African Journal of Biotechnology Vol. 10(79), pp. 18256-18265, 12 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1340
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Changes over time in muscle fatty acid composition of Malaysian mahseer, *Tor tambroides*, fed different dietary lipid percentage

Ehsan Ramezani-Fard, Mohd Salleh Kamarudin*, Che Roos Saad and Sharr Azni Harmin

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Accepted 1 September, 2011

The effects of four isonitrogenous diets containing different lipid levels on the muscle fatty acid profiles of Malaysian mahseer were evaluated over a period of six weeks from June to July, 2010. A general increase of monounsaturated fatty acid contents in the muscle of fish that were fed the test diets was detected after two weeks. Despite the high total content of long chain *n*-3 polyunsaturated fatty acid (PUFA) in the formulated diets, the levels of these fatty acids in the muscle did not increase and it may be speculated that the content of long chain *n*-3 PUFAs in this species was determined by desaturation and elongation of shorter chain fatty acids rather than direct absorption from the diet. Moreover, reduction of muscle *n*-3 PUFA content after six weeks of feeding showed that a diet containing high level of *n*-3 PUFA may not be able to provide all the essential fatty acid requirements of *T. tambroides*.

Key words: Fatty acid, lipid, muscle, Malaysian mahseer, *Tor tambroides*.

INTRODUCTION

The content of long chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs) differentiates fish from the other food products. These fatty acids are important beneficial nutrients for the prevention of human coronary disease, reducing triacylglycerols level and promoting cardio-vascular functions (Moreira et al., 2001; Akpinar et al., 2009). Eicosapentaenoic acid (EPA, C20:5*n*3) is also helpful in brain disorder and cancer treatment (Özogul et al., 2007). Since the human body is not able to synthesize *n*-3 PUFA, fish play a significant role in the supply of these functional nutrients in the diet. Freshwater fishes have a greater capacity to desaturate and elongate short chain fatty acids than marine fishes (Moreira et al., 2001). However, marine fish contain relatively higher amount of EPA and docosahexaenoic acid (DHA, C22:6*n*3) due to high amounts of these fatty acids in their natural diets (Justi et al., 2003). Nevertheless, consumption of freshwater fish significantly contributes to providing EPA and DHA in the diets of population living far from coastal areas (Rasoarahona et al., 2005).

Whether from freshwater or marine species, the fatty acid compositions vary between wild and cultured fish (Aslan et al., 2007). Dietary lipid, which extremely influences the fatty acid composition of fish, is considered as the main reason for such difference (Alasalvar et al., 2002). Decrease of EPA and DHA levels among the farmed fish is generally attributed to the substitution of fish oil by vegetable oil in the diet of commercial fish (Cahu et al., 2004). Despite the abundance of information on the effect of dietary lipid level on the fatty acid content of cultured fish, the changes of fish fatty acid profiles over time have not been fully understood (Jobling, 2004). It has been shown that the effect of a change in fish diet is perceptible on the tissue fatty acid profiles within 2 to 6 weeks (Dos Santos et al., 1993; Skonberg et al., 1994; Jobling, 2004).

In the current study, the changes over a six-week time of muscle fatty acid composition of Malaysian mahseer fed varying dietary lipid levels were evaluated. Mahseers (*Tor* spp.) are an important group of riverine cyprinids occurring in mountainous rivers and lakes of most trans-Himalayan countries and about 30 known species of this fish are distributed throughout East and Southeast Asia (Ng et al., 2008). Mahseer is omnivorous and feeds on

*Corresponding author. E-mail: msalleh@agri.upm.edu.my. Tel: +603 89464103. Fax: +603 89464102.

Table 1. Ingredients and proximate analysis of the formulated diets.

Ingredient (g/100 g)	Dietary lipid level (%)			
	5	10	15	20
Fish meal[a]	38.0	38.0	38.0	38.0
Soy meal	13.0	13.0	13.0	13.0
Casein	14.8	14.8	14.8	14.8
Corn meal	32.2	28.4	23.1	17.6
Fish oil[b]	0	3.8	9.1	14.6
Vitamin premix[c]	1.0	1.0	1.0	1.0
Mineral premix[d]	1.0	1.0	1.0	1.0
Proximate composition (% as a fed basis)				
Crude protein	40.4	40.1	39.7	39.6
Crude lipid	4.7	9.4	14.8	19.6
Ash	10.4	10.8	10.6	10.9
Gross energy (kJ g^{-1})	17.3	18.0	19.2	20.1
Dry weight	87.6	88.9	90.1	89.2

[a]Malaysian fish meal including 63% crude protein; [b]cod liver oil (Seven seas); [c]Vitamin premix (g kg^{-1} premix); ascorbic acid, 45; myo-inositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiamin mononitrate, 0.92; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.083; vitamin K menadione, 1.67; α-tocopheryl acetate (500 IU/g), 8; biotin, 0.02; folic acid, 0.09; vitamin B_{12}, 0.001; cellulose, 845.11; [d]Mineral premix (g kg^{-1} premix): KCL, 90; KI, 0.04; $CaHPO_4.2H_2O$, 500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H_2O$, 4; $CoSO_4$, 0.02; $FeSO_4$ $7H_2O$, 20; $MnSO_4.H_2O$, 3; $CaCo_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

green filamentous algae, slimy matter encrusted the rocks, aquatic weeds of all sorts and the seeds and fruits of many trees that hang over the river (Dinesh and Nandeesha, 2007). Although, insects, shrimps, mollusks and small fish were recorded from mahseer stomach content, they prefer vegetative matter in case of availability (Rahman et al., 2005; Dinesh and Nandeesha, 2007). Malaysia has two different species of mahseer, *Tor tambroides* and *T. douronensis*. These species live in headwaters of most major river systems from Indonesia to southern China (Nguyen et al., 2006). *T. tambroides* is locally important food fish that can fetch a market price exceeding 80 USD kg^{-1}. Due to the high market value and a successful hatchery production (Ingram et al., 2007), this species has a high potential in aquaculture.

MATERIALS AND METHODS

Rearing and sampling

In total, 144 wild *T. tambroides* juveniles (mean initial weight, 6.7 ± 0.5 g) were obtained from a local supplier and transferred immediately to the Aquaculture Experimental Station, Universiti Putra Malaysia. Fish were randomly distributed into rectangular-shaped glass aquaria with a stocking density of 12 fish per aquarium (volume: 65 L). Each aquarium was supplied with de-chlorinated public utility water and equipped with a recirculating system (flow rate of approximately 3 L/min) that continuously purified water through a series of physical and biofilter system. Continuous aeration by an air-stone per aquarium kept oxygen level always above 7 mg/L. Water temperature was found between 27.5 and 29°C, while pH was between 8 and 8.8. Four isonitrogenous (40% crude protein) diets containing 5, 10, 15 and 20% crude lipid (as fed basis) were used. Feeds were prepared by mixing of dry ingredients (Table 1) in a vertical mixer, and subsequently adding of dietary oil and distilled water to produce soft and homogenous dough. The moist dough was pelleted by a commercial meat grinder through a 2 mm die. Diets were dried in a convection oven for 8 h at 65°C (López et al., 2009), cooled and stored frozen at -20°C. The diets were thawed weekly during experimental period and kept refrigerated at 4°C until use. The fish were fed twice per day until visual satiety. All treatments were conducted in a controlled rearing condition, each of which was triplicated. Prior to the feeding trial, six fish per dietary lipid level (two per aquarium) were sacrificed. The same numbers of fish were also sampled and sacrificed at 2, 4 and 6 weeks after beginning the experiment. The sacrificed fish were individually weighed, dissected and dressed. The fish muscle between lateral and dorsal line was sampled and immediately frozen and stored at -80°C for further analyses.

Analytical methods

At the end of the experiment, crude protein, crude lipid and ash content of experimental diets, as well as crude lipid content of fish muscle were determined according to AOAC (1997) method. Gross energy was measured by direct combustion in an adiabatic bomb calorimeter. Prior to chemical analysis, fillet samples were freeze dried in triplicate group for 48 h. Lipid from feed and freeze dried fillet were extracted with a chloroform: methanol (2:1 v:v) mixture (Folch et al., 1957), saponated by KOH (Ibeas et al., 1994) and transesterified with methanolic boron triflouride according to methods in AOAC (1997). Fatty acid methyl ester (FAME) were then separated and quantified on a fused silica capillary column (Supelco SP-2330: 30 m × 0.25 mm, film thickness 0.20 µM) in a gas chromatograph (Agilent 7890N) equipped with a split/splitless injector and flame ionization detector. The carrier gas was high purity nitrogen with a flow rate of 40 ml/min. The detector temperature was 300°C, while the injector temperature was 250°C.

Table 2. Fatty acid composition (% of total fatty acids) of the formulated diets.

Fatty acid	Dietary lipid (%)			
	5	10	15	20
14:0	1.1	3.1	3.5	4.4
16:0	25.8	22.8	21.2	18.4
16:1	3.5	4.4	5.5	7.0
18:0	6.4	5.6	5.2	4.4
18:1	31.9	26.3	23.3	21.4
18:2*n*-6	19.7	12.0	6.3	3.8
18:3*n*-3	0.9	0.8	0.6	0.5
20:0	0.7	0.1	0.2	0.3
20:1	0.3	3.9	6.3	8.3
20:2*n*-6	-	1.2	0.9	0.7
20:4*n*-6	-	0.2	0.5	0.6
22:0	0.3	0.2	0.2	0.2
22:1	-	3.8	6.9	8.7
20:5*n*-3	4.4	6.3	7.9	8.2
22:5*n*-3	1.1	2.3	2.43	2.9
22:6*n*-3	4.0	6.7	9.0	10.2
Total saturated	34.2	32.1	30.4	27.5
Total monoenes	35.7	38.4	41.9	45.4
Total PUFA *n*-3	10.4	16.0	20.0	21.9
Total PUFA *n*-6	19.7	13.4	7.7	5.1
n-3/*n*-6	0.5	1.2	2.6	4.2

The column temperature was 100°C for 2 min, warmed up to 170°C, held for 2 min and finally increased to 200°C and held for 20 min to facilitate optimal separation. Fatty acids were identified by comparing the relative retention time with those of the reference standards (37 component FAME Mix and menhaden oil, Supelco). Fatty acid compositions of experimental diets are presented in Table 2.

Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA). Mean differences were tested using Duncan's multiple range test. The changing trends of muscle fatty acids content over time were estimated by regression analysis. Significance was accepted at probabilities of 0.05 or less. All statistical analyses were computed using SPSS 15 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Fatty acid composition of test diets is shown in Table 2. The concentrations of arachidonic acid were not different between the treatments, while the concentration of DHA and EPA increased with the increase of dietary lipid level. Muscle crude lipid content and fatty acid profiles of fish fed different dietary lipid levels are presented in Table 3. There were no significant differences ($P>0.05$) in muscle lipid content of fish fed different dietary lipid level over a six-week experiment. The main fatty acids component of *T. tambroides* muscle were myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1*n*-9), linoleic acid (18:2*n*-6), arachidic acid (20:0), eicosenoic acid (20:1*n*-9), arachidonic acid (20:4*n*-6), EPA, docosapentaenoic acid (22:5*n*-3) and DHA. The 16:0 and 18:1*n*-9 were the primary saturated and monounsaturated fatty acids, while 18:2*n*-6 and DHA were the most abundant *n*-6 and *n*-3 PUFA, respectively. After two weeks, there were a significant increase ($P<0.05$) of muscle monounsaturated fatty acid (MUFA) and significant reduction ($P<0.05$) of muscle saturated fatty acid (SFA) contents for all the fish. The polynomial trends of changes over time for MUFA content of fish fed different dietary lipid level are shown in Figure 1. After two weeks of feeding, muscle *n*-3 PUFA content of fish significantly decreased ($P<0.05$) (Table 4). However, no significant correlation ($P>0.05$) between the changing trends and time was found.

In terms of individual fatty acid, the muscle 18:1*n*-9 content increased from initial values of 23.8, 22.8, 23.4 and 23.6% to the final amounts of 32.3, 31.0, 31.6 and 30.8% in fish fed 5, 10, 15 and 20% dietary lipid, respectively (Table 3). However, the major elevation occurred during two weeks (Table 4 and Figure 2). Moreover, all the experimental diets significantly reduced ($P<0.05$) muscle 20:0 and 20:4*n*-6 percentage. The interaction between dietary lipid and time had significant impact ($P<0.05$) on 18:2*n*-6, 20:1*n*-9 and 22:1*n*-11 contents of *T. tambroides* muscle. The changes over time trends for muscle 18:2*n*-6 contents of fish fed different dietary lipid levels are shown

Table 3. Muscle fatty acid composition (% of total fatty acid) and total crude lipid content (% of muscle wet weight) of *T. tambroides* fed different dietary lipid content over a six-week duration.

FA	5% dietary lipid				10% dietary lipid				Statistical analysis		
	I	W2	W4	W6	I	W2	W4	W6	*T*	*L*	*T*× *L*
14:0	3.4 ± 0.2	3.7 ± 0.3	3.5 ± 0.1	3.4 ± 0.1	3.2 ± 0.2	3.4 ± 0.3	3.1 ± 0.5	3.7 ± 0.1	*ns*	*	*ns*
16:0	25.9 ± 1.0	28.2 ± 0.7	26.5 ± 0.3	26.9 ± 0.1	26.1 ± 1.2	26.3 ± 0.7	24.6 ± 1.2	27.1 ± 0.1	*	***	*ns*
16:1	4.9 ± 0.4	5.9 ± 0.3	5.1 ± 0.2	4.7 ± 0.5	4.9 ± 0.4	5.4 ± 0.3	4.7 ± 0.4	6.0 ± 0.3	**	*	*ns*
18:0	10.8 ± 0.4	9.2 ± 0.4	8.5 ± 0.4	9.6 ± 0.3	10.3 ± 0.5	8.9 ± 0.3	8.5 ± 0.4	8.8 ± 0.4	***	*ns*	*ns*
18:1	23.8 ± 1.0	30.4 ± 1.0	30.9 ± 1.3	32.3 ± 1.3	22.8 ± 0.6	28.6 ± 0.8	28.5 ± 1.1	31.0 ± 0.6	***	*	*ns*
18:2*n*-6	7.6 ± 0.2	7.9 ± 0.4	8.0 ± 0.2	8.6 ± 0.2	8.3 ± 0.4	8.4 ± 0.1	8.7 ± 0.3	7.4 ± 0.4	**	***	**
18:3*n*-3	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	**	*ns*	*ns*
20:0	2.6 ± 0.0	1.2 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	2.0 ± 0.3	1.3 ± 0.3	0.6 ± 0.0	0.6 ± 0.0	***	*ns*	*ns*
20:1	1.8 ± 0.5	1.5 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	2.4 ± 0.2	2.2 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	*	***	***
20:3	0.8 ± 0.2	0.8 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	0.6 ± 0.3	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	*	***	*ns*
20:4*n*-6	5.8 ± 0.2	2.4 ± 0.4	3.1 ± 0.1	2.7 ± 0.4	6.4 ± 0.5	2.3 ± 0.3	2.8 ± 0.4	2.1 ± 0.2	***	*ns*	*ns*
22:1	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	-	0.8 ± 0.1	0.8 ± 0.0	1.1 ± 0.1	0.7 ± 0.1	***	**	**
24:1	0.1 ± 0.0	0.1 ± 0.0	-	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.3 ± 0.2	-	*ns*	*ns*	*ns*
20:5*n*-3	3.1 ± 0.5	1.8 ± 0.1	1.5 ± 0.2	1.2 ± 0.1	3.5 ± 0.3	2.6 ± 0.1	2.4 ± 0.3	1.7 ± 0.1	**	*ns*	*ns*
22:5*n*-3	1.4 ± 0.3	1.1 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.3	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	*ns*	*ns*	*ns*
22:6*n*-3	6.5 ± 0.7	4.8 ± 0.5	6.0 ± 0.8	4.9 ± 0.6	6.3 ± 0.5	6.3 ± 0.5	8.1 ± 1.5	5.6 ± 0.6	*	**	*ns*
SFA	43.0 ± 1.3	42.6 ± 1.2	39.9 ± 0.5	40.6 ± 0.3	41.9 ± 1.3	40.4 ± 0.8	37.3 ± 1.3	40.4 ± 0.2	***	***	*ns*
MUFA	31.3 ± 0.7	38.2 ± 1.4	38.2 ± 1.6	39.2 ± 1.5	31.1 ± 0.6	37.4 ± 0.6	37.4 ± 1.4	40.3 ± 0.8	***	**	*ns*
PUFA *n*-3	11.2 ± 1.2	7.9 ± 0.6	9.0 ± 1.0	7.7 ± 0.8	11.4 ± 0.1	10.5 ± 0.7	12.2 ± 1.9	9.0 ± 0.5	**	**	*ns*
PUFA *n*-6	14.5 ± 0.5	11.3 ± 0.8	12.7 ± 0.4	12.5 ± 0.5	15.5 ± 0.5	11.7 ± 0.3	12.8 ± 0.8	10.2 ± 0.6	***	***	*
n-3/*n*-6	0.78 ± 0.97	0.74 ± 0.96	0.73 ± 0.98	0.63 ± 0.04	0.73 ± 0.08	0.89 ± 0.04	0.94 ± 0.09	0.88 ± 0.04	*ns*	***	*ns*
Crude lipid	5.2 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	5.0 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	*ns*	*ns*	*ns*

FA	15% dietary lipid				20% dietary lipid				Statistical analysis		
	I	W2	W4	W6	I	W2	W4	W6	*T*	*L*	*T*× *L*
14:0	3.6 ± 0.1	4.2 ± 0.2	4.0 ± 0.1	3.5 ± 0.3	3.2 ± 0.1	3.7 ± 0.3	2.7 ± 0.5	3.4 ± 0.1	*ns*	*	*ns*
16:0	26.6 ± 0.7	26.0 ± 0.4	25.2 ± 0.8	24.9 ± 0.4	25.3 ± 0.4	24.6 ± 0.3	22.8 ± 0.6	22.5 ± 0.8	*	***	*ns*
16:1	4.4 ± 0.3	6.5 ± 0.4	6.3 ± 0.2	6.6 ± 0.2	5.1 ± 0.3	6.2 ± 0.4	4.9 ± 0.4	6.3 ± 0.3	**	*	*ns*
18:0	10.4 ± 0.2	8.2 ± 0.4	8.0 ± 0.4	8.5 ± 0.5	11.0 ± 0.2	8.4 ± 0.4	9.1 ± 0.5	8.4 ± 0.3	***	*ns*	*ns*
18:1	23.4 ± 0.4	29.5 ± 0.8	30.1 ± 0.5	31.6 ± 0.4	23.6 ± 0.9	27.9 ± 1.2	26.7 ± 1.0	30.8 ± 1.2	***	*	*ns*
18:2*n*-6	7.7 ± 0.2	7.1 ± 0.1	6.8 ± 0.3	6.0 ± 0.1	7.8 ± 0.3	7.0 ± 0.2	7.1 ± 0.3	6.7 ± 0.1	**	***	**
18:3*n*-3	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.3 ± 0.1	**	*ns*	*ns*
20:0	2.6 ± 0.2	1.5 ± 0.1	1.1 ± 0.3	0.8 ± 0.1	2.3 ± 0.4	1.5 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	***	*ns*	*ns*
20:1	1.9 ± 0.5	3.6 ± 0.2	4.2 ± 0.4	4.3 ± 0.3	1.7 ± 0.3	4.1 ± 0.4	3.7 ± 0.4	4.9 ± 0.2	*	***	***
20:3	0.7 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.7 ± 0.1	0.4 ± 0.1	*	***	*ns*
20:4*n*-6	5.6 ± 0.3	1.9 ± 0.1	2.1 ± 0.1	2.6 ± 0.3	6.0 ± 0.7	2.2 ± 0.4	4.0 ± 0.9	2.4 ± 0.2	***	*ns*	*ns*
22:1	0.4 ± 0.1	1.8 ± 0.2	2.0 ± 0.3	1.8 ± 0.3	0.5 ± 0.1	2.3 ± 0.2	1.6 ± 0.8	2.5 ± 0.2	***	**	**
24:1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	-	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	*ns*	*ns*	*ns*
20:5*n*-3	3.3 ± 0.3	2.1 ± 0.2	1.8 ± 0.2	1.7 ± 0.1	3.4 ± 0.2	2.9 ± 0.4	3.0 ± 0.3	1.9 ± 0.3	**	*ns*	*ns*
22:5*n*-3	1.6 ± 0.3	1.2 ± 0.1	1.1 ± 0.0	1.3 ± 0.1	1.6 ± 0.2	1.4 ± 0.2	1.9 ± 0.3	1.7 ± 0.2	*ns*	*ns*	*ns*
22:6*n*-3	7.1 ± 0.4	5.0 ± 0.5	5.1 ± 0.1	5.4 ± 0.2	7.0 ± 0.4	6.7 ± 1.2	9.5 ± 1.1	6.4 ± 0.7	*	**	*ns*
SFA	43.0 ± 1.3	40.2 ± 0.6	38.6 ± 1.2	37.7 ± 0.5	41.4 ± 0.3	38.4 ± 0.8	35.9 ± 0.6	35.4 ± 1.0	***	***	*ns*
MUFA	31.3 ± 0.7	41.7 ± 1.5	42.9 ± 1.3	44.3 ± 1.1	31.6 ± 0.3	40.7 ± 2.2	37.1 ± 2.3	44.6 ± 1.5	***	**	*ns*
PUFA *n*-3	11.2 ± 1.2	8.4 ± 0.7	8.3 ± 0.2	8.7 ± 0.3	12.2 ± 0.4	11.0 ± 1.7	14.5 ± 1.6	10.3 ± 0.9	**	**	*ns*
PUFA *n*-6	14.5 ± 0.5	9.8 ± 0.2	9.9 ± 0.1	9.1 ± 0.4	14.7 ± 0.9	9.9 ± 0.6	12.1 ± 1.2	9.6 ± 0.1	***	***	*

Table 3. Contd.

n-3/*n*-6	0.80 ± 0.10	0.87 ± 0.06	0.83 ± 0.03	0.95 ± 0.01	0.83 ± 0.06	1.10 ± 0.13	1.19 ± 0.04	1.07 ± 0.07	*ns*	***	*ns*
Crude lipid	5.0 ± 0.0	4.8 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	5.0 ± 0.1	4.7 ± 0.0	4.7 ± 0.1	4.6 ± 0.0	*ns*	*ns*	*ns*

Mean ± SE (*n*=3); I, initial amounts; W2, week 2; W4, week 4; W6, week 6; T, time; L, dietary lipid percentage; ns, non-significant; *, $P<0.05$; **, $P<0.01$, ***$P<0.001$.

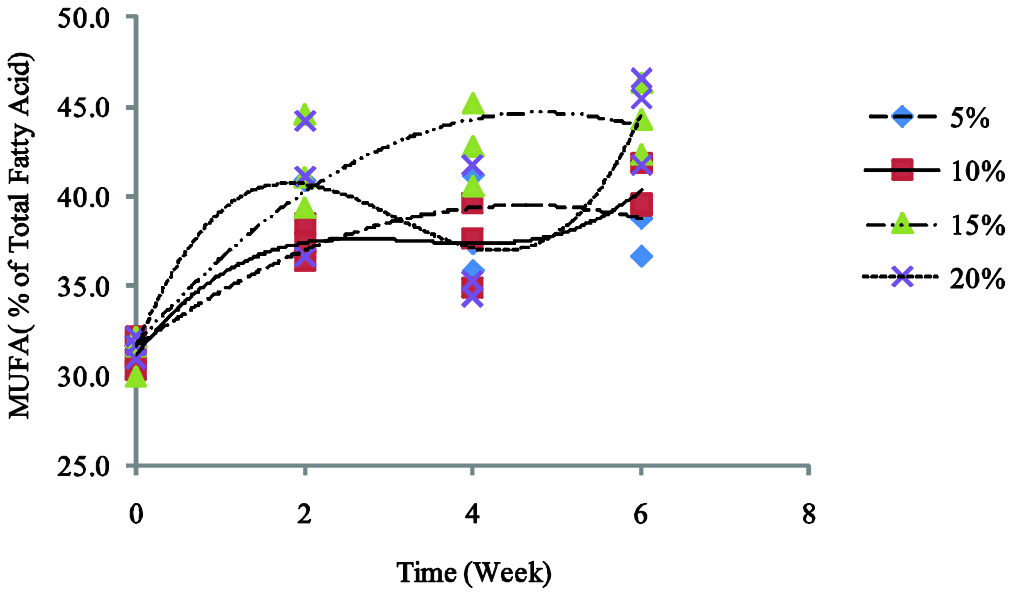

Figure 1. Polynomial relation of fillet total monounsaturated fatty acid (MUFA) content of *T. tambroides* fed different dietary lipid percentage and time. 5% Dietary lipid: $y = -0.3677x^2 + 3.3854x + 31.696$; $R^2 = 0.676$; 10% dietary lipid: $y = 0.1938x^3 - 1.95x^2 + 6.2481x + 31.167$; $R^2 = 0.878$; 15% dietary lipid: $y = -0.5577x^2 + 5.3611x + 31.777$; $R^2 = 0.861$; 20% dietary lipid: $y = 0.4915x^3 - 4.5221x^2 + 11.595x + 31.633$; $R^2 = 0.784$.

Table 4. Mean comparison of muscle fatty acid composition (% of total fatty acid) of *T. tambroides* over a six-week duration.

Fatty acid	Time (week)			
	Initial	W2	W4	W6
14:0	3.35	3.76	3.30	3.52
16:0	25.97^{a}	26.27^{a}	24.78^{b}	25.34^{ab}
16:1	4.83^{a}	6.00^{b}	5.24^{a}	5.90^{b}
18:0	10.63^{a}	8.65^{b}	8.52^{b}	8.80^{b}
18:1*n*-9	23.40^{a}	29.10^{b}	29.05^{b}	31.41^{c}
18:3*n*-3	0.18^{a}	0.09^{b}	0.17^{a}	0.25^{a}
20:0	2.38^{a}	1.38^{b}	0.88^{c}	0.80^{c}
20:3	0.73^{ab}	0.61^{a}	0.90^{b}	0.72^{ab}
20:4*n*-6	5.94^{a}	2.18^{c}	3.00^{b}	2.46^{c}
24:1	0.10	0.10	0.14	0.11
20:5*n*-3	3.33^{a}	2.34^{b}	2.18^{b}	1.66^{c}
22:5*n*-3	1.50	1.32	1.51	1.48
22:6*n*-3	6.73^{ab}	5.69^{a}	7.17^{b}	5.56^{a}
SFA	42.32^{a}	40.40^{b}	37.95^{c}	38.52^{c}
MUFA	31.35^{a}	39.49^{b}	38.88^{b}	42.10^{c}
PUFA *n*-3	11.51^{a}	9.44^{b}	11.03^{a}	8.95^{b}
n-3/n-6	0.79	0.88	0.92	0.88

n=12; Values within the same row with different superscript are significantly different at $P<0.05$.

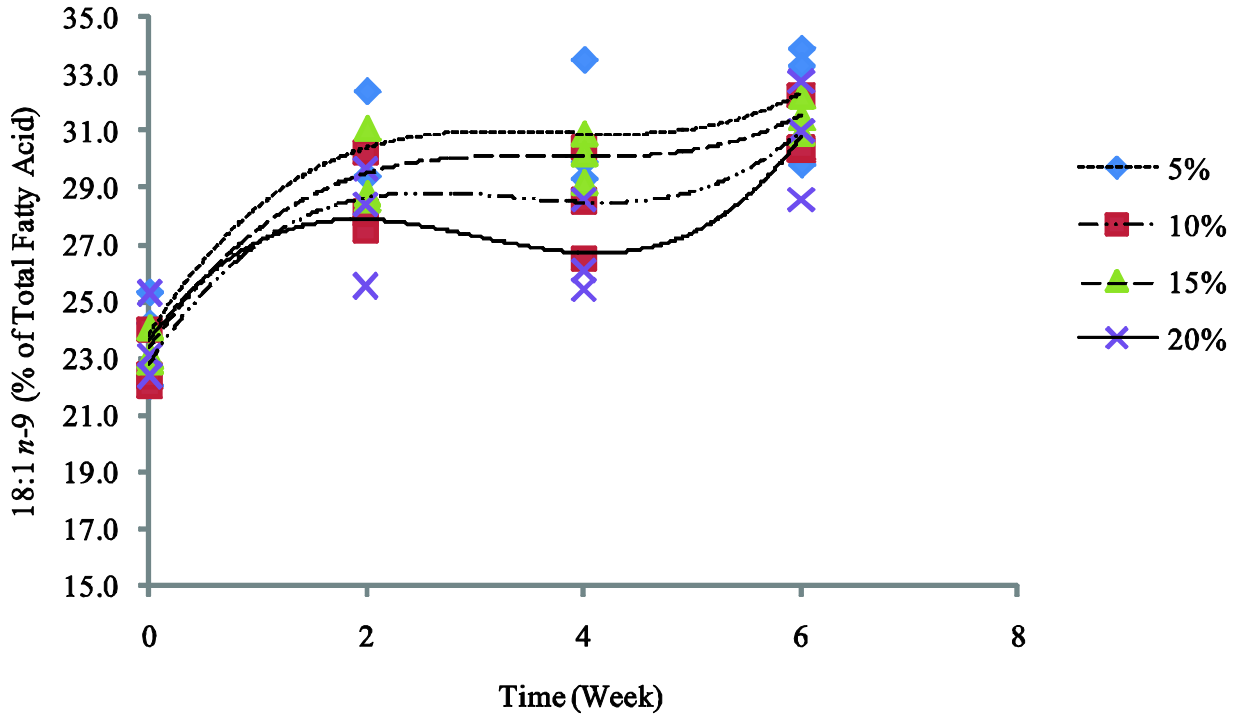

Figure 2. Polynomial relation of fillet 18:1*n*-9 (OA) content of *T. tambroides* fed different dietary lipid percentage and time. 5% Dietary lipid: $y=0.1458x^3-1.6333x^2+5.9667x+23.833$; $R^2=0.802$; 10% dietary lipid: $y = 0.1801x^3 - 1.83x^2 + 5.8594x + 22.8$; $R^2 = 0.870$; 15% dietary lipid: $y = 0.1331x^3 - 1.49x^2 + 5.5144x + 23.367$; $R^2 = 0.944$; 20% dietary lipid: $y = 0.2226x^3 - 2.0154x^2 + 5.2756x + 23.6$; $R^2 = 0.7405$.

in Figure 3. However, no significant correlation (P>0.05) between the muscle 18:2*n*-6 content and time was observed. Regression analyses showed significant polynomial correlations (P<0.05) between muscle 20:1*n*-9 and 22:1*n*-11 and time (Figures 4 and 5). After two weeks, the 20:1*n*-9 and 22:1*n*-11 contents of muscle were generally higher in fish fed 15 and 20% dietary lipid than those fed 5 and 10% lipid. At the end of the experiment, muscle EPA content of fish fed different dietary lipid level decreased to 1.2 to 1.9% of the initial amounts of 3.1 to 3.5%. The polynomial trends of muscle EPA reduction over time are shown in Figure 6. The DHA levels in the muscle of fish after four weeks were significantly higher (P<0.05) than those obtained after the second week. However, the amounts of this fatty acid in the muscle of fish after six weeks were not significantly different (P>0.05) from the initial amounts (Table 4). The muscle DHA content significantly increased (P<0.05) with the increase of dietary lipid percentage (Table 5).

DISCUSSION

T. tambroides with a muscle crude lipid of 4.6 to 5.2% should be classified as a semi-fatty fish since fatty fish usually contain a minimum of 5 to 8% lipid in their edible tissue (Özogul and Özogul, 2007). Major fatty acid contents of freshwater species are 14:0, 16:0, 16:1, 17:0, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:3n-6, EPA and DHA (Özogul et al., 2007). Different ratios of these fatty acids except for 17:0 were detected in *T. tambroides* muscle. The 20:1n-9 and 22:1n-11 found in the muscle of *T. tambroides* should be associated with the high amount of these fatty acids in the diets.

In this study, combined increase of muscle 18:1n-9, 20:1n-9 and 22:1n-11 was responsible for the increase of muscle MUFA content of fish fed different diets. High concentrations of 20:1n-9 and 22:1n-11 in the diets with 15 and 20% lipid, resulting from high inclusion of fish oil, led to notable increase of muscle MUFA content of fish fed these diets after two weeks of feeding. However, the lower amounts of 20:1n-9 and 22:1n-11 in fish muscle compared to the diets may show fish preference to utilize these fatty acids as the source of energy. In agreement with earlier study that showed a rapid accumulation of 18:1n-9 in salmonid muscle within two weeks (Skonberg et al., 1994), this study also indicated that accumulation of 18:1n-9 in *T. tambroides* muscle was fast. Despite a wide range of 18:1n-9 content from 21.4 to 31.9% in the diets, a relatively uniform level of muscle 18:1n-9 from 27.9 to 30.4% after two weeks suggested that this fatty acid was selectively retained by *T. tambroides*. The selective retention of 18:1n-9 by yellow catfish, *Pelteobagrus fulvidraco*, was previously reported (Tan et al., 2009).

The high percentage of long chain n-3 PUFA in diets 15

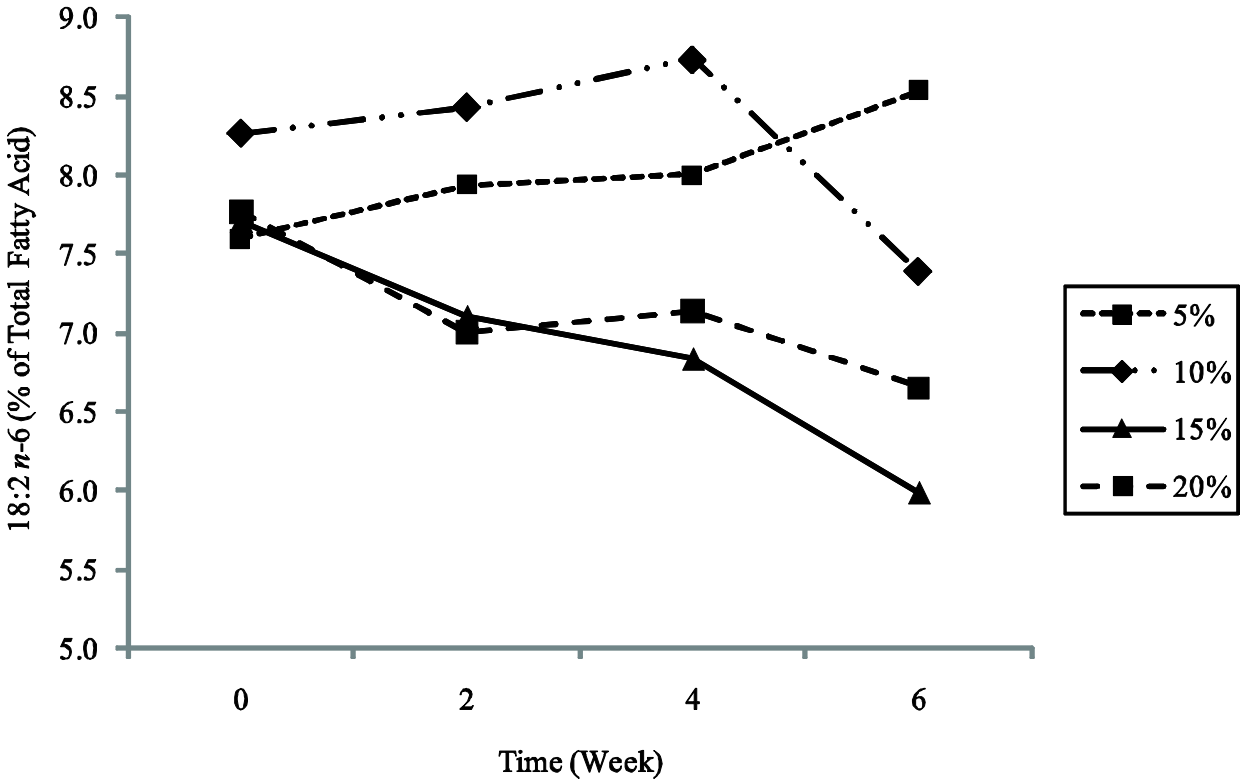

Figure 3. The changing trends of 18:2*n*-6 (LA) content in the fillet of fish fed different dietary lipid percentage over a six-week time.

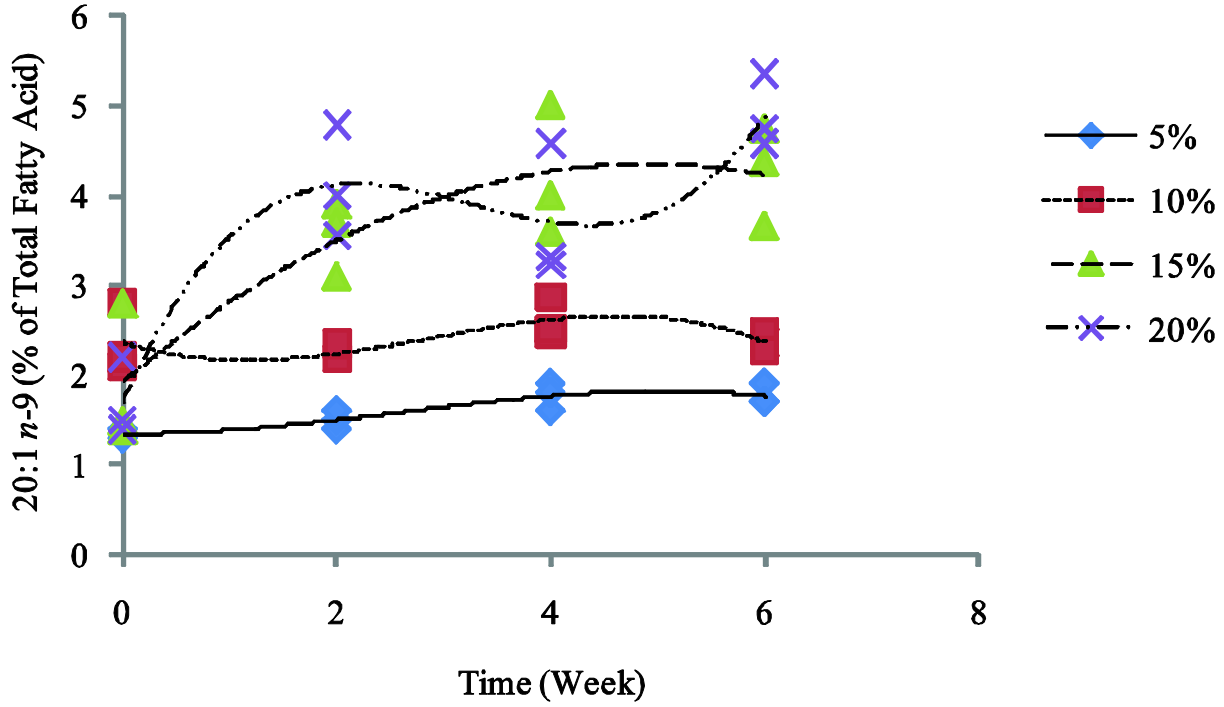

Figure 4. Polynomial relation of fillet 20:1*n*-9 content of *T. tambroides* fed different dietary lipid percentage and time. 5% Dietary lipid: $y = -0.008x^3 + 0.0625x^2 - 0.0181x + 1.35$; R^2=0.764; 10% dietary lipid: $y = -0.019x^3 + 0.15x^2 - 0.1789x + 2.15$; $R^2 = 0.701$; 15% dietary lipid: $y = -0.1006x^2 + 0.9889x + 1.9228$; $R^2 = 0.770$; 20% dietary lipid: $y = 0.0923x^3 - 0.9075x^2 + 2.6542x + 1.7$; $R^2 = 0.863$.

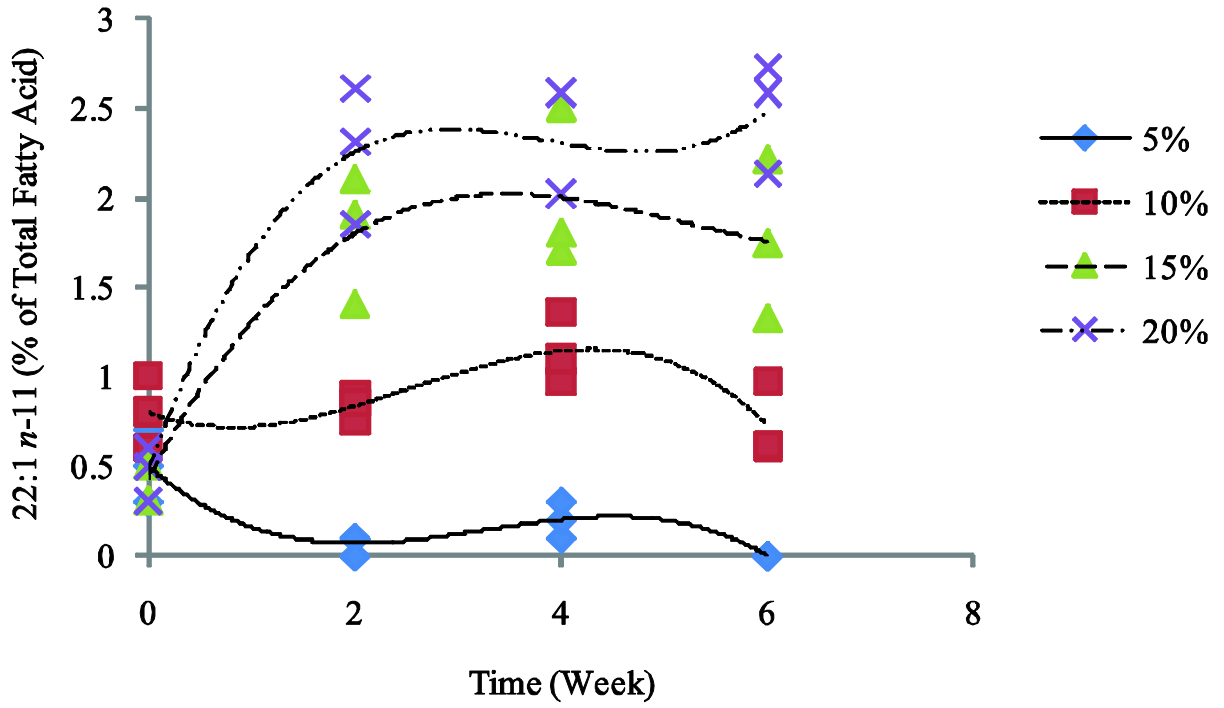

Figure 5. Polynomial relation of fillet 22:1*n*-11 content of *T. tambroides* fed different dietary lipid percentage and time. 5% Dietary lipid: $y = -0.0188x^3 + 0.1833x^2 - 0.5083x + 0.5$; R^2=0.806; 10% dietary lipid: $y = -0.0208x^3 + 0.1592x^2 - 0.2186x + 0.8$; $R^2 = 0.540$; 15% dietary lipid: $y = 0.0151x^3 - 0.2363x^2 + 1.0956x + 0.4333$; $R^2 = 0.813$; 20% dietary lipid: $y = 0.039x^3 - 0.4517x^2 + 1.6424x + 0.4667$; $R^2 = 0.918$.

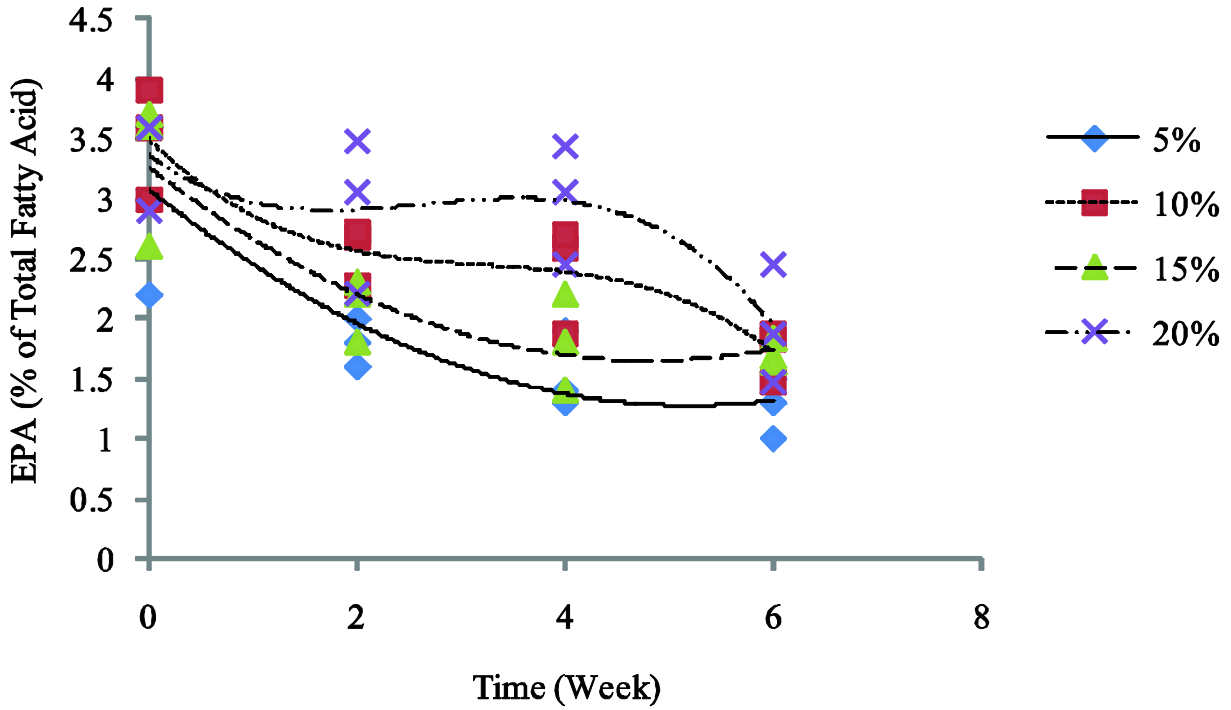

Figure 6. Polynomial relation of fillet 20:5*n*-3 (EPA) content of *T. tambroides* fed different dietary lipid percentage and time. 5% Dietary lipid: $y = 0.0667x^2 - 0.6933x + 3.08$; R^2=0.761; 10% dietary lipid: $y = -0.0263x^3 + 0.2529x^2 - 0.8692x + 3.5$; $R^2 = 0.822$; 15% dietary lipid: $y = 0.0696x^2 - 0.6705x + 3.2657$; $R^2 = 0.787$; 20% dietary lipid: $y = -0.0342x^3 + 0.2713x^2 - 0.6339x + 3.3667$; $R^2 = 0.612$.

Table 5. Mean comparison of muscle fatty acid composition (% of total fatty acid) of *T. tambroides* fed different dietary lipid percentage.

Fatty acid	Dietary lipid (%)			
	5	10	15	20
14:0	3.51^{ab}	3.35^{a}	3.82^{b}	3.25^{a}
16:0	26.87^{a}	26.02^{ab}	25.68^{b}	23.78^{c}
16:1	5.14^{a}	5.26^{a}	5.96^{b}	5.61^{ab}
18:0	9.51	9.11	8.78	9.20
18:1*n*-9	29.37^{a}	27.73^{bc}	28.63^{ab}	27.24^{c}
18:3*n*-3	0.19	0.13	0.20	0.17
20:0	1.34	1.16	1.49	1.46
20:3	1.08^{a}	0.71^{b}	0.62^{b}	0.55^{b}
20:4*n*-6	3.49	3.41	3.03	3.66
24:1	0.09	0.16	0.12	0.09
20:5*n*-3	1.94	2.54	2.22	2.80
22:5*n*-3	1.30	1.57	1.29	1.65
22:6*n*-3	5.54^{a}	6.57^{ab}	5.64^{a}	7.41^{b}
SFA	41.53^{a}	39.98^{b}	39.88^{b}	37.79^{c}
MUFA	36.70^{a}	36.57^{a}	40.05^{b}	38.50^{ab}
PUFA *n*-3	8.95^{a}	10.82^{b}	9.16^{a}	12.00^{b}
n-3/*n*-6	0.70^{a}	0.87^{b}	0.86^{b}	1.05^{c}

n=12; Values within the same row with different superscript are significantly different at *P*<0.05.

and 20% lipid did not increase muscle n-3 PUFA content. It may be speculated that the long chain n-3 PUFA content of *T. tambroides* muscle was determined by desaturation and elongation of shorter chain fatty acids rather than direct absorption from the diet. Fresh water species are able to elongate and desaturate de novo C_{18} to longer chain PUFAs (Cahu et al., 2004; Turchini et al., 2006). However, this capability highly depends on the water temperature (Kayama et al., 1986; Tocher et al., 2004). Farkas (1984) showed that high water temperature reduces the level of PUFA in carp tissues. Accordingly, reduction of total n-3 PUFA observed in fish fed all dietary lipid levels in this study after two weeks may be associated with the effect of temperature on the PUFA de novo synthesis. The water temperature (28.23 ± 0.68) of experimental aquaria was about 10°C higher than water from where the fish were caught (about 18°C). Moreover, reduction of long chain n-3 PUFA in fish tissues, particularly the EPA content, has been considered as a sign of essential fatty acid deficiency (Watanabe, 1982). Therefore, it is suggested that a diet containing high level of n-3 PUFA with low amounts of other fatty acid classes cannot meet all the essential fatty acid requirements of *T. tambroides*.

Although, notable reduction of 20:4n-6 after two weeks trial can be explained by the retarded PUFA de novo synthesizing, it may also be related to the permanent competition between the n-3 and n-6 families for using the elongase and desaturase enzymes (Jankowska et al., 2010). Desaturases, Δ6 and Δ5, more readily utilize the n-3 than n-6 PUFA (Turchini et al., 2006). Therefore, the 20:4n-6 amounts usually determined by feed inclusion instead of bioconversion of 18:2n-6 (Jankowska et al., 2010). In this study, the muscle 20:4n-6 contents of fish fed diet containing 5% lipid (with higher amounts of 18:2n-6 than the other diets) also reduced after two weeks. The similar 20:4n-6 content of fish fed different diets supported that the content of this fatty acid is determined by feed inclusion rather than bioconversion of 18:2n-6. Low level of 20:4n-6 in the fish muscle is important for good cardiovascular health of human consumer because of antagonistic effect of 20:4n-6 to health benefit of the n-3 PUFA (Özogul and Özogul, 2007).

Tropical fruits fallen from the trees grown along the riverbanks are one of the major foods incorporated *into T. tambroides* diets (Siraj et al., 2007). Some of these fruits contain relatively high amount of lipid with moderate to high level of SFA. Therefore, the initial amount of muscle SFA was higher than the experimental diets fed fish. Alasalvar et al. (2002) reported that wild fish contain relatively higher amounts of SFA than farmed fish. Zenebe et al. (1998) also expressed that tropical fish contain higher SFA level than temperate species. The lower amount of SFA in the experimental diets decreased the level of muscle SFA after two to four weeks of feeding.

The impact of experimental diets on the muscle SFA, MUFA and n-3 PUFA were detectable after two weeks of feeding. Moreover, the major fatty acid alterations occurred within two weeks except for muscle EPA, which was continuously reduced by end of the experiment.

Jobling (2004) expressed that the effect of diet on tissue fatty acid is perceptible within 2 to 6 weeks. The result of this study suggested that reduction of EPA and DHA in farmed freshwater fish is not always as a result of the lack of these fatty acids in the commercial diets. Despite using fish oil as the major source of the oil in this study, the n-3 PUFA content of fish muscle reduced compared to its initial amounts. Therefore, it can be concluded that a diet containing high level of long chain n-3 PUFA cannot meet all the essential fatty acid requirements of *T. tambroides*. Moreover, a diet with a balanced ratio of different fatty acid classes as well as appropriate environmental conditions, water temperature in particular, have vital roles in the retention of muscle n-3 PUFA content of freshwater fish for the final human consumer.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science, Technology and Innovation of Malaysia through an E-Science Grant no. 05-01-04-SF0209.

REFERENCES

Akpinar MA, Görgün S, Akpinar AE (2009). A comparative analysis of the fatty acid profiles in the liver and muscles of male and female *Salmo trutta macrostigma*. Food Chem. 112(1): 6-8.

Alasalvar C, Taylor KDA, Zubcov E, Shahidi F, Alexis M (2002). Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. Food Chem. 79(2): 145-150.

AOAC (1997). Official Methods of Analysis, 16th Ed. AOAC International. Maryland, USA.

Aslan S, Guven K, Gezgin T, Alpaslan M, Tekinay A (2007). Comparison of fatty acid contents of wild and cultured rainbow trout *Onchorhynchus mykiss* in Turkey. Fish. Sci. 73(5): 1195-1198.

Cahu C, Salen P, De Lorgeril M (2004). Farmed and wild fish in the prevention of cardiovascular diseases: Assessing possible differences in lipid nutritional values. Nutr. Metab. Cardiovasc. Dis. 14(1): 34-41.

Dinesh K, Nandeesha MC (2007). Status of mahseers, the king of freshwater systems in india - A review. In: Siraj SS, Christianus A, Ng CK and De Silva SS (eds.), Proceeding of the international symposium on the mahseer Kuala Lumpur: Malaysian Fish. Soc. pp. 3-35.

Dos Santos J, Burkow IC, Jobling M (1993). Patterns of growth and lipid deposition in Cod (*Gadus morhua* L.) fed natural prey and fish based feeds. Aquaculture, 110(2): 173-189.

Farkas T (1984). Adaptation of fatty acid composition to temperature. a study on carp (*Cyprinus carpio* L.) liver slices. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 79(4): 531-535.

Folch J, Lees M, Stanley GHS (1957). A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226(1): 497-509.

Ibeas C, Izquierdo MS, Lorenzo A (1994). Effect of different levels of *n*–3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). Aquaculture, 127(2-3): 177-188.

Ingram BA, Sungan S, Tinggi D, Sim SY, Gooley GJ, De Silva SS (2007). Observation on the growth of cage and pond reared *Tor tambroides* and *T. douronensis* in sarawak, malaysia. In: Siraj SS, Christianus A, Ng CK, De Silva SS (eds.), Proceeding of the international symposium on the mahseer Kuala Lumpur: Malaysian Fish. Soc. pp. 145-159.

Jankowska B, Zakęś Z, Żmijewski T, Szczepkowski M (2010). Fatty acid profile of muscles, liver and mesenteric fat in wild and reared perch (*Perca fluviatilis* L.). Food Chem. 118(3): 764-768.

Jobling M (2004) Are modifications in tissue fatty acid profiles following a change in diet the result of dilution?: Test of a simple dilution model. Aquaculture, 232(1-4): 551-562.

Justi KC, Hayashi C, Visentainer JV, De Souza NE, Matsushita M (2003). The influence of feed supply time on the fatty acid profile of nile tilapia (*Oreochromis niloticus*) fed on a diet enriched with *n*-3 fatty acids. Food Chem. 80(4): 489-493.

Kayama M, Hirata M, Hisai T (1986). Effect of water temperature on the desaturation of fatty acids in carp. Bull. Jpn. Soc. Sci. Fish. 52: 853-858.

López LM, Durazo E, Viana MT, Drawbridge M, Bureau DP (2009). Effect of dietary lipid levels on performance, body composition and fatty acid profile of juvenile white seabass, *Atractoscion nobilis*. Aquaculture, 289(1-2): 101-105.

Moreira AB, Visentainer JV, De Souza NE, Matsushita M (2001). Fatty acids profile and cholesterol contents of three Brazilian brycon freshwater fishes. J. Food Comp. Anal. 14(6): 565-574.

Ng WK, Abdullah N, De Silva SS (2008). The dietary protein requirement of the malaysian mahseer, *Tor tambroides* (bleeker), and the lack of protein sparing action by dietary lipid. Aquaculture, 284(1-4): 201-206.

Nguyen TTT, Ingram B, Sungan S, Gooley G, Sim SY, Tinggi D, De Silva SS (2006). Mitochondrial DNA diversity of broodstock of two indigenous mahseer species, *Tor tambroides* and *T. douronensis* (cyprinidae) cultured in Sarawak, Malaysia. Aquaculture, 253(1-4): 259-269.

Özogul Y, Özogul F (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black seas. Food Chem. 100(4): 1634-1638.

Özogul Y, Özogul F, Alagoz S (2007). Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: A comparative study. Food Chem. 103(1): 217-223.

Rahman MA, Mazid MA, Rahman MR, Khan MN, Hossain MA, Hussain MG (2005). Effect of stocking density on survival and growth of critically endangered mahseer, *Tor putitora* (hamilton), in nursery ponds. Aquaculture, 249(1-4): 275-284.

Rasoarahona JRE, Barnathan G, Bianchini J, Gaydou EM (2005). Influence of season on the lipid content and fatty acid profiles of three tilapia species (*Oreochromis niloticus*, *O. macrochir* and *Tilapia rendalli*) from Madagascar. Food Chem. 91(4): 683-694.

Siraj SS, Daud SK, Keong RBP, Ng CK (2007). Characterisation of the Malaysian mahseer (kelah), *Tor tambroides*. In: Siraj SS, Christianus A. Ng CK, De Silva SS (eds.), Proceeding of the international symposium on the mahseer Kuala Lumpur: Malaysian Fish. Soc. pp. 179-201.

Skonberg DI, Rasco BA, Dong FM (1994). Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet. J. Nutr. 124(9): 1628-1638.

Tan X, Luo Z, Xie P, Liu X (2009). Effect of dietary linolenic acid/linoleic acid ratio on growth performance, hepatic fatty acid profiles and intermediary metabolism of juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture, 296(1-2): 96-101.

Tocher DR, Fonseca-Madrigal J, Dick JR, Ng W, Bell JG, Campbell PJ (2004). Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 137(1): 49-63.

Turchini GM, Francis DS, De Silva SS (2006). Fatty acid metabolism in the freshwater fish murray cod (*Maccullochella peelii peelii*) deduced by the whole body fatty acid balance method. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 144(1): 110-118.

Watanabe T (1982). Lipid nutrition in fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 73(1): 3-15.

Zenebe T, Ahlgren G, Boberg M (1998). Fatty acid content of some freshwater fish of commercial importance from tropical lakes in the Ethiopian Rift Valley. J. Fish. Biol. 53(5): 987-1005.