Effect of Bacillus spp. and Nitrosomonas sp. in commercial feed as a probiotic agent to increase growth performance and feed efficiency of sangkuriang satfish (Clarias gariepinus)

A A Yaquín¹, Sudarno¹, and Rozi¹, ²

¹Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, Indonesia

* corresponding author E-mail: rozi@fpk.unair.ac.id

Abstract. The purpose of this research was to know the combination effect of Bacillus spp., Nitrosomonas sp., that added to the commercial feed to feed conversion and growth on Sangkuriang Catfish. The method used in this experiment was design with 3 treatments and 3 replications. Samples (24.49±1.21 g) were a stocking density of 1,500 fish/pond and maintained for 30 days. The treatment applied was P0 treatment (feed without probiotic), P1 (5.0 x 10⁷ CFU/gram feed of probiotic was sprayed), and treatment P2 (5.0 x 10⁹ CFU/gram feed of probiotic is sprayed). The best treatment was probiotic in treatment P1 quantitatively made a real effect in growth and made a better feed efficiency than other treatments, with FCR (0.7±0.57), SGR (4.33±0.11), EPP (109.61±9.69), SR (96.91±0.45), DWG (2.07±0.18). Water quality of culture media was in the range of reasonable level of cultivation C. gariepinus. Adding probiotic P1 could be an effective alternative to increase growth performance and feed efficiency on C. gariepinus.

1. Introduction

Sangkuriang catfish (Clarias gariepinus) is one of superior commodity of consumable freshwater fish that has a promising prospect and starts to take an interest to cultivation businessmen. Catfish production in Indonesia during 2005-2009 shows an increase in production in an average of 31.55% per year [1]. According to [2], the probiotic is a product composed of microbe culture or natural microscopic feed which is beneficial and makes effects on microbe balance in the host intestinal tract.

One of the efforts to increase fish physiological function, especially its ability to digest feed, is adding probiotics in the feed in hope that the probiotic can be taken along into the digestion tract. According to [3] and [4] stated that probiotics are useful in managing the microbial environment in the intestine, preventing pathogenic microorganism and fixing feed efficiency by releasing enzymes which help in the food digesting process. Hence, the present experiment was conducted to study the effect of the combination of Bacillus spp. and Nitrosomonas sp. in commercial feed to increase growth performance and feed efficiency of C. gariepinus.

2. Materials and methods

2.1 Sampling fish

The fish used in this experiment was Sangkuriang Catfish (C. gariepinus) with 15.82 ± 0.38 cm in size and the average weight of 24.49 ± 1.21 gram which was taken from UK BAT Wonocatur. The
research was performed in UK BAT Wonocatur, Aquaculture Technology Development Center in Cangkringan Village, Yogyakarta. The test was done in a concrete pond with a diameter of 2.6 m and 2 m high with a water level of 1.5 m and the density level used was 1,500 fish/pond. There were nine ponds used in this research. The pond set up consisted of two steps. The first step was cleaning the pond area and drying for ± 2-3 hours. The second step was filling the pond with a volume of 5,500 liters of water.

2.2 Culture and fermentation
The bacteria in commercial probiotic was activated by culturing feed fermentation. It is done by mixing all ingredients used in the probiotic fermentation, they were 10 liters of water, 10 grams of probiotic feed powder, 2.5 grams of yeast, and 130 ml molasses. This solution was sprayed using the sprayer on 4 kg of fish food (protein level 34%, lipid 6%, crude fiber oil 4.3%, ash 11%, and water 12%), then it was kept in the closed bucket and let sit for 2 days. The feed from the fermentation would be soft in texture, smelled like fermented cassava, and the colour was white because of the fungi.

2.3 Bacteria probiotics
The method used in the procedure of this research was experimental with Completely Randomized Design of three treatments and three replications. Two different kinds of probiotics (CV. Nutrindo Jaya Abadi, Indonesia) were used in the treatments, and the other treatment was without probiotics (control). P0 was the treatment without adding probiotics (control); P1 was the treatment by adding Herbac aquatic probiotics (Bacillus subtilis, Bacillus meganterium, Bacillus polimyxa, Nitrosomonas sp., dan enzym amylase) with a concentration of 5x10^7 cfu/g; P2 is a treatment by adding Growbac probiotics (Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium logum, dan Streptococcus faecalis) with a concentration of 5x10^9 cfu/g. The fish were fed with probiotic feed twice a day (at 08.00 in the morning and 4.00 in the afternoon) for 30 days with a 4% of feeding rate (FR) of biomass per day.

2.4 Analysis Data
Variance Analysis was applied to evaluate which probiotic was different from feed conversion ratio (FCR). Specific growth rate (SGR), Efficiency of feed utilization (EPP), Survival rate (SR), Absolute length, Absolute growth, and Daily weight gain (DWG) were analyzed using Analysis of Variance (ANOVA) with a confidence interval of 95% (p < 0.05). If there is a difference, the analysis is then continued to Duncan’s Multiple Range Test (DMRT).

3. Results and discussion
The result of the experiment showed that treatment P1 made the best result among other treatments. With the addition of probiotic P1 (Bacillus spp., Nitrosomonas sp.) and (P< 0.05), there was a significant difference to treatment P1 compared to treatment P0 and P2. They were FCR, SGR, EPP, SR, and Absolute length (P< 0.05), while Absolute growth and DWG were not significantly different (P> 0.05) on Sangkuriang Catfish (C. gariepinus) (Table 1).
Figure 1. The average growth in terms of Length (a) and Weight (b) in sangkuriang catfish *C. gariepinus*. The treatment of different probiotic applied sangkuriang catfish (*C. gariepinus*) for 30 days of ponds culture. P0 treatment (feed without probiotic), P1 (5.0 x 10^7 CFU/gram feed of probiotic was sprayed), and treatment P2 (5.0 x 10^9 CFU/gram feed of probiotic is sprayed).

This result is in accordance with the previous research that an addition of *Bacillus* sp. in the feed is proven to increase DWG, WG, FCR, EPP, and SGR on nile tilapia [5], gold fish [6], and groper fish [7]. Probiotic P1 addition in commercial feed can trigger activities of digestion enzyme and increase food absorption for fish growth (Figure 1). In accordance with Gomez [8] it explains that *Bacillus* spp. will increase food absorption and takes part in the weight gain. The increase of food absorption is caused by microbe balance in digestion tract. It is in accordance with [9] which explains that bacterial activities in digestion will change rapidly if the microbes get in along with food or water and cause a change in bacterial balance in the digestion tract. According to [10], *Bacillus* sp. is one type of bacteria which is believed to be able to increase digesting ability of fish. It also takes part digesting process to break nutrition such as carbohydrate, protein, and lipid by producing an extracellular enzyme [11].

**Table 1.** Mean and standard deviations of feed conversion ratio (FCR), specific growth rate (SGR), efficiency of feed utilization (EPP), survival rate (SR), absolute length, absolute growth, daily weight gain (DWG) of sangkuriang catfish (*C. gariepinus*) in different probiotic treatments after 30 days of ponds culture.

| Parameter | The result of treatments |
|-----------|--------------------------|
|           | P0          | P1          | P2          |
| FCR       | 1.60±0.10^a | 0.70±0.57^a | 1.00±0.57^b |
| SGR       | 3.17±0.09^a | 4.33±0.11^c | 3.90±0.09^b |
| EPP       | 39.77±2.54^a | 109.61±9.69^c | 80.71±2.70^b |
| SR (%)    | 84.16±2.22^a | 96.91±0.45^c | 90.57±2.40^b |
Absolute length 3.73 ±0.14<sup>a</sup> 6.23 ±0.22<sup>b</sup> 3.83 ±0.13<sup>a</sup>
Absolute growth 35.00±2.24<sup>a</sup> 57.88±5.00<sup>b</sup> 52.46±1.75<sup>b</sup>
**DWG** 1.25±0.08<sup>a</sup> 2.07±0.18<sup>b</sup> 1.87±0.06<sup>b</sup>

Note: Average values with letter (a, b, c) show significant difference on **Duncan’s Multiple Range Test (DMRT)** with confidence level of 5% (p<0.5). Average values with the same letter show insignificant difference, while the values with different letter show significant difference.

**Table 2.** Observation of water quality during the enlargement of sangkuriang catfish (*C. gariepinus*).

| Parameter | Result | References |
|-----------|--------|------------|
| P0 | P1 | P2 |
| pH | 7.3 | 7.1 | 7.2 | 6.5 - 8.5 | [12] |
| Temperature (°C) | 26 | 26 | 28 | 27-30 °C | [12] |
| DO (mg/ L) | 5.4 | 5.9 | 5.5 | > 5 mg/ L | [12] |
| Ammonia (mg/ L) | 1.2 | 0.12 | 0.3 | <0.2 mg/ L | [13] |

Water quality of cultivation media is in the range of suitable for sangkuriang catfish (*C. gariepinus*) cultivation. All treatments (P0, P1, P2) has pH between 7.23 - 7.5; temperature between 25°C - 27°C; DO between 5.4 - 5.9 mg/l; and ammonia 0.08 – 0.12 ppm (Table 2). However, probiotic P1 produces a better quality of water than probiotic P0 and P2, especially the decrease in ammonia. It is caused by *Nitrosomonas* sp. in probiotic P1 can degrade organic substance on cultivation media optimally, so that ammonia level in the cultivation media in treatment P1 is lower than the other treatments, which is about 0.12 mg/L. The decreasing level of ammonia in treatment P1 is caused by nitrification process by *Nitrosomonas* sp. in probiotic P1, so that ammonia reduction process becomes faster than the treatment without probiotic bacteria. On the other side, there aren’t any nitrification bacteria in probiotic P0 and P2. Ammonia is used as an energy source for nitrification bacteria (*Nitrosomonas* sp.) and oxidized into nitrite [14]. In treatment P1, it can grow well because it uses nitrate in cultivation media, therefore organic substance in cultivation media can be well accumulated and stabilized water quality. This accord with the earlier experiment by [15], which explains that poor water condition in fish cultivation affects biomass and fish health.

**4. Conclusion**

Adding probiotic P1 (*Bacillus* spp., and *Nitrosomonas* sp.) with the concentration of 5x10<sup>7</sup>cfu/g feed had a positive effect on growth, feed utilization and water quality on sangkuriang catfish (*Clarias gariepinus*) (p < 0.05). The result showed that by adding probiotic P1 could be an effective alternative to increase growth performance and feed efficiency on *C. gariepinus*.

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Acknowledgments
We are thankful to our team of researchers, specially technicians in the ponds for their valuable helps with fieldwork and sample collection.