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The potential of probiotics of malondialdehyde and gonadosomatic index of Tilapia (*Oreochromis Niloticus*) after exposure of cadmium

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Abstract. Cadmium pollution originating from factory waste and human activities is very dangerous because it has high toxicity and becomes a threat for aquatic organisms including tilapia as an aquatic animal that is sensitive to heavy metal pollution. Cadmium contained in water will enter the body of fish and damage organs, one of which is the testes. It will affect the productivity of tilapia and harm the farmers. Therefore, in this study, supplementation of probiotics and vitamin C was provided to repair testicular damage due to cadmium exposure. The parameters evaluated included the level of malondialdehyde (MDA) and the gonadosomatic index (GSI). Based on data analysis of MDA levels and GSI values, it is known that supplementary feeding (probiotics, vitamin C, and a combination of both) has a positive effect on the value of GSI, but testicular MDA levels only feed with vitamin C that can provide a positive effect.

1. Introduction

Heavy metal is potential to be carcinogenic and mutagenic in the body. It is released into aquatic environments by industries and human activities [1]. Its nature to be affected by mutagenic or carcinogenic compounds, heavy metal pollution in pond waters becomes problem in tilapia cultivation. Pond which is affected by heavy metal decreases the fish cultivation productivity. Based on its carcinogenic nature, International Agency for Research on Cancer of USA places cadmium in the first level as very dangerous carcinogenic material [2]. Previous research proves that oxidative stress is the main mechanism of cadmium toxicity. Cadmium exposure inhibits antioxidant performance and cause production of Reactive Oxygen Species (ROS) which is not normal so that it causes lipid and oxidative damage in DNA [3]. Oxidative stress is a condition where the number free radicals is greater compared to antioxidant. If the amount of free radicals exceeds the ability of antioxidants in neutralizing them, then the excess of free radicals has the potential to cause cell death [4].

Determination of Malondialdehyde (MDA) illustrates free radical activity which indirectly becomes an indicator of oxidative stress. This measurement of MDA by Thiobarbituric Acid Reactive substances test (TBARs test) [5]. One of the organs that can be measured for MDA levels is the testes. The testes are very sensitive to Cd toxicity, because there are many blood vessels, where blood vessels are the main target of Cd toxicity [6]. Some research has shown that intense exposure of Cd in in vivo manner caused



problem of blood testis barrier (BTB), germ cell loss, testicular edema, bleeding, necrosis, and sterility in some species [7].

In addition to using MDA levels to determine changes or damage that occur in the testes, quantitative observations can be conducted by counting the gonad somatic index (GSI). Similar to hematological and biochemical parameters that can change according to environmental conditions, GSI is also often used to describe and monitor the toxicity of a heavy metal in aquatic animals. In general, GSI reflects on the development, metabolism and reproductive status of an organism in a respectful manner. GSI is an important parameter that reflects the survival of a generation and changes in organisms under the influence of heavy metals [8].

The oxidative stress caused by heavy metal exposure is by increasing the antioxidant in the fish body. One of the substances that can be antioxidant is vitamin C. In the testicle, vitamin C functions to protect the process of spermatogenesis which is by maintaining the number of spermatogonium or its quality, maintain the spermatozoa membrane integrity, and improve the level of testosterone [9]. Vitamin C has ability to catch and non-activate the free radicals or ROS resulted from heavy metals. Beside using vitamin C, several types of *Lactobacillus* has potential as probiotic to reduce and treat toxicity caused by heavy metal. Research of Zhai *et al.* [10] shows that *Lactobacillus plantarum* CCFN8610 as probiotic with ability to bind cadmium and as antioxidant, can significantly inhibit the absorption in digestive tract. This can be seen by the decrease of cadmium accumulation and ease the induction of cadmium in the system [11].

This research was conducted to find out the influence of supplement feed (probiotic and vitamin C) to GSI and MDA concentration of *Tilapia* testicles which is exposed by cadmium.

2. Methods

2.1. Food Supplement

The feed used is a commercial feed. The feed with probiotics was conducted by spraying 25 ml probiotic solution and spraying it into 1 kg of commercial feed and then dried. Feed with vitamin C was made by mixing 100 mg of vitamin C into 1 kg of pellet. Feed with probiotics and Vitamin C was made by spraying 25 ml of probiotic solution and mixing of vitamin C 100 mg/kg feed

2.2. Preparation of Cadmium Sulphate

Cadmium sulfate main solvent with a concentration of 500 ppm was prepared by diluting 0.92 grams of CdSO₄ into 500 mL of distilled water. The variation of concentration 0; 0.3; and 0.6 ppm CdSO₄ used in this research.

2.3. Animal Experiment

Thirty-six tilapia (*Oreochromis niloticus*), weighing 250-300 grams, obtained from freshwater fish farms at Pandaan, Pasuruan, East Java, Indonesia. Each fish was kept in a tank in fresh water by aeration at 25 °C ± 1.5 in the animal laboratory at Airlangga University. The fish was kept in light for 12 h and in the dark for 12 h. The fish were divided into 12 equal groups each comprising of three fish. Each group was kept in separate glass tank. The first group was kept in a tank contained normal water without any treatment, this group was used as negative control. The fish of test groups were exposed to variation Cd (0, 0.3, and 0.6 ppm) with variation type of feed (feed, feed containing vitamin C, feed containing probiotics, and feed containing combination of both vitamin C and probiotics), and the study was conducted for 15 days. The fish were acclimatized at the two weeks.

2.4. Malondialdehyde (MDA) Determination

Around 10 grams' fish testis dilution on 150 mL of saline was added into 300 mL of ice-cold phosphate buffered saline (PBS) and homogenized by brief Sonication on ice (20 s). Malondialdehyde concentration was analyzed by QuantiChrom™ TBARS Assay Kit (DTBA-

100) (BioAssay Systems, USA) according to the manufacturer's protocol. The absorbance was measured at 535 nm.

2.5. Gonadosomatic index-(GSI)

The Tilapia to be operated is weighed its weight first, as well as the gonad which has been obtained from operation result. Then the *Gonadosomatic Index* (GSI) is calculated using the formula 1:

$$\% \text{ GSI} = \frac{\text{Weight of gonad (g)}}{\text{Weight of fish (g)}} \times 100\% \quad (1)$$

2.6. Data Analysis

The statistical analysis calculation using Statistical Package for the Social Sciences (SPSS version 17.0; SPSS, Inc., Chicago, IL). The statistic Test used is normality data and homogeneity test which then continued with One-Way ANOVA test with posthoc Duncan to examine the difference between treatments. The significant value used is $P < 0.05$.

3. Results and Discussion

Morphology of tilapia has a round body shape oval, high back, the body and caudal fin found a straight line (vertical). The dorsal fin was found extending straight lines. Tilapia live in freshwater and tails to move, stomach fins, pectoral fins and hard gill covers to support the body. Tilapia has five fins, namely dorsal fin, pectoral fin, ventral fin, anal fin, and caudal fin. The dorsal fin extends from the top of the girdle lid to the top of the caudal fin. A pair of pectoral fins and a small pelvic fin and anus fin that are only one fruit elongated in shape and the tail fin is only one fruit with a round shape and has a pair of testicles located in the abdomen (Figure 1).

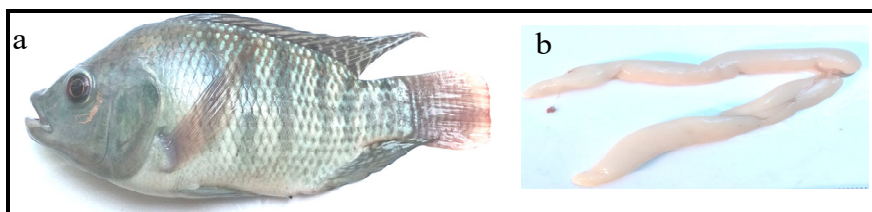


Figure 1. Morphology of tilapia (a) and a pair of testicles (b)

3.1. MDA Concentration

Heavy metal Cd had effect of increasing MDA significantly at 0.6 ppm ($P < 0.05$) while a concentration of 0.3 ppm did not significant ($P > 0.05$). Cd 0 ppm and supplementary feeding have not a significant different for MDA ($P > 0.05$) compare the control. At 0.3 ppm Cd and vitamin C was significantly increased MDA ($P < 0.05$) while other feeding did not a significant different ($P > 0.05$). At 0.6 ppm Cd with supplement of probiotics and its combination probiotic-vitamin C were significant reduced MDA ($P < 0.05$), but supplementary vitamin C significantly increased of MDA ($P < 0.05$) (Figure 2).

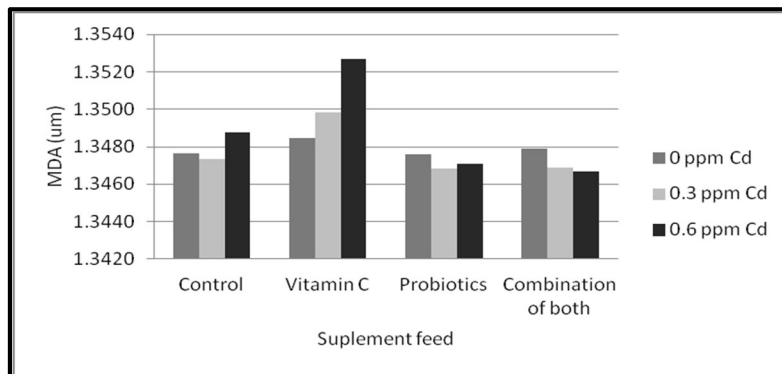


Figure 2. MDA of Tilapia's gonad with supplement feeds I different concentration of Cd (0.3 and 0.6 ppm)

3.2. Gonadosomatic Index (GSI)

The results of data analysis showed that in controls, exposure of 0.3 ppm Cd decreased GSI insignificantly ($P > 0.05$) compared to 0 ppm, but exposure of 0.6 ppm significantly increased GSI compared to 0.3 ppm. Insignificant vitamin C supplementation ($P > 0.05$) reduced GSI exposed to 0.03 ppm, but there was a significant decrease ($P < 0.05$) after exposure to 0.06 ppm compared to 0 ppm Cd. This is different when adding probiotic supplements that can increase fish GSI. The combination of probiotics-vitamin C did not significantly affect GSI ($P > 0.05$) (Figure 3).

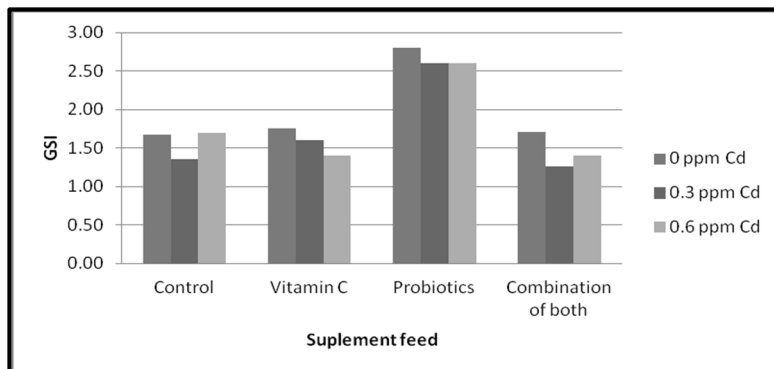


Figure 3. The percentage of Tilapia's GSI with supplement feeds in different concentration of Cd

Cadmium (Cd) is one of the most toxic heavy metals that inhibit physiological processes on the organism body. This heavy metal increase environmental concern due to its wide variety of adverse effects. The organisms are exposed to Cd through the intake of water, food and contaminated air. Cadmium is absorbed from the digestive tract and mainly accumulated in the tissues and organs, especially liver where it is bound to metallothionein (MT). When Cd concentration exceeds the binding capacity of MT causes toxicity possibly due to free radical induction and lipid peroxidation [12]. Oxidative stress by lipid peroxidation which changes the membrane cell permeability and DNA integrity on the cell nucleus. They caused abnormality in cells function [13]. In this research, Cd with concentration of 0.6 ppm can increase the level of MDA because the exposure of Cd inhibits the antioxidant performance and causes production reactive oxygen species (ROS) which is abnormal that cause lipid peroxidation and oxidation damage to DNA [3].

Cd with a concentration of 0.6 ppm also can increase the value of GSI due to histological damage caused by Cd in the form of a disruption in Tight Junction in blood vessels and cause leakage of

erythrocytes into the interstitial space, causing bleeding and edema. It is this bleeding and edema that causes the testicles to become enlarged and more severe [7].

Supplementary feeding in the form of probiotics and probiotics-vitamin C combination can reduce MDA levels because probiotics can decrease the toxicity of Cd in the fish body by acting as an antioxidant and helping the work of vitamin C to protect intestinal epithelial cells from ROS to remain intact so that cadmium cannot enter into the blood circulation through the intestine [14]. Whereas supplementation in the form of vitamin C alone can significantly increase MDA because vitamin C has a special role in the redox reaction of metal ions and activates these ions to exacerbate the effects of oxidative stress [15]. In the concentration of Cd 0.6 ppm the supplement feed is in the form of probiotics, vitamin C and combination of probiotic-vitamin C significantly decreases the GSI value where this means that supplement feed can reduce the effect of bleeding and edema caused by Cd in the testicle.

4. Conclusion

Cd exposure increase MDA and reduced GSI. The addition of a probiotic supplement is better than the vitamin C supplement and its combination.

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