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APPLICATION OF MICROENCAPSULATED SYNBIOTIC TO IMPROVE THE GROWTH PERFORMANCE AND HEALTH STATUS OF COMMON CARP (*Cyprinus carpio*) CULTURED IN THE PONDS

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ABSTRACT

This study aimed to determine the best dose of microencapsulated synbiotic to improve the growth performance and the health status of common carp (*Cyprinus carpio*) cultured in the ponds. Synbiotic microencapsulation was performed using the spray drying technique. The microencapsulated synbiotic was then administered through feed to common carp (4.81±0.25 g) reared in ponds using hapa net cages sizing 1x1x1 (m^3) (30 fish/hapa net cage) for 30 days. This study used the Completely Randomized Design, consisting of four treatments with three replications, a control and microencapsulated synbiotic supplementation at doses of 5, 10 and 20 g/kg feed. Supplementation of the microencapsulated synbiotic could improve the growth performance and the health status of common carp with the best results obtained at a dose of 10 g/kg feed.

Keywords: microencapsulated synbiotic, growth performance, health status, common carp

INTRODUCTION

The application of probiotic bacteria in aquaculture has demonstrated many positive effects on the host. Guardiola *et al.,* (2016) reported that dietary supplementation of date palm fruit extracts and probiotic *Shewanella putrefaciens* Pdp11 administered alone or in combination had beneficial effects on antioxidant status and innate immune parameters of European seabass (*Dicentrarchus labrax*). *Bacillus* sp. NP5 which was isolated from the gastrointestinal tract of tilapia has been proven to increase the activity of digestive enzymes, induce natural immune system, improve the pathogen inhibition activity, increase growth rate and increase survival in several fish species (Putra and Widanarni, 2015; Djauhari *et al*., 2016).

The role of probiotic bacteria could be increased through the application of prebiotics, feed materials that cannot be digested, but have beneficial effects on the host by stimulating growth and activity of beneficial bacteria in the intestines that would improve the host's health status (Cerezuela *et al*., 2011). The principal prebiotics used as immunostimulants in aquaculture include mannanoligosaccharides (MOS), fructooligosaccharides (FOS) and inulin generally called as immunosaccharides due to their abilities to directly enhance different innate immune responses (Carbone and Faggio, 2016). The application of natural immunostimulants is a promising field to be developed, because they are biodegradable, biocompatible, and safe for enviroment or human health (Ortuño *et al.*, 2002). The administration of gum arabic as a natural immunostimulant demonstrated a protective effect against oxidative damage and a slight immunostimulatory effect indicated by the increase of thrombocyte count in *Mugil cephalus* (Faggio *et al*., 2015). The sweet potato (*Ipomoea batatas* L.) extract contains oligosaccharides, including FOS, galactooligosaccharides (GOS) and inulin, which have potential as a prebiotic, which has been proven to improve growth performance and disease resistance of several species (Widanarni and Tanbiyaskur, 2015; Djauhari *et al.*, 2017).

If probiotic and prebiotic are combined in a single product (synbiotic), the benefits will increase (Lisal, 2005). A combination between fresh culture of *Bacillus* sp. NP5 and prebiotic from sweet potato extract has been tested for its ability to improve growth performance (Putra *et al*., 2015) and to control streptococcosis in tilapia (Widanarni and Tanbiyaskur, 2015). However, the main obstacle in the application of fresh culture probiotic is the viability that quickly decreases during preparation and storage (Wang *et al.*, 2008). The microencapsulation technique is useful to protect and maintain the probiotic cells from external environmental conditions when they pass the fish's digestive tract, such as acidic condition and exposure to bile salts (Del Piano *et al*., 2010; Solanki *et al*., 2013), heat shock in spray drying process, cold shock in deep freezing and freeze drying process (Shah and Ravula, 2000).

One of factors that affects the performance of probiotic, prebiotic, and synbiotic is dose. The doses of probiotic, prebiotic, and synbiotic are usually determined based on their ability to improve growth and protect the host. The optimum

dose of probiotic is depending on the host and the immune parameters expected to be induced; therefore, the dose of each probiotic must be determined for each host species (Nayak, 2010). Studies related to the application of microencapsulated synbiotic in the aquaculture industry have still received little attention, so this study was conducted to determine the best dose of microencapsulated synbiotic to improve the growth performance and the health status of common carp (*Cyprinus carpio*) cultured in the ponds.

MATERIALS AND METHODS

Synbiotic preparation: Synbiotic preparation was done in two phases, prebiotic preparation and probiotic preparation. Prebiotic preparation was done in several steps according to the method described by Marlis (2008), including production of sweet potato starch, extraction of oligosaccharides using ethanol 70%, and measurement of total dissolved solids. Probiotic used was *Bacillus* sp. NP5 marked with rifampicin (*Bacillus* sp. NP5 Rf^R) as a molecular marker. The bacteria were grown in TSB (Trypticase Soy Broth) medium and were incubated in a waterbath shaker at 29-30 ^oC, 160 rpm for 24 hours. Bacterial cells pellet was then rinsed twice with Phosphate Buffer Saline (PBS) solution. Synbiotic was prepared by mixing probiotic with prebiotic to the optimum dose of fresh culture synbiotic, 1% (v/w) probiotic with a density of 10^{10} CFU/mL and 2% (v/w) prebiotic.

Preparation of microencapsulated symbiotic: The preparation of microencapsulated synbiotic consisted of three steps: 1) preparation of coating materials for the synbiotic microencapsulation process, 2) synbiotic microencapsulation, and 3) enumeration of probiotic count contained in microencapsulated synbiotic before and after the microencapsulation process, and then after one month of storage.

Coating materials used were whey protein and maltodextrin (Munaeni *et al*., 2014). Whey protein was obtained by separating casein protein and whey protein from cow's milk using rennet enzyme. Fresh cow's milk was heated to 70-80 ^oC then cooled down. Separation of protein and fat from the cow's milk was done using 0.05 g/L rennet enzyme and 0.4 mL/L $CaCl_2$, homogenized for 10 minutes and incubated at 37 ^oC for 45 minutes, those process produced a clear liquid (whey protein), which was then used as the coating material.

The microencapsulation process was done through the spray drying technique with the following materials: synbiotic suspension, whey protein and maltodextrin at a ratio of 1:1:10% (v/v/w) (Zubaidah *et al*., 2015). The synbiotic suspension was mixed with coating materials, was homogenized with a mixer for 30 minutes and was dried with a spray dryer. The temperature used during the microencapsulation process was 165 ^oC at the inlet and 70 ^oC at the outlet with a flow rate of 15 mL per minute. The product of microencapsulation process was micro powder (microencapsulated synbiotic) which was stored in a refrigerator at 4 ^oC.

The probiotic count in microencapsulated synbiotic was enumerated by counting the number of probiotic cells through the total plate count method on TSA (Trypticase Soy Agar) medium with rifampicin supplementation (50 μg/mL). The probiotic count before microencapsulation process was 9.67±0.05 log CFU/mL, while those after microencapsulation process and one month of storage were 8.83±0.03 log CFU/g and 8.79±0.02 log CFU/g, respectively.

Experimental feed preparation: Experimental feed preparation was done by mixing commercial feed with 31% protein content and microencapsulated synbiotic at doses of 0, 5, 10, and 20 g/kg feed. The feed and microencapsulated synbiotic were mixed by adding 2% (v/w) egg white as a binder, while the control feed was only added 2% (v/w) egg white.

Rearing condition and experimental design: This study was conducted for 30 days. The experimental animals were common carps obtained from fish farmer in Bogor, West Java, Indonesia. The fish were acclimatized to the feed and the environmental condition for 2 weeks. Common carps (4.81±0.25 g) were randomly stocked into hapa net cages sizing 1x1x1 (m^3) (30 fish/hapa net cage). The study used the Comp-letely Randomized Design, consisting of four treatments with three replications, the control (feed without microencapsulated synbiotic), A (feed with microencapsulated synbiotic suppleme-ntation at a dose of 5 g/kg feed), B (feed with microencapsulated synbiotic supplementation at a dose of 10 g/kg feed), and C (feed with micro-encapsulated synbiotic supplementation at a dose of 20 g/kg feed). The feed was given through *ad satiation* method three times a day (08.00, 12.00, and 16.00 Western Indonesia Time). The water quality parameters were monitored during the rearing period with the following ranges: tempe-rature at 28-29 ^oC, DO at 7.4-7.5 mg/L, pH at 7.34-7.67, and TAN at 0.0079-0.0098 mg/L.

Experimental parameters: The experimental parameters observed were total viable bacterial count and total probiotic count in the fish intestines that were observed through total plate

count method, the growth performance and the fish's immune response. The growth performance parameters observed included survival, daily growth rate (DGR), feed conversion ratio (FCR), protein retention and fat retention. The immune response parameters measured were total leukocyte count, phagocytic activity, hematocrit, hemoglobin and total erythrocyte count. All of these parameters were directly evaluated after 30 days of treatment with microencapsulated synbiotic supplementation.

Statistical analysis: The data obtained were analyzed statistically using the SPSS Statistic 17.0 and were then tested with the Duncan's test for significant difference test ($P<0.05$).

RESULTS

The growth performance of common carp supplemented with microencapsulated synbiotic at various doses is presented in Table 1.

Table-1: Total viable bacterial count (TVBC), total probiotic count (TPC), feed intake (FI), survival (SR), daily growth rate (DGR), feed conversion ratio (FCR), protein retention (PR), and fat retention (FR) of common carp fed microencapsulated synbiotic at various doses

Parameter	Control	A	B	C
TVBC (log CFU/g intestine)	7.59 ± 0.08^{a}	8.17 ± 0.04^{b}	9.85 ± 0.08^{c}	8.09 ± 0.04^{b}
TPC (log CFU/g intestine)	n.d.^{a}	7.96 ± 0.03^{c}	8.32 ± 0.06^{d}	7.73 ± 0.06^{b}
FI (g)	692.83 ± 9.95^{a}	689.55 ± 9.45^{a}	685.73 ± 8.93^{a}	651.46 ± 9.33^{b}
SR (%)	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
DGR (%/day)	5.02 ± 0.04^{a}	5.50 ± 0.17^{b}	5.99 ± 0.05^{c}	5.19 ± 0.05^{a}
FCR	1.39 ± 0.02^{d}	1.15 ± 0.03^{b}	0.97 ± 0.02^{a}	1.22 ± 0.01^{c}
PR %)	32.99 ± 0.2^{a}	39.95 ± 0.22^{c}	51.82 ± 0.37^{d}	38.95 ± 0.32^{b}
FR (%)	58.38 ± 0.12^{a}	70.67 ± 0.7^{c}	88.66 ± 0.77^{d}	65.78 ± 0.99^{b}

Note: n.d. = not detected. Different superscript letters on the same row indicate significant different results (Duncan's test; $P<0.05$). The values shown in the table are mean and standard deviation.

Administration of microencapsulated synbiotic at different doses demonstrated significantly different results ($P<0.05$) from the control for total viable bacterial count and total probiotic count in the fish intestines. Supplementation of microencapsulated synbiotic also affected feed intake, daily growth rate, feed conversion ratio, protein and fat retention in common carp, that were all significantly different ($P<0.05$) from the control. The best results were demonstrated by supplementation of microencapsulated synbiotic at a dose of 10 g/kg feed. The survival in all treatments reached 100%, verifying that the supplementation of microencapsulated synbiotic had no adverse effects on survival of common carp. The common carp's health status during the rearing period was represented by the values of the immunity parameters in Table 2. The positive influences of microencapsulated synbiotic could be seen from the values of hemoglobin and phagocytic activity in the blood of common carp that were significantly different ($P<0.05$) from the control.

Table-2: The immunity parameters of common carp after 30 days of microencapsulated synbiotic supplementation

Parameter	Control	A	B	C
Total leukocyte count (10^4 cells/mm^3)	6.05 ± 0.82^{a}	6.67 ± 0.63^{a}	7.16 ± 1.48^{a}	6.94 ± 1.3^{a}
Total erythrocyte count (10^6 cells/mm^3)	1.24 ± 0.2^{a}	1.17 ± 0.09^{a}	1.36 ± 0.21^{a}	1.32 ± 0.08^{a}
Hematocrit (%)	32.35 ± 0.99^{a}	32.05 ± 3.27^{a}	31.55 ± 1.12^{a}	33.25 ± 2.02^{a}
Hemoglobin (g%)	7 ± 0.21^{b}	6.5 ± 0.27^{a}	7.3 ± 0.21^{b}	6 ± 0.00^{a}
Phagocytic activity (%)	10.07 ± 1.19^{a}	32.29 ± 3.74^{bc}	39.66 ± 7.21^{c}	26.89 ± 1.92^{b}

Note: Different superscript letters on the same row indicate significant different results (Duncan's test; $P<0.05$). The values shown in the table are mean and standard deviation.

DISCUSSION

Supplementation of microencapsulated synbiotic in this study had a positive effect on the bacterial population in the intestines of common carp. Based on the ratio of total probiotic count to total viable bacterial count in the intestines of

common carp, it could be concluded that *Bacillus* sp. NP5 could replace most of other bacteria in the intestines of common carp. Microencapsulation of probiotic or synbiotic has great potential in protecting and releasing various beneficial microorganisms into the intestines. The improvement in the common carp's growth was closely related to the increased number of *Bacillus* sp. NP5 in the intestines. After colonizing, *Bacillus* sp. NP5 utilized the various nutrients in the prebiotic (FOS, GOS and inulin) for proliferation and its growth that were simultaneous with the release of digestive enzymes which facilitate nutrient assimilation, resulting in high protein and fat retention, that then led to a high daily growth rate. Fish are commonly thought to have a limited capability to utilize carbohydrate compared to mammals (Faggio *et al.*, 2014), so they need a help from external sources to improve their capability to utilize carbohydrate. *Bacillus* sp. NP5 has been known to produce amylase which could increase feed digestibility (carbohydrate digestibility) and improve the growth of tilapia (Putra and Widanarni, 2015). Common carp fingerlings fed with feed containing microencapsulated synbiotic for 30 days at a dose of 10g/kg feed demonstrated a higher daily growth rate and a lower FCR than those on 20 g/kg feed. This was in line with the results of a previous study by Utami *et al.* (2015) who declared that the administration of dried probiotic at higher doses (1% and 2%) did not show a significant effect on the host's growth performance compared to the dose of 0.5%. The effects of the exposure of xenobiotic compounds or foreign materials (e.g. probiotics and synbiotics) are highly influenced by exogenous factors, such as environmental factors and their concentrations (Burgos-Aceves *et al.*, 2016). Probiotic at very high doses can cause disturbances on the microbiota in the digestive tract and interfere the immune response through an excessive inflammatory response that leads to the loss of energy that can be used for growth (Li *et al.*, 2012; Ramos *et al.*, 2013).

The measurement of hematological parameters of cultured fish is a preventative effort that is commonly performed to observe the fish health status (Fazio *et al.*, 2013). In teleosts, probiotics can positively stimulate several immuno-hematological parameters, including mononuclear phagocytic cells (monocytes and macrophages), polymorphonuclear leukocytes (neutrophils) and NK cells (Balcazar, 2003). This was in line with the result of the present study that showed positive effects of the supplementation of microencapsulated synbiotic containing probiotic on the fish's health status or hematological parameters that were demonstrated by the higher hemoglobin and phagocytic activity in microencapsulated synbiotic treatments compared to the control. The higher hemoglobin values in microencapsulated synbiotic treatments related to the probiotic's ability to improve the hematological parameters as the results of stimulation on the blood formation (Renuka *et al.*, 2014). The higher values on phagocytic activity in microencapsulated synbiotic treatments happened, because the microencapsulated synbiotic supplemented to common carp was an immunogenic material (Aly *et al.*, 2008), which stimulated the fish's immune response through the production and the activation of phagocytic cells. The better cellular immune response would then play a role in the improvement of the fish's protection against stress due to infections by pathogens or environmental stressors.

CONCLUSION

Supplementation of microencapsulated synbiotic through feed could improve the growth performance and the health status of common carp with the best results obtained at a dose of 10 g/kg feed.

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