Eco. Env. & Cons. 26 (June Suppl. Issue) : 2020; pp. (S160-S164)
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ISSN 0971–765X

Dietary supplements of herbs and lactic acid bacteria to improve the quality of fish sperm exposed to mercury

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(Received 1 March 2020; Accepted 20 April 2020)

ABSTRACT

Water pollution by heavy metals waste from industries, agriculture, and other human activities affects the reproductive health of aquatic biota, especially freshwater fish. The purpose of this study was to evaluate the potential of various herbal plants and lactic acid bacteria to improve the quality of sperm of Nile tilapia (*Oreochromis niloticus*) contaminated with mercury (Hg). A total of 36 mature male gonad fish, received various treatments by distinguishing three types of Hg doses (0, 0.1, and 0.5 ppm) and six kinds of feed, namely commercial feed; commercial feed mix with a suspension consortium composed of a lactic acid bacterium (LAB) 100 mL/kg of feed, pure vitamin C 100 mg/kg of feed, *I. aquatica* powder 15%, *A. sativum* powder 3.2%, and fermentation powder *O. sativa* Bran 10%. The results of the study showed that supplementation of dietary supplements did not alter fish GSI, except for *A. sativum* supplements which significantly reduced GSI; increases the duration of motility and viability of fish sperm exposed to Hg.

Key words: *Sperm quality, Fish, Lactic acid bacteria, I. aquatica, A. sativum, O. sativa Bran*

Introduction

Herbaceous plants contain phytochemical compounds; therefore, they are widely used as sources of antioxidants in various organisms. These plants include *Ipomoea aquatica*, Allium sativum, *Oryza Sativa* (Rice) Bran, and others (Awad and Awaad, 2017) are used as an adjunct in the manufacture of fish feeds. The plants are easily found in tropical and subtropical regions of the world. Ipomoea aquatica contains several phytochemical compounds such as polyphenols (22.7 mg/g), saponins (51.2 mg/g), and flavonoids (37.9 mg/g) (Dewanjee, 2015) and ascorbic acid (3.1 mg/g). Also, it contains myricetin, quercetin, and apigenin (Saikat, 2015) as well as 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O-β-D-glucopyranoside which has antioxidant properties (Prasad *et al.*, 2005). *A. sativum* contains allicin decomposed of various compounds such as S-allylmercaptocysteine, allylmercaptan, dialyl disulfide, allylmethyl disulfide, vinyldithiins, and ajoene. *A. sativum* in fish feed can increase growth rates, reduce mortality, and fish productivity. *O. Sativa* (Rice) Bran contains many antioxidants (Chanphrom, 2007) such as tocopherols, tocotrienols and oryzanols (Chen and Bergman, 2005).

Many plants contain flavonoids that act as antioxidants (Banjarnahar and Artanti, 2015) to prevent the generation of free radicals and can ward off oxidants such as reactive oxygen species (ROS)

(Akhlaghi, 2009). Heavy metals such as mercury (Hg) are known to cause the production of the ROS once they are consumed in the body (Bubber, 2019). Mercury compounds are often found in waters such as rivers, as pollutants originating from the disposal of industrial waste and human activities thereby increasing the risk of fish contamination. This heavy metal Hg pollution results in polluted aquatic biota and accumulates in body tissues (Ebrahimi and Taherianfard, 2010). Hg accumulation changes several parameters of fish sperm quality by decreasing motility, viability, and fertilization ability of the sperms, increasing DNA fragmentation and the levels of malondialdehyde (MDA) and DNA fragmentation, as well as alters the morphological structure of the sperm head and interferes with the development of fish embryos (Hayati *et al.,* 2019). Hg at high concentrations inhibits the metabolic processes of aquatic biota, especially fish, which can cause death (Gosar, 008) and affect the quality and function of sperm. The decrease in fish sperm quality determines the low success of the fertilization process so that it can threaten the presence of fish in their habitat (Hayati *et al.,* 2017). In several previous studies, the uses of lactic acid bacteria (LAB) as a probiotic in fish *O. niloticus* has been examined. However, there are still no studies that use LAB in a consortium with specific abilities to remedy the decline in fish sperm quality.

This study determined to investigate the effect of herbal and probiotic variations on the recovery of sperm quality in Nile tilapia at the age of sexual maturity after exposure to Hg at various concentrations.

Materials and Methods

Experiments

Thirty-six (36) mature male O. *niloticus* fish with body weights 110 ± 10 g were obtained from the Freshwater Aquaculture Hatchery Unit Pandaan, Pasuruan, Indonesia. The acclimation was done for two weeks, with commercial feed daily as much as 3-5% of body weight. The fish were treated with variations of distinguishing three types of Hg doses (0, 0.1 and 0.5 ppm) and six types of feed, namely commercial feed; commercial feed mixed with a suspension of a lactic acid bacterial consortium (*Lactobacillus buchneri* (DSM 20057); *Lactobacillus casei (DSM 20011); Lactobacillus* bulgaricus (NBRC13953); and *Lactobacillus fermenum* (ME3) 100 mL/kg of feed, pure vitamin C / 100 mg / mg / mg kg of feed, *I. aquatica* powder 15%, *A. sativum* powder 3.2%, and fermentation powder *O. sativa* Bran 10%.

Gonadal index calculation and test of spermatozoa quality

The gonad index (GSI) of tilapia was done by weighing the weight of fish and weight of the fish testes (%). Sperm collection was done by cutting the testicles, then sperm suspension was dissolved in 0.9% NaCl. Then sperm quality testing were done by observing the sperm motility includes the duration of mass motility and duration of individual motility. Measuring the duration of sperm mass motility was done by calculating the movement of the initial sperm until all sperms stop to move in one field of view of a microscope. The duration of individual sperm motility was determined by calculating the time of movement of one sperm cell from the beginning until it stops moving in one field of view, under a microscope, 100x. Observation of sperm viability was done by using 1% Eosin dyes and 10% Nigrosin under a light microscope(100x) where a dead sperm was colored purplish or red while alive sperm was not colored. The viability of sperm was presented in percentage (%).

Data analysis

To test the effectiveness of the plant's herbs and lactic acid bacteria on the quality of sperm of tilapia intoxicated with Hg, One-Way ANOVA statistical test was used and $P<0.05$ was considered significant.

Results and Discussion

There were no significant changes in GSI in *O. niloticus* exposed to 0.1 and 0.5 ppm Hg for 14 days. Supplementary feeding (a mixture of commercial and probiotic feed, vitamin C, *I. aquatica* and *O. sativa Bran*) did not affect GSI, but only on 0.5 ppm Hg exposure significantly decreased GSI compared with controls on *A. sativum* supplementation (Figure 1, P <0.05%).

The results of GSI during the study revealed different patterns among the variations of the feed studied. The GSI was not affected by variations in the concentration of Hg in the variation of supplementary feeding, except in the supplementary feed with *A. sativum* mixture there was a significant difference (Figure 1, P<0.05). High Hg concentrations can reduce the GSI of fish given *A. sativum* supplements. This decrease is thought to be more influenced by gonad maturity. Antioxidants contained in herbal plants can optimize survival and physiological recovery to accelerate spawning (Dabrowski and Ciereszko, 2001). These antioxidants also regulate reproduction, gonadal development and support the work of the hypothalamus in the regulation of fish gonadotropin hormones (Maitra and Hasan, 2016; Zuo *et al.,* 2018).

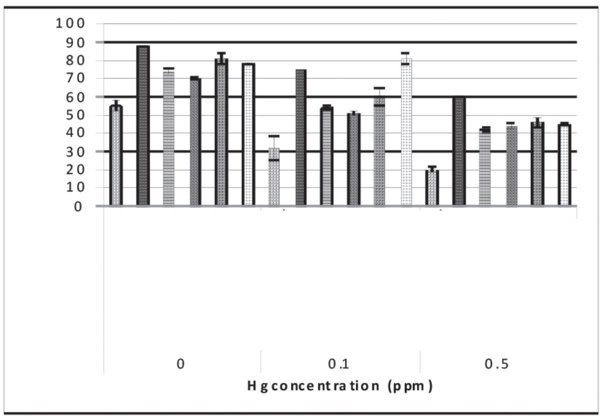

Fig. 1. Potential variations in supplement feed to GSI exposed to heavy metal Hg

Mercury exposure affects the motility duration of individual fish sperm. The results showed the sperm duration of the control group had the longest significant duration (P <0.05) (323 ± 7.7 seconds) compared to other treatments (0.1 and 0.5 ppm) respectively 241 ± 8.3 and 121 ± 5.1 seconds. In line with the duration of individual motility, the duration of group motility decreases when the concentration of Hg increased. There was a significant decrease in all treatments compared to controls (P <0.05) (Fig. 2A and 2B).

Supplementation of dietary supplements can restore sperm motility exposed to Hg. Probiotic supplements provide the best results for both individual (950 ± 7.6 second) and mass sperm motility (1229 ± 1.6 seconds) than vitamin C, *I.aquatica*, *O.sativa* (rice) Bran and *A. sativum* which showed individual sperm motility of 695 ± 3.9; 701 ± 1.3; 712 ± 1.3 and 718 ± 1.1 seconds respectively and mass sperm 989 ± 4.7; 1003 ± 3.0; 1116 ± 1.3 and 1026 ± 1.8 seconds respectively.

Fish as aquatic organisms, their reproductive system is vulnerable to the side effects of heavy metal Hg. Hg accumulation can reduce sperm quality, especially sperm motility (Hayati *et al.,* 2019). The results showed that Hg exposure could reduce the motility and viability of fish sperm. This decrease is due to an increase in ROS caused by Hg that causes damage to sperm cell organelles including mitochondria (Authman *et al.,* 2015). Sperm mitochondria are located in the middle piece of the flagellum. This organelle encloses the axoneme complex of the outer solid fiber in species-specific ways forming a cylindrical mitochondrial sheath. Mitochondria through cellular respiration produce energy (ATP) needed for sperm tail motion (Srivastava and Pande, 2016). Disturbances in mitochondrial function results in decreased time and speed of the sperms.

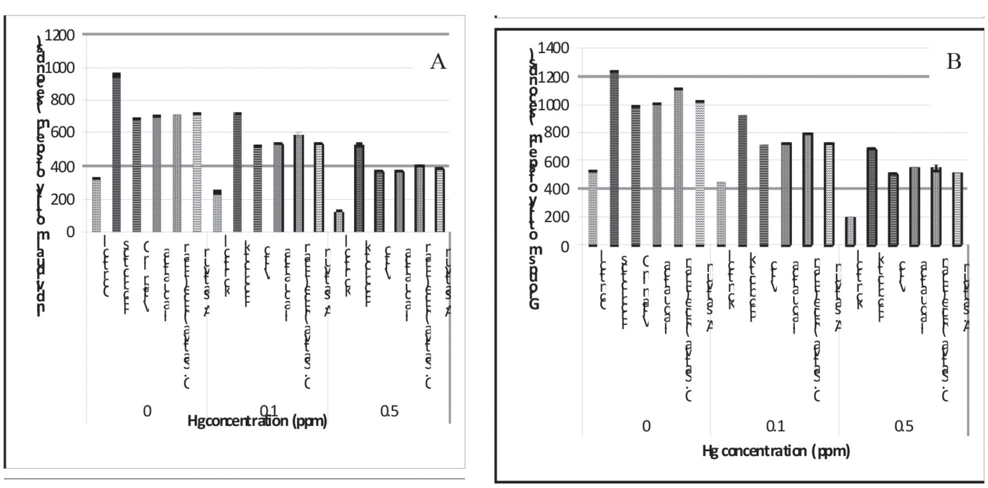

Fig. 2. Potential variations in supplementary feed on the duration of individual motility (A) and duration of mass spermmotility (B) of fish exposed to heavy metal Hg

The sperm viability calculation results showed that the control contain viable sperm (55 ± 3.0%), but not all of these sperms were continued to live. This percentage of viability was higher than the viability of 0.1 and 0.5 ppm Hg exposed fish that showed viability of 31.75 ± 6.5 and 20.25 ± 1.5% respectively. The percentage of live sperm was determined by identifying sperm with intact cell membranes. Living cells were clear, but non-living cells absorbed the dye. Exposure to Hg decreases membrane integrity so that dyes can enter into the sperms. Variations in the concentration of Hg exposure significantly reduced the sperm viability compared to the control group ($P < 0.05$). ROS from heavy metal cause lipid peroxidation that occurs in cell membranes, especially at unsaturated fatty acids which are important components of cell membrane constituents (Powers and Jackson, 2008). Supplementary feeding increased the viability of fish sperm exposed to Hg (0.1 and 0.5 ppm). The best provided supplements were probiotics that showed viability of 75 ± 0.2 and 60 ± 0.7% in fish groups exposed to 0.1 Hg and 0.5 Hg respectively (Fig. 3).

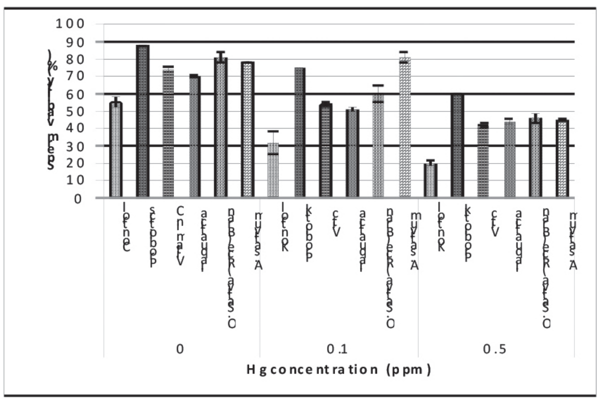

Fig. 3. Potential variations in supplementary feed on sperm viability exposed to heavy metal Hg

Conclusion

Probiotic supplements and herbal plants able to repair the damaged sperm quality (motility and viability) of sperm *O. niloticus* exposed to heavy metal Hg.

Acknowledgement

The authors would like to thank the Faculty of Science and Technology, Airlangga University, Indonesia, which provided funding for Higher Education Research Activities in 2020.

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