The Effectiveness of Turmeric Enriched Pellets in Curing the Gill of *Clarias batrachus* Infected with *Aeromonas hydrophila*

M Riauwaty¹, Windarti²

¹Department of Aquaculture, Fisheries and Marine Science Faculty, Riau University, Pekanbaru
²Department of Aquatic Resources Management, Fisheries and Marine Science Faculty, Riau University, Pekanbaru

*morina.riauwati@lecturer.unri.ac.id

**Abstract.** Curcumin is an antibiotic agent extracted from *Curcuma longa*. To understand the effectiveness of curcumin in curing the gill of *Clarias batrachus* infected with *Aeromonas hydrophila*, a study was conducted from August to November 2020. There were 3 treatments applied, namely turmeric enriched pellets 0.7g/kg (P1); 0.9g/kg (P2) and 1.1g/kg (P3). Prior to the treatment, the fish was infected with *A. hydrophila* (0.1 m of *A. hydrophila* culture, 1.0x10⁸ cells/ml). The infected fish was kept for 6 days until the clinical signs of MAS disease occurred. By the 7th day, the fish was feed with turmeric enriched pellets. For negative control (Cn) the fish was not infected with *A. hydrophila* nor treated with turmeric, while the positive control (Cp) was infected, but no turmeric treatment. The fish was reared for 30 days and by the end of the experiment, the gill was removed and processed for histological study (5 μm sliced and HE stained). Result indicated that the gill of the negative control fish was normal, while that of the positive control shown abnormalities such as hemorrhages, necrotic cells, fused lamellae and enlarged cartilaginous cells. The turmeric treated fish shown less damage in the gill. The best results were obtained in C3, as the gill structure was almost normal with slightly hemorrhage. In the C1 and C2 treated fishes, the gill shown light abnormality such as fused lamella and hemorrhage. Data obtained shown that the consumption of turmeric enriched pellet was effective to cure the *A. hydrophyla* infected gill.

1. Introduction

Catfish farming business in Riau, Indonesia at this time has experienced significant improvement in quality and quantity. Cultivation systems carried out by cultivators in Riau mostly with high stocking densities, but the water changes might be limited. This condition resulted in poor water quality that trigger development of pathogen and leads to infectious diseases in fish. Pathogen attacks is very serious problem in cultivation business, as it reduces production, and even lead to deadly outbreaks and mass death [2].

*Clarias batrachus* is a fishery commodity that is widely cultivated in Indonesia. Catfish production from 2017 – 2018 increased from 841.75 thousand tons to 1.81 million tons or 114.82% increase [12]. In african catfish intensive farming, the *Aeromonas hydrophila* attacks causes MAS diseases that causes loss not only because of the death of fish but also the wounded fish that has no economic value. The MAS disease outbreaks causes mortality up to of 80-100% within 1-2 weeks [10]. Economic
losses do to pathogen attacks may be difficult to be overcome, but the disease can be prevent through improving the immunity system of the fish.

One of the natural ingredients that can be used as an antibacterial as well as boosting the immunity system is turmeric. The main compounds contained in the turmeric rhizome are essential oils and curcuminoids [3]. The use of turmeric is known to have several pharmacological effects such as anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, and anticancer [1].

The sensitivity of Aeromonas sp. toward turmeric was 13 mm with LD$_{50}$ was around 1.54 g/l [7]. In fish that was feed with turmeric enriched pellet (0.7 g/kg feed), increase the immunity of Pangasius hypophthalmus and C. batrachus toward Edwarsiela tarda attack [9]. In these fish, the abnormality in gill structure that is caused by the bacteria attack was recovered after being treated with turmeric enriched pellets.

So far, the MAS disease commonly attack the cultured C. batrachus, however, information on preventing and curing efforts conducted to solve the problems were limited. To understand the effectiveness of turmeric in curing the gill wound due to A. hydrophila attack, a study titled “The effectiveness of turmeric enriched pellets in curing the gill of Clarias batrachus infected with Aeromonas hydrophila” was conducted.

2. Methodology
This research has been done in August to November 2020 in the Parasite and Fish Diseases Laboratory, Aquaculture Department, Fisheries and Marine Science Faculty, Riau University, Indonesia. The experiments were carried out within the ethical guidelines provided by the research institution and national or international regulations.

2.1. Experimental design
The method used in this study is an experimental method with Completely Randomized Design (CRD). There were five treatments applied, namely:

- CN : Negative control (no treatment)
- CP : Positive control (infected with A. hydrophyla, no turmeric)
- C1 : The fish was fed with 0.5 g turmeric enriched pellets
- C2 : The fish was fed with 0.7g turmeric enriched pellets
- C3 : The fish was fed with 0.9 g turmeric enriched pellets

2.2. Rearing of fish
Clarias batrachus obtained from the hatchery of the Riau Province’s Marine Fisheries and Department in Tibun, Pekanbaru, Riau, Indonesia. The fish used were 7-8 cm total length (TL) and 5-7 g body weight (BW). The fingerlings were acclimatized under laboratory conditions for 4 days. The fish were then reared in aquaria (30 x 40 x 40 cm$^3$,10 fish/aquarium) completed with aerators and filters. There were 150 fish used in this study (30 fish/treatments for five treatments). The feed given was commercial feed with 35% protein content and the turmeric enriched feed was given for 30 days, three times per day (morning, noon and afternoon), ad satiation.

2.3. Fish feed preparation
Turmeric was obtained from traditional markets in Riau, Indonesia. The turmeric was cleaned, peeled, thinly sliced and dried. The turmeric was then blended and then sifted. The fish feed used was commercial feed type F-999 with 35% protein content. The addition of turmeric in feed was done by diluting turmeric into a table spoon (around 5 ml) of heated distilled water and the solution was sprayed evenly into commercial 1 kg of feed using a sprayer, mixed well and then the feed was dried. The turmeric enriched pellets was ready to be given to the treated fish.

2.4. Aeromonas hydrophila infection
Prior to treatment, the fish was infected with *A. hydrophila* (0.1 ml of *A. hydrophila* culture, 1.0x10^7 cells/ml). To avoid stress, the fish was sedated using clove oil and as they were unconscious, they were injected using a 1 ml syringe intramuscularly. The infected fish was kept for 6 days until the clinical signs of MAS disease occurred. By the 7th day, the fish was feed with turmeric enriched pellets for 30 days.

2.5. Histological study
For histological study, by the 30th day, 3 fishes/ aquarium were sacrificed and the gill was removed. The tissue samples were processed according to standard of histological technique [12]. The gills were fixed in 10% formalin for 24 hours and in 4% formalin for three days. Then the gills were processed using alcohol series and paraffin, 5 µ sliced and Hematoxylin-Eosin (HE) stained [12]. Histological slides were observed using a binocular microscope Olympus CX-21 for evaluating the gill condition and it was analyzed descriptively.

3. Results and Discussion
Result shown that the gill structure in the turmeric fed fish and in the fish with no turmeric was different. As the fish were infected with *A. hydrophila* bacteria, the bacteria were multiply in the fish tissue and produce toxic (aerolysin) that damage the tissue, including the gill tissue. In the control positive (CP) fish, the *A. hydrophila* attack causing damage in the gill structure and there was no sign of curing. This fact indicates that the immunity of the fish did not able to combat the bacteria attack and as a result, the damage signs such as hemorrhages, necrotic cells, fusion of epithelial cells and enlarged cartilaginous cells occur (Figure 1). As the gill tissue was infected by *A. hydrophila*, this bacteria produced aero and hyla genes which are responsible for producing aerolysin and hemolysin toxins. Aerolysin is an extracellular protein produced by several strains of *A. hydrophila* which is soluble, hydrophilic and has hemolytic and cytolytic properties [11]. The Aerolysin from *A. hydrophila* may damage the fish cells through their hemolytic and cytolytic action. This poison may attack epithelial cells as well as causing gastroenteritis in fish [11].

The gill of fish is consisted of epithelial and other supporting tissues [12]. As the epithelial cells are target of *A. hydrophila* poison, several abnormalities signs present in the gill of the *A. hydrophila* infected fish. In this study, the abnormalities present in the gill of fish infected with *A. hydrophila* were hemorrhages, necrotic cells, fusion of epithelial cells and enlarged cartilaginous cells. This results were similar to gill structure of the infected fish studied by [10], [11]. The gill in general or the secondary lamella were damage and the shape of the lamelleae were no longer perfect [13]. The fish with Aeromonas infection shown severe damages such as complete injury of gill filaments, mucous deposition, vacuolization, aneurysm, loss of secondary lamella and loss of chloride cells. In fish, gill is the primary target organ of many toxicants as the absorption of all contaminants occurs mainly through the gills that are continuously in contact with water [4].

The weakness of the immunity system in the infected fish in this recent study may be due to the lack of supplement that empowers the self-defense system in the fish. In the turmeric treated fishes, in contrast, the gill structure shown curing process as indicated by less of abnormality symptoms. The curing of the gill structure indicates that the immune system responses well toward the attack of the bacteria and it may reduce the bacteria population as well as the negative impact caused by the attack. The only abnormality present in the gill of turmeric feed fish was light hemorrhage. This abnormality was categorized as the 1st category of gill damage [12] and it may be cured as the water quality improved and the bacteria vanished.

This fact suggests that turmeric improves the condition of the gill tissue of infected fish. It is also strongly alleged that turmeric is able to improve the digestion and nutrient metabolism [1]. Turmeric feeding may elevate the non-specific immune system and provide long term defense toward pathogenic bacteria attack.

Beside containing curcumin, turmeric contain an active compound that is able to improve immunity as well as increases the appetite of the fish toward feed provided [8]. Curcuminoids serve as
The curcumin was also contain flavonoids that is able to protect the cell structure, improve the effectiveness of Vitamin C absorption, anti-inflammatory, and antibiotic agents. By consuming curcumin, the fish response well to feed provided and thus improve the feed consumption as well as feed digestibility [5],[9]. Similar results were obtained in gold fish that was fed with turmeric enriched feed. The fish shown highest acid protease, alkaline protease and lipase activity that enhanced the growth rate of the fish. In the fish that was fed with turmeric extract, there was significantly higher specific activities of digestive enzymes and it may enhanced the growth [5]. The curcumin improve the immune stimulatory response of Clarias batrachus [8]. This fact indicates that curcumin had antibacterial effects and played a role in inhibition of the growth of the bacteria trough improving the ability of the non-specific immune system to overcome the destroying effect of the bacteria and preventing septicemia signs.

Figure 1. Histological structure of the gills of Clarias batrachus. CN (Negative control). CP (Positive control, C3 (Gill structure of turmeric treated fish (1.1g/kg). PL: Primary lamellae, SL: Secondary Lamellae, H: Haemorrhage, Hp: Hyperplasia, N:Necrosis, Ecc: Epithel enlarged cartilaginous. HE Staining, 400x magnification).

4. Conclusion
The gill structure of the fish that was infected with A. hydrophila and was fed with turmeric enriched pellets is showing less abnormalities than that of the fish with no turmeric treatment. The turmeric is able to improve the non-specific immune system in the fish and the fish was able to face the Aeromonas hydrophila attack. The best result was obtained in C3 (1.1g turmeric in 1 kg fish feed pellets). It is proved that turmeric consumption is able to improve the health of fish in general and it is able to cure the damage in the gill of Clarias batrachus infected with A. hydrophila.

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