Effect of garlic extract (Allium sativum) on the non-specific immune response of carp (Cyprinus carpio)

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Abstract. One natural ingredient that can enhance non-specific immune responses and fish growth is garlic powder. Garlic is one of the natural plants that contain active ingredients, such as sulfur compounds: allicin, disulfide, trisulfide; enzymes: alinase, pectinase; and amino acids: arginine and minerals like selenium. This study investigated the effect of garlic (Allium sativum) extract on the non-specific immune response of Carp (Cyprinus carpio). 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 observed parameter was an increase in non-specific immune responses, including total leukocytes and phagocytic activity and absolute growth parameters. Based on the result, garlic extracts increased the non-specific immune response of carp.

1. Introduction
Carp fish is accounted for a high economically fish in Java Island. This fish is popular due to its taste, which has triggered farmer to massively cultured carp species. Intensive cultivation using high densities and feed harms water quality. Hence, it will affect carrying ponds capacity [1]. The unbalance in the carrying capacity of a pond triggers many problems in fish cultivation. The decrease in water quality due to high stocking density and feed resulted in fish stressed. Hence, 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 made by using vaccines and antibiotics. However, vaccines are high cost and too specific against pathogens. Meanwhile, antibiotics have long been used in the treatment of fish diseases. Continued use of antibiotics and other chemotherapy agents can cause resistance to pathogenic microorganisms and accumulate in fish and their environment. Furthermore, the use of an antibiotic can disrupt the fish cell organ [3]. Hence, other prevention efforts, such as immunostimulants is a promising approach. 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 [2].

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 bacteria, such as Aeromonas sp. [5]. Furthermore, allicin can improve the fish's immune system so that garlic can be used as an efficient immunostimulant [4]. Therefore, in this study, garlic powder (Allium sativum) investigated for an immunostimulant to increase non-specific immune response in carp (Cyprinus carpio).

2. Research material
2.1. Materials
Garlic and fish feed were purchased from a local market in Malang, East Java, Indonesia. Carp fish with sizes of 3-5 cm with ten fish/tank. The tools used in this study include 15 fish tanks with a size of 20 x 16 cm² with a volume of 18 liters of water.

2.2. Garlic extraction
The garlic was extracted based on the method of Abubakar [6]. The garlic was crushed using mortar at room temperature. Garlic was dried and extracted using distilled water for 24 hours at a temperature of about 60 °C. The extract was then concentrated using a rotary evaporator.

2.3. Feed preparation
The addition of garlic in this study was as follows: 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 feed was mixed and dried at room temperature. For maintaining the extract in feed, the fish feed was coated with egg yolk.

2.4. Rearing fish
Fish were acclimatized for two days before treatments. During the acclimation process, the fish are given commercial feed pellets 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. Fish were then transferred to glass fish tank (80 cm x 40 cm x 40 cm) with a density of 15 fish/tank. The treatments applied for four weeks.

2.5. Blood sampling and immunological analysis
The blood sampling and immunological analysis were done following the method of Rachmawati and Prihanto [7]. Fish blood was collected with needles and stored at 4°C. Phagocytic and leucocyte analysis was carried out using the Giemsa staining method and calculated using a light microscope.

2.6. Absolute growth analysis
The absolute growth rate was calculated based on the method of Soedibya et al. [8]. The formula for absolute growth was as follow:

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\text{Absolute growth (g)} = \text{Final weight (g)} - \text{Initial weight (g)} \tag{1}
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3. Results and discussion
3.1. Total leucocyte
The results showed 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 was 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 (C. carpio) can be seen in Figure 1.
The result showed that the addition of garlic in the diet during the study increased the total leukocytes of carp (Cyprinus carpio L). Garlic extract has been reported increasing either leukocyte or several blood parameters on Rainbow trout [9, 10].

3.2. Phagocytic 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 phagocytosis activity followed a linear regression equation (Figure 2).

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 (C. carpio) can be seen in Figure 3. In this data, we can confirm that the increase in immune response improves the growth performance of fish [12].
Figure 3. Absolute growth of carp fish (C. carpio).

4. Conclusion
Garlic extract increases the leukocyte and phagocytosis activity of carp fish. Hence, the garlic extract has potential as an immunostimulatory agent on Carp fish (C. carpio).

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