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Replacement of Fish Oil with Linseed Oil or Soybean Oil in Feeds for Japanese Seabass, *Lateolabrax japonicus*: Effects on Growth Performance, Immune Response, and Tissue Fatty Acid Composition

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Abstract

A 66-d feeding experiment was conducted to investigate the utilization of vegetable oils in Japanese seabass (initial weight: 10.09 ± 0.70 g). In experimental diets, linseed oil (LO) or soybean oil (SO) was used to replace 1/3, 2/3, and 3/3 of fish oil (FO) (Diets F_2L_1, F_1L_2, and LO, respectively, or Diets F_2S_1, F_1S_2, and SO, respectively). A diet with FO alone was used as the control diet. LO or SO supplementation did not reduce the specific growth rate (3.06–3.29%/d) and feed efficiency ratio (0.75–0.83) of fish, but group F_2L_1 showed significantly better growth ($P < 0.05$) and feed utilization ($P < 0.01$) than group SO. Total replacement of FO with LO or SO significantly reduced certain non-specific innate immune responses. Total replacement of FO by LO significantly increased the lipid content of fish. Concentrations of long-chain polyunsaturated fatty acids (LC-PUFAs) in whole fish and tissues were significantly reduced by LO or SO supplementation. In conclusion, LO or SO supplementations did not reduce the growth of Japanese seabass but reduced the immune responses and LC-PUFA concentrations. LO was a better lipid source than SO for Japanese seabass in terms of fish growth and immune responses.

Over the past decade, the expansion of marine fish culture has rapidly increased the demand of fish oil (FO), which supplies long-chain polyunsaturated fatty acids (LC-PUFAs), that is, arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). However, the supply of FO has been decreasing due to overfishing. Thus, there is increasing interest in alternatives to FO (Mourente et al. 2005; Piedecausa et al. 2007; Peng et al. 2008; Tacon and Metian 2008; Martins et al. 2009, 2011; Seierstad et al. 2009; Asdari et al. 2011; Ganga et al. 2011; Karalazos et al. 2011; Trushenski et al. 2011; Friesen et al. 2013a, 2013b).

Linseed oil (LO) and soybean oil (SO) are the most commonly used alternative lipid sources for their stable supply and high contents of 18-carbon PUFAs, α-linolenic acid (ALA, 18:3n-3) in LO and linoleic acid (LNA, 18:2n-6) in SO. Compared to freshwater fish which has relatively high capacity to synthesize LC-PUFAs from ALA and LNA (Tocher 2003), marine fish could only use SO and LO at a limited level (Glencross 2009; Tocher 2010). Replacement of FO with LO or SO in diets of marine fish could reduce the growth and survival, and modulate a number of physiological processes such as lipid metabolism, energy utilization, and immune response (Bell et al. 1999; Tocher et al. 2000; Ringø et al. 2002; Mourente et al. 2005; Martins et al. 2009; Ganga et al. 2011; Friesen et al. 2013a). However, other studies reported that total replacement of FO with SO or LO did not reduce the growth of marine

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fish (Grisdale-Helland et al. 2002; Francis et al. 2007; Piedecausa et al. 2007). Thus, it appeared that the tolerance to SO or LO of marine fish varies according to species, making specific studies necessary to evaluate the utilization of SO and LO in certain cultured species. This study was conducted to investigate the effects of partial or total replacement of FO with SO or LO on Japanese seabass, which is one of the most important marine culture species in western Pacific areas.

A previous study on Japanese seabass, *Lateolabrax japonicus*, with alternative lipid sources such as pork lard, beef tallow, poultry fat, and corn oil have indicated the potential of this fish in using alternative lipid sources, showing that replacing half of the dietary FO with these alternative lipid sources did not reduce the growth and feed utilization (Xue et al. 2006). This potential was confirmed by one of our previous studies which observed that Japanese seabass has a low ARA requirement (Xu et al. 2010). Also, our recent studies have investigated the gene expression of Δ6 fatty acyl desaturase in Japanese seabass and its response to alternative lipid sources (Xu et al. 2014). The results suggested that Japanese seabass has the capacity of LC-PUFA biosynthesis to some degree and this capacity could be stimulated by alternative lipid sources.

Based on the promising potential of Japanese seabass in utilizing alternative lipid sources, the aim of this study was to evaluate and compare the utilization of LO and SO, two of the most important alternative lipid sources for aquaculture stated above, in Japanese seabass. Growth performance, health status, and body composition were used as evaluating indicators in this study. Non-specific immunity, which is more important for disease resistance than specific immune system in fish (Anderson 1992), was used as the indicator of health status as it has been widely demonstrated that non-specific immune responses were closely related to dietary lipids and fatty acids (Calder 2001, 2008; Grisdale-Helland et al. 2002; Wu et al. 2002; Lin and Shiau 2003; Montero and Kalinowski 2003; Montero et al. 2008, 2010; Glencross 2009; Wu and Chen 2012).

Materials and Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations in the Guide for the Use of Experimental Animals of Ocean University of China. The protocols for animal care and handling used in this study were approved by the Institutional Animal Care and Use Committee of Ocean University of China (Permit Number: 20001013). Before sacrificing and handling, experimental fish were first anesthetized with eugenol (1:10,000) (Shanghai Reagent, Shanghai, China), and all efforts were made to minimize suffering.

Diet Formulation

The basal practical diet was formulated to contain approximately 42% crude protein and 12% lipid, which have been shown to be sufficient to support the optimum growth of Japanese seabass (Table 1). Oil was included at 9% of dry matter, in which the proportion of FO to LO or SO was 2:1, 1:2, and 0:3 (Diets F_2L_1, F_1L_2, and LO, respectively, or Diets F_2S_1, F_1S_2, and SO, respectively). A diet with 9% FO (Diet FO) was added as the control diet (Table 1). Fatty acid profiles of the experimental diets are presented in Table 2.

Ingredients were ground into fine powder through a 200-μm mesh. All ingredients were thoroughly mixed with the oils, and water was added to produce a stiff dough. The dough was then pelleted with an experimental feed mill and dried for approximately 12 h in a ventilated oven at 45 C. The dried diets were broken up, sieved into proper pellet size (1.5×5.0 mm, 2.5×5.0 mm), and stored at −15 C until use.

Feeding Procedure

Japanese seabass were obtained from a commercial farm in Ningbo, China. Prior to starting the experiment, the juvenile seabass were reared in floating sea cages ($3.0 \times 3.0 \times 3.0$ m), and fed a low-lipid commercial diet for 2 wk to acclimate to the experimental conditions.

At the onset of the feeding trial, the fish were allowed to fast for 24 h and weighed after being

TABLE 1. *Formulation and proximate chemical composition of the experimental diets (% dry matter).*

	Diets						
Ingredient	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO
Fish meal[1]	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Soybean meal[2]	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Wheat meal	25.9	25.9	25.9	25.9	25.9	25.9	25.9
FO	9.0	6.0	3.0		6.0	3.0	
LO		3.0	6.0	9.0			
SO					3.0	6.0	9.0
Mold inhibitor[3]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[4]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premix[5]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Proximate composition							
Crude protein	42.30	42.27	41.71	42.14	42.09	42.61	42.17
Crude lipid	11.95	11.89	11.70	11.17	11.74	12.02	12.03
Ash	13.40	12.43	13.35	12.20	13.37	12.36	12.19

FO = fish oil; LO = linseed oil; SO = soybean oil.

[1] Fish meal: crude protein 74.3% dry matter, crude lipid 6.63% dry matter.

[2] Soybean meal: crude protein 49.4% dry matter, crude lipid 0.95% dry matter.

[3] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

[4] Mineral premix (per kg of diet): $MgSO_4 \cdot 7H_2O$, 1200 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $FeSO_4 \cdot H_2O$, 80 mg; $MnSO_4 \cdot H_2O$, 45 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $NaSeSO_3 \cdot 5H_2O$ (1%), 20 mg; $Ca(IO_3)_2 \cdot 6H_2O$ (1%), 60 mg; $CaH_2PO_4 \cdot H_2O$, 10 g; and zoelite, 8.485 g.

[5] Vitamin premix (per kg of 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 acid, 200 mg; folic acid, 20 mg; biotin (2%), 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol (50%), 240 mg; ascorbic acid, 2000 mg; choline chloride (50%), 4000 mg; ethoxyquin 150 mg, and wheat middling, 12.32 g.

anesthetized with eugenol (1:10,000) (Shanghai Reagent). Fish of similar sizes (body weight: 10.09 ± 0.70 g) were randomly distributed into 21 sea cages ($1.5 \times 1.5 \times 2.0$ 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 (0500 and 1700 h). The feeding trial lasted 66 d. During the feeding trial, temperature ranged from 23.5 to 30.5 C, salinity from 28 to 32 g/L, and the dissolved oxygen content from 6 to 7 mg/L.

Sampling and Collection of Growth Data

At the end of the feeding trial, the fish were allowed to fast for 24 h before harvest. Then, the experimental fish were anesthetized with eugenol (1:10,000) (Shanghai Reagent), and after that the total fish number and body weights of fish individuals in each cage were measured. A sample of 10 fish per cage were collected and stored at −20 C for the determination of proximate carcass composition and whole-body fatty acid composition. Liver and muscle samples were collected from three fish per cage and stored at −80 C for the assay of fatty acid compositions. Blood (serum) samples were collected from five fish per cage and after the blood samples were taken, these five fish were sacrificed and subjected to the isolation of head kidney macrophages immediately. The detailed procedures of serum collection and isolation of head kidney macrophages are available in our previous publications (Xu et al. 2010, 2015).

Immune Function Assays

The assay of immune parameters, such as phagocytic index (PI) and respiratory burst (RPB) activity of head kidney macrophages, and the activities of lysozyme (LYZ), alternative complement pathway (ACP), superoxide dismutase (SOD), and catalase (CAT) in serum, are available in our previous publications (Xu et al. 2010, 2015). Briefly, the phagocytic activity was determined by assaying the phagocytosis of

TABLE 2. *Fatty acid composition of the experimental diets for Japanese seabass (% total fatty acids, means of duplicate assays).*[1]

	Diets						
Fatty acid	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO
C14:0	2.92	2.29	1.41	0.85	2.18	1.78	0.91
C16:0	13.80	12.44	9.73	8.32	12.84	13.89	12.70
C18:0	3.55	3.68	3.00	2.96	3.70	4.49	4.73
∑SFA	20.28	18.41	14.15	12.13	18.71	20.16	18.33
C16:1n-7	4.28	3.60	2.38	1.55	3.21	2.59	1.34
C18:1n-9	16.31	19.00	16.40	16.62	18.91	21.38	21.40
C20:1n-9	2.12	2.16	2.04	2.16	1.70	1.55	0.95
∑MUFA	22.70	24.76	20.83	20.33	23.82	25.52	23.69
C18:2n-6	13.72	13.99	12.65	13.18	20.39	30.24	38.26
C20:4n-6	0.51	0.43	0.25	0.15	0.41	0.37	0.17
∑n-6	14.23	14.42	12.90	13.33	20.80	30.61	38.43
C18:3n-3	1.77	9.26	15.98	23.38	2.53	3.21	3.78
C18:4n-3	0.62	0.48	0.30	0.19	0.45	0.37	0.19
C20:5n-3	4.35	3.45	2.12	1.31	3.24	2.63	1.33
C22:5n-3	1.75	1.31	0.69	0.32	1.20	0.89	0.32
C22:6n-3	5.63	4.60	3.11	2.29	4.32	3.67	2.21
∑n-3	14.12	19.10	22.19	27.49	11.74	10.77	7.82
∑PUFA	28.35	33.52	35.09	40.82	32.54	41.38	46.24
∑n-3 LC-PUFA	11.73	9.36	5.91	3.92	8.76	7.19	3.85
∑n-3/∑n-6	0.99	1.32	1.72	2.06	0.56	0.35	0.20
C18:1n-9/∑n-3	1.16	0.99	0.74	0.60	1.61	1.98	2.74

FO = fish oil; LO = linseed oil; MUFA = monounsaturated fatty acid; n-3 = n-3 unsaturated fatty acid; n-6 = n-6 unsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; SO = soybean oil.

[1] 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, and C20:3n-6, were not listed in the table.

yeast into head kidney macrophages. The production of intracellular superoxide anion (O_2^-) was evaluated using nitroblue tetrazolium. The LYZ activity was determined by measuring the decrease of the absorbance of *Micrococcus lysodeikticus* suspension caused by fish serum. The ACP activity was assayed by measuring the hemolysis of rabbit red blood cells by fish serum. The SOD activity was measured spectrophotochemically by the ferricytochrome C method using xanthine/xanthine oxidase as the source of superoxide radicals. The CAT activity was determined by measuring the decrease of H_2O_2 concentration caused by fish serum.

Proximate Composition Analysis and Fatty Acid Composition Analysis

Proximate composition analysis on experimental diets and fish body was performed using the standard methods of AOAC (1995). Samples of diets and fish were oven-dried at 105 C to a constant weight for moisture assay. Protein was determined by measuring nitrogen ($N \times 6.25$) using the Kjeldahl method; lipid by ether extraction using Soxhlet; and ash by combustion at 550 C. The fatty acid compositions of diets and fish tissues were determined with gas chromatography (GS, HP6890; Agilent, Santa Clara, CA, USA), as described by Mourente et al. (1999) (with minor modifications). Results are expressed as the percentage of each fatty acid with respect to total fatty acids (TFAs).

Calculations and Statistical Analysis

The following variables were calculated:

$$\text{Specific growth rate (SGR)} = \left(\ln W_t - \ln W_0\right) \times 100/t;$$

$$\text{Feed efficiency ratio (FER)} = \text{wet weight gain in g/dry feed feding;}$$

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

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

$$\text{PI} = \text{Number of yeast ingested} / \text{number of macrophages observed},$$

where W_t and W_0 were final and initial fish weight, respectively; N_t and N_0 were final and initial number of fish, respectively; and t was the experiment duration.

All data were subjected to one-way ANOVA and correlation analysis where appropriate in SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). All percentage data were arcsine transformed before analysis. Differences between the means were examined by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$ and the results are presented as mean ± SE of the mean.

Results

Dietary Fatty Acid Profile

In experimental diets, the replacement of FO by LO or SO decreased the contents of LC-PUFAs, 18:4n-3, and 16:1n-7, but increased the contents of ALA or LNA (Table 2). The content of ALA increased from 1.77% of TFA in Diet FO to 23.38% of TFA in Diet LO and the content of LNA increased from 13.71% of TFA in Diet FO to 38.26% of TFA in Diet SO.

With increasing level of FO replacement by LO (Diets FO, F_2L_1, F_1L_2, and LO), dietary contents of saturated fatty acid (14:0, 16:0, and 18:0) slightly decreased, while with increasing level of FO replacement by SO (Diets FO, F_2S_1, F_1S_2, and SO), the contents of 14:0 and 20:1n-9 slightly decreased but the contents of 18:0 and 18:1n-9 slightly increased.

Growth Performance

No significant difference in SGR was observed either among groups FO, F_2L_1, F_1L_2, and LO or among groups FO, F_2S_1, F_1S_2, and SO (Table 3). Group F_2L_1 showed significantly higher ($P < 0.05$) SGR than group SO. The final weight followed the same pattern with SGR. The FER in group SO was significantly lower ($P < 0.01$) than that in groups FO, F_2L_1, and F_1L_2. Group F_2L_1 showed the highest FER among dietary treatments, significantly higher than that in group F_1S_2 and SO. No significant difference in FER was observed either among groups FO, F_2L_1, F_1L_2, and LO or among groups FO, F_2S_1, F_1S_2, and SO. No significant difference in HSI was observed among dietary groups. The survival was high (88–94%) in all groups and no significant difference was observed among dietary treatments (Table 3).

Immune Parameters

The PI of head kidney macrophage in groups LO and SO was significantly lower ($P < 0.01$) compared to group FO (Table 4). No significant difference in PI was observed either between the LO and SO groups or among other groups. No significant difference in RPB activity of head kidney macrophage was observed among all dietary treatments.

The activity of serum LYZ in group LO was significantly lower ($P < 0.05$) compared to groups FO and F_1S_2 (Table 4). No significant difference was observed among groups F_2S_1, F_1S_2, SO, and FO. No significant difference in serum activity of ACP was observed among all dietary treatments.

The activity of serum SOD in group SO was significantly lower ($P < 0.01$) compared to groups FO and F_2L_1 (Table 4). Group F_2L_1 showed the highest serum SOD activity, significantly higher compared with other treatments except the control group. No significant difference in serum SOD was observed among groups F_2L_1, LO, F_2S_1, F_1S_2, and FO. The activity of serum CAT showed a similar trend with the activity of SOD. Groups F_2S_1, F_1S_2, and SO showed significantly lower ($P < 0.01$) activities of serum CAT compared with group F_2L_1. No significant difference in serum CAT activity was observed either among groups FO, F_2L_1, F_1L_2, and LO or among groups FO, F_2S_1, F_1S_2, and SO.

TABLE 3. *Growth performance of Japanese seabass fed the experimental diets (means ± SEM, n = 3).*[1]

Growth response	Diets							*P*-value
	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	
Initial weight (g)	10.09	10.09	10.09	10.09	10.09	10.09	10.09	
Final weight (g)	83.4 ± 3.2^{ab}	88.8 ± 3.3^{a}	85.6 ± 3.5^{ab}	84.7 ± 1.1^{ab}	87.3 ± 2.2^{ab}	77.2 ± 3.3^{ab}	76.0 ± 1.6^{b}	0.034
SGR (%/d)	3.20 ± 0.06^{ab}	3.29 ± 0.06^{a}	3.24 ± 0.06^{ab}	3.22 ± 0.02^{ab}	3.27 ± 0.04^{ab}	3.08 ± 0.06^{ab}	3.06 ± 0.03^{b}	0.028
FER	0.82 ± 0.01^{ab}	0.83 ± 0.01^{a}	0.81 ± 0.01^{ab}	0.78 ± 0.01^{abc}	0.80 ± 0.01^{abc}	0.77 ± 0.02^{bc}	0.75 ± 0.02^{c}	0.003
HSI (%)	1.20 ± 0.08	1.11 ± 0.02	1.07 ± 0.12	1.46 ± 0.08	1.09 ± 0.11	1.12 ± 0.06	1.29 ± 0.13	0.090
Survival (%)	91.2 ± 4.5	93.1 ± 3.5	94.1 ± 0.0	94.1 ± 1.7	90.2 ± 3.5	96.1 ± 1.0	92.2 ± 2.6	0.798

FER = feed efficiency ratio; FO = fish oil; HSI = hepatosomatic index; LO = linseed oil; SGR = specific growth rate; SO = soybean oil.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$).

Chemical Composition of Fish

The whole-body moisture content in group LO was significantly lower ($P < 0.05$) compared with groups FO, F_2L_1, and F_1L_2 (Table 5). The whole-body lipid content showed an opposite trend with the moisture content, with the value in group LO significantly higher ($P < 0.05$) compared with groups FO, F_2L_1, and F_1L_2. No significant difference in whole-body contents of ash and protein was observed among all dietary treatments.

The fatty acid composition of whole body, liver, and muscle is presented in Tables 6, 7, and 8, respectively. With increasing level of dietary LO supplementation, the contents of 14:0, 16:0, 16:1n-7, 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3 in whole body and tissues significantly decreased while the contents of 20:1n-9 and 18:3n-3 significantly increased. With increasing level of dietary SO supplementation, the contents of 14:0, 16:0, 16:1n-7, 18:1n-9, 20:1n-9, 20:4n-6, 20:5n-3, 22:5n-3, and 22:6n-3 in whole body and tissues significantly decreased while the contents of 18:0, 18:2n-6, and 18:3n-6 significantly increased. The n-3 : n-6 fatty acid ratio significantly increased with increasing level of dietary LO supplementation and significantly decreased with increasing level of dietary SO supplementation.

With the contents of most fatty acids in fish tissue reflecting closely those of the diets, the 18:4n-3 was an exception. Significant positive correlations were not observed between fish 18:4n-3 concentrations and dietary 18:4n-3 content (the correlation coefficient [*r*] and *P* value were −0.127 and 0.556, respectively, for whole body, and −0.469 and 0.021, respectively, for liver) but observed between tissue 18:4n-3 contents and the dietary 18:3n-3 content (*r* and *P* value were 0.827 and 0.000, respectively, for whole body, and 0.929 and 0.000, respectively, for liver).

Discussion

In this study, partial or total replacement of FO with LO or SO did not reduce the growth of juvenile Japanese seabass. This was consistent with previous findings in other marine or euryhaline fish such as sharpsnout seabream (Piedecausa et al. 2007), Murray cod (Francis et al. 2007), and Atlantic salmon (Grisdale-Helland et al. 2002). 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, other studies on marine fish such as turbot (Bell et al. 1999; Regost et al. 2003), gilthead sea bream (Montero et al. 2008), starry flounder (Lee et al. 2003), cobia (Trushenski et al. 2011), and black seabream (Peng et al. 2008) suggested that total replacement of FO with LO or SO significantly decreased the growth rate, and only when LO or SO replaced dietary FO at a proportion of 0–80% were there no negative effects on growth (Figueiredo-Silva et al. 2005; Mourente et al. 2005; Francis et al. 2006; Menoyo et al. 2007; Peng et al. 2008; Martins et al. 2011; Trushenski et al. 2011; Friesen et al. 2013a). Although these discrepancies could be related to different dietary protein sources, levels of residual FO in fish meal, and durations of feeding trials, tolerance of species was probably the main influencing factor. The relatively high tolerance to alternative lipid sources of Japanese seabass has been evidenced

TABLE 4. *Immune response of Japanese seabass fed the experimental diets (means ± SEM, n = 3).*[1]

Immune response	Diets							*P*-value
	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	
Phagocytic index	1.42 ± 0.07^{a}	0.99 ± 0.15^{ab}	1.31 ± 0.10^{ab}	0.85 ± 0.13^{b}	1.34 ± 0.09^{ab}	1.16 ± 0.11^{ab}	0.84 ± 0.10^{b}	0.008
Respiratory burst activity (OD630)	0.73 ± 0.11	0.56 ± 0.17	0.62 ± 0.11	0.40 ± 0.14	0.92 ± 0.28	0.47 ± 0.17	0.52 ± 0.17	0.469
Lysozyme activity (U/mL)	106.0 ± 3.2^{a}	100.5 ± 4.1^{ab}	85.9 ± 3.5^{ab}	77.1 ± 2.7^{b}	94.8 ± 4.8^{ab}	108.0 ± 8.8^{a}	101.8 ± 5.3^{ab}	0.025
ACP activity (ACH_{50} U/mL)	43.5 ± 7.1	58.5 ± 13.2	61.7 ± 11.9	52.5 ± 12.7	80.3 ± 8.1	59.5 ± 8.4	68.3 ± 7.9	0.409
SOD activity (U/mL)	116.7 ± 2.6^{ab}	119.4 ± 1.6^{a}	104.4 ± 1.3^{bc}	104.1 ± 1.2^{bc}	103.4 ± 3.7^{bc}	103.6 ± 3.4^{bc}	101.6 ± 2.2^{c}	0.002
Catalase activity (U/mL)	6.24 ± 0.19^{ab}	7.84 ± 0.39^{a}	7.14 ± 0.14^{ab}	6.30 ± 0.14^{ab}	6.10 ± 0.20^{b}	6.09 ± 0.56^{b}	5.54 ± 0.53^{b}	0.007

ACP = alternative complement pathway; ACH_{50} = 50% hemolysis; FO = fish oil; LO = linseed oil; SO = soybean oil; SOD = superoxide dismutase.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$).

by a previous study of Xue et al. (2006) which showed that replacement of half of the dietary FO in diets of Japanese seabass with a series of alternative lipid sources such as pork lard, beef tallow, poultry fat, and corn oil did not reduce the growth and feed utilization. One of our previous studies also observed that Japanese seabass needed relatively low dietary levels of ARA (0.32% of dry matter) to support the optimal growth (Xu et al. 2010). Another study of ours on Δ6 fatty acyl desaturase of Japanese seabass partly explained the relatively high tolerance to alternative lipid sources and low dependence on LC-PUFAs in Japanese seabass (Xu et al. 2014). This study suggested that Japanese seabass has the capacity of LC-PUFA biosynthesis to some degree and this capacity could be stimulated by alternative lipid sources.

In this study, although the replacement of FO by LO or SO did not significantly reduce the growth, the diet with 2/3 FO and 1/3 LO led to significantly higher SGR than the diet with total replacement of FO by SO. This indicated that Japanese seabass probably could utilize ALA better than LNA given that ALA and LNA were the characteristic fatty acids of LO and SO, respectively. This was similar with grouper (Wu and Chen 2012) and Murray cod (Senadheera et al. 2010) yet different from some freshwater fish such as iridescent shark (Asdari et al. 2011), Eurasian perch (Blanchard et al. 2008), and Nile tilapia (El-Husseiny et al. 2010) which require more LNA than ALA in the diet. Even silver perch did not respond to dietary ALA while its growth rate increased with increasing dietary LNA content in a certain range (Smith et al. 2004).

Besides growth performance, replacement of FO by vegetable oils could also exert regulations on health status of fish, especially on immune responses (Glencross 2009). LO and SO have been reported to reduce certain immune responses in several marine or euryhaline fish, such as European seabass (Mourente et al. 2005), gilthead seabream (Montero and Kalinowski 2003; Ganga et al. 2005; Montero et al. 2008, 2010), and Atlantic salmon (Bell et al. 1996). This is in agreement with what was observed in this study. In this study, non-specific

TABLE 5. *Body composition of Japanese seabass fed the experimental diets (% wet weight, means ± SEM, n = 3).*[1]

Body composition	Diets							*P*-value
	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	
Moisture	72.5 ± 0.2^{a}	72.3 ± 0.3^{a}	72.2 ± 0.5^{a}	70.7 ± 0.4^{b}	71.7 ± 0.2^{ab}	71.9 ± 0.2^{ab}	71.9 ± 0.2^{ab}	0.048
Crude protein	16.73 ± 0.10	16.44 ± 0.13	16.69 ± 0.09	16.53 ± 0.06	16.75 ± 0.04	16.59 ± 0.13	16.69 ± 0.09	0.963
Crude lipid	6.55 ± 0.28^{b}	6.83 ± 0.23^{b}	6.84 ± 0.40^{b}	8.26 ± 0.35^{a}	7.29 ± 0.22^{ab}	7.05 ± 0.20^{ab}	6.97 ± 0.08^{ab}	0.046
Ash	4.50 ± 0.06	4.59 ± 0.08	4.63 ± 0.02	4.46 ± 0.13	4.53 ± 0.08	4.62 ± 0.11	4.55 ± 0.08	0.926

FO = fish oil; LO = linseed oil; SO = soybean oil.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$).

immune parameters were assayed to evaluate the immunoregulating effects of alternative lipid sources. These parameters included phagocytosis of head kidney macrophages, LYZ and ACP in serum, and the redox-related immune parameters such as RPB, SOD, and CAT, which play critical roles in controlling the balance of release and clear of reactive oxygen species in immune cells (Holmblad and Söderhäll 1999; Campa-Córdova et al. 2002).

Total replacement of dietary FO by LO or SO in this study significantly reduced certain immune responses such as PI of head kidney macrophages, serum LYZ activity, and serum SOD activity. In marine or euryhaline fish, the reduction of immune responses by replacing FO with alternative lipids normally occurred with the reduction of the LC-PUFA contents in fish tissues, especially in leukocytes (Ganga et al. 2005). When FO was replaced by vegetable oil, the alterations in amount and type of highly bioactive eicosanoids produced from LC-PUFAs, such as prostaglandins, thromboxanes, and leukotrienes, were supposed to be a main mechanism involved in immune function alternations (Bell et al. 1996).

Other immune parameters analyzed in this study, RPB activity of head kidney macrophage and serum ACP activity, however, were not influenced by dietary supplementations of LO and SO. This indicated a common characteristic of the immune modulation by dietary lipid sources, that is, the responses of immune parameters to alternative dietary lipid sources may vary as observed in previous studies (Montero and Kalinowski 2003; Montero et al. 2008). This phenomenon might be related to different fatty acids in dietary lipid sources potentially regulating immune responses in different ways, such as altering membrane property and cell membrane interactions, regulating the eicosanoid profile, and altering the redox environment (Hwang 1989, 2000; Farndale et al. 1999; Calder 2001; Delaporte et al. 2006; Weldon et al. 2007; Gorjão et al. 2009; Furne et al. 2013). The exact mechanisms involved in the immune regulation by different lipid sources in fish need to be elucidated by further studies.

In this study, total replacement of FO with LO significantly decreased the moisture content while it significantly increased the lipid content of whole fish body compared to the control group fed FO. This is in accordance with what was observed for other marine fish species such as European sea bass (Mourente et al. 2005), gilthead seabream (Caballero et al. 2003), turbot (Bell et al. 1999), and Arctic char (Olsen et al. 2000), which showed that high dietary LO promotes lipid droplet accumulation in hepatocytes or enterocytes. The increase of body lipid content by dietary LO in fish may be due to the preference for specific fatty acids (e.g., 18:3n-3 and 18:1n-9) as energy sources, which subsequently resulted in storage of surplus fatty acids in lipid droplets in fish tissues (Mourente et al. 2005). The tendency for lipid accumulation may be also related to the decreased dietary n-3 LC-PUFA contents, which would result in impaired lipoprotein synthesis and subsequent impaired lipid transportation (Watanabe 1982; Sargent et al. 1989; Olsen et al. 1999, 2000).

The relationship between tissue fatty acid compositions and dietary fatty acid contents has been well revealed in previous studies (Blanchard et al. 2008; El-Husseiny et al. 2010; Asdari et al. 2011; Trushenski et al. 2011; Wu and Chen 2012). It has been reported that the fatty acid profiles of fish tissues reflected those of

TABLE 6. *Whole-body fatty acid composition of Japanese seabass fed the experimental diets (% total fatty acids, means ± SEM, n = 3).*[1]

	Diets							
Fatty acid	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	*P*-value
C14:0	2.97 ± 0.03^{a}	2.36 ± 0.02^{b}	1.77 ± 0.04^{c}	1.17 ± 0.09^{d}	2.26 ± 0.03^{b}	1.71 ± 0.04^{c}	1.07 ± 0.05^{d}	0.000
C16:0	14.85 ± 0.10^{a}	13.54 ± 0.10^{bc}	12.25 ± 0.24^{d}	10.67 ± 0.37^{e}	13.93 ± 0.04^{ab}	13.42 ± 0.13^{bc}	12.92 ± 0.20^{cd}	0.000
C18:0	7.64 ± 0.37^{b}	6.58 ± 0.20^{b}	7.22 ± 0.34^{b}	6.34 ± 0.72^{b}	7.16 ± 0.66^{b}	8.77 ± 0.88^{b}	12.44 ± 0.35^{a}	0.000
∑SFA	25.46 ± 0.41^{ab}	22.49 ± 0.10^{bc}	21.24 ± 0.56^{c}	18.18 ± 0.94^{d}	23.35 ± 0.63^{ab}	23.90 ± 0.86^{abc}	26.43 ± 0.47^{a}	0.000
C16:1n-7	5.57 ± 0.09^{a}	4.50 ± 0.02^{b}	3.38 ± 0.08^{c}	2.36 ± 0.14^{d}	4.42 ± 0.06^{b}	3.35 ± 0.09^{c}	2.24 ± 0.12^{d}	0.000
C18:1n-9	21.46 ± 0.30^{a}	22.28 ± 0.39^{a}	21.07 ± 0.42^{a}	21.85 ± 0.51^{a}	22.08 ± 0.76^{a}	19.99 ± 0.74^{a}	17.02 ± 0.42^{b}	0.000
C20:1n-9	2.46 ± 0.00^{b}	2.46 ± 0.01^{b}	2.54 ± 0.04^{b}	2.72 ± 0.02^{a}	1.88 ± 0.01^{c}	1.56 ± 0.05^{d}	1.13 ± 0.04^{e}	0.000
∑MUFA	29.50 ± 0.38^{a}	29.24 ± 0.40^{a}	26.99 ± 0.37^{ab}	26.93 ± 0.50^{ab}	28.37 ± 0.82^{a}	24.90 ± 0.79^{b}	20.39 ± 0.42^{c}	0.000
C18:2n-6	17.71 ± 0.16^{d}	18.30 ± 0.26^{d}	18.03 ± 0.34^{d}	17.32 ± 0.33^{d}	26.12 ± 0.30^{c}	32.12 ± 0.63^{b}	38.76 ± 0.69^{a}	0.000
C18:3n-6	0.36 ± 0.02^{bc}	0.43 ± 0.04^{bc}	0.44 ± 0.03^{bc}	0.27 ± 0.02^{c}	0.48 ± 0.07^{bc}	0.62 ± 0.08^{b}	1.01 ± 0.08^{a}	0.000
C20:4n-6	0.71 ± 0.01^{a}	0.61 ± 0.03^{ab}	0.48 ± 0.01^{c}	0.40 ± 0.02^{cd}	0.58 ± 0.02^{b}	0.49 ± 0.02^{c}	0.32 ± 0.01^{d}	0.000
∑n-6	18.78 ± 0.13^{d}	19.35 ± 0.30^{d}	18.95 ± 0.31^{d}	17.98 ± 0.32^{d}	27.18 ± 0.27^{c}	33.23 ± 0.56^{b}	40.09 ± 0.76^{a}	0.000
C18:3n-3	2.80 ± 0.19^{d}	9.74 ± 0.28^{c}	16.95 ± 0.72^{b}	24.54 ± 1.10^{a}	3.13 ± 0.09^{d}	3.29 ± 0.06^{d}	3.72 ± 0.28^{d}	0.000
C18:4n-3	0.67 ± 0.02^{b}	0.70 ± 0.03^{b}	0.96 ± 0.04^{a}	0.99 ± 0.08^{a}	0.55 ± 0.02^{bc}	0.45 ± 0.02^{c}	0.38 ± 0.01^{c}	0.000
C20:5n-3	3.89 ± 0.10^{a}	2.92 ± 0.02^{b}	2.12 ± 0.08^{c}	1.34 ± 0.08^{d}	2.87 ± 0.10^{b}	2.07 ± 0.02^{c}	1.19 ± 0.07^{d}	0.000
C22:5n-3	2.87 ± 0.04^{a}	2.20 ± 0.16^{b}	1.50 ± 0.04^{c}	0.72 ± 0.06^{d}	2.13 ± 0.07^{b}	1.54 ± 0.07^{c}	0.72 ± 0.03^{d}	0.000
C22:6n-3	6.84 ± 0.16^{a}	5.51 ± 0.15^{b}	4.39 ± 0.24^{c}	3.25 ± 0.29^{d}	5.19 ± 0.04^{bc}	4.42 ± 0.11^{c}	2.75 ± 0.15^{d}	0.000
∑n-3	17.07 ± 0.12^{d}	21.08 ± 0.60^{c}	25.92 ± 0.73^{b}	30.84 ± 0.84^{a}	13.87 ± 0.14^{e}	11.77 ± 0.17^{e}	8.76 ± 0.49^{f}	0.000
∑PUFA	35.84 ± 0.12^{d}	40.42 ± 0.31^{c}	44.87 ± 0.77^{b}	48.83 ± 1.11^{a}	41.05 ± 0.27^{c}	45.00 ± 0.42^{b}	48.85 ± 0.27^{a}	0.000
∑n-3 LC-PUFA	13.60 ± 0.29^{a}	10.63 ± 0.33^{b}	8.01 ± 0.36^{c}	5.31 ± 0.43^{d}	10.19 ± 0.12^{b}	8.02 ± 0.13^{c}	4.66 ± 0.22^{d}	0.000
∑n-3/∑n-6	0.91 ± 0.01^{d}	1.09 ± 0.05^{c}	1.37 ± 0.05^{b}	1.71 ± 0.03^{a}	0.51 ± 0.01^{e}	0.35 ± 0.01^{f}	0.22 ± 0.02^{f}	0.000
C18:1n-9/∑n-3	1.26 ± 0.01^{c}	1.06 ± 0.05^{cd}	0.81 ± 0.03^{de}	0.71 ± 0.02^{e}	1.59 ± 0.05^{b}	1.70 ± 0.05^{ab}	1.96 ± 0.13^{a}	0.000

FO = fish oil; LO = linseed oil; MUFA = monounsaturated fatty acid; n-3 = n-3 unsaturated fatty acid; n-6 = n-6 unsaturated fatty acid; SFA = saturated fatty acid; SO = soybean oil.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$). Some fatty acids, of which the contents are minor, trace amount, or not detected, were not listed in the table.

TABLE 7. *Liver fatty acid composition of Japanese seabass fed the experimental diets (% total fatty acids, means ± SEM, n = 3).*[1]

	Diets							
Fatty acid	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	*P*-value
C14:0	1.80 ± 0.10^{a}	1.55 ± 0.09^{ab}	1.29 ± 0.04^{bc}	1.04 ± 0.01^{cd}	1.63 ± 0.09^{a}	1.18 ± 0.03^{cd}	0.88 ± 0.05^{d}	0.000
C16:0	13.70 ± 0.20^{a}	12.23 ± 0.65^{abc}	10.62 ± 0.70^{c}	10.59 ± 0.34^{c}	13.05 ± 0.39^{ab}	12.53 ± 0.30^{abc}	11.18 ± 0.12^{bc}	0.001
C18:0	6.41 ± 0.23^{b}	4.76 ± 0.67^{b}	4.73 ± 0.81^{b}	6.56 ± 1.21^{b}	4.93 ± 0.33^{b}	8.27 ± 1.38^{ab}	12.54 ± 1.00^{a}	0.001
∑SFA	21.91 ± 0.39^{ab}	18.53 ± 1.38^{ab}	16.64 ± 1.54^{b}	18.19 ± 1.55^{ab}	19.62 ± 0.72^{ab}	21.99 ± 1.65^{ab}	24.50 ± 0.79^{a}	0.017
C16:1n-7	4.61 ± 0.01^{a}	3.85 ± 0.20^{b}	3.10 ± 0.14^{c}	2.75 ± 0.12^{cd}	3.95 ± 0.17^{b}	3.07 ± 0.10^{c}	2.32 ± 0.04^{d}	0.000
C18:1n-9	21.05 ± 0.62^{ab}	21.76 ± 1.48^{a}	21.17 ± 1.07^{ab}	21.88 ± 0.83^{a}	21.87 ± 0.05^{a}	19.83 ± 1.03^{ab}	16.22 ± 1.71^{b}	0.026
C20:1n-9	2.26 ± 0.02^{b}	2.25 ± 0.08^{b}	2.42 ± 0.07^{b}	2.76 ± 0.06^{a}	1.79 ± 0.05^{c}	1.60 ± 0.03^{c}	1.21 ± 0.01^{d}	0.000
∑MUFA	27.92 ± 0.65^{a}	27.86 ± 1.75^{a}	26.69 ± 1.28^{a}	27.39 ± 0.74^{a}	27.61 ± 0.26^{a}	24.50 ± 0.99^{ab}	19.75 ± 1.74^{b}	0.002
C18:2n-6	16.75 ± 0.38^{de}	15.87 ± 0.60^{de}	17.37 ± 0.15^{d}	14.87 ± 0.32^{e}	22.73 ± 0.63^{c}	30.34 ± 0.35^{b}	37.47 ± 0.47^{a}	0.000
C18:3n-6	0.74 ± 0.05^{b}	0.76 ± 0.11^{b}	0.74 ± 0.07^{b}	0.97 ± 0.04^{b}	0.95 ± 0.05^{b}	1.16 ± 0.14^{b}	2.45 ± 0.20^{a}	0.000
C20:4n-6	1.00 ± 0.08^{a}	0.77 ± 0.01^{ab}	0.47 ± 0.02^{c}	0.35 ± 0.04^{c}	0.80 ± 0.04^{ab}	0.58 ± 0.03^{bc}	0.35 ± 0.08^{c}	0.000
∑n-6	18.49 ± 0.37^{d}	17.39 ± 0.52^{de}	18.58 ± 0.09^{d}	16.19 ± 0.35^{e}	24.48 ± 0.69^{c}	32.09 ± 0.44^{b}	40.20 ± 0.58^{a}	0.000
C18:3n-3	1.77 ± 0.08^{d}	6.84 ± 0.58^{c}	14.70 ± 0.56^{b}	16.49 ± 0.30^{a}	2.36 ± 0.10^{d}	2.18 ± 0.05^{d}	2.41 ± 0.10^{d}	0.000
C18:4n-3	0.31 ± 0.03^{c}	0.61 ± 0.10^{c}	1.03 ± 0.02^{b}	1.87 ± 0.15^{a}	0.39 ± 0.02^{c}	0.31 ± 0.02^{c}	0.41 ± 0.04^{c}	0.000
C20:5n-3	3.08 ± 0.37^{a}	2.20 ± 0.17^{ab}	1.66 ± 0.13^{bc}	0.91 ± 0.09^{c}	2.28 ± 0.10^{ab}	1.46 ± 0.11^{bc}	0.92 ± 0.22^{c}	0.000
C22:5n-3	3.08 ± 0.32^{a}	2.11 ± 0.26^{b}	1.71 ± 0.22^{b}	0.58 ± 0.07^{c}	2.11 ± 0.13^{b}	1.45 ± 0.11^{bc}	0.74 ± 0.12^{c}	0.000
C22:6n-3	10.10 ± 1.09^{a}	7.29 ± 0.76^{ab}	5.59 ± 0.56^{bc}	3.53 ± 0.30^{c}	7.33 ± 0.35^{ab}	5.01 ± 0.28^{bc}	3.63 ± 0.78^{c}	0.000
∑n-3	18.34 ± 1.72^{bc}	19.05 ± 1.74^{abc}	24.67 ± 1.41^{a}	23.39 ± 0.77^{ab}	14.47 ± 0.52^{cd}	10.40 ± 0.49^{de}	8.12 ± 1.23^{e}	0.000
∑PUFA	36.83 ± 1.77^{b}	36.44 ± 2.21^{b}	43.25 ± 1.49^{ab}	39.58 ± 1.03^{b}	38.95 ± 1.20^{b}	42.49 ± 0.66^{ab}	48.32 ± 1.06^{a}	0.000
∑n-3 LC-PUFA	13.19 ± 1.44^{a}	9.49 ± 0.93^{ab}	7.24 ± 0.69^{bc}	4.43 ± 0.39^{c}	9.61 ± 0.45^{ab}	6.46 ± 0.38^{bc}	4.56 ± 1.00^{c}	0.000
∑n-3/∑n-6	0.99 ± 0.10^{c}	1.09 ± 0.07^{bc}	1.33 ± 0.07^{ab}	1.44 ± 0.04^{a}	0.59 ± 0.01^{d}	0.32 ± 0.02^{de}	0.20 ± 0.03^{e}	0.000
C18:1n-9/∑n-3	1.18 ± 0.16^{c}	1.18 ± 0.19^{c}	0.87 ± 0.10^{c}	0.94 ± 0.06^{c}	1.52 ± 0.05^{ab}	1.92 ± 0.16^{a}	2.04 ± 0.22^{a}	0.000

FO = fish oil; LO = linseed oil; MUFA = monounsaturated fatty acid; n-3 = n-3 unsaturated fatty acid; n-6 = n-6 unsaturated fatty acid; SFA = saturated fatty acid; SO = soybean oil.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$). Some fatty acids, of which the contents are minor, trace amount, or not detected, were not listed in the table.

TABLE 8. *Muscle fatty acid composition of Japanese seabass fed the experimental diets (% total fatty acids, means ± SEM, n = 3).*[1]

	Diets							
Fatty acid	FO	F_2L_1	F_1L_2	LO	F_2S_1	F_1S_2	SO	*P*-value
C14:0	1.64 ± 0.09^{a}	1.55 ± 0.08^{ab}	1.26 ± 0.11^{b}	0.78 ± 0.03^{c}	1.40 ± 0.09^{ab}	1.21 ± 0.04^{b}	0.82 ± 0.01^{c}	0.000
C16:0	17.58 ± 0.88^{a}	17.38 ± 0.44^{a}	16.85 ± 0.32^{a}	13.84 ± 0.49^{b}	17.69 ± 0.23^{a}	16.90 ± 0.35^{a}	16.03 ± 0.17^{ab}	0.001
C18:0	6.44 ± 0.10	6.13 ± 0.16	6.28 ± 0.29	6.03 ± 0.20	6.62 ± 0.29	5.91 ± 0.17	6.27 ± 0.29	0.389
∑SFA	25.66 ± 0.92^{a}	25.06 ± 0.52^{a}	24.40 ± 0.50^{a}	20.65 ± 0.67^{b}	25.72 ± 0.43^{a}	24.02 ± 0.49^{a}	23.12 ± 0.41^{ab}	0.000
C16:1n-7	4.10 ± 0.19^{a}	3.73 ± 0.05^{ab}	3.11 ± 0.15^{bc}	2.05 ± 0.12^{e}	3.53 ± 0.07^{abc}	2.91 ± 0.12^{cd}	2.28 ± 0.17^{de}	0.000
C18:1n-9	21.55 ± 0.61	22.43 ± 0.34	22.12 ± 0.20	22.30 ± 0.27	21.97 ± 0.17	21.28 ± 0.17	21.71 ± 0.58	0.357
C20:1n-9	1.94 ± 0.10^{bc}	2.18 ± 0.09^{b}	2.24 ± 0.08^{ab}	2.51 ± 0.07^{a}	1.72 ± 0.01^{c}	1.39 ± 0.04^{d}	1.06 ± 0.03^{e}	0.000
∑MUFA	27.60 ± 0.69^{ab}	28.34 ± 0.38^{a}	27.48 ± 0.42^{ab}	26.86 ± 0.22^{abc}	27.22 ± 0.22^{abc}	25.58 ± 0.33^{bc}	25.05 ± 0.71^{c}	0.002
C18:2n-6	13.56 ± 0.29^{e}	14.98 ± 0.05^{d}	14.83 ± 0.25^{de}	15.30 ± 0.05^{d}	20.26 ± 0.39^{c}	26.70 ± 0.43^{b}	31.34 ± 0.30^{a}	0.000
C20:4n-6	1.96 ± 0.12^{a}	1.70 ± 0.07^{abc}	1.50 ± 0.15^{abcd}	1.24 ± 0.07^{cd}	1.78 ± 0.07^{ab}	1.32 ± 0.04^{bcd}	1.08 ± 0.11^{d}	0.000
∑n-6	15.52 ± 0.20^{d}	16.68 ± 0.09^{d}	16.33 ± 0.35^{d}	16.54 ± 0.08^{d}	22.04 ± 0.37^{c}	28.01 ± 0.39^{b}	32.42 ± 0.33^{a}	0.000
C18:3n-3	1.80 ± 0.24^{d}	6.61 ± 0.34^{c}	11.37 ± 0.30^{b}	18.20 ± 0.09^{a}	2.18 ± 0.04^{d}	2.31 ± 0.05^{d}	2.26 ± 0.05^{d}	0.000
C20:5n-3	4.32 ± 0.25^{a}	3.97 ± 0.03^{a}	3.17 ± 0.12^{b}	2.22 ± 0.09^{cd}	3.94 ± 0.04^{a}	2.77 ± 0.15^{bc}	1.81 ± 0.04^{d}	0.000
C22:5n-3	3.05 ± 0.16^{a}	2.90 ± 0.09^{a}	2.13 ± 0.15^{b}	1.14 ± 0.08^{c}	2.88 ± 0.05^{a}	2.13 ± 0.02^{b}	1.18 ± 0.04^{c}	0.000
C22:6n-3	13.14 ± 0.92^{a}	12.59 ± 0.42^{ab}	10.74 ± 0.96^{abc}	7.73 ± 0.27^{c}	12.71 ± 0.15^{ab}	9.99 ± 0.59^{bc}	7.76 ± 0.59^{c}	0.000
∑n-3	22.31 ± 1.08^{b}	26.07 ± 0.22^{a}	27.41 ± 1.17^{a}	29.30 ± 0.32^{a}	21.72 ± 0.11^{b}	17.19 ± 0.70^{c}	13.00 ± 0.59^{d}	0.000
∑PUFA	37.83 ± 0.93^{b}	42.75 ± 0.25^{a}	43.74 ± 1.52^{a}	45.84 ± 0.37^{a}	43.76 ± 0.39^{a}	45.21 ± 0.32^{a}	45.41 ± 0.72^{a}	0.000
∑n-3 LC-PUFA	20.51 ± 1.27^{a}	19.46 ± 0.45^{ab}	16.03 ± 1.18^{bc}	11.09 ± 0.37^{d}	19.54 ± 0.12^{ab}	14.89 ± 0.74^{c}	10.74 ± 0.60^{d}	0.000
∑n-3/∑n-6	1.44 ± 0.09^{c}	1.56 ± 0.01^{bc}	1.68 ± 0.04^{ab}	1.77 ± 0.02^{a}	0.99 ± 0.02^{d}	0.61 ± 0.03^{e}	0.40 ± 0.02^{f}	0.000
C18:1n-9/∑n-3	0.97 ± 0.07^{bc}	0.86 ± 0.02^{c}	0.81 ± 0.04^{c}	0.76 ± 0.02^{c}	1.01 ± 0.01^{bc}	1.24 ± 0.06^{b}	1.68 ± 0.11^{a}	0.000

FO = fish oil; LO = linseed oil; MUFA = monounsaturated fatty acid; n-3 = n-3 unsaturated fatty acid; n-6 = n-6 unsaturated fatty acid; SFA = saturated fatty acid; SO = soybean oil.

[1] Means in the same row sharing a same superscript letter are not significantly different, determined by Tukey's test ($P > 0.05$). Some fatty acids, of which the contents are minor, trace amount, or not detected, were not listed in the table.

the diets, as observed in this study for 14:0, 16:0, 18:0, 16:1n-7, 20:1n-9, 18:2n-6, 20:4n-6, and 18:3n-3. Particularly, the LC-PUFA (ARA, EPA, and DHA) contents in fish tissues significantly decreased with increasing level of dietary FO replacement, suggesting that the influences of dietary LO or SO supplementation on flesh quality, in terms of LC-PUFA concentrations, should not be neglected. However, on the other hand, exceptions were also observed in that tissue 18:4n-3 contents did not positively correlate with dietary 18:4n-3 content but had a significantly positive correlation with dietary 18:3n-3 content. This again proved the possible biosynthesis of LC-PUFAs (Xu et al. 2014) in Japanese seabass, at least indicating the existence of the first step of LC-PUFA biosynthetic pathway to some extent, that is, the desaturation from 18:3n-3 to 18:4n-3.

Finally, it can be concluded that (1) LO and SO were both good potential alternative lipid sources for juvenile Japanese seabass in terms of effects on growth performance but total replacement of FO by LO or SO caused reductions in certain immune responses and tissue LC-PUFA concentrations and (2) LO was a better lipid source than SO for Japanese seabass in terms of fish growth and immune responses.

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