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Effects of dietary *Bacillus* NP5 and sweet potato extract on growth and digestive enzyme activity of dumbo catfish *Clarias* sp.

Efek *Bacillus* NP5 dan ekstrak ubi jalar pada pakan terhadap pertumbuhan dan aktivitas enzim pencernaan ikan lele dumbo *Clarias* sp.

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ABSTRACT

This study aimed to investigate the effects of probiotic (*Bacillus* NP5), prebiotic (extracted from *Ipomoea batatas* var. sukuh,), and synbiotic on growth and digestive enzyme activity of dumbo catfish (*Clarias* sp.). Four treatments with 3 replications were used in the experiment, i.e; control, probiotic (1% *Bacillus* NP5 in feed), prebiotic (2% sweet potato extract in feed), and synbiotic (1% probiotic and 2% prebiotic in feed). After 45 days of rearing, catfish that were given probiotic, prebiotic, and synbiotic showed significantly (P<0.05) higher growth than that of the control. The average results on digestive enzyme activities in probiotic, prebiotic, and synbiotic treatments were significantly different (P<0.05) from that of the control. The addition of synbiotic to diets showed the best result on final weight, specific growth rate, feed conversion ratio, protein retention, protein efficiency ratio, and protease activities among other treatments.

Keywords: catfish, growth, probiotic, prebiotic, symbiotic

ABSTRAK

Penelitian ini bertujuan untuk menginvestigasi pengaruh dari probiotik (*Bacillus* NP5), prebiotik (ekstrak ubi jalar varietas sukuh), dan sinbiotik terhadap pertumbuhan dan aktivitas enzim perncernaan ikan lele dumbo (*Clarias* sp.). Penelitian terdiri dari 4 perlakuan dan 3 kali ulangan, yakni: probiotik (*Bacillus* NP5 sebanyak 1% dalam pakan), prebiotik (ekstrak ubi jalar sebanyak 2% dalam pakan), dan sinbiotik (*Bacillus* NP5 1% + ekstrak ubi jalar sebanyak 2% dalam pakan). Setelah pemeliharaan selama 45 hari, ikan lele pada perlakuan dengan suplementasi probiotik, prebiotik, dan sinbiotik dalam pakan secara signifikan (P<0,05) menghasilkan pertumbuhan yang lebih baik dibandingkan dengan kontrol. Nilai aktivitas enzim pencernaan pada perlakuan probiotik, prebiotik, dan sinbiotik secara signifikan berbeda (P<0,05) dari kontrol. Penambahan sinbiotik pada pakan memberikan hasil terbaik pada parameter bobot akhir, laju pertumbuhan harian, FCR, retensi protein, rasio efisiensi protein, dan aktivitas enzim protease dibandingkan dengan perlakuan lainnya.

Kata kunci: ikan lele, pertumbuhan, probiotik, prebiotik, sinbiotik.

INTRODUCTION

Catfish stand the world's most important group of aquaculture species in terms of production. In Indonesia, *Clarias* sp. is one of popular catfish with 873.716 tons production rate in 2016 with intensive cultivation system is being used (DJPB, 2016). However, problems arise in the intensive cultivation system since this system requires high stocking density and amounts of feed (Widanarni *et al*., 2012). This certainly brings a negative impact on fish growth due to the high concentrations of organic compounds, which are dominated by nitrogenous wastes (Ekasari *et al*., 2010).

Supplementation of probiotic, prebiotic, and synbiotic in catfish diet is expected to increase growth performance of fish. Probiotic is an additional microbe which potentially provides beneficial effects for the host (Nayak, 2010). Probiotics can increase aquatic animal feed efficiency by stimulating digestive enzymes and maintaining the balance of intestinal microbes (Dawood & Koshio, 2016). The influences of probiotic for fish and shrimp have been reported in various studies (Geng *et al*., 2012; Arig *et al*., 2013; Giri *et al*., 2013; Wang *et al*., 2014; Hauville *et al*., 2015, Imanpoor & Roohi, 2015; Dawood *et al*., 2015; Ibrar *et al*., 2017; Dawood *et al*., 2017). *Bacillus* NP5 is probiotic bacteria which has beneficial effects in aquaculture. The application of *Bacillus* NP5 in fish diets improved growth performance and increase diseases resistance in tilapia *Oreochromis niloticus* (Utami *et al*., 2015; Putra & Widanarni, 2015; Agung *et al*., 2015), white shrimp *Litopenaeus vannamei* (Widanarni *et al*., 2014), and *Pangasianodon hypophthalmus* (Tamamdusturi *et al*., 2016).

Prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon (Ringgø *et al*., 2010). Some studies have shown the benefits of using prebiotic for increasing growth performance (Buentello *et al*., 2010; Ringø *et al.,* 2014; Selim & Reda, 2015) and resistance of pathogen in aquaculture (Geraylou *et al*., 2013; Wang *et al*., 2014; Anuta *et al.,* 2014). Prebiotic mainly consist of oligosaccharides (Merrifield *et al*., 2010) and could be found in various tuber plants, such as sweet potatoes *Ipomoea batatas* L (Moongngram *et al*., 2011). The utilization of sweet potatoes as prebiotic in tilapia fed improved growth performance and feed digestibility of tilapia (Putra, 2014; Putra *et al*. 2015). Marlida *et al*. (2014) reported that dietary sweet potato extract as a prebiotic can increase growth, digestive enzyme activity and health status of humpback grouper *Cromileptes altivelis*. Sweet potato is potential as a prebiotic source due to its oligosaccharide which is functional and indigestible by digestive enzymes (Marlida *et al*., 2014; Putra *et al*., 2015).

A synbiotic is defined as a combination of a probiotic and a prebiotic (Song *et al*., 2014; Akhter *et al*., 2015). It refers to nutritional supplements combining probiotics and prebiotics in the form of synergism (Kumar *et al*., 2017). Applications of synbiotic in the diet have been reported on growth performance, digestive enzyme activities and health status of several fish and shellfish species (Cerezuela *et al*., 2011; Lin *et al*., 2012; Zhang *et al*., 2013; De *et al*., 2014; Ringø *et al*., 2014; Putra *et al*., 2015; Widanarni *et al*., 2016). However, limited data are available regarding the application of synbiotics in catfish. This study aimed to investigate the effects of probiotic (*Bacillus* NP5), prebiotic (extracted from *Ipomoea batatas* var. sukuh), and synbiotic in improving growth and digestive enzyme activity of dumbo catfish (*Clarias* sp.).

MATERIALS AND METHODS

Preparation of probiotic, prebiotic, and synbiotic

Probiotic bacteria used in the present study was *Bacillus* NP5 isolated from the digestive tracts of tilapia (Putra & Widanarni, 2015). Isolates of *Bacillus* NP5 were cultured in trypticase soy broth medium for 18 hours at a temperature of 29°C. The fresh culture was then harvested by centrifugation at speed of 1000 rpm for 10 min to obtain the probiotic biomass. Probiotic were homogenized into phosphate buffered saline (PBS) for the addition to the feed. This study used a probiotic dose of 1% (g/100g), referring to Putra *et al*. (2015).

The experimental prebiotic was extracted from sweet potatoes var. sukuh (*Ipomoea batatas*). The extraction process of the prebiotic was conducted in several stages referred to the method by Muchtadi (1989). A 10 g of steamed sweet potato meal was suspended in 100 ml of 70% ethanol and stirred for 15 h using magnetic stirred at room temperature. The filtrate obtained was concentrated using a vacuum evaporator at 40°C. In the present study, the probiotic dose was

Table 1. Formulation and proximate composition of the experimental diets

Ingredients (%)	Diet			
Commercial feed	100			
Tapioca	3			
Pro/pre/synbiotic	*			
Proximate analysis (% dry matter)	**Treatments**			
	Control	Feed with 1% probiotic	Feed with 2% prebiotic	Feed with synbiotic
Crude protein	31.33	31.17	31.17	31.17
Crude fat	5.04	5.07	5.15	5.12
Nitrogen-free extract	34.65	34.86	35.49	34.94
Energy (kcal/kg feed)**	237.12	237.29	239.50	237.92
Moisture	10.98	10.91	9.86	9.77

* Control, 1% Probiotic, 2% prebiotic, and synbiotic (1% probiotic and 2% prebiotic) (g/100 g feed)

** DE: digestible energy = carbohydrate: 2.5 kcal DE; protein: 3.5 kcal DE, fat: 8.1 kcal DE (NRC, 1993).

according to Putra (2014), i.e 2% (g/100 g) sweet potato extract. Then, the composition of synbiotic in this study referred to the previous dose of Putra *et al.* (2015), i.e 1% *Bacillus* NP5 (g/100 g) and 2% sweet potato extract (g/100 g).

Diets and experimental design

The composition of the experimental diets is described in Table 1. The commercial feed used in this study contained identical nutrient content (protein, lipid, energy, moisture) for all treatments. The present study consisted of four treatments and three replications, including control, feed with probiotic 1%, prebiotic 2% and, synbiotic (probiotic 1% + prebiotic 2%). The commercial feed was mashed into the meal, then 3% of tapioca added as a binder in each feed. Furthermore, sweet potato extracts of 2% were added to prebiotic and synbiotic feed before the pelleting process. Probiotic added to the feed by spraying using a syringe with adding egg yolk of 2% (Putra *et al*., 2015). Feeding was done three times a day using at satiation feeding method. The tested fish (*Clarias* sp.) used in this study had an average weight of 10.03 ± 0.05 g at a density of 200 fish per tank. The experimental fish were reared in a round tank with a diameter of 200 cm for 45 days. Replacement of water in the rearing aquarium as much as 50% of total volume (200 L) were conducted every 2 days to maintain water quality.

Experimental parameters

Samples (diets and fish) were analyzed for dry matter, protein, lipid, moisture, nitrogen-free extract (NFE), and ash using a standard method (AOAC, 1995). Samples of fish were dried to a constant weight at 105°C for 6 h to determine moisture. Protein was determined by measuring nitrogen using the Kjeldahl system method, lipid by ether extraction using Soxhlet, and ash by combustion at 550°C for 24 h. All chemical analysis was performed in duplicate and averaged. The data obtained from the trial were analyzed for survival rate (SR) and specific growth rate (SGR), that were determined according to Huisman (1987). Feed conversion ratio (FCR), nutrient (protein and fat) retention, and protein efficiency ratio (PER) (Takeuchi, 1988) using the following formula.

$$FCR = \frac{\text{Feed consumption}}{\text{Weight gain}} \times 100$$

$$\text{Nutrient retention} = 100 \times \frac{\text{Final body nutrient-initial body nutrient}}{\text{protein intake (g)}}$$

$$PER = \frac{\text{Wet weight gain}}{\text{Protein intake}} \times 100$$

$$SGR\ (\%/day) = \frac{100\ (\text{Ln final weight - Ln initial weight})}{\text{days}} \times 100$$

$$SR = \frac{\text{Total individuals at the final}}{\text{Total individuals at the initial}} \times 100$$

Digestive enzymes activity

The enzyme activities measured were the activity of protease and lipase according to the method by Bergmeyer and Grassi (1983). To measure digestive enzyme activity, intestine samples of catfish were homogenized in an ice-cold buffer. At the end of the experiment, five

Table 2. Effects of dietary *Bacillus* NP5 and sweet potato extract on growth of dumbo catfish.

Parameter***	Treatments (g/100 g)*			
	Control	1% probiotic	2% prebiotic	Synbiotic**
IW (g)	10.04 ± 0.05	10.07 ± 0.04	10.04 ± 0.05	10.05 ± 0.05
FW (%)	97.40 ± 1.28[a]	115.04 ± 1.05[b]	114.67 ± 2.08[b]	139.90 ± 0.17[c]
PR (%)	32.10 ± 1.09[a]	38.70 ± 1.86[b]	40.22 ± 1.53[b]	48.02 ± 1.30[c]
FR (%)	53.51 ± 2.12[a]	62.41 ± 1.34[b]	62.37 ± 0.57[b]	62.63 ± 1.47[b]
SGR (%/day)	4.87 ± 0.02[a]	5.25 ± 0.03[b]	5.26 ± 0.04[b]	5.67 ± 0.03[c]
FCR	1.50 ± 0.02[c]	1.31 ± 0.01[b]	1.30 ± 0.02[b]	1.09 ± 0.01[a]
PER (%)	2.13 ± 0.03[a]	2.45 ± 0.02[b]	2.46 ± 0.05[b]	2.95 ± 0.04[c]
SR (%)	92.33 ± 0.29[a]	92.50 ± 0.50[a]	92.50 ± 0.50[a]	92.33 ± 0.58[a]

*The value in the same row followed by similar superscript letters are not significantly different (P>0.05).
**(1% probiotic *Bacillus* NP5 + 2% prebiotic)
***Initial weight (IW), final weight (FW), protein retention (PR), fat retention (FR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate (SR) of dumbo catfish.

catfish from each tank were taken. The entire alimentary tract was quickly removed and placed on ice. The intestines were washed thoroughly with chilled saline and then dried quickly on a piece of filter paper and weighed. Furthermore, they were homogenized into 10 volumes (w/v) of ice-cold distilled water at 1,000 ×*g* for 5 min, respectively. Homogenates were centrifuged at 10,000 ×*g* for 30 min at 4°C to analyze protease and amylase activity and, 1,660 ×*g* for 20 min at 4°C to analyze lipase activity.

Statistical analysis

All statistical analysis was carried out using the statistical package for the social sciences (SPSS 16.00) program for Windows. Data were expressed as the average of three replicates and was compared using ANOVA. Where significant differences were found, the means within each treatment and among treatments were compared using Duncan of multiple comparisons with a 95% significant level.

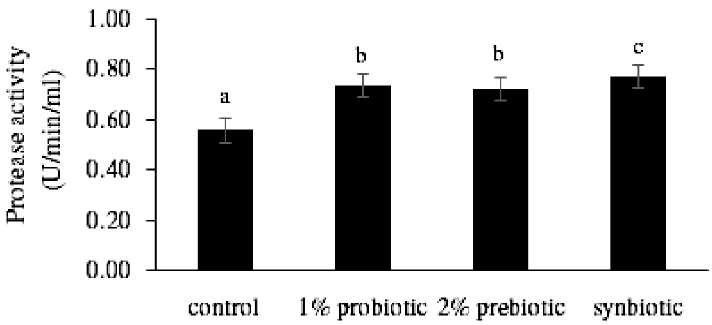
RESULTS AND DISCUSSION

Growth

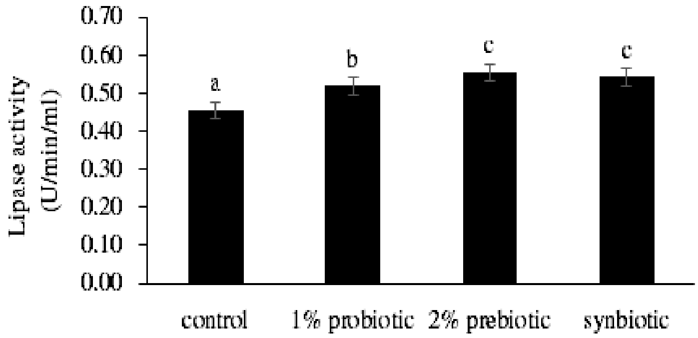
Growth performance of dumbo catfish fed experimental diet is shown in Table 2. The final weight of fish in synbiotic treatment was of 139.90 ± 0.17 g and it was significantly higher (P<0.05) than the other treatments. The protein retention was highest in synbiotic (48.02 ± 1.30%) and the lowest was in control (32.10 ± 1.09%). Furthermore, there was no difference in protein retention between probiotic (38.70 ± 1.86) and prebiotic (40.22 ± 1.5%) treatment. Fat retention was significantly higher in synbiotic treatment (62.63 ± 1.47%) compared to the control (53.51 ± 2.12%), but there was no difference between probiotic, prebiotic, and synbiotic treatment. The addition of synbiotic to diets also produced the best specific growth rate, with statistically values better than the other treatments (P<0.05). The value of specific growth rate was significantly highest (P<0.05) in synbiotic (5.67 ± 0.03 %/day), followed by prebiotic (5.26 ± 0.04 %/day), probiotic (5.25 ± 0.03 %/day) and the lowest in the control (4.87 ± 0.02 %/day). The value of feed conversion ratio (FCR) in synbiotic (1.09 ± 0.01) was lower (P<0.05) than that of the other treatments. Protein efficiency ratio (PER) of fish in probiotic, prebiotic and synbiotic treatments showed significant higher (P<0.05) than the control one. The survival rate of feeding trial in controls, probiotic, prebiotic and synbiotic showed no significant different (P>0.05).

Enzyme activity

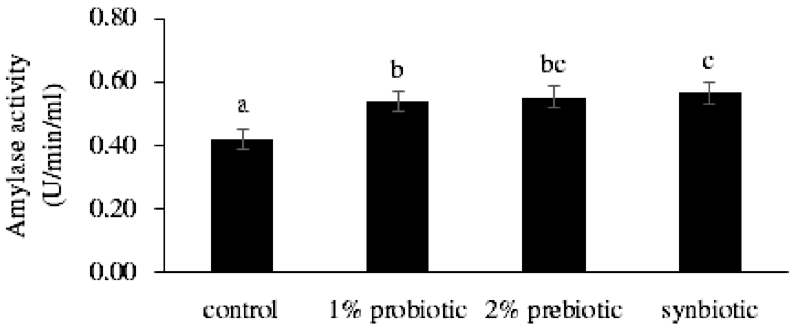
After cultured for 45 days, the average digestive enzyme activities in probiotic, prebiotic, and synbiotic treatment were significantly different from that of the control (Figure 1). The protease activity was significantly higher in synbiotic (0.77 ± 0.01 U/min/mL) compared with probiotic (0.73 ± 0.02 U/min/mL), prebiotic (0.72 ± 0.01 U/min/mL), and control (0.56 ± 0.01 U/min/mL) treatment, but there was no significant (P>0.05) difference between probiotic and prebiotic (Figure 1). Lipase activity was significantly (P<0.05) higher in synbiotic (0.54 ± 0.01 U/min/mL) and prebiotic (0.56 ± 0.02 U/min/mL) compared with probiotic (0.52 ± 0.01 U/min/mL) and control (0.46 ± 0.01 U/min/mL). Amylase enzyme showed higher activity (P<0.05) in probiotic, prebiotic, and synbiotic treatment compared with control. However, there



(i)



(ii)



(iii)

was no difference (P>0.05) between probiotic and prebiotic treatment.

Discussion

Probiotic, prebiotic, and synbiotic have been proven to improve growth and physiological aspects in various aquaculture species. Probiotic, prebiotic, and synbiotic supplemented diets resulted in better growth performances than that of the control (Table 2). Similar results were observed by Daniels *et al*. (2010) in the European lobster (*Homarus gammarus* L.) larvae, Ai *et al*. (2011) in juvenile large yellow croaker, Putra *et al*. (2015) in tilapia, Nurhayati *et al*. (2015) in white shrimp, and Kumar *et al*. (2017) in *Cirrhinus mrigala* (Ham.) fingerling. In the present study, the combination of *Bacillus* NP5 and prebiotic of extracted sweet potatoes var. sukuh (*Ipomoea batatas*) has appeared to improve growth performance in terms of FW and SGR, and also for PR, FCR, PER, and protease activity in dumbo catfish.

Nutrients retention is the amount of nutrient which could be absorbed from the feed and stored in the fish body. In the present study, the supplementation of synbiotic showed higher protein retention as compared to the control and other treatments. Similarly, fat retention value was higher than the control. This result is supported by the study of Putra *et al.* (2015) who observed a positive effect of synbiotic on growth performance in tilapia. Marlida *et al*. (2015) noted that synbiotic application can improve growth, digestive enzyme activity and health status of humpback grouper. The addition of probiotic and prebiotic in feed intended to increase the population of microbiota in the digestive tract of host, so that the action mechanism of microbiota in producing enzymes for digestion will increase (Merrifield *et al*., 2010), and will improve the amount of nutrient which could be absorbed by fish. In this study, there was no difference in fat retention between prebiotic and synbiotic treatment, indicating that the amount of lipase enzyme activity in the digestive tract has no difference. This is indicated that prebiotic and synbiotic treatment have a similar effect to increase lipolytic bacteria on the digestive tract of catfish. This is shown by the activity of the lipase enzyme which was no significance between prebiotic and synbiotic treatments. One potential mechanism of probiotic and prebiotic is the colonization of beneficial bacteria (Buentello *et al.,* 2010). The beneficial bacteria can produce digestive enzyme such as protease, lipase, and amylase which have to function is to digest macronutrients from feed into a form that can be absorbed by fish (Nimrat *et al*., 2013).

The increased activity of retention nutrient has a positive correlation to SGR, FCR, and PER. Synbiotic treatment showed the best value in SGR, FCR, and PER compared with other treatments. Kumar *et al*. (2017) also showed that the combined supplementation of *Bacillus subtilis* (100 g/kg) and MOS (4 g/kg) as synbiotic enhanced % weight gain, SGR, FCR, and PER of about 75.80, 0.94, 2.66, and 1.06%, respectively, compared to the control, in *Cirrhinus mrigala* (Ham.) fingerling. These findings were also similar to the previous study by Daniels *et al*. (2010) who showed that application of a commercial probiotic (*Bacillu*s spp.) and mannan oligosaccharides (MOS) as synbiotic, could increase SGR of European lobster (*Homarus gammarus* L.) larvae. The increased activity of the digestive enzymes could help the host in degrading nutrients, improving digestibility and increasing feed efficiency (Cerezuele *et al.*, 2011). The study of Nurhayati *et al*. (2015) in white shrimp showed that after 30 days, the combination of 1% probiotic and 2% prebiotic supplemented with the feed to the shrimp culture improved the daily growth rate and decreased FCR.

The combined application of probiotic and prebiotic is known as synbiotic, which work by improving the survival and colonization of live microbial dietary supplements (probiotic) in the gastrointestinal tract which can promote health and thus improve the welfare of the host (Daniels *et al*., 2010). In this study, there was no difference in survival rate among treatments, indicating that the addition of probiotic, prebiotic and synbiotic to feed did not affect to fish health. The similar result reported by Akrami *et al*. (2015) who found the addition of synbiotic showed no difference in the survival rate of beluga (*Huso huso*) compared to the control.

The combined use of probiotic and prebiotic is a new concept in aquaculture and the present study has not only highlighted significantly improved catfish growth performance in comparison to unsupplemented diets, but also demonstrated even greater success in digestive enzyme activity (protease, lipase, and amylase activity). In the present study, the addition of probiotic, prebiotic, and synbiotic to feed resulted in better digestive enzyme activity than that of the control (Figure 1). A similar result has been reported by Putra *et al*. (2015) in tilapia and Munir *et al*. (2016) in snakehead *Channa striata* fingerlings. The supplementation of synbiotic showed higher protease activity as compared to the control and the other treatments. A similar result also occurred in the study on the addition of synbiotic to feed which could improve protease activity in *Cirrhinus mrigala* (Ham.) fingerling (Kumar *et al*., 2017). The higher level of enzyme activity obtained with diets containing probiotic improved the digestion of protein, starch, and fat which might, in turn, explain the better growth performance observed with the synbiotic supplemented diets.

Probiotic and prebiotic beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Dawood & Koshio, 2016). *Bacillus* NP5 is probiotic bacteria isolated from the digestive tracts of tilapia which has the activity of amylase (Putra & Widanarni, 2015), protease, and lipase (Putra *et al*., 2015). In this study there was no difference in growth performance parameter (FW, PR, FR, SGR, FCR, and PER) and digestive enzyme activity (protease, lipase, and amylase activity) between probiotic and prebiotic treatment, indicating that the addition of probiotic and prebiotic to fed did a similar effect to increase the population of microbiota in the digestive tract of catfish. The population of these microbiota depends on the survival capability, colonization, and nutrient competition, and environmental changewhich caused bacteria living in the digestive system be washed out

(Goubeyre *et al*., 2011). Similar effects had been reported by Daniels *et al*. (2010) that no difference in FW, live weight gain, SGR, and FCR between probiotic and prebiotic treatment in European lobster *Homarus gammarus* (Linn.) larvae.

CONCLUSION

In conclusion, the addition of probiotic, prebiotic, and synbiotic to diets showed significant result on final weight, specific growth rate, feed conversion ratio, protein retention, protein efficiency ratio, and enzyme activities compared to the control (P<0.05). These results revealed that the combination of *Bacillus* NP5 1% and prebiotic 2% (extracted from sweet potatoes *Ipomoea batatas* var. sukuh) as synbiotic in feed showed the best result compared to other treatments and it can be applicated for dumbo catfish culture.

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