Blood condition of *Pangasius hypophthalmus* fed with turmeric enriched pellets and infected with *Aeromonas hydrophila*

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Abstract. Turmeric is commonly used as stimulant and it is an important ingredient in traditional herbal medication. A study aims to understand the blood condition of *Pangasius hypophthalmus* fed on turmeric enriched pellets and were infected with *Aeromonas hydrophila* has been conducted from August to October 2018. There were 3 treatments applied; namely P1: Fishes were fed with turmeric enriched pellets of 0.5 g/Kg feed, P2 (0.7 g/Kg feed) and P3 (0.9 g/Kg feed). The negative control (Pn) were the fish that was not receive any treatment, while the positive control (Pp) was the fish that was infected with *A. hydrophila*, and was not given turmeric powder. Fourteen days after being fed on turmeric enriched pellets, the fishes were infected with *Aeromonas hydrophila* (0.1 ml of 10⁸ of *A. hydrophila* culture). Totally 15 fishes from each treatments were studied. Blood were taken from vena caudalis using 1 ml syringe wet with 10% EDTA and then were checked using binocular microscope for erythrocyte and leucocyte number. Haematocrite and leukocrite levels and types of leucocyte were also studied. Results shown that the blood condition of turmeric treated fishes were different. The total erythrocyte of the negative control (Pn) fishes was 2.136.670 cells/mm³, the positive control (Pp) was 1.423.300 cells/mm³ and that of the treated fishes ranged from 1.520.000 to 2.886.700 cells/mm³. While the number of the leucocytes of the negative control fishes was 153.200 cells/mm³, the positive control (Pp) showing infectious condition with 726.000 leucocyte cells/mm³. The leucocyte number of the turmeric treated fishes (P1, P2 and P3) showing normal values, ranged from 107.000 to 2.069.000 cells/mm³. Based on data obtained, it can be concluded that the turmeric enriched pellets improved the ability of the fish to cope the problems caused by *A. hydrophila* infection.

1. Introduction

*Pangasius hypophthalmus* is one of freshwater fish that commonly inhabit in Riau Province, Indonesia. This fish is belonged to the members of Pangasidae family. In Riau, *Pangasius hypophthalmus* has relatively high economical values. Due to high demand of the fish, many farmers culture that fish in ponds as well as in floating cages. However, there are some problems occurred in development of aquaculture of *Pangasius hypophthalmus* due to *Aeromonas* infection in Riau [1].

*Aeromonas hydrophila* and other motile aeromonads are among the most common bacteria in freshwater habitats throughout the world, and are frequently associated with severe disease among cultured and feral fishes [2,3]. Determinations of the etiology of diseases involving aeromonad infections has been complicated by the genetic, biochemical, and antigenic heterogeneity of members of this group. Consequently, motile aeromonads consist of a complex of disease organisms that are associated with bacterial hemorrhagic septicemias in fishes [4]. The bacterial etiology of these early reports was often inconclusive; however, the pathology described was analogous to that of red leg...
disease in frogs, in which *A. hydrophila* was also implicated as the causal organism. In many isolations of bacteria from hemorrhagic septicemias in fish. There are many factor for the predisposing the diseases such as malnutrition, low temperature, overcrowding and high organic pollution [5].

Motile Aeromonad Septicaemia (MAS) is acute or sub acute or chronic infectious disease in all freshwater fishes caused by motile aeromonad bacteria. The disease caused about 80% mortality in fish farming especially when the fish held under stress [6]. MAS diseases can be transmitted by discharge from the intestinal tract and external lesions on the skin. On the other hand, the parasitic damage and fungal infection of the epidemic may allow the entry and spread of infection among fish. Carrier fish also play an important role in transmission of the MAS infection. This disease is characterized by rapidly fatal septicemia with few gross signs, exophthalmia, ascitis and ulcer formation [5]. This poor performance causing low market price or even can not be sold.

The epidemy of this diseases caused great lost. To solve the MAS diseases relative problem, the farmer tend to use antibiotics. However, the use of chemicals and antibiotics causes negative effects such as bacterial resistance and environmental pollution. To avoid the negative effect of antibiotics, alternative treatment such as the use of turmeric can be applied. The most important chemical components of turmeric are a group of compounds called curcuminoids, which include curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin [7]. The best studied compound is curcumin, which constitutes 3.14% of powdered turmeric [8]. In addition, other important volatile oils include turmerone, atlantone, and zingiberene. Some general constituents are sugars, proteins, and resins. The active compound curcumin is believed to have a wide range of biological effects including anti inflammatory, antioxidant, antitumour, antibacterial, and antiviral activities, which indicate potential in clinical medicine [9].

The use of hematological parameter values to understand the health status of fish is limited. The hematological parameter values are very important to detect fish condition, whether in the cultured fish nor in fish living in the polluted area. Even the information on hematological parameter values in normal fish is almost none. These parameters are very important, as it can be used as a basis to compare the hematological parameters of the unhealthy fish.

Research on blood condition of *Pangasius hypophthalmus* that were fed with turmeric enriched pellets in Riau especially is rare. To obtain information on blood condition *Pangasius hypophthalmus* that were infected with *Aeromonas hydrophila* and were fed with turmeric enriched pellets, this research is needed.

2. Materials and Methods
In this study, fish samples (*Pangasius hypophthalmus*) were obtained from hatchery in Bangkinang, Riau Province, Indonesia. Fish samples were varied from 80 - 120 mm TL and 11 to 16 g BW. The experiment were performed in the Parasite and Fish Diseases Laboratory of Fishery and Marine Science Faculty of Riau University, Pekanbaru. In this experiment, 15 aquaria (25 L) were used and 150 fishes were adapted. *Aeromonas hydrophila* strain (ATCC 35654) used in this study was obtained from Fish Quarantine in Pekanbaru. The fishes were infected with *A. hydrophila* (0.1 ml of 10⁸ of *A. hydrophila* culture). Curcumin was extracted from *Curcuma longa* based on Harjanti [10]. There were 3 treatments applied; namely P1 (Fishes were fed with turmeric enriched pellets of 0.5 g/Kg feed), P2 (0.7 g/Kg feed) and P3 (0.9 g/Kg feed). The negative control (Pn) were the fish that was not receive any treatment, while the positive control (Pp) was the fish that was infected with *A. hydrophila*, and was not given turmeric powder. The fishes were infected with *A. hydrophila* (0.1 ml of 10⁸ of *A. hydrophila* culture). Totally 15 fishes from each treatment were studied. Fish sampled was then anesthetized using clove oil (5 drops/ L water). Blood was then collected from the unconsciously fish, using a tuberculin spuit that was moistened with EDTA. Blood was taken from the artery caudalis (under the *linea lateralis*, in the base of the tail) [11]. The blood sample was then kept in the EDTA moistened vial, placed in a cool box that was filled with crushed ice and was transported to laboratory for further analysis. Hematological parameters studied were as follows: hematocryte and leucocryte level, the number of erythrocyte and leucocyte. The hematology analysis was following [12]. This treated and control bood sample were used to estimate the hematological parameters of *Pangasius hypophthalmus*.
2.1. Determination of Total Red Blood Cells Counts

Total Red blood cells were counted by using haemocytometer. Blood was diluted 1:2000 with Hayem fluid. Erythrocytes were counted in the loaded haemocytometer chamber and the total erythrocyte numbers were reported as \(10^4\)/mm\(^3\).

2.2. Determination of Total White Blood Cells Counts

Total White blood cells were counted by using haemocytometer. Blood was diluted 1:20 with Turk diluting fluid and placed in haemocytometer. 4 large corner square of haemocytometer were counter under the binocular microscope (Olympus CX 21) at 1000X. The total number of white blood cells were calculated in \(10^4\)/mm\(^3\).

2.3. Statistical analysis

Hematological data were analysed using one-way analysis of variance (ANOVA) and significant differences among treatment means were compared using Duncan’s multiple range test (DMRT). Significance was tested at 5% level.

3. Results and Discussions

In this study, the hematological condition of *Pangasius hypophthalmus* were studied. The total erythrocyte count of the treated fishes (P1, P2 and P3) increased, when compared to control fishes. The decrease was highly significant in control fishes than the fishes in all treatment (Table 1).

| Treatments | Hematocryte level (%) | Leucocryte level (%) | Number of erythrocyte \((x10^4\) cells/mm\(^3\)) | Number of leucocyte \((x10^4\) cells/mm\(^3\)) |
|------------|------------------------|----------------------|--------------------------------|------------------|
| Pn         | 31.00 ± 1.00\(^b\)     | 2.3± 1.2             | 213.67 ± 11.37\(^b\)          | 15.32±0.80\(^a\) |
| Pp         | 29.33 ± 1.52\(^a\)     | 3.0± 0.2             | 142.33 ± 6.11\(^a\)          | 7.26±0.49\(^b\)  |
| P1         | 32.67 ± 1.15\(^bc\)    | 2.5 ± 0.5            | 275.67 ± 12.34\(^c\)         | 10.70±0.22\(^b\) |
| P2         | 33.67 ± 0.57\(^c\)     | 2.3± 0.5             | 288.67 ± 9.50\(^c\)          | 20.92±0.76\(^b\) |
| P3         | 30.67 ± 1.15\(^a\)     | 2.5± 0.5             | 152.00 ± 8.88 \(^a\)         | 20.69±0.60\(^b\) |

The number of red blood cells in *P. hypophthalmus* was various, ranged from 1.423.300 – 2,880,000 cells/mm\(^3\). The number of red blood cells in this fish can be categorized as normal. The number of erythrocyte of normal fishes ranged from 1,000.000 – 3,000.000 cells/ mm\(^3\)[12]. The number of leucocyte was also normal, 1 – 2 million cells/ mm\(^3\). In this study, all of the treated fishes shown high hematocryte level, around 30 – 33% and it is predicted that this fish having high hematocryte level naturally. According to Raa et.al [13], the high hematocryte level (27%) was also found in healthy female of *Pangasius* sp. The hematocryte levelof healthy fish is around 30 – 50%. This fact indicates that the hematocryte level of the treated fishes in this study is normal.

The hamatocryte and leucocryte levels, and the number of erythrocyte in control positive in this study can be categorized as normal. However, the number of leucocyte is slightly higher than that of the healthy fishes. The normal fish has 20,000 -150,000 cells/ mm\(^3\) [12]. It is predicted that the fish might be infected or has been infected with microorganism. Due to the infection, defence system in fish become active and it is reflected in the high number of leucocyte. Moreover, leucocyte levels of infected fishes (Pp) was relatively high (3%) and it indicates that there is any infection or microorganism attract like Aeromonas infection.

Result shown that the leucocyte level level in treated fishes was relatively low (2.5 %) and it may be caused by the high number of white blood cells, which was 107,000 – 209,000 cells/mm\(^3\) (Table 2). In all of fish sampled, the leucocyte level and the number of white blood cells were high and these might be the characteristics of *Pangasius* sp. in Pekanbaru [15]. Leucocyte number was significantly higher in experimentally infected fish at 1.9x10\(^6\) and 7.3x10\(^6\) CFU ml which indicates that *A. allosaccharophila* could induce the non specific innate defense mechanism [11].
Study on the type white blood cells indicates that all of white blood cells type were present in *Pangasius hypophthalmus*, there were lymphocyte, monocyte and neutrophyl (Table 2). The most common cell was lymphocyte (around 72 – 83%), monocyte was around 8-13% and 8-14% respectively. The number of granulocyte was lower that of the other type of cells. According to [15] the number of granulocyte was various, around 4 – 60% from total leucocyte population.

Table 2. Percentage of white blood cells present in *Pangasius hypophthalmus*

| Treatments | Lymphocyte (%) | Monocyte (%) | Netrophyl (%) |
|------------|----------------|--------------|--------------|
| Pn         | 77,00±1,00\(^b\) | 12,00±1,00\(^a\) | 11,00±1,00\(^c\) |
| Pp         | 72,33±0,57\(^a\) | 13,33±0,57\(^a\) | 14,33±0,57\(^b\) |
| P1         | 80,00±1,00\(^c\) | 9,66±0,57\(^a\)  | 10,33±1,15\(^b\) |
| P2         | 83,00±1,00\(^d\) | 8,33±0,57\(^b\)  | 8,66±0,57\(^a\)  |
| P3         | 79,66±2,08\(^c\) | 10,00±1,00\(^b\) | 10,33±2,51\(^a\) |

Note: Pn (negative control), Pp (Positive control), P1 (fish were fed with 0.5g/Kg), P2 (0.7 g/kg feed), P3 (0.9g/Kg feed).

Martins et.al [17], stated that an increase in the number of circulating lymphocyte is observed in bacterium infected fish. Increased number of lymphocytes is an integral part of defense mechanism of any living organism. However, there are reports of lymphopenia under certain conditions, especially when fishes are intraperitoneal injected with bacterium.

The lymphopenia in intraperitoneal injected fishes might be caused by localized migration of lymphocytes to tissues. Neutrophils are the first line of innate immune against infectious diseases. Apart from releasing various antimicrobial molecules, neutrophils also release neutrophil extracellular traps (NETs) for the containment of infection. Activated neutrophils provide signals for the activation and maturation of macrophages as well as dendritic cells. Neutrophils are also involved in the regulation of T-cell immune response against various pathogens. The number of neutrophils in fish injected with bacterium are not significantly different from the un-injected and PBS injected fish, which is in concurrence with the earlier finding while few other researchers found significant increase in the number of neutrophils and monocytes after bacterial infections [17].

In this study, monocytes were higher in infected fish in comparison to control fish. Phagocytosis is one of the important processes in poikilothermic animals because it is the process that is least influenced by temperature. Phagocytic cells are the important cellular components of the innate immune system of fish and their phagocytic activity is a defence mechanism, which is an important characteristic of the non specific immune system. The main cells involved in phagocytosis in fish are neutrophils and macrophages [18]. In this study the higher percentage of phagocytosis by leukocytes was observed in infected fishes (Table 1), as well as the highest phagocytosis was found in fish injected with 10^8 CFU ml-1 of *A. hydrophyla*. This bacteria pathogen is a rare bacterium, known to cause disease in fish. However, so far there are no reports of the effect of this bacterium on innate immune cells of catfish. The results showed that, there is significant difference in some of the studied immune related blood parameters of catfish, experimentally infected with *A. hydrophila*, which indicates that this virulent bacterium could induce the migration of immune cells [19].

In this study, the fishes that were fed with turmeric enriched pellets of 0.7 g/Kg feed shown the good hematological condition. *A. Anguilla* were treated with traditional Chinese medicines (TCMs) increased significantly their resistance to common infectious diseases [19]. According to Wu et.al [20] observed that traditional Chinese medicines had a beneficial effect on the growth and on the prevention and treatment of common diseases in *C. carpio*. Neem leaves, garlic and turmeric powder induced diseases resistance of fry of carp [19]. In this study, we found that herbal medicine (curcumin) can be use as alternative medicine for practical use in diseases management strategy in fish. However, the MAS symptoms in the fish in this study was not completely cured. The blood condition of the fish was taken by the 14th day after being treated with turmeric supplemented in feeding. This time period may not be enough for the fish organ for recover from the damage cause by the MAS diseases.
fish was reared for longer time, the condition of teh blood might be better. Organ recovery will be completed by around 30 days [22]. The use of natural immunostimulants in aquaculture has been increasing rapidly for the prevention of diseases and also to avoid the indiscriminate use of hazardous antibiotics. Herbal immunostimulants are biocompatible, biodegradable and safe for the environment and human health [17]. The blood condition of curcumin enriched pellets 0.7g/Kg feed (P2) shown in normal condition, because the curcumin and its analogues demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC) can protect the endothelial cells from Abeta-induced oxidative stress, and these compounds were better antioxidants than alpha-tocopherol [2]. As curcumin showing great potensial for curing the symptoms, the use of curcumin for curing the MAS diseases is recommended. So, it can be concluded that the use of curcumin with 0.7 g/Kg feed concentration is able in curing motile aeromonads septicaemia diseases in Pangasius hypophthalmus.

4. Conclusion
The best result shown by the addition of 0.7gr turmeric in 1 kg fish feed pellets. The fish that being infected with A. hydrophila and then was feed with turmeric enriched pellets do not shown any clinical signs of MAS diseases, indicate that the fish was able to face the Aeromonas hydrophila attack.

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