The effect of *Chaetoceros calcitrans* extract on hematology common carp (*Cyprinus carpio*) infected by *Aeromonas salmonicida*

Maftuch\(^1,4\), N D A Wulan\(^1\), H Suprastyani\(^1\), E Wijayanto\(^1\), M Noercholis\(^1\), A A Prihanto\(^2,4\) and A Kurniawan\(^3,4\)

\(^1\)Dept. Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Jl. Veteran, Malang 65145, East Java, Indonesia
\(^2\)Dept of Fishery Product Technology, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, Jl. Veteran, Malang 65145, East Java, Indonesia
\(^3\)Dept Aquatic Resources Management, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, East Java, Indonesia
\(^4\)Coastal and Marine Research Center, Brawijaya University, Malang, East Java, Indonesia

E-mail: maftuch@ub.ac.id

**Abstract.** The application of *C. calcitrans* extract in carp (*C. carpio*) is expected to inhibit the growth of *A. salmonicida*. *A. salmonicida*-infected common carp (*C. carpio*) were treated with the extract of *C. calcitrans*. Hematology, erythrocyte, leukocyte, hematocrit and hemoglobin test analysis was observed. The result indicated that the extract can be used to treat the infected fish. The best dose was treatment of D with 45.3 ppm.

1. **Introduction**

The fishery and marine sectors is leading sectors for promoting economic growth in Indonesia [1]. Aquaculture is an endeavor to increase the fisheries production. According to Suseno [2], carp (*Cyprinus carpio*) is an example of freshwater fishery which has an economical important value. Hence, this species is one of important species to be intensively cultured.

One draback for the aquaculture is bacterial infection, such as *A. salmonicida*. *A. salmonicida* is a gram-negative bacterium categorized as must Quarantine Disease and Pest of Fish. This bacterium is designated as a quarantine pest of fish-bacteria class II based on Ministerial Decree No.03/MEN/2010 [3]. The attack of this bacterium is extremely severe.

An alternative agent for combating this infection is by using a natural product. Anti-bacterial agent which are natural and effective to kill and inhibit the growth of bacteria. The extract of *Chaetoceros calcitrans* contains many metabolites and antibacterial compounds composed of fatty acids, alkaloids, and terpenoid [4]. Hematology analysis is a very useful diagnostic method to determine the health status of fish. Blood tests can be used as an indicator of the severity of a particular disease. The
parameters that can show pathological changes in blood are the number of leukocytes, leukocyte differential, and erythrocytes [5].

This study was aimed to determine the effect of *C. calcitrans* extract on the hematology of common carps (*C. carpio*) and to prescribe the best dose of *C. calcitrans* extract for common carps (*C. carpio*) infected by *A. salmonicida*.

### 2. Methodology

#### 2.1. Materials

The research was conducted from February 20, 2017–April 20, 2017 at Fish Cultivation Laboratory, Division of Disease and Health of Fish, Faculty of Fisheries and Marine Sciences and Central Laboratory of Life Sciences of Brawijaya University.

The following materials were used in this research: *C. calcitrans* extract and *A. salmonicida* were obtained from BPBAP (Center of Brackish Water Aquaculture) Situbondo. Common carps (*C. carpio*) in the size of 5–7 cm with the weight of 6–10 grams obtained from local breeder. The research design used was complete randomized design (CRD). This research consisted of four treatments and control with three replications.

#### 2.2. Preparation and infection of animal test

The fish used in this research were common carps (*C. carpio*) that were obtained from fish farmers in Malang. As many as 210 fish in the size of 5–7 cm were required in this research. According to [10], during the study, the carps were given pellet feed as much as 4% of their biomass weight 2 times (morning and evening) in a day. After being acclimatized, the fish were ready to use.

#### 2.3. Infected common carp (*C. carpio*) with *A. salmonicida*

Bacteria *A. salmonicida* were immersed in an aquarium containing the carps (*C. carpio*). The infection process followed [11]. The bacterial density of $10^7$ cells/mL was applied for the infection. Fish was soaked for 2 hours.

#### 2.4. Treatment of *C. Calcitrans* extract in carps (*C. carpio*)

The administration of *C. calcitrans* was done by immersion method. The *C. calcitrans* extract was administered at doses of 0.3, 15.3, 30.3, and 45.3 ppm (consistent with in vitro dosage). Then, the carps were kept for 7 days to allow the extract to function as an antibacterial agent. Then, blood sample was taken as a negative control.

#### 2.5. Hematology analysis

Fish blood sampling was performed after 12 hours of infection. Blood was taken from caudal peduncle of carps (*C. carpio*) using a syringe needle. 3.8% citrate was added to the blood as anti-coagulant.

**a. Erythrocytes**

The number of erythrocytes was counted according to Blaxhall and Daisley [14]. Blood was dropped onto the hemocytometer (Neubauer type) and covered with a glass cover. The total erythrocyte was counted in five small boxes and the amount was calculated using the following formula:
\[ Total \text{ erythrocytes} = \sum N \times 10^4 \text{ cell/mm}^3 \]  

(1)

b. Leukocytes

The number of leukocytes was measured according to Blaxhall dan daisley [14]. Then, turk solution was added up to scale 11. After that, the first two drops of blood solution from the pipette were removed, and the next drop was dropped into the solution on the hemocytometer. The total leukocyte count was calculated following the formula:

\[ Total \text{ leukocytes} = \sum n \times 50 \text{ cell/mm}^3 \]  

(2)

Note:

\[ \sum n : \text{Total number of leucocytes in 4 field of view} \]

50 : Dilution factor

c. Hematocrit

The level of hematocrit was measured according to Anderson and Siwicki [15]. The end of the bloodfilled tube was covered with crytoceal by placing the tip of the tube into crytoceal approximately 1 mm deep to form a crytoceal plug. The microhematocrit tube was centrifuged for 5 minutes at a speed of 12,000 rpm with the position of the tube having the same volume to face for a balanced centrifuge rotation. The length of the deposited blood portion (a) and the total volume of blood contained in the tube (b) were measured using a microhematocrit tube. Measurement of hematocrit grade values was done by comparing the volume of red blood cell solids with total blood volume using hematocrit scale.

d. Hemoglobin

The calculation of hemoglobin followed Wedemeyer and Yasutake method [16]. Sahlinometer tube was filled with HCl 0.1 N solution until number 10 showed (the lowest scale line on the sahlinometer tube). Then, the tube was placed between 2 tubes in standard color. Next, 0.02 mL fish blood was taken from the tube with sahli pipette. The next steps were cleaning the tip of the pipette, putting the blood into the Sahli tube, and waiting for 3 minutes. Lastly, distilled water was added gradually by using a dropper, and it was stirred by using a stirrer glass until the color turned exactly the same as the standard color.

3. Results and Discussion

3.1. Erythrocytes

Diagram of erythrocyte observation results during observation and in figure 1. From the result it can be seen that the highest erythrocytes (2.4 cell/ml) was obtained from the extract of 45.3 ppm. The lowest cell was from 12 hours and the dose of 0.3 ppm extract.
Regression analysis suggested that the erythrocytes was followed of $y = 2.12 + 0.005x$ function, which had a coefficient determination of 0.8266. This suggested that the higher the dose given, the higher the number of erythrocytes. Erythrocytes in the body play a role in the transfer of oxygen throughout the body. The decrease of the number of erythrocytes leads to lower oxygen supply to all organs and reduces food supply to the cells.

### 3.2. Leukocytes

The average results obtained from leukocyte observation can be seen in figure 2.

**Figure 1.** Number of Erythrocyte of infected-Common Carp (*C. carpio*). (Blue is 12 hours, Orange is 24 hours, Grey is 36 hours).

**Figure 2.** Number of Leukocytes in infected-Common Carp (*C. carpio*). (Blue is 12 hours, Orange is 24 hours, Grey is 36 hours).

Orthogonal polynomial analysis showed that the result followed the function of $y = 1.256 - 0.004x$, which has a coefficient determination of (R2) of 0.740. The higher the dose of *C. calcitrans* extract given, the lower the number of leukocytes in the blood of carp. Leukocyte is one of blood components...
that has a function in the non-specific body defense system that will localize and minimize pathogens through phagocytosis [17].

3.3. Hematocrit
The average results obtained from hematocrit observations during the observation can be seen in figure 3.

Regression analysis revealed that the hematocrit followed the function of $y = 19.86 + 0.217x$, which had a coefficient determination of 0.801. The extract affected the hematocrit levels of the fish (C. carpio). This is shown by the fact that the higher dose of extract C. calcitrans given, the higher the hematocrit level in carp blood (C. carpio). This is in line with Wahjuningrum et al. [18], which revealed that a hematocrit level of less than 30% indicates that the fish has vitamin deficiency, low protein in the feed, and infection. If the hematocrit is lower than normal fish, the fish is in anaemia condition. Contrastingly, if the hematocrit level is above normal, the fish is stress or infected.

3.4. Hemoglobin
The average results obtained from hemoglobin observation can be seen in figure 4.

Regression analysis revealed that the hemoglobin followed the function of $y = 19.86 + 0.217x$, which had a coefficient determination of 0.801. The extract affected the hemoglobin levels of the fish (C. carpio). This is shown by the fact that the higher dose of extract C. calcitrans given, the higher the hemoglobin level in carp blood (C. carpio). This is in line with Wahjuningrum et al. [18], which revealed that a hemoglobin level of less than 30% indicates that the fish has vitamin deficiency, low protein in the feed, and infection. If the hemoglobin is lower than normal fish, the fish is in anaemia condition. Contrastingly, if the hemoglobin level is above normal, the fish is stress or infected.
The hemoglobin resulted in the function of $y = 7.107 + 0.020x$, which had a coefficient determination of 0.730. According to Sukenda et al. [17], the hemoglobin contained in red blood cells has the function in the transport of oxygen from the gills to the tissues. Research from Serapse et al. [19] suggested that the extract of Chaetoceros sp. which is dissolved with ethanol and various organic materials, produces terpenoid compounds. The results of the study also showed that Chaetoceros sp. extract had a wide spectrum of activity. Hence, C. calcitrans extract could be used to inhibit bacterial growth.

4. Conclusion
Treatment of C. calcitrans extract affected the hematology of infected common carp (C. carpio). Based on the result C. calcitrans extract has a potency to be used as the treatment agent for A. salmonica.

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