# Analysis of the Effectiveness of Mango Leaves (*Mangifera indica*) as a Phytoimunostimulant on Common Carp (*Cyprinus carpio*) in Brackish Water

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#### ABSTRACT

The problems that are often experienced when cultivating goldfish are diseases caused by pathogens that attack cultivated fish. The cause of disease in fish is caused by interactions between fish, pathogens and their habitat environment that can cause death in fish. The solution to this problem is to provide a phytoimmunostimulant derived from mango leaves (*Mangifera indica*). The purpose of this study was to determine the effect of mango leaves on the hematology and physiology of common carp. This research was conducted in January-April 2022. The method used in this research is an experimental method by comparing data. Parameters observed were total erythrocytes, hemoglobin, hematocrit, blood glucose, absolute body weight growth, survival rate, and water quality. Based on the research that has been done, it can be concluded that the addition of 1.5% mango leaf powder into fish feed as a phytoimmunostimulant affects the hematological and physiological effects of common carp. Common carp treated with phytoimmunostimulants in fresh water had higher mean values (total erythrocytes  $1.75 \times 10^6$  cells/mm<sup>3</sup>, hematocrit 33.66%, and hemoglobin 8.13 g/dL) compared to data in brackish water (total erythrocytes  $1.54 \times 10^6$  cells/mm<sup>3</sup>, hematocrit 30.66%, and hemoglobin 7.76 g/dL).

Keywords: Mango Leaves, Common Carp, Phytoimmunostimulants

#### 1. INTRODUCTION

Common carp (*Cyprinus carpio*) is a freshwater fish that can adapt to water conditions that have salinity. This is because the common carp is a freshwater fish that can tolerate salinity. According to Alam *et al.* (2020), common carp can tolerate salinity <10 ppt. The problem that is often encountered in efforts to cultivate carp, namely death caused by disease, which causes losses.

The cause of disease in fish is caused by the interaction between fish, pathogens, and their habitat environment. Conditions for limited cultivation containers, high seed stocking densities, overfeeding, and inappropriate management of water quality can result in disturbed environmental balance. This causes fish to become stressed and makes it easier for infection from pathogens to result in death (Sari *et al.*, 2018).

To overcome diseases caused by pathogens, farmers use antibiotics as the main solution. Widespread use of antibiotics has been carried out mainly because of their performance that can work selectively in inhibiting and killing pathogenic organisms without damaging the treated host with the right dose (Sari et al., 2012). Antibiotics are commonly used in aquaculture to prevent and treat disease. However, antibiotic therapy is not always cheap and successful, in some cases; it has detrimental effects on fish (Hoseini & Yousefi, 2019). A promising solution is to administer immunostimulant substances as an alternative to antibiotics.

Parts of the mango plant (*Mangifera indica*) that can be used as an immunostimulant are the leaves, bark, stems, roots, and fruit. However, the part of the mango plant that has the highest chemical content is found in the leaves, namely alkaloids, saponins, tannins, flavonoids, and steroids (Nugraha *et al.*, 2017). Phytoimmunostimulants can be given to fish through several methods, one of which is by mouth. These chemical compounds are mixed into the feed, and then fish eat the feed so that the body can absorb the extracts from the plant

material eaten.

The provision of medicinal plant materials or stimulant phytochemicals in the form of mango leaves mixed into this feed is an effort that can be made to overcome the problem of mass mortality of cultivated common carp caused by bacterial infectious disease factors.

Based on this description, we are interested in conducting research on the effectiveness of mango leaves as a phytoimmunostimulant in common carp reared in brackish waters.

### 2. RESEARCH METHODS

#### Time and Place

This research was conducted in April– May 2022. The research was conducted at the Floating Net Cages in the Koto Panjang Hydroelectric Generator (PLTA) Reservoir, and at the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine, Universitas Riau.

#### Methods

The method used in this study is an experimental method, by comparing data, in hydropower reservoirs (freshwater) and laboratories (brackish water) which are used as research locations. The effectiveness of mango leaves on common carp immunity as an object of research using 2 controls, namely positive control (fish given a mixture of mango leaf powder at 1.5%/100g; negative control (fish fed Hi-Provit 781-2 commercial). The use of these two controls is useful to determine the effect of additional mango leaf powder on common carp hematology.

#### Procedure

This research began with the manufacture of fish feed in the form of pellets which were given mango leaf extract as a natural Phytoimmunostimulant. Then proceed with the maintenance of carp which is carried out in two different places, namely the hydropower reservoir with a container measuring 2m x 2m x 2m, and in the laboratory using container with a diameter of 1 m with a volume of 100 L of 4 units. Next, 3 fish blood was taken from each container. Fish blood sampling was carried out at the beginning (day 1) and 30 days of rearing (end).

#### Parameters of Observed Total Erythrocytes

The total number of erythrocytes is counted as many as five small squares on the haemocytometer with the formula:

#### Number of erythrocytes = $\Sigma$ NX 10<sup>4</sup> cells/mm<sup>3</sup>

Information:

N = Number of counted erythrocytes in 5 fields of view

 $10^4$  = dilution factor

#### Hemoglobin

Calculation of hemoglobin levels was carried out with reference to the Sahli method. The Salinometer tube is placed between 2 tubes with standard color. The Sahlinometer tube was filled with 0.1 N HCl solution until the number 0 (the bottom scale line on the Sahlinometer tube), then fish blood was taken from the microtube with a Sahli pipette as much as 0.02 mL and put into the Sahlinometer tube. Then let it stand for 3 minutes, before cleaning the pipette tip first. Then add distilled water with a pipette little by little while stirring with a glass stirrer until the color is the same as the standard color. Hemoglobin levels are expressed in g/dL or g%.

#### Hematocrit

The blood sample is inserted into the hematocrit capillary tube until approximately 2/3 of the length of the capillary tube (by touching the red striped end of the capillary tube to the blood, the other end of the tube is closed with the fingers and make an open-closing motion to draw blood into the capillaries).

#### **Blood Glucose**

The procedure carried out to check blood glucose in fish is carried out using a glucose test kit for humans, namely the Gluco DR brand with a range of 20-600 mg/dl. Fish blood is dripped onto a strip that has been attached to the Gluco DR tool. The results of fish blood glucose levels will be displayed on the Gluco DR screen (Philipson *et al.*, 2010).

### Absolute Weight Growth

Absolute weight growth is calculated using the formula (Effendie, 2002). As follows:

$$\mathbf{GR} = \mathbf{Wt} - \mathbf{Wo}$$

#### Information:

GR = Absolute growth (g) Wt = Final average weight (g)

Wo = initial average weight (g)

### Survival Rate

The survival rate or survival rate can be calculated by the formula according to Effendie (2002) as follows:

$$\mathbf{SR} = \frac{Nt}{No} \mathbf{X} \ \mathbf{100\%}$$

Information:

SR = Survival rate (%) Nt = Final number of fish (fish)

No = Initial number of fish (fish)

### Data Analysis

Data analysis used in this study was made in tabular form, and then the results of the analysis were obtained descriptively based on haematological and physiological observed. Statistical analysis was carried out using the SPSS application version 23.

## 3. RESULT AND DISCUSSION

### Haematological Profile

In this study, the hematology of the fish tested and the results observed were the of the fish erythrocyte profiles (total erythrocytes, hematocrit levels, and hemoglobin levels) with average total erythrocytes of  $1.75 \times 10^{6}$ cells/mm<sup>3</sup>, а hematocrit of 33.66%, and hemoglobin of 8.03 g/dL in fresh water. While in brackish water the average total erythrocytes were  $1.63 \times 10^6$ cells/mm<sup>3</sup>, the hematocrit 30.66% and the hemoglobin 7.76 g/dL. The results of this study indicate that the addition of mango leaf powder as a phytoimmunostimulant to artificial feed can improve the physiological profile of fish reared in fresh water and 5 ppt salinity for 30 days.

Total erythrocytes of fish in KJA  $(1.75 \times 10^6 \text{ cells/mm}^3)$  and in the laboratory  $(1.63 \times 10^6 \text{ cells/mm}^3)$  are still within the normal range. The normal range of total common carp erythrocytes ranges from 1.43-1.88 x 106 (Sezgin & Aydin, 2021). This is caused by the level of salinity that affects the osmotic levels and body fluids of fish. Rohman *et al.* (2017) said that fish that experience osmotic pressure would experience erythrocyte cell creation starting from 36 hours of rearing.

The increase in goldfish erythrocyte cells is thought to be caused by the content of secondary metabolites from mango leaves, such as the presence of Fe or iron content that plays a very important role in increasing the number of erythrocytes. Suci et al. (2018) said that mango leaves that had been processed into powder contained Fe of 437 ppm.

The hematocrit in maintenance at the Koto Panjang hydropower reservoir is in the range of 33-66%, while in laboratory maintenance it is in the range of 30-66%. In this study in two locations, it was shown that there was an increase in hematocrit levels in carp fed with the addition of mango leaf powder.

Observations of the average hemoglobin level in this study were carried out on carp fed with the addition of mango leaf powder in fresh water at the Koto Panjang hydropower reservoir, which was 8.03 g/dL, which was classified as normal. While the maintenance carried out in the laboratory had a lower hemoglobin value range namely 7.76 g/dL, but still categorized as normal. The increase in hemoglobin due to the addition of mango leaf powder is due to the presence of iron that plays a role in increasing erythrocytes along with hemoglobin and mango leaves also contain high triterpenoids that can facilitate blood flow (Sutardi, 2016).

## Physiological condition of fish

Other variables tested in this study, namely absolute weight, blood glucose, and survival rate were obtained respectively 10.68 g, 79 mg/dL, and 94.67% for fish reared in freshwater. While 9.70 g, 74.66 mg/dL, and 92% in carp reared in media with salinity.

Blood glucose in carp fed with a mixture of mango leaf powder in media with salinity had an average of 60.33 mg/dL. In freshwater has an average value of 74.66 mg/dL. This shows that the blood glucose value of fish is still normal, namely 40-90 mg (Malini *et al.*, 2011). The growth of common carp was lower in media with salinity; this was thought to be caused by an increase in salinity. According to Saputra *et al.* (2017), common carp require more energy for the osmoregulation process than somatic growth.

The survival rate in freshwater and brackish water can be categorized as good. This is because the management of water and feed quality in the maintenance container is well maintained. Agus *et al.* (2020) stated that the ability of fish to receive feed depends on the type of feed and the size of the feed. This condition illustrates that feed with the addition of mango leaf powder as a phytoimmunostimulant gives good survival to common carp reared in fresh water and brackish water.

#### 4. CONCLUSION

Based on the research that has been

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done, it can be concluded that the addition of 1.5% mango leaf powder to fish feed is considered effective as a phytoimmunostimulant in common carp reared both in freshwater and brackish water because the results obtained can improve the hematological and physiological effects of fish.