Increased Immune Response of Carp (*Cyprinus carpio L*) by Giving Garlic (*A. sativum*) powder extract

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**Abstract.** Cultivation by utilizing the stocking density and feed used is a driving force for the emergence of diseases due to decreased water quality due to the deposition of organic material from leftover food or fish excretion. Meanwhile, fish become stressed so they are vulnerable to disease attacks, especially infectious diseases such as those caused by bacteria or viruses. One of the natural ingredients that can enhance non-specific immune responses and fish growth is garlic powder. garlic is one of the natural plants which contains active ingredients such as sulfur compounds: alliin, allicin, disulfide, trisulfide; Enzymes such as Alinase, perinase; amino acids like arginine, and minerals like selenium. Allicin is one of the active substances that can kill pathogens (antibacterial) such as Aeromonas bacteria. While alliin contained in the white bottom can significantly improve the fish's immune system so that garlic can be used as an efficient immunostimulant. The study was conducted using the experimental method. The addition of garlic in this study was: treatment A (0 g/kg of feed), treatment B (5 g/kg of feed), treatment C (10 g/kg of feed), treatment D (15 g/kg of feed), Treatment E (20 g/kg of feed) and Treatment F (25 g/kg of feed). The design used was a completely randomized design (CRD) with 6 treatments and 3 replications. The main parameters observed were the increase in non-specific immune responses including total leukocytes and phagocytic activity and added with absolute growth parameters. As supporting data water quality measurements are also carried out.

1.Introduction

Carp (*Cyprinus carpio L*) is one type of freshwater aquaculture that is most widely cultivated by farmers both in hatchery cultivation, enlargement in pond ponds, or jetted water. Like a fish that is quite popular with the community, the increase in carp production has increased significantly from year to year. Increased production of goldfish mentioned above, due to intensive use of aquaculture technology, both in the pool media, floating cages to tarpaulin ponds. The application of intensive cultivation is done by maximizing high stocking densities. However, on the other hand, the intensive cultivation system also has negative implications in the form of disruption to the health of the fish being kept.

Intensive cultivation by using stocking densities and high doses of feed will have an impact on the decline in the quality of aquaculture water due to the increasing level of waste from the rest of the feed and feces. Increased density will be followed by a decrease in growth so that at a certain density growth will cease because it has reached the point of carrying capacity (environmental carrying capacity) [1]. To obtain optimal results, an increase in density must also be followed by an increase in carrying capacity.
Also, the high stocking density and feed used are driving factors for the onset of disease due to decreased water quality due to the deposition of organic material from leftover feed and fish excretion. Meanwhile, fish become stressed so they are vulnerable to disease attacks, especially infectious diseases such as those caused by bacteria and viruses [2].

Efforts to prevent disease in fish can be done by using vaccines and antibiotics. However, vaccines are specific in that they are effective against certain pathogens. Vaccines are also not widely available, and although there are already quite expensive prices. Antibiotics have long been used in the treatment of fish diseases. But now it has been found that the continued use of antibiotics and other chemotherapy materials can cause resistance to pathogenic microorganisms and accumulate in fish and their environment. Other prevention efforts can be made using immunostimulants. Immunostimulant is a substance that can enhance the non-specific immune system of fish and is an alternative to the use of chemicals or drugs [3].

One of the natural ingredients that can enhance non-specific immune responses and fish growth is garlic powder. Garlic is a natural plant that contains active ingredients such as sulfur compounds: alliin, allicin, disulfide, trisulfide; Enzymes such as Alinase, perinase; amino acids like arginine, and minerals like selenium [4]. Allicin is one of the active substances that can kill pathogens (antibacterial) such as Aeromonas bacteria [5]. While alliin contained in the white bottom can significantly improve the fish's immune system so that garlic can be used as an efficient immunostimulant [4]. Therefore, in this study using garlic powder (A. sativum) with different doses as an immunostimulant to increase non-specific immune response in carp.

2.Research Material

2.1. Place and time of research
This research was conducted in September - October 2018 at UPT-BAT Punten, Bate. Observation of samples in the Laboratory of Parasites and Fish Diseases, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya.

2.2. Tools and materials
The test material used was garlic in powder form. While fish feed used is commercial pellets (confessed) obtained from fish feed sales, Goldfish with sizes of 3-5 cm with 10 fish stocking densities. The tools used in this study include 15 aquariums with a size of 20 x 16 cm with a volume of 18 liters of water, scales, thermometer, pH, aerator.

2.3. Procedure of research preparation

2.3.1. Research Preparation
Prepare the test material weighed according to the dose used in the study, then the garlic powder is dissolved in 100 ml of water and mixed with 1 kg of feed. Furthermore, the solution is put into a sprayer and then sprayed into the feed evenly, then the feed is dried at room temperature.

After drying, the feed that has been mixed with the test material is coated with egg yolk (coating) evenly, then re-dried. The dried feed is then put in a plastic bag and kept in the refrigerator until it will be used.

2.3.2. Research Implementation
Before conducting the research, the test fish were kept for 2 (two) weeks in a 2-meter diameter and 20 cm high fiber tub equipped with an aerator for the acclimation process. During the acclimation process, the fish are given commercial feed pellets (Comfeed) at a dose of 5% of body weight/day with a frequency of giving twice a day at 8:00 a.m. and 4:00 p.m. WIB. Water quality during the acclimation period is controlled every day so that it remains stable, using the impurities that stick to the aquarium in the form of leftover food or fish droppings are removed using spraying which is done every day before feeding. Whereas the change of water is carried out once every 2-3 days depending on the condition of the water with a water turnover volume of 30%.

After the acclimation process is completed, the fish are transferred into a glass aquarium measuring 80 cm x 40 cm x 40 cm (p x l x t) with a density of 15 fish/aquarium, but previously weighing was done initially. Each aquarium is equipped with an aerator. Feeding the treatment lasted for 4 weeks.
During the trial period, fish were fed with a dose of 5% of body weight/day with a frequency of feeding 2 (two) times a day at 8:00 a.m. and evening at 4:00 p.m. WIB. During the treatment feeding period, the water quality was kept in good condition in the same way during acclimatization, i.e. the removal of dirt and settling at the bottom of the aquarium was cleaned and the water replacement was 30%. Siphon was carried out at 07.00 and 15.00 WIB before feeding.

2.4. Data analysis
To find out the effect of different dosages of garlic on the increase in non-specific immune responses of carp (Cyprinus carpio L), an analysis of the data collected using the F test was carried out, if:
1. If $F_{count} < F_{table}$ means the result is not significantly different (ns)
2. If $F_{table} 5\% < F_{count} < F_{table} 1\%$ means the results are significantly different (*)
3. If $F_{count} > F_{table} 1\%$ means the results are very really different (**) 
If the F value is significantly different followed by the least significant difference test (LSD) which is done by comparing the difference in treatment values with LSD values 0.05 and LSD 0.01 obtained with the help of the t table and continued with regression tests.

3. Result and Discussion
3.1. Total Leukocyte
From the results of the study note that the highest total leukocytes found in treatment E (20 g/kg of feed) with an average value of 1255.33 cells/µl, then followed by treatments D, C, F, B and the lowest in treatment A (0 g/kg of feed) with an average value of 1034 cells/µl. A graph of the linear regression equation of the increase in the average leukocyte rate of carp (Cyprinus carpio L) can be seen in Figure 1 below.

![Graph of Total Leukocytes](image)

Figure 1. Graph of Average Results of Total Goldfish Leukocytes Each - Every Treatment During Research

The data obtained are then performed the calculation of variance analysis. To find out whether the total leukocytes are significantly different, significantly different, even does not affect (ns), it can be done with a variance test. The results of the total leukocyte statistical calculation can be seen in the following Table 1.

| Source Variation | db | JK        | KT       | Fhit     | F Table |
|------------------|----|-----------|----------|----------|---------|
| Treatment        | 5  | 93893.33  | 18778.67 | 624.80 **| 3.11    |
| Error            | 12 | 360.67    | 30.06    |          | 5.06    |
| Total            | 17 |           |          |          |         |

Information : * = Significantly different ( F Count > F Table 1% )
As noted in Table 1 above, the ANOVA test showed that the addition of garlic in the diet during the study had a very significant effect on the increase in total leukocytes of carp (*Cyprinus carpio* L). This is evidenced by F Count > F Table 1%.

3.2. Phagocytosis Activity

The highest increase in phagocytic activity was at treatment F (25 g/kg of feed) with an average value of 15.11%, then successively followed by treatments E, D, C, B and the lowest in treatment A (0 g/kg feed) with an average value of 9.96%. The complete data can be seen in Appendix 2. The graph of the linear regression equation of the increase in total leukocytes in average carp (*Cyprinus carpio* L) can be seen in Figure 2 below.

![Figure 2. Graph of Average Results of Phagocytic Activities of Goldfish Each - Every Treatment During the Study](image)

The data obtained are then performed the calculation of variance analysis. To find out whether the phagocytic activity is significantly different even does not affect (ns) can be done with a test of variance. The results of the statistical calculation of phagocytic activity can be seen in the following Table 2.

| Source        | Variation | db | JK     | KT     | Fhit     | F Table 5% | F Table 1% |
|---------------|-----------|----|--------|--------|----------|------------|------------|
| Treatment     |           | 5  | 55.45  | 11.09  | 11472.07 | **         | 3.11       | 5.06       |
| Error         |           | 12 | 0.01   | 0.001  | 0.056    |            |            |            |
| Total         |           | 17 |        |        |          |            |            |            |

Information : * = Significantly different (F Count > F Table 1%)

As shown in Table II above, the ANOVA test showed that the addition of garlic in the diet during the study had a very significant effect on the increase in the activity of goldfish (*Cyprinus carpio* L). This is evidenced by F Count > F Table 1%.

3.3. Absolute Growth

The highest absolute growth was in treatments E (20 g/kg of feed) and F (25 g/kg of feed) with an average value of 6.71 g, then followed by treatments D, C, B, and the lowest in the treatment. A (0 g/kg of feed) with an average value of 5.33 g. The graph of the linear regression equation of absolute growth of carp (*Cyprinus carpio* L) can be seen in Figure 3 below.
The data obtained are then performed the calculation of variance analysis. To find out the absolute growth of whether the difference is very real, significantly different, even does not affect (ns) can be done with the test of variance. The results of absolute growth statistical calculations can be seen in the following Table 3.

Table 3. Analysis of Absolute Growth Variance During Research

| Source Variation | db  | JK  | KT  | Fhit | F Table  |
|------------------|-----|-----|-----|------|----------|
| Treatment        | 5   | 4.22| 0.84| 1028.22 ** | 3.11      | 5.06      |
| Error            | 12  | 0.010| 0.001|       |          |           |
| Total            | 17  |     |     |       |          |           |

Information: * = Significantly different (F Count > F Table 1%)

As noted in Table 4.6 above, the ANOVA test showed that the addition of garlic in the diet during the study had a very significant effect on the absolute growth of carp (Cyprinus carpio L). This is evidenced by F Count > F Table 1%.

4. Conclusion

From the treatment of adding garlic in the feed, optimum dosage of garlic was produced in treatment E (20 g/kg of feed) and F (25 g/kg of feed). The addition of garlic in the feed gives a very significant effect on the increase in the non-specific immune resonance of carp (Cyprinus carpio L). This was then shown by several parameters, namely: The best results of the total parameters of goldfish leukocytes were obtained in Treatment E (20 g/kg of feed) which was 1255.33 cells/µl, then followed by Treatment D (15 g/kg of feed) of 1222, 67 cells/µl; Treatment C (10 g/kg of feed) of 1207 cells/µl; F treatment (1155, 33 cells/µl); Treatment B (5 g/kg of feed) was 1135, 67 cells/µl and Treatment A (0 g/kg of feed) was 1034 cells/µl.

The best results of the phagocytic activity parameters of carp were found in Treatment F (25 g/kg of feed) of 15, 11%, then followed by Treatment E (20 g/kg of feed) of 14, 9% and Treatment D (15 g/kg of feed) of 11.81%; Treatment C (10 g/kg of feed) of 11.06%; Treatment B (5 g/kg of feed) of 10.77%; and Treatment A (0 g/kg of feed) of 9.96%.

The best results of the absolute growth parameters of carp were obtained in Treatment E (20 g/kg of feed) and Treatment F (25 g/kg of feed) of 6, 71 g, then followed by Treatment D (15 g/kg of feed) respectively. in the amount of 6, 46 g; Treatment C (10 g/kg of feed) 6, 14 g; Treatment B (5 g/kg of feed) of 5, 95 g; and Treatment A (0 g/kg of feed) of 5, 33 g.
5. References
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