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# Effect of Feed Supplement on Sperm Quality and Total Intestinal Bacteria of Fish Exposed by Cadmium

**Alfiah Hayati*, Farah Annisa Nurbani, Meirizka Amira, Windy Seftiarini, Aken Puti Wanguyun and Bayyinatul Muchtaromah[1]**

Department of Biology, Airlangga University, Surabaya, Indonesia
[1]Department of Biology, State Islamic University, Maulana Malik Ibrahim Malang, Indonesia
✉ alfiah-h@fst.unair.ac.id

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**Abstract:** In the ecological environment, cadmium, a heavy metal produced from human activities and industry toxic material, has polluted the water and affects the reproductive health of aquatic biota. Many fish farmers use water from the river for freshwater fish cultivation. This study examined the effects of supplementation feed (probiotics and vitamin C) on sperm quality and total bacteria in fish intestine, *Oreochromis niloticus*, after Cadmium (Cd) exposure. We found that probiotics did not seem to colonise fish intestine or change the overall amount of the intestinal microbiota. However, probiotic supplementation actually changed the total amount of bacteria in the fish intestine. In particular, the administration of probiotics in fish exposed to Cd has increased more intestinal bacteria compared to the administration of vitamin C. The addition of vitamin C as a supplement did not affect the total amount of bacteria in the fish intestine. These results indicated that probiotics have a significant capacity to recover sperm motility in freshwater fish exposed to Cd pollution.

**Key words:** Fish, probiotic, sperm quality, *intestinal bacteria*, cadmium.

## Introduction

Fish are nutritional sources of proteins, minerals and vitamins. During the last few decades, industrial development has resulted in heavy metals contamination of freshwaters. They can either be adsorbed or accumulated by aquatic organisms, including fish (Hayati et al., 2017a; Sujata. 2015). Heavy metals enter the aquatic ecosystem where they pose a serious threat because of their toxicity, bioaccumulation and biomagnifications in the food chain (Hamada et al., 2018). Industrial discharges such as coal and oil combustion are considered as the major sources of heavy metals pollutants of water. Fish absorb heavy metals from water through the digestive tract, skin and gills. The heavy metals which spread concern to aquatic biotic health are lead (Pb), mercury (Hg) and cadmium (Cd) (Rahman et al., 2018). Uptake of heavy metals through food chain in aquatic organisms may cause various disorders related to growth, reproduction rate, mortality and physiological functions (Nursanti et al., 2017; Putranto et al., 2014; Valko et al., 2006; Usman et al., 2013). Freshwater fish are vulnerable to Cd toxicity. The toxic effects of Cd on fish sperm can reduce the duration of motile and immotile sperm in vitro (Hayati et al., 2017b).

Bacterial bioremediation could be a good alternative to remediate heavy metal pollution in water. The use of bacteria for remediation of heavy metals Cd is capable to transforming them into less toxic forms. There are several types of microbes to remediate heavy metals,

*Corresponding Author

including lactic acid bacteria (LAB) (Wanguyun et al., 2019). LAB microbial groups can remediate heavy metals by forming metal bonds in their cells (Jin et al., 2018). In addition to probiotic supplements, vitamin C in fish feeds can reduce the ROS produced due to heavy metals exposure (Argawal and Sekhon, 2010). Antioxidant vitamin C plays a role in protecting the sperm membrane from ROS (Shabanian et al., 2017). The study aimed to determine the effect of feeding supplements on the quality of Tilapia (*Oreochromis niloticus*) sperm and the number of bacteria in its intestine after Cd exposure.

## Materials and Methods

### Animal Experiment

Male tilapia was obtained from the Pandaan Freshwater Aquaculture, East Java, Indonesia. Acclimation was done for one week. Thirty-six fish were divided into 12 groups. In the control, fish were not given Cd (0 ppm) but had varieties of feeds (commercial feed only, P1-0 ppm; plus probiotics, PP1-0 ppm; plus vitamin C, PC1-0 ppm; and added a combination of both probiotics and vitamin C, PPC1-0 ppm). The treatment groups were given different concentrations of Cd (0.3 and 0.6 ppm), each fish group was given commercial feed (P2-0.3 ppm and P3-0.3 ppm) and supplementary (probiotics and vitamin C) feed (PP2-0.3 ppm; PC2-0.3 ppm; PPC2-0.3 ppm; PP3-0.6 ppm; PC3-0.6 ppm; and PPC3-0.6 ppm). Feeding was carried out for 14 days, two times daily as much as 1% of body weight of fish. The commercial feed with 25 mL/kg probiotics and 100 mg/kg vitamin C were used in this study.

### Sperm Analysis

Analysis of fish sperm quality was the duration of motility and the percentage of viability. Sperm motility was observed under a light microscope and determined by calculating the duration of individual and mass motility (seconds). Sperm viability analysis was done by using Eosin 1% and Nigrosin 10% stain.

### The Number of Intestinal Bacteria

Intestines taken from fish were washed using 0.85% NaCl and homogenised to isolate bacteria from the intestines. The result of dilution of 1 mL was flattened over the agar plate count and incubated for 24-72 hours at 37°C. The calculation of the number of intestinal bacteria was displayed by log CFU/mL (Giri et al., 2018).

### Statistical Analysis

Statistical analysis used the normality test and homogeneity test followed by the ANOVA test to check the differences between treatments ($p<0.05$).

## Results

### Effect of Cadmium on Sperm Motility

Exposure of Cd (0, 0.3 and 0.6 ppm) had no significant effect ($p> 0.05$) on individual and mass motility. Supplement addition to fish food affected the sperm motility. The administration of supplement feed to the control and treatment group (0.6 ppm) significantly affected individual motility as well as mass motility ($p<0.05$). The given supplement feed has significantly affected the individual motility ($p<0.05$) in fish exposed with 0.3 ppm Cd, but no significant changes in mass motility ($p>0.05$). (Figure 1).

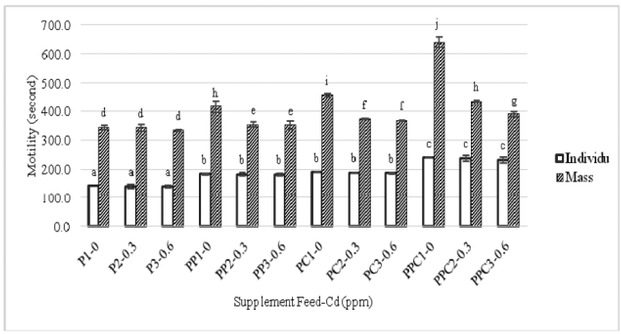


**Figure 1: The duration of individual and mass motility with supplement feed in different concentrations of Cd (0, 0.3 and 0.6 ppm).**

### Effect of Cadmium on Sperm Viability

Feed supplements in a control showed no significant difference in viability ($p>0.05$), but in treatment groups they showed a significant increase in sperm viability ($p>0.05$) (Figure 2).

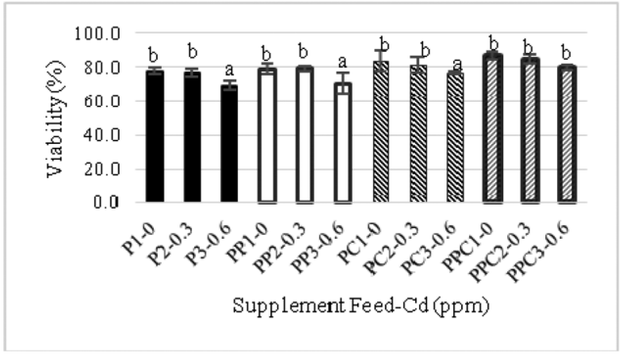


**Figure 2: The percentage of sperm viability with supplement feed in different concentrations of Cd (0, 0.3 and 0.6 ppm)**

**Total of Intestinal Bacteria**

The results showed that the total bacteria in fish intestines did not have a significant effect ($p > 0.05$) in control with the addition of probiotics, but the total bacteria decreased significantly ($p < 0.05$) after the addition of vitamin C (Figure 3).

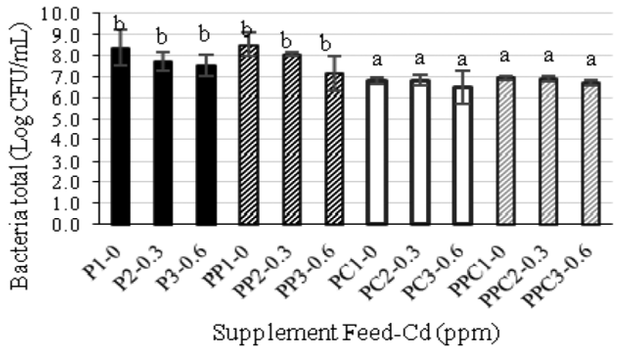


**Figure 3: The effect of supplement addition on fish feed in different concentrations of Cd on total bacteria in intestine of tilapia.**

## Discussion

Cadmium is a heavy metal that has received considerable attention as a threat to the environment. This metal has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to Cd causes toxic effects due to its accumulation over time in a variety of tissues, including in reproductive systems. Toxicity of Cd also affects cell proliferation, differentiation and apoptosis. These activities interact with the generation of ROS such as superoxide ion, hydrogen peroxide, and hydroxyl radicals, DNA fragmentation mechanism and the induction of apoptosis (Rani et al., 2014).

In this study, we found that the exposure to Cd (0.3 and 0.6 ppm) cannot effect the duration of mass motility of fish (Figure 1). According to Hayati et al. (2017b), heavy metal Cd at low concentration does not affect fish sperm motility, but at high concentrations (>50 ppm) it can reduce the duration of individual and mass motility. Decreased motility is due to impaired sperm mitochondrial function in respiration to produce motion energy. When the concentration of Cd is high, it can cause a decrease in sperm quality and result in apoptosis for the sperm.

LAB was able to interact with a range of toxic metals, including Cd, Cu, Fe, Mg and Pb. This ability was attributed to differences between the net negative charge of bacteria and the cationic charge of many metals (Fein et al., 2001). The theory stated that nucleation sites on the cell surface had the ability to bind metals of opposite charge. Once bound to the cell wall, this resulted in a nucleation site where a large concentration of metals could bind and precipitate on the cell wall. The neutral pH 7 had the optimum binding potential of cationic metal species, because at this pH reactive functional groups would not be ionised (Fein et al., 2001). However, this mechanism does not apply in all bacterial species or all bacteria interactions toward metals. The formation of bacterial bonds with heavy metals can reduce toxic from heavy metals, thus the addition of probiotics can restore cell-damage caused by toxic materials.

Dietary probiotics supplements added during the study increased individual and mass sperm motility. Vitamin C that was contained in dietary supplements acts as an antioxidant that protects sperm from oxidizing agents (Cd). Vitamin C increases the fish's immune response and prevents side effects from heavy metals (El-Greisy and El-Gamal, 2015).

Viability is the ability of sperm to survive until it arrives at the fertilisation site. The result shows that Cd (0.6 ppm) significantly decreased sperm viability ($p < 0.05$), while the combination of both probiotic and vitamin C increased the sperm viability (Figure 2). This shows that the concentration of Cd is low and the potential of combination of probiotics and vitamin C are needed to recover sperm viability.

Lactic acid bacteria can help increase the digestive enzymes such as amylases, lipases and proteases. Results by other researchers have reported an increase in the total bacteria when probiotics are added to it. Wanguyun et al. (2018) found that probiotic in food can increase total intestinal bacteria than vitamin C and the combination of feed (probiotics and vitamin C).

## Conclusion

Probiotics and vitamin C as an additional supplement in the feeds were able to recover sperm motility after Cd exposure. Probiotics increased the total number of intestinal bacteria, however, the addition of vitamin C did not affect the total number of intestinal bacteria in fish.

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## References

Argawal, A. and L.H. Sekhon (2010). The role of antioxidant therapy in the treatment of male infertility. *Human Fertility*. **13(4):** 217-225.

El-Greisy, Z.A. and A.H.A. El-Gamal (2015). Experimental studies on the effect of cadmium chloride, zinc acetate, their mixture and the mitigation with vitamin C supplementation on hatchability, size and quality of newly hatched larvae of common carp, *Cyprinus carpio*. *Egyptian Journal of Aquatic Research*, **41(2):** 219-226.

Fein, J.B., Martin, A.M. and P.G. Wightman (2001). Metal adsorption onto bacterial surfaces: Development of a predictive approach. *Geochimica et Cosmochimica Acta*, **65(23):** 4267-4273.

Giri, S.S., Yun, S., Jun, J.W., Kim, H.J., Kim, S.G., Kang, J.W., Kim, S.W., Han, S.J., Sukumaran, V. and S.C. Park (2018). Therapeutic effect of intestinal autochthonous *Lactobacillus reuteri* P16 against waterborne lead toxicity in *Cyprinus carpio*. *Frontiers in Immunology*, **9:** 1-13.

Hamada, M.G., Elbayoumi, Z., Khader, R. and A. Elbagory (2018). Assessment of heavy metal concentration in fish meat of wild and farmed Nile Tilapia (*Oreochromis niloticus*), Egypt. *Alexandria Journal of Veterinary Sciences*, **57**(**1):** 30-37.

Hayati, A., Pratiwi, H., Khoiriyah, I., Winarni, D. and Sugiharto (2017a). Histopathological assessment of cadmium effect on testicles and kidney of *Oreochromis niloticus* in different salinity. AIP Conference Proceedings. **1854(00014):** 1-8.

Hayati, A., Giarti, K., Winarsih, Y. and Amin, M.H.F. (2017b). The effect of cadmium on sperm quality and fertilization of *Cyprinus carpio* L. *Journal of Tropical and Biotechnology*, **2:** 45-50.

Jin, Y., Luan, Y., Ning, Y. and L. Wang (2018). Effects and mechanisms of microbial remediation of heavy metals in soil: A critical review. *Applied Sciences*, **8(8):** 1-17.

Nursanti, L., Nofitasari, E., Hayati, A., Sariyanto, S., Irawan, B. and A. Soegianto (2017). Effects of cadmium on metallothionein and histology in gills of tilapia (*Oreochromis niloticus*) at different salinities. *Toxicological and Environmental Chemistry*, **99(5-6):** 926-927.

Putranto, T.W.C., Andriani, R., Munawwaroh, A., Irawan, B. and A. Soegianto (2014). Effect of cadmium on survival, osmoregulation and gill structure of the Sunda prawn, *Macrobrachium sintangense* (de Man), at different salinities. *Marine and Freshwater Behaviour and Physiology*, **47(5):** 349-360.

Rahman, Z., Ahmad, I. and I. Rashid (2018). Effects of cadmium exposure on growth and survival and accumulation in various organs of Nile Tilapia (*Oreochromis niloticus*, Linnaeus). *Journal of Agriculture and Aquaculture*, **1(1):** 2-10.

Rani, A., Kumar, A., Lal, A. and M. Pant (2014). Cellular mechanisms of cadmium-induced toxicity: A review. *International Journal of Environmental Health Research*. **24:** 378-399.

Shabanian, S., Farahbod, F., Rafieian, M., Ganji, F. and A. Adib (2017). The effects of vitamin C on sperm quality parameters in laboratory rats following long-term exposure to cyclophosphamide. *Journal of Advanced Pharmaceutical Technology Research*, **8(2):** 73-79.

Sujata, K. (2015). Impact of cadmium on the biochemical contents in the reproductive organs of freshwater fish, *Channa punctatus* (Bloch). *International Journal of Science and Research (IJSR)*, **4:** 114-118.

Usman, N., Irawan, B. and A. Soegianto (2013). Effect of copper on survival and osmoregulation in different life stages of white shrimp *Litopenaeus vannamei* Boone, 1931. *Cahiers de Biologie Marine*, **54(2):** 191-197.

Valko, M., Christopher, J.R., Moncol, J., Izakovič, M. and M. Mazúr (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, **160:** 1-40.

Wanguyun, A.P., Hayati, A. and B. Utomo (2019). The effects of probiotics feed supplementation on Tilapia (*Oreochromis niloticus*) in copper-tainted Water. *Ecology, Environment and Conservation*, **25:** S13-S17.