The effect of diet supplement on Oreochromis niloticus (L.) morphometrics in environments contaminated with Cadmium

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Abstract. The use of probiotic supplements from a consortium of microorganisms is a new alternative approach to improve and utilize the bioremediation of heavy metals. Some of the mechanisms of protection against heavy metal resistance by microorganisms include extracellular barriers, active transport of metal ions (efflux), and reduction of heavy metal ions. A study of the benefits of dietary supplements on morphometry of tilapia exposed to heavy metal cadmium has been carried out. This study wants to analyze the potential of vitamin C, probiotic (lactic acid bacterial consortium, LAB), and the combination of both to changes in the morphometric character of fish due to exposure to heavy metals cadmium. Thirty-six samples were examined for morphometric characteristics based on variations in treatment, namely variations in Cd concentrations (0, 0.3, and 0.6 ppm) and variations in the supplementary feed. The results of the study showed that there were many changes in fish morphometry. Significant cadmium exposure reduced bodyweight and morphometric size (total length, standard length, operculum length, chest fins to dorsal fins, and fishtail height). The administration of dietary supplements increased the growth of the fish body weight and other morphometry exposed to cadmium. Probiotic supplementation from the LAB consortium showed the best performance in increasing the fish morphometry, followed by vitamin C supplementation and a combination of both. This study gave valuable information for broodstock farmers or hatcheries that use water sources from rivers or reservoirs that are contaminated with heavy metals.

1. Introduction

Probiotic is a collection of safe in the gut microbiota, that mainly help in the immune system. Many bacteria act as probiotics, but only a few are able to remediate heavy metals, including the lactic acid bacteria (LAB) group. The groups of LAB categorized as probiotics, among which are Lactobacillus, Streptococcus, Lactococcus, Leuconostoc, Pediococcus, Enterococcus, Bifidobacterium and Weissella. Lactic acid bacteria are able to remediate heavy metals by interacting with various toxic metals, including cadmium and copper [1]. This potency is the difference between the positive metals and the negative charge of bacteria. The cell surface of probiotic bacteria has the ability to bind to the metal with the opposite charge then settle on the cell wall. Thus probiotics can convert toxic heavy metals into non-toxic ones [2].

The probiotic potential of the consortium from LAB in the remediation process has been proven effective, and this is very beneficial for living things exposed to heavy metals, including fish. Aquatic fish populations are strongly influenced by water quality. Polluted waters have an impact on the life of the biota in them. Heavy metal contamination decreases the reproductive health of fish, including testicular damage, decreased sperm quality, decreased fertility, and development of fish embryos [3,4].
The pollution of the river or reservoir caused by heavy metals. They are poisonous and disturb the ecology of the waters. The main sources of heavy metals in waters come from industry, agriculture, and human activities including mining, smelting, and manufacturing synthetic compounds which cause an increase in the number of heavy metals released into water bodies [5,6]. This results in the large number of aquatic biota being exposed to heavy metals and accumulating in the body, thus affecting growth and development, including morphometry [7].

Morphometric studies are important in analyzing the growth, development, and morphological variations of various fish species in populations or between populations. Therefore, this information is important for the future management plan for fish resources.

2. Material and methods
   2.1. Preparation of the probiotics for fish diet supplementation
   The probiotics were the mixed cultures consisting of Lactobacillus bulgaricus (NBRC13953), Lactobacillus fermentum (ME3), Lactobacillus buchneri (DSM 20057), and Lactobacillus casei (DSM 20011) at the ratios of 1:1:1:1. The solution of bacteria (1x10⁸CFU/ml) was sprayed at the ratio of 200 ml/kg of the fish diet and then air-dried. The fish diet used in this study was a commercial fish diet (Prima Feed, PF- 500), containing 25.57% crude protein, 6.72% crude fat, crude fibre 3.49%, ash 9.16%, and energy 2974.80 Kcal/Kg. The feed was prepared weekly and stored at 4°C.

   2.2. Experimental design
   Tilapia male with approximate age around three months and weight around 150 g were obtained from a local fish farm in Pandaan, Pasuruan, East Java. The fishes were acclimatized for two weeks and fed with basal diet. The physicochemical parameter of the water was monitored twice a week. Tilapia (O. niloticus) as many as 36, weighing about 250-300 g divided into 12 groups. Each group is kept in a separate glass tank. The first group (n = 3), each fish was kept in a separate tank containing plain water without any treatment, this group was used as a negative control. The fish in the treatment group were given heavy metal Cd (concentration variations 0, 0.3, and 0.6 ppm) with variations in the type of feed, namely commercial feed, feed containing vitamin C, feed containing probiotics, and feed containing a combination of both. The study was conducted for 15 days. The fish were acclimatized in the tank in two weeks.

   2.3. Morphometry analysis
   Measurements and calculations have been carried out on standard methods. Body composition measurements were made with a scale and a digital scale determined fish body weight. Six morphometric characters were used to analyze the growth and development of each fish sampled in this study. Fish body weight is expressed in grams, while fish morphometric characters are expressed in centimetres (Figure 1).
2.4. Statistical analysis
Data analysis used analysis of variance (ANOVA) to see the interaction between treatments. Statistical significance was accepted at a level of $P < 0.05$ using statistical tests (SPSS version 21).

3. Result and discussion
The result indicated that Cd exposure significantly reduced fish body weight ($P < 0.05$). The addition of probiotic feed supplements and other supplements significantly increased fish body weight ($P < 0.05$), except for the group given probiotic supplements and exposed to 0.6 ppm Cd. The highest weight gain, respectively, was after the vitamin C, the combination, and probiotics (Figure 2).

Exposure to Cd at various concentrations decreased fish morphometry significantly ($P < 0.05$). Probiotic supplementation significantly increased ($P < 0.05$) the total body length, the standard length, and the head length of tilapia. The difference in morphometric sizes before and after treatment is presented in Figure 3, indicating that the administration of probiotics and vitamin C was better than the combination of the two.
Figure 3. The difference in total body length, the standard length, and the head length of fish before and after treatment with various Cd concentration and various feed supplements.

The distance between the dorsal and pectoral fins was also affected by Cd concentration variations. The greater the Cd concentration the shorter the distance between the two fins. This condition also occurred at the height of the base of the fish's tail. Supplementary feeding improved the morphometry of fish exposed to Cd. The distance of the two fish scales given probiotics and a combination supplement was higher than the fish given only vitamin C. However, the feeding of probiotics, vitamin C, and their combination increased the height of tilapia that were exposed to 0.3 and 0.6 ppm Cd concentrations. The difference in morphometric sizes before and after treatment is presented in Figure 4.

Figure 4. The difference in the length of pectoral and dorsal fin as well as the height of fishtail before and after treatment with various Cd concentration and various feed supplements.

The data on the effect of Cd exposure with various concentrations for 15 days on fish body weight (Figure 2) shows a decrease. The higher the concentration, the smaller the fish body weight. This was because Cd, one of the toxic heavy metals, entered the fish body through its food and entered the cells through the calcium channels of the cell plasma membrane. Accumulation of Cd caused oxidation stress which leads to DNA damage, chromosomal aberrations, and changes in gene expression [8]. It also caused cell and tissue damage [9], morphological abnormalities and even cell death [10,11]. Cd toxicity occurs due to the formation of reactive oxygen species (ROS) which causes oxidative damage to cell constituent molecules [12]. When the ROS is higher than the antioxidant capacity in cells, it will cause oxidative stress which results in oxidative damage that can lead to cell damage [13].
Cadmium-induced cell damage causes necrosis and/or apoptosis [14]. This tissue damage can result in weight loss.

The addition of a variety of feed supplements successfully increased the bodyweight of fish exposed to Cd. Probiotics are composed of a consortium of lactic acid bacteria (LAB). These bacteria are Gram-positive bacteria that have a role in decontaminating and neutralizing the toxins of heavy metal in water or foods [15]. Lactic acid bacteria, especially the order of Lactobacillales, reduced the absorption of toxic substances by the fish digestive tract [16]. The LAB in the remediation process of heavy metals has been proven effective. These bacteria are able to bind to metals due to the specific structure found in their cell walls. The bacterial cell wall is composed of peptidoglycan acid, lipoteichoic acid, and neutral polysaccharide structures allowing adhesion to macromolecules.

Vitamin C plays a role in antioxidants which reduce the toxic properties of heavy metal oxidants. Cd exposure also decreased fish morphometrics. It was presented in Figure 4 that shows the difference in total length and standard length between before and after treatment, as well as the difference in the length of pectoral and dorsal fin as well as the height of the fishtail. Probiotic and vitamin C supplements and their combinations have increased all lengths of fish. In addition to neutralizing toxins from Cd, supplements can also improve cell health so that it can trigger cell growth and development. Probiotics, which promote fish growth, remain in the intestines to help balance healthy bacteria and play an important role in maintaining a healthy immune system and digestion of fish. At the same time, vitamin C works after being absorbed into the bloodstream. Vitamin C, as an antioxidant, will bind to ROS caused by Cd.

4. Conclusion
Supplementary feeding containing LAB, vitamin C, and their combinations has successfully increased the morphometry of tilapia exposed to cadmium.

5. References
[1] Huet M and Puchooa D 2017 J App Biol Biotech 5(6), 14-23.
[2] Monachese M, Burton J P, and Reid G 2012 Appl Environ Microbiol 78(18), 6397–6404.
[3] Hayati A, Taufiq A P, Setiarini W, Wangyuyun A P, Amira M, Putra P A D, and Muchtaromah B 2020 IOP Conference Series: Earth & Env Sci, 1-6.
[4] Hayati A, Wulansari E, Armando D S, Sofiyanti A, Amin M H F, Pramudya M 2019 Egypt. J. Aquat. Res. 45, 189-195.
[5] Rasyad M F M, Widianti A, Pebriani N, Fadliyay S, Ainiyah R K, Pratama W N, Putra A J, Wahyunindita V, Wahyuni H I, Dewangga E P, and Hayati A 2020 Ecol. Environ. Conserv. 26, 69-76.
[6] Mc Connell J R, and Edwards R 2008 PNAS USA. 105, 12140-12144.
[7] Hayati A, Rasyad M F M, Putra I D S, Nurhariyati T, Amin M H F, Putranto T WC, Wangyuyun A P, Sugiharto and Affandi M 2019 Ecol. Environ. Conserv. 25, 57-61.
[8] Tully D B, Collins B J, Overstreet J D, Smith C S, Dinse G E, Muntaz M M, and Chapin R E 2000 Toxicol. Appl. Pharmacol. 168, 79-90.
[9] Kaoud H A, Zaki M M, El-Dahshan A R, Saed S E I, Zorba H Y 2011 J. Life Sci. 8, 185-195.
[10] Matz C J, and Krone P H 2007 Environ. Sci. Technol. 41, 5143-5148.
[11] Yang P M, Chen H C, Tsai J S, Lin L Y 2007 Chem. Res. Toxicol. 20, 406-415.
[12] Kefaloyianni E, Gourgou E, Ferle V, Kotsakos E, Gaitanaki C, and Beis I 2005 J Exp Biol. 208, 4427-4436.
[13] Li Y, Li M, Shi J, Yang X, and Wang Z 2012 Aquat. Toxicol. 120-121, 90-98.
[14] Kim S C, Cho M K, and Kim S G 2003 Toxicol. Lett. 144, 325-336.
[15] Ameen F A, Hamdan A M, El-Naggar M Y 2000 Sci. Rep. 10, (314).
[16] Meriluoto J, Guemonde M, Haskard CA, Spoel L, Sjövall O, and Salminen S 2005 Toxicon 46, 111-114.
6. Acknowledgment
The author would like to thank Airlangga University, Indonesia, for providing funds and facilities under the University's Excellent Basic Research grants for 2019 to 2020.