Effect of Adding BIOM-S Probiotic on Survival and Growth Rate of Nile Tilapia (*Oreochromis niloticus*)

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Authors’ contributions

This work was carried out in collaboration between all authors. Author YA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TIK and IZ managed the analyses of the study. Author IZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research was conducted in May-June 2017 in Hatchery Indoor Maksudi, Astanaanyar, Bandung. This research aims to find out the optimal BIOM-S probiotic on culture media of Nile tilapia, and the influence to survival rate and growth rate of Nile tilapia. This research was conducted by an experiment using Completely Randomized Design (CRD). This research consisted of five treatments and three replications, which were treatment A (control), treatment B (giving probiotic with 0.6 ml/L concentration), treatment C (giving probiotic with 0.8 ml/L concentration), treatment D (giving probiotic with 0.0 ml/L concentration), and treatment E (giving probiotic with 1.2 ml/L concentration). The parameters in this research were the survival rate and specific growth rate. Survival rate and specific growth rate used data analysis with F test to find out the influence of each treatment. The concentration of optimal probiotic based on the results was 0.8 ml/L, it produced the highest survival rate for 81.67% and the specific growth rate for 0.039%.

Keywords: Growth rate; Nile tilapia; probiotic; survival rate.
1. INTRODUCTION

Tilapia is one of the main commodities contributes to increased production of aquaculture fishery. The production of tilapia in Indonesia in 2014 was 999,695 ton [1]. In supporting the success of tilapia fishery and improving the production, it needs innovations to manage the culture media. One of the innovations which can be applied to water and give good effects on the cultivation is adding probiotic on culture media.

Probiotic is defined as additional living microbes which give benefits by ensuring the improving of feeding or improving the nutritional value, improving the response to diseases, or by improving the quality of environment [2]. The adding probiotic on culture media can be accidentally digested by fish orally. The most bacteria found in the probiotic are Bacillus sp., Saccharomyces sp., and Lactobacillus sp. [3]. Bacillus sp. is believed to be able to improve digestibility on fish. According to [4] Bacillus sp. can secrete some enzymes that play a role in digestion such as a protease. Saccharomyces is also potential as an immune stimulant that is a material capable of improving the fish immune system by directly interacting with cells that activate the immune system [5]. Lactobacillus produces lactase enzyme which can break lactose into glucose and galactose. Then, glucose used in the lactic acid fermentation process to produce lactic acid and energy. Lactic acid can inhibit the growth of pathogen microorganism [6].

The use of probiotics in tilapia cultivation shows the growth rate and improvement of feed conversion ratio [7,8,9]. BIOM-S is one of the local probiotic products produced from microbes isolated from vegetable and fruit waste containing microorganisms such as Bacillus sp., Lactobacillus sp., and Saccharomyces sp. The application in fish farming requires research to determine the right concentration of probiotic to increase the growth and survival rate of Nile tilapia.

2. MATERIALS AND METHODS

2.1 Materials

Tools used in this research were 20 boxes (volume 40 litre) of Styrofoam as containers of culture, aerator, hose, aeration stones, a digital scale, measuring cup, and drain. Materials used in the present research were 800 Nile tilapia fry with average length 3 - 5 cm and weight 3-5 g, PF-1000 feed protein content 39 – 41%, and probiotic trademark BIOM-S in liquid preparations containing Bacillus sp. with density 1,04 x 10^9 CFU/ml, Saccharomyces sp. density 8,20 x 10^6 CFU/ml, and Lactobacillus sp. density 8,00 x 10^4 (Laboratory of Soil Fertility and Soil Nutrition, Faculty of Agriculture, University of Padjadjaran in 2016).

2.2 Methods

The research method was conducted experimentally by using Completely Randomized Design (CRD). The present research consisted of five treatments and three replications:

- Treatment A: Control/without giving probiotic on culture media.
- Treatment B: Giving probiotic with 0.6 ml/L concentration
- Treatment C: Giving probiotic with 0.8 ml/L concentration
- Treatment D: Giving probiotic with 1.0 ml/L concentration
- Treatment E: Giving probiotic with 1.2 ml/L concentration

Linear model used from Completely Randomized Design [10] was:

\[ X_{ij} = \mu + \tau_{ij} + \epsilon_{ij} \]

where:

- \( X_{ij} \): The result of observation in \( i^{th} \) treatment and \( j^{th} \) replication
- \( \mu \): Overall mean
- \( \tau_{ij} \): The effect of the \( i^{th} \) treatment
- \( \epsilon_{ij} \): The effect of a random factor in \( i^{th} \) treatment and \( j^{th} \) replication

2.3 Procedures

The research was conducted for 30 days, in accordance with the nursery period of Nile tilapia in middle age. Fish reared at a density of 20 fish/media. On the first day, probiotic was started to be added on water as culture media. Probiotic was added once in three days and the concentration was based on the treatments. Every 10 days, sampling was done to measure the weight of fish and measure water quality.
parameters such as temperature (°C), pH, Dissolved Oxygen (mg/L) and ammonia (mg/L). Observation of fish survival rate was done every day. During the research, syphon on culture media was done every 10 days (based on preliminary test) and water was added to culture media if there was water reduction due to evaporation. During the research, feeding was done three times a day at 08.00, 12.00, and 16.00. The amount of the feed was 5% of fish total biomass.

2.4 Parameter

2.4.1 Survival rate

Calculating data from survival rate parameter could be done by using this formula [11].

\[ \text{SR} = \left( \frac{N_t}{N_0} \right) \times 100\% \]

Note:

\( \text{SR} = \) Survival Rate (%)
\( N_t = \) The number of fishes at the end of the observation
\( N_0 = \) The number of fishes test at the beginning of the observation

2.4.2 Specific growth rate

According to [11] to calculate specific growth rate of fish was by using this formula:

\[ G = \left( \frac{\ln W_t - \ln W_0}{t} \right) \times 100\% \]

Note:

\( G = \) Growth Rate
\( t = \) Duration of the research (day)
\( W_0 = \) The weight of biomass at the beginning of the research (g)
\( W_t = \) The weight of biomass at the end of the research (g)

2.5 Data Analysis

The effect of treatments on survival and specific growth rate to fish test was analysed by using diversity analysis and F test to find out the influence of each treatment. To find out the concentration of optimum probiotic to survival and specific growth rate quadratic regression analysis was used. Then, to find out the difference between treatments it was continued by Duncan's multiple range test with 95% confident interval [12].

3. RESULTS AND DISCUSSION

3.1 Survival Rate

Based on the result of the research, survival rate on Nile tilapia fish fry had a different rate of each treatment. The highest survival rate was from treatment C (0.8 ml/L) with the survival rate was 81.67%, while the lowest survival rate was from treatment A (control) with the survival rate was 53.33%.

Based on the result of variance analysis, survival rate of treatment C (0.8 ml/L) had significant difference with survival rate of treatment A (control), D (1.0 ml/L), and E (1.2 ml/L) but there was no significant difference with treatment B (0.6 ml/L). The survival rate of Nile tilapia fish fry can be seen in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>53.33 ± 0.02 a</td>
</tr>
<tr>
<td>B (0.6 ml/L)</td>
<td>76.67 ± 0.02 bc</td>
</tr>
<tr>
<td>C (0.8 ml/L)</td>
<td>81.67 ± 0.06 c</td>
</tr>
<tr>
<td>D (1.0 ml/L)</td>
<td>68.33 ± 0.05 b</td>
</tr>
<tr>
<td>E (1.2 ml/L)</td>
<td>66.67 ± 0.06 b</td>
</tr>
</tbody>
</table>

*Note: The rate followed by different alphabet was significantly based on Duncan's multiple range test with 95% confident interval

The high survival rate on treatment C was also caused by the influence of microorganisms contained in additional probiotic. Probiotic used in this research contained Bacillus sp., Saccharomyces sp., and Lactobacillus sp. microorganism. Saccharomyces and Lactobacillus were known to be able to give good directly influence to fish survival rate. According to [5] Saccharomyces had potential to be immunostimulant that is a material which can activate the immune system. Lactobacillus had the ability to produce lactic acid from fermentation. The production of lactic acid could inhibit the growth of pathogen microorganism [6]. The activity of the two microorganisms (Saccharomyces and Lactobacillus) caused Nile tilapia fish fry on treatment C had a good immune system and on the culture media, the growth of pathogen microorganism could be inhibited so that it produced the best survival rate.

On treatment D and E, the survival rate produced were lower than C, it caused by high concentration of given probiotic. The giving
probiotic with high concentration was assumed to cause the high competition of probiotic microbe in getting oxygen and nutrition so that the activity of probiotic microbe was inhibited. This assumption was based on the statement of [13] that giving probiotic with high concentration causes ineffective probiotic bacteria and too many probiotics in culture media so that there is a negative competition such as competition in using nutrition and space.

On treatment A (control) it was not added probiotic into the culture media so that it produced the lowest survival rate. The culture media with no additional probiotic caused no microorganism that was capable to inhibit the growth of pathogen microorganism. Immunity of fish was also poor because there was no additional Saccharomyces sp. which could improve fish immunity. The culture media with no probiotic was assumed to be dominated by pathogen microorganism. It was because there was no Lactobacillus bacterium on the culture media which could inhibit the growth of pathogen microorganism.

The dominance of pathogenic microorganisms in media can impact to disease as seen in some fish in treatment A. Disease that attacks fish with treatment A is suspected Saprolegniasis, caused by fungi Saprolegnia sp. This can be seen with the characteristics of fish in the form of branched brown threads attached to the fish's body parts. The fry of Nile tilapia attacked by Saprolegnia sp. can be seen in Fig. 1.

According to Husni et al. [14] Saprolegniasis (white cotton growth) is a disease of fish and fish eggs caused by Saprolegnia molds in the form of threads resemble cotton, white to grey and brown. According to Kurniawan et al. [15] Saprolegnia sp. attack almost all types of freshwater fish such as Osphronemus goramy, Cyprinus carpio, Barbonymus gonionotus Bleeker, Nile tilapia, and ornamental fish, both fry and eggs. It attacks the external organs such as the head, gill cover, fins, and other external body parts.

The use of probiotic can stimulate the immune system, provided disease protection by activating both the cellular and humoral immune defences, as well as presumably providing competitive exclusion in the shrimp's gut. The addition of yeast-glucan, yeast zymosan and dead bacterial cells have also stimulated immune responses in shrimp (Penaeus monodon) [16]. According to Panigrahi et al. [17] reported that the immune response was induced by different forms of the probiotic Lactobacillus rhamnosus (JCM 1136) in the rainbow trout (Oncorhynchus mykiss).

According to Khatun and Saha [9] the addition of probiotics at 5 mg/kg in feed monosex Nile tilapia produce the highest survival rate of 92.35%. While based on [18], the addition of EM4 probiotics of 0.8 ml/L in the medium was able to produce the highest survival rate of the BEST tilapia fry (Oreochromis niloticus) 63.33%.

3.2 Specific Growth Rate

Growth is a length of a period of time or growth of weight according to the simple term, while if it is seen further growth is a complex biological process influenced by some factors [19]. Based on the result of the research on specific growth rate, the highest specific growth rate was from treatment C with the mean of specific growth rate was 3.93%. The lowest specific growth rate was from treatment A or treatment without additional probiotic into the culture media (3.0%). The specific growth rate of Nile tilapia fish fry can be seen in Table 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific growth rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (Control)</td>
<td>3.00 ± 0.0017 a</td>
</tr>
<tr>
<td>B (0.6 ml/L)</td>
<td>3.60 ± 0.0010 bc</td>
</tr>
<tr>
<td>C (0.8 ml/L)</td>
<td>3.90 ± 0.0035 c</td>
</tr>
<tr>
<td>D (1.0 ml/L)</td>
<td>3.40 ± 0.0020 ab</td>
</tr>
<tr>
<td>E (1.2 ml/L)</td>
<td>3.30 ± 0.0027 ab</td>
</tr>
</tbody>
</table>

Note: The rate followed by a different alphabet was significantly based on Duncan's multiple range test with 95% confident interval.
Based on the result of variance analysis, treatment C (0.8 ml/L) was significantly different with treatment D (1.0 ml/L), E (1.2 ml/L), and A (control), while on treatment B (0.6 ml/L) was only significantly different with treatment A. The high specific growth rate on treatment C shows that probiotic bacteria were able to work optimally on water with 0.8 ml/L concentration. The highest growth rate was assumed to be caused by the role of Bacillus sp. bacterium. The adding probiotic on culture media could make Bacillus sp. got into fish digestion accidentally though fish's oral. Bacillus sp. bacterium was believed to be able to improve digestibility of fish. According to [4] Bacillus sp. can secrete some enzymes that play a role in digestion such as a protease. Bacillus sp. bacterium can improve protease enzyme activity that accelerated the reaction of protein hydrolysis and cut off the peptide bond so that it can produce well growth. The growth rate was influenced by optimum water quality during the study. Water quality data during the study showed temperatures in the range of 25.6-25.8°C, pH 6.8-7.6, DO 6.8-7.9 mg/L and ammonia 0.014-0.109 mg/L, where the value is in accordance with the water quality standards required in tilapia cultivation. Among all the routes of probiotic administration in aquaculture, supplementation of rearing water is the only method which is applicable for all ages of fish. The administration via feeding (dry feed) definitely has limitations during early larval stages due to immature digestive tracts of fish in that stage of development [3].

The lowest growth rate was from treatment A (control) with the growth rate was 3.00%. The low growth rate on treatment A caused by there was no probiotic bacterium which was able to improve digestibility of fish. According to [20] the activity of protease enzyme on intestine of tilapia fish was low so that if they were over feeding that contained quite high protein, the process of protein absorption would not be optimal and caused the low growth rate on fish.

Some research suggests probiotics can improve fish growth. [21] state the probiotic-supplemented diets resulted in the growth of nile tilapia higher than the control. Best growth rate, food consumption, and food conversion (P < 0.05) were in the group fed a cocktail of the three bacteria Lactobacillus acidophilus, Streptococcus thermophilus, and Bifidobacterium bifidum. Dietary administration of 2 g probiotic mg/kg can be used as a probiotic agent in O. niloticus culture to enhance fish health, survival, feed efficiency and growth performance [22].

4. CONCLUSION

Based on the research it can be concluded that the best concentration of probiotic for survival and growth rate of Nile tilapia was 0.8 ml/L, it produced the highest survival rate 81.67% and specific growth rate 3.9%.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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