UNIVERSITI PUTRA MALAYSIA

GROWTH PERFORMANCE AND FATTY ACID PROFILES OF MALAYSIAN MAHSEER, Tor tambroides (Bleeker, 1854) FED WITH DIFFERENT TYPES OF PALM OIL AND RIVERINE FRUIT OIL

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By

MAHKAMEH LASHKARIZADEH BAMI

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

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DEDICATION

"THE BEST COMES AT THE END"

This thesis is dedicated to my beloved mother and father, Mahnoush and Mahmoud, whose supports have always been with me since the beginning of my life and taught me patience and strength as precious gifts of the life.

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

GROWTH PERFORMANCE AND FATTY ACID PROFILES OF MALAYSIAN MAHSEER, *Tor tambroides* (Bleeker, 1854) FED WITH DIFFERENT TYPES OF PALM OIL AND DIFFERENT RIVERINE FRUIT OIL

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February 2017

Chairman : Professor Mohd Salleh Kamarudin, PhD
Faculty : Agriculture

This study was carried out to investigate the effects of various dietary oil sources containing different types of palm oil and riverine fruit oils on the growth performance, proximate composition and tissue fatty acid profile of juvenile Malaysian mahseer, *Tor tambroides*. Domesticated wild caught *T. tambroides* juveniles were used in all feeding trials. Each feeding trial was conducted for a period of 12 weeks and treatments were triplicated. In Experiment 1, four isocaloric and isonitrogenous diets with 5% lipid from different types of palm oil [crude palm oil (CPO), refined bleached deodorized palm oil (RBDO), refined bleached deodorized palm olein (RBDPO) and refined bleached deodorized palm stearin (RBDPS)] were tested. Although the growth performance of fish fed CPO was similar to those fed refined products, its muscle contained significantly higher n-3 fatty acids. Thus, CPO was used as the control in the subsequent feeding trials.

In Experiment 2, five isocaloric and isonitrogeous diets containing 5% lipid with different illipe oil-CPO ratios (100:0, 75:25, 50:50, 25:75 and 0:100) were evaluated. Juveniles fed on different dietary illipe oil-CPO ratios showed similar growth performances. However, juveniles fed on 100% dietary CPO had the best tissue fatty acid profile. In Experiment 3, five isocaloric and isonitrogeous diets containing 5% lipid with different canarium oil-CPO ratios as in the previous experiment were tested. Juveniles fed on 100% dietary CPO achieved the best growth performance and tissue fatty acid profile. The dietary inclusion of canarium oil significantly affected the fish growth. In Experiment 4, five isocaloric and isonitrogeous diets containing 5% lipid with different kembayau oil-CPO ratios as in the previous two experiments were assessed. Although fish fed 100% dietary

CPO gave the best growth performance, kembayau oil could be included in the mahseer diet up to 50% replacement. In addition, the best muscle fatty acid profile was observed among those fed 50% dietary kembayau oil and 50% CPO.

In terms of fish growth, the cheaper CPO performed as well as illipe oil and refined palm oil products. CPO, with high antioxidative activity along with high amounts of 16:0 and 18:1n-9 and moderate 18:2n-6, was also efficient in maintaining the muscle n-3 PUFAs. However, a better muscle fatty acid composition could be achieved when mahseer was fed a 50:50 combination of CPO-kembayau oil. In conclusion, CPO and a 50:50 mixture of kembayau oil-CPO were respectively recommended as a lipid source in the grow-out and finishing diets for juvenile *T. tambroides*.

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

PRESTASI PERTUMBUHAN DAN PROFIL ASID LEMAK KELAH, *Tor tambroides* (Bleeker, 1854) YANG DIBERI MAKAN MINYAK SAWIT DAN MINYAK BUAH SUNGAI YANG BERBEZA

Oleh

MAHKAMEH LASHKARIZADEH BAMI

Februari 2017

Pengerusi : Profesor Mohd Salleh Kamarudin, PhD
Fakulti : Pertanian

Kajian ini telah dijalankan untuk menyiasat kesan pelbagai sumber minyak yang terdiri dari beberapa jenis minyak sawit dan minyak buah sungai terhadap prestasi pertumbuhan, komposisi proksimat dan profil asid lemak tisu kelah, *Tor tambroides.* Juvana *T. tambroides* liar yang telah dijinakkan telah digunakan dalam kesemua ujian pemakanan. Setiap ujian pemakanan dijalankan selama 12 minggu. Kesemua rawatan dilakukan dalam tiga replikat. Dalam Eksperimen 1, sebanyak empat diet isokalori dengan kandungan protein dan lipid yang sama tetapi berbeza jenis minyak kelapa sawit [minyak sawit mentah (CPO), minyak sawit bertapis yang diluntur dan dinyahbau (RBDO), minyak sawit olein bertapis yang diluntur dan dinyahbau (RBDPO), dan minyak sawit stearin bertapis yang diluntur dan dinyahbau (RBDPS) telah diuji. Walaupun prestasi pertumbuhan ikan yang diberi makan CPO serupa dengan ikan yang diberikan produk sawit bertapis, ototnya mengandungi asid lemak n-3 yang ketara lebih tinggi. CPO telah digunakan sebagai rawatan kawalan dalam kajian makanan yang seterusnya.

Dalam Ekperimen 2, sebanyak lima diet isokalori dan isonitrogen yang mengandungi 5% lipid dengan nisbah minyak engkabang-CPO yang berbeza (100:0, 75:25, 50:50, 25:75 dan 0:100) telah dinilai. Juvana diberi makan nisbah dietari minyak engkabang dan CPO yang berlainan menunjukkan prestasi pertumbuhan yang serupa. Bagaimanapun ikan yang diberi 100% CPO mempunyai profil tisu asid lemak yang terbaik. Dalam Eksperimen 3, lima diet isokalori dan isonitrogen mengandungi 5% lipid dengan nisbah minyak dabai-CPO yang berbeza seperti eksperimen sebelumnya telah diuji. Juvana yang diberi makan 100% CPO mencapai prestasi pertumbuhan dan profil asid lemak tisu terbaik. Kemasukan minyak dabai dalam makanan ketara mengganggu pertumbuhan ikan. Dalam Eksperimen 4, lima diet isokalori dan isonitrogen

mengandungi 5% lipid dengan nisbah minyak kembayau-CPO berbeza seperti dua eksperimen sebelumnya telah dinilai. Walaupun ikan yang diberi makan 100% CPO memberikan pertumbuhan terbaik, minyak kembayau boleh dimasukkan dalam makanan kelah sehingga 50% penggantian. Selain itu, profil asid lemak otot terbaik dilihat antara ikan yang diberi makan 50% minyak kembayau dan 50% CPO.

Dari segi pertumbuhan ikan, CPO yang lebih murah memberikan prestasi sebaik minyak engkabang dan produk sawit bertapis. CPO, dengan aktiviti antioksida yang tinggi serta kandungan 16:0 dan 18:1n-9 yang tinggi dan kandungan 18:2n-6 yang sederhana, juga cekap mengekalkan jumlah n-3 PUFA dalam otot. Bagaimanapun, profil asid lemak otot yang lebih baik boleh diperoleh apabila kelah diberi makan campuran 50:50 minyak kembayau-CPO. Kesimpulannya, CPO dan campuran 50:50 kembayau-CPO, masing-masing, dicadangkan sebagai sumber minyak makanan dalam fasa pertumbuhan dan pengakhir juvana*T. tambroides*.

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I certify that a Thesis Examination Committee has met on 10 February 2017 to conduct the final examination of Mahkameh Lashkarizadeh Bami on her thesis entitled "Growth Performance and Fatty Acid Profiles of Malaysian Mahseer, *Tor tambroides* (Bleeker, 1854) Fed with Different Types of Palm Oil and Riverine Fruit Oil" 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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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

Acetyl-CoA	Acetyl Coenzyme A
ANOVA	Analysis of Variance
CLE	Cyclolinopeptide
CLO	Cod Liver Oil
CO	Canola Oil
CPKO	Crude Palm Kernel Oil
CPO	Crude Palm Oil
DAH	Days After Hatching
DFI	Daily Feed Intake
DO	Dissolved Oxygen
EFA	Essential Fatty Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
FCR	Feed Conversion Ratio
HSI	Hepato-somatic Index
LC-PUFA	Long-chain Polyunsaturated Fatty Acid
MPOB	Malaysian Palm Oil Board
MUFA	Monounsaturated Fatty Acid
n-3	Omega 3
n-6	Omega 6
PER	Protein Efficiency Ratio
PFAD	Palm Fatty Acid Distillates
PUFA	Polyunsaturated Fatty Acid
RBDO	Refined, Bleached and Deodorized Palm Oil
RBDPO	Refined, Bleached and Deodorized Palm Olein
RBDPS	Refined, Bleached and Deodorized Palm Stearin
SBC	Spent Bleaching Clay
SD	Standard Deviation
SE	Standard Error
SFA	Saturated Fatty Acids
SGR	Specific Growth Rate
UFA	Unsaturated Fatty Acid
VLDL	Very Low Density Lipoprotein
VSI	Viscera-somatic Index
WG	Weight Gain

CHAPTER 1

INTRODUCTION

Tor tambroides and *T. douronensis* are the two most sought after mahseer species in Malaysia. These species inhabit most large river systems in Peninsular Malaysia, Sabah and Sarawak (Ingram *et al*., 2005). The over-exploitation of mahseers as well as the damages and destructions to their natural habitats have led to a rapid decline in their natural stocks in recent years and a sharp increase in their market prices which can go to as high as US $257 kg^{-1} (DOF, 2014). The total mahseer catch in Malaysia and Indonesia were 13.71 and 60 tons in 2004 and have raised to 72.48 and 569 tons in 2011. Thus, serious efforts have been made to increase their artificial reproduction for their conservation and aquaculture production. To date, three government hatcheries are actively involved in the fry production while the number of private hatcheries is not available. However, the production is still low with only 2897 fry produced in 2013 (DOF, 2014). In the meanwhile, the mahseer aquaculture production in Malaysia has increased from 15 tons in 2012 to 24.70 tons in 2015 (DOF, 2013, 2014, 2015, 2016).

To ensure optimal fish growth and a sustainable increase in the production, it is essential to provide the mahseers with a specific balanced diet that meets all or most of their nutritional requirements. Fish require dietary lipid as a major energy source (Tocher, 2003). Lipids are generally preferred over carbohydrates to serve as an energy resource in fish in the natural environment (Tocher, 2003). Lipids can also spare dietary protein for growth purposes (Watanabe, 2002) although there are new evidences that lipids may not do that in some fish (Ng *et al*., 2008; Ng & Andin, 2011). Protein is a high-cost nutrient and therefore, maximizing protein utilization by animals will be economical. Moreover, lipids are a significant source of essential fatty acids (EFAs), which are needed for the maintenance and function of cell membranes (Coway & Sargent, 1977). Fish require both n-3 and n-6 series of polyunsaturated fatty acids (PUFAs) with carbon chain lengths of 18 as well as long-chain polyunsaturated fatty acids (LC-PUFAs) with carbon chain lengths of 20 and 22 as EFAs in their diets for their growth and health (Coway & Sargent, 1977; Smith *et al*., 2004). To produce C_{18} PUFAs from 18:1n-9, Δ12 and Δ15 desaturases are required, but fish lack in them. The continuous growth of the aquaculture industry in recent years has led to an increasing demand for fish oil as a sole practical EFA source (Tan *et al*., 2009). The stagnant fish oil production can be a limiting factor for the aquaculture production in the future. Therefore, replacing fish oil with a suitable substitute that retains EFAs, especially 20:5n-3 (EPA) and 22:6n-3 (DHA), in fish tissue is essential for fish growth and health as well as for human health as the fish consumer.

High levels of EPA and DHA retention in fish tissues require minimal catabolism of these fatty acids (FA) by the fish. There are few vegetable oils containing large amounts of some saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), which are readily oxidized over n-3 LC-PUFAs (Sargent *et al*., 2002). These oils, such as palm oil (with high amounts of SFAs and MUFAs), coconut oil (with high SFA content), rapeseed and olive oil (with high MUFA contents) (Turchini *et al*., 2010), can be potential substitutes for fish oil. These vegetable oils have been reported to spare dietary n-3 LC-PUFAs in fish tissues (Kamarudin *et al*., 2012; Ng *et al*., 2003b; Ng *et al*., 2001; Ramezani-Fard *et al*., 2012b; Torstensen *et al*., 2005; Turchini *et al*., 2011). Recently, the vegetable oils rich in n-6 PUFAs such as cottonseed oil have also been observed to spare dietary n-3 LC-PUFAs in fish species with the ability to use dietary n-6 PUFAs as an efficient energy source such as European sea bass (*Dicentrarchus labrax*) (Eroldoğan *et al*., 2013; Yilmaz & Eroldoğan, 2015).

Palm oil seems to be more advantageous than other vegetable oils to be included in aquafeed. In addition to its high amounts of 16:0 as a SFA and 18:1n-9 as a MUFA, it is the most available vegetable oil in the world (FAO, 2015). Refined, bleached and deodorized palm olein (RBDPO) has been successfully used to substitute fish oil in *T. tambroides* nutrition (Kamarudin *et al*., 2012; Ramezani-Fard *et al*., 2012b). As LC-PUFAs are highly susceptible to be oxidized, crude palm oil (CPO) which contains high carotenoids and vitamin E as antioxidants (Ng *et al*., 2003b), may have a higher potential than RBDPO to be utilized successfully in *T. tambroides* nutrition. Moreover, CPO as well as refined, bleached and deodorized palm oil (RBDO) are cheaper than RBDPO (Ng *et al*., 2003b). Refined, bleached and deodorized palm stearin (RBDPS), another type of palm oil, may also be used as a highly efficient oil in Malaysian mahseer diet. Although palm oil has been observed to successfully substitute fish oil in the aquaculture industry, no literature in comparing the efficiency of different palm oil products in the nutrition of fish including *T. tambroides* was found.

In the wild, this omnivorous fish has also been observed to feed on the fruits that drop into the rivers from certain species of trees that grow along the riverbanks (Misieng *et al*., 2015; Siraj *et al*., 2007). Some of these fruits contain relatively high lipid percentages with moderate to high SFA contents. In spite of the importance of these riverine fruits in the natural diet of Malaysian mahseer, no studies on their roles in the nutrition of this fish species have been found. These fruits may be potential to be used as cheaper indigenous oil sources in *T. tambroides* feed. Thus, the general objective of this study was to evaluate the oils of some of these riverine fruits as the dietary lipid sources for the Malaysian mahseer. In addition, the specific objectives of this study were:

- To investigate the effects of different types of palm oil on the growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer.
- To study the effects of illipe oil and its combination with the best type of palm oil on the growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer.
- To determine the effects of canarium oil and its combination with the best type of palm oil on the growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer.
- To observe the effects of kembayau oil and its combination with the best type of palm oil on the growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer.

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