Histopathology of gill tissue koi carp (Cyprinus carpio) infected Myxobolus sp. with treatment of diflubenzuron

U Yanuhar¹*, M Musa¹, R D Putri¹, N R Caesar¹ and N S Junirahma¹

¹Faculty of Fisheries and Marine Science, University of Brawijaya Jl. Veteran, Malang, 65145, Indonesia

*E-mail: doktoruun@ub.ac.id

Abstract. Along with the development of aquaculture activities, certainly, there are many problems that can interfere with the cultivation process one of the causes is because of parasite attacks on fish. One of the parasites that often attacks koi fish is Myxobolus sp. The purpose of this study was to determine the histological picture and the effect of giving Diflubenzuron on the damage to the gill tissue of koi fish (Cyprinus carpio) with the treatment of Diflubenzuron. The method used in this study is an experimental method using 5 treatments namely (A) healthy fish, (B) fish infected with Myxobolus sp., (C) fish infected with Myxobolus sp. and Diflubenzuron dose 0.01 mg, (D) of fish infected with Myxobolus sp. and Diflubenzuron dose 0.02 mg and (E) fish infected with Myxobolus sp. and Diflubenzuron dose 0.03 mg. Histopathological observations were made on gill tissue. The level of gill tissue damage can be determined by the scoring method. Based on the results of the study, there were three tissue damages found in histopathological observations of gill tissue, namely oedema, hyperplasia, and lamella fusion. Treatment of Diflubenzuron with different doses has a different effect on the level of gill tissue damage. The conclusion of this study, the treatment of Diflubenzuron 0.02 mg (D) has the best effect compared to other doses, evidenced by the reduction in damage to the gill tissue.

1. Introduction

One of the freshwater potentials of high economic value is Koi fish (Cyprinus carpio). Koi fish (C. carpio) are in high demand because of the beauty of the body shape and body-colour [1]. Besides Koi fish (C. carpio) has a stable price in the international market. That potential makes Koi (C. carpio) as one of the leading ornamental fish commodities, that grow bigger lately. The condition above encourages farmers to increase their cultivation business of Koi (C. carpio) [2].

Nevertheless, success in freshwater fish farming activities to produce fish that have high selling power is still meagre. This is due to the lack of adequate fish seed both in quality and quantity. Good quality fish are those that are resistant to disease and resistant to changes in water quality conditions.

One of the infectious agents that is harmful is myxosporea from the genus Myxobolus sp. Myxobolus sp. is a common parasite on various types of freshwater fish, including Koi (C. carpio) [3]. Myxobolus included in the list of Quarantine Fish Diseases (HPIK) group I. Myxobolus sp. is a parasite in fish Koi (C. carpio) that can interfere with the survival of Koi fish (C. carpio) and even can cause death [4]. Fish attacked by Myxobolus sp. usually characterized by damage to body tissue one of the gills. Gills infected with Myxobolus sp. will experience swelling caused by reddish-white nodules and gills appear pale red. This parasite forms a cyst on the gill sheet of the fish so that the fish will obstruct the process of oxygen absorption [5].
Myxobolus sp. parasites which develop rapidly can lead to genetic changes that affect mutations in the host immune system. High mutation rates result in high genetic diversity, as well. Most mutations are destructive and cause loss [3]. To overcome the outbreak of Myxobolus sp. is by administering synthetic chemicals such as twisted [6]. Twisted or commonly called diflubenzuron is a pesticide that can interfere with the formation of the outer skin (exoskeleton) during the growth and development [7]. Therefore, the authors interested in researching the immune response in the gill tissue of Koi fish (C. carpio) infected with myxosobolus sp with the treatment of dimyiline as an effort to control Myxobolus infection.

2. Methods

An experimental method was applied in this research with 7-12 cm size of fishes were tested as a sample. Then the fish were categorized into 5 treatment groups which are: (A) healthy fish, (B) fish infected with Myxobolus sp., (C) fish infected with Myxobolus sp. and Diflubenzuron dose 0.01 mg, (D) of fish infected with Myxobolus sp. and Diflubenzuron dose 0.02 mg and (E) fish infected with Myxobolus sp. and Diflubenzuron dose 0.03 mg, each treatment groups is containing 10 fish. This research utilizes the chemical Diflubenzuron to be tested on Koi (C. carpio) fish infected with Myxobolus sp. The use of Diflubenzuron as a chitin inhibitor in parasites is used as an active ingredient to reduce infections that occur due to the parasite Myxobolus sp. A sampling of koi fish infected with Myxobolus sp. done by observing the clinical symptoms that show disease. Lom and Dykova [8], states that the examination can be done by looking at clinical signs in koi fish, namely the presence of nodules in the gills. Another clinical symptom that is seen is that the operculum cannot close when the fish is heavily infected [9].

2.1. Histopathology of Gills Infected by Myxobolus sp.

Histopathological analysis can be used to determine the health condition of fish which is seen based on structural changes that occur in organ tissues like gills. Histopathological examination can also be used as a biomarker in monitoring the environment by observing organs that have essential functions in the body's metabolic processes so that it can be used as an initial diagnosis of health disorders in an organism [10]. The damage caused by Myxobolus infection in this study is Edema, Hyperplasia, and Lamella Fusion.

2.2. Tissue Damage Scoring Method

The level of damage to fish gill tissue that has been treated with Dimilin can be determined by statistical analysis of scoring. With a semi-qualitative method, which is used to calculate the number of coloured areas and is done manually by calculating the percentage. In the semi-quantitative method seen from 5 broad fields of view to get the maximum results on the level of tissue damage. Each area of view observed the degree of tissue damage with the criteria of hyperplasia, fusion, and necrosis (cell damage), then in percentage by giving a score of 1-4. Percentage of damage in each field of view is calculated based on the number of cells damaged. According to Raza'i [11] with the formula:

$$\text{Damage Percentage} = \frac{\text{total damaged cell}}{\text{Total analysed cell}} \times 100\%$$

Then the percentage that generated is scored from numbers 1 to 4. Number 1 (mild) has a percentage level of tissue damage 0 - 5%, number 2 (moderate) percentage of tissue damage 6-25%, number 3 (severe) percentage level of damage network 26 - 50%, and number 4 (very heavy) the percentage of tissue damage is more than > 50%.

3. Results and Discussion

Histopathological analysis can be used to determine the health condition of fish which is seen based on structural changes that occur in organ tissues like gills. Histopathological examination can also be used as a biomarker in monitoring the environment by observing organs that have essential functions in the body's metabolic processes so that it can be used as an initial diagnosis of health
disorders in an organism [10]. The results showed a picture of koi fish (C. carpio) gills with different fish conditions treated with different dimilin doses showing different histopathological results (Figure 1). Although administration of twisted can affect tissue recovery is not directly visible, the difference in damage that occurs in gill tissue with varying conditions of fish can be demonstrated through scoring.

![Image of fish gills with different conditions treated with different dimilin doses showing different histopathological results](image)

**Figure 1.** Gills of koi fish (C. carpio) (A) healthy fish, (B) fish infected with Myxobolus sp; (C) fish infected with Myxobolus sp. and Diflubenzuron dose 0.01 mg; (D) of fish infected with Myxobolus sp. and Diflubenzuron dose 0.02 mg; (E) fish infected with Myxobolus sp. and Diflubenzuron dose 0.03 mg (note: black arrow: Oedema; yellow arrow a: Hyperplasia; and blue arrow lamella fusion).

### 3.1. Calculation of Oedema Damage Scoring

The treatment of twisting during the study of koi fish gave a different average result of damage due to oedema that occurred in the gills of koi fish (C. carpio). Based on the results of research that has been carried out, the average value of oedema scoring from gill damage can be seen in Table 1.

| Number | Treatment | Repetition | Total | Average |
|--------|-----------|------------|-------|---------|
| 1.     | a         | 0.4        | 0.4   | 0.8     | 0.27    |
| 2.     | b         | 3.2        | 3.2   | 3       | 9.4     | 3.13    |
| 3.     | c         | 1.6        | 1.6   | 1.8     | 5       | 1.67    |
| 4.     | d         | 2          | 2.2   | 1.8     | 6       | 2.00    |
| 5.     | e         | 2.4        | 2.8   | 2.6     | 7.8     | 2.60    |

Based on Table 1, the average value of damage to koi gill tissue oedema in adverse control treatments was 0.27, and in the positive control, treatments were 3.13. In procedure (c), namely koi fish that were attacked by Myxobolus by giving dimilin a dose of 0.02 mg obtained an average of 1.67. In treatment (d) the koi fish attacked by Myxobolus sp. by giving dimilin dose 0.01 mg achieved an average of 2.00. In therapy (e) the koi fish attacked by Myxobolus sp. by giving dimilin the dose of 0.03 mg obtained an average of 2.60. In the negative control, the average value of oedema damage in koi gill tissue is in the low damage category and the positive control. The average oedema damage value is in the weight category. After giving it, the koi were infected with Myxobolus sp. and healthy
koi, the results of the level of oedema damage to healthy fish, are still in the mild category whereas in fish infected with *Myxobolus* sp. the degree of damage is in the moderate category. Based on the 3 doses given namely dose A (0.02 mg), B (0.01 mg), and C (0.03 mg), dose A is the optimal dose for gill tissue oedema damage, because it has a lower level of damage than administration of dose B and C. That is because the entry of foreign material in the form of twisted at suboptimal doses which causes increased levels of tissue damage to characterize stressed fish.

Oedema or coagulation is an excessive accumulation of fluid in a tissue so that the tissue is enlarged and does not function properly [12]. Oedema will reduce the efficiency of gas diffusion because the absorptive surface area of the secondary lamella is narrowed [13]. Increased hydrostatic pressure tends to force fluid into the body's interest space so that oedema can occur. Oedema can cause swelling in tissue that is inflamed due to accumulation of fluid.

3.2. Hyperplasia

Based on Table 2, it is known the average value of damage to hyperplasia of koi gill tissue in negative control treatments is 0.27 and in positive control treatments are 3.00. In procedure (c), the koi fish that were attacked by *Myxobolus* by giving dimilin dose of 0.02 mg obtained an average of 1.53. In treatment (d), namely koi fish which were infected by *Myxobolus* by giving dimilin dose of 0.01 mg earned an average of 1.67. In treatment (e) that is, the koi fish attacked by *Myxobolus* by giving dimilin dose of 0.03 mg obtained an average of 2.20.

| No | Treatment | Repetition | Total | Average |
|----|-----------|------------|-------|---------|
|    |           | 1  | 2  | 3  |     |
| 1. | a         | 0.2| 0.2| 0.4| 0.8 | 0.27 |
| 2. | b         | 3  | 2.8| 3.2| 9   | 3.00 |
| 3. | c         | 1.4| 1.6| 1.6| 4.6 | 1.53 |
| 4. | d         | 1.8| 1.8| 1.4| 5   | 1.67 |
| 5. | e         | 2.2| 2.4| 2  | 6.6 | 2.20 |

After giving it, the koi were infected with *Myxobolus* sp. and healthy koi obtained the level of damage to hyperplasia of healthy fish is still in the mild category while in fish infected with *Myxobolus* sp. the level of damage is in the moderate category. Based on the 3 doses given namely dose A (0.02 mg), B (0.01 mg), and C (0.03 mg), dose A is the optimal dose for gill tissue hyperplasia damage, because it has a lower level of damage than administration of dose B and C. That is because the entry of foreign material in the form of twisted at suboptimal doses which causes increased levels of tissue damage to characterize stressed fish. Hyperplasia is the addition of a body part or organ due to an increase in the number of new cells. Hyperplasia damage will occur if the fish are in a continuously polluted environment [14]. Secondary lamella hyperplasia in gills results from uncontrolled epithelial cell division, whereas the excessive division of chloride cells causes primary lamellae. Hyperplasia of gill lamella cells begins with several events including oedema, cell death and release of epithelium cells in gill lamellae [15]. The process of gill defence against parasitic infections can result in bleeding. The defence reaction to the lamella will stimulate the growth of gill epithelial cells very quickly. The infection causes the gills to become irritated and secrete mucus (mucus) as protection against the parasitic attack. However, the resulting mucus covers the surface of the gill lamella so that it will inhibit the exchange of O2 and CO2. As a result, there is no oxygen binding by blood haemoglobin. That may cause blood transportation throughout the body is not smooth. So the parasites that grow in the gills cause hyperplasia to get worse [16].

3.3. Lamella Fusion

Based on Table 3, it is known that the average value of damage to lamella Fusion of koi fish gill tissue in the negative control treatment was 0.27, and the positive control treatment was 3.13. In treatment
(c), the koi fish which were attacked by Myxobolus by giving dimilin at a dose of 0.02 mg obtained an average of 1.80. In treatment (d), namely koi fish that were attacked by Myxobolus by giving dimilin dose of 0.01 mg obtained an average of 2.60. In treatment (e), the koi fish which were attacked by Myxobolus by giving dimilin dose of 0.03 mg obtained an average of 2.93.

| Number | Treatment | Repetition | Total | Average |
|--------|-----------|------------|-------|---------|
| 1.     | a         | 0.2        | 0.2   | 0.4     | 0.8     | 0.27   |
| 2.     | b         | 3.4        | 3.2   | 2.8     | 9.4     | 3.13   |
| 3.     | c         | 2          | 1.8   | 1.6     | 5.4     | 1.80   |
| 4.     | d         | 2.4        | 2.6   | 2.8     | 7.8     | 2.60   |
| 5.     | e         | 2.8        | 3     | 3       | 8.8     | 2.93   |

Table 3. Scoring Results of Lamella Fusion Damage to Gill Tissue

After administration of dimilin to both infected and healthy koi the results of the level of Lamella fusion damage in healthy fish, are still in the mild category while in fish infected with Myxobolus sp. the level of damage is in the moderate category. Based on the 3 doses given, namely dose A (0.02 mg), B (0.01 mg), and C (0.03 mg), dose A is the optimal dose for lamella fusion damage due to gill tissue, because it has a lower level of damage than administration of dose B and C. That is because increasing levels of tissue damage characterize the entry of foreign material in the form of twisted at suboptimal doses which causes stressed fish. Lamella fusion results from an increase in the pathology of hyperplasia continuously and causes the filling of space between secondary lamellae by new cells which then triggers attachment to both sides of the lamella [15]. Fusion or fusion is the attachment of secondary lamellae to one another. Fusion occurs because the lamella has swelling or hyperplasia so that the breathing process is disrupted. This situation causes the size of the cavity (capillary lumen) to narrow, and the cells in the middle of the secondary lamella are shifted to the other end of the secondary lamella, causing a close. According to Aliza [17], the fusion of the lamella occurs due to the proliferation of secondary lamella epithelial cells, so that the distance between secondary lamellae shortens and eventually fuses.

4. Conclusion
The results of the study concluded that Dimilin administration influenced the level of tissue damage caused by the parasite Myxobolus sp. based on analysis of variance of 0.02 mg dose is the most optimal dose, evidenced by the reduction in damage to the gill tissue

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