Biotropia 2020

by Achmad Noerkhaerin Putra

 DOI: 10.11598/btb.2020.27.1.1102

EFFECTS OF DIETARY PROBIOTIC *Bacillus* NP5 ON THE GROWTH PERFORMANCES OF CATFISH (*Clarias* sp.)**

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Received 18 July 2018 / Accepted 27 May 2019

ABSTRACT

Probiotic have long been applied to aquaculture and produce positive effects on fish and shrimp. This research aimed to evaluate the effect of probiotic *Bacillus* NP5 to promote the growth of catfish (*Clarias* sp.). Five doses *Bacillus* NP5 with 3 replicates, namely 0% probiotic (control), 0.5 % probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic (g/100 g feed) were used. The result showed that application of probiotic in catfish feed can promote better growth performance compared to control. Total digestibility and protease enzyme activites were significantly highest in 1% probiotic. The value of specific growth rate showed in 1% probiotic (2.67±0.18% day^{-1}), followed by 2% probiotic (2.63±0.02% day^{-1}), 1.5% probiotic (2.42±0.07% day^{-1}), 0.5% probiotic (2.29±0.14% day^{-1}) and control (1.60±0.01% day^{-1}). The addition of 1% *Bacillus* NP5 as probiotic in catfish feed showed the best result on protease enzyme activities, protein digestibility, total digestibility, final weight, specific growth rate, weight gain, feed efficiency and, the protein efficiency ratio than other probiotic doses.

Keywords: *Bacillus* NP5, catfish, growth performance, probiotic

INTRODUCTION

Aquaculture is an important economic activity because it can be carried out by all levels of society in many countries. Rapid aquaculture has an important role to increase fish production for the last decades. Aquaculture is estimated to have supplied 47.6% of total world fish production in 2011 (Mathiesen 2012). Catfish (*Clarias* sp.) is an economical freshwater aquaculture commodity in several Asian countries including Indonesia. However, the intensive cultivation of catfish are faced many problems, which one is the high feed price, pathogen outbreaks (Ulkhaq *et al.* 2014), low environmental quality (Yusuf *et al.* 2015), and low feed digestibility (Afrilasari *et al.* 2016). Feed costs about 50-60% of the total production costs in aquaculture, so the efficiency of feeding will reduce the cost of production (Nates 2016). Several methods in intensive aquaculture system have been applied to improve feed digestibility, fish growth, water quality and response immune such as bio-Floch technology (Widanarni *et al.* 2012; Dauda *et al.* 2017), and dietary supplements with immuno-stimulant, anti-microbial and probiotic (Cheng *et al.* 2017).

Probiotic can be defined as additional microbe which have a positive influence on the host (Nayak 2010). In aquaculture, its application has been considered more environmentally friendly and effective to improve feed digestibility, growth, water quality and immune response (Mohapatra *et al.* 2012; Dawood *et al.* 2015; Kumar *et al.* 2016). In addition, the positive effect of probiotics is to produce inhibitory compounds to decrease the growth of pathogenic bacteria (Perez-Sanchez *et al.* 2013), water quality improvement of fish rearing media (Chumpol *et al.* 2017), enhancement fish resistance against disease (Newaj-Fyzul *et al.* 2014), and produce digestive

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**This paper was presented at the International Conference on Food Security Innovation (ICFSI) 2017, 18-20 October 2017, Serang, Banten, Indonesia

enzymes (Zheng & Wang 2016). Amylase, protease and lipase are exogenous enzyme that can produce by probiotics (Dawood & Koshio 2016). The exogenous enzymes can lead to an endogenous enzyme to hydrolyzes nutrients from host feeding.

In this study, *Bacillus* NP5 was used as probiotic which were added in feed. The bacteria were isolated by Putra and Widanarni (2015) from digestive tract of tilapia. The previous studies have reported that the administration of *Bacillus* NP5, could increase the growth, feed efficiency, enzyme activity of tilapia (Putra *et al.* 2015). Utami *et al.* (2015) proved that dietary dried *Bacillus* NP5 Rf^{R} could improve growth performance of tilapia. This research aimed to evaluate the effect of probiotic *Bacillus* NP5 to promote the growth catfish (*Clarias* sp.).

MATERIALS AND METHODS

Probiotic Preparation

Bacillus NP5 was cultured in 500 mL of TSB (Tryptic soy broth) in aseptic condition and incubated at room temperature (29°C) for 18 hours. Furthermore, culture of probiotic was centrifuged at 1000 rpm for 10 min to obtain the probiotic biomass. After that, probiotic was diluted in 1000 mL PBS (Phosphate Buffered Saline) before it was added the feed.

Experimental Feed Preparation

Commercial feed (protein of 29.75-30%, lipid of 8.02-8.57% and free nitrogen extract of 32.75-30%) was used in this study. These values are in accordance with the nutrient requirement of catfish (Robinson *et al.* 2001). Feed were mashed into a meal and 0.5% Chromium oxide (Cr_2O_3) as indicator of digestibility and 3% tapioca asa feed binder were added to the meal before the pelleting process. The feed was molded with a pellet machine at diameters of 1-2 mm and was dried using an oven at 40°C for 24 hours. The experimental feeds were manufactured by adding different doses of 0%, 0.5%, 1%, 1.5% and 2% (g/100 g feed) of *Bacillus* NP5 as probiotic into the feed. *Bacillus* NP5 was mixed with 2% egg yolk as a binder and was added to the feed by spraying using a syringe according to Putra *et al.* (2015). The formulation and proximate feed in this study presented in Table 1.

Experimental Design and Procedure

The fish rearing was carried out at the Laboratory of Aquaculture, Department of Fisheries, University of Sultan Ageng Tirtayasa, Indonesia. Juveniles of catfish were obtained from Tri Farm, Anyer Banten Province, Indonesia. A total of 225 juvenile of catfish (initial individual weight of 5.03±0.05 g) were used. They were reared in five treatments (0% probiotic as a control, 0.5% probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic) with each consisting of three replicates (15 fishes/replicate). 15 aquariums (50 x 40 x 30 cm) were used in this study and equipped with an aeration point. Fish were reared for 45 days and before weighing the weight of fish, fish were fasted for 24 hours. The weight of catfish was evaluated once every 2 weeks. Feeding was done three times daily at 08:00, 12:00 and 17:00 hours with satiation. Siphon is carried out on the remaining feed and feces in morning before feeding time. 50% of the water volume in the rearing aquariums was remove every 3 days to maintain water quality. The daily water quality monitoring was conducted and in the present study, we obtained the range value of temperature of 27-30.1°C, dissolved oxygen of 5.48-6.95 ppm and pH of 6.77-7.29.

Table 1 Formulation and proximate composition of the experimental diets

Ingredients (%)	Diet (%)				
Commercial feed	100				
Tapioca	3				
Cr_2O_3	0.5				
Probiotic *Bacillus* NP5	[a]				
Proximate analyses (% dry matter)	Probiotic diet (g/100 g)				
	0 (control)	0.5	1	1.5	2
Crude protein	29.95	29.95	29.75	30.00	30.00
Crude lipid	8.04	8.57	8.55	8.17	8.02
NFE	32.75	33.00	33.00	33.00	33.00
Energy (Kcal/kg feed)[b]	251.78	256.74	255.88	253.64	252.42
Moisture	6.41	6.57	6.60	6.45	6.41

Notes: [a]Probiotics: 0%, 0.5%, 1%, 1.5%, 2% (g/100 g feed)
[b]energy in digestible energy value according to NRC (1993) where protein of 3.5 Kcal, lipid of 8.1 Kcal, carbohydrate of 2.5 Kcal.

Growth Parameters

The growth parameters of the juvenile catfish were SR: survival rate (1), WG: weight gain (2), SGR: specific growth rate (3) and FE: feed efficiency (4) and were determined according to Huisman (1987). While PER: protein efficiency ratio (5) was determined according to Takeuchi (1988), by the following equations:

SR (%) = NT/N0 x 100 (1)
WG (%) = 100 x (Ft-Fo)/Fo (2)
SGR (% day^{-1}) = 100 x (lnWt-lnWo) / time (days) (3)
FE (%) = 100 x [W/ F]. (4)
PER = W/Protein feed (5)

Where;
NT is total of ndividuals at the end experiment, N0 is total of individuals at the beginning, Ft (g) is final weight, Fo is initial weight, Wt is total biomass of final fish (g), Wo is total biomass on initial fish (g), W is weight gain (g) and F is the dry feed intake (g).

Digestibility Parameters

The value of nutrient digestibility is calculated by the indirect method using chromium oxidase of 0.5% (NRC 2011). Faeces collection was conducted on the day-7 after stocking by siphoning out the faecal material from the bottom of the aquarium. Fish faeces from each treatment were collected and were stored at -4 °C until analysis. The nutrient digestibility (ND) (6) including protein digestibility (PD), fat digestibility (FD), and total digestibility (7) were calculated refer to Takeuchi (1988):

ND = 100 - [1-c/c'x d'/d] (6)
TD = 100 – [1-c/c'] (7)

Where, c is the value of chromium in the feed, c' is chromium in the faeces, d is the value of nutrient in the feed, and d' is the value of nutrient in the faeces.

Digestive Enzymes Activity

Enzyme activities (protease, lipase and amylase) of digestive tract of catfish were analyzed based on the method presented by Bergmeyer and Grassi (1983). At the end of the fish rearing, catfish intestine from 5 individual per treatment were taken under low temperature condition (4 °C) and rinsed with cold water. Then, at a dose of 1 g/10 mL, it is mixed and homogenized within phosphate buffer at pH 7.5 and temperature of 4 °C. Furthermore, it is centrifuges for 15 minutes at temperature of 4 °C and speed of 15000 rpm. The supernatant formed is stored at temperature of 4 °C before being analyzed.

Proximate Composition Analysis

The standard method from AOAC (1995) was used to determine the proximate composition of catfish and feces. The value of protein was determined by measuring the nitrogen content according to the Kjeldahl method. Lipid content in feed and feces was analyzed based on folch method using Soxhlet. Feed and feces were dried at 105°C for 6 hours to obtain the value of moisture. Nitrogen extract was obtained by subtracting 100% of the ingredient with percentage of other nutrients.

Statistical Analyses

All was analyzed by statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) program for Windows (v. 16.0). Duncan's multiple range with 95% significant level was used as post hos test in the present study.

RESULTS AND DISCUSSION

The effects of probiotics have been evaluated in aquaculture commodities and many researches recorded that the growth of, cobia *Rachycentron canadum* (Geng *et al* 2012), black tiger shrimp, *Penaeus monodon* Fabrucius 1798 (Hasan *et al*. 2012), carp, *cyprinus carpio* (Xu *et al*. 2014), Pangasius catfish (Doan *et al*. 2016), Florida pompano, common snook and red drum larvae (Hauville *et al*. 2016), grass carp (Li *et al*. 2017), giant freshwater prawn *Macrobrachium Rosenbergii* (DeMan, 1879) (Ghosh *et al*. 2016), parrot fish *Oplegnathus fasciatus* (Liu *et al*. 2018), and the whiteleg shrimp *Litopenaeus vannamei* (Madani *et al*. 2018) were significantly increased. In this study, all probiotic treatment can promote better growth performance of catfish compared to control. Growth performance of catfish with different probiotic doses were presented in Table 2. In the beginning, no difference (p>0.05) was obtained in feed intake. In the beginning, no difference (p>0.05) was obtained in feed intake between control (143.67±2.08) and 0.5-2% probiotic treatments (148.00±7.27, 146.67±2.08, 144.67±3.06, and 140.33±2.08, respectively). This is showing that dietary *Bacillus* NP5 not affect to palatability. Similar result has been found by Putra *et al*. (2015) and Elsabagh *et al*. (2018), the addition of probiotic in feed could not significantly (p>0.05) on the consumption of tilapia feed. The same results were also found d in grouper (*Efinephelus coioides*), where supplementation of *Lactococcus lactis* and *Enterococcus faecium* could not increase feed consumtion (Sun *et al*. 2012).

After the 45 days fish rearing, probiotic treatments showed the higher of digestive enzyme compared to control. The value of protease enzyme activity in 1% probiotic (0.70±0.04 U/min/mL) was different higher than the other treatments. but there were no significant differences among the 0.5%, 1.5% and 2% probiotic treatments (0.58±0.05, 0.60±0.02, and 0.62±0.01 U/min/mL, respectively). The best value of lipase activity was found in 1% probiotic (0.58±0.03 U/min/mL) compared to control (0.33±0.03 U/min/mL), 0.5% probiotic (0.44±0.03 U/min/mL) and 2% probiotic (0.52±0.01 U/min/mL). However, the value was no difference (P>0.05) between 1% probiotic and 1.5% probiotic. Furthermore, the value of amylase activity in probiotic treatment was greater (P<0.05) than control. There was no difference value of amylase activity in probiotic treatment with doses of 1-2%.

Tabel 2 Effect of dietary probiotic *Bacillus* NP5 on growth performance of catfish[a]

Parameters[b]	Probiotic treatments (%)				
	0 (control)	0.5	1	1.5	2
IW (g)	5.03 ± 0.00	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01	5.00 ± 0.01
FI (%)	143.67 ± 2.08	148.00 ± 7.27	146.67 ± 2.08	144.67 ± 3.06	140.33 ± 2.08
PA (U/min/mL)	0.48 ± 0.07^{a}	0.58 ± 0.05^{b}	0.70 ± 0.04^{c}	0.60 ± 0.02^{b}	0.62 ± 0.01^{b}
LA (U/min/mL)	0.33 ± 0.03^{a}	0.44 ± 0.03^{b}	0.58 ± 0.03^{d}	0.57 ± 0.01^{d}	0.52 ± 0.01^{c}
AA (U/min/mL)	0.22 ± 0.01^{a}	0.36 ± 0.02^{b}	0.45 ± 0.05^{c}	0.44 ± 0.03^{c}	0.43 ± 0.02^{c}
PD (%)	52.95 ± 1.71^{a}	69.78 ± 0.74^{b}	76.97 ± 0.98^{d}	75.17 ± 1.57^{cd}	74.54 ± 0.67^{c}
FD (%)	63.10 ± 1.13^{a}	72.82 ± 4.13^{b}	77.63 ± 2.94^{b}	75.94 ± 4.49^{b}	71.95 ± 1.41^{b}
TD (%)	44.10 ± 1.35^{a}	51.04 ± 2.39^{b}	56.03 ± 1.13^{c}	52.36 ± 2.14^{b}	52.16 ± 1.40^{b}
FW (g)	10.33 ± 0.07^{a}	11.76 ± 0.10^{b}	12.78 ± 0.56^{d}	12.02 ± 0.34^{bc}	12.38 ± 0.1^{cd}
WG (g)	79.55 ± 1.00^{a}	102.28 ± 1.59^{b}	116.62 ± 8.34^{d}	105.23 ± 5.11^{bc}	110.62 ± 1.61^{cd}
SGR (% day^{-1})	1.60 ± 0.01^{a}	2.29 ± 0.14^{b}	2.67 ± 0.18^{d}	2.42 ± 0.07^{bc}	2.63 ± 0.02^{cd}
FE (%)	55.37 ± 0.23^{a}	68.57 ± 4.30^{b}	79.50 ± 5.54^{c}	72.71 ± 1.98^{b}	78.83 ± 0.63^{c}
PER (%)	1.85 ± 0.01^{a}	2.29 ± 0.14^{b}	2.67 ± 0.18^{c}	2.42 ± 0.07^{b}	2.63 ± 0.02^{c}
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

Notes: [a] The value in the same row followed by similar superscript are not significantly different (p>0.05).
[b] Initial weight (IWB), (FW), feed intake (FI), protease activity (PA), lipase activity (LA), amylase activity (AA), protein digestibility (PD), fat digestibility (FD), total digestibility (TD), final weight (FW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), protein efficiency ratio (PER), survival rate (SR) of catfish.

Probiotic produces some extracellular/ exogenous enzymes (protease, lipase, and cellulose) which can improve feed utilization (Zheng & Wang 2016). The exogenous enzyme could lead endogenous enzyme to hydrolyze nutrients from host feeding. *Bacillus* NP5 are proven to produces digestive enzyme that support the nutrients absorption (Putra & Widanarni 2015). The endogenous enzyme activity will increase because probiotic can increase the colonization of beneficial bacteria in the digestive tract digestive tract (Dawood & Khosio 2016; Sankar *et al.* 2016). The result indicated that *Bacillus* NP5 could improve enzyme activities in catfish intestine. Similar effects had been reported by Nimrat *et al.* (2013) where supplementation of *B. subtilis* and *Enterococcus* sp. in black tiger shrimp *Penaeus monodon* postlarvae and Adel *et al.* (2017) with the addition of *Pediococcus pentosaceus* spp. in white shrimp, *Litopenaeus vannamei*.

The value of protease enzyme was difference in 1% probiotic than the other treatments. This is suggested that the addition of 1% probiotic in feed stimulated the growth of intestinal microbiota in the catfish intestine. Digestive enzyme were secreted by beneficial bacteria in the colon to digest the nutrient contained in the fed (Buentello *et al.* 2010). This result also confirms the similar hypothesis that all probiotic treatments can produce the better protease enzyme than control. The results were similar to the finding in freshwater prawn prawn (*Macrobrachium rosenbergii*) that supplementation of probiotic could increase the protease activities in the colon (Gupta *et al.* 2016; Sumon *et al.* 2018, Valipour *et al.* 2019). In this study, probiotic treatment has the higher value of lipase activity. This result in line with findings from Allameh *et al.* (2015) in Javanese carp that the best value of enzymes lipase in probiotic treatment. The result showed that the increase in the probiotic dose was not followed by an increase in the value of enzyme activity. The value of digestive enzyme was the highest in the 1% probiotic than 1.5-2% probiotic. This result is might to be due to the limitations of bacteria conducting colonization in the intestine. Therefore, the higher probiotic doses not caused positive effect for the host (Talpur *et al.* 2013; Zhang *et al.* 2018).

Probiotic addition to feed is aimed to increase the population of probiotic in the catfish tract so that the action mechanism of the probiotic in producing exogenous enzymes for digestion will increase (Merrifield 2010). Subsequent digestibility of fish diets is important because it influences the energy and nutrient availability, absorption and utilization. The protein digestibility in control (52.95±1.71%) was lower than that of the 0.5-2% probiotic treatments (69.78±0.74%, 76.97±0.98%, 75.17±1.57%, 74.54±0.67%, respectively). Furthermore, fat digestibility also differed among the treatments. Total digestibility was significantly higher in the 1% probiotic (56.03±1.13%) as compared with the other treatments but no significant difference existed among the 0.5% probiotic, 1.5% probiotic and 2% probiotic.

Digestibility of fish diets is important because it can influence energy and nutrient availability, absorption and utilization. The value of protein digestibility in control (52.95±1.71%) was lower ($p<0.05$) than that of the 0.5-2% probiotic treatments (69.78±0.74%, 76.97±0.98%, 75.17±1.57%, 74.54±0.67%, respectively). Furthermore, difference in fat digestibility between 0.5% probiotic, 1% probiotic, 1.5% probiotic and 2% probiotic. Total digestibility was significantly higher in 1% probiotic (56.03±1.13%) compared to the other treatment but there was no difference between 0.5% probiotic, 1.5% probiotic and 2% probiotic. The result showed that the digestibility of protein, fat and dry matter in the 0.5-2% probiotic were greater than control ($p<0.05$). This result was caused the value of digestive enzyme in probiotic treatments that are greater than control. Similar results were found in white shrimp (*Litopeaneus vannamei*) that probiotic *Bacillus subtilis* in their feed can increase the digestibility of protein, lipid dan dry matter/total digestibility (Tsai *et al.* 2019).

The value of final weight and weight gain in control were lower ($p<0.05$) than that of the 0.5-2% probiotic. The result showed that feed efficiency of the control is 55.37±0.23%, and the value was smaller than that obtain by 0.5-2% probiotic (68.57±4.30, 79.50±5.54, 72.71±1.98, 78.83±0.63, respectively) and there was no difference between 1% probiotic and 2% probiotic. The increased nutrient digestibility (protein, fat and total digestibility) in catfish has a positive correlation to final weight, weight gain, PER, and SGR. The high value of digestive enzyme and nutrient digestibility has affected growth and feed efficiency (Afrilasari *et al.* 2016). The highest (P<0.05) of SGR was found in 1% probiotic (2.67±0.18% day-1), followed by 2% probiotic (2.63±0.02% day-1), 1.5% probiotic (2.42±0.07% day-1), 0.5% probiotic (2.29±0.14% day-1) and control (1.60±0.01% day-1). In the previous study, probiotic could stimulate the growth of intestinal microbial and secretion of digestive enzyme to help feed digestion (Wang *et al.* 2012; Zokaeifar *et al.* 2012). Therefore, growth increased of catfish was strongly related to be caused by the increased of digestive enzyme activities in the colon. It is showed with the higher value of digestive enzyme and nutrient digestibility in probiotic treatment than that control. In the present study, the high concentrations of probiotic-supplemented (1.5%-2% probiotic treatments) in diets may not further promote the SGR of catfish. This might be related to the lack of enzyme activity (protease, lipase, and amylase) in catfish intestine of 1.5%-2% probiotic compared to 1% probiotic treatment. Similar results had been reported by Sun *et al.* (2010) in the addition of *Bacillus* spp. in *Epinephelus coioides*. Probiotic in the very high dose will cause the negative effect on the microflora in the digestive tract (Ramos *et al.* 2013). In this study, supplementation of probiotic not influence survival rate, which indicated that administration of *Bacillus* NP 5 not impact on fish physiologies of catfish. Similarly, Kumar *et al.* (2013) found that the survival rate of giant freshwater prawn with the addition of *B. licheniformis* in the diet and Garcia-Bernal *et al.* (2018) also reported that supplementation of as probiotic could no decrease the survival rate of *Litopenaeus vannamei*.

CONCLUSION

The addition of probiotics to feed can promote better growth performance of catfish compared to control. The specific growth rate, protease activity and protein digestibility were significantly highest (P<0.05) in 1% probiotic (2.67±0.18% day^{-1}, 0.70±0.04 U/min/mL, 76.97±0.98%, respectively) than other treatments. The addition of 1% probiotic in feed showed the best result on enzyme protease activities, protein digestibility, total digestibility, the final weight, specific growth rate, weight gain, feed efficiency and protein efficiency ratio among other treatments.

ACKNOWLEDGEMENTS

The authors want to thank to Mr Heri Endra from Tri Farm in Anyer, Banten, Indonesia, for the guidance in catfish culture.

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