

# Effect of exposure to palm oil mill effluent on reproductive impairment of male Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758)

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**Abstract.** One of the negative impacts arising from the existence of palm oil mill industries is the increase of pollution from Palm Oil Mill Effluent (POME), particularly for the aquatic environment. This study was conducted to investigate the reproductive impairment of male Nile tilapia (*Oreochromis niloticus* Linnaeus 1758) after exposed to POME. An experiment of a chronic test was carried out using a completely randomized design consisting four treatments with five replication: control (0 mg/L POME), treatment A (1.565 mg/L POME), treatment B (2.347 mg/L POME), and treatment C (3.130 mg/L POME). The exposure of POME was performed for 45 days. Reproductive hormone concentration, gonadosomatic index and spermatocrite value in each treatment statistically analyzed by using one-way analysis of variance (ANOVA). Results showed that the POME exposure had no significant impact on estradiol and testosterone concentrations ( $p > 0.05$ ), but, it triggered a decline in the progesterone concentration ( $p < 0.05$ ). Progesterone concentration decreased significantly from  $0.57 \pm 0.24$  ng/mL in control to  $0.28 \pm 0.04$  ng/mL in treatment C. The hormonal decline resulted in a significant decrease in gonadosomatic index and spermatocrite of male Nile Tilapia ( $p < 0.05$ ). In conclusion, exposure to POME induced reproductive impairment in male Nile Tilapia.

Keywords: estradiol, progesterone, testosterone, gonadosomatic index, spermatocrite

## 1 Introduction

Indonesia is the world's largest producer of palm oil, reaching 54% of total world palm oil production [1]. Indonesia's palm oil production in 2014 touched 29.27 million tons with a production value reaching 15.4 billion USD in 2015 [2,3]. However, the palm oil management sector in Indonesia is inseparable from various controversies, especially those related to environmental problems such as deforestation [4,5], damage to ecosystems [6,7] and water pollution [8].

Palm Oil Mill Effluent (POME) is an inevitable by-product of the palm oil industry that causes serious environmental hazards if discharged directly to the environment. This is mainly due to its high concentrations of nutrient and organic matter. Abram et al. stated that contamination of POME can result in serious water pollution and disturb aquatic ecosystem [9]. Exposure to COD & BOD harm aquatic organisms through hypoxic conditions [10,11]. Our previous research also revealed that contamination of POME decreases phytoplankton diversity and led to a disruption of the fish liver and gill performances [12,13,14].

Reproductive performance is the key factor in analyzed the population dynamics of fish that have implications for fish sustainability. Several reports have shown that pollutants can induce reproductive impairment both in male and female fish [8,15]. Mc-Master et al. documented reduced plasma testosterone in male brown bullhead (*Ictalurus nebulosus*) exposed to PAH-contaminated sediments [16]. Extensive distribution of Nile Tilapia (*Oreochromis niloticus*) in Indonesian waters, make this species becomes potentially impacted by POME [17]. However, to date, information regarding the effect of POME on the reproduction performance of male tilapia is still rare. Furthermore, this study was conducted to investigate the reproductive impairment of male Nile Tilapia (*Oreochromis niloticus* Linnaeus 1758) after exposed to Palm Oil Mill Effluent (POME).

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## 2 Materials and Methods

### 2.1 Fish and POME

As many as 300 male Nile tilapia (weight:  $9.46 \text{ g} \pm 1.16$ ; length:  $7.75 \text{ cm} \pm 1.67$ ) were purchased from Fish Hatchery Center (*Balai Benih Ikan*) Batee Iliak, Bireuen District, Indonesia and transferred in the oxygenated container to the Aquaculture Laboratory, Almuslim University. Acclimatization of fish in laboratory conditions (DO:  $5.2 \text{ mgO}_2 / \text{L}$ ; temperature:  $28.5 \text{ }^\circ\text{C}$  and pH: 7.1) lasts for seven days (one week). A total of 30 liters of POME wastewater was collected from the palm oil mill factory (Bireuen District, Indonesia) and diluted into the required concentration for the toxicological test. Fish exposure media were  $50 \times 30 \times 40 \text{ cm}$  glass aquaria of about a 25 liters capacity. The water was obtained from municipal tap water and aerated for 24 hours before being used.

### 2.2 Experimental setup

LC<sub>50</sub>-96 hours of POME on Nile tilapia ( $15.65 \text{ mg/L}$ ) has used as the reference for determining the chronic toxicity dose [18]. The male Nile Tilapia were divided into five treatment (n=10) concerning their different POME doses (control, 0% POME:  $0 \text{ mg/L}$ , Treatment A, 10% of LC<sub>50</sub>-96 hour:  $1.565 \text{ mg/L}$ , Treatment B 15% of LC<sub>50</sub>-96 hour:  $2.347 \text{ mg/L}$ , and Treatment C 20% of LC<sub>50</sub>-96 hour:  $3.130 \text{ mg/L}$ ). The exposure period lasts for 45 days. Fish were fed twice daily with commercial food and water in each aquarium was completely renewed every 15 days.

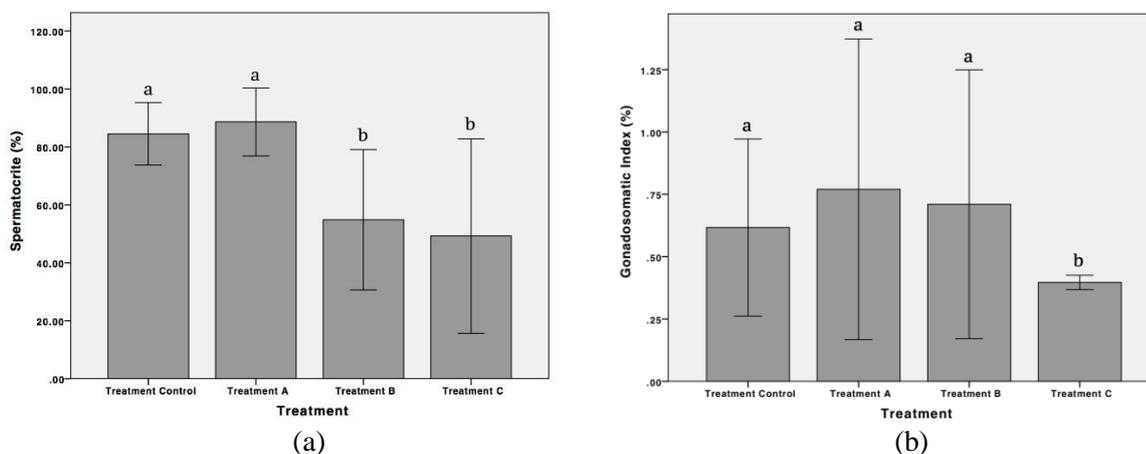
At the end of the exposure period, all fish from each replicate were collected and sacrificed by using overdose clove oil for reproductive hormone concentration (estradiol, testosterone, and progesterone) and biometric measured. Hormone measurements were performed by the ELISA method at the Laboratory of Physiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala using a commercial ELISA kits produced by DRG

Instruments GmbH, Marburg, Germany (Cat, No. EIA-2693 for Estradiol, Cat. No. EIA-1561 for Progesterone, Cat. No. EIA-1559 for Testosterone). These assays have been validated for animals [19]. The concentrations of estradiol, testosterone, and progesterone hormones were calculated by using the MPM 6 program. Gonadosomatic index (GSI) and spermatocrite were calculated using Bolger and Connolly (1989) formula. Differences in GSI, spermatocrite and reproductive hormone concentration between treatment were evaluated using a one-way analysis of variance (ANOVA). The level of significance was set to  $p < 0.05$ . All data analyses were conducted using SPSS 23 software for Macintosh.

## 3 Results and Discussion

Results showed that exposure to POME caused a decrease in GSI and spermatocrite value of male Nile Tilapia. The highest GSI value observed in control while the lowest observed in treatment C was  $0.61 \pm 0.14\%$  and  $0.39 \pm 0.11\%$ , respectively. Statistically, GSI value decreased significantly in treatment C ( $p < 0.05$ ) (Figure 1). The lower value of GSI is thought to occur due to the disturbance development of male fish gonads during spermatogenesis. Several studies also reported a decrease in the value of GSI in male fish due to exposure to various types of pollutants, for instance, mercury, cadmium, and  $17\alpha$ - ethinylestradiol [20,21,22].

In female fish, the lower value of GSI is affected by the reduced yolk size, while in male fish, the lower value of GSI is thought to have a strong correlation with the lower value of spermatocrite. Spermatocrite is the ratio between the density of sperm cells in semen after the centrifugation process so that if the fish sperm becomes thicker, the spermatocrit value will also increase [23]. The results showed that exposure to POME caused the spermatocrite levels of fish in C treatment decreased significantly ( $p < 0.05$ ). The spermatocrit value in the control was  $84.4 \pm 4.34\%$  while in the treatment C was  $49.22 \pm 13.50\%$  (Figure1).



**Figure 1.** Spermatocrit (a) and Gonadosomatic index (GSI) (b) Tilapia in each treatment. (\*the same letter above each data block means not significantly different)

Reproductive hormones are chemical compounds produced by both male and female reproductive organs. This hormone plays an important role in the stages of

reproduction such as gonad maturation, producing gamete cells and affect fish sexual behavior. Estradiol, testosterone, and progesterone are three important

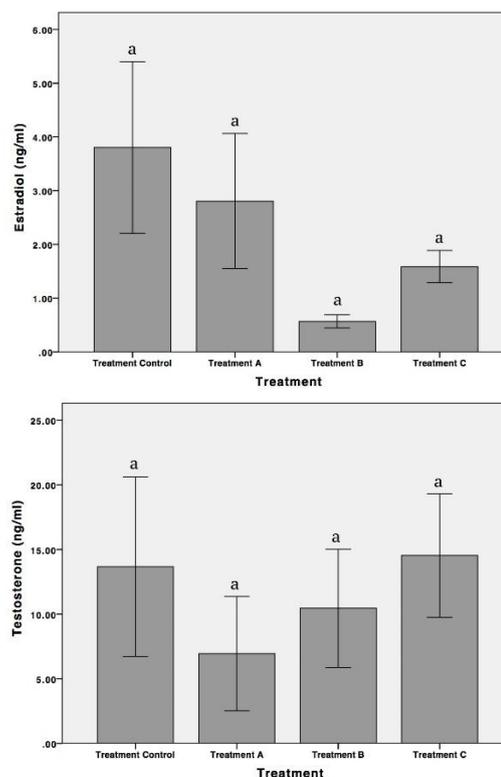
hormones in the process of gonad development [24,25,26]. In female fish, the impairment of performance from three hormones has an impact on the disruption of the process of vitellogenesis, cause low lipid content in egg yolks, shrinking the size of the egg diameter to cause low hatchability [27,28]. The hormone estradiol plays an important role in supporting the process of spermatogenesis, especially in the stages of development and division of spermatogonia [28]. Statistical analysis showed that exposure to POME did not cause a significant difference in the concentration of estradiol hormone between treatments ( $p > 0.05$ ) (Figure 2). These results tend to be similar to some previous studies. Foran et al. revealed that cadmium exposure did not result in a decrease in the concentration of the hormone estradiol in fish *Oryzias latipes* [29]. Estradiol and testosterone levels are exposed to tebuthiuron, resulting in increased levels of the aromatase enzyme resulting in changes in spermatogenesis and gonad size reduction [30]. In male *Pimephales promelas* exposed to metformin, no difference was found in plasma testosterone levels between controls thought to be due to the absence of insulin signaling in the steroid synthesis pathway and no difference in mRNA levels for steroidogenic enzymes [31].

The hormone testosterone is an important regulator in the process of spermatogenesis, especially in the stages of proliferation and differentiation of spermatogonia [32]. Similar to the hormone estradiol, statistical analysis shows that exposure to POME also does not cause a significant difference in the concentration of testosterone between treatments ( $p > 0.05$ ) (Figure 2). These results tend to be similar to some previous studies such as the *Cyprinus carpio* and *Capoeta* sp male fish at a low concentration of 20% exposure to heavy waste such as arsenic (As), cadmium (Cd) and lead (Pb) did not show significant differences, this is due to no serious pathological lesions or damage to organs [15]. However, there are several types of exposure to pollutants that can cause a decrease and increase in the hormone testosterone, such as cadmium exposure in *Pimephales promelas* [23] and bleached kraft pulp mill effluent (BKME) exposure in *Catostomus commersoni* fish [33].

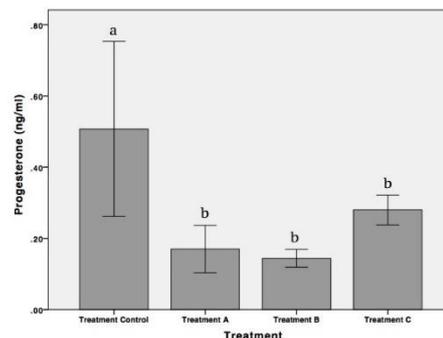
POME tends to have lower toxicity compared to heavy metals and pesticides. Thus, the exposure of POME is thought to have not able to intervene in the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) which play a role in secreting the hormone testosterone. In addition, Ohno et al argued that the lack of influence on the hormone testosterone is caused by the lack of effects of pollutants in suppressing the activity of  $17\beta$ -hydroxysteroid dehydrogenase which plays an important role in the synthesis of testosterone hormone [34].

The hormone progesterone plays an important role in increasing the motility and swimming ability of fish sperm [35]. The hormone progesterone also functions to bind to androgen hormone receptors in fish [36]. Statistical analysis showed that exposure to POME waste caused significant differences in the concentration of the hormone progesterone between treatments ( $p > 0.05$ ). The

highest concentration of the hormone progesterone was in the control while the lowest concentration was detected in treatment B which was  $0.50 \pm 0.24$  ng/ml and  $0.28 \pm 0.04$  ng/ml, respectively (Figure 3). The results of Ebrahimi and Taherianfard's research also revealed a decrease in the concentration of the hormone progesterone in male *Cyprinus carpio* fish after exposure to heavy metals such as cadmium, lead, mercury, and arsenic [15]. The toxic effects of pollutants that can damage the hypothalamus-pituitary system and inhibit the production of the hormone proesterester [37]. Furthermore, inhibition of the performance of the hormone progesterone can disrupt the stage of steroidogenesis, cell proliferation and sperm motility in the testes [38].



**Figure 2.** Concentrations of estradiol and testosterone in the blood plasma of male tilapia. (\*the same letter above each data block means not significantly different)



**Figure 3.** The concentration of the hormone progesterone in the blood plasma of male tilapia. (\*the same letter above each data block means not significantly different).

## 4 Conclusion

The Exposure to POME significantly reduces spermatocyte, GSI, and progesterone hormone of male Nile tilapia (p <0.05). On the other side, exposure to POME does not have a negative effect on the concentration of estradiol and testosterone hormone in male Nile tilapia.

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