UNIVERSITI PUTRA MALAYSIA

DIETARY OMEGA-3 OIL SUPPLEMENTATION TO INCREASE OMEGA-3 POLYUNSATURATED FATTY ACIDS IN THE RED TILAPIA (*Oreochromis* HYBRID) AND CATFISH (*Clarias gariepinus)*

MAR MAR KYI

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By

MAR MAR KYI

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

September 2007

Dedicated To

My late mother “MAY MAY” and my beloved father “PHAY PHAY”,

for their loving kindness which has brought me this far in my life and career.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

DIETARY OMEGA-3 OIL SUPPLEMENTATION TO INCREASE OMEGA-3 POLYUNSATURATED FATTY ACIDS IN THE RED TILAPIA (*Oreochromis* HYBRID) AND CATFISH (*Clarias gariepinus)*

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September 2007

Chairman: **Professor Mohamed Ali Rajion, PhD**

Faculty: **Veterinary Medicine**

A study was carried out to determine the essential polyunsaturated fatty acid (PUFA) profiles of the red tilapia (*Oreochromis* hybrid) and catfish (*Clarias gariepinus*) and an attempt was made to increase the omega-3 polyunsaturated fatty acids by dietary omega -3 oil supplementation. The fatty acid profiles of commercially farmed adult fish were determined using standard extraction, fatty acid methylation and gas liquid chromatographic procedures and the fatty acid concentration of dried fish tissues was expressed in absolute amounts (mg/g) as a measure of the actual fatty acid content in the fish tissues. The levels of total fatty acids, SFA, UFA, monoenes, total n-6, total n-3, 18:2n-6 and 18:3n-3 were higher in the catfish compared to the red tilapia. The higher (17-20% of the total fatty acids) n-6 PUFA found in both fish compared to n-3 PUFA (1.0 – 9.5 %) was characteristic for freshwater fish.

The absolute amounts of total n-6 and n-3 PUFA increased as the age of the fish increased for both species of fish when measured from 10 to 75 days of age although they decreased when expressed as a percentage of total fatty acids. The absolute

amounts of total n-6 PUFA in the red tilapia increased from 10.0 ± 0.6 mg/g at 10 days to 26.6 ± 2.4 mg/g at 75 days of age. The absolute amounts of total n-3 PUFA in the red tilapia increased from 3.6 ± 0.2 mg/g at 10 days to 9.4 ± 0.3 mg/g at 75 days of age. Similarly, the absolute amounts of total n-6 PUFA in the catfish increased from 15.1 ±1.0 mg/g at 10 days to 36.5 ± 2.5 mg/g at 75 days of age whilst the absolute amounts of total n-3 PUFA increased from 8.1 ± 0.2 mg/g at 10 days to 21.8 ± 1.5 mg/g at 75 days of age. The results were suggestive of a combined effect of accumulation, desaturation and elongation and oxidation of the PUFA in the fish tissues.

The Δ6 desaturase enzyme activity in the liver microsomes was measured in six of each species of fish employing radiolabelled linoleic acid [1-^{14}C] and argentation thin layer chromatography. Desaturase activities were detected in both species but the activity in the red tilapia (3.55 + 0.2%; 1.19 + 0.1 pmol/min/mg protein) was higher, although not significant ($p>0.05$), than the catfish (3.07 ± 0.2%; 1.02 + 0.1 pmol/min/mg protein).

The antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) and the lipid peroxidation value of malonaldehyde (MDA) were measured in ten of each species of fish. The antioxidant activity was higher in the catfish whilst the lipid peroxidation value was higher in the red tilapia. The activity of SOD (1.54 ± 0.1 U/g) and GSH-Px (0.37 ± 0.1 U/g) in the red tilapia was lower than the activity of SOD (2.48 ± 0.1 U/g) and GSH-Px (1.18 ± 0.1 U/g) in the catfish. The MDA values were 21.39 ± 0.5 nmol/g for the red tilapia compared to 19.15 ± 0.2 nmol/g for the catfish.

The dietary omega-3 oil supplementation trial was carried out for eight weeks where the fish was reared in glass aquariums in under three dietary treatments, in triplicate. The treatment diets were the control diet CON (with no oil added), or diets containing added 10% or 20% flaxseed oil (10% FLAX or 20% FLAX) or added 10% or 20% cod-liver oil (10% COD, 20% COD). The desirable n-3 PUFA were not increased by the 10% FLAX or 10% COD diets where the total n-3 PUFA concentrations in the red tilapia were 3.2 + 0.1 mg/g (CON), 2.6 ± 0.2 mg/g (10% FLAX) and 3.4 ± 0.2 mg/g (10% COD). The n-3 PUFA concentrations in the catfish were 7.1 ± 0.4 mg/g (CON), 6.4 ± 0.1 mg/g (10% FLAX) and 6.4 ± 0.4 mg/g (10%COD). However, the n-3 PUFA concentrations were significantly increased ($p<0.05$) when fed the 20% FLAX and 20% COD diets. In the red tilapia the n-3 PUFA concentrations were 3.4 ± 0.1 mg/g (CON), 4.7 ± 0.1 mg/g (20% FLAX) and 3.8 ± 0.2 mg/g (20% COD). The n-3 PUFA concentrations in the catfish were 6.5 ± 0.3 mg/g (CON), 8.5 ± 0.6 mg/g (20% FLAX) and 9.0 ± 0.6 mg/g (20% COD). However high mortality rates up to 60% were encountered when the 20% FLAX and 20% COD diets were used suggesting that the levels of the oils used in these diets were toxic to the fish. Histological examinations carried out at *post-mortem* confirmed the toxicological condition by the occurrence of several histopathological lesions in the liver, kidney and small intestine.

In conclusion, the essential PUFA profiles of the red tilapia which has a herbivorous mode of nutrition and the catfish which is more omnivorous, with different desaturase and oxidative enzyme activities are somewhat different, where the former represents a better source of desirable essential PUFA to the human consumer. The concentrations of the desirable essential PUFA in both fishes can be increased by

modifying their diets to contain added oils such as flaxseed or cod-liver oil but the percentage of the added oils have to be between 10-20% of the diet to avoid toxicity and high mortalities.

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

PENAMBAHAN MINYAK OMEGA-3 DALAM DIET UNTUK MENINGKATKAN KANDUNGAN ASID LEMAK POLITAKTEPU OMEGA-3 PADA IKAN TILAPIA MERAH (*Oreochromis* HYBRID) DAN IKAN KELI (*Clarias gariepinus*)

Oleh

MAR MAR KYI

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Pengerusi: **Profesor Mohamed Ali Rajion, PhD**

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Kajian telah dijalankan untuk menentukan profil asid lemak politaktepu perlu dalam ikan tilapia merah (*Oreochromis* hybrid) dan keli (*Clarias gariepinus*) dan satu percubaan dijalankan untuk meningkatkan asid lemak politaktepu omega-3 melalui penambahan minyak omega-3 dalam diet. Profil asid lemak ikan dewasa ternakan komersial ditentukan menggunakan metodologi ekstraksi, methilasi dan kromatografi gas-cecair biasa. Kepekatan asid lemak dalam tisu kering ikan (mg/g) menunjukkan kepekatan asid lemak sebenarnya dalam ikan. Tahap jumlah asid lemak, asid lemak tepu dan taktepu, jumlah asid lemak politaktepu n-6, jumlah asid lemak politaktepu n-3, 18:2n-6 dan 18:3n-3 adalah lebih tinggi dalam ikan keli berbanding dengan ikan tilapia merah. Tahap tinggi (17-20% daripada jumlah asid lemak) asid lemak politaktepu n-6 terdapat dalam kedua-dua spesis ikan berbanding dengan asid lemak politaktepu n-3 (1.0 – 9.5 %) adalah ciri biasa bagi ikan air tawar.

Jumlah asid lemak politaktepu n-6 dan n-3 meningkat apabila umur ikan meningkat bagi kedua-dua spesis ikan apabila diukur dari umur 10 hingga 75 hari sugguhpun peratusan daripada jumlah asid lemak menurun.. Kandungan mutlak jumlah asid lemak politaktepu n-6 dalam tilapia merah meningkat daripada 10.0 ± 0.6 mg/g pada umur 10 hari kepada 26.6 ± 2.4 mg/g pada umur 75 hari. Kandungan mutlak jumlah asid lemak politaktepu n-3 dalam tilapia merah meningkat daripada 3.6 ± 0.2 mg/g pada umur 10 hari kepada 9.4 ± 0.3 mg/g pada umur 75 hari. Kandungan mutlak jumlah asid lemak politaktepu n-6 dalam ikan keli meningkat daripada 15.1 ±1.0 mg/g pada umur 10 hari kepada 36.5 ± 2.5 mg/g pada umur 75 hari. Manakala kandungan mutlak jumlah asid lemak politaktepu n-3 meningkat daripada 8.1 ± 0.2 mg/g pada umur 10 hari kepada 21.8 ± 1.5 mg/g pada umur 75 hari. Keputusan menyarankan kesan bergabung pengumpulan, desaturasi dan elongasi dan oksidasi asid lemak politaktepu dalam tisu ikan tersebut.

Aktiviti enzim Δ6 desaturase dalam mikrosom hati telah diukur dalam enam ekor setiap spesis menggunakan asid linoleik radioaktif [1-^{14}C] dan kromatografi lapisan halus argentasi. Aktiviti desaturase dikesan dalam kedua-dua spesis tetapi aktiviti dalm tilapia merah (3.55 ± 0.2%; 1.19 ± 0.1 pmol/min/mg protin) adalah lebih tinggi berbanding dengan keli (3.07 ± 0.2%; 1.02 ±0.1 pmol/min/mg protin sungguhpun tiada bererti ($p>0.05$).

Enzim antioksidan, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) dan nilai peroksidasi lipid malonaldehyde (MDA) diukur dalam 10 daripada setiap spesis ikan. Aktivati antioksidan adalah lebih tinggi dalam keli manakala peroksidasi lipid adalah lebih tinggi dalam tilapia merah. Aktivati SOD (1.54 ± 0.1

U/g) dan GSH-Px (0.37 ± 0.1 U/g) dalam tilapia merah adalah lebih rendah daripada aktiviti SOD (2.48 ± 0.1 U/g) and GSH-Px (1.18 ± 0.1 U/g) dalam ikan keli. Nilai MDA adalah 21.39 ± 0.5 nmol/g untuk tilapia merah berbanding dengan 19.15 ± 0.2 nmol/g untuk ikan keli.

Ujian manipulasi diet telah dijalankan selama lapan minggu di mana ikan dipelihara dalam akuarium gelas diberi tiga jenis makanan tiga kumpulan setiap jenis makanan. Jenis diet adalah diet kawalan CON (tanpa tambahan minyak), atau diet mengandungi tambahan 10% or 20% minyak flaxseed (10% FLAX or 20% FLAX) atau 10% or 20% minyak hati kod (10% COD, 20% COD). Asid lemak politaktepu n-3 yang baik tidak ditingkatkan oleh diet 10% FLAX atau 10% COD di mana jumlah kepekatan asid lemak politaktepu n-3 dalam tilapia merah adalah 3.2 ± 0.1 mg/g (CON), 2.6 ± 0.2 mg/g (10% FLAX) dan 3.4 ± 0.2 mg/g (10% COD). Kepekatan asid lemak politaktepu n-3 dalam ikan keli adalah 7.1 ± 0.4 mg/g (CON), 6.4 ± 0.1 mg/g (10% FLAX) dan 6.4 ± 0.4 mg/g (10% COD). Bagaimanapun, kepekatan asid lemak politaktepu n-3 meningkat ($p<0.05$) apabila ikan diberi diet 20% FLAX dan 20% COD. Dalam tilapia merah kepekatan asid lemak politaktepu n-3 adalah 3.4 ± 0.1 mg/g (CON), 4.7 ± 0.1 mg/g (20% FLAX) dan 3.8 ± 0.2 mg/g (20% COD). Kepekatan asid lemak politaktepu n-3 dalam ikan keli pula adalah 6.5 ± 0.3 mg/g (CON), 8.5 ± 0.6 mg/g (20% FLAX) and 9.0 ± 0.6 mg/g (20% COD). Bagaimanapun, kadar kematian ke tahap 60% berlaku dengan diet 20% FLAX dan 20% COD menunjukkan bahawa tahap minyak yang digunakan dalam diet adalah terlalu tiggi. Peperiksaan *post-mortem* histologi yang dijalankan yang menunjukkan beberapa lesi histopatologi dalam hati, ginjal dan intestine kecil ikan mengesahkan berlakunya keracunan.

Dalam kesimpulannya, profil asid lemak politaktepu perlu dalam ikan tilapia merah yang mempunyai jenis pemakanan herbivor dan ikan keli yang mempunyai jenis pemakanan lebih omnivor adalah berbeza dengan adanya aktiviti enzim desaturase and oksidasi yang berbeza. Ikan tilapia merah merupakan sumber asid lemak politaktepu yang lebih baik untuk pengguna manusia. Kepekatan asid lemak politaktepu yang baik boleh ditingkatkan dengan manipulasi diet melalui penambahan minyak flaxseed atau minyak hati kod tetapi peratusan yang boleh digunakan mestilah di antara 10-20% untuk mengelakkan masaalah keracunan dan kadar kematian tinggi.

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I certify that an Examination Committee has met on **11 September 2007** to conduct the final examination of **Mar Mar Kyi** on her **Doctor of Philosophy** thesis entitled **"Dietary omega-3 oil supplementation to increase omega-3 polyunsaturated fatty acids in the red tilapia (*Oreochromis* hybrid) and catfish (*Clarias gariepinus*)"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

MAR MAR KYI

Date.11 September 2007

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13	Normal Liver Parenchyma of Catfish Fed a CON Diet. Hepatocytes (H); Central Hepatic Duct (CHD). H & E stain, Mag. X 400	114
14	Liver of Catfish Fed a 20% COD Diet for 60 days. Focal Infiltration of Mononuclear Inflammatory Cells in Hepatic Vessel (IM) and Fatty Infiltration (FI) in Liver Parenchyma. H & E stain, Mag. X 400.	114
15	Normal Kidney of Red Tilapia Fed a CON Diet. Glomerulus (G); Renal Tubules (RT) H & E stain, Mag. X 400	115

16	Kidney of Red Tilapia Fed a 20% COD for 60 days. Increased Glomerular Capsule Space (IBS); Degenerated Renal Tubules (DRT) and Congestion in Capillaries of Bowman's Capsule (CG). H & E stain, Mag. X 400	116
17	Normal Kidney of Catfish Fed a CON Diet. Glomerulus (G); Renal Tubules (RT). H & E stain, Mag. X 400	117
18	Kidney of Catfish Fed a 20% COD Diet for 60 days. Infiltration of Mononuclear Inflammatory Cells (IF), Dilated Bowman's Capsule (DB), Degenerated Renal Tubules (DRT) and Increased Presence of MMC (Melanomacrophage Centres). H & E stain, Mag. X 400	117
19	Normal Intestine of Red Tilapia Fed a CON Diet. Columnar Epithelium (CE); Lamina Propria (LP); Muscularis Layer (MS); Serosa (S). H & E stain, Mag. X 400	118
20	Intestine of Red Tilapia Fed a 20% FLAX Diet for 60 days. Degenerated Hepatopancreas (HS); Increased Secretion of Mucus (M) by Mucosal Cells in Lamina Propria; Mucosal Cells (MC). H & E stain, Mag. X 200	119
21	Normal Intestine of Catfish Fed a CON Diet. Columnar Epithelium (CE); Lamina Propria (LP); Muscularis Layer (MS); Serosa (S). H & E Stain, Mag. X 400	120
22	Intestine of Catfish Fed a 20% COD Diet for 60 Days. Increased Mucus Secretion (M) by Mucosal Cells; Mucosal Cells (MC). H & E stain, Mag. X 200	120