Effects of Probiotic Treatment on Histopathology of Koi Carp (Cyprinus carpio) Infected by Myxobolus sp.

U Yanuhar1*, N S Junirahma1, K Susilowati1, N R Caesar1 and M Musa1

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

*Corresponding author’s email: doktoruun@ub.ac.id

Abstract. Koi fish (Cyprinus carpio) is one of the ornamental fish of a high economic value. However, raising koi can be troublesome due to poor water quality and high levels of stress that they are attacked easily by parasites. One of the parasites often attacking the gill of koi is Myxobolus sp. The presence of Myxobolus sp. can be observed through a histopathological observation of gill tissues. The purpose of this study was to determine the histological effect of probiotic administration to the damage on the gill tissue of Koi fish (Cyprinus carpio) infected with Myxobolus sp. The method used in this study was an explorative experimental method using factorial RAL. The observation of gill tissue was carried out on samples of koi fish consisting of two (2) control treatments, namely positive control (K+) and negative control (K-). Then, six (6) treatments, consisting of fish infected with Myxobolus and healthy fish, were done by applying a dose treatment of 1.1 ml/30 litters of water, 0.55 ml/30 litters of water, and 1.65 ml/30 litters of water. Treatments on control groups were carried out in a controlled tank by maintaining water quality parameters, which include temperature, pH, DO, and CO2. The level of gill tissue damage treated with probiotic formulations was determined by scoring analysis. The results of histopathological observations commonly showed tissue damage, namely damage to Edema, Hyperplasia, and Lamella Fusion. The treatment having the best influence according to the 5% BNT test was the PB treatment (infected fish with a dose of probiotic formulation of 0.55 ml/30l).

1. Introduction
Koi fish (Cyprinus carpio) is one of the ornamental fish of a high economic value. However, raising koi can be troublesome due to extreme weather, changes in aquatic environment like poor water quality, and high stress levels in fish; these problems increase the number of cases of disease attacks on koi fish. Parasites are a disease that often attacks fish. Unstable environmental conditions can cause fish to have stress. So that its self-defense mechanism becomes weak and ultimately vulnerable to parasites [1]. Myxobolus has been known to be one of the parasites that often attacks the gills and sheets of koi.

Myxosporeasis or Myxobolusis is a parasitic disease caused by Myxobolus sp. The life cycle of this parasite is horizontal (indirect) involving invertebrates (Oligochaeta) and a number of vertebrates (fish or amphibians). The species has developed quite rapidly and need to be aware because it can cause economic losses. Clinical symptoms caused by the parasite Myxobolus sp. depending on the type of fish attacked, generally they have white nodul in their gills (operculum). These nodules are collection of thousand spores [2]. Diagnosis of the presence of Myxobolus sp. can be done through visual observations of behavior and clinical symptoms. Further observations can be carried out microscopically through histopathological observation of tissues of organs susceptible to Myxobolus sp.
Appropriate management of water quality and observation of tissues are the preventive measures to do to decrease the possibility of deaths. Water quality plays an important role in raising koi. In addition, to support the survival of fish, probiotic formulations are generally possible as an effort to manage water quality and as anti-parasites. The purpose of this study was to examine the histopathological damage in gill tissues of koi fish (Cyprinus carpio) infected with Myxobolus sp. and to determine the effect of probiotic formulations toward the damage. By knowing the histopathological damage and the effect of probiotic formulations, it is expected that we can provide better water quality for koi fish so the death rate due to parasites, especially Myxobolus sp., can be reduced.

2. Methods
The study was experimental. Samples were koi fish showing symptoms of being infected with Myxobolus sp. The samples were 7-12 cm long. As many as 288 koi were used in the experiment and were put into 24 treatment tanks, so there were 12 fish on each tank. The koi fish were soaked in the tanks and were given a probiotic formulation.

The doses of probiotic formulation administered to each healthy and infected fish to each tank were as follows: PA (infected fish with a dose of probiotic formulation of 1.1 ml/30 liters), PB (infected fish with a dose of probiotic formulation of 0.55 ml/30 liters), PC (infected fish with a dose of probiotic formulation of 1.65 ml/30 liters), PD (healthy fish with a dose of probiotic formulation of 1.1 ml/30 liters), PE (healthy fish with a dose of probiotic formulation of 0.55 ml/30 liter), and PF (healthy fish with a dose of probiotic formulation of 1.65 ml/30 liters). The treatment was carried out for 6 hours in water and probiotic formulations were given every other day for two weeks.

Koi fish were observed to determine the clinical symptoms. Infected fish that showed the most severe clinical symptoms were dissected and gills (lamella) were used for histopathological analysis. Then, the observation using microscope to analyse gill tissue damage. Histopathological analysis using the scoring method. Scoring data that obtained from observation were processed and the results were analysed by using ANOVA. Sampling was done from January to March 2019.

3. Results and Discussion
3.1. Clinical Symptoms of Koi Fish Infected with Myxobolus sp.
Koi fish infected with Myxobolus sp. showed clinical symptoms that could be observed directly, i.e. the existence of a reddish white cyst or bulge. The cyst contained thousands of spores covering the gills of the fish that the gill cover appeared open and made it difficult for fish to breathe. Infected fish were found to swim slowly, to stay more at the bottom of the water, and to show low feeding responses; many were dead [3]. Koi fish have clinical symptoms in the form of open or unable to close the operculum completely caused by swelling of the Myxobolus sp. reddish white color making it difficult for fish to do the process of respiration as in general. Furthermore, the health status of fish can be known from the body and behavior—healthy fish have no wound on their body, have bright color, are actively moving, and do not swim on the surface of the water [4].

3.2. Measuring the Damage of Koi Fish Gills
3.2.1. Edema. Based on observations of gill tissue (lamella) of koi fish infected with Myxobolus sp., the damage to the gill tissue was different in each treatment (Figure 1). The damage to gill (lamella) of koi fish infected with Myxobolus sp. can be seen from the presence of edema, which is indicated by an arrow in the observation Figure 1. Edema is cell swelling or excessive accumulation of fluid in tissues. The accumulation of fluid causes the cells to be irritative, which causes the cells to swell [5]. Edema can be characterized by the loosing of the basement membrane and the narrow of lacuna cells leading to deficiencies in the function of the gills and difficulties in the respiratory process, and then the metabolism begins to be disturbed [6].
Based on the findings, the highest average score of edema damage was found in the PC treatment (infected fish with a dose of probiotic formulation of 1.65 ml/30 L) and the lowest was in the PB treatment (infected fish with a dose of probiotic formulations of 0.55 ml/30 L) and PE (healthy fish with a dose of probiotic formulation of 0.55 ml/30 L). Based on the 5% BNT test, the administration of the probiotic formulation of 0.55 ml/30 L showed the best effect to edema damage in the gill tissue of koi fish.

Table 1. Analysis of Variance Analysis (ANOVA) Edema Damage.

| Sources of Diversity | Db  | JK     | KT     | F Count  | F 5%  | F 1% |
|----------------------|-----|--------|--------|----------|-------|------|
| Treatment            | 7   | 14,58  | 2,083  | 63,314** | 2,66  | 4,03 |
| A                    | 1   | 1,71   | 1,707  | 68,267** | 4,49  | 8,53 |
| B                    | 3   | 2,43   | 0,811  | 32,444** | 3,24  | 5,29 |
| AB                   | 3   | 10,44  | 3,480  | 139,2**  |       |      |
| Error                | 16  | 0,4    | 0,025  |          |       |      |
| Total                | 23  | 14,98  |        |          |       |      |

Information : (***) = Very Real Difference, (TN) = Not Real

3.2.2. Hyperplasia. Based on observations of gill tissue (lamella) of koi fish infected with Myxobolus sp., the damage to hyperplasia is presented in Figure 2, as indicated by the arrow [7] hyperplasia is excessive tissue formation due to the increase in the number of cells. This is a physiological response to protect tissues from toxic substances. Hyperplasia of gill lamella begins with several case including edema, death cells and release of epithelium cells in gill lamellae [8]. Lamella with hyperplasia results in thickening of the epithelial tissue at the end of the filament or thickening of the epithelium tissue located near the base of the lamella. Hyperplasia occurs with the addition of a number of secondary lamella epithelium, so the secondary lamella gets bigger and coincides; consequently, the secondary lamella sticks together and converges [9]. This makes the gill lamella appears larger than normal and differences between the primary and secondary lamella cannot be seen clearly.

Figure 1. The damage of edema.

Figure 2. The damage of hyperplasia.
Based on the findings, the highest average score of hyperplasia damage was found in the PA treatment (infected fish with a dose of probiotic formulation of 1.11 ml/30 L) and PC treatment (infected fish with a dose of probiotic formulation of 1.65 ml/30 L) and the lowest was in the PB treatment (infected fish with a dose of probiotic formulation of 0.55 ml/30 L) and PE (healthy fish with a dose of probiotic formulation of 0.55 ml/30 L). Based on the 5% BNT test, the administration of probiotic formulations of 0.55 ml/30 L showed the best effect to hyperplasia damage in the gill tissue of koi fish.

Table 2. Analysis of Variance Analysis (ANOVA) of Hyperplasia Damage.

| Sources of Diversity | Db | JK   | KT   | F Count   | F 5% | F 1% |
|----------------------|----|------|------|-----------|------|------|
| Treatment            | 7  | 17.23| 2.461| 67.177**  | 2.66 | 4.03 |
| A                    | 1  | 0.67 | 0.667| 18.182**  | 4.49 | 8.53 |
| B                    | 3  | 3.05 | 1.018| 27.758**  | 3.24 | 5.29 |
| AB                   | 3  | 13.51| 4.502| 122.788** |      |      |
| Error                | 16 | 0.59 | 0.037|           |      |      |
| Total                | 23 |      |      | 17.81     |      |      |

Information : (***) = Very Real Difference, (TN) = Not Real

3.2.3. Lamella Fusion. Based on observations of gill tissue (lamella) of koi fish infected with Myxobolus sp., the damage to lamella fusion is presented in Figure 3, as indicated by the arrow. The fusion of two secondary lamellae can be caused by a parasitic wound on the secondary lamella forcing the organ to release much mucus to cover the wound. Lamella fusion occurs by hyperplasia that extends to basal cells and epithelium so secondary lamella will coalesce. This event results in the obstruction of the respiration and expiration processes [10]. Lamella fusion represents severe damage because it is an advanced stage of hyperplasia damage.

![Figure 3. Lamella Fusion.](image)

Based on the findings, the highest average score of lamella fusion damage was found in the PC treatment (infected fish by administering a dose of probiotic formulation of 1.65 ml/30 L) and the lowest was in the PB treatment (infected fish with a dose of probiotic formulation of 0.55 ml/30 L). Based on the 5% BNT test, the administration of probiotic formulations of 0.55 ml/30 L showed the best effect to lamella fusion damage in the gill tissue of koi fish.

Table 3. Analysis of Variance Analysis (ANOVA) of Lamella Fusion Damage.

| Sources of Diversity | Db | JK   | KT   | F Count   | F 5% | F 1% |
|----------------------|----|------|------|-----------|------|------|
| Treatment            | 7  | 15.06| 2.151| 56.124**  | 2.66 | 4.03 |
| A                    | 1  | 2.16 | 2.160| 56.348**  | 4.49 | 8.53 |
| B                    | 3  | 2.73 | 0.909| 23.710**  | 3.24 | 5.29 |
| AB                   | 3  | 10.17| 3.391| 88.464**  |      |      |
Error  16  0.61  0.038
Total  23  15.67
Information: (***) = Very Real Difference, (TN) = Not Real

3.3. Molecular Response of Probiotic Formulations on the Survival of the Body of Koi Infected with Myxobolus sp. This study used Bacillus spp., Lactobacillus sp., and Nitrosomonas sp. as the starters of probiotic formulation, with a quantity of 108 cfu/ml each. The use of Bacillus spp. with the right concentration and dosage as an ingredient in probiotic formulations will be able to increase the number of red blood cells and hemoglobin levels—these are believed to be one indicator of an increase in the ability of fish to supply nutrients to the entire body and tissue repair to increase fish growth [11].

The effect on fish related to the administration of probiotic formulations is the formation of an adaptive immune response. Fish have an innate and adaptive immune defense system. Congenital parameters are at the forefront of immune defense and are an important factor in disease resistance. Adaptive responses are usually delayed but are very important for long-term immunity and are a key factor in successful treatment [12]. This response is characterized by the formation of IGM in koi fish.

Fish only synthesizes one class of immunoglobulin (IgM). IgM serum is tetrameric in teleosts and is pentameric in cartilaginous fish. IgM is more efficient than IgG in complement activation, opsonization, viral neutralization, and agglutination. IgM is found in fish mucus and is cell-mediated immunity. T-cytotoxic cells help kill viral-infected cells and abnormal cells [13].

4. Conclusion
Histopathological observations on gill tissue of koi fish not infected with Myxobolus sp. shows damage to different gill tissue (lamella). The damage to the gill tissue of koi fish infected with Myxobolus sp. is edema, hyperplasia, and lamella fusion. The treatment administered to tackle Myxobolus sp. with probiotic formulations at a dose of 1.1 ml/30l, 0.55 ml/30l, and 1.65 ml/30l of water in koi fish of different sizes give different effects on the level of gill tissue damage. The best effect of probiotic formulations on the level of histopathological damage to gill tissue, i.e. damage to edema, hyperplasia, and lamella fusion, is the one with the dose of probiotic formulation of 0.55 ml/30l according to 5% BNT.

References
[1] Pujiastuti N and Setiati N 2015 Identifikasi dan Prevalensi Ektoparasit Pada Ikan Konsumsi di Balai Benih Ikan Siwarak UNNES Journal of Life Science 4 (1) 9-15
[2] Mahasri G and Kismiyati 2011 Buku Ajar Parasit dan Penyakit Ikan I (Ilmu Penyakit Protozoa pada Ikan dan Udang) (Surabaya: Universitas Airlangga) hal. 3-4
[3] Deriyanti A 2016 Korelasi Kualitas Air dengan Prevalensi Myxobolus Pada Ikan Koi (Cyprinus carpio) di Sentra Budidaya Ikan Koi Kabupaten Blitar, Jawa Timur Skripsi Fakultas Perikanan dan Kelautan Universitas Airlangga Surabaya
[4] Rahmaningsih S 2016 Hama & Penyakit Ikan (Yogyakarta: Deepublish) hal. 352
[5] Tandjung S 1982 The Toxicity of Alumunium for Organs of Salvalinus Fontanalis Mitchell In Acid Water Jakarta
[6] Fitriawan F Sutarno and Sunarto 2011 Perubahan mikroanatomi pada insang dan ginjal kerang air tawar (Anodonta woodiana) terhadap paparan kadmium Bioteknologi 8 (1) 42-52
[7] Setyawan N 2013 Gambaran Mikroanatomi Pada Insang Ikan Sebagai Indikator Pencemaran Logam Berat di Perairan Kaligaran Semarang Skripsi Jurusan Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Negeri Semarang
[8] Widayati E D 2008 Studi Histopatologi Insang Ikan Mujair (Oreochromis mossambicus) Pada Konsentrasi Sublethal Air Lumpur Sidoarjo. Skripsi. Institut Teknologi Sepuluh Nopember Surabaya
[9] Yuniar V 2009 Toksisitas Merkuri (Hg) Terhadap Tingkat Kelangsungan Hidup Pertumbuhan, Gambaran Darah dan Kerusakan Organ Pada Ikan Nila Skripsi Institut Pertanian Bogor, Bogor
[10] Suparjo M N 2010 Kerusakan Jaringan Insang Ikan Nila (Oreochromis niloticus L) Akibat Deterjen Jurnal Saintek Perikanan 5 (2) 1-7
[11] Rajikkannu M, Natarajan N, Santhanam P, Deivasigamani B, Ilamathi J and Janani S 2015 Effect of probiotics on the haematological parameters of Indian major carp (Labeo rohita) International Journal of Fisheries and Aquatic Studies 2 (5) 105-109
[12] Secombes C J and Wang T 2012 The innate and adaptive immune system of fish Infectious disease in aquaculture Woodhead Publishing Limited 2012
[13] Ode I 2013 Kajian Sistem Imunitas Untuk Pengendalian Penyakit Pada Ikan dan Udang Jurnal Ilmiah agribisnis dan Perikanan (agrikan UMMU-Ternate) 6 (2) 41-43