UNIVERSITI PUTRA MALAYSIA

CHANGES IN NUTRITIONAL VALUES IN DIPLOID AND TRIPLOID AFRICAN CATFISH (Clarias gariepinus BURCHELL, 1822) EXPOSED TO CHLORPYRIFOS AND BUTACHLOR

SAMANEH SADAT KARBALAEI SEYEDJAVAD

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By

SAMANEH SADAT KARBALAEI SEYEDJAVAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2017

DEDICATION

This dissertation is specifically dedicated to my lovely mother (Zahar Gholami), my lovely father (Seyed Ahmad Karbalaei Seyedjavad), my lovely husband (Abbas Abdollahi), and my beloved family members (Hamid Reza, Majid, and Reza) for their encouragement, faith and belief me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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Faculty : Medicine and Health Sciences

Despite the widespread distribution of pesticides in aquatic environments, very little is known about the effects of contaminants on nutritional parameters in fish. Also limited information is known about the impacts of environmental stressors on polyploid organisms. This study investigated the effects of two commonly used pesticides, chlorpyrifos and butachlor, on the nutritional values of juvenile full-sibling diploid and triploid African catfish (*Clarias gariepinus*). In two separate experiments, diploid and triploid *C. gariepinus* were exposed for 21 days to three nominal concentrations of chlorpyrifos (50, 100, 150 µg/L) or butachlor concentrations (50, 100, 150 µg/L). The effect of chlorpyrifos on the skin gelatine yield and amino acid (AA) composition, and AA, fatty acid (FA), and proximate composition in the muscle of juvenile diploid and triploid *C. gariepinus* were investigated in the chlorpyrifos experiment. Similarly, the effect of butachlor on the skin gelatine yield and AA composition, and AA, FA, and proximate composition in the muscle of juvenile diploid and triploid *C. gariepinus* were investigated in the butachlor experiment. This study also compared the skin gelatine yield and AA composition, and muscle AA, FA, and proximate composition between unexposed (control) groups of juvenile diploid and triploid fish.

No significant difference ($p>0.05$) was shown for the skin gelatine yield and AA composition, and muscle AA, FA and proximate composition between diploid and triploid *C. gariepinus* in the control groups of both experiments. However, the triploids contained higher levels of myristic acid (C14:0) and lower levels of docosahexaenoic acid (DHA; 22:6n-3) and total omega-3 FA in the muscle as compared to diploids only in the control groups of the chlorpyrifos experiment. For the chlorpyrifos-exposed groups (150 µg/L), there were significant reductions ($p<0.05$) of AA composition and protein content in the muscle of diploids while the skin gelatine yield remained unchanged. On the other hand, the skin gelatine yield of triploids exposed to chlorpyrifos was affected while the changes in AA composition

of gelatine, muscle AA composition, protein, fat and ash content of the triploids were not significant ($p>0.05$). In addition, chlorpyrifos changed the moisture content of the diploids and the ash content of the triploids. In the chlorpyrifos-exposed groups (50 and 100 µg/L), the triploids showed higher levels of stearic acid (C18:0), docosapentaenoic acid (DPA; 22:5n-3), DHA, and total omega-3 FAs, and lower levels of palmitic acid (C16:0) in the muscle. The diploids, however, contained higher levels of palmitic acid and total saturated fatty acid (SFA). However, the level of total unsaturated fatty acid (UFA) was lower. The butachlor treatments did not alter the skin gelatine yield and AA composition in both the diploid and triploid fish. However, the changes in concentrations of some AAs, FAs, and protein content in the muscle of the diploids were more significant ($p<0.05$) than those of the control group. The changes of AA, most FA composition, moisture, protein, fat and ash content in the muscle of the triploids exposed to butachlor were not significant ($p>0.05$).

This study showed the different biochemical responses of diploid and triploid *C. gariepinus* upon exposure to pesticides such as chlorpyrifos and butachlor. Significant ($p<0.05$) changes in AA, FA and protein content in the muscle of diploids following chlorpyrifos or butachlor exposure may indicate a lower adaptability of diploid *C. gariepinus* than triploids to environmental stressors. Meanwhile, parameters such as AA, FA, and protein content were suggested as reliable biomarkers due to their sensitivities to pesticides exposure. Triploids were heavier, longer and exhibited fewer changes in nutritional values under chlorpyrifos and butachlor exposure. Also, despite comparable gelatine yield and protein per unit of skin and muscle, heavier body weight and longer body length in triploid *C. gariepinus* should produce higher total gelatine, flesh and protein yield compared to diploids. Therefore, triploid *C. gariepinus* is more attractive in the aquaculture industry and fisheries management.

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PERUBAHAN PARAMETE PEMAKANAN DIPLOID DAN TRIPLOID IKAN KELI AFRICA (*Clarias gariepinus* BURCHELL, 1822) YANG DIDEDAHKAN KEPADA CHLORPYRIFOS DAN BUTACHLOR

Oleh

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Walaupun telah diketahui wujudnya taburan racun di persekitaran akuatik, sangat sedikit maklumat yang diketahui tentang impak bahan pencemar keatas parameter nutrisi dalam ikan. Hanya sedikit maklumat diketahui tentang kesan pemberi tekanan alam sekitar ke atas organisma poliploid Kajian ini mengkaji kesan-kesan dua jenis racun perosak yang sering digunakan, klorpirifos dan butachlor ke atas nilai-nilai nutrisi pada diploid dan triploid juvenil ikan keli Afrika (*Clarias gariepinus*). Dalam dua eksperimen yang berbeza, *C. gariepinus* diploid and triploid didedahkan selama 21 hari kepada kepekatan nominal klorpirifos (50, 100, 150 µg/L) atau butachlor (50, 100, 150 µg/L). Kesan klorpirifos ke atas hasil gelatin kulit dan komposisi asid amino (AA) dan AA, asid lemak (FA), dan komposisi tepat otot diploid dan triploid juvenil *C. gariepinus* telah dikaji dalam eksperimen klorpirifos. Seterusnya, kesan butachlor ke atas hasil gelatin kulit dan komposisi AA dan AA, FA, dan komposisi tepat dalam otot juvenil diploid and triploid *C. gariepinus* dikaji dalam eksperimen butachlor. Kajian ini membandingkan hasil gelatin kulit dan komposisi AA dan otot AA, asid lemak (FA), dan komposisi tepat kumpulan tidak terdedah (kawalan) ikan diploid dan triploid juvenil.

Tidak ada perbezaan yang signifikan ($p>0.05$) terbukti pada hasil gelatin kulit dan komposisi AA, dan otot AA, FA dan komposisi tepat *C. gariepinus* diploid dan triploid dalam kumpulan kawalan bagi kedua-dua eksperimen tersebut. Walaubagaimanapun, triploid didapati mengandungi asid miristik aras tinggi (C14:0) dan aras rendah asid docosahexaenoic (DHA; 22:6n-3) dan omega-3 FA dalam otot berbanding dengan diploid hanya dalam kumpulan kawalan eksperimen klorpirifos. Untuk kumpulan yang terdedah kepada klorpirifos (150 µg/L), terdapat pengurangan yang signifikan ($p<0.05$) bagi komposisi AA dan kandungan protin dalam otot diploid manakala hasil gelatin kulit adalah kekal tidak berubah. Sebaliknya, hasil gelatin kulit triploid yang terdedah kepada klorpirifos terjejas sementara perubahan dalam komposisi gelatin AA, otot komposisi AA, protin, lipid dan kandungan abu triploid

tidak signifikan ($p>0.05$). Tambahan lagi, klorpirifos mengubah kandungan kelembapan diploid dan kandungan abu triploid. Dalam kumpulan yang terdedah dengan klorpirifos (50 and 100 µg/L), triploid menunjukkan asid stearik pada aras tinggi, asid docosapentaenoic (DPA; 22:5n-3), DHA, FA omega-3, dan aras asid Palmitik (C16:0) yang rendah dalam otot. Walaubagaimanapun, diploid mengandungi aras asid palmitik yang tinggi dan jumlah asid lemak tepu. Namun demikian, didapati aras asid lemak tidak tepu adalah lebih rendah. Rawatan butachlor tidak mengubah hasil gelatin kulit dan komposisi AA dalam ikan diploid dan triploid. Namun demikian, perubahan dalam kepekatan AA, FA, dan kandungan protin dalam otot diploid adalah lebih signifikan ($p<0.05$) dari perubahan dalam kumpulan kawalan. Perubahan AA, kebanyakan komposisi FA, kelembapan, protin, lipid dan kandungan abu dalam otot triploid terdedah kepada butachlor tidak signifikan ($p>0.05$).

Kajian ini menunjukkan respon biokimia *C. gariepinus* diploid and triploid yang berbeza ke atas pendedahan racun perosak, seperti klorpirifos dan butachlor. Perubahan yang signifikan ($p<0.05$) dalam AA, FA dan kandungan protin dalam otot diploid berikutan pendedahan klorpirifos dan butachlor mungkin menunjukkan kebolehsuaian *C. gariepinus* diploid yang lebih rendah berbanding triploid terhadap pemberi tekanan alam sekitar. Sementara itu, parameter seperti AA, FA dan protin dicadangkan sebagai bio-penanda yang baik dalam ikan diploid disebabkan oleh kepekaannya terhadap pendedahan racun perosak. Triploid adalah lebih berat, lebih panjang dan memaparkan sedikit sahaja perubahan dalam nilai nutrisi di bawah pendedahan klorpirifos dan butachlor. Juga terdapat perbandingan di antara hasil gelatin dan protin per unit kulit dan otot, badan yang lebih berat dan lebih panjangan pada *C. gariepinus* triploid sepatutnya menghasilkan jumlah gelatin, daging dan protin yang tinggi berbanding dengan diploid. Oleh itu, *C. gariepinus* triploid lebih dikira menarik dalam industri akua-kultur dan pengurusan perikanan.

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I certify that a Thesis Examination Committee has met on 26 May 2017 to conduct the final examination of Samaneh Sadat Karbalaei Seyedjavad on her thesis entitled "Changes in Nutritional Values in Diploid and Triploid African Catfish (*Clarias gariepinus* Burchell, 1822) Exposed to Chlorpyrifos and Butachlor" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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LIST OF ABBREVIATIONS

AA	Amino Acid
AChE	Acetylcholinesterase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ANPU	Apparent Net Protein Utilization
ARA	Arachidonic Acid
ASTM	American Society for Testing and Materials
CF	Condition factor
cyp19a2	Brain aromatase
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DHA	Docosahexaenoic Acid
DMSO	Dimethylsulfoxide
DPA	Docosapentaenoic acid
E2	17-β estradiol
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAC	Fluorescent Aromatic Compound
foxl2	Forkhead box L2
ftz-f1	Fushi tarazu-factor 1
PUFA	Polyunsaturated Fatty Acid
GnRH	Gonadotropinreleasing hormone
GST	Glutathione *S*-transferase
GDP	Gross Domestic Product
HIS	Hepatosomatic index

MANOVA	Multivariate Analysis of Variance
mg/L	Milligrams per liter
MUFA	Monounsaturated Fatty Acid
OECD	Organisation for Economic Cooperation and Development
PAH	Polynuclear Aromatic Hydrocarbon
PBS	Phosphate Buffer Saline
PC	Phosphatidylchlonine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositole
PI	Propidium Iodide
PS	Phosphatidylserine
SFA	Saturated Fatty Acid
SDS	Sodium Dodecly Sulphate
T	Testosterone
TEMED	Tetramethylethylenediamine
tph2	Tryptophan hydroxylase2
UFA	Unsaturated Fatty Acid
US EPA	United States Environmental Protection Agency
VSI	Visceral-somatic index
11β-hsd2	11 β-hydroxysteroid dehydrogenase type 2
6-DMAP	6-dimethylaminopurine
µg/L	Microgram per liter
^{o}C	Degrees Celsius

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Contamination of aquatic environments with pesticides is a global concern (Stehle & Schulz, 2015; Yadav, Devi, Syed, Cheng, Li, Zhang, & Jones, 2015). Tens of thousands of chemicals are regularly released into the environment with only partially understood impacts (Groner & Relyea, 2011). Over the past few decades, the application of pesticides has grown exponentially around the globe (Edwards, 2013). Aquatic ecosystems are particularly vulnerable to the influx of pesticides via aerial drift and run-off events (Tankiewicz, Fenik, & Biziuk, 2010). These ecosystems provide a habitat for aquatic mammals, water birds, fish, amphibians, aquatic plants, insects, zooplankton, and phytoplankton. Despite the great effort that has been expended to understand the impact of pesticides and other pollutants on non-target organisms, a great degree of variation in their impact can be observed (Biga, 2013).

Pesticides are among the most dangerous environmental contaminants due to their mobility and long-term impact on living organisms (Tankiewicz et al., 2010). Carriger, Rand, Gardinali, Perry, Tompkins, & Fernandez, (2006) reported that around 0.1 % of applied pesticides reach the intended target, while the remaining amount finds its way into the surrounding environment. Aquatic ecosystems are usually the last receptacle for pesticide residues (Pereira, Antunes, Castro, Marques, Gonçalves, Gonçalves, & Pereira, 2009; Boithias, Sauvage, Taghavi, Merlina, Probst, & Pérez, 2011). Chlorpyrifos, an organophosphate insecticide, and butachlor, an organochlorine herbicide, are extensively applied in Asian countries to control insects and weeds, respectively (Zhang, Shen, Yu, Liu, 2012; Zhu, Li, Zha, Wang, Yuan, & Wang, 2014). Earlier studies have reported the toxicities of these two pesticides to aquatic organisms (Ajimoko, & Adelowo, 2008; Narra, Begum, Rajender, & Rao, 2011; Deb & Das, 2013; Farombi Baorong, Ling, & Qiujin, 2015).

Biomarkers are defined as any biological changes which represent a deviation from the normal condition of an organism in response to stress (Van Gestel and Van Brummelen, 1996). They are worldwide-recognised tools for detection of environmental contaminants (Van der Oost, Beyer, & Vermeulen, 2003). The application of biomarkers has drawn intensive attention over recent years (Hamza Chaffai, 2014). Biochemical parameters such as protein and lipid are among the sensitive biomarkers of fish exposed to organophosphate and organochlorine pesticides (Kavitha & Rao, 2008; Karami Mohajeri & Abdollahi, 2010). However, limited studies have investigated nutritional parameters as reliable biomarkers of environmental contaminants in fish.

Fish are a promising source of nutritional compounds such as amino acids (AA), fatty acids (FA), protein, vitamins, minerals, and trace elements, which have shown therapeutic effects in humans and animals (Zuraini, Somchit, Solihah, Goh, Arifah, Zakaria, Somchit, Rajion, Zakaria, & Jais, 2006). Also, fish processing by-products such as skin and bone are a significant source for gelatine production. Fish gelatine is a promising alternative to mammalian gelatine because there are no consumer concerns due to religious dietary restrictions or safety considerations regarding bovine spongiform encephalopathy (Kittiphattanabawon, Benjakul, Sinthusamran, & Kishimura, 2016). Amino acids are the building blocks of vital proteins and peptides, which play important roles in nutrition and the homeostasis of organisms. Fatty acids are essential constituents of complex lipids. Long-chain polyunsaturated fatty acids (LC-PUFA), particularly eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) play a crucial role in human nutrition, disease prevention, and health promotion (Dunbar, Bosire, & Deckelbaum, 2014). The proximate composition of fish affects fish appetite, and growth (Breck, 2014).

Interest in the production of triploid animals is increasing in the aquaculture industry (Cleveland & Weber, 2013). Triploid organisms contain three sets of chromosomes in their cells. The presence of 50 % more chromosomes in a triploid organism increases cell size and decreases cell number (Benfey, 1999). These significant changes may cause physiological differences between diploid and triploid organisms. Sterility and reduction of gonadal growth through triploid induction (Benfey, 1999; Piferrer, Beaumont, Falguière, Flajšhans, Haffray, & Colombo, 2009) proposes several advantages of their usage in the aquaculture industry. For example, the negative effects of sexual maturation on fillet quality and growth rate of aquatic organisms can be avoided by sterility (Salem, Kenney, Rexroad, & Yao, 2006; Aussanasuwannakul, Kenney, Weber, Yao, Slider, Manor, & Salem, 2011). Previous studies showed a higher growth rate (Felip, Piferrer, Zanuy, & Carrillo, 2001; Burke, Sacobie, Lall, & Benfey, 2010), superior flesh quality (Bjørnevik, Espe, Beattie, Nortvedt, & Kiessling, 2004; Poontawee, Werner, Müller-Belecke, Hörstgen-Schwark, & Wicke, 2007) and higher disease resistance (Parsons, Busch, Thorgaard, & Scheerer, 1986; Nichols, 2009) of triploids compared to their diploid counterparts. Secondly, the use of sterile fish is required for farming purposes to minimise the genetic impact of escaped cultured fish on wild populations (Benfey, 2015). Presently, most studies regarding the effect of polyploidisation on nutritional values in organisms are on plants, while information about the impact of polyploidisation on the nutritional values in animals is limited.

Notwithstanding the advantages of triploid fish for the environment and aquaculture industry, the effects of environmental contaminants on nutritional values in polyploid fish have not been investigated. Therefore, this study was aimed to investigate the changes in skin gelatine yield and AA composition, muscle AA, FA and proximate composition of diploid and triploid African catfish (*Clarias gariepinus*) following exposure to chlorpyrifos or butachlor. The data reported in this study would be provide biomarker tools in monitoring environmental quality.

1.2 Problem Statement

Pesticides play a major role in modern agriculture to increase crop yields by protecting the agricultural production from pest attack and weeds (Arias Estévez, López Periago, Martínez Carballo, Simal Gándara, Mejuto, & García Río, 2008; Stehle & Schulz, 2015). Despite their numerous merits, pesticides are some of the most toxic substances contaminating the environment today (Tankiewicz et al., 2010). They are also mobile and have potential to bioaccumulate in organisms (Gavrilescu, 2005). On top of this, they can take part in different physical, chemical and biological processes (Gavrilescu, 2005). Particularly the presence of pesticides in water is dangerous. Exposure to such polluted water is harmful to the health and the life of both humans and living organisms (Tankiewicz et al., 2010).

In Peninsular Malaysia, the oil palm plantation areas have increased from 96,900 hectares in 1965 to 4.48 million hectares in 2008 (Malaysia Palm Oil Board, 2009). Likewise, rice is the major cereal crop in the economy of Malaysia (Shitan, Ng, & Karmokar, 2015). These areas use a huge amount of pesticides for controlling pests, weeds and plant pathogens (Sapari & Ismail, 2012), which released into the aquatic environment via water runoff to canals, streams, and main rivers (Ueji & Inao, 2001). On the other hand, the presence of fish farming areas in the water ecosystems especially close to agricultural fields could affect the heath of fish. Chlorpyrifos and butachlor are widely used pesticides in palm oil areas and paddy fields, respectively (Ismail, Prayitno, & Tayeb, 2015; Leong, Tan, & Mustafa, 2007; Zhang et al., 2012; Halimah, Ismail, Nashriyah, & Maznah, 2016). Leong et al. (2007) reported the contamination levels of some selected organochlorine and organophosphate pesticides in the Selangor River in Malaysia between 2002 and 2003.

The toxicological impact of pesticide on non-target organisms in the aquatic environment can be investigated by detecting changes in organisms at the physiological, biochemical or molecular levels (Joseph & Raj, 2011). The African catfish is a valuable source of protein, lipid and mineral in the human diet in tropical and subtropical countries (Rosa, Bandarra, & Nunes, 2007). In Malaysia, most of the catfish farms are located in and around the agriculture fields such as paddy fields in which lot of pesticides, herbicides and insecticides are commonly used (Marimuthu, Muthu, Xavier, Arockiaraj, Rahman, & Subramaniam, 2013). Therefore, the presence of different classes of pesticides in aquatic ecosystems could adversely affect the health of this species (Marimuthu et al., 2013). Also, despite the benefits of triploid fish in the aquaculture industry and environment, no studies have been conducted on the effect of environmental contamination on the nutritional values of triploid fish. Thus, this study investigated the impact of chlorpyrifos and butachlor on skin gelatine yield and AA composition, and AA, FA, protein, fat, moisture and ash in the muscle of diploid and triploid *C. gariepinus.*

1.3 Significance of the Study

Induced triploidy has attracted the attention of aquaculturists as the most effective method for producing sterile fish (Benfey, 1999). However, many aspects of triploid biology remain uncertain. The findings of the present study can open new insights for the food industry, aquaculture and fisheries management to the better understanding the physiological differences between triploid and diploid fish. Also, to date no studies have investigated the changes in nutritional values of triploid fish under environmental contaminants. This research can expand the knowledge concerning responses of triploid fish following exposure to contaminants. This study also provided an effective biomarkers for environmental biomonitoring.

1.4 Research Hypotheses

There are no significant differences in skin gelatine yield and AA composition, muscle AA, FA, protein, fat, moisture, or ash content between juvenile diploid and triploid *C. gariepinus* in chlorpyrifos or butachlor experiments.

1.5 Objectives of the Study

1. To determine changes in skin gelatine yield and AA composition in juvenile diploid and triploid *C. gariepinus* following exposure to chlorpyrifos or butachlor.
2. To determine changes in muscle AA, FA, and proximate composition in juvenile diploid and triploid *C. gariepinus* following exposure to chlorpyrifos or butachlor.
3. To investigate the reliability of skin gelatine yield and AA composition, muscle AA, FA, and proximate composition as sensitive biomarkers of environmental pesticides.

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