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Total replacement of dietary fish oil with alternative lipid sources in a practical diet for mandarin fish, *Siniperca scherzeri*, juveniles

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Abstract

A 12-week feeding trial was designed to evaluate the effect of total replacement of fish oil (FO) with terrestrial alternative oils on growth, feed utilization, body composition, hematological parameters, and fillet fatty acid profile of mandarin fish juveniles. Four iso-nitrogenous (56% crude protein) and iso-lipidic (13% crude lipid) practical diets were formulated. A control diet contained 6% FO and three other experimental diets were prepared by replacing FO with linseed oil, soybean oil, and lard (designed as FO, LO, SO, and lard, respectively). Each diet was randomly allocated to triplicate groups of 25 fish (1.8 ± 0.03 g/fish) in a circular tank. Complete replacement of FO by three tested alternative oils had no remarkable impact on growth performance, feed utilization efficiency, and morphological and hematological parameters of juvenile mandarin fish. However, daily feed intake was found to be significantly higher for fish fed the SO diet compared with those fed the FO and LO diets. Fish fed LO and SO diets exhibited significantly higher levels of the whole body lipid compared to fish fed diet containing FO. Fillet fatty acid composition reflected dietary fatty acid profile. The highest level of α-linolenic acid, linoleic acid, and oleic acid was observed in fish fillet fed LO, SO, and lard, respectively. Although the eicosapentaenoic acid level of fish fillet fed diet FO was higher than other treatments, no significant difference was found in docosahexaenoic acid content among all dietary groups. The results of the present study clearly demonstrate that the complete replacement of FO in mandarin fish diets is achievable. These findings are useful in dietary formulation to reduce feed costs without compromising mandarin fish growth.

Keywords: Growth performance, Feed utilization efficiency, Fillet fatty acid composition, Fish oil replacement, Mandarin fish, *Siniperca scherzeri*

Background

Feed ingredients of marine origin such as fish meal (FM) and fish oil (FO) have been extensively used as the main protein and lipid sources in the aquafeeds. Fish oil is particularly popular in aquafeed industry because of its high proportions of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) that play an important role in supporting normal growth, health, and nutritional quality of farmed aquatic animals (Turchini et al. 2011b). However, it is clearly evident that the aquafeed-manufacturing industry cannot continue to rely on this highly palatable and nutritious marine ingredient. Indeed, the sustainability

issues and rising costs linked with FO have exerted and continue to exert substantial pressure on global aquafeed sector to find economically viable and environmentally sustainable substitutions. In this regard, terrestrial oils, particularly vegetable oils, have been considered as the prime candidates for FO replacement in aquafeeds due to their high availability and relatively lower prices (Turchini et al. 2011b). In comparison to FO, however, oils of terrestrial origin are typically rich in C_{18} fatty acids, mainly linoleic (LA, 18:2n-6), α-linolenic (ALA, 18:3n-3), and oleic (OA, 18:1n-9) acids, but lack or have a very limited content of the n-3 LC-PUFA, such as eicosapentaenoic (EPA, 20:5-3) and docosahexaenoic (DHA, 22:6n-3) acids, which are regarded as undesirable nutritional properties (Bureau and Meeker 2011; Nasopoulou and Zabetakis 2012). Consequently, numerous studies have investigated the efficacy

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of various oils of terrestrial origin in feeds for cultured fish. Overall, the literature evidence suggests that most alternative oil sources are able to replace FO to some extent depending upon the species studied and also the type and fatty acid content of the alternative oil used (Turchini et al. 2011b). It is also now generally recognized that partial or full substitution of FO is more feasible for freshwater fish than marine species which apparently lack the ability to desaturate and elongate C_{18} PUFA and therefore are very prone to n-3 LC-PUFA deficiency (Tocher 2010). Indeed, a review of previous experiments with freshwater species such as Murray cod, *Maccullochella peelii peelii* (Turchini et al. 2011a); pikeperch, *Sander lucioperca* (L.) (Kowalska et al. 2012); snakehead, *Channa striatus* (Aliyu-Paiko and Hashim 2012); darkbarbel catfish, *Pelteobagrus vachelli* (Jiang et al. 2013); Nile tilapia, *Oreochromis niloticus* (Peng et al. 2015; Apraku et al. 2017); gibel carp, *Carassius auratus gibelio* (Zhou et al. 2016); silver catfish, *Rhamdia quelen* (Lazzari et al. 2016); and rainbow trout, *Oncorhynchus mykiss* (Gause and Trushenski 2013; Yıldız et al. 2018); has shown that it is possible to replace FO by single or a mixture of terrestrial oils (both vegetable and animal oils) without compromising growth or feed efficiencies.

Mandarin fish, *Siniperca scherzeri*, is a strict freshwater piscivore mainly found throughout East China, Korea, and Northern Vietnam (Zhou et al. 1988; Li 1991). The growing interest in mandarin fish culture has been triggered by increasing demand on its shrinking wild stock (Wu et al. 1997; Chu et al. 2013). Yet, so far, there is relatively little scientific information available concerning mandarin fish nutrition (Zhang et al. 2009; Lee et al. 2012; Sankian et al. 2017, 2018, 2019), and its commercial production still relies on expensive live prey. Hence, one of the most important steps in developing and implementing a profitable and sustainable culture practice for this species is to formulate a nutritionally balanced and cost-effective commercial feed. A recent feeding trial with mandarin fish juveniles, of < 9 g, in our laboratory demonstrated that a diet containing 55% crude protein and 14% crude lipid yielded the best feeding and growth performance (Sankian et al. 2017). However, there is no information available on the use of alternative lipid source in a practical diet of this species. Therefore, the overall aim of this study was to investigate the possible effects of total substitution of dietary FO with alternative lipid sources including linseed oil, soybean oil, or lard on mandarin fish juveniles in terms of growth performance, efficiency of feed utilization, whole body proximate composition, biochemical indices, and fillet fatty acid profile. It is likely that the main findings in this study could be useful in formulating a cost-effective practical diet for this emerging species of increasing interest in the South Korean freshwater aquaculture sector.

Methods

Experimental diets

Fatty acid composition of the tested oil sources are shown in Table 1 and the formulation and proximate composition of the experimental diets are presented in Table 2. Four iso-nitrogenous (approximately 56% crude protein) and iso-lipidic (approximately 13% crude lipid experimental diets, varying only in the added lipid source) were formulated using anchovy FM as the main source of protein. In all the experimental diets, ~ 6% lipid was provided from the residual oil in the FM and other dietary ingredients, while the other 6% lipid was achieved through separate addition of four different oil sources including fish oil (FO), linseed oil (LO), soybean oil (SO), and lard to the diet, and the resultant experimental diets were named accordingly. For preparing each experimental diet, all the dry ingredients were thoroughly mixed with oil and distilled water to form a sticky dough, which was then passed through a mincer (SMC-32, SL Co., Incheon, South Korea) to produce 3.0-mm-diameter feed strands. The moist feed strands were then chopped into pellets of a desired length (approximately 15 to 18 mm), dried on wire racks at 25 °C in a forced air oven overnight, and kept frozen at − 20 °C until used. A pilot study in our laboratory has shown that the size, shape, and texture of the feed pellets play an important role in the acceptance of artificial diets by mandarin fish which is well known for its very unique food preference (Liang et al. 1998; Li et al. 2017). The fish was found to be most attracted to the 15 to 18-mm-long moist pellets, which contained more than 30% moisture.

Table 1 Major fatty acid composition (% total fatty acids) of the tested oil sources

	Oil sources			
	FO	LO	SO	Lard
14:0	9.8	0.1	0.1	2.1
16:0	19.6	5.6	11.9	22.6
18:0	3.9	3.6	4.1	12.2
16:1n-7	10.7	0.1	0.1	2.1
18:1n-9 (OA)[1]	7.0	16.0	26.1	43.3
18:2n-6 (LA)[2]	1.5	15.5	48.2	11.2
18:3n-3 (ALA)[3]	0.6	48.7	5.8	0.1
20:4n-6	–	–	–	–
20:5n-3 (EPA)[4]	15.4	–	–	–
22:5n-3	–	–	–	–
22:6n-3 (DHA)[5]	5.7	–	–	–

FO fish oil, *LO* linseed oil, *SO* soybean oil
[1]Oleic acid
[2]Linoleic acid
[3]α-Linolenic acid
[4]Eicosapentaenoic acid
[5]Decosahexaenoic acid

Table 2 Ingredients and proximate composition of the experimental diets (% DM)

	Experimental diets			
	FO	LO	SO	Lard
Ingredients (% DM)				
Anchovy FM[1]	55.0	55.0	55.0	55.0
Casein	5.0	5.0	5.0	5.0
Wheat gluten	10.0	10.0	10.0	10.0
Wheat flour	20.0	20.0	20.0	20.0
Fish oil	6.0	–	–	–
Linseed oil	–	6.0	–	–
Soybean oil	–	–	6.0	–
Lard	–	–	–	6.0
Vitamin premix[2]	1.5	1.5	1.5	1.5
Mineral premix[3]	1.8	1.8	1.8	1.8
Stay-C (50%)	0.3	0.3	0.3	0.3
Vitamin E (25%)	0.2	0.2	0.2	0.2
Choline (50%)	0.2	0.2	0.2	0.2
Proximate composition (% DM)				
Dry matter	70.5	63.7	66.8	60.4
Crude protein	56.0	56.2	56.8	56.9
Crude lipid	13.0	13.2	13.2	13.3
Ash	11.0	11.0	11.0	11.2
Fatty acids (% total fatty acids)				
14:0	8.5	1.7	1.6	3.0
16:0	21.2	11.2	15.1	22.7
18:0	4.5	4.2	4.5	10.1
16:1n-7	8.3	0.9	0.8	2.2
18:1n-9 (OA)[4]	8.3	14.3	20.7	32.8
18:2n-6 (LA)[5]	1.6	11.2	32.7	8.1
18:3n-3 (ALA)[6]	0.7	33.7	4.2	0.4
20:4n-6	0.8	0.7	0.7	0.7
20:5n-3 (EPA)[7]	14.5	3.7	3.6	3.6
22:5n-3	11.4	0.6	0.6	0.6
22:6n-3 (DHA)[8]	16.6	12.4	12.1	12.3

FO fish oil, *LO* linseed oil, *SO* soybean oil
[1]Pesquera Bahia Caldera, Caldera, Chile; with crude protein 69.1%, crude lipid 12.6%, ash 14.1% dry matter
[2]Vitamin premix contained the following amounts which were diluted in cellulose (g/kg premix): DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003
[3]Mineral premix contained the following ingredients (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0
[4]Oleic acid
[5]Linoleic acid
[6]α-Linolenic acid
[7]Eicosapentaenoic acid
[8]Decosahexaenoic acid

Fish and experimental design

Mandarin fish juveniles were obtained from Inland Fisheries Research Institute (Chungcheongbuk-do, South Korea). The fish were acclimated to the experimental condition in a 5000-L glass tank, connected to a recirculation system, at the GWNU Marine Biology Center in ambient freshwater temperature (24 ± 0.1 °C, mean ± SE), feeding on a repelleted commercial feed (50% crude protein and 13% lipid; Woosung, Daejeon, South Korea) with the same size as the experimental diets. Following the 1-week acclimation procedure, 25 fish with an initial mean body weight of 1.8 ± 0.03 g were randomly stocked into each 65-L polyvinyl circular tank supplied with filtered and dechlorinated tap water using a freshwater recirculating (closed) system. Triplicate groups of fish were fed one of the formulated diets to apparent satiation twice a day at 09:00 and 17:00 for 12 weeks. The uneaten feed was siphoned out, dried to constant weight, and then weighed to estimate the amount of feed consumed. The mean water temperature during the experimental period was 24 ± 0.1 °C. The photoperiod was maintained on a 12:12-h (light/dark) schedule. Fish were deprived of feed for 16 h prior to weighing or sampling to minimize handling stress on fish.

Sample collection

At the end of the experiment, all the survived fish in each tank were counted and weighed for calculation of growth performance, feed utilization parameters, and survival rates. Total body length was measured for each individual fish to the nearest 0.1 mm. A random sample of 5 fish per tank was collected and stored at − 43 °C for subsequent whole body proximate composition analyses.

Blood samples were collected from the caudal veins of six anesthetized (200 mg/L MS-222, Sigma, St. Louis, MO, USA) individual fish per tank (nine fish/dietary treatment) using heparinized syringes. Blood samples were kept on ice prior to plasma separation by centrifugation at 7500 RPM for 10 min using a high-speed refrigerated microcentrifuge (HanilBioMed Inc., Gwangju, South Korea). Plasma samples were immediately stored at − 80 °C until used in subsequent hematological analyses including total protein (TP), total cholesterol (TCHO), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin (ALB), and total bilirubin (TBIL). Immediately after blood sampling, fish (6 fish/tank; 18 fish/dietary treatment) were dissected to obtain their liver and visceral weights for calculating the hepatosomatic (HSI) and viscerosomatic (VSI) indices, respectively. Fillet samples were subsequently dissected out of the same fish and stored at − 80 °C until proximate and fatty acid composition analyses.

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Analytical methods
Chemical composition analyses

Chemical composition of the experimental diets, whole body, and fillet samples was determined according to the standard methods (AOAC 2005). Moisture content was estimated by oven drying at 105 °C for 6 h. Crude protein content was determined by an automated Kjeldahl system (Buchi, Flawil, Switzerland). Crude lipid was measured using a Soxhlet extractor (VELP Scientifica, Milano, Italy), and ash content by a Thermolyne™ combustion in a muffle furnace at 600 °C for 4 h.

Hematological analysis

Plasma samples were analyzed for TP, TCHO, AST, ALT, ALP, ALB, and TBIL concentrations using an automated blood analyzer (DRI-CHEM NX500i, FUJIFILM Corporation Tokyo, Japan).

Fatty acid analyses

Total lipids in oil sources, experimental diets, and fillet samples were extracted following the Folch et al. (1957) method using a chloroform and methanol mixture (2:1 v/v). Extracted lipids were then submitted to acid-catalyzed transmethylation using BF_3-MeOH (Sigma, St. Louis, MO, USA) to obtain fatty acid methyl esters (FAMEs). Then, FAMEs were analyzed using a PerkinElmer Clarus 600 gas chromatograph (Shelton, CT, USA) equipped with a flame-ionization detector and a SP-2560 capillary column (100 m × 0.25 mm i.d., 0.2-μm film thickness; Supelco, Bellefonte, PA, USA) using helium as the carrier gas and temperature programmed operation from 140 to 240 °C in increments of 5 °C/min. The temperature of both injector and detector was adjusted at 240 °C. Fatty acids were identified by comparison with standard FAME mixtures (FAME 37 and PUFA 3; Supelco, Bellefonte, PA, USA) and data analyzed using TotalChrom software (version 6.3.1; PerkinElmer Inc., Shelton, CT, USA).

Formulae, calculations, and statistical analysis

Weight gain (WG, %) =
$$[(\text{final body weight}-\text{initial body weight})/\text{initial body weight}] \times 100$$

Specific growth rate (SGR, %/day) =
$$[(\ln \text{final body weight}-\ln \text{initial body weight})/\text{days}] \times 100$$

Daily feed intake (DFI, %) =
$$\{\text{total dry feed consumed(g)}/[(\text{initial fish weight}+\text{final fish weight}+\text{dead fish weight}) \times \text{days}/2]\} \times 100$$

Daily protein intake (DPI, %) =
$$\{\text{total protein consumed(g)}/[(\text{initial fish weight}+\text{final fish weight}+\text{dead fish weight}) \times \text{days}/2]\} \times 100$$

Feed efficiency (FE, %) =
$$(\text{wet weight gain (g)}/\text{total dry feed consumed(g)}) \times 100$$

Protein efficiency ratio (PER) =
$$\text{wet weight gain (g)}/\text{total protein consumed(g)}$$

Condition factor (CF, %) =
$$[\text{fish body weight}/(\text{total length of fish}) (\text{cm})^3] \times 100$$

Hepatosomatic index (HSI, %) =
$$(\text{liver weight (g)}/\text{fish body weight}) \times 100$$

Viscerosomatic index (VSI, %) =
$$(\text{viscera weight (g)}/\text{fish body weight}) \times 100$$

Data were analyzed as a completely random design with the tank as the experimental unit, using one-way ANOVA in SPSS program version 22.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, Tukey's honest significant difference multiple range test was performed to detect statistically significant differences between mean responses at a significance level of $P < 0.05$. Data were checked for normal distribution (Shapiro-Wilk's test) and homogeneity of variances (Levene's test) and when necessary arcsine transformed. Data were presented as mean ± standard error (SE.) of the triplicate groups.

Results

The results of growth, feed utilization, and morphological parameters of mandarin fish juveniles are presented in Table 3. The total replacement of dietary FO by different lipid sources showed no significant negative effect on growth performance in terms of final body weight (10.3–11.6 g), WG (499–549%), and SGR (2.13–2.23%). Although growth rates were not significantly affected, fish received FO and LO diets grew slightly better and were numerically larger than those fed the other two diets. The DFI of fish fed the SO diet was significantly higher than those of fish fed FO and LO diets. No remarkable differences on DPI, FE, and PER were observed among any of the treatments ($P > 0.05$). The survival rate was higher than 97%, and there was no significant difference among all experimental groups. Similarly, the total substitution of FO with LO, SO, or lard had no significant effects on fish morphological parameters.

The results of whole body proximate composition are presented in Table 4. No significant differences were observed in whole body composition in terms of moisture, crude protein, and ash contents among all experimental groups. Total replacement of dietary FO with three different oil sources resulted in increased fat levels in mandarin fish body. Fish fed the LO and SO diets had significantly higher crude lipid level in the whole body than that of fish fed FO diet.

The results of hematological parameters are reported in Table 5. There was neither a considerable difference

Table 3 Growth performance, feed utilization efficiency, and morphological parameters of mandarin fish fed the four experimental diets for 12 weeks

	Experimental diets			
	FO	LO	SO	Lard
IBW[1]	1.8 ± 0.02	1.8 ± 0.02	1.7 ± 0.01	1.8 ± 0.02
FBW[2]	11.6 ± 0.3	10.9 ± 0.3	10.3 ± 0.03	10.7 ± 0.4
WG[3]	549 ± 20	524 ± 18	503 ± 1	499 ± 13
SGR[4]	2.23 ± 0.04	2.18 ± 0.04	2.14 ± 0.00	2.13 ± 0.03
DFI[5]	2.54 ± 0.10[a]	2.50 ± 0.06[a]	2.81 ± 0.02[b]	2.72 ± 0.04[ab]
DPI[6]	1.44 ± 0.06	1.42 ± 0.03	1.57 ± 0.01	1.53 ± 0.02
FE[7]	69.1 ± 3.2	69.1 ± 2.3	61.4 ± 0.8	62.5 ± 1.2
PER[8]	1.44 ± 0.06	1.45 ± 0.04	1.31 ± 0.01	1.34 ± 0.02
Survival (%)	98.7 ± 1.33	97.3 ± 1.33	98.7 ± 1.33	98.7 ± 1.33
CF[9]	1.14 ± 0.11	1.41 ± 0.06	1.17 ± 0.02	1.14 ± 0.11
HSI[10]	2.06 ± 0.10	3.04 ± 0.57	2.47 ± 0.28	2.24 ± 0.09
VSI[11]	9.93 ± 0.73	10.42 ± 0.50	9.65 ± 0.74	8.95 ± 0.30

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscripts in the same row are significantly different (*P* < 0.05). The lack of superscript letter indicates no significant differences among treatments *FO* fish oil, *LO* linseed oil, *SO* soybean oil
[1]Initial mean body weight (g)
[2]Final mean body weight (g)
[3]Weight gain (%) = [(final bodyweight − initial bodyweight)/initial body weight] × 100
[4]Specific growth rate (%/day) = [(ln final bodyweight − ln initial bodyweight]/days] × 100
[5]Daily feed intake (%) = {total dry feed consumed (g)/[(initial fish weight + final fishweight + dead fishweight) × days/2]} × 100
[6]Daily protein intake (%) = {total protein consumed (g)/[(initial fishweight + final fishweight + dead fishweight) × days/2]} × 100
[7]Feed efficiency (%) = (wet weight gain (g)/total dry feed consumed (g)) × 100
[8]Protein efficiency ratio = wet weight gain (g)/total protein consumed (g)
[9]Condition factor (%) = [fish body weight/(total length of fish) (cm)[3]] × 100
[10]Hepatosomatic index (%) = (liver weight (g)/fish body weight) × 100
[11]Viscerosomatic index (%) = (viscera weight (g)/fish body weight) × 100

nor any discernible trend among dietary groups regarding plasma hematological parameters.

Results on fillet proximate composition and fatty acid profile are given in Table 6. No significant effect nor any defined trend was found in fillet proximate composition among dietary treatments. However, the addition of vegetable oil or animal fat in fish diet significantly

Table 4 Whole body proximate composition of mandarin fish fed the four experimental diets for 12 weeks (% wet weight)

	Experimental diets			
	FO	LO	SO	Lard
Moisture	79.1 ± 1.5	77.0 ± 0.6	76.7 ± 1.7	77.8 ± 1.52
Crude protein	11.3 ± 0.6	11.8 ± 0.5	13.4 ± 0.9	12.4 ± 1.8
Crude lipid	3.85 ± 0.17[a]	5.95 ± 0.34[b]	5.80 ± 0.12[b]	4.84 ± 0.67[ab]
Ash	3.34 ± 0.12	3.36 ± 0.21	3.55 ± 0.48	3.51 ± 0.24

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscripts in the same row are significantly different (*P* < 0.05). The lack of superscript letter indicates no significant differences among treatments *FO* fish oil, *LO* linseed oil, *SO* soybean oil

Table 5 Hematological parameters of mandarin fish fed the four experimental diets for 12 weeks

	Experimental diets			
	FO	LO	SO	Lard
TP[1]	4.33 ± 0.17	4.17 ± 0.20	4.03 ± 0.32	4.80 ± 0.12
TCHO[2]	205 ± 8	190 ± 13	187 ± 13	219 ± 9
AST[3]	174 ± 16	224 ± 61	172 ± 22	274 ± 11
ALT[4]	10.0 ± 0.6	10.0 ± 0.0	8.7 ± 0.3	11.0 ± 6.7
ALP[5]	412 ± 30	342 ± 30	341 ± 40	489 ± 34
ALB[6]	0.77 ± 0.03	0.67 ± 0.03	0.70 ± 0.06	0.83 ± 0.03
TBIL[7]	0.53 ± 0.09	0.60 ± 0.12	0.77 ± 0.09	0.73 ± 0.17

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscripts in the same row are significantly different (*P* < 0.05). The lack of superscript letter indicates no significant differences among treatments *FO* fish oil, *LO* linseed oil, *SO* soybean oil
[1]Total protein (g/dL)
[2]Total cholesterol (mg/dL)
[3]Asparatet aminotransferase activity (U/L)
[4]Alanine aminotransferase activity (U/L)
[5]Alkaline phosphatase (U/L)
[6]Albumin (g/dL)
[7]Total bilirubin (mg/dL)

altered fillet fatty acid profile. Regarding saturated fatty acids (SFAs), the highest content of myristic acid (14:0) was noted in fish fed the FO diet, which differed significantly from the other three dietary treatments. Fillets of fish fed diets containing lard in place of FO had significantly higher levels of palmitic acid (16:0) than do fillets of fish fed the other experimental diets. While no statistically significant differences were found among LO, SO, and lard groups, the stearic acid (18:0) content of fillet in lard group was significantly higher than that of FO-fed fish. With respect to monounsaturated fatty acids (MUFAs), fillets of fish fed FO and lard diets contained significantly higher palmitoleic acid (16:1n-7) compared to fish fed on LO and SO diets. A significantly greater level of OA was found in the fillet of fish fed lard diet compared to the other dietary groups. By evaluation of n-6 fatty acid levels in the fish fillet, the highest concentration of LA was found in fish fed on diets containing SO. Alternative oil treatments had no significant impact on fillet arachidonic acid (AA, 20:4n-6) content. With respect to the fillet n-3 fatty acid composition, fish fed the LO diet had significantly higher levels of ALA than that of fish fed the FO-based diet, which was itself significantly higher than those found in the SO and lard groups. Fillets of fish fed diets containing FO had significantly higher concentrations of EPA than those fed diets containing the other three oil sources. The 22:5n-3 content of fillet in FO fish was significantly higher than that of lard diet-fed fish, while no significant differences in fillet 22:5n-3 among LO, SO, and lard diet-fed fish were observed. The DHA contents of the fish fillets were numerically lower in the LO, SO, and lard groups compared to fish fed the FO diet.

Table 6 Fillet proximate and fatty acid composition of mandarin fish fed the four experimental diets for 12 weeks

	Experimental diets			
	FO	LO	SO	Lard
Proximate composition (% wet weight)				
Moisture	77.9 ± 0.8	79.1 ± 0.4	78.7 ± 1.2	77.6 ± 0.4
Crude protein	18.1 ± 0.5	18.1 ± 0.8	17.6 ± 0.9	18.8 ± 0.6
Crude lipid	1.44 ± 0.36	1.70 ± 0.27	1.36 ± 0.17	1.32 ± 0.24
Ash	1.37 ± 0.16	1.03 ± 0.11	1.14 ± 0.09	1.46 ± 0.02
Fatty acid (% total fatty acid)				
14:0	2.54 ± 0.17[b]	1.84 ± 0.12[a]	1.45 ± 0.02[a]	1.68 ± 0.03[a]
16:0	21.8 ± 0.5[a]	20.8 ± 0.1[a]	20.8 ± 0.2[a]	23.4 ± 0.7[b]
18:0	5.30 ± 0.12[a]	5.91 ± 0.14[ab]	6.01 ± 0.28[ab]	6.64 ± 0.25[b]
16:1n-7	5.61 ± 0.58[b]	2.42 ± 0.18[a]	2.49 ± 0.37[a]	4.94 ± 0.12[b]
18:1n-9 (OA)[1]	21.5 ± 1.7[a]	21.2 ± 0.7[a]	20.3 ± 0.1[a]	27.98 ± 1.2[b]
18:2n-6 (LA)[2]	4.90 ± 0.31[a]	7.38 ± 0.20[b]	15.86 ± 0.45[c]	6.29 ± 0.39[ab]
18:3n-3 (ALA)[3]	4.90 ± 0.28[b]	10.47 ± 0.48[c]	2.78 ± 0.31[a]	1.67 ± 0.02[a]
20:4n-6	1.92 ± 0.32	1.09 ± 0.04	1.09 ± 0.02	1.42 ± 0.04
20:5n-3 (EPA)[4]	6.20 ± 0.31[b]	4.96 ± 0.05[a]	4.49 ± 0.13[a]	4.59 ± 0.38[a]
22:5n-3	1.56 ± 0.15[b]	1.42 ± 0.05[ab]	1.30 ± 0.03[ab]	1.04 ± 0.13[a]
22:6n-3 (DHA)[5]	18.2 ± 1.8	16.4 ± 1.0	15.7 ± 0.2	14.0 ± 1.9

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscripts in the same row are significantly different (P < 0.05). The lack of superscript letter indicates no significant differences among treatments

FO fish oil, LO linseed oil, SO soybean oil

[1]Oleic acid
[2]Linoleic acid
[3]α-Linolenic acid
[4]Eicosapentaenoic acid
[5]Decosahexaenoic acid

Discussion

The growth rate values observed in the present study were much higher than those previously reported for the same species by our research team using practical diets with relatively similar protein and lipid contents (Sankian et al. 2017, 2018). This is probably due to the higher initial weight of the fish in the previous studies (8–20 vs. 1.8 g in the present study). To our knowledge, so far, there is no previous record on the effects of different dietary lipid source on growth, feed utilization, hematological indices, and body/fillet nutritional traits of mandarin fish. The findings of the present study showed that FO in a practical diet with 13% lipid for juvenile mandarin fish can be completely replaced by LO, SO, or lard without markedly compromising growth performance and feed efficiency. Our results are in agreement with the previous studies in which it was demonstrated that various alternative oils could be used to completely replace FO in freshwater fish diets without any adverse effect on the nutrient utilization or growth rates (Turchini et al. 2011a; Kowalska et al. 2012; Jiang et al. 2013; Han et al. 2013; Zhou et al. 2016). However, the DFI value in fish fed with SO diet was significantly higher than those of fish fed the FO and LO

diets. Similarly, Du et al. (2008) found markedly higher feed intake in grass carp, Ctenopharyngodon idella, fngerlings fed lard or a blend of vegetable oils in comparison with a FO-based diet. A large body of literature provides strong evidence to suggest that fish are able to regulate feed intake in order to meet their metabolizable energy demands (De la Higuera 2001). Within the context of FO replacement in aquafeed, if FO is replaced with an equal amount of alternative oil source, minimal differences in the total dietary energy content are expected. However, since different oils may have different digestibility, it is possible that the total digestible/metabolizable energy of feed can be partially changed, resulting in variations in feed intake. This may partly explain the DFI differences observed in the present study.

The replacement of the dietary FO by three different oils, in the present study, did not affect the morphological indices of fish, which is often used to indicate the nutritional status of fish. This was in agreement with previous studies in other freshwater species, which reported that the replacement of dietary FO with vegetable oils and rendered animal fat did not affect the morphological parameters of snakehead, Murray cod, rainbow

trout, darkbarbel catfish, Nile tilapia, and gibel carp (Figueiredo-Silva et al. 2005; Turchini et al. 2011a; Aliyu-Paiko and Hashim 2012; Jiang et al. 2013; Peng et al. 2015; Zhou et al. 2016).

In the present study, the whole body lipid content of mandarin fish fed the LO and SO diets were significantly higher than that of fish fed the FO diet. In accordance with the present study, significantly high fat was recorded by Aliyu-Paiko and Hashim (2012) in whole body proximate composition of snakehead fingerlings, fed palm oil as a FO replacer. Similarly, in hybrid tilapia, *Oreochromis niloticus × O. aureus*, fed tea see oil for 10 weeks, significantly higher body lipid levels were obtained compared to fish fed FO (Han et al. 2013). It has been suggested that diets enriched in n-3 LC-PUFA may suppress fatty acids synthesis, simulate fatty acid β-oxidation, activate lipoprotein lipase, and reduce tri-acylglycerol synthesis, thereby leading to an overall reduction in lipid deposition and suppressing the development of obesity in mammals (Al-Hasani and Joost 2005; Madsen et al. 2005). Indeed, Todorčević et al. (2009) reported that feeding FO to Atlantic salmon, *Salmo salar*, for 21 weeks reduced white adipose tissue fat content and increased fatty acid β-oxidation activity compared to fish fed rapeseed oil.

Hematological parameters are important indicators of fish's physiological and health status (Maita 2007). Hematological responses of juvenile mandarin fish were unaffected by the complete substitution of FO in this study. There are conflicting reports concerning the effects of FO substitution by other oil sources on hematological indices, with some studies suggesting marked modifications (Ferreira et al. 2011; Peng et al. 2015; Mozanzadeh et al. 2016), while others show no discernible effects (Figueiredo-Silva et al. 2005; Twibell et al. 2012). This discrepancy can be attributed to various factors including fish species, life stage, composition and nutrient content of the diet, source or quality of the alternative oil source, and experimental conditions, mainly water temperature. However, since dietary FO replacement with alternative oils caused no detectable change either in morphological indices or in hematological parameters of juvenile mandarin fish in the present study, it can be concluded that fish were in overall good health and nutritional status.

The fatty acid composition of mandarin fish fillets closely resembled those of the experimental diets, with fish fed the LO, SO, and lard having remarkably elevated levels of ALA, LA, and OA, respectively. It is well documented that the fatty acid profile of farmed-fish fillets reflects the fatty acid composition of the dietary oil used (Bell et al. 1994). Although replacement of FO with alternative oils resulted in reduced levels of EPA and DHA, the levels of these fatty acids in the fish fillets were still higher than their respective levels in diets. This could be due either to selective retention and/or active synthesis of these fatty acids (Turchini et al. 2011b). Similar results were observed in other freshwater fish species such as rainbow trout (Caballero et al. 2002; Turchini and Francis 2009), Murray cod (Turchini et al. 2006), common carp (Ren et al. 2012), tilapia (Al-Souti et al. 2012, Li et al. 2016), Chinese long snout catfish, *Leiocassis longirostris* Günther (Choi and Lee 2015), and silver barb, *Puntius gonionotus* (Nayak et al. 2017). It is widely believed that freshwater fish can convert C_{18} PUFA of both n-6 and n-3 series to their corresponding LC-PUFA via a series of desaturation and elongation reactions (NRC 2011; Turchini et al. 2011b). However, the capacity of C_{18} PUFAs desaturation/elongation to n-3 LC-PUFAs is depended on the species (Sargent et al. 2002). Therefore, the decreased deposition of dietary ALA, LA, and OA along with good growth, feed utilization, and survival in this study is suggestive of the ability of mandarin fish to meet their essential fatty acid requirement by the use of C_{18} PUFA. It could also be assumed that residual fish oil from dietary FM was probably adequate to provide essential fatty acids for normal growth and development of juvenile mandarin fish. The results of this experiment presented a higher retention of DHA in fish fed vegetable oil-based or lard diets. Previous studies in which fish were fed vegetable oil-based diets reported high DHA retention in the muscles of salmonids (Bell et al. 2001, 2003; Caballero et al. 2002; Thanuthong et al. 2011). The preferred retention of DHA over EPA may also indicate a selective utilization of EPA over DHA when dietary levels decrease, as a means of meeting the requirements for tissue membrane integrity and function (Fountoulaki et al. 2009).

Conclusion

This study indicates that total FO replacement with LO, SO, or lard can be tolerated by juvenile mandarin fish without any pronounced adverse effects on fish performance and health. The abundance of C_{18} PUFAs provided by tested alternative oil sources coupled with some n-3 LC-PUFA from the residual lipid of FM in the basal diet appeared to meet the essential fatty acid requirements of ~ 1.8 g mandarin fish under the conditions of the present study. This probably stemmed from the ability of this species to bioconvert C_{18} PUFA to their corresponding LC-PUFA and selectively retain EPA and DHA to some extent. Our findings therefore suggest that mandarin fish juveniles have high tolerance to diets that differ markedly in fatty acid composition.

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Availability of data and materials
All datasets generated and/or analyzed during the current study are available
from the corresponding author on reasonable request.

Authors' contributions
ZS and SKH manufactured the experimental feed, performed the analyses,
and drafted the manuscript. YOK conducted the feeding trial. SML conceived
and designed the study and experimental facility and also revised the
manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Experimental protocols followed the guidelines approved by the Animal
Care and Use Committee of GWNU (GWNU-2017-9).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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