



***EFFECTS OF IRON OXIDE NANOPARTICLES (FeO-NPs) ON HISTOLOGY  
(LIVER) AND ELECTRON MICROSCOPE ULTRASTRUCTURE (GILL) OF  
KERAI LAMPAM LARVAE***

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This is to certify that I have examined the final project report and all corrections have been made is recommended by the panel examiners. This project complies with the recommended format stipulated in the AKU4999 project guidelines, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia.

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## ABSTRACT

### EFFECTS OF IRON OXIDE NANOPARTICLES (FeO-NPs) ON HISTOLOGY (LIVER) AND ELECTRON MICROSCOPE ULTRASTRUCTURE (GILL) OF KERAI LAMPAM LARVAE

Rapid development of the industrial sector especially the nanotechnology sector is a new threat to the fish species. Iron oxide nanoparticles (FeO-NPs) which are widely used in anthropogenic activities are one of the heavy metals that released into the environment. The aim of this study is to study the effects of iron oxide nanoparticles on growth performances, histopathology on liver and gill ultrastructure of the Kerai Lampam, new hybrid species that have been successfully breed. The fingerling of Kerai Lampam were exposed to different concentrations of iron oxide nanoparticles (0, 10, 50, and 100ppm) for a period of 28 days. The total length of the larvae was measured every weeks to calculate the growth performances. After 28 days, alterations in gill and liver were studied by using hematoxylin-eosin staining and scanning electron microscope. As a results, there was significantly different ( $P < 0.05$ ) on growth performances between control group and treatments. The histopathological changes can be scored as moderate severity due to the present of necrotic liver tissue, melanomacrophage pigmentation and formation of vacuolation. As for the concentration of 50ppm, the lamellea become hypertrophy and excess mucus were secreted. Besides that, lamellar ruptures were observed at concentration of 100ppm. Based on the observation, larvae of Kerai Lampam were able to survive during the experiment and no mortality was detected. The concentrations of iron oxide nanoparticles that been used were considered as non - lethal concentration. Liver and gill are targeted organ to pollutant that can be used as biomarkers on environmental effects.

**KEYWORDS:** Kerai Lampam, iron oxide nanoparticles (FeO-NPs), liver, gill, histology, scanning electron microscope

## ABSTRAK

Perkembangan pesat sektor perindustrian terutamanya sektor nanoteknologi adalah ancaman baru kepada sepsis ikan. Salah satu logam berat yang dilepaskan ke alam sekitar ialah nanopartikel ferum oksida (FeO-NPs) yang kini banyak digunakan. Kajian ini bertujuan untuk melihat kesan nanopartikel ferum oksida pada pertumbuhan, histopatologi pada hati dan insang dari spesies hibrid baru iaitu Kerai Lampam yang telah berjaya dibiakkan. Fingerling Kerai Lampam didedahkan kepada kepekatan nanopartikel ferum oksida yang berbeza (0, 10, 50, dan 100ppm) untuk tempoh 28 hari. Ikan diukur panjangnya setiap minggu untuk mengira prestasi pertumbuhan. Selepas 28hari, perubahan ultrastruktur histologi dan insang dikaji dengan menggunakan pewarnaan haematoxylin-eosin dan pengimbasan mikroskop elektron. Sebagai hasilnya, terdapat perbezaan ( $P < 0.05$ ) kesan ke atas pertumbuhan antara kumpulan kawalan dan rawatan. Perubahan histopatologi dapat dijadikan keparahan yang sederhana disebabkan adanya jaringan hati nekrotik, pigmentasi melanomacrophage dan pembentukan vaksinasi. Lamella pada ultrastruktur insang menjadi hipertropi dan lendir berlebihan dirembeskan pada kepekatan 50ppm. Pecah lamaran dikesan pada kepekatan 100ppm. Berdasarkan pemerhatian, ikan dapat bertahan semasa eksperimen dan tiada kematian dikenalpasti. Kepekatan nanopartikel ferum oksida yang digunakan adalah dianggap sebagai kepekatan yang menjadi punca kematian ikan. Hati dan insang adalah organ sasaran kepada pencemar yang boleh digunakan sebagai biomarker terhadap kesan alam sekitar.

**KEYWORDS:** Kerai Lampam, nanopartikel ferum oksida (FeO-NPs), hati, insang, histologi, scanning electron microscope

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## LIST OF ABBREVIATIONS

FeO-NPs	Iron oxide nanoparticles
NPs	Nanoparticles
Ag	Silver
ppm	Parts per million
nm	Nanometer
ml	Milliliter
Mmol/l	Millimoles per liter
cm	Centimeter
min	minute
°C	Degree celcius
SEM	Scanning electron microscope
40x, 500x	Magnification

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Aquaculture is one of the rapidly growing sectors which is in line with the other agriculture sectors. Aquaculture is essential to supply protein for human consumption besides supporting the rapidly depleting number wild fisheries. Currently aquaculture provides around 30% of the fish supply in ASEAN nations. Aquaculture can help improve countries economic value and create various job opportunities for the people. Aquaculture in Malaysia sector have a high potential because this country has large coastal and inland area for aquaculture activities (Ang, 1990).

Production of improved brood stock through hybrid technology is one of the research that have been done to increase certain traits in fish such as growth, susceptibility towards disease, and improve food conversion ratio. One of hybrid species that have been successfully breed is Kerai Lampam (Suharmili *et. al.*, 2015). Further research should be done on his hybrid species in order to test its resilience ability towards environmental factors.

One of the environmental factor that threaten the aquaculture industry is pollution from heavy metal. Rapid development from the industry especially nanotechnology sector causing a new threat to the fish. The environmental pollution from heavy metals has

been intensively examined in freshwater ecosystems due to the bioaccumulation and toxicity of these metals.

Kerai Lampam is a new hybrid that has high potential to be cultured due to its fast growth and high breeding rate. The taste is good as its flesh basically has similar to local species. This species can be one of the main commercial species for freshwater farming in Malaysia. Therefore, to ensure the success of this new hybrid strain, the study on its growth performances towards heavy metal such as iron oxide nanoparticles should be considered.

## 1.2 Problem statement

The embryonic stage in respect to heavy metal has been extensively studied. Chemical and biological qualities of water influence the optimum fish production (Bhatnagar & Devi, 2013). Toxicity studies showed that the exposure of silver to Medaka embryo was able to induce various malformations, including underdevelopment of eyes and brain, short spinal cord, ischemia, blood clots, pericardiovascular edema, tubular heart and vascular defects. As for the new hybrid species, lack of study and information on effects of Iron oxide Nanoparticles (FeO-NPs) on the growth performances, resilience ability and effect on histology. Since Kerai Lampam is a new hybrid species and the demand is increasing, study on the effects of (FeO-NPs) on development of larvae is important for better production in future. By conducting this experiment, Kerai Lampam could be the model organism for the effect of heavy metal which is might be similar to other experimental species.

### 1.3 Objective of Study

The objectives of this study are:

- a. To study the effect of different concentration of iron oxide nanoparticles (FeO-NPs) on growth performance of Kerai Lampam.
- b. To study the effect of different concentration of iron oxide nanoparticles (FeO-NPs) on liver and gills of Kerai Lampam.



## REFERENCES

- Abalaka, S. E. (2015). Heavy metals bioaccumulation and histopathological changes in *Auchenoglanis occidentalis* fish from Tiga dam, Nigeria. *Journal of Environmental Health Science & Engineering* (2015) 13:67. DOI 10.1186/s40201-015-0222-y
- Agius, C., Robert, R. J. (2003). Melano-macrophage centres and their role in fish pathology, *J. Fish Dis.* 26 (2003) 499-509
- Alijani, R.A., Movahedinia A, Rastgar S. (2017). Fish Liver Biomarkers for Heavy Metal Pollution: A Review Article *American Journals of Toxicology* 2017, 2:18
- Alvarado, N. E., Quesada K, Hylland I, Marigómez, L, Soto M. (2006). Quantitative changes in metallothionein expression in target cell types in the gills of turbot (*Scophthalmus maximus*) exposed to Cd, Cu, Zn and after a depuration treatment. *Aquatatic Toxicology* 77: 64-77
- Ang, K. (1990). Status of aquaculture in malaysia. *Aquaculture in Asia. Asian Fisheries Society, Indian Branch, Mangalore*, 265-279.
- Baskurt, O. K., Meiselman, H. J. (2010) Role of red blood cell aggregation in tissue perfusion:new findings, *STV22* (2010) 137143, <http://dx.doi.org/10.1684/stv.2010.0466>
- Bhatnagar, A., & Devi, P. (2013). Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Sciences*, 3(6), 1980.
- Cambier S., Røgeberg, M, Georgantzopoulou, A., Serchi, T., Karlsson, C., Verhaegen, S., Iversen, T-G., Guignard, C., Kruszewski, M., Hoffmann, L., Audino, J-N., Ropstad, E., Gutleb Arno C. et al. Science of the total Environment 610-611 (2017). Fate and effects of silver nanoparticles on early life-stage development of zebrafish (*Danio rerio*) in comparison to silver nitrate



- Dutta, H. M., 1996. A composite Approach for Evaluation of the Effects of Pesticides on Fish. In : Fish Morphology, Munshi, J.S.D. and H.M. Dutta (Eds.). Science Publishers Inc., India
- Griffitt, R. J., Weil R, Hyndman, K. A., Denslow, N. D., Powers, K., Taylor, D., Barber, D. S. (2007). Exposure to copper nanoparticles causes gill injury and acute lethality in zebrafish (*Danio rerio*). *Environ Sci Technol* 2007; 41, 8178–86
- Griffitt, R. J., Hyndman, K. A., Denslow, N. D., Barber, D. S. (2009). MOLECULAR AND HISTOLOGICAL CHANGES IN ZEBRAFISH GILLS EXPOSED TO METALLIC NANOPARTICLES TOXICOLOGICAL SCIENCES 107(2),404–415 (2009)
- Handy, R. D., Maunder, R. J.(2009). The biological roles of mucus: importance for osmoregulation and osmoregulatory disorders of fish health. In *Osmoregulation and Ion Transport: Integrating Physiological, Molecular and Environmental Aspects*, Vol. 1 (ed. R. D. Handy, N. R. Bury and G. Flik), pp. 203-35. Cambridge: Society for Experimental Biology. 2009.
- Hermenean, A., Damache, G., Albu, P., Ardelean, A., Ardelean, G., Ardelean, D. P.(2015). Histopathological alterations and oxidative stress in liver and kidney of *Leuciscus cephalus* following exposure to heavy metals in the Tur River, North Western Romania. *Ecotoxicol Environ Saf.* 2015,119:198205
- Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., Muller, R.N., 2010. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chem. Rev.* 100. <http://dx.doi.org/10.1021/cr900g>, pp.2574-2574.
- Linhua, H., Zhenyu, W., Baoshan, X.(2009). Effect of subacute exposure to TiO<sub>2</sub> nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (*Cyprinus carpio*). *J Environ Sci* 2009;21:145966.
- Mahboob, S., Kausar, S., Jabeen, F., Sultana, S., Sultana, T., Al Ghanim, K. A., Hussain, B., Al-Misned, F., Ahmed, Z. (2016). Effect of Heavy Metals on

Liver, Kidney, Gills and Muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus. *Braz. Arch. Biol. Technol.* v.59: e16150275, Jan/Dec 2016

Mitra, V., and Metcalf, J. (2009). Metabolic functions of the liver. *Anaesth Intensive Care Med.* 2009, 10(7):334-335

Mohamed, M. M. (2008). Effects of cryopreservation on performance and fertilizing ability of sperm from *kerai (puntiuss daruphani)*

Myers, S. M., & Linda D. R. (1988). Pathobiology Task, Environmental Conservation Division, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, Seattle, WA 98112, U.S.A.

Postic, C., Dentin, R., Girard, J. (2004). Role of the liver in the control of carbohydrate and lipid homeostasis *Diabet Metab.* 2004, 30(5):398-408

Safahieh, A., Hedayati, A., Savari, A., Movahedinia, A. (2011). Effect of sublethal dose of mercury toxicity on liver cells and tissue of yellowfin seabream. *Toxicol Industrial Health.* 2011, 28(7):583-593

Sanderson, S., Stebar, M., Ackermann, K., Jones, S., Batjakas, I. I., and Kaufman, L. (1996). Mucus entrapment of particles by a suspension feeding tilapia (Pisces: Cichlidae). *J. Exp. Biol.* 199,1743–1756.

Sfakianakis, D.G, E.Renieri , M.Kentouri , A.M.Tsatsakis 2014. Effect of heavy metals on fish larvae deformities : A review

Simmonds, M. J., Christy, R., Marshall-Gradisnik, S. M., Meiselman, H.J., Baskurt, O.K.(2011). Red blood cell aggregation parameters measured by capillary tube aggregometer using venous and capillary blood samples, *Korea-Aust. Rheol. J.* 23 (2011)

Suharmili, R., Kamarudin, M. S., Saad, C. R., Ina-Salwany, M. Y., Ramezani Fard, E., &Mahmud, M. H. 2015. Effects of varying dietary protein level on the growth, feed efficiency and body composition of lemon fin barb hybrid fingerlings. *Iranian Journal of Fisheries Sciences*,14(2),425 435

Tao, S., Liu, C., Dawson, R., Cao, J., and Li, B. (1999). Uptake of particulate lead via the gills of fish (*Carassius auratus*). *Arch. Environ. Contam. Toxicol.* 37, 352–357.

