Application of environmental probiotic on rearing snakehead fish (Channa striata)

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Abstract. This study aims to evaluate the application of environmental probiotic on production performance (survival rate, absolute weight and length, biomass weight and specific growth rate and blood condition level in snakehead fish (Channa striatus) rearing. The research was conducted at the Cibalagung Environmental Research and Toxicology Installation, Bogor, Indonesia. The experiment was conducted in a completely randomized design with three treatments, including RIFA probiotic, commercial probiotic, and without probiotic application repeated three times each experiment. Fish (weight: 6.5-6.9 ± 0.04 g) were reared in 300 L tanks with density of about 30 fish/tank. Fish were fed with commercial feed (31% protein content) about 5% biomass per day. The parameters observed were production performance, hematology, and water quality. All data were analyzed using ANOVA and continued by the Duncan test. Water quality parameters were analyzed descriptively. The results showed that RIFA probiotic treated on snakehead fish produced the best production performance compared to commercial probiotic and without probiotic applications. These results were supported by much better water quality parameters and hematology performance of the fish.

1. Introduction
Excessive feeding in intensive aquaculture of the raceway system is the main problem for the environment as it commonly produces feed waste that pollutes aquatic habitat. The use of probiotics in aquaculture media can be utilized to reduce the level of pollution and improve water quality [1-2] as well as increase production [3]. The use of probiotics is one alternative to overcome water quality problems in aquaculture adapted from conventional domestic waste treatment techniques [4]. Probiotics are live and beneficial bacteria that are deliberately implemented to ponds to improve fish growth.

Snakehead (Channa striatus) is a native freshwater fish commodity that has high economic value [5]. Aside from being a fish food, this species contains albumin commonly used for pharmaceuticals row materials, especially in the process of healing wounds. Snakehead albumin can be used as an alternative source to replace Human Serum Albumin (HSA), which is very expensive [6]. However, there are only a few farmers involve in snakehead aquaculture as the aquaculture technology for this species has not been well established. Thus, to support the aquaculture program, it is necessary to produce seeds continuously. Seed production can be increased by an intensive fish breeding program, including using environmental probiotics for improving water quality. Several studies have shown that the applications of probiotics in aquaculture play vital roles in improving water quality, biosecurity, productivity, feed efficiency, and decrease cost production through feed cost reduction [4,7-9].
RIFA probiotic has been successfully isolated and well-identified. This probiotic can be used for controlling nitrogen compounds in the fish pond. The nitrification and denitrification bacteria are *Pseudomonas aeruginosa* and *Achromobakter xylosoxidans* [10]. This study aims to evaluate the application of environmental probiotic on production performance (survival rate, absolute weight and length, biomass weight and specific growth rate) and blood condition level in snakehead (*Channa striatus*) rearing.

2. Materials and methods
The study was conducted at the Cibalagung Environmental and Toxicology Research Installation, Bogor, Indonesia. The experiment was conducted using a completely randomized design with three treatments, namely: (1) RIFA probiotics, (2) commercial probiotics, and (3) without probiotics. Each treatment was repeated three times. The dose of probiotic was 10 ml/m³ applied to snakehead fingerlings (6.5-6.9 ± 0.04 g) every 10 days. One milliliter probiotic contained 10¹⁰ CFU/ml. The stocking density was about 30 fish/tank. No water exchange was conducted during the culture period, but water was added only to replace evaporation. The fingerlings were reared in circular fiber tank of 300 liters. Fish were fed with commercial feed (31% protein content) about 5% biomass per day given three times a day (08.00 am; 12.00 am; and 17.00 pm). The parameters observed were production performance (survival rate and growth rate), hematology, and water quality. Data on fish length and weight were obtained by weighing the fish every 10 days, and weight adjustment was conducted every 10 days. Measurement of water quality and stocking of probiotics was done every 10 days. The calculation of fish survival rate was conducted at the end of the study. Hematology parameters included blood glucose, hemoglobin, hematocrit, erythrocyte, and leukocytes [11]. Water quality parameters (temperature, pH, dissolve oxygen DO, ammonia, nitrite, and nitrate) were measured every 10 days. Blood samples were collected using a syringe from the caudal vessels about 10 ppm then were given anticoagulants. Blood glucose was measured through an enzymatic process using a blood glucose test meter tool kit [12]. All the data were analyzed using ANOVA, and if any differences, then continued to Duncan test. Water quality parameters were descriptively analyzed using Excel program.

3. Results
The production performance of snakehead (absolute body length, biomass weight, survival, specific growth rate and feed efficiency) treated by RIFA probiotics, commercial probiotic, and without probiotic applications for 60 experiments are shown in Table 1.

| Parameters                  | Treatments                  |
|-----------------------------|-----------------------------|
|                             | RIFA Probiotic | Commercial Probiotic | No probiotic |
| Absolute body Length (cm)   | 11.95 ± 0.79a | 11.17 ± 0.61a | 10.8 ± 0.74a |
| Biomass Weight (g)          | 303.0 ± 18.16a | 206.7 ± 5.15b | 148.2 ± 11.92c |
| Survival Rate (%)           | 89.50 ± 4.95a | 76.5 ± 4.95b | 58 ± 2.33c |
| Specific Growth Rate (%)    | 3.20 ± 0.13c | 2.83 ± 0.17b | 1.51 ± 0.05a |

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

Figure 1 shows the snakehead fish survival rate in all treatment. The survival rate of snakehead treated with RIFA probiotics was statistically different from commercial probiotics, and without probiotics applications (p <0.05). The largest survival rate of snakehead fingerlings observed in the treatment of RIFA probiotic was about 89.50 ± 4.95%, followed by the commercial probiotic about 76.5 ± 4.95%, and the lowest was without probiotic application about 58±2.33%.
The blood chemistry level shows that the final values of blood parameters were higher as compared with the initial experiment (Figure 3). The initial blood glucose was similar in all treatments, about 55 mg DL$^{-1}$. After 60 days experiment, the lowest blood glucose occurred in the treatment of RIFA application about 84.5 mgDL$^{-1}$, the commercial probiotic application treatment was 86.5 mgDL$^{-1}$ and the highest blood glucose was without probiotic application with 96 mgDL$^{-1}$. The conditions show that fish with no probiotic treatment were more stressful than fish with application probiotic.
Figure 3. The blood glucose measurement results of snakehead fish  
Note : RIFA probiotic; CP= commercial probiotic; NP= Non probiotic

The blood erythrocyte levels in snakehead measured at the initial and final study in all treatments are shown in Figure 4. The erythrocyte concentration of snakehead fingerlings at the initial study was equal to 3.170,000 cells mm\(^{-3}\). After 60 days of the experiment, the blood erythrocyte concentration of snakehead treated by RIFA probiotic performed the highest level at 4,990,000 cells mm\(^{-3}\) followed by commercial probiotic applications, and the lowest was 3,340,000 cells mm\(^{-3}\) from treatments without the application of probiotics.

Figure 4. The results of the measurement of blood erythrocyte snakehead fish  
Note : RIFA probiotic; CP= commercial probiotic; NP= Non probiotic

Figure 5 shows total leukocytes concentration of snakehead fingerlings at the initial and final study. The total leukocytes of the initial study were similar in all treatment calculated around 10,000 cells/mm\(^3\). After 60 days experiment, the highest leukocyte concentration was without probiotic application around 16,500 cells/mm\(^3\), followed by the commercial probiotic was 14,025 cells/mm\(^3\), and the lowest leukocyte concentration was 13,850 cells/mm\(^3\) occurred at the RIFA probiotic application.
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Figure 5. The value of the total leucocytes of snakehead fish
Note: RIFA probiotic; CP= commercial probiotic; NP= Non probiotic

The hemoglobin (Hb) content of snakehead fingerlings at the initial and final study was presented in Figure 6. The Hb content of snakehead fingerlings at the initial study was the same among treatments (10.5%). After 60 days of the experiment, the highest Hb value was 12.25% observed from the fish treated by the RIFA probiotic application, while the treatment of commercial probiotic and without probiotics were similar values at around 10.75%.

Figure 6. The value of the Hb measurement of snakehead fish
Note: RIFA probiotic; CP= commercial probiotic; NP= Non probiotic

Figure 7 shows the comparison of hematocrit level of snakehead fingerlings at the initial and final study in all treatments. The hematocrit content at the initial and final study was the same among treatments (30%). After 60 days of study, the hematocrit value observed in fish treated by RIFA and without probiotic applications increased to 59% and 62%, respectively. The lowest hematocrit value was 41% observed in fish with the commercial probiotic treatment.
Table 2 shows water quality parameters treated in different probiotics applications. Temperature, DO, pH, nitrite, nitrate, and ammonia with probiotic applications were still in the normal range. Ammonia and nitrite content in fish reared using RIFA probiotic application had the lowest concentration as compared with other treatments.

Table 2. Water quality parameters during the study

| No | Parameter     | RIFA Probiotic | Commercial Probiotic | No Probiotic | Note                  |
|----|---------------|----------------|----------------------|--------------|-----------------------|
| 1  | Temperature °C| 29.0±0.5       | 29.5±0.5             | 29.0±0.5     | 27.0-32.0 [14]        |
| 2  | pH            | 7.0±0.5        | 7.5±0.5              | 6.25±0.5     | 6-9 [14]              |
| 3  | DO (ppm)      | 6.25±0.05      | 5.0±0.8              | 4.0±0.5     | <3 [14]               |
| 4  | Nitrite (ppm) | 0.57±0.09      | 0.64±0.05            | 1.05±0.3    | <006 [14]             |
| 5  | Nitrate (ppm) | 0.97±0.29      | 1.99±0.95            | 5.40±0.07   | <10 [14]              |
| 7  | Ammonia (ppm) | 0.02±0.005     | 0.03±0.025           | 0.6±0.15    | <0.02 [14]            |

4. Discussion
Survival rate is a measure of the survival of a population for a certain period of time after a diagnosis or experiment. Probiotics contain beneficial microorganisms such as Lactobacillus, Bacillus, Nitrosomonas and Nitrobacter that increase waste decomposition and improve water quality [15]. The results of the study show that the highest survival rate was obtained from the treatment of the RIFA probiotic. RIFA probiotic contains Pseudomonas aeruginosa, and Achromobakter xylosoxidans for water quality improvement and optimal environment condition as compared to commercial probiotic. In this study, the lowest survival rate was observed in the experiment without probiotics application. The probiotic application in fish was able to improve the growth and survival rate [16]. Similar result has been reported in tilapia (Oreochromis niloticus) that the use of fresh probiotic can improve the growth performance of the fish [17]. Other studies also reported that the application of probiotics can improve water quality compared to without probiotics application. Probiotics reduce nitrite, nitrate, and ammonia concentrations in Sangkuriang catfish rearing and increase its survival [18]. Furthermore, in optimum water quality, the energy used to adjust in poor environmental conditions
due to feed waste was likely converted to growth. Thus, the growth of snakehead treated by RIFA probiotic became greater as compared to commercial probiotic and without probiotics applications.

Using probiotics in fish culture is able to maintain the balance of pathogenic and non-pathogenic microbes both in the digestive tract and in the environment [19]. Therefore, the use of probiotics can increase the digestibility of feed and growth. The provision of a nitrification and denitrification bacterial flock in aquaculture pond of tiger prawns provided positive results such as improving water quality and increasing growth and production [20]. The increase of total length in snakehead during 60 days rearing between treatments was not different. It was indicated that the energy from the feed used for small increments of length increased for larger fish weight gain. The use of probiotics in Sangkuriang catfish culture increases the length and weight of fish [21]. The highest biomass of snakehead was produced from the tanks treated with RIFA probiotic, while the lowest biomass was observed from the tanks without probiotics application.

Based on the results of the blood condition level, almost all values of the blood parameters at the final experiment were higher than the initial study. The change in water quality occurred over 60 days of experiments. Water quality is one of the main factors responsible for fluctuations in the value of blood performance in fish. Water quality decreases due to the presence of many toxic substances or physical changes from water affecting fish hematology [22].

Measurement of blood glucose levels is one method to determine the stress response of fish from different treatment. At the end of the study, the highest increase in blood glucose occurred in the treatments without probiotics. This indicated that fingerlings were likely stressful by the changes of environment conditions. The lowest blood glucose observed in the snakehead was treated by RIFA probiotic. It is assumed that the condition of water quality during fish rearing is better than other treatments. Thus, fish experienced pressured conditions and tent to smaller in body weight. In conditions that are not in accordance with the biological conditions of animals or stress conditions, chromafine cells release the hormone catecholamines, adrenaline and noradrenaline into blood vessels. Furthermore, these stress hormones combine with cortisol to move and increase blood sugar levels through the process of glucogenesis and glycogenolysis [23].

The erythrocyte (Hb) content in fish treated by RIFA environmental probiotic treatment performed the highest concentration in all treatments. It is presumably due to the condition of snakehead fish treated by RIFA tend to be healthier than other treatments. Hb is a protein that is able to bind oxygen, so high Hb indicates healthy fish. However, as Hb content in fish rises significantly high, this indicates that stress response occurred in hypoxic fish due to increasing the blood affinity of oxygen [24]. Increasing Hb levels will be followed by an increase in hematocrit levels as the erythrocyte is the most substances (98-99%) contained in the blood of animal [25]. The amount of Hb in the body is affected by stress and dissolved oxygen content in water. Erythrocytes is known as the largest part of the blood cells ranging from 1.05 to 3.0 (× 106 mL−1 cells) [26]. The number of erythrocytes in healthy Cyprinus carpio carp ranged from 0.79 to 2.90 × 106 cells mm−3.

In this study, the lowest leukocytes occurred in fish treated by RIFA probiotic and the highest leukocytes content observed in fish without probiotics treatment. It is suspected that the highest leukocyte content in the fish blood indicated disease infection. White blood cells are active cell types in the body's defense system [27]. Leukocytes were produced in the thymus and kidney organs and the substances were then transported in the blood throughout the body. Furthermore, the calculation of white blood cells composition can be used for the initial diagnosis of fish disease infection. Infection symptoms will increase fish immunity by increasing leukocytes content so that the concentration will increase from normal levels.

Hematocrit is the percentage of red blood cells in total blood. The hematocrit level is very related to Hb content. The results of this study show that the highest hematocrit content occurred in fish treated by RIFA probiotic application, this is in accordance with the statement of Maheswaran et al [11]. Fish treated by RIFA probiotic treatment showed that the higher hematocrit levels would be followed by the higher Hb concentration. On the other hand, fish treated by the treatment of commercial probiotic showed that the hematocrit level of fish was low, while the hemoglobin content was high. In the treatment without probiotics, the hematocrit level of fish was high, but the hemoglobin content was low. Hematocrit levels were influenced by nutrition, body size, health status, and the environment, and hematocrit levels dropped dramatically due to the presence of mercury (Hg) exposure [11].
5. Conclusion
The application of RIFA probiotic in snakehead fingerlings provided the highest production performance (survival rate, absolute weight and length, biomass weight, and specific growth rate) compared with commercial probiotic and without probiotics application. The blood performance of fish treated with RIFA probiotic was the greatest in this experiment (blood glucose, erythrocytes, hemoglobin, leucocytes, and hematocrit).

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