameters on day 43, then increased on day 46 and 50. The highest values of EC, Hb and Ht after challenge test was found in B treatment.

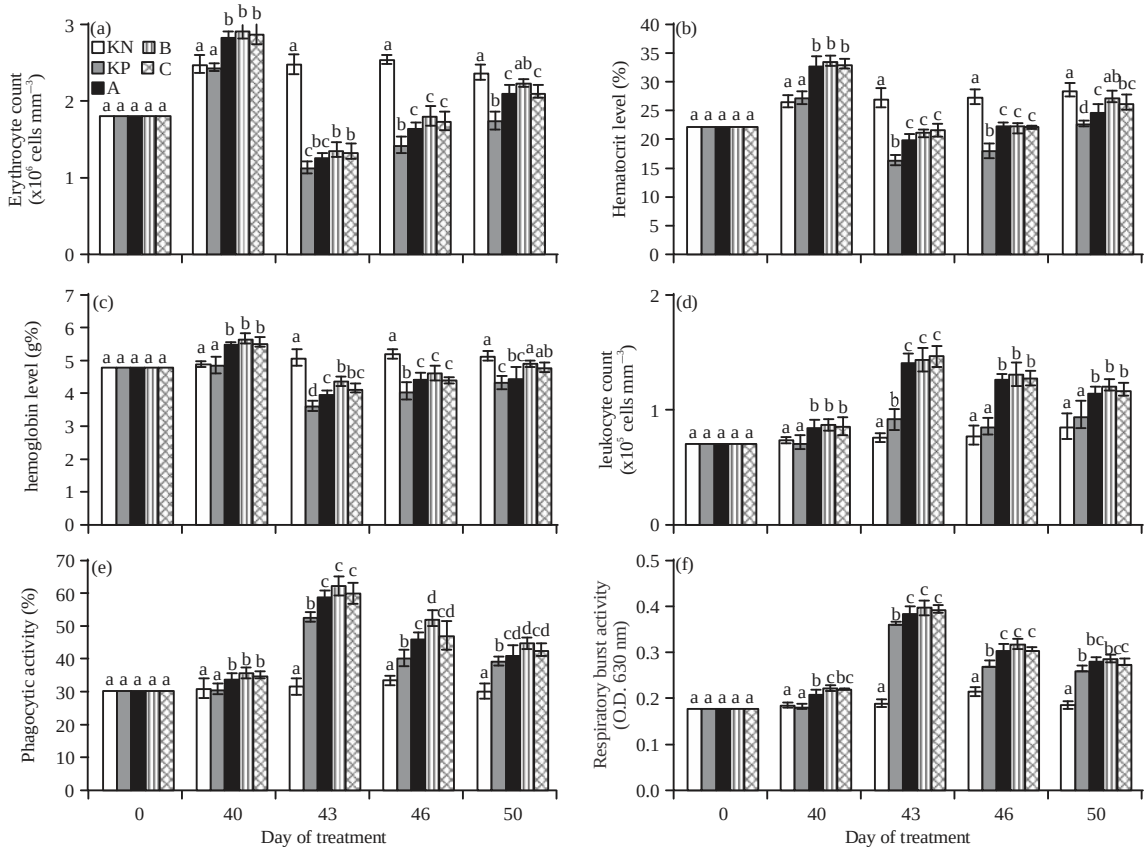


Fig. 2(a-f): (a) Erythrocyte count, (b) Hematocrit level, (c) Hemoglobin level, (d) Leukocyte count, (e) Phagocytic activity and (f) Respiratory burst activity of tilapia during the study. The different letters on each bar in the same day of treatment indicate significantly different results (Duncan; $p<0.05$), negative (KN), positive (KP) control, administration of 0.5% micro-encapsulated probiotic (A), 0.4% prebiotic (B), synbiotic (C)

The results showed that A, B and C treatment gave significant effect ($p<0.05$) to LC, PA and RB of tilapia on day 40 compared to KN and KP treatment. The highest leukocyte count at the end of feeding trial (day 40) was found in B treatment ($0.87\times10^5$ cells $mm^{-3}$). Fluctuations of LC, PA and RB in tilapia occurred on day 43, 46 and 50; there were increases in these parameters on day 43 and then declined on day 46 and 50. The LC of B treatment increased after the challenge test on day 43 that was significantly different from positive and negative control ($p<0.05$), then declined on day 46 and 50. The highest phagocytic activity and respiratory burst activity after the challenge test was also found in B treatment with the highest value on day 43 (62±3%; 0.398±0.016 O.D 630 nm).

**Total *Streptococcus agalactiae* in target organs:** The observation results of total *S. agalactiae* in target organs presented in Fig. 3(a-d). The highest total *S. agalactiae* in target organs (brain, eyes, kidneys and liver) was found on day 45, then decreased on day 50. On day 45, total *S. agalactiae* of A, B and C treatment were lower compared to KP ($p<0.05$). On day 50, there were the decreases in total *S. agalactiae* in all target organs that were significantly different from

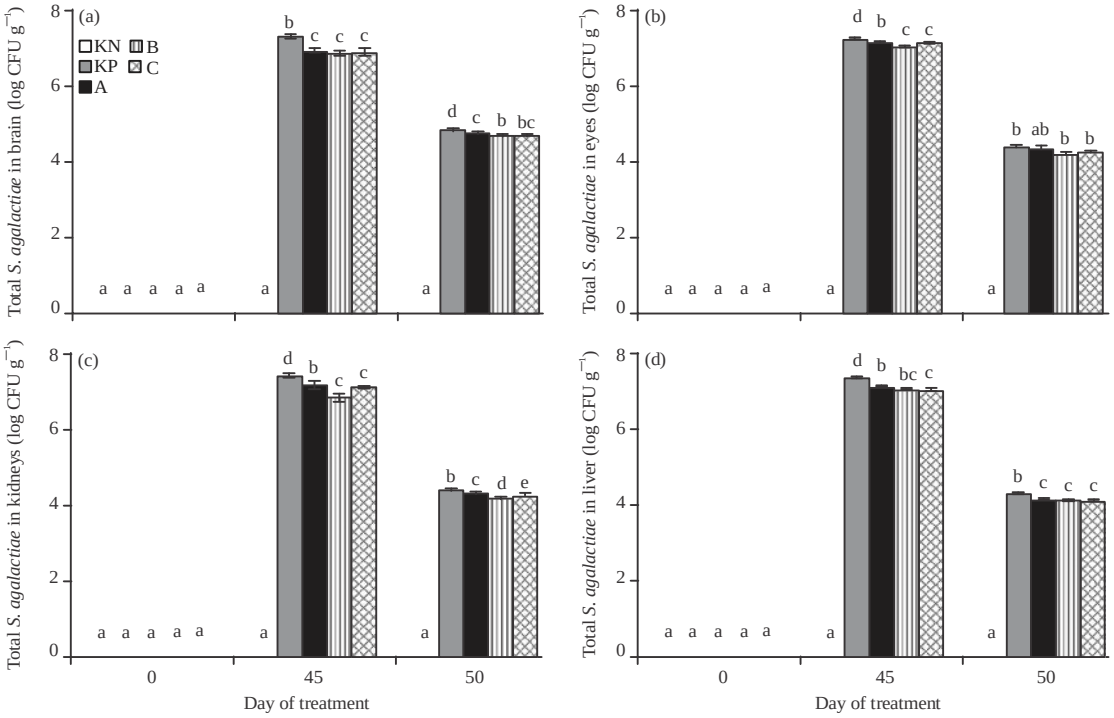


Fig. 3(a-d): Total *S. agalactiae* in the (a) Brain, (b) Eyes, (c) Kidneys and (d) Liver of tilapia during the challenge test. The different letters on each bar in the same day of treatment indicate significantly different results (Duncan; $p<0.05$), negative (KN), positive (KP) control, administration of 0.5% micro-encapsulated probiotic (A), 0.4% prebiotic (B), synbiotic (C)

positive control ($p<0.05$). The B treatment showed the lowest value of total *S. agalactiae* compared to A, C and KP in all target organs (brain, eyes, kidney and liver).

## DISCUSSION

The results showed that administration of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$ (A), prebiotic MOS (B) and synbiotic were able to increase specific growth rate and feed conversion ratio which showed the lower values than negative control (KN) and positive control (KP). This was supported by the presence of probiotic *Bacillus* NP5 $Rf^R$ and total intestinal bacteria data that were higher than positive and negative control. Administration of probiotic is known able to promote the host growth by producing digestive enzymes which help nutrient utilization and host digestion (Geng *et al*., 2011). *Bacillus* NP5 $Rf^R$ is able to produce amylase which can increase growth and improve feed efficiency in tilapia (Putra and Widanarni, 2015). While, prebiotic is capable of giving beneficial effects to the host by modulation of beneficial bacteria selectively in the gastrointestinal tract of tilapia. According to Titapoka *et al*. (2008), beneficial bacteria which have mannanase can utilize mannan oligosaccharide for growth and bacteria replication which contributes to the growth and digestibility of tilapia through exogenous digestive enzyme secretion. In addition, prebiotics are known can inhibit the adhesion of pathogenic bacteria in the intestine, improve growth, feed efficiency and composition of beneficial micro-flora in the intestine (Mahious *et al*., 2006).

The administration of synbiotic could improve growth performance of tilapia. This indicated that *Bacillus* NP5 $Rf^R$ which was given in the form of synbiotic was able to produce digestive enzymes that improved Specific Growth Rate (SGR) and resulting the lower Feed Conversion Ratio (FCR) than controls (KN and KP). But in this study, administration of synbiotic showed no significant differences on Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) compared the administration of micro-encapsulated probiotic treatment (A) and prebiotic MOS treatment (B). Furthermore, total probiotic count on synbiotic treatment (C) and probiotic treatment (A) did not show significant differences. This was presumably due to *Bacillus* NP5 $Rf^R$ could not utilize prebiotic MOS, so there was no synergistic action between probiotic and prebiotic. This caused synbiotic application did not provide significant differences on Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) of tilapia when compared to administration of micro-encapsulated probiotic (A) and prebiotic MOS (B).

Administration of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and synbiotic were able to increase survival rate of tilapia against *S. agalactiae*. The B treatment produced the best survival rate (65±4.3%). *Bacillus* NP5 $Rf^R$ was able to increase survival rate of infected-tilapia by inducing GALT (gut associated lymphoid tissue) system in the digestive tract of tilapia which can enhance the fish immune responses through non-specific immune stimulation (Nayak, 2010). Whereas prebiotic MOS can work directly and indirectly in increasing fish non-specific immunity (Song *et al*., 2014). Prebiotic MOS works directly by interacting with mannose receptor in macrophages, in which it can activate the phagocytic activity of macrophages and it works indirectly by modulating the beneficial bacteria in the digestive tract of tilapia that can activate the fish immune responses. Synbiotic ability to increase survival rate of infected-tilapia associated with the high viability of *Bacillus* NP5 $Rf^R$ in the fish intestines. According to Nayak (2010), probiotic works by passing through the intestinal epithelial cells and interacting with lymphoid tissue, then activating the fish immune system. In this study, administration of synbiotic (C) did not show significant differences on survival rate of tilapia when compared to administration of micro-encapsulated probiotic (A) and prebiotic (B). This was in line with study by Ai *et al*. (2011) who reported that the survival rate of yellow croakers (*Larimichthys crocea*) which were given a combination of *B. subtilis* and fructo oligosaccharide after infected with *Vibrio harveyi*, showed no significant differences when compared to the administration of *B. subtilis*.

There were increases in EC, Hb and Ht of tilapia after feeding trial. These results were in line with the study by Renuka *et al*. (2014) who found that EC, Hb and Ht of *Catla catla* increased after administration of *Lactobacillus acidophilus*. Similarly with prebiotic application, Ahmdifar *et al*. (2011) reported that administration of inulin could increase erythrocyte count on sturgeon. This relates to the ability of probiotic, prebiotic and synbiotic in improving hematological parameters as a response of blood production stimulation (haematopoietic). Furthermore, Takashima and Hibiya (1995) reported that erythrocyte count in fish ranges between $1\text{-}3\times10^6$ cells $mm^{-3}$. EC values at the end of feeding trial were still in the normal range. This showed that the administration of probiotic, prebiotic and synbiotic were safe for fish and able to improve the health of tilapia. The increases in EC, Hb and Ht after the challenge test related to fish recovery phase.

The LC, PA and RB of tilapia in A, B and C treatment showed higher values compared to negative control (KN) and positive control (KP) on day 40 of feeding trial. This related to the fish immune responses in responding immunogenic foreign substances which enter the fish body

through feed (Aly *et al*., 2008), i.e., micro-encapsulated probiotic, prebiotic and synbiotic. Fluctuations of LC, PA and RB occurred on day 43, 46 and 50; there were increases in these parameters on day 43 and then decreased on day 46 and 50. The increases in LC, PA and RB in this study indicated that the immune system worked against pathogen infection, in which leukocytes played a role in phagocytosis.

Total *S. agalactiae* increased on day 45 and then decreased on day 50 in all target organs of *S. agalactiae* (brain, eyes, kidneys, liver), while the lowest total *S. agalactiae* was found in B treatment. The high values of total *S. agalactiae* on day 45 in all target organs related to the incubation period and the growth of *S. agalactiae* resulting in fish mortality. The total *S. agalactiae* in A, B and C treatment were lower than the positive control on day 45. This related to the ability of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and synbiotic in enhancing the fish immune responses through increased phagocytic activity and respiratory burst activity. Phagocytic activity is the non-specific immune system that is mediated by mononuclear cells (monocytes and macrophages) and polymorphonuclear cells. The cells act as the cells which catch, process and present antigens to T cells. Respiratory burst activity produces $H_2O_2$ superoxide and anion ($OH^-$) that are highly toxic to bacteria thereby the ability of phagocyte cells to kill pathogens (Rawling *et al*., 2012). The decrease in total *S. agalactiae* resulted in decrease of *S. agalactiae* infection level, so, it will increase the fish survival rate.

## CONCLUSION

Application of micro-encapsulated probiotic *Bacillus* NP5 $Rf^R$, prebiotic MOS and synbiotic were able to increase growth performance, immune response and survival rate of tilapia infected by *S. agalactiae*.

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## REFERENCES

Ahmdifar, E., R. Akrami, A. Ghelichi and A.M. Zarejabad, 2011. Effects of different dietary prebiotic inulin levels on blood serum enzymes, hematologic, and biochemical parameters of great sturgeon (*Huso huso*) juveniles. Comp. Clin. Pathol., 20: 447-451.

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

Aly, S.M., Y.A.G. Ahmed, A.A.A. Ghareeb and M.F. Mohamed, 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol., 25: 128-136.

Anal, A.K. and H. Singh, 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends Food Sci. Technol., 18: 240-251.

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.

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

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

Geng, X., X.H. Dong, B.P. Tan, Q.H. Yang, S.Y. Chi, H.Y. Liu and X.Q. Liu, 2011. Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia, *Rachycentron canadum*. Fish Shellfish Immunol., 31: 400-406.

Huisman, E.A., 1987. Principles of fish production. Department of Fish Culture and Fisheries, Wageningen Agriculture University, pp: 1-170.

Li, Y.W., L. Liu, P.R. Huang, W. Fang and Z.P. Luo *et al*., 2014. Chronic streptococcosis in Nile tilapia, *Oreochromis niloticus* (L.), caused by *Streptococcus agalactiae*. J. Fish Dis., 37: 757-763.

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.

Mahious, A.S., F.J. Gatesoupe, M. Hervi, R. Metailler and F. Ollevier, 2006. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquacult. Int., 14: 219-229.

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

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

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.

Reid, G., M.E. Sanders, H.R. Gaskins, G.R. Gibson and A. Mercenier *et al*., 2003. New scientific paradigms for probiotics and prebiotics. J. Clin. Gastroenterol, 37: 105-118.

Renuka, K.P., M. Venkateshwarlu and A.T.R. Naik, 2014. Effect of probiotic (*Lactobacillus acidophilus*) on haematological parameters of *Catla catla* (Hamilton). Int. J. Curr. Microbiol. Applied Sci., 3: 326-335.

Samrongpan, C., N. Areechon, R. Yoonpundhan and P. Srisapoome, 2008. Effects of mannan-oligosaccharide on growth, survival and disease resistance of Nile Tilapia (*Oreochromis niloticus* L.) fry. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-18, 2008, Cairo, Egypt, pp: 345-353.

Song, S.K., B.R. Beck, D. Kim, J. Park, J. Kim, H.D. Kim and E. Ringo, 2014. Prebiotics as immunostimulants in aquaculture: A review. Fish Shellfish Immunol., 40: 40-48.

Takashima, F. and T. Hibiya, 1995. An Atlas of Fish Histology: Normal and Pathological Features. 2nd Edn., Kondansha Ltd., Tokyo, ISBN-13: 9781560814122, Pages: 195.

Takeuchi, T., 1988. Laboratory Work-Chemical Evaluation of Dietary Nutrients. In: Fish Nutrition and Mariculture, Watanabe, T. (Ed.). Kanagawa International Fisheries Training Center, Japan International Cooperation Agency (JICA), Japan, pp: 179-233.

Tanbiyaskur, Widanarni and A.M. Lusiastuti, 2015. Administration of *Bacillus* NP5 and oligosaccharide to enhance the immune response in Tilapia *Oreochromis niloticus* towards Streptococcosis. Int. J. Sci.: Basic Applied Res., 20: 304-315.

Taukhid and U. Purwaningsih, 2011. [Screening of *Streptococcus* spp. isolates as an antigen candidate in vaccine development and its efficacy to prevent streptococcosis on tilapia, *Oreochromis niloticus*]. Jurnal Riset Akuakultur, 6: 103-118, (In Indonesian).

Titapoka, S., S. Keawsompong, D. Haltrich and S. Nitisinprasert, 2008. Selection and characterization of mannanase-producing bacteria useful for the formation of prebiotic manno-oligosaccharides from copra meal. World J. Microbiol. Biotechnol., 24: 1425-1433.

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.A. and W.T. Yasutake, . Clinical methods for the assessement of the effects of environmental stress on fish health. U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC., USA.