The effect of garlic *Allium sativum* addition in feed to the growth performance and immune response of tilapia *Oreochromis niloticus*

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Abstract. Garlic is a type of medicinal plant known to act as an immunostimulant. The purpose of this research is to examine the percentage of garlic extract on growth, survival, and blood profile. The research was conducted at the Research Station for Environmental Technology and Toxicology Freshwater Aquaculture, Cibalagung, Bogor, West Java. Fish reared using aquarium with a size of 60 x 50 x 40 cm, using an aeration system with a stocking density of 20 fish/aquarium. A completely randomized design (CRD) with three replications were performed. The treatments were as followed: A. 0% without garlic in the feed (control); B. 1% garlic in feed; C. 3% garlic in feed, and D. 5% garlic in the feed. The results showed that the addition of garlic extract to the feed had a significant effect on absolute growth and daily growth rate (p>0.05), where the addition of 1% showed the highest value in weight gain (17.75 ± 0.48 g) and daily growth rate (0.42 ± 0.01 g/day), erythrocytes (1.74 x 10⁶ cells/mm³), and hemoglobin (6.04 g%). This study can be concluded that adding garlic extract to feed at a dose of 1% or 10 g/kg of feed resulted in the highest growth.

Keywords: *Allium sativum*; immunostimulant; *Oreochromis niloticus*

1. Introduction

To grow, fish requires a balance of nutrients such as adequate feed so that the increase in weight and length of fish is fulfilled from the availability of carbohydrates so that the fish grows healthily. Apart from the feed, disease problems are a major obstacle that needs attention in fish farming. Disease expansion control must be carried out as early as possible so that disease outbreaks that cause fish mortality do not occur. The use of antibiotics is a disease control method that has long been applied and is the most widely applied in aquaculture activities. However, the administration of antibiotics in ponds has resulted in the emergence of antibiotic-resistant pathogens.

The use of immunostimulants is an alternative option to reduce the use of drugs that able to increase the performance of fish growth and disease control by increasing non-specific defense mechanisms and disease resistance in cultured fish [1] and immunostimulants work by activating leucocyte cells. The use of immunostimulants leaves no residue in the fish body, is safe for the environment, and increases resistance to infectious diseases [2].

The use of immunostimulants in cultivation can come from natural or synthetic ingredients. Natural ingredients do not cause negative side effects on the environment of the fish live media. One of the natural ingredients that can be used is garlic (*Allium sativum*). Garlic can improve fish survival and can
help control pathogens, especially bacteria and fungi [3]. Garlic (*Allium sativum*) is a medicinal plant known as an immunostimulant [4]. Garlic in fish farming can stimulate a response to eating so that it encourages growth and boosts the immune system. The effect of garlic is associated with the presence of organosulfur compounds, such as allicin [5].

Combining immunostimulants with feed is an effective method to improve non-specific immune systems [6]. The addition of garlic extract to feed will increase the body's immune system. Allicin content can activate non-specific immunity by increasing cytokine gene expression [7].

The fish used in this study was tilapia. Tilapia is a commodity that has high economic value. The demand for year after year continues to increase because this fish is very popular and is also known as an aquatic chicken, even now it is known as aquatic turkey [8]. The use of garlic as an anti-oxidant in the second nursery has never been carried out for this purpose. This study aims to examine the effect of using different doses of garlic in artificial feeds on non-specific immune responses and biological factors of tilapia in the second nursery.

2. Materials and methods

The research was conducted at the Aquaculture and Toxicology Research Installation in Cibalagung Bogor. This study was conducted on a laboratory scale for 30 days. The fish that was used was tilapia (*Oreochromis niloticus*) with an average weight range of 4.85 ± 0.090 g to 5.44 ± 0.414 g. Fish reared using aquarium with a size of 60 x 50 x 40 cm, using an aeration system with a stocking density of 20 fish/aquarium. The remaining feed and feces were removed by siphoned and wasted water was replaced with new water. The fish rearing period was carried out for 30 days.

The treatment consisted of three treatments and one control with a completely randomized design (CRD) with three replication. The different dosages of garlic used were A. 0% without garlic in the feed (control); B. 1% garlic in feed; C.3% garlic in feed; and D. 5% garlic in the feed. Feed preparation begins by weighing the test material and weighing it according to the determined dose, namely 10, 30, and 50 g/kg of feed. The garlic dissolved in 100 ml of distilled water (10%/kg feed weight) then mixed with commercial feed PF 1000 by spraying the test material evenly using a sprayer, then aerated at room temperature. The dried feed is put in a plastic bag and stored in the refrigerator until it was used. Satiation was given based on 5% weight of biomass and given three times a day.

The main variables observed were fish weight, blood profiles (leucocytes, erythrocytes, and hemoglobin levels). The calculation of variables was carried out on the test parameters: daily growth rate (LPH), survival rate (SR). Data were analyzed for variance (ANOVA) to determine the effect of the interaction between the treatments given, if there were differences, the Duncan test was performed to determine which treatment gave the highest and lowest results.

2.1. Absolute weight growth

Absolute weight growth is the weight gain of fish (difference in final weight and initial weight) during the rearing time. Absolute weight growth can be calculated by the formula:

\[ W = \bar{W}_t - \bar{W}_0 \]  

Notes:
- \( W \) = absolute weight growth (g)
- \( \bar{W}_t \) = average fish weight at the end of treatment (g)
- \( \bar{W}_0 \) = average of fish initial weight (g)

2.2. Daily growth rate (DGR)

The daily growth rate is the percentage of fish weight gain each day during fish rearing. The daily growth rate can be calculated using the formula:

\[ \text{DGR (G)} = \frac{W_2 - W_1}{t} \]  

Notes:
- \( W_1 \) and \( W_2 \) = body weight of fish at the beginning and end of the experimental period (g)
- \( t \) = time (day)
Notes:
G = daily growth rate (g/day)
W₂ = average weight of fish at the end of rearing time (g)
W₁ = average of fish initial weight (g)
t = rearing time (days)

2.3. Survival rate (SR)
Survival rate is the ratio of the number of fish that are alive until the end of the rearing with the number of fish at the beginning of rearing. Survival rate observations are done every day during the rearing period.

\[
SR = \frac{N_t}{N_0} \times 100 \%
\]  

(3)

Notes:
SR = survival rate (%)
Nₜ = number of live fish at the end of rearing (individual)
N₀ = number of fish at initial of rearing (individual)

3. Results and discussion

3.1. Absolute weight growth
The calculation of absolute weight growth for 30 days of tilapia rearing with the addition of garlic in the feed can be seen in figure 1. The results of the analysis of variance after 30 days of tilapia rearing with the addition of garlic in the feed had a very significant effect on the growth of tilapia (p<0.05). The highest growth increase was achieved by treatment B with a weight gain of 17.75 ± 0.48 g followed by treatment C 15.91 ± 0.71 g, treatment D 11.92 ± 0.21 g, and treatment A 11.97 ± 0.42 g respectively (figure 1).

![Figure 1. The absolute weight growth of tilapia which was given garlic.](image)

The results of Duncan's continued test showed that treatment B was significantly different compared to treatment C, D, and A. Treatment C was significantly different from treatment D and A, but treatment D was not significantly different from treatment A. The use of garlic in fish feed significantly increased growth, because garlic has active ingredients, such as allicin. Allicin in garlic acts as an
immunostimulant to increase the immune system of the fish body [9], regulates the performance of microorganisms in the intestine to improve food digestion, and affect fish growth [3].

3.2. Daily growth rate
The daily growth rate calculation of tilapia rearing (second nursery phase) for 30 days with the addition of garlic in feed can be seen in figure 2. Daily growth rates in tilapia maintenance with different doses in feed ranged from 0.24 to 0.42 g/day. In the treatment of a 1% (B) dosage of garlic, the highest daily growth rate was obtained, namely 0.42 ± 0.01 g/day. This shows that the optimal use of a dosage of 1% garlic can support the growth of tilapia in the second nursery phase. Meanwhile, the lowest daily growth rate was found in the control treatment, namely 0.24 ± 0.02 g/day (figure 2).

![Figure 2. The daily growth rate of tilapia after given garlic.](image)

The results of the analysis of variance showed that the use of garlic doses had a significant effect on the daily growth rate (p<0.05). According to Lengka et al. [10], the use of garlic in fish feed can increase growth, because garlic contains active ingredients, such as allicin. The allicin contained in garlic acts as an immunostimulant to increase the fish’s immune system. Where the allicin content in garlic provides odor stimulation to increase the response of tilapia feed. Allicin is an organosulphur compound that is water-soluble and volatile. Allicin is a compound that is responsible for boosting immunity. Appears when garlic is crushed, a strong odor that arises indicates the presence of allicin content [11].

3.3. Survival rate
The survival rate calculation of tilapia rearing (second nursery phase) for 30 days with the addition of garlic in feed can be seen in figure 3. There was no difference between treatments on the survival rate of tilapia for 30 days of rearing with the addition of garlic (figure 3). This means that the use of garlic in feed does not harm the survival of tilapia. It even looks like garlic plays a role in boosting immunity.

Fish that have good immunity can survive without any health problems or death. Fish survival is related to organosulfur compounds, including the allicin found in garlic extract which can increase growth, feed response, play an important role as an immunostimulant so that fish are not stressed [12]. According to Marentek et al. [2], garlic can increase fish survival and stimulate the immune system and organ functions related to the formation of blood cells.
3.4. Non-specific immune response

3.4.1. Leucocytes. The leucocyte's value of tilapia after 30 days of rearing (second nursery phase) with garlic treatment was shown in figure 4. Feeding with the addition of garlic has no effect on the value of tilapia leucocytes in this research. However, the leucocytes value in this study was still in the normal range, which means the fish were in good health, the result showed that leucocytes value of each treatment were A (9.07 x 10^4 cells/mm^3), B (9.18 x 10^4 cells/mm^3), C (9.26 x 10^4 cells/mm^3) and D (8.47 x 10^4 cells/mm^3) (figure 4). According to Sasongko [13], the normal leucocytes value in tilapia ranges from 20,000–150,000 cells/mm^3.

![Figure 3. The survival rate of tilapia after given garlic.](image1)

![Figure 4. The leucocytes of tilapia after given garlic.](image2)

3.4.2. Erythrocytes. The results of the tilapia erythrocytes profile for 30 days of rearing (second nursery phase) with garlic treatment were shown in figure 5. The erythrocytes value of tilapia in this study was still in the normal range. According to Hartika et al. [14], the number of normal erythrocytes in tilapia ranges from 20,000–3,000,000 cells/mm^3.

The highest erythrocyte value was shown in treatment B of 1.74 x 10^6 cells/mm^3 (figure 5). The high erythrocyte value in treatment B was thought to be caused by the role of garlic as an antioxidant that can...
ward off free radicals. Free radicals are compounds that can damage erythrocyte membranes and other cells. Garlic has compounds that can act as an antioxidant, in the blood cells this antioxidant can act as a reservoir for free radicals to protect the erythrocyte membrane, as Astawan and Made [15] stated that antioxidants can protect a substance from oxidation attacks, including free radicals.

**Figure 5.** The erythrocytes of tilapia after given garlic.

### 3.4.3. Hemoglobin

The tilapia hemoglobin profile for 30 days of rearing (second nursery phase) with garlic treatment was shown in figure 6. The highest hemoglobin level at the end of fish rearing was found in treatment B of 6.04 g% (figure 6). Fish blood profile, which is one of the non-specific immune indicators such as hemoglobin levels, in normal conditions ranges from 5.05 to 8.33 g% [16]. Hemoglobin determines the level of resistance in fish due to its relationship which is very close to the presence of oxygen-binding capacity by the blood [17]. The ability to bind oxygen in the blood depends on the amount of hemoglobin contained in red blood cells. The low hemoglobin level causes the rate of metabolism to decrease and the energy produced becomes low [18].

**Figure 6.** The hemoglobin of tilapia after given garlic.
4. Conclusion
The addition of 1% garlic extract (B treatment) that equal to 10g/Kg to the feed is the optimal dose for increasing the weight gain (17.75 ± 0.48 g) and daily growth rate (0.42 ± 0.01 g/day) and also increase the erythrocytes 1.74 x 10^6 cells/mm^3 and hemoglobin 6.04 g%.

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