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Riverine Fruit *Dacryodes rostrata* Crude Oil as a Potential Dietary Lipid Source for Malaysian Mahseer, *Tor tambroides*

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ABSTRACT

Riverine fruits, including lipid-rich *Dacryodes rostrata*, are commonly consumed by the omnivorous Malaysian mahseer (*Tor tambroides*) and other tropical riverine cyprinids in their natural habitats. To increase its aquaculture production, feeding the fish with an optimized diet that meets all its nutritional requirements is of crucial importance. This study was performed to investigate the effects of varying levels of *D. rostrata* oil on the growth performance, body composition and fatty acid profile of juvenile Malaysian mahseer. Juveniles (1.81 g ± 0.11) were fed the test diets (0, 1.25, 2.5, 3.75 and 5% *D. rostrata* oil) for 12 weeks in triplicated groups. Crude palm oil (CPO) was used as the control. Fish given 0% *D. rostrata* oil showed the greater growth performance, while juveniles fed 2.5% *D. rostrata* oil demonstrated the highest muscular retention of long-chain polyunsaturated fatty acids (both n-3 and n-6 PUFAs), which have beneficial health effects for human consumers. From the results, it was suggested that *T. tambroides* juveniles be fed with 5% CPO for the grow-out period. A finishing diet containing 2.5% *D. rostrata* oil was suggested for the fish towards the end of its culture period to achieve the highest concentration of long-chain PUFAs (both n-3 and n-6) in the muscle tissue, which is an important criteria for the health of humans as fish consumers.

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INTRODUCTION

Malaysian mahseer (*Tor tambroides* Bleeker) is a riverine cyprinid fish with lots of socio-economic importance in fisheries and conservation. It has also a tremendous potential for freshwater aquaculture (Anon, 2005; Ingram et al., 2005). Similar to other mahseers worldwide, its natural stock has rapidly declined in the last decades (Ingram et al., 2007; Ramezani-Fard et al., 2011). Mahseer catch in Malaysia has dropped by almost 60% from 65,771 kg in 2011 to 28,213 kg in 2015 according to the reports made by Department of Fisheries (Department of Fisheries Malaysia [DOF], 2012, 2013, 2014, 2015, 2016). Since the success of its artificial breeding (Ingram et al., 2005), efforts to culture this species has increased as there is a continuous high demand for this fish. However, its aquaculture production has remained below 22,680 kg a year (DOF, 2012, 2013, 2014, 2015, 2016).

To increase its aquaculture production, feeding the fish with an optimized diet that meets all its nutritional requirements is of crucial importance. Recent studies have shown that *T. tambroides* requires 40-48% dietary protein and 5% dietary lipid (Misieng, Kamarudin, & Musa, 2011; Ng & Andin, 2011; Ramezani-Fard, Kamarudin, Harmin, Saad, & Goh, 2012a). A dietary lipid level of more than 10% suppresses its growth performance and survival (Ramezani-Fard et al., 2012a). Lipids and their constituent fatty acids are the main sources of energy for growth purposes in fish (Tocher, 2003). Fish are the major sources of long-chain polyunsaturated fatty acids of n-3 series (n-3 LC-PUFAs), which are health-beneficial fatty acids for humans as fish consumers (Tocher, 2003). The long-chain polyunsaturated fatty acids with 20 carbons in their chain lengths, especially 20:4n-6 (an n-6 LC-PUFA), have an important role in providing eicosanoids, the cyclic or linear compounds with significant biological activities. Since 18:3n-3 and 18:2n-6 can be converted to n-3 and n-6 LC-PUFAs in freshwater fish and humans, both of them have crucial roles as essential n-3 and n-6 polyunsaturated fatty acids (n-3 and n-6 PUFAs) in their diets (Sargent, Tocher, & Bell, 2002; Tapiero, Nguyen Ba, Couvreur, & Tew, 2002). Therefore, much effort needs to be made to maintain the amounts of these fatty acids in fish tissues. Saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) are considered to spare n-3 LC-PUFAs in fish tissues (Trushenski, Crouse, & Rombenso, 2015; Turchini, De Smet, & Francis, 2011). Better growth and n-3 LC-PUFA maintenance in the tissues of *T. tambroides* have been reported when fed diets containing low n-3 PUFAs and high SFAs compared to when fed diets including high n-3 PUFAs and low SFAs (Kamarudin, Bami, Arshad, Saad, & Ebrahimi, 2018; Ramezani-Fard, Kamarudin, Harmin, & Saad, 2012b).

Riverine fruits, including oily *Dacryodes rostrata*, are commonly eaten by the Malaysian mahseer and other riverine carps in their natural habitats (Siraj, Daud,

Keong, & Ng, 2007; Tan, 1980). *D. rostrata* is an indigenous fruit of Peninsular Malaysia (Hoe & Siong, 1999; Saw, LaFrankie, Kochummen, & Yap, 1991), Brunei (Tinggal, 1992), Borneo, Thailand, Indo-China, the Philippines, the Indonesian islands of Sumatra and Celebes (Ashton, 1995). This fruit is known as kembayau in Sarawak. The fruit is available from October to February. This white or yellow small fruit turns pinkish and then black when ripe (Saw et al., 1991; Tinggal, 1992). Fresh *D. rostrata* contains the lipid levels of 12.5-14.0%, 11.5-21.3% and 7.9-17.8% in its seeds, pulps and peels, respectively (Kong et al., 2011). Its pulp and seed oils contain 42.2% and 59.5% of 16:0 and 18:0, respectively (Ibrahim, Lim, Salimah, & Mariani, 2007; Tee et al., 2014).

Although this riverine fruit is one of the major food components in the natural diet of Malaysian mahseer and other riverine carps (Misieng, Kamarudin, & Saad, 2015; Siraj et al., 2007; Tan, 1980), its specific roles in the nutrition of riverine cyprinids have not been studied. This study was performed to investigate the effects of dietary *D. rostrata* oil inclusion on growth performance, feed efficiency, body composition, and fatty acid profile of muscle and liver in *T. tambroides* juveniles.

METHOD

Diet Preparation

The *D. rostrata* fruits were procured from a local supplier and transferred to the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia. The fruits were first sun-dried for 1 week to prevent molding. The fruits were then peeled, and about 100 g of the dried peeled fruits (the flesh and seed) was ground using a hammer mill with 600 μm mesh screen. The powder was then moisturized by adding distilled water and using a vertical mixer until about 80% moisture content was achieved. After that, the fruit oil was extracted using the Bligh and Dyer (1959) method.

Five isonitrogenous (40% protein) and isocaloric (17.2 kJ/g) diets containing 5% oil with different levels of *D. rostrata* oil were formulated and prepared (Tables 1 and 2). Crude palm oil (CPO) was used as the control (Bami et al., 2017; Kamarudin, Ramezani-Fard, Saad, & Harmin, 2012; Ramezani-Fard et al., 2012b). Table 3 presents the fatty acid compositions of the oils and lipid of fishmeal and soybean meal. A kitchen mixer was used to mix dry ingredients. A homogeneous mixture was produced after adding distilled water and test oils. A single screw laboratory-scale extruder (Brabender KE-19) was used to extrude the moist mixture through a 2-mm die. The pellets were oven-dried at 45 ˚C for 4 h, cooled to the room temperature, bagged and stored with dehumidifying agents until used.

Juvenile Rearing

T. tambroides juveniles (mean ± SD initial body weight = 1.81 ± 0.11 g) were purchased from a local supplier and transferred to the

Table 1
Feed and chemical compositions of the experimental diets fed to juvenile T. tambroides

	Dietary *D. rostrata* oil (%)				
Diet parameter	0	1.25	2.5	3.75	5
	Ingredient (g/100 g as fed basis)				
Fishmeal[1]	35.8	35.8	35.8	35.8	35.8
Soybean meal	37.8	37.8	37.8	37.8	37.8
Tapioca starch	19.4	19.4	19.4	19.4	19.4
D. rostrata oil	0.0	1.2	2.5	3.8	5.0
CPO[2]	5.0	3.8	2.5	1.2	0.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0
Mineral premix[4]	1.0	1.0	1.0	1.0	1.0
	Proximate composition (% as fed basis)				
Crude protein	39.98 ± 1.64	40.03 ± 1.40	39.95 ± 0.93	39.96 ± 1.43	40.02 ± 1.04
Crude lipid	7.84 ± 0.34	7.76 ± 0.26	7.75 ± 0.35	7.76 ± 0.25	7.79 ± 0.34
Ash	11.89 ± 0.26	11.86 ± 0.40	11.85 ± 0.55	11.87 ± 0.34	11.86 ± 0.19
Carbohydrate[5]	28.61 ± 0.81	28.68 ± 1.03	28.95 ± 1.07	28.76 ± 0.90	28.56 ± 1.34
Gross energy (kJ/g)	17.23 ± 0.29	17.22 ± 0.34	17.20 ± 0.08	17.19 ± 0.60	17.18 ± 0.99
Dry matter	88.32 ± 0.88	88.33 ± 0.46	88.50 ± 0.86	88.35 ± 0.65	88.23 ± 0.56

Note. Mean ± SE (n = 3). [1] Malaysian fishmeal (59.6% crude protein). [2] Crude palm oil supplied by Malaysian Palm Oil Board (MPOB). [3] Vitamin premix (g/kg 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. [4] Mineral premix (g/kg premix): KCl, 90; Kl, 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. [5] Carbohydrates = Dry matter – [protein + lipid + ash]

Table 2
Fatty acid compositions (% of total fatty acids) of the experimental diets fed to juvenile T. tambroides

	Dietary *D. rostrata* oil (%)				
	0	1.25	2.5	3.75	5
Fatty acid					
14:0	2.15 ± 0.13	2.07 ± 0.12	1.97 ± 0.12	1.81 ± 0.10	1.72 ± 0.10
16:0	38.70 ± 0.34	35.50 ± 1.35	32.56 ± 1.37	28.93 ± 1.40	26.14 ± 1.36
16:1n-7	2.24 ± 0.13	2.24 ± 0.13	2.30 ± 0.13	2.30 ± 0.13	2.36 ± 0.14
18:0	6.22 ± 0.36	8.19 ± 0.47	9.97 ± 0.57	12.74 ± 0.68	13.94 ± 0.80
18:1n-9	32.80 ± 0.19	34.22 ± 1.97	35.50 ± 2.05	36.59 ± 2.11	38.96 ± 2.24
18:2n-6	10.91 ± 0.63	10.68 ± 0.62	10.26 ± 0.59	9.78 ± 0.57	9.29 ± 0.54
18:3n-3	1.29 ± 0.08	1.34 ± 0.08	1.41 ± 0.09	1.36 ± 0.14	1.31 ± 0.08
20:4n-6	1.06 ± 0.06	1.12 ± 0.06	1.18 ± 0.07	1.28 ± 0.08	1.29 ± 0.08
20:5n-3	1.35 ± 0.08	1.34 ± 0.07	1.43 ± 0.08	1.58 ± 0.09	1.50 ± 0.09
22:5n-3	0.57 ± 0.03	0.63 ± 0.03	0.62 ± 0.02	0.65 ± 0.02	0.67 ± 0.04
22:6n-3	2.71 ± 0.14	2.66 ± 0.16	2.81 ± 0.21	2.99 ± 0.17	2.82 ± 0.17
Σ SFA[1]	47.07 ± 0.11	45.77 ± 1.69	44.50 ± 1.82	43.48 ± 1.97	41.80 ± 2.06
Σ MUFA[2]	35.04 ± 0.32	36.46 ± 2.10	37.79 ± 2.18	38.89 ± 2.25	41.12 ± 2.38
Σ n-3 PUFA[3]	5.92 ± 0.26	5.97 ± 0.27	6.27 ± 0.30	6.58 ± 0.36	6.30 ± 0.29
Σ n-6 PUFA[4]	11.97 ± 0.69	11.80 ± 0.68	11.44 ± 0.66	11.06 ± 0.64	10.58 ± 0.61
n-3/n-6	0.49 ± 0.05	0.51 ± 0.05	0.55 ± 0.06	0.59 ± 0.08	0.60 ± 0.07
PUFA/SFA	0.38 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01

Note. Mean ± SE (n = 3). [1] Saturated fatty acids (Sum of 14:0 + 16:0 + 18:0). [2] Monounsaturated fatty acids (Sum of 16:1n-7 + 18:1n-9). [3] The n-3 series of polyunsaturated fatty acids (Sum of 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3). [4] The n-6 series of polyunsaturated fatty acids (Sum of 18:2n-6 + 20:4n-6)

Table 3
Fatty acid compositions (% of total fatty acids) of different lipid sources

	Type of the lipid source			
Fatty acid	D. rostrata	CPO	Fishmeal[1]	Soybean meal[2]
14:0	0.00	1.53 ± 0.44	4.87 ± 0.06	0.00
16:0	34.00 ± 4.00	43.94 ± 0.32	31.77 ± 0.20	19.51 ± 2.42
16:1n-7	0.00	0.00	5.37 ± 0.25	0.00
18:0	10.79 ± 4.96	4.17 ± 0.56	11.27 ± 0.12	4.69 ± 0.20
18:1n-9	45.63 ± 2.32	39.98 ± 1.18	19.72 ± 0.22	17.07 ± 2.08
18:2n-6	9.36 ± 1.37	10.18 ± 0.21	2.53 ± 0.03	50.85 ± 3.44
18:3n-3	0.23 ± 0.01	0.21 ± 0.06	0.93 ± 0.44	7.90 ± 0.85
20:4n-6	0.00	0.00	3.93 ± 0.13	0.00
20:5n-3	0.00	0.00	5.51 ± 0.02	0.00
22:5n-3	0.00	0.00	2.40 ± 0.05	0.00
22:6n-3	0.00	0.00	11.73 ± 0.16	0.00
Σ SFA[3]	44.79 ± 0.96	49.63 ± 1.33	47.90 ± 0.39	24.19 ± 2.22
Σ MUFA[4]	45.63 ± 2.32	39.98 ± 1.18	25.08 ± 0.04	17.07 ± 2.08
Σ n-3 PUFA[5]	0.23 ± 0.01	0.21 ± 0.06	20.55 ± 0.20	7.90 ± 0.85
Σ n-6 PUFA[6]	9.36 ± 1.37	10.18 ± 0.21	6.47 ± 0.15	50.85 ± 3.44
n-3/n-6	0.02 ± 0.01	0.02 ± 0.01	3.18 ± 0.05	0.16 ± 0.01
PUFA/SFA	0.21 ± 0.03	0.21 ± 0.01	0.57 ± 0.02	2.47 ± 0.40

Note. Mean ± SE (n = 3). [1] 7.9% lipid. [2] 0.6% lipid. [3] Saturated fatty acids (Sum of 14:0 + 16:0 + 18:0). [4] Monounsaturated fatty acids (Sum of 16:1n-7 + 18:1n-9). [5] The n-3 series of polyunsaturated fatty acids (Sum of 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3). [6] The n-6 series of polyunsaturated fatty acids acids (Sum of 18:2n-6 + 20:4n-6)

Wet Laboratory, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia and acclimatized in a 1,000-L fiberglass tank for 2 weeks. Juveniles were given a 32% crude protein commercial tilapia starter feed (StarFeed (M) Sdn. Bhd., Malaysia) during the acclimatization period. Fish were then randomly distributed into 15 glass aquaria (60 L) at 20 fish per aquarium. Each aquarium was supplied with continuous aeration. Water quality was monitored every 3 weeks; a thermometer, a portable pH meter (YSI 60, Yellow Springs Instruments) and a DO meter (YSI DO200, Yellow Springs Instruments) were used for the measurement of water temperature, pH and dissolved oxygen. Water temperature ranged between 27.1-29.4 ˚C, and pH averaged approximately 6.5 and 7.4. The ammonia (NH^{3+}) level was retained below 0.2 mg/L. Dissolved oxygen levels in each aquarium was maintained above 7 mg/L. Fish were fed twice daily (0900 and 1600 h) close to visual satiety during the trial. The feeding trial was conducted for 12 weeks.

Fish Sampling

All 20 juveniles from each aquarium were individually weighed at the start and end of the feeding trial as well as every 3 weeks. Weight gain, specific growth rate (SGR), daily feed intake (DFI) and feed conversion ratio (FCR) were measured and estimated at the end of the experiment.

At the start of feeding trial, 20 fish were also sacrificed following anesthetization by MS222 and individually weighed. About 15 of them were kept frozen at -20 ˚C for subsequent whole body composition analysis, and the muscle and liver tissue of other five juveniles were removed and stored at -80 ˚C for fatty acid analysis. At the end of the experiment, 5 fish per aquarium were also sacrificed, individually weighed and dissected for the hepatosomatic index (HSI) and viscera-somatic index (VSI) estimation. The fish were starved for 24 h to facilitate the liver collection and VSI estimation. The dissected fish were then dressed, and muscle from the area between the lateral and dorsal line was removed for fatty acid analyses (Ahlgren, Blomqvist, Boberg, & Gustafsson, 1994). The liver and muscle samples were stored at -80 ˚C instantly for later fatty acid analyses. Another 15 fish per aquarium were sacrificed, weighed individually and stored at -20 ˚C for subsequent whole body composition analysis.

Biochemical Analysis

Before the biochemical analysis, the whole fish (before and after the experiment) were dried in the oven at 40 ˚C for 48 h, and moisture loss was estimated. After that, the samples were ground into fine powder. Moisture contents of experimental diets were estimated using an infrared moisture balance (A&D, AD-4715). The crude protein, crude lipid and crude fiber of experimental diets and the whole body of fish samples were respectively determined by the Kjeldahl method (Foss Kjeltec™ 8000), Soxhlet extraction (Foss Soxtec™ 8000) and hot extraction (Foss Fibertec™ 2010) according to Association of Official Analytical Chemists (AOAC) methods

(1997). The ash content was estimated by cauterizing the dry sample at 600 ˚C for 4 h, and the gross energy was measured by a direct incineration in an adiabatic bomb calorimeter (Leco AC-350).

Lipid from the fishmeal, soybean meal, feed, liver and muscle was extracted using a chloroform : methanol (2:1, v:v) mixture, saponated by KOH and transesterified with methanolic boron trifluoride according to AOAC methods (1997). After that, separation and quantification of fatty acid methyl esters (FAMEs) were performed using 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. High purity nitrogen was used as the carrier gas with a flow rate of 40 mL/min. The injector temperature was 250˚C, while the detector temperature was 300˚C. The column temperature was programmed at 100˚C for 2 min, warmed up to 170 ˚C at 10 ˚C/min, held for 2 min and then warmed to 220˚C at 7.5 ˚C/min for 20 min to facilitate optimal separation. Fatty acids were identified by comparison of the relative FAME peak retention time with the internal standard, heneicosanoic acid (21:0), obtained from Sigma (St. Louis, MO, USA) and expressed as the area percentage of FAMEs.

Statistical Analysis

All data analyses were performed using one-way analysis of variance (ANOVA). Duncan's multiple range test was conducted if ANOVA indicated there were significant differences among the treatment means. All percentage data were arcsin transformed prior to being analyzed statistically. All analysis was performed using SPSS 18 for Windows (SPSS Inc., Chicago, IL, USA), and the difference was considered significant at $P < 0.05$. Results were reported as mean values ± SE.

RESULTS

Survival, final weight, weight gain, daily feed intake, specific growth rate, feed conversion ratio, viscera-somatic index, hepato-somatic index, protein efficiency ratio and lean percentage of *T. tambroides* fed varying levels of *D. rostrata* oil are shown in Table 4. The dietary *D. rostrata* oil level did not have any significant effects ($P > 0.05$) on the survival of juvenile *T. tambroides*. Juveniles fed 0% *D. rostrata* oil had a significantly higher ($P < 0.05$) growth than those fed 3.75-5% *D. rostrata* oil. Significant differences ($P < 0.05$) were found in FCR, DFI, PER and HSI among the treatments. The highest FCR was observed among fish fed 5% *D.rostrata* oil, while the lowest FCR was found among fish fed 0 and 1.25% *D. rostrata* oil. Fish fed 5% *D. rostrata* oil had the highest DFI and the lowest PER, while those fed 2.5% *D. rostrata* oil had the lowest DFI and the highest PER. Juveniles fed 3.75% *D. rostrata* oil had the highest HSI. VSI was not significantly affected ($P > 0.05$) by various dietary *D. rostrata* oil concentrations.

The *D. rostrata* oil level also did not significantly affect ($P < 0.05$) the body

Table 4
Survival rate, growth performance, feed utilization efficiency and body indices of juvenile T. tambroides *fed varying* D. rostrata *oil levels for 12 weeks*

	Dietary *D. rostrata* oil (%)				
Parameters	0	1.25	2.5	3.75	5
Survival (%)	100.00	100.00	100.00	100.00	100.00
Final Weight (g)	3.98 ± 0.09^{b}	3.76 ± 0.09^{ab}	3.85 ± 0.07^{ab}	3.70 ± 0.09^{a}	3.63 ± 0.05^{a}
Weight Gain (%)	119.89 ± 5.01^{b}	107.55 ± 4.83^{ab}	112.52 ± 3.63^{ab}	104.60 ± 4.78^{a}	100.55 ± 2.55^{a}
SGR[1]	0.87 ± 0.02^{b}	0.81 ± 0.03^{ab}	0.84 ± 0.02^{ab}	0.79 ± 0.02^{a}	0.77 ± 0.01^{a}
FCR[2]	1.89 ± 0.05^{a}	1.98 ± 0.07^{ab}	1.89 ± 0.19^{a}	2.37 ± 0.16^{bc}	2.49 ± 0.08^{c}
DFI[3]	1.57 ± 0.03^{ab}	1.53 ± 0.06^{a}	1.50 ± 0.12^{a}	1.80 ± 0.07^{bc}	1.85 ± 0.07^{c}
PER[4]	1.32 ± 0.04^{c}	1.27 ± 0.04^{bc}	1.35 ± 0.12^{c}	1.07 ± 0.07^{ab}	1.01 ± 0.03^{a}
HSI[5]	2.16 ± 0.14^{ab}	2.14 ± 0.17^{ab}	1.81 ± 0.13^{a}	2.36 ± 0.13^{b}	1.92 ± 0.06^{a}
VSI[6]	7.83 ± 0.23	7.91 ± 0.59	7.65 ± 0.95	8.30 ± 0.20	7.38 ± 0.10
Lean (% wet weight)	60.00 ± 0.64	60.00 ± 0.70	60.00 ± 0.72	60.00 ± 0.17	60.00 ± 0.89

Note. Mean ± SE (n = 3); means within the same row with different superscripts are significantly different at $P < 0.05$. [1] Specific growth rate (%/d) = $\left[\frac{\ln \text{final weight} - \ln \text{initil weight}}{\text{Experimental days}}\right] \times 100$. [2] Feed conversion ratio = $\frac{\text{Total feed consumed (g)}}{\text{Wet weight gain (g)}}$. [3] Daily feed intake (%/d) = $\frac{\text{Total feed consumed}}{[(\text{final weight} + \text{initial weight}) \times (\text{Experimental days}/2)]} \times 100$. [4] Protein efficiency ratio = $\frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}}$. [5] Hepatosomatic index (%) = $\frac{\text{liver weight (g)}}{\text{body weight (g)}} \times 100$. [6] Viscerosomatic index (%) = $\frac{\text{visceral weight (g)}}{\text{body weight (g)}} \times 100$

proximate composition of mahseer except fiber and ash (Table 5). Major components in mahseer body were water (67-68.6%), protein (15.1-16.1%) and lipid (12.5-13.4%). Fish fed 3.75% *D. rostrata* oil had a significantly lower ($P < 0.05$) body ash compared to those fed 1.25-5% *D. rostrata* oil. Fish given 0% *D. rostrata* oil had a significantly higher ($P < 0.05$) body fiber content than fish fed 2.5% *D. rostrata* oil. Nevertheless, the fish body fiber remained extremely low (0.01-0.13%).

Juveniles given 5% *D. rostrata* oil had significantly lower ($P < 0.05$) protein and gross energy retention values than those given 0 or 2.5% *D. rostrata* oil (Table 6). Juveniles fed 5% *D. rostrata* oil had also a significantly lower ($P < 0.05$) lipid retention

Table 5
Whole body proximate composition (% wet weight) of juvenile T. tambroides *fed varying* D. rostrata *oil levels for 12 weeks*

		Dietary kembayau oil (%)				
	Initial	0	1.25	2.5	3.75	5
Dry matter	34.33	33.10 ± 0.40	32.97 ± 0.28	32.60 ± 0.11	32.30 ± 0.36	31.40 ± 0.69
Crude protein	14.49	16.15 ± 0.37	15.49 ± 0.72	15.65 ± 0.10	15.36 ± 0.39	15.06 ± 0.52
Crude lipid	11.12	13.40 ± 0.50	13.02 ± 0.57	12.71 ± 0.28	12.95 ± 0.45	12.53 ± 0.26
Ash	4.16	2.83 ± 0.08^{ab}	2.87 ± 0.04^{b}	2.82 ± 0.05^{ab}	2.54 ± 0.07^{a}	2.98 ± 0.15^{b}
Fiber	0.20	0.13 ± 0.06^{b}	0.05 ± 0.01^{ab}	0.01 ± 0.01^{a}	0.06 ± 0.03^{ab}	0.10 ± 0.00^{ab}
NFE[1]	4.35	0.60 ± 0.31	1.55 ± 0.37	1.41 ± 0.22	1.40 ± 0.39	0.72 ± 0.08
Carbohydrate	4.55	0.73 ± 0.37	1.60 ± 0.38	1.42 ± 0.22	1.46 ± 0.42	0.82 ± 0.08
Gross energy (kJ/g)	11.85	8.68 ± 0.08^{b}	8.18 ± 0.16^{a}	8.36 ± 0.25^{ab}	8.49 ± 0.09^{ab}	8.10 ± 0.05^{a}

Note. Mean ± SE (n = 3); means within the same row with different superscript are significantly different at $P < 0.05$. [1] Nitrogen free extract = Dry matter – (protein + lipid + ash + fiber)

than those given 0-2.5% *D. rostrata* oil. Fish fed 0% *D. rostrata* oil showed a significantly higher ($P < 0.05$) energy retention than those given 1.25, 3.75 or 5% *D. rostrata* oil. The results showed that the *D. rostrata* oil level did not have any significant effects ($P > 0.05$) on carbohydrate retention. However, the total carbohydrate amount in the fish was less than the initial amount after the 12-week feeding.

Table 7 presents the effects of *D. rostrata* oil level on the muscle fatty acid composition of *T. tambroides* juveniles. The most dominant fatty acid in the muscle of *T. tambroides* was 18:1n-9 and followed by 16:0, 18:2n-6, 18:0 and 16:1n-7. However, muscle 18:1n-9, 16:0 and 18:2n-6 were not significantly affected ($P > 0.05$) by the dietary *D. rostrata* oil level. The increased dietary *D. rostrata* oil content significantly

Table 6
Estimated protein, lipid, carbohydrate and energy retention (%) of juvenile T. tambroides *fed varying* D. rostrata *oil levels for 12 weeks*

Retention	Dietary *D. rostrata* oil (%)				
	0	1.25	2.5	3.75	5
Crude protein[1]	27.93 ± 2.74^{c}	22.59 ± 3.26^{abc}	25.58 ± 3.26^{bc}	18.30 ± 2.93^{ab}	15.90 ± 1.64^{a}
Crude lipid[2]	124.05 ± 5.39^{c}	102.81 ± 2.00^{bc}	109.89 ± 9.89^{c}	84.28 ± 9.75^{ab}	72.24 ± 2.25^{a}
Carbohydrate[3]	-5.52 ± 1.18	-2.31 ± 1.31	-2.76 ± 0.85	-2.31 ± 1.09	-4.06 ± 0.18
Gross energy[4]	22.27 ± 1.70^{c}	15.24 ± 2.44^{ab}	18.67 ± 2.81^{bc}	13.77 ± 1.68^{ab}	10.32 ± 0.78^{a}

Mean ± SE (n = 3); means within the same row with different superscripts are significantly different at $P < 0.05$

[1] $\text{Protein retention (\%)} = \frac{[(\%\text{ final body crude protein} \times \text{final body weight}) - (\%\text{ initial body crude protein} \times \text{initial body weight})]}{(\text{food intake} \times \%\text{ diet crude protein})} \times 100$

[2] $\text{Lipid retention (\%)} = \frac{[(\%\text{ final body crude lipid} \times \text{final body weight}) - (\%\text{ initial body crude lipid} \times \text{initial body weight})]}{(\text{food intake} \times \%\text{ diet crude lipid})} \times 100$

[3] $\text{Carbohydrate retention (\%)} = \frac{[(\%\text{ final body carbohydrate} \times \text{final body weight}) - (\%\text{ initial body carbohydrate} \times \text{initial body weight})]}{(\text{food intake} \times \%\text{ diet carbohydrate})} \times 100$

[4] $\text{Gross energy retention (\%)} = \frac{[(\text{final body gross energy} \times \text{final body weight}) - (\text{initial body gross energy} \times \text{initial body weight})]}{(\text{food intake} \times \text{diet gross energy})} \times 100$

increased ($P < 0.05$) the 18:0 percentage in the mahseer muscle. Total muscle SFAs, MUFAs were not significantly affected ($P > 0.05$) by dietary *D. rostrata* oil. Although juveniles fed 0% *D. rostrata* oil contained significantly lower ($P < 0.05$) 20:4n-6 than those fed 2.5 and 5% *D. rostrata* oil, total n-6 PUFA content was not significantly affected ($P > 0.05$) by dietary *D. rostrata* oil. Fish fed 0% *D. rostrata* oil had significantly lower ($P < 0.05$) muscle total n-3 PUFA content than those fed 2.5-5% *D. rostrata* oil. Nevertheless, dietary *D. rostrata* oil level did not significantly affect ($P > 0.05$) the n-3/n-6 ratio in the muscle of mahseers. Muscle 18:2n-6, 20:4n-6, 22:5n-3, 22:6n-3 and total n-6 PUFAs were lower at the end of the trial compared to those at the beginning of the trial. The juveniles had higher SFAs and MUFAs in their muscle following the feeding than their initial amounts.

The liver fatty acid compositions of *T. tambroides* juveniles before and after the feeding are shown in Table 8. The most abundant fatty acid in the fish liver was 18:1n-9 and followed by 16:0, 18:0, 16:1n-7 and 18:2n-6. The liver of fish fed 5% *D. rostrata* oil had a significantly lower ($P < 0.05$) 16:1n-7 and a significantly higher ($P < 0.05$) 18:0 than those fed 0% *D. rostrata* oil.

Table 7

Fatty acid composition (% of total fatty acids) of muscle tissue of juvenile T. tambroides *at the beginning and end of the 12-week experimental period*

		Dietary *D. rostrata* oil (%)				
	Initial	0	1.25	2.5	3.75	5
14:0	2.51	2.98 ± 0.17	3.04 ± 0.17	2.70 ± 0.16	2.93 ± 0.17	2.65 ± 0.16
16:0	26.47	33.09 ± 1.91	32.53 ± 1.88	28.87 ± 1.67	29.87 ± 1.73	27.77 ± 1.61
16:1n-7	3.42	5.56 ± 0.32^{ab}	5.42 ± 0.31^{ab}	4.80 ± 0.28^{a}	5.97 ± 0.35^{b}	5.22 ± 0.30^{ab}
18:0	6.99	6.28 ± 0.36^{a}	7.22 ± 0.42^{ab}	7.97 ± 0.46^{bc}	8.18 ± 0.47^{bc}	9.11 ± 0.53^{c}
18:1n-9	33.71	37.44 ± 2.11	37.75 ± 2.08	39.16 ± 1.99	38.52 ± 1.92	39.61 ± 1.88
18:2n-6	18.76	8.91 ± 0.51	8.06 ± 0.47	9.07 ± 0.53	7.65 ± 0.44	7.95 ± 0.46
18:3n-3	1.30	1.14 ± 0.06^{a}	1.28 ± 0.08^{ab}	1.43 ± 0.08^{b}	1.32 ± 0.08^{ab}	1.52 ± 0.09^{b}
20:4n-6	1.86	0.96 ± 0.06^{a}	1.00 ± 0.06^{a}	1.25 ± 0.08^{b}	1.16 ± 0.07^{ab}	1.31 ± 0.08^{c}
20:5n-3	0.54	0.53 ± 0.03	0.53 ± 0.03	0.60 ± 0.03	0.57 ± 0.03	0.63 ± 0.03
22:5n-3	0.45	0.31 ± 0.02^{a}	0.32 ± 0.02^{ab}	0.40 ± 0.02^{c}	0.38 ± 0.02^{bc}	0.34 ± 0.02^{abc}
22:6n-3	4.00	2.79 ± 0.16^{a}	2.86 ± 0.17^{ab}	3.75 ± 0.22^{c}	3.44 ± 0.20^{bc}	3.87 ± 0.23^{c}
Σ SFA[1]	35.96	42.35 ± 2.10	42.79 ± 2.12	39.54 ± 2.97	40.98 ± 2.03	39.53 ± 1.97
Σ MUFA[2]	37.13	43.00 ± 2.44	43.17 ± 2.39	43.96 ± 2.26	44.49 ± 2.26	44.83 ± 2.18
Σ n-3 PUFA[3]	6.30	4.78 ± 0.24^{a}	4.99 ± 0.25^{ab}	6.19 ± 0.31^{c}	5.72 ± 0.28^{bc}	6.37 ± 0.33^{c}
Σ n-6 PUFA[4]	20.61	9.87 ± 0.57	9.06 ± 0.53	10.31 ± 0.60	8.81 ± 0.51	9.26 ± 0.54
n-3/n-6	0.31	0.49 ± 0.05	0.56 ± 0.06	0.61 ± 0.07	0.66 ± 0.07	0.70 ± 0.08
PUFA/SFA	0.75	0.35 ± 0.01^{a}	0.33 ± 0.01^{a}	0.42 ± 0.01^{b}	0.36 ± 0.01^{a}	0.40 ± 0.01^{b}

Note. Mean ± SE (n = 3); means within the same row with different superscripts are significantly different at $P < 0.05$. [1] Saturated fatty acids (Sum of 14:0 + 16:0 + 18:0). [2] Monounsaturated fatty acids (Sum of 16:1n-7 + 18:1n-9). [3] The n-3 series of polyunsaturated fatty acids (Sum of 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3). [4] The n-6 series of polyunsaturated fatty acids (Sum of 18:2n-6 + 20:4n-6)

Table 8

Fatty acid composition (% of total fatty acids) of liver tissue of juvenile T. tambroides *at the beginning and end of the 12-week experimental period*

		Dietary *D. rostrata* oil (%)				
	Initial	0	1.25	2.5	3.75	5
14:0	2.24	3.21 ± 0.18	3.34 ± 0.19	3.27 ± 0.19	3.36 ± 0.20	3.15 ± 0.18
16:0	26.87	30.49 ± 1.76	29.33 ± 1.69	28.91 ± 1.67	30.86 ± 1.78	27.52 ± 1.59
16:1n-7	4.71	7.21 ± 0.42^{b}	6.73 ± 0.39^{ab}	6.48 ± 0.38^{ab}	6.52 ± 0.38^{ab}	5.81 ± 0.33^{a}
18:0	7.79	6.75 ± 0.39^{a}	8.11 ± 0.47^{ab}	7.89 ± 0.46^{ab}	8.42 ± 0.48^{b}	8.91 ± 0.51^{b}
18:1n-9	31.65	41.11 ± 1.75	41.78 ± 1.74	42.46 ± 1.72	40.99 ± 1.78	42.73 ± 1.74
18:2n-6	14.97	6.16 ± 0.36^{b}	5.72 ± 0.33^{b}	5.79 ± 0.33^{b}	4.60 ± 0.27^{a}	6.21 ± 0.36^{b}
18:3n-3	1.28	1.35 ± 0.08	1.44 ± 0.08	1.51 ± 0.09	1.60 ± 0.09	1.46 ± 0.09
20:4n-6	2.15	0.88 ± 0.05	0.84 ± 0.05	0.85 ± 0.05	0.83 ± 0.05	0.92 ± 0.05
20:5n-3	0.80	0.45 ± 0.03^{a}	0.41 ± 0.02^{a}	0.49 ± 0.03^{a}	0.41 ± 0.02^{a}	0.59 ± 0.03^{b}
22:5n-3	1.08	0.26 ± 0.02	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
22:6n-3	6.47	2.14 ± 0.12	2.07 ± 0.12	2.14 ± 0.12	2.17 ± 0.13	2.46 ± 0.14
Σ SFA[1]	36.89	40.45 ± 1.97	40.77 ± 1.97	40.07 ± 1.93	42.64 ± 2.07	39.58 ± 1.92
Σ MUFA[2]	36.36	48.32 ± 2.17	48.52 ± 2.13	48.94 ± 2.10	47.51 ± 2.15	48.54 ± 2.07
Σ n-3 PUFA[3]	9.63	4.20 ± 0.21	4.16 ± 0.21	4.36 ± 0.23	4.41 ± 0.23	4.75 ± 0.25
Σ n-6 PUFA[4]	17.12	7.03 ± 0.41^{b}	6.56 ± 0.38^{ab}	6.64 ± 0.39^{ab}	5.44 ± 0.31^{a}	7.13 ± 0.41^{b}
n-3/n-6	0.56	0.61 ± 0.06	0.64 ± 0.07	0.66 ± 0.07	0.82 ± 0.09	0.68 ± 0.08
PUFA/SFA	0.73	0.28 ± 0.01^{bc}	0.26 ± 0.01^{b}	0.27 ± 0.01^{bc}	0.23 ± 0.01^{a}	0.30 ± 0.01^{c}

Note. Mean ± SE (n = 3); means within the same row with different superscripts are significantly different at $P < 0.05$. [1] Saturated fatty acids (Sum of 14:0 + 16:0 + 18:0). [2] Monounsaturated fatty acids (Sum of 16:1n-7 + 18:1n-9). [3] The n-3 series of polyunsaturated fatty acids (Sum Of 18:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3). [4] The n-6 series of polyunsaturated fatty acids (Sum of 18:2n-6 + 20:4n-6)

Table 9

Estimated fatty acid retention (% dietary respective fatty acids) of muscle of juvenile T. tambroides *fed varying* D. rostrata *oil levels*

	Dietary *D. rostrata* oil (%)				
	0	1.25	2.5	3.75	5
14:0	107.58 ± 5.03	97.75 ± 7.59	90.52 ± 10.60	84.90 ± 5.04	66.54 ± 6.24
16:0	68.91 ± 8.49	61.22 ± 5.99	59.35 ± 9.08	54.89 ± 10.86	45.78 ± 5.29
16:1n-7	217.98 ± 8.32^{c}	180.73 ± 13.12^{b}	158.72 ± 17.59^{b}	163.08 ± 8.27^{b}	116.97 ± 8.05^{a}
18:0	67.37 ± 9.69^{b}	53.05 ± 5.92^{ab}	54.94 ± 8.27^{ab}	34.89 ± 6.81^{a}	32.25 ± 3.34^{a}
18:1n-9	85.80 ± 4.01^{c}	70.05 ± 5.41^{bc}	76.27 ± 8.19^{c}	54.82 ± 3.35^{ab}	47.01 ± 3.20^{a}
18:2n-6	18.45 ± 6.94^{b}	4.66 ± 4.66^{ab}	12.84 ± 7.39^{b}	2.83 ± 2.83^{ab}	0.00^{a}
18:3n-3	57.08 ± 3.31^{a}	55.09 ± 4.89^{a}	67.85 ± 7.81^{b}	46.88 ± 3.06^{a}	53.73 ± 4.44^{a}
20:4n-6	27.15 ± 8.08	19.58 ± 6.99	43.27 ± 9.85	24.84 ± 8.16	28.05 ± 4.68
20:5n-3	27.34 ± 1.35^{b}	22.92 ± 1.87^{ab}	28.13 ± 3.28^{b}	17.80 ± 1.15^{a}	19.16 ± 1.54^{a}
22:5n-3	28.53 ± 4.91^{ab}	21.35 ± 3.52^{ab}	37.51 ± 6.28^{b}	25.17 ± 5.90^{ab}	15.76 ± 2.14^{a}
22:6n-3	53.02 ± 4.47^{a}	45.06 ± 5.13^{a}	79.85 ± 9.88^{b}	48.07 ± 3.70^{a}	54.97 ± 5.61^{a}
Σ SFA[1]	70.47 ± 8.04^{b}	61.41 ± 5.38^{ab}	50.85 ± 5.11^{ab}	45.23 ± 5.42^{ab}	42.12 ± 4.20^{a}
Σ MUFA[2]	94.25 ± 4.26^{c}	76.85 ± 5.88^{bc}	81.28 ± 8.76^{c}	61.22 ± 3.64^{ab}	51.26 ± 3.49^{a}
Σ n-3 PUFA[3]	42.52 ± 2.37^{a}	39.12 ± 3.46^{a}	51.60 ± 6.59^{b}	38.17 ± 2.36^{a}	40.38 ± 3.62^{a}
Σ n-6 PUFA[4]	19.22 ± 7.04	5.43 ± 5.17	15.97 ± 7.61	4.08 ± 4.08	2.38 ± 1.21

Note. Mean ± SE (n = 3); means within the same row with different superscripts are significantly different at $P < 0.05$. Fatty acid retention (%) = $\frac{(\text{g final muscle fatty acid} - \text{g initial muscle fatty acid})}{\text{g total fatty acid fed}} \times 100.$

[1]Saturated fatty acids.

[2]Monounsaturated fatty acids. [3] The n-3 series of polyunsaturated fatty acids.

[4]The n-6 series of polyunsaturated fatty acids.

Total SFAs and MUFAs in the liver were not significantly affected ($P > 0.05$) by dietary *D. rostrata* oil. Although the increment of dietary *D. rostrata* oil concentration significantly lowered ($P < 0.05$) the 20:5n-3 concentration in the liver of mahseer juveniles, total n-3 PUFAs and n-3/n-6 ratios were not significantly affected ($P > 0.05$) in the liver of juveniles fed different dietary *D. rostrata* oil levels. The concentrations of liver SFA and MUFA were higher upon feeding than the initial levels while most of the PUFAs (except 18:3n-3) showed the opposite trends.

High dietary 16:1n-7 retention (>100%) was found among all treatment groups (Table 9). The retention significantly increased ($P < 0.05$) with the increase of *D. rostrata* oil concentration in the diet. Significantly higher ($P < 0.05$) dietary 18:0, 18:1n-9, 18:2n-6, 20:5n-3, total SFA and total MUFA were retained in the muscle of fish fed 0% *D. rostrata* oil than in those fed 5% *D. rostrata* oil. Meanwhile, juveniles fed on 2.5% *D. rostrata* oil showed significantly higher ($P < 0.05$) muscular 22:5n-3 and 22:6n-3 retention compared to those fed on 5% *D. rostrata* oil. The highest 18:3n-3, 22:6n-3 and total n-3 PUFA muscular retention values were found in juveniles given 2.5% *D. rostrata* oil.

DISCUSSION

Lower growth performance and feed efficiency were observed in juveniles given higher than 2.5% *D. rostrata* oil compared to those given less than 2.5% *D. rostrata* oil in spite of similar survival and lean portions. This could be as a result of higher 18:0 content in the former diets than the latter ones. The mentioned fatty acid is considered a low digestible and low absorbable fatty acid in both fish (Francis, Turchini, Jones, & De Silva, 2007; Menoyo, Lopez-Bote, Bautista, & Obach, 2003; Turchini et al., 2005) and mammals (Kritchevsky, 1994), which can influence growth performance. On the other hand, the fatty acids 16:0 and 18:1n-9 are known as predominant energy sources in fish to spare dietary protein for better growth (Henderson, 1996; Lim, Boey, & Ng, 2001; Sargent et al., 2002; Tocher, 2003). MUFAs with a *cis* double bond as well as PUFAs have a strong potential to inhibit other fatty acids to be used in the β-oxidation process during the intramitochondrial NADPH inadequacy (Gurr, Harwood, & Frayn, 2002; Osmundsen & Bjornstad, 1985). However, 16:0 may be even more preferred than 18:1n-9 in β-oxidation as the multi regression results (%WG = -1.34 KEM + 119.63) also demonstrated that higher dietary 16:0 concentration was responsible for the higher growth in juvenile Malaysian mahseer.

Moreover, SFAs and MUFAs have been found to spare PUFAs and to increase the retention of these fatty acids in fish tissues (Mishra & Samantaray, 2004). Feeding fish with high SFA diets produces a readily oxidized lipid source to provide the energy needed and to spare protein for better growth. No protein sparing activity has been observed in the Malaysian mahseer fed a diet with lipid containing low SFA

(Ng, Abdullah, & De Silva, 2008). In contrast, other researchers (Kamarudin et al., 2012; Kamarudin et al., 2018) noted that *T. tambroides* obtains a better growth when palm oil with high SFA is included in its diet than when cod liver oil, linseed oil, sunflower oil, or illipe oil are fed. They demonstrated that the better growth is due to the protein-sparing action facilitated by higher amounts of SFAs. This group of researchers also found higher 22:6n-3 concentration in the liver of juveniles fed on the diet with 50% replacement of cod liver oil with palm oil compared to fish fed on the diets with 50% replacement of cod liver oil with linseed oil or sunflower oil. Ramezani-Fard et al. (2012b) suggested that SFAs spare n-3 PUFAs in the tissue of Malaysian mahseer and provide high retention of n-3 PUFAs as well as a desirable growth performance in this fish species. Therefore, higher protein, lipid and energy retention as well as higher n-3 PUFA retention in juveniles fed 2.5% or less *D. rostrata* oil could be as a result of higher 16:0 contents in their diets than those fed 3.75-5% *D. rostrata* oil. However, some anti-nutritional factors might also be responsible for the lower growth in juveniles fed more than 2.5% *D. rostrata* oil despite higher feed intake in these groups of juveniles. Such factors have been reported in the pulp and the seeds of African plum or safou (*D. edulis*) (Hanson, 2009; Omogbai & Ojeaburu, 2010).

In general, negative carbohydrate, low protein and high lipid retention together with higher tissue concentrations of SFAs and MUFAs at the end of this feeding trial compared to their initial concentrations demonstrated that juvenile *T. tambroides* utilized non-lipid nutrients to provide most of its required energy as well as to convert some of those non-lipid nutrients into body lipid (Hepher, 1988). High dietary SFA and MUFA contents could be another reason of higher tissue concentrations of these fatty acids at the end of the feeding trial than their initial amounts. Lower protein and lipid retention in juveniles given 3.75-5% *D. rostrata* oil compared to those given 2.5% and less *D. rostrata* oil demonstrated the insufficiency of SFAs and MUFAs in diets containing more than 2.5% *D. rostrata* oil to spare protein and n-3 PUFAs in the bodies of these groups of juveniles, which suppressed their growth performance in spite of higher food intake in these groups of fish. The high VSI values showed that *T. tambroides,* in general, had a high tendency to accumulate lipids in its visceral cavity, which had been previously observed in this fish (Bami et al., 2017; Kamarudin et al., 2018; Ng et al., 2008; Ramezani-Fard et al., 2012b).

Ishak, Kamarudin, Ramezani-Fard, Saad and Yusof (2016) have reported *T. tambroides* dietary carbohydrate requirement of about 25%. The SGR (0.9-1.1) and WG (83.2-155.5 %) values obtained by these researchers were higher than those observed in the current research with the diets including 28 % carbohydrates, especially in the fish fed on more than 2.5 % kembayau oil. Actually, these values in the current study were nearer to those fish fed on 30 % carbohydrate obtained by that group of researchers. This could be as the

result of using corn starch in that research which is the best carbohydrate source for *T. tambroides* followed by taro, sago and tapioca starch (Kamarudin et al., 2014). The SGR and WG values obtained in the current research, especially for those juveniles fed 2.5 % or less kembayau oil, were higher than those found in the studies used diets containing lower SFAs and higher PUFAs (Misieng et al., 2011; Ramezani-Fard et al., 2014; Ramezani-Fard et al., 2012b). The reasonably low FCR values obtained in this study, especially for those juveniles fed 2.5 % or less kembayau oil, may demonstrate appropriate feeding schedule, high digestibility of feed ingredients and proper feed utilization. The FCR values calculated in the current study, were comparable to those obtained in other studies (Ishak et al., 2016; Misieng et al., 2011; Ng et al., 2008; Ramezani-Fard et al., 2012a, 2012b; Ramezani-Fard et al., 2014).

Increased activities of proteolytic enzymes, trypsin and chymotrypsin have been observed in juvenile *T. tambroides* feeding on 0.10 % *Spirulina* as a feed additive in its diet (Jalal, Abmbak, Abol, Hassan, & Zahangir Alam, 2005). According to this research, growth performance and body composition of Malaysian mahseer might be promoted by the addition of *Spirulina* in its diet. Moreover, an improvement in the growth performance and body composition of *T. tambroides* fry fed with a phototrophic purple bacteria, *Marichromatium* sp. as a probiotic in their diet has been reported (Chowdhury, Zakaria, Zainal Abidin, & Rahman, 2016). Their study showed that there were trends of increased growth, better survival rate and improved FCR when fed with diet 1 (*Marichromatium* sp.) compared to other diets. There was a significant difference ($P < 0.05$) between the sampling days. The specific growth rate and weight gain of the fish fed with diet 1 were 0.49 % and 4.92 g, respectively, compared to 0.42 % and 4.11 g from the control. This study suggested that purple bacteria could be used in feed formulation as a supplement to promote growth and survival of freshwater fishes in Malaysia.

In addition to different growth performances, food efficiencies, and protein, lipid and energy retention values, different fatty acid compositions were observed in the muscle and liver tissues of juveniles fed on various dietary *D. rostrata* oil levels. The 16:0 in both the muscle and liver of the mahseer were relatively at constant levels (27.52-30.49% and 27.52-30.86%, respectively) despite a wide dietary range of this fatty acid concentration (26.14-38.70%). The findings supported the notion of selective 16:0 retention by *T. tambroides*, which had been previously reported in this fish (Bami et al., 2017; Kamarudin et al., 2018; Ramezani-Fard et al., 2012b). Mahseer has been demonstrated to selectively retain this fatty acid because of its important role in phosphatidylcholine, the significant phospholipid required for the structure and function of biomembranes (Ng, Lim, & Boey, 2003). These findings also explained the significance of a high concentration of 16:0 provided by CPO. Moreover, narrower ranges of 18:0 observed

in juveniles muscle (6.28-9.11%) and liver (6.75- 8.91%) compared to those of the experimental diets (6.22-13.94%) reconfirmed the notion of selective retention of this SFA in *T. tambroides* by other researchers (Bami et al., 2017; Kamarudin et al., 2018; Ramezani-Fard et al., 2012b). Freshwater species have been reported to have a tendency to maintain tissue total SFAs at a constant level regardless of the dietary SFA content (Bahurmiz & Ng, 2007; Greene & Selivonchick, 1990; Ng et al., 2003). Juveniles fed on diets containing higher *D. rostrata* oil accumulated higher 18:0 in their tissues. In general, lesser SFA and higher MUFA concentrations were found in both liver and muscle tissues of all fish compared to their dietary contents. This suggested that *T. tambroides* utilized both SFAs and MUFAs for β-oxidation, and there was a bioconversion of 16:0 and 18:0 to 16:1n-7 and 18:1n-9 by the Δ9 desaturase enzyme in fish tissues. SFAs and MUFAs are preferred over PUFAs for β-oxidation in fish (Lim et al., 2001; Ng et al., 2003). Mishra and Samantaray (2004) observed that vegetable oils had adequate dietary SFA and MUFA amounts to perform a PUFA sparing activity and maintained the levels of PUFAs in the body of some freshwater fish species.

Fish prefer to use 20:5n-3 as an energy source rather than 22:6n-3 as they can readily β-oxidize 20:5n-3 but need to β-oxidize 22:6n-3 proxisomally and mitochondrially (Tocher, 2003). This evidence together with 20:5n-3 bioconversion to 22:6n-3 in fish tissues could be the reason of lower 20:5n-3 than 22:6n-3 in the tissues of juvenile *T. tambroides* of all groups. Less n-3 PUFAs were found in the muscle of fish fed on 0-1.25% *D. rostrata* oil compared to those fed on 2.5% or more *D. rostrata* oil. This could be as a result of slightly lower n-3 PUFA in the diets containing more than 2.5% CPO than the diets including 2.5% or more *D. rostrata* oil. Moreover, as mentioned earlier, 16:0 and 18:1n-9 are preferred over other fatty acids for β-oxidation in fish (Henderson, 1996; Sargent et al., 2002; Tocher, 2003). Although *D. rostrata* oil contained lower 16:0 than CPO, higher 18:0 and 18:1n-9 were found in *D. rostrata* oil. The 18:0, despite having low absorbability and digestibility in animals such as fish (Kritchevsky, 1994), is the precursor for 18:1n-9 production. Therefore, a 2.5:2.5 combination of *D. rostrata* oil and CPO could have resulted in a balance in the dietary concentrations of 16:0, 18:1n-9 and 18:0 to be used as the energy sources, which could have led to a higher retention of n-3 PUFAs in the muscle of juvenile *T. tambroides*. This evidence could also explain that why lower muscular 20:4n-6 content was observed in fish given lower *D. rostrata* oil percentages (0-1.25%) than the other treatments. A similar observation has been reported in our previous studies related to Malaysian mahseer (Bami et al., 2017; Kamarudin et al., 2018).

Ramezani-Fard et al. (2012a) suggested that muscular n-3 PUFA content in *T. tambroides* depends on *de novo* synthesis more than on a direct absorption from the diet. The lower 20:5n-3 and 22:5n-3

observed in the muscle of juveniles fed on test diets compared to their dietary percentages could be as a result of bio-conversion of both 20:5n-3 and 22:5n-3 to 22:6n-3. Nevertheless, the lower concentrations of n-3 LC-PUFAs were observed in the fish muscle when compared to their initial concentrations could also be due to their low dietary contents. Moreover, the higher water temperature in the aquaria (Farkas, 1984), which was about 10 ˚C higher than that of its natural habitat, could also decrease the muscular concentrations of n-3 LC-PUFAs. This notion has been previously suggested by Ramezani-Fard et al. (2012a). Farkas (1984) has also observed a higher PUFA concentration in the tissue of common carp (*Cyprinus carpio*) when reared in a lower water temperature.

The muscular and liver 18:2n-6 percentages in juveniles were in the ranges of 7.65-9.07% and 4.60-6.21%, respectively, despite being fed on diets containing higher levels of 18:2n-6 (9.29-10.91%). Ramezani-Fard et al. (2012b) and made a similar observation and proposed that the selective depletion of this fatty acid in *T. tambroides* tissues demonstrates the high tendency of this fish to mobilize and catabolize the fatty acid. Similar findings have been achieved in other studies on Malaysian mahseer (Bami et al., 2017; Kamarudin et al., 2018).

Lower 20:4n-6 percentages in both liver and muscle of juveniles compared to its initial percentages at the start of the study might also be related to both the decline in PUFA *de novo* synthesis and the preference of desaturase enzymes to use n-3 PUFAs than n-6 PUFAs that could have caused the decrease in 18:2n-6 conversion to 20:4n-6 (Jankowska, Zakęś, Żmijewski, & Szczepkowski, 2010). Moreover, conversion of the dietary 20:4n-6 to the tissue 20:4n-6 is preferred to that of the dietary 18:2n-6 (Jankowska et al., 2010). This could be the reason why tissue 20:4n-6 percentage was as low as its dietary percentage.

Nevertheless, although juveniles fed on various dietary *D. rostrata* oil levels showed muscle n-3/n-6 ratios higher than the 0.1-0.2 ratio recommended by the World Health Organization (Tanamati et al., 2009) at the end of the experiment, the highest retention of n-3 LC-PUFAs was observed in the muscle of juveniles given 2.5% *D. rostrata* oil. A balance between dietary 16:0 and 18:1n-9 concentrations in the mixture might be responsible in sparing PUFAs and maintaining their concentrations in *T. tambroides* body. Moreover, the fish fed on 2.5% *D. rostrata* oil had similar growth and feed efficiency to those fed 0% *D. rostrata* oil. Ramezani-Fard et al. (2012b) suggested that a diet containing 2.5% n-3 PUFA and an SFA/n-3 PUFA ratio of 15.3 could efficiently spare n-3 PUFA concentration in the tissue of *T. tambroides* as well as a satisfactory growth performance of this fish. These researchers added that n-3 PUFA concentrations of less than 2.5% and SFA/n-3 PUFA ratios of higher than 15.3 in the diet of Malaysian mahseer may reduce n-3 PUFA concentration in its tissues. However, they did not suggest the appropriate dietary MUFA level for this fish. No information on the exact dietary

fatty acid requirements of *T. tambroides* has been published. Even by using 2.5% *D. rostrata* oil, a high muscular concentration of 18:0 was found in juveniles with high muscular PUFA concentrations. High 18:1n-9 production from 18:0 as well as a balanced 16:0 concentration in the diet with 2.5% *D. rostrata* oil could be the reason of sparing more n-3 PUFAs in the tissue of juveniles fed on the mentioned diet than juveniles fed on the other test diets. Therefore, there might be a limited dietary requirement of the fatty acid 18:0 for *T. tambroides*. There could be a possibility that the fish given a diet with an 18:0 concentration higher than its nutritional requirement has a high tissue concentration of this fatty acid over a long-term culture period.

CONCLUSIONS

This study showed that *D. rostrata* oil could be used up to 2.5% as a dietary lipid source for mahseer juvenile without affecting the survival, growth, feed efficiency and major body nutrients. From the findings of this study, the use of a grow-out diet containing 5% CPO and a finisher diet containing a combination of 2.5% *D. rostrata* oil and 2.5% CPO were suggested for the mahseer culture to achieve a maximum growth performance and a desired flesh fatty acid profile, especially in the case of n-3 and n-6 LC-PUFAs. The optimal finishing period of juvenile *T. tambroides* needs to be determined. The effects of *D. rostrata* oil on the taste and shelf life of mahseer flesh should also be investigated. Dietary lipid source has been shown to affect the flesh taste, texture and smell in seabream (*Spaurus aurata*) and seabass (*Dicentrarchus labrax*). However, these effects could not be determined in this study as the fish had not reached its minimum commercial size. *D. rostrata* is a seasonal fruit, and no commercial production of the fruit or its oil has been reported. Research on the commercial farming and production of *D. rostrata* fruit and oil should also be conducted as its crude oil showed a high potential as a good source of dietary lipid for the Malaysian mahseer and possibly other highly sought after riverine carps. The addition of *Spirulina* or *Marichromatium* sp. to its diet is also suggested as it may result in an improved growth performance and body composition of this species.

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