Aquaculture 461 (2016) 40–47

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Response of juvenile Japanese seabass (*Lateolabrax japonicus*) to different dietary fatty acid profiles: Growth performance, tissue lipid accumulation, liver histology and flesh texture

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ARTICLE INFO

Article history:
Received 21 March 2016
Received in revised form 24 April 2016
Accepted 25 April 2016
Available online 26 April 2016

Keywords:
Japanese seabass
Dietary fatty acid
Growth performance
Lipid accumulation
Liver histology
Flesh texture

ABSTRACT

A 10-week feeding experiment in offshore floating cages was conducted to investigate the effects of dietary fatty acid profiles on growth, tissue lipid accumulation, liver histology and flesh texture of juvenile Japanese seabass (mean initial weight 29.53 ± 0.86 g). Six low-fish meal diets were prepared with different lipid sources (10% in the diets), each with a characteristic fatty acid: Diet MCA: medium-chain fatty acid, C8:0 + C10:0; Diet PA: palmitic acid, C16:0; Diet SA: stearic acid, C18:0; Diet OA: oleic acid, C18:1n−9; Diet LNA: α-linolenic acid, C18:3n−3; Diet N-3 LC-PUFA: n−3 LC-PUFA, DHA + EPA. A diet with 10% fish oil was used as the control diet (Diet FO). Each diet was fed triplicate groups of 30 fish. The results showed that the compared to group FO, the final body weight (FBW), specific growth rate (SGR) and feed efficiency ratio (FER) were significantly reduced in groups MCA, PA, and SA, but not in groups OA, LNA, and N-3 LC-PUFA. The feed intake (FI) in group MCA was much lower compared to other groups, while group PA showed the highest FI, significantly higher than that in group N-3 LC-PUFA. Diets MCA, PA, and SA leaded to much lower lipid content in muscle, gut, and whole fish body, while the liver lipid content was significantly higher in groups PA, SA, OA, and LNA than that in groups FO, MCA, and N-3 LC-PUFA. Obvious hepatocytes swelling, lipid vacuolization and nucleus polarization were observed in liver from groups OA, SA, PA, and LNA, while in group MCA, the hepatic tissue was in disorder with indistinguishable hepatic cell outline and dissolved nuclei. Fish fed MCA, PA, and SA tended to have firmer flesh than fish fed OA, LNA, and N-3 LC-PUFA. These results showed that Japanese seabass has a relatively high tolerance to oils rich in C18:1n−9 and C18:3n−3 but high levels of C16:0 or C18:0 enriched lipid sources could reduce the feed efficiency and growth rate. High levels of medium chain fatty acids (C8:0 and C10:0) in the diets could drastically reduce the feed intake. All the alternative lipid sources leaded to impairment to liver histology and modified the flesh texture, probably via altering tissue lipid concentrations.

Statement of relevance: This study provided useful data for the application of a range of alternative lipid sources in fish feed. Also, the present results are helpful in formulating a balanced dietary fatty acid profile for Japanese seabass.

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1. Introduction

At present, limited by the shortage and consequently the high price of fish oil, the replacement of fish oil by alternative lipid sources in fish feed has been widely evaluated in both scientific studies and aquaculture practices. Compared to freshwater teleosts or anadromous fish which have a capacity to desaturate-elongate 18C fatty acids (FA) into 20–22C long chain-polyunsaturated fatty acids (LC-PUFA), marine or euryhaline fish which have limited capacity to synthesize LC-PUFA have been demonstrated to require relatively higher fish oil levels in diets to meet the LC-PUFA requirements (Tocher, 2003). A number of previous studies have observed that 0–100% fish oil in diets for marine or euryhaline fish can be replaced by alternative lipid sources such as vegetable oils and animal fat without reduction of fish growth (Turchini et al., 2011; Tocher, 2015), depending upon fish species and type of alternative lipid source. Vegetable oils, such as soybean oil, rapeseed oil, linseed oil, and palm oil, and animal fat such as poultry oil, beef tallow and lard are the most commonly used alternative lipid sources in aquafeed (Francis et al., 2007; Grant et al., 2008; Martins et al., 2009; Ng and Wang, 2011; Nogales-Mérida et al., 2011; Mozanzadeh et al., 2016). Oils containing medium chain fatty acids (MCA) such as coconut oil is also gaining more and more attention since beneficial properties of MCA, such as high digestibility (Røsjø et al., 2000) and positive effects on nitrogen retention (Nordrum et al., 2000), anti-parasite (Hirazawa et al., 2001) and energy utilization (Smith et al., 2005) have been

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http://dx.doi.org/10.1016/j.aquaculture.2016.04.023
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observed in fish studies. Meanwhile, besides growth performance, the response of fish bio-functions to fish oil replacement now is being more and more studied since the focus of aquaculture industry is gradually moving from quantity to quality.

Japanese seabass is one of the most commercially valuable aquaculture species in Asia. It is carnivorous and has the ability to adapt to a wide range of salinity. Our previous studies have found that Japanese seabass reared in seawater has a low dependence on LC-PUFAs (Xu et al., 2010) and the total replacement of fish oil by linseed oil or soybean oil in the diets did not compromise the growth performance (Xu et al., 2015). Also, other studies of ours have suggested that Japanese seabass has the capacity of LC-PUFA biosynthesis to some degree and this capacity could be stimulated by alternative lipid sources (Xu et al., 2014; Dong et al., 2015). These studies indicated the relatively high tolerance of Japanese seabass to alternative lipid sources. However, on the other hand, these studies also showed that although the growth performance of Japanese seabass was not reduced by the alternative lipid sources, the immunity were impaired and LC-PUFA composition was lowered. Therefore, it is necessary and important to evaluate the properties of alternative lipid sources not only in term of fish growth. The present study was designed to evaluate the impacts of fish oil replacement on Japanese seabass in terms of both growth performance and biological properties such as liver health and flesh quality, which are important dimensions of high quality aquaculture. On the other hand, considering that the alternative lipid sources commonly have complex fatty acids profiles and thus it is difficult to elucidate the precise mechanisms related to a certain fatty acid from previous studies using common natural alternative lipid sources, the alternative lipid sources used in the present study were purified fatty acid esters or oils highly enriched with a certain fatty acid, i.e., tricaprin/tricaprylin, tripalmitin, tristearin, oleic acid enriched rapeseed oil, α-linolenic acid enriched oil and n−3 LC-PUFA enriched oil. These lipid sources have characteristic fatty acids with different chain lengths and desaturation degrees, from medium-chain fatty acids to LC-PUFA, and mechanisms involved in the modulation of fish physiological processes by these fatty acids was expected to be indicated in this study. Moreover, to make the results more useful for the aquaculture practices, a sustainable low-fish meal basal diet was used in this study.

2. Materials and methods

2.1. Experimental diets

To lower the lipid content in the basal diet, fish meal used in the experiment was defatted first with ethanol (fish meal:ethanol = 1:2 w/v, 6 h with intermittent shaking at 45 °C water bath, three times), obtaining the lipid concentration of 1.8% dry matter. Seven low-fish meal experimental diets with similar proximate compositions (42% crude protein and 11% lipid) were formulated, differing only in the lipid source (Table 1). Six different lipid sources, tricaprin/tricaprylin (50% tricaprin + 50% tricaprylin; Big Sense Trading Co., Ltd., Guangzhou, China), tripalmitin (Shanghai Zhixin Chemical Co., Ltd., Shanghai, China), tristearin (Hudong article of everyday use Co., Ltd., Jiaxing, China), rapeseed oil, linolenic acid enriched oil (blended oil of linseed oil and perilla oil at a ratio of 3:1), and n−3 LC-PUFA enriched oil (in the form of triglyceride; Hebei Haiyuan Health Biological Science and Technology Co., Ltd., Changzhou, China), were supplemented separately to the basal diet at a ratio of 10% to obtain six diets with different characteristic fatty acids, respectively: Diet MCA, medium chain fatty acid (C8:0 + C10:0); Diet PA, palmitic acid (C16:0); Diet SA, stearic acid (C18:0); Diet OA, oleic acid (C18:1n−9); Diet LNA, α-linolenic acid (C18:3n−3); and Diet N-3 LC-PUFA, n−3 LC-PUFA (C20:5n−3 + C22:6n−3). The seventh diet, formulated with 10% fish oil (Diet FO), was used as the control diet. The diets were made, packed and stored following the common procedures in our laboratory (Xu et al., 2010). The fatty acid profiles of the lipid sources and experiment diets were presented in Tables 2 and 3 respectively.

Table 1
Formulation and proximate composition of the experiment diets (% dry matter).

Ingredients	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA
Defatted fish meal[a]	26.00	26.00	26.00	26.00	26.00	26.00	26.00
Soybean meal[a]	34.00	34.00	34.00	34.00	34.00	34.00	34.00
Wheat meal	25.91	25.91	25.91	25.91	25.91	25.91	25.91
Fish oil	10.00						
Tricaprin/tricaprylin[b]		10.00					
Tripalmitin[c]			10.00				
Tristearin[d]				10.00			
Rapeseed oil					10.00		
C18:3n−3 enriched oil[e]						10.00	
n−3 LC-PUFA enriched oil[f]							10.00
Mineral premix[g]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[h]	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Mold inhibitor[i]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Attractant[j]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Yttria	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Proximate composition							
Crude protein	42.18	42.17	41.67	41.54	42.17	42.29	42.25
Crude lipid	11.51	11.43	11.36	11.16	10.93	10.89	11.26
Ash	11.25	11.11	11.06	10.47	11.16	10.46	10.84

[a] Defatted fish meal: crude protein 75.6% dry matter, crude lipid 1.8% dry matter; soybean meal: crude protein 51.7% dry matter, crude lipid 2.0% dry matter.
[b] Tricaprin/tricaprylin: 50% tricaprin + 50% tricaprylin; Big Sense Trading Co., Ltd., Guangzhou, China.
[c] Shanghai Zhixin chemical Co., Ltd., Shanghai, China.
[d] Hudong article of everyday use Co., Ltd., Jiaxing, China.
[e] Blended oil of linseed oil and perilla oil (Linseed oil:perilla oil = 3:1).
[f] n−3 LC-PUFA enriched oil: in the form of triglyceride; Hebei Haiyuan Health Biological Science and Technology Co., Ltd., Changzhou, China.
[g] Mineral premix (mg or g/kg diet): $MgSO_4·7H_2O$, 1200 mg; $CuSO_4·5H_2O$, 10 mg; $ZnSO_4·H_2O$, 50 mg; $FeSO_4·H_2O$, 80 mg; $MnSO_4·H_2O$, 45 mg; $CoCl_2·6H_2O$ (1%), 50 mg; $NaSeSO_3·5H_2O$ (1%), 20 mg; $Ca(IO_3)_2·6H_2O$ (1%), 60 mg; $CaH_2PO_4·H_2O$, 10 g; zoelite, 8.485 g.
[h] Vitamin premix (mg or g/kg diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12} (1%), 10 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol (50%), 240 mg; ascorbic acid, 2000 mg; choline chloride (50%), 4000 mg; wheat middling, 8.47 g.
[i] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.
[j] Attractant: glycine and betaine.

2.2. Feeding trial

Japanese seabass were obtained from Haiwan Hatchery Co., Ltd., Ningbo, China. Prior to the start of the feeding trial, the juveniles were reared in floating sea cages (3.0 × 3.0 × 3.0 m), and fed a low-lipid commercial diet for 2 weeks to acclimate to the experimental conditions.

At the onset of the feeding trial, the fish were fasted for 24 h and weighed after being anesthetized with eugenol (1:10,000) (Shanghai Reagent, China). Fish of similar sizes (29.53 ± 0.86 g) were randomly distributed into 21 offshore floating cages (1.5 × 1.5 × 2.0 m) and each cage was stocked with 30 fish. Each diet was randomly assigned to triplicate cages. Fish were hand-fed to apparent satiation twice daily (05:00 and 17:00). The feeding trial lasted 10 weeks. At the end of the feeding trial, the fish were fasted for 24 h before harvest. After anesthetized with eugenol, fish in each cage was weighed individually and the total number was counted.

2.3. Analysis and measurement

2.3.1. Sampling

At the end of feeding trial, after fasted and anesthetized, 5 fish per cage were collected and stored frozen (−20 °C) for determination of the proximate composition of fish body. Another 5 fish from each cage

Table 2
Fatty acid profiles of the experimental lipids (% total fatty acid).

Fatty acid	Fish oil	Mixture of tricaprin and tricaprylin	Tripalmitin	Tristearin	Rapeseed oil	Mixture of linseed oil and perilla oil	n−3 LC-PUFA enriched oil
C8:0 + C10:0	–	78.01	–	–	–	–	–
C14:0	5.85	2.60	0.42	2.43	0.06	0.04	0.20
C16:0	18.13	4.62	71.59	27.97	4.53	5.97	2.74
C18:0	3.39	3.23	21.75	62.04	2.15	3.10	1.36
C16:1n−7	6.90	–	0.55	0.35	0.05	0.20	0.60
C18:1n−9	16.43	1.25	12.07	3.11	43.48	24.91	8.42
C20:1n−9	2.58	–	–	–	5.98	–	0.86
C22:1n−11	3.03	–	–	–	–	–	0.79
C22:1n−9	–	–	–	–	15.70	–	–
C18:2n−6	7.24	–	–	–	19.13	12.90	10.28
C20:4n−6	0.81	–	–	–	–	–	3.20
C18:3n−3	1.98	–	–	–	7.46	49.36	1.65
C18:4n−3	2.63	–	–	–	–	–	1.22
C20:5n−3	8.29	–	–	–	–	–	20.76
C22:5n−3	0.84	–	–	–	–	–	3.18
C22:6n−3	11.38	–	–	–	–	–	37.13

Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:2n−6, C20:3n−6, were not listed in the table. –: trace amount or non-detected.

were dissected to collect the liver, muscle, gut and serum, for determination of the lipid accumulation. Raw fillet samples for texture analysis and liver samples for histological structure analysis were also collected from 3 fish per cage. After collected, the tissue samples were frozen with liquid nitrogen immediately and then stored at −80 °C before usage.

2.3.2. Proximate composition, lipid concentration, and fatty acid composition

Proximate composition analysis on experimental diets and fish body were performed by the standard methods of AOAC (1995). For lipid analyses of liver, muscle and gut, extraction was done with chloroform-methanol method according to Folch et al. (1957). The triacylglycerol (TAG) and cholesterol in serum was analyzed with commercial kits (Dongou Diagnosis Co., Ltd., Wenzhou, Zhejiang, China) according to the manufacturer's instructions. The fatty acid compositions of diets were analyzed with high-performance gas chromatography. Fatty acids in freeze-dried samples were esterified first with KOH-methanol and then with HCL-methanol, on 72 °C water bath. Fatty acid methyl esters were extracted with hexane and then subjected to gas chromatography (HP6890, Agilents Technologies Inc., Santa Clara, California, USA) with a fused silica capillary column (007-CW, Hewlett Packard, Palo Alto, CA, USA) and a flame ionization detector. The column temperature was programmed to rise from 150 °C up to 200 °C at a rate of 15 °C min^{-1}, and then from 200 °C to 250 °C at a rate of 2 °C min^{-1}. Both the injector and detector temperature was 250 °C.

Table 3
Fatty acid profiles of experimental diets (% total fatty acid).[a]

Fatty acid	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA
C8:0 + C10:0	nd	63.60	0.12	0.15	0.05	0.05	nd
C14:0	4.13	3.30	0.79	1.94	0.29	0.38	0.65
C16:0	20.92	9.90	56.30	25.64	7.69	10.08	6.17
C18:0	14.25	5.87	18.60	55.78	3.62	4.99	7.13
∑SFA[b]	39.31	82.67	75.69	83.36	11.60	15.45	13.95
C16:1n−7	4.89	1.67	1.09	0.32	tr	tr	2.59
C18:1n−9	8.96	3.12	10.03	2.62	41.06	29.00	7.05
C20:1n−9	4.40	1.93	1.36	0.89	6.64	1.11	2.36
C22:1n−11	4.93	tr	0.64	0.59	tr	0.22	0.57
C22:1n−9	nd	nd	nd	nd	15.97	nd	nd
∑MUFA[b]	23.18	6.72	13.12	4.42	63.66	30.33	12.57
C18:2n−6	10.69	5.31	6.37	5.88	16.74	14.81	14.91
C20:4n−6	0.73	0.04	0.07	0.08	0.10	0.10	2.73
∑n−6[b]	11.64	5.35	6.54	6.32	16.85	14.91	17.86
C18:3n−3	2.13	0.86	0.80	1.04	5.48	35.38	2.17
C18:4n−3	1.36	0.09	0.11	0.09	0.11	0.15	0.93
C20:5n−3	6.03	0.73	0.74	0.63	0.67	0.87	15.19
C22:5n−3	0.70	0.06	0.08	0.07	0.08	0.09	1.19
C22:6n−3	9.12	0.87	0.87	0.84	0.83	1.18	28.80
∑n−3[b]	19.33	2.61	2.60	2.67	7.18	37.68	48.28
∑PUFA[b]	30.97	7.96	9.15	9.00	24.02	52.59	66.14
∑n−3LC-PUFA	15.84	1.66	1.69	1.55	1.58	2.15	45.18
∑n−3/∑n−6	1.66	0.49	0.40	0.42	0.43	2.53	2.70
C18:1n−9/∑n−3	0.46	1.20	3.85	0.98	5.72	0.77	0.15

[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:2n−6, C20:3n−6, were not listed in the table. tr: trace amount; nd: non-detected.
[b] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; n−6: n−6 unsaturated fatty acid; n−3: n−3 unsaturated fatty acid; PUFA: polyunsaturated fatty acid.

2.3.3. Histological structure of liver

Samples were fixed in Bouin's fluid. Following fixation, the samples were dehydrated in a graded ethanol series and embedded in paraffin. Sections of 4 μm were stained with hematoxylin and eosin (H&E), and then examined and digitally photographed with Nikon eclipse Ti–S microscope.

2.3.4. Flesh texture

The texture of raw skinned fillet was assayed immediately after collection. The fillet texture was analyzed with TA-XT2i Texture Analyser (Stable Micro System Ltd, UK). A texture profile analysis (TPA) was made using a cylindrical compression detector with a 3 mm diameter. The compression rate was set to 2 mm/s and the strain to 50%. Samples were compressed twice, 60 s apart (Tryggvadottir and Olafsdottir, 2000). Eight texture parameters were calculated according to Ginés et al. (2004).

2.4. Calculations and statistical methods

The following variables were calculated:

$$\text{Specific growth rate (SGR)} = (\text{Ln}\,W_t - \text{Ln}\,W_0) \times 100/t$$

$$\text{Feed efficiency ratio (FER)} = \text{Wet weight gain in g/dry feed fed in g}$$

$$\text{Feed intake (FI)} = 100 \times I_d \times 2/((W_t + W_0) \times t)$$

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

$$\text{Viscerosomatic index (VSI)}\,(\%) = \text{visceral wet weight} \times 100/\text{body wet weight}$$

$$\text{Survival}\,(\%) = N_t \times 100/N_0$$

Table 4
Growth performance of juvenile Japanese seabass fed experimental diets (means ± S.E.M., n = 3).[1]

Parameter	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA	*P* value
IBW[2] g	29.68 ± 0.45	28.72 ± 0.18	28.96 ± 0.76	29.92 ± 0.67	29.60 ± 0.77	29.87 ± 0.38	29.57 ± 0.31	0.655
FBW[2] g	115.46 ± 2.51[a]	43.31 ± 2.98[d]	89.09 ± 3.62[c]	88.85 ± 3.01[c]	95.84 ± 2.44[bc]	106.19 ± 5.23[ab]	109.29 ± 2.09[ab]	0.000
SGR[2] g d^{-1}	1.95 ± 0.00[a]	0.58 ± 0.11[d]	1.60 ± 0.05[bc]	1.55 ± 0.05[c]	1.68 ± 0.03[abc]	1.81 ± 0.06[abc]	1.87 ± 0.01[ab]	0.000
FER[2]	0.67 ± 0.04[a]	0.38 ± 0.10[bc]	0.31 ± 0.02[c]	0.42 ± 0.02[bc]	0.58 ± 0.03[abc]	0.59 ± 0.07[ab]	0.65 ± 0.05[ab]	0.004
FI[2] % d^{-1}	2.45 ± 0.08[ab]	0.79 ± 0.04[c]	2.63 ± 0.10[a]	2.46 ± 0.13[ab]	2.42 ± 0.08[ab]	2.29 ± 0.10[ab]	2.16 ± 0.07[b]	0.000
HSI[2] %	1.27 ± 0.03[bc]	1.15 ± 0.05[c]	1.63 ± 0.07[ab]	1.57 ± 0.16[ab]	1.62 ± 0.10[ab]	1.71 ± 0.02[a]	1.28 ± 0.04[bc]	0.001
VSI[2] %	9.28 ± 0.70[ab]	6.12 ± 0.18[c]	7.78 ± 0.33[bc]	7.69 ± 0.24[bc]	10.93 ± 0.08[a]	10.81 ± 0.41[a]	9.98 ± 0.59[a]	0.000
Survival %	90.67 ± 5.81	88.00 ± 4.62	85.33 ± 9.33	85.33 ± 1.33	93.33 ± 3.53	88.00 ± 6.93	90.67 ± 5.81	0.947

[1] Values in the same row with different superscript letters are significantly different ($P < 0.05$). S.E.M.: standard error of means.
[2] IBW: initial body weight; FBW: final body weight; SGR: specific growth rate; FER: feed efficiency ratio; FI: feed intake; HSI: hepatosomatic index; VSI: viscerosomatic index.

where W_t and W_0 were final and initial fish weight, respectively; I_d is feed intake as dry matter; t was duration of experimental days; N_t and N_0 were final and initial number of fish, respectively.

All data were subjected to one-way analysis of variance and correlation analysis (Two-tailed Pearson correlation analysis) where appropriate in SPSS 16.0 for Windows. All percentage data were arcsine transformed before analysis. Differences between the means were tested by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$ and the results are presented as means ± S.E.M. (standard error of the mean).

3. Results

3.1. Growth performance and survival

Fish fed MCA showed the lowest final body weight (FBW) and SGR, significantly lower ($P < 0.01$) compared to other groups (Table 4). The FBW and SGR in group PA and SA were significantly lower compared to group FO, but no significant difference was observed among groups FO, LNA, and N-3 LC-PUFA. The FBW of group OA was significantly lower compared to group FO, but the SGR of group OA was not significantly different from that of group FO. The lowest FER was observed in group PA, followed by group SA, both significantly lower ($P < 0.01$) compared to group FO. No significant difference in FER was observed among groups FO, OA, LNA, and N-3 LC-PUFA. The FI in group MCA was much lower ($P < 0.01$) compared to other groups. The highest FI was observed in group PA, significantly higher ($P < 0.01$) than that in groups MCA and N-3 LC-PUFA. Compared to group FO, the HSI was significantly increased ($P < 0.01$) only in group LNA. Compared to group FO, the VSI was reduced only in group MCA, but groups PA and SA showed significantly lower ($P < 0.01$) VSI than groups OA, LNA, and N-3 LC-PUFA. The survival was not significantly different among all dietary groups.

3.2. Body composition and tissue lipid accumulation

The whole-body moisture concentration in group MCA and SA was significantly higher ($P < 0.01$) than that in groups OA, LNA and N-3 LC-PUFA (Table 5). No significant difference in whole-body protein content was observed among all the dietary groups. Compared to group FO, groups PA and SA showed significantly lower whole-body lipid content, but group MCA showed a further lower ($P < 0.01$) value. NO significant difference was observed in lipid concentration among groups FO, OA, LNA, and N-3 LC-PUFA. Fish fed MCA showed significantly higher ash content than fish fed other diets and fish fed LNA showed the lowest ash content, significantly lower ($P < 0.01$) compared to fish fed MCA, PA, and SA.

Fish fed diet PA, SA, OA, and LNA had significantly higher ($P < 0.01$) lipid concentration in liver than fish fed FO, MCA, and N-3 LC-PUFA (Table 6). The muscle lipid in group MCA was significantly lower ($P < 0.01$) compared to other groups. Fish fed SA had significantly lower muscle lipid content than fish fed FO, LNA, and N-3 LC-PUFA. The highest muscle lipid content was observed in fish fed N-3 LC-PUFA. The gut lipid content significantly ranked as follows: FO, OA, LNA, and N-3 LC-PUFA > PA and SA > MCA ($P < 0.01$). The serum TAG concentration in groups PA and MCA was significantly lower ($P < 0.01$) compared to group FO. Compared to the control group FO, the total cholesterol (TC) concentration in serum was significantly reduced ($P < 0.05$) only in group MCA. The low density lipoprotein-cholesterol (LDL-C) in serum of fish fed MCA, PA, and OA was significantly lower ($P < 0.01$) compared to group FO. The high density lipoprotein-cholesterol (HDL-C) in serum was not significantly different among all the experimental groups.

3.3. Histological structure of liver

Hepatocytes from group FO and N-3 LC-PUFA showed normal histology (Fig. 1). In group MCA, the hepatic tissue was in disorder with indistinguishable hepatic cell outline. Some nuclei were fractured and dissolved. Obvious hepatocytes swelling, nucleus polarization and lipid vacuolization were observed in groups PA, SA, OA and LNA.

3.4. Flesh texture

Fish fed MCA, PA, and SA showed significantly higher ($P < 0.01$) flesh hardness than fish fed LNA and N-3 LC-PUFA (Table 7). The hardness in fillet from group OA was significantly lower compared to groups MCA and SA. The fracture ability of flesh from groups MCA and SA was significantly higher ($P < 0.01$) compared to groups N-3 LC-PUFA and LNA. The springiness in groups MCA and PA was significantly higher ($P < 0.01$) than that in groups OA and LNA. The chewiness and cohesiveness of fillet from group OA was significantly lower ($P < 0.01$) compared to groups MCA and PA. The flesh gumminess of group OA was significantly lower ($P < 0.01$) than that of group MCA, PA, and SA. No significant difference was observed in resilience among experimental groups.

Table 5
Whole-body proximate composition of juvenile Japanese seabass fed experimental diets (% wet weight; means ± S.E.M., $n = 3$).*

Proximate composition	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA	*P* value
Moisture	70.01 ± 0.68[bc]	74.69 ± 0.49[a]	72.38 ± 0.41[ab]	74.14 ± 0.18[a]	69.03 ± 0.50[c]	68.44 ± 0.12[c]	69.67 ± 0.86[c]	0.000
Crude protein	17.80 ± 0.18	17.44 ± 0.51	18.68 ± 0.13	18.01 ± 0.26	17.87 ± 0.23	18.35 ± 0.24	17.79 ± 0.37	0.198
Crude lipid	8.06 ± 0.72[a]	2.50 ± 0.26[c]	4.64 ± 0.33[b]	3.53 ± 0.22[bc]	8.80 ± 0.33[a]	8.73 ± 0.36[a]	7.16 ± 0.47[a]	0.000
Ash	4.52 ± 0.04[bc]	5.71 ± 0.18[a]	4.79 ± 0.03[b]	4.79 ± 0.16[b]	4.49 ± 0.13[bc]	4.17 ± 0.15[c]	4.56 ± 0.04[bc]	0.000

S.E.M.: standard error of means.
* Values in the same row with no common superscript letters are significantly different ($P < 0.05$).

Table 6
Tissue lipid concentrations in juvenile Japanese seabass fed experimental diets (means ± S.E.M., $n = 3$).[1]

Parameter	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA	P value
Liver lipid % W.W.[2]	12.96 ± 1.21^{b}	15.24 ± 1.02^{b}	23.42 ± 3.16^{a}	26.63 ± 0.74^{a}	28.07 ± 1.39^{a}	26.12 ± 1.35^{a}	10.15 ± 0.88^{b}	0.000
Muscle lipid % W.W.[2]	2.97 ± 0.09^{ab}	1.10 ± 0.33^{d}	2.10 ± 0.22^{bc}	1.73 ± 0.13^{c}	2.50 ± 0.23^{bc}	2.84 ± 0.19^{ab}	3.58 ± 0.21^{a}	0.001
Gut lipid % W.W.[2]	47.70 ± 1.41^{a}	28.95 ± 0.33^{c}	42.48 ± 1.32^{b}	42.60 ± 0.34^{b}	48.05 ± 0.73^{a}	48.10 ± 0.50^{a}	47.60 ± 0.87^{a}	0.001
Serum TAG[2] mmol L^{-1}	6.52 ± 0.73^{a}	2.11 ± 0.75^{c}	3.58 ± 0.43^{bc}	4.19 ± 0.41^{abc}	5.20 ± 0.87^{ab}	4.77 ± 0.26^{abc}	4.51 ± 0.42^{abc}	0.004
Serum TC[2] mmol L^{-1}	8.06 ± 0.93^{a}	3.31 ± 0.96^{b}	3.66 ± 0.86^{ab}	5.38 ± 0.19^{ab}	6.30 ± 1.55^{ab}	6.33 ± 0.43^{ab}	7.65 ± 1.16^{ab}	0.023
Serum HDL-C[2] mmol L^{-1}	2.49 ± 0.19	1.48 ± 0.40	1.83 ± 0.27	2.35 ± 0.15	2.27 ± 0.21	2.30 ± 0.17	2.16 ± 0.31	0.163
Serum LDL-C[2] mmol L^{-1}	5.02 ± 0.35^{a}	2.03 ± 0.37^{d}	2.76 ± 0.44^{cd}	3.48 ± 0.11^{abcd}	3.25 ± 0.26^{bcd}	4.33 ± 0.30^{abc}	4.58 ± 0.54^{ab}	0.000

[1] Values in the same row with no common superscript letters are significantly different ($P < 0.05$). S.E.M.: standard error of means.
[2] W.W.: wet weight; TAG: triacylglycerol; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol.

4. Discussion

In the present study, the most remarkable result in fish growth was that MCA (tricaprin and tricaprylin) drastically reduced the growth rate of juvenile Japanese seabass. The growth reducing effects of MCA has also been observed in another study on common carp larvae, which showed that fish fed the diet with 10% tricaprylin showed reduced growth rate than fish fed the diet with 10% triolein or 10% coconut oil (Fontagné et al., 1999). However, beneficial effects of MCA on nutrient utilization and health status of fish have also been observed by other workers (Nordrum et al., 2000; Røsjø et al., 2000; Hirazawa et al., 2001; Smith et al., 2005). Fontagné et al. (2000) observed that both tricaprylin and tricaproin can initially stimulate growth of common carp larvae during the first week. These discrepancies could mainly be related to the dietary MCA concentration. Another study by Fontagné et al. (2001) found that high levels of caprylic acid (˃2%) decreased fish growth but that low levels were well utilized by carp larvae, irrespective of the form of supply. With respect to the cause of the growth reduction in the MCA fed fish, in the present study it was observed that the feed intake was drastically reduced by dietary MCA. An obvious

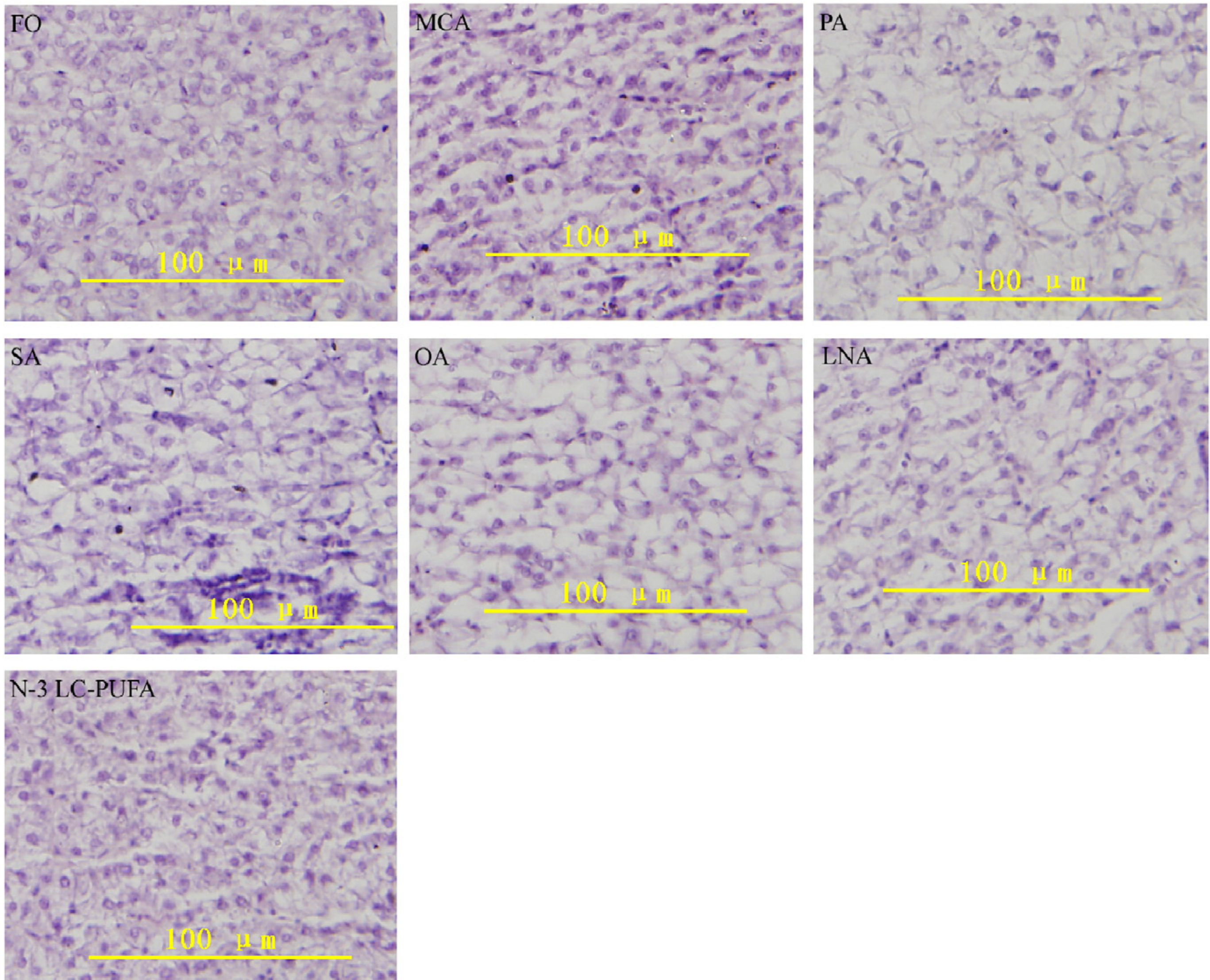

Fig. 1. Histological structure of liver from juvenile Japanese seabass fed experimental diets (HE staining, ×40).

Table 7
Fillet texture of juvenile Japanese seabass fed experimental diets (means ± S.E.M., $n = 3$).*

Parameter	FO	MCA	PA	SA	OA	LNA	N-3 LC-PUFA	*P* value
Hardness g	326.21 ± 14.11^{abc}	361.66 ± 4.64^{a}	354.73 ± 3.21^{ab}	359.00 ± 11.72^{a}	305.95 ± 9.11^{bc}	303.75 ± 14.25^{c}	302.73 ± 9.10^{c}	0.001
Fracture ability	281.55 ± 6.55^{abcd}	303.38 ± 6.26^{a}	287.50 ± 4.97^{abc}	295.28 ± 2.70^{ab}	277.69 ± 3.37^{bcd}	267.52 ± 4.53^{cd}	261.24 ± 4.66^{d}	0.000
Springiness	1.02 ± 0.01^{bc}	1.39 ± 0.09^{a}	1.27 ± 0.05^{ab}	1.06 ± 0.03^{bc}	0.94 ± 0.04^{c}	0.94 ± 0.04^{c}	1.03 ± 0.09^{bc}	0.001
Chewiness g	109.29 ± 12.41^{abc}	166.34 ± 0.92^{a}	146.07 ± 20.91^{ab}	120.94 ± 17.45^{abc}	80.29 ± 4.77^{c}	93.57 ± 7.79^{bc}	98.14 ± 10.22^{bc}	0.003
Gumminess g	105.13 ± 3.98^{abc}	121.66 ± 6.84^{a}	112.72 ± 2.86^{ab}	108.31 ± 5.81^{ab}	84.33 ± 1.31^{c}	99.43 ± 2.79^{abc}	92.01 ± 6.67^{bc}	0.001
Cohesiveness	0.32 ± 0.01^{ab}	0.34 ± 0.01^{a}	0.34 ± 0.02^{a}	0.32 ± 0.02^{ab}	0.27 ± 0.02^{b}	0.33 ± 0.01^{ab}	0.30 ± 0.01^{ab}	0.028
Resilience	0.11 ± 0.02	0.09 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.15 ± 0.04	0.11 ± 0.01	0.369

S.E.M.: standard error of means.
* Values in the same row with no common superscript letters are significantly different ($P < 0.05$).

feed rejection of MCA diets was observed during the feeding trial. A previous study on juvenile Atlantic salmon has also showed the reduced feed intake by fish oil replacement (10% of the diet) with medium chain triglycerides (Nordrum et al., 2000). In the present study, considering the rejection of MCA diets by Japanese seabass, the MCA group was actually a starvation group. Thus, using the present data it was no more reasonable to compare the effects of MCA with other fatty acids on fish bio-functions. The high dietary MCA concentration was probably the primary factor causing feed rejections. Thus, the amount of MCA integrated into commercial diets should be considered carefully.

Not only MCA, glycerides of palmitic acid (PA, C16:0) and stearic acid (SA, C18:0), i.e., tripalmitin and tristearin, also reduced the growth rate of Japanese seabass. Few previous studies have been conducted on application of purified palmitic acid (C16:0) or stearic acid (C18:0) in fish feed. In fish studies, alternative lipid sources containing high levels of PA or SA, such as palm oil (rich in C16:0), beef tallow (rich in C18:0) and lard (rich in C18:0) showed various efficacy depending on fish species and dietary lipid concentration. Palm oil could totally replace the fish oil in diets for some freshwater fish (Bahurmiz and Ng, 2007; Ng and Wang, 2011) but could only replace <70% fish oil in diets for marine fish without reducing fish growth (Fountoulaki et al., 2009), in particular only 40–50% for Japanese seabass (Han et al., 2012). Lard could totally replace fish oil even in some marine species (Nogales-Mérida et al., 2011) but the effective levels of beef tallow in marine fish diets were much lower, generally at most replacing 50% dietary fish oil (Mozanzadeh et al., 2016). The worse performance of beef tallow compared to lard might be attributed to the higher concentration of C16:0 + C18:0 in beef tallow (approx. 50% of TFA Vs approx. 40% in lard). Nevertheless, although the concentration of C16:0 + C18:0 in palm oil is also very high (approx. 50% of TFA), palm oil shows a relatively better potential as alternative lipid source in fish diets. This might be explained by the relatively higher concentration of oleic acid (OA, C18:1n−9) and linoleic acid (LA, C18:2n−6) in palm oil. Overall, high levels of dietary PA and SA were detrimental to growth performance of marine or euryhaline fish. In addition, based on the present data on Japanese seabass, no significant difference was observed between the effects of PA and SA on fish growth.

Different from MCA, PA and SA reduced Japanese seabass growth via reducing feed efficiency in this study. Studies on Atlantic salmon and yellowtail reported that dietary palm oil lead to reductions in FA apparent digestibility and lipid absorption (Torstensen et al., 2000; Khaoian et al., 2014), which consequently lead to lower lipid accumulation in muscle, intestine and whole body (Olsen et al., 2000). In the present study, it was also observed that the PA inclusion lead to reduced lipid accumulation in muscle, gut, serum and whole body. However, the liver lipid concentration was significantly increased in the PA or SA fed Japanese seabass, in accordance with what observed in sea bream fed lard and beef tallow (Montero et al., 2001; Nogales-Mérida et al., 2011). The lipid accumulation in liver probably consequently leaded to the increased HSI and liver steatosis of Japanese seabass.

In this study, the oleic acid (OA, C18:1n−9) or linolenic acid (LNA, C18:3n−3) enriched vegetable oil did not compromise the growth of Japanese seabass. Oils rich in OA or LNA, such as rapeseed oil, canola oil, linseed oil and flaxseed oil are widely used alternative lipid sources in fish feed. In Murray cod, total replacement of fish oil with linseed oil did not reduce the fish growth (Francis et al., 2007). Martins et al. (2009) even found that the apparent digestibility coefficient of flaxseed oil was higher than that of herring oil in Atlantic halibut. However, in other marine or euryhaline species such as European sea bass (Mourente et al., 2005), sablefish (Friesen et al., 2013), and Atlantic salmon (Menoyo et al., 2007), only when linseed oil replaced dietary FO at a proportion of 0–75% were there no negative effects on growth. The effective levels of OA rich oils such as canola oil, rapeseed oil, or even oleine oil in fish diets also vary with different fish species, i.e., e.g., 100% replacement for spring chinook salmon parr (Huang et al., 2008), brook charr (Guillou et al., 1995), post-smolt Atlantic salmon (Bell et al., 2003), and brown trout (Turchini et al., 2003), 70% for red sea bream (Huang et al., 2007), 60% for European sea bass (Mourente et al., 2005) and gilthead seabream (Caballero et al., 2003), and 50% for fall chinook salmon (Grant et al., 2008). It seemed that these discrepancies were probably mainly attributed to the different tolerance to alternative lipid sources of different fish species. Results of the present study suggested that Japanese seabass has a high tolerance to OA and LNA enriched oil, as evidenced by our previous studies with linseed oil and soybean oil on this fish (Xu et al., 2015). The certain capacity of LC-PUFA biosynthesis from 18C precursor fatty acids in Japanese seabass, as well as the stimulation of this capacity by alternative lipid sources, as suggested in our studies on Δ6 fatty acyl desaturase of Japanese seabass, could partly contribute to this high tolerance of Japanese seabass to OA and LNA diets.

Although the growth of Japanese seabass was not affected by LNA and OA, the liver lipid concentration was significantly increased. The increase of lipid accumulation in fish liver by LNA or OA rich oils has also been observed in studies on Arctic char (Pettersson et al., 2009) and European sea bass (Mourente et al., 2005). Liver lipid accumulation caused by alternative lipid sources was assumed to be a main factor leading to the histopathological lesions in fish liver. In this study, all the treatments with PA, SA, OA and LNA showed increased liver lipid concentration and accompanied impairments of liver histological structure, although at different levels. In these groups, obvious hepatocytes swelling, lipid vacuolization, and nucleus polarization and were observed. In other studies on gilthead seabream and turbot, concomitant promoted lipid accumulation and liver histopathology have also been observed when fish was fed high levels of alternative lipid sources such as beef tallow, palm oil, soybean oil, linseed oil, olive oil, sunflower oil, and rapeseed oil (Montero et al., 2001; Caballero et al., 2004; Wassef et al., 2009; Fountoulaki et al., 2009). Imbalanced fatty acid profiles in alternative lipid sources were assumed to be the main factor inducing lipid metabolism disturbance in fish liver. Deficiency of essential fatty acids, i.e., LC-PUFA for marine fish, especially DHA, has been reported to be the preferential factor inducing metabolism disorder and consequently liver histopathology (Bransden et al., 2005). It would result in impaired lipoprotein synthesis and subsequent impaired lipid transportation (Sargent et al., 1989; Olsen et al., 2000). Besides, a suitable n−3/

n − 6 fatty acid ratio has also been reported to be important in maintaining normal liver histology of fish (Robaina et al., 1998). In the present study, the histological alterations induced by the LNA diet was relatively milder compared to diets with other alternative lipids. This might be attributed to a more balanced n − 3/n − 6 fatty acid ratio in the LNA diet for Japanese seabass. In addition, besides the fatty acid profile, sufficient dietary polar lipid is necessary for the normal lipid transport and consequently beneficial to the regular lipid accumulation and normal liver histology (Robaina et al., 1998). The precise mechanisms involved in the modulation of lipid accumulation and liver histology by alternative oils was still not clear up to date, but the impairment to lipid metabolism and liver health by high levels of vegetable oils or animal fat in fish diets should be considered carefully.

Besides the histological modulations, the alterations in lipid accumulation and fatty acid profiles in fish tissues probably also mediates the effects of alternative lipid sources on another fish biological process, i.e., flesh texture. In this study, groups PA and SA generally showed higher values of flesh texture attributes, such as hardness, springiness, chewiness, gumminess, and cohesiveness, than groups OA and LNA. Since the muscle fatty acid composition reflected closely those of the diets (Supplementary Table 5 in Xu et al., 2014, online at doi: 10.1371/journal.pone.0087726), this result indicated that C16:0 and C18:0 enriched oils tend to lead to firmer flesh than oils rich in C18:1n − 9 or C18:3n − 3. Studies by Stejskal et al. (2011) and Fuentes et al. (2010) also showed that fish having a higher content of saturated fatty acids exhibited higher values of hardness, springiness, cohesiveness, and gumminess in raw fillet than fish having a higher content of monounsaturated fatty acids. However, a number of studies have demonstrated that the lipid content other than the fatty acid profile was a more important chemical basis for flesh texture forming (Venugopal and Shahidi, 1996; Johnston et al., 2006). Many investigations have showed that the increase of fat content leads to a softening of fish flesh, i.e., a decrease in firmness (Grigorakis et al., 2003; Ginés et al., 2004; Johnston et al., 2006; Suárez et al., 2014), and this was confirmed by the present study, which showed that groups MCA, PA and SA having lower lipid content in muscle exhibited higher values of texture attributes than groups OA, LNA and N-3 LC-PUFA having higher muscle lipid contents. A significant negative correlation ($r = -0.700$, $P = 0.000$) between muscle lipid concentration and flesh hardness was observed in the present results. Although there are many other properties of muscle components influencing flesh texture such as the myofibrillar, connective tissue proteins and moisture content (Venugopal and Shahidi, 1996; Johnston et al., 2006), they were not discussed in the present study since these components was not assayed in this study.

Additionally, in this study, besides the control group with fish oil, another group with n − 3 LC-PUFA enriched oil, of which the n − 3 LC-PUFA concentration was three times that of fish oil, was included in the present study in order to evaluate whether excess LC-PUFA has negative effects on juvenile Japanese seabass. However, the present results showed that none of the parameters was significantly different between the fish oil group and the group with n − 3 LC-PUFA enriched oil, indicating that Japanese seabass has a relatively high tolerance to LC-PUFA. However, this tolerance was limited to n − 3 LC-PUFA, since our previous studies have suggested that excess dietary arachidonic acid (ARA, C20:4n − 6) exerted negative effects on both growth performance and immune responses of Japanese seabass (Xu et al., 2010).

In conclusion, Japanese seabass has a relatively high tolerance to oils rich in C18:1n − 9 and C18:3n − 3 and total fish oil replacement (10% in the diet) with these oils did not compromise the growth performance. However, high levels of C16:0 or C18:0 enriched lipid sources in the diets can reduce the feed efficiency and growth rate. High levels of medium chain fatty acids (C8:0 and C10:0) in the diets drastically reduced the feed intake. All the alternative lipid sources leaded to impairment to liver histology and modified the flesh texture, probably via altering tissue lipid concentrations.

Acknowledgments

This work was supported by Special Fund for Agro-scientific Research in the Public Interest (201003020).

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