The growth performance and survival rate of catfish (Clarias sp.) that given probiotic controlling nitrogen

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Abstract. The water quality can be improved by using probiotics as bioremediation. The probiotics are a consortium of nitrification and denitrification bacteria (Pseudomonas aeruginosa and Achromobacter insuavis), which both of the indigenous bacteria are the products of the Research Institute for Freshwater Aquaculture and Fisheries Extension (RIFA). The objective study is to determine the adding frequency of RIFA probiotic into the water fish pond of catfish culture concerning growth performance and survival. The catfish fingerling with a size of 7-8 cm long and weighing 16.45 ± 0.3 g was used. The stocking density was 750 fish/pond. Nine plastic sheet ponds with a water volume of 1.6 m³ and completed with aeration were used. A dose of probiotics was 10 mL/m³. The difference in adding the frequency of probiotic as a treatment was as followed: A) every 5 days; B) every 10 days, and C) without probiotics. The parameters measured survival rate (SR), absolute weight growth, specific growth rate (SGR), feed conversion ratio (FCR), water quality. One way ANOVA with three treatments and three replications were performed. The results showed that the use of probiotics could control nitrogen compounds in catfish cultivation, where the frequency of administration every 5 days gives the best results.

1. Introduction
Fish as a protein source is one of the commodities to support national development. The capture fishing production has declined and unstable. Therefore, fish production from fish culture is mainly considered to keep the fish demand to fulfill food safety in Indonesia. The main freshwater fish species in Indonesia are tilapia, common carp, gourami, and catfish. The catfish production was achieved about 873,716 ton in 2016 [1].

Some efforts have been made to improve catfish production. The culture system of catfish, Clarias in Indonesia consists of three systems, i.e. traditional, semi-intensive, and intensive systems [2]. The three systems have an advantage and disadvantage in terms of fish production. The conventional system is low stocking density and low production, but the waste from the culture activity is also low. Thus this system has an advantage concerning environmental impact. The semi-intensive system is in the middle, and it means that the stocking density is higher than the extensive system. Thus, fish production is growing better. However, the semi-intensive system has a small contribution to the environmental impact because of waste and uneaten feed and degraded by nature, even though the
waste of a semi-intensive system is higher than the traditional system. An intensive system is the most used by the fish farmers due to high fish production. Based on the economic perspective, an intensive system has a good income [3].

Nevertheless, the negative impact on the environment, especially, aquatic environment such as water surface and groundwater, also the land of the surrounding area, will be a direct impact on destructions. Thus, the culture system should be sustainable and environmentally friendly. The waste products from intensive aquaculture are nitrogen (N) and phosphor (P). The nitrogen waste derived from the metabolic of fish such as fesses and urine, and the other is from uneaten food. All those materials will be accumulated in the fish pond and make the water quality decrease. When the water and sediment from the fish pond throw out directly into the river, eutrophication might have happened. This condition appears because the water from the fish pond contains a high concentration of nitrogen and phosphorus that make the algae bloom rapidly. Nitrogen compounds in the water can be formed as ammonia, nitrite, and nitrate, where ammonia and nitrite are toxic to aquatic organisms, including fish.

Implementation of microorganisms in the aquaculture to mitigate the pollution is called probiotic. The bacteria has a function to control a nitrogen compound in the water [4]. The RIFA probiotic has been isolated and identified. This probiotic can be used for controlling nitrogen compounds in the fish pond. The nitrification and denitrification bacteria are Pseudomonas aeruginosa and Achromobacter xylosoxidans [5]. The utility of local nitrification bacteria is better than other sources to decrease the nitrogen compound because of its adaptability [6]. Pseudomonas sp is the dominant bacteria as a nitrification process. Thus, this species is the most used for probiotic, even in the wastewater treatment industry [7]. On the other hand, this bacteria has resistance to antibiotic and Cd heavy metal [8] and also can oxidize the ammonia in shrimp and freshwater giant prawn [9, 10].

The objective of this experiment is to determine the adding frequency of the RIFA probiotic into the water fish pond of catfish culture concerning the growth performance and survival rate.

2. Materials and Methods
The experiment was carried out from July to August 2018 on the fish farm at Palasari Village, Cijeruk District, Bogor, West Java, Indonesia. Nine fish ponds were used. Each pond was covered by a plastic sheet and completed by aeration. The fish pond was filled by water ground up to 1.6 m³. No water exchanged during the culture period, except due to the evaporation. Fish size ranged from 7-8 cm in total length and 16.45 ±0.3 g in body weight. The stocking density was 750 fish/pond. Fish were fed by an artificial diet with a feeding rate of 4% a day. The artificial diet contained 35% protein. The feeding frequency is three times a day (08.00 am; 12.00 am; and 05.00 pm). The catfish were cultivated for 60 days. 50 fish were sampled every 10 days to measure body length and body weight. The dose of RIFA probiotic was 10 mL/m³. One milliliter probiotic contained 10¹⁶ CFU/mL. Applying probiotic was conducted every 10 days. The parameters observed were survival (SR), absolute weight growth, specific growth rate (SGR), and feed conversion ratio (FCR). Water quality parameters (temperature, pH, dissolved oxygen/DO, ammonia, nitrite, and nitrate) were measured every ten days as supporting data.

The treatments were adding the frequency of probiotics to the fish pond i.e., A) every 5 days; B) every 10 days; and C) without probiotic. Each treatment consisted of three replications. The completed randomized design applied with three treatments and three replications. All the data were analyzed using ANOVA, and if any differences found, then continued to Duncan test. The water quality parameters were analyzed descriptively.

3. Results and discussion
The survival rate, absolute weight growth, SGR, and FCR of catfish cultured with a different adding frequency of probiotic presents in Table 1.
Table 1. The survival rate, absolute weight, SGR, and FCR of catfish cultured for 60 days

| Parameter          | Frequency of adding probiotic |   |   |
|--------------------|-------------------------------|---|---|
|                    | Every 5 days                  | Every 10 days | Control |
| Survival rate (%)  | 89.91±1.59<sup>b</sup>        | 82.40±8.77<sup>a</sup> | 80.76±7.11<sup>a</sup> |
| Absolute weight (g)| 68.98±3.10<sup>a</sup>        | 57.80±5.10<sup>b</sup> | 41.32±3.99<sup>c</sup> |
| SGR (%)            | 2.67±0.19<sup>a</sup>         | 2.45±0.14<sup>b</sup> | 2.26±0.24<sup>c</sup> |
| FCR                | 0.83±0.13<sup>a</sup>         | 1.03±0.052<sup>b</sup> | 1.7±0.283<sup>c</sup> |

Remarks: The values followed by the same superscript are not significantly different (P>0.05).

The result (Table 1) showed that the survival rate (89.91±1.59%) on every 5 days adding probiotic was the highest, followed by every 10 days (82.40±8.77%), and without probiotic (80.76±7.11%). The highest of absolute weight (68.98±3.10 g) was found on every 5 days adding probiotic and then followed by 10 days adding probiotic (57.80±5.10 g) and without probiotic (41.32±3.99 g). The specific growth rate (SGR) every 5 days adding probiotic was the highest (2.67±0.19%), while the lowest (2.26±0.24%) found at control (without adding probiotic). The feed conversion ratio on every 5 days adding probiotic was the lowest (0.83±0.13) while the highest found at control (1.7±0.283). Statistical analysis revealed that all the data such as the survival rate, absolute weight, SGR, and FCR were significantly different among the treatments, except for the survival rate showed that adding probiotic on every 10 days was not significantly different compared to control.

Table 2. Water quality parameters during the cultured catfish period

| Parameter | unit | Every 5 days | Every 10 days | control | Recommendation |
|-----------|------|--------------|---------------|---------|----------------|
| Temperature | °C  | 23.7 - 27.7  | 23.7 - 27.5   | 23.7 - 27.8 | 25-30 [11]       |
| pH        | mg/L | 6.3 - 7.19   | 6.3 - 7.42    | 6.4 - 7.34 | 6.5-8.0 [11]     |
| DO        | mg/L | 3.11 - 4.12  | 3.21 - 4.12   | 2.04 - 3.12 | ≥ 3.0 [11]       |
| Ammonia   | mg/L | 0.001 - 0.09 | 0.001 - 0.013 | 0.24 - 0.3  | < 0.1 [11]       |
| Nitrite   | mg/L | 0.001 - 0.06 | 0.001 - 0.07  | 0.57 - 1.15 | < 0.06 [12]      |
| Nitrate   | mg/L | 1.79 - 2.85  | 1.79 - 2.87   | 1.79 - 5.79 | < 20 [12]        |

The water quality parameters such as temperature, pH, DO, ammonia, nitrite, and nitrate showed that the DO, ammonia, nitrite, and nitrate at the control were exceeded the threshold for catfish culture while at adding probiotic every 5 and 10 days were within in the recommended range (Table 2).

4. Discussion

Probiotic is containing most of the microorganisms such as *Lactobacillus*, *Bacillus*, *Nitrosomonas*, and *Nitrobacter* that increasing waste decomposition and improving water quality [13]. Adding probiotic in the water pond can increase the immune response against diseases, improve the digestive system, accelerate the nitrification and denitrification processes [14] and also improve growth and survival rate [15]. The present experiment showed that the survival rate, absolute weight, SGR was the highest in treatment A, but the FCR was the lowest (Table 1). This matter indicated that the probiotic could improve survival, growth, and feeding conversion ratio. A similar result has been reported that *Pseudomonas synxantha* and *Pseudomonas aeruginosa* can improve growth, survival, and decrease the FCR of western king prawn (*L. vannamei*) [16]. The probiotic utility was able to improve the survival rate and decreased the FCR up to 0.8 [17].

The water quality parameters such as temperature, DO, ammonia, nitrite, and nitrate at the pond with applying probiotic were within the optimal range while the pond without probiotic (control)
(Table 2). This condition indicated that the probiotic had played an important role in water quality parameters. The application of probiotics in the water pond can improve the water quality because it can convert the toxic compound such as ammonia and nitrite to non-toxic compounds such as nitrate [18]. The present experiment shows that adding probiotic in the water pond can reduce the toxic compound derived from uneaten food, feces, and urine. *Pseudomonas tolaasii* bacteria would be able to the removal of ammonium, nitrite, and nitrate, where this bacteria had tolerated water temperature [19]. Thus, the present experiment shows that the probiotic used that containing *Pseudomonas* sp. could reduce the ammonia and nitrite concentration in the pond.

5. Conclusion

The adding of RIFA probiotic contains *Pseudomonas* sp. bacteria in the water pond with frequently on every 5 days is the best way for improving survival, growth, and water quality of catfish culture. Besides, applying probiotics can also reduce the FRC value up to 0.8.

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