

# Effect Of Deltamethrin Administration On Koi Fish (*Cyprinus Carpio*) Infected By *Myxobolus* Sp.

Muhammad Sumsanto, Uun Yanuhar, Asus Maizar Suryanto H.

**Abstract**— Some parasites that attached to the koi fish's body can be treated by administering chemicals. The treatment with organophosphate group has been done by some of ornamental fish farmers. In this study, the effect of deltamethrin administration on koi fish (*Cyprinus carpio*) infected by *Myxobolus* sp is presented. The treatments were divided into 5 groups with 3 replications and the administration of deltamethrin was mixed into feed. The dose of deltamethrin in this study was  $0.5 \mu\text{l g}^{-1}$ ,  $1 \mu\text{l g}^{-1}$ , and  $1.5 \mu\text{l g}^{-1}$ . Deltamethrin administration had a significant effect ( $p < 0.05$ ) on hematologic levels, including hematocrit and hemoglobin, as well as CD8. Furthermore, this study showed that the expression of CD8 significantly decreased after deltamethrin administration.

**Keywords** : Hematocrit, Hemoglobin, CD8, Deltamethrin, *Myxobolus* sp.

## 1 INTRODUCTION

Fish disease defined as anything that can disturb organs function or structure, both directly and indirectly. The disease also interpreted as an organism that lives and develops in the fish's body, so the fish organs are disturbed [1,2]. One of the diseases that can attack koi fish (*Cyprinus carpio*) is myxosporeasis. Koi fish infected with *Myxobolus* sp. usually interfere the respiratory process, besides the presence of nodules on the gills can cause imbalance. The fish will swim in a spiral mode from the bottom to the surface of the water [3]. The disease attacks on koi fish according is due to unbalanced interactions between fish as hosts, water as the environment and disease-causing agents (pathogens). Unbalanced interactions cause stress on the fish, so the body's defense mechanism decreases and is susceptible to disease [4].

Many methods are carried out by koi fish farmers to avoid myxobolus sp. infection. They were using natural and chemical treatments. The koi fish treatment can be done by administering chemicals such as the organophosphate group. This treatments have been done by ornamental fish farmers. The use of a botanical insecticide called Pyrethrum is quite effective in controlling fish parasites [5]. However, natural pyrethrums are quite expensive and unstable to light. To resolve those problems, we can use synthetic pyrethrum, commonly called pyrethroids [6,7]. Pyrethroids are nerve poisons that binding to protein in the nerve called  $\text{Na}^+$  ion channel [8].

Pyrethroid insecticide that can be used is deltamethrin. Deltamethrin easily degraded in the soil and cannot be traced to microflora or microfauna [9,10]. Deltamethrin is a synthetic pyrethroid insecticide and one of the most widely used as plant protection. Deltamethrin is a broad-spectrum insecticide acting as a contact poison and stomach poison [11,7]. The purpose of this study is to analyze the effect of deltamethrin administration on koi fish (*Cyprinus carpio*) infected by *Myxobolus* sp.

## 2 MATERIALS AND METHODS

### 2.1 Sampling of Koi Fish (*Cyprinus Carpio*) Infected with *Myxobolus* Sp.

Koi fish were obtained from farmer's ponds in the Kemloko and Nglegok, Blitar. This study used 180 fishes and they were measured in 7-12 cm. Koi fish was selected by observing the clinical symptoms. The examination can be done by looking at clinical symptoms like nodules in the gills [12]. Another clinical symptom that can be seen is the operculum. It cannot close when the fish is heavily infected [13].

### 2.2 Deltamethrin In-vivo Test on Koi Fish (*Cyprinus carpio*)

All of the fish were acclimatized for 24 hours before treatments to avoid stress so they will be able to adjust in a new environment. This study was used 5 treatments with 3 repetitions. The treatment was carried out by feeding method. Deltamethrin was added to the feed and then gave to koi fish infected by *Myxobolus* sp. in 3 different doses:  $0.5 \mu\text{l g}^{-1}$  (P1),  $1 \mu\text{l g}^{-1}$  (P2), and  $1.5 \mu\text{l g}^{-1}$  (P3).

### 2.3 Hematocrit

The examination of hematocrit was performed by microhematocrit method. Microhematocrit along with heparin was inserted into the blood sample, less than three-quarters (3/4) of the capillary tube. In addition, the end of the capillary tube was blocked by sticking it in the wax stopper. Then, it was centrifuged for 5 minutes using a microhematocrit centrifuge with a speed of 1,500 rpm. Furthermore, a hematocrit was used to read the results and it is stated in % [14].

### 2.4 Hemoglobin

Hemoglobin measurement was done by the sahli method. The principle of this method was converted hemoglobin in the blood into hemotin acid by hydrochloric acid. The blood was

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sucked using a sahli pipette up to a scale of 20 mm<sup>3</sup> and transferred into a hemoglobin tube containing 0.1 N HCL to 10 scale (yellow), keep it 3-5 minutes so that hemoglobin works with HCL and become hemotin acid. Then aquades was added and stirred little by little by the same color as the standard color. Gram / 100 ml means the amount of hemoglobin in grams per 100 ml of blood.

## 2.5 Immunohistochemistry (IHC)

Immunohistochemistry staining includes several stages of preparation, including the preparation of glass slides, coating glass slides with neufon (attachment agents), transferring the sample tissue to glass slides and the immunohistochemistry staining procedure itself. Positive reaction in immunohistochemistry staining was shown by the appearance of a brown color on the part of the cell that has specificity with the primary antibody (anti-CD8). Furthermore, the sample was carried out under a microscope, then a quantitative analysis was conducted using the ImmunoRatio (IR) analysis by ImageJ software. ImmunoRatio segmented the core area of diaminobenzidine (DAB) and hematoxylin from the microscope image, calculates the labeling index (percent of the DAB-colored area of the total nuclear area), and produces matching images of segmentation staining [15].

## 3 RESULTS AND DISCUSSION SECTIONS

### 3.1 Hematocrit Levels

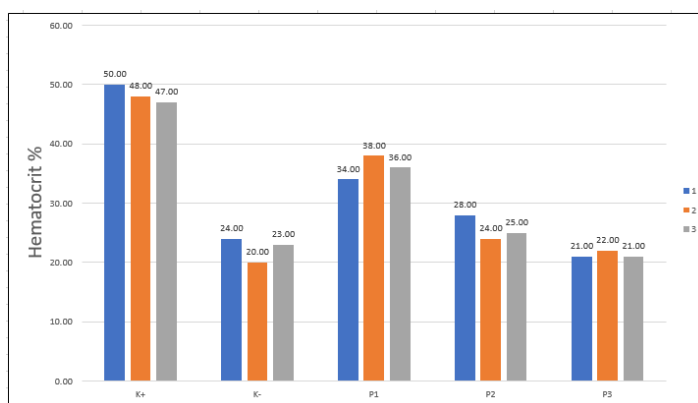
Hematocrit shows the percentage of solids in the blood towards the blood fluids. Hematocrit is used to measure the ratio between erythrocytes and plasma, so hematocrit provides the ratio of total erythrocytes to the total volume of blood in the body. The hematocrit value is influenced by the size and the amount of erythrocytes [16]. Hematocrit levels are the percentage of the volume of red blood cells in the blood from total blood samples in capillary tubes. The enhancement of erythrocytes can increase the hematocrit value [17]. Determination of hematocrit levels in blood fluid is used to see the health of fish and the relationship between blood and hormones in fish. Hematocrit levels are the percentage of red blood cell volume in the goldfish range from 28-40 % [18]. Hematocrit levels can be an indicator of feed with low protein content, deficiency of vitamins or infections in fish. Meanwhile, hematocrit and erythrocytes enhancement show the state of stress [19].

Based on the Table 1, the negative control has normal hematocrit levels (22.33%) and the positive control has very high levels of hematocrit (48.33%). It shows that *Myxobolus sp.* infected the fish's body. Furthermore, the level of hematocrit decrease along with the enhancement of deltamethrin in each treatment P1 (36.00%) > P2 (25.67%) > P3 (21.33%). These results show that the infection of *Myxobolus sp.* on koi fish (*Cyprinus carpio*) is weakened by deltamethrin treatment. If the value of hematocrit in the fish is less than 20%, then the fish has anemia and if the hematocrit value of the fish is more than 60% it indicates that the fish is experiencing stress on its environment [20]. The decreased on hematocrit values can be used as a clue about low protein content, vitamin deficiency or infections in fish such as disease [21].

**Table 1.**  
*Hematocrit Levels in Koi Fish*

Treatments	Repetition			Average (%)
	A (%)	B (%)	C (%)	
K+	50.00	48.00	47.00	48.33
K-	24.00	20.00	23.00	22.33
P1	34.00	38.00	36.00	36.00
P2	28.00	24.00	25.00	25.67
P3	21.00	22.00	21.00	21.33

Remarks: K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5  $\mu\text{g}^{-1}$  deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1  $\mu\text{g}^{-1}$  deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5  $\mu\text{g}^{-1}$  deltamethrin.



**Fig 1.** Graph of Hematocrit Levels in Koi Fish (K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5  $\mu\text{g}^{-1}$  deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1  $\mu\text{g}^{-1}$  deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5  $\mu\text{g}^{-1}$  deltamethrin).

### 3.2 Hemoglobin Levels

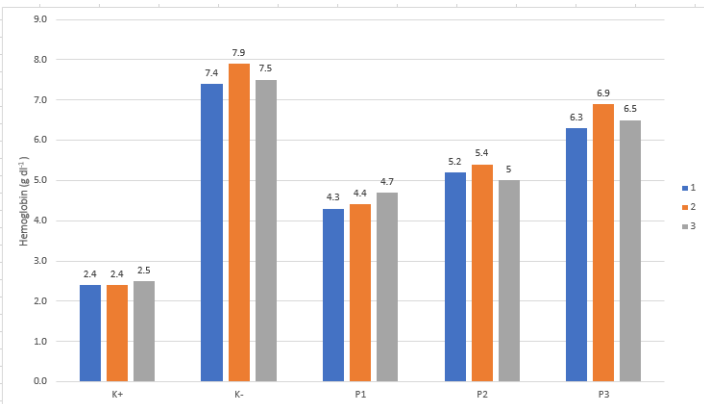
The function of hemoglobin test in blood fluids is for fish health and to find out the relation between blood and hormones in fish. Hemoglobin level is the amount of hemoglobin gram / 100 ml of blood [18]. The state of stress can affect physiological activity and hemoglobin levels in fish. The physiological state of fish blood varies greatly, depending on environmental conditions such as humidity, temperature, and pH [22].

**Table 2.**  
*Hemoglobin Levels in Koi Fish*

Treatments	Repetition			Average (g dl <sup>-1</sup> )
	A (g dl <sup>-1</sup> )	B (g dl <sup>-1</sup> )	C (g dl <sup>-1</sup> )	
K+	2.4	2.4	2.5	2.4
K-	7.4	7.9	7.5	7.6
P1	4.3	4.4	4.7	4.5
P2	5.2	5.4	5	5.2
P3	6.3	6.9	6.5	6.6

Remarks: K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5  $\mu\text{g}^{-1}$  deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1  $\mu\text{g}^{-1}$  deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5  $\mu\text{g}^{-1}$  deltamethrin.

Table 2 shows that the infected fish are lower in hemoglobin level rather than in healthy fish. After deltamethrin administration in each treatment the hemoglobin value increased respectively by 4.5 g dl<sup>-1</sup> (P1), 5.2 g dl<sup>-1</sup> (P2) and 6.6 g dl<sup>-1</sup> (P3). Normal hemoglobin levels in fish range from 5.05 to 8.33 g dl<sup>-1</sup> of blood (Safitri et al., 2013). Under normal conditions, fish will capable to bind oxygen well. The main function of hemoglobin is binding oxygen. It is used for the process of catabolism so the energy is produced and it's prevented high blood acidity [23].



**Fig 2.** Graph of Hemoglobin Levels in Koi Fish (K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5 μl g<sup>-1</sup> deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1 μl g<sup>-1</sup> deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5 μl g<sup>-1</sup> deltamethrin).

**3.3 CD8 expression**

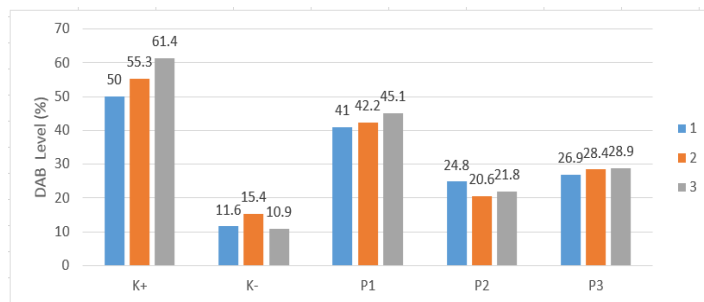
T cells are categorized into two general populations according to their function, cytotoxic T cells (CTL) and helper T cells (Th). CTL expresses CD8 molecules involved in interactions with MHC class I, while helper T cells express CD4 that interacts with MHC class II. Recently, in humans and mice, helper T cells are subdivided into several populations, Th1, Th2, Th17 and Treg which play different roles in the immune response. The enhancement of CD4 T cells is larger and faster than CD8. Pathological changes similar to those that occur in humans are observed in lymphoid organs as well as target organs such as skin, liver and intestines, including destruction of cells and tissues and massive leukocyte infiltration [24].

**Table 3.**  
DAB value of CD8 expression

Treatments	Repetition			Average (%)
	A (%)	B (%)	C (%)	
K+	50	55.3	61.4	55.56
K-	11.6	15.4	10.9	12.63
P1	41	42.2	45.1	42.76
P2	24.8	20.6	21.8	22.4
P3	26.9	28.4	28.9	28.06

Remarks: K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5 μl g<sup>-1</sup> deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1 μl g<sup>-1</sup> deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5 μl g<sup>-1</sup> deltamethrin.

The fish infected with *Myxobolus sp.* without any deltamethrin treatment is used to investigate the CD8 response. It appears as an immune response in koi fish (*Cyprinus carpio*) that attacks by pathogens. Based on the results of IR in the intestinal tissue obtained a DAB value of 55.56%. The DAB value of the control treatment was high because of an infection from *Myxobolus sp.* It is occurring because the immune response from the fish will be active to deal with the attack. The value of CD8 expression in each treatment is being decreased, at P1 42.76%, P2 22.4% and P3 28.06%. It is happening may be because of deltamethrin treatments. The infection of *Myxobolus sp.* is weakened, then CD8 expression is being decreased and the immune system in fish returns to normal. CD8 is a transmembrane glycoprotein and a co-receptor for T-cell receptors (TCR). Like TCR, CD8 binds to the Main Histocompatibility Complex (MHC) molecule that is specific to MHC Class I proteins. In order to work, CD8 forms a dimer, which consists of a pair of CD8 chains. The most common form of CD8 consists of CD8-α and CD8-β chains, the two members of the immunoglobulin superfamily with extracellular domains like variable immunoglobulin (IgV) connected to membranes with thin stems, and intracellular tails. CD8 is a T-cell marker for detection of cytotoxic cells / suppressors of blood lymphocytes. CD8 is also detected in NK cells, mostly thymocytes, sub-populations of zero cells and bone marrow cells. These antibodies are used to differentiate between reactive and neoplastic T cells [25].



**Fig 3.** Graph of DAB value (K+: Infected Fish (*Myxobolus sp.*), K-: healthy fish, P1: Infected Fish (*Myxobolus sp.*) + 0.5 μl g<sup>-1</sup> deltamethrin, P2: Infected Fish (*Myxobolus sp.*) + 1 μl g<sup>-1</sup> deltamethrin, and P3: Infected Fish (*Myxobolus sp.*) + 1.5 μl g<sup>-1</sup> deltamethrin).

**4 CONCLUSION**

*Myxobolus sp.* infections weakened at a dose 1.5 μl g<sup>-1</sup> deltamethrin. Hematocrit and CD8 expression was significantly decreased after deltamethrin administration in 1.5 μl g<sup>-1</sup>. Along with that, the amount of hemoglobin in koi fish was significantly increased.

**ACKNOWLEDGMENT**

Thank you to Dr. Uun Yanuhar has guided research at the Aquaculture Laboratory, Fish Disease and Health Division, Faculty of Fisheries and Marine Sciences, Brawijaya University.

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