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Dietary docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio influenced growth performance, immune response, stress resistance and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier)

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Abstract

A 10-week feeding experiment was conducted to investigate the effects of dietary DHA/EPA ratio on juvenile Japanese seabass reared in sea floating cages. Six practical diets were formulated differing only in DHA/EPA ratio: 0.55 (Diet D/E0.55), 1.04 (D/E1.04), 1.53 (D/E1.53), 2.08 (D/E2.08), 2.44 (D/E2.44) and 2.93 (D/E2.93). All diets had the same contents of total n-3 long-chain polyunsaturated fatty acid (LC-PUFA) and arachidonic acid (ARA). The results showed that the final weight and specific growth rate significantly increased with increasing dietary DHA/EPA ratio from 0.55 to 2.08 and thereafter declined. Activities of lysozyme and superoxide dismutase in serum in groups with DHA/EPA of 1.53–2.93 was significantly higher compared to group D/E0.55 while the activity of serum alternative complement pathway in group D/E2.93 was significantly lower compared with group D/E1.53. However, no difference was observed in activities of both respiratory burst of head kidney macrophage and serum catalase among dietary treatments. The per cent survival after air exposure in group D/E1.53, D/E2.08 and D/E2.93 was significantly higher compared with group D/E0.55. The fatty acid composition of whole body and tissues reflected closely those of diets, while fish accumulated more DHA than EPA in fish tissues, especially in muscle. These results suggested that at the same dietary contents of n-3 LC-PUFA (appr. 18% of TFA) and ARA (appr. 0.7% of TFA), moderate dietary DHA/EPA ratios of 1.53–2.08 significantly enhanced growth performances, certain innate immune responses, and the stress tolerance of Japanese seabass, in accordance with the preferential incorporation of DHA into fish tissues.

Keywords: *Lateolabrax japonicus*, docosahexaenoic acid to eicosapentaenoic acid ratio, growth performance, immunity, stress resistance, fatty acid composition

Introduction

Docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), the so-called n-3 long-chain polyunsaturated fatty acids (LC-PUFAs), are essential fatty acids of marine fish since marine fish lacks the ability to synthesize LC-PUFAs from their 18-carbon precursor fatty acids such as linolenic acid (18:3n-3) and linoleic acid (18:2n-6) (Tocher 2003). It has been reported that DHA and EPA play important roles in various physiological functions such as functions of visual and neural systems (Bell, Batty, Dick, Fretwell, Navarro & Sargent 1995; Furuita & Takeuchi 1998; Ishizaki,

Uematsu & Takeuchi 2000; Ishizaki, Masuda, Uematsu, Shimizu, Arimoto & Takeuchi 2001; Benítez-Santana, Masuda, Carrillo, Ganuza, Valencia, Hernández-Cruz & Izquierdo 2007; Noffs, Martino, Trugo, Urbinati, Fernandes & Takahashi 2009), bone development (Gapasin & Duray 2001; Roo, Hernández-Cruz, Socorro, Fernández-Palacios, Montero & Izquierdo 2009), pigmentation (Villalta, Estévez, Bransden & Bell 2008; Vizcaíno-Ochoa, Lazo, Barón-Sevilla & Drawbridge 2010) and stress resistance (Kanazawa 1997; Liu, Caballero, Izquierdo, Ali, Hernández-Cruz, Valencia & Fernández-Palacios 2002), and thus are essential for normal growth and development of marine fish, especially for larvae (Takeuchi, Toyota, Satoh & Watanabe 1990; Watanabe 1993; Bransden, Battaglene, Morehead, Dunstan & Nichols 2005; Bransden, Cobcroft, Battaglene, Morehead, Dunstan, Nichols & Kolkovski 2005; Glencross 2009; Glencross & Rutherford 2011; Hossain, Almatar & James 2012; Matsunari, Hashimoto, Oda, Masuda, Imaizumi, Teruya, Furuita, Yamamoto, Hamada & Mushiake 2013).

Besides the individual effects of DHA and EPA, the ratio of these two LC-PUFAs in diets has also been demonstrated to exert significant influences on a range of physiological processes in marine fish (Ibeas, Cejas, Fores, Badía, Gómez & Lorenzo Hernández 1997; Rodríguez, Pérez, Díaz, Izquierdo, Fernández-Palacios & Lorenzo 1997; Wu, Ting & Chen 2002; Dantagnan, Bórquez, Hernández & Izquierdo 2010; Trushenski, Schwarz, Bergman, Rombenso & Delbos 2012). However, its importance was relatively neglected compared to the total amount of n-3 LC-PUFAs (Sargent, Bell, Mcevoy, Tocher & Estevez 1999; Kim, Lee, Park, Bai & Lee 2002; Lee, Lee & Kim 2003; Skalli & Robin 2004; Hamre & Harboe 2008; Wilson 2009; Lund & Steenfeldt 2011; ØStbye, Kjær, Rørå, Torstensen & Ruyter 2011; Zuo, Ai, Mai, Xu, Wang, Xu, Liufu & Zhang 2012b). The limited amount of work performed to date has suggested that dietary DHA and EPA had different efficiency in influencing bio-functions of fish such as vision (Navarro, McEvoy, Bell, Amat, Hontoria & Sargent 1997; Noffs *et al.* 2009), pigmentation (Villalta *et al.* 2008; Vizcaíno-Ochoa *et al.* 2010) and reproduction (Mazorra, Bruce, Bell, Davie, Alorend, Jordan, Rees, Papanikos, Porter & Bromage 2003), whereas little information was available regarding the difference between DHA and EPA in modulation of fish health such as immunity and stress resistance (Wu, Ting & Chen 2003; Zuo, Ai, Mai, Xu, Wang, Xu, Liufu & Zhang 2012a). Most comparison studies on immunomodulatory activities of fatty acids focused on the n-3/n-6 ratio (Chow, Sisfontes, Björkhem & Jondal 1989; Bell, Ashton, Secombes, Weitzel, Dick & Sargent 1996; Thompson, Tatner & Henderson 1996; Farndale, Bell, Bruce, Bromage, Oyen, Zanuy & Sargent 1999; Lauridsen, Stagsted & Jensen 2007; Berge, Witten, Baeverfjord, Vegusdal, Wadsworth & Ruyter 2009), and the previous studies on fatty acids were primarily conducted in larvae, which were not appropriate targets for immunity study. Regarding stress resistance, relevant studies on DHA/EPA were not only limited but also inconsistent in results (Bell, Tocher, Farndale & Sargent 1998; Furuita, Konishi & Takeuchi 1999; Tago, Yamamoto, Teshima & Kanazawa 1999; Liu *et al.* 2002). Thus, there is large necessity to study the modulation of fish immunity and stress resistance by dietary DHA/EPA. It will not only be worthwhile for elucidating the difference between DHA and EPA in affecting animal immunity and stress resistance but also provide more information for the modulation of fish health by essential nutrients, which was further important for aquaculture since the expansion of aquaculture and high culture density have caused more diseases and stress but the use of antibiotics leads to various adverse impacts (Burridge, Weis, Cabello, Pizarro & Bostick 2010).

The present study investigated the effects of dietary DHA/EPA ratio on innate immune responses and air exposure resistance in Japanese seabass, which was the most important marine culture species in south China. Effects of dietary DHA/EPA ratio on growth performance and fatty acid compositions were also studied in the present study since the lipid and fatty acid nutrition of this fish has not been comprehensively studied. The present study was conducted in sea floating net cages, which was commonly used in the industry of Japanese seabass culture. This made the present study more worthwhile for the farmers since the difference between DHA and EPA in their effects on fish was always species-specific and easily influenced by rearing conditions (Koven, Tandler, Sklan & Kissil 1993; Tago *et al.* 1999; Villalta & Estévez 2005; Ding, Xu, Zhang, Wang, Chen & Sun 2009; Dantagnan *et al.* 2010; Trushenski *et al.* 2012).

Materials and methods

Experimental diets

The basal experimental diet was formulated to contain approximately 430 g kg^{-1} crude protein and 120 g kg^{-1} crude lipid, which have been shown to be sufficient to support the optimum growth of Japanese seabass (Table 1). An arachidonic acid (ARA)-enriched oil (ARA content, 41% of total fatty acid (TFA); in form of triglyceride; Hubei Youzhiyou Biotechnology Co., Ltd., Wuhan, Hubei, China) was supplemented to the basal diet to meet the requirement of ARA in Japanese seabass. DHA-enriched oil (DHA content, 42% of TFA; in the form of triglyceride; Hubei Youzhiyou Biotechnology Co., Ltd.) and EPA-enriched oil (EPA content, 49% of TFA; DHA content, 25% of TFA; both in form of triglyceride; Hebei Haiyuan Health biological Science and Technology Co., Ltd., Cangzhou, Hebei, China) were supplemented to the basal diet to obtain six different dietary DHA/EPA ratios (0.50, 1.00, 1.50, 2.00, 2.50 and 3.00) (Table 2). The soybean oil contents in diets were adjusted to make the formulations 100%. The accurate dietary DHA/EPA ratios, analysed by high-performance gas chromatography (GS, HP6890, Santa Clara, CA, USA), were 0.55, 1.04, 1.53, 2.08, 2.44 and 2.93, respectively and the corresponding diets were named D/E0.55, D/E1.04, D/E1.53, D/E2.08, D/E2.44 and D/E2.93 respectively. All diets had the same contents of total n-3 LC-PUFA and ARA.

Ingredients were ground into fine powder through a 200 μm mesh. All ingredients were thoroughly mixed with the oils (Table 1), and water was added to produce stiff dough. The dough was then pelleted with an experimental feed

Table 1 Formulation and proximate chemical composition of experimental diets (g kg^{-1} dry matter)

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio					
Ingredient	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**
Fish meal*	400.0	400.0	400.0	400.0	400.0	400.0
Soybean meal*	220.0	220.0	220.0	220.0	220.0	220.0
Wheat meal	246.0	246.0	246.0	246.0	246.0	246.0
Soybean oil	27.3	25.8	21.7	19.0	17.0	15.6
DHA-enriched oil†	0.0	7.3	14.8	19.8	23.4	26.1
EPA-enriched oil‡	17.7	11.9	8.5	6.2	4.6	3.3
ARA-enriched oil§	20.0	20.0	20.0	20.0	20.0	20.0
Attractant¶	3.0	3.0	3.0	3.0	3.0	3.0
Mold inhibitor**	1.0	1.0	1.0	1.0	1.0	1.0
Lecithin	25.0	25.0	25.0	25.0	25.0	25.0
Mineral premix††	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin premix‡‡	20.0	20.0	20.0	20.0	20.0	20.0
Proximate composition						
Crude protein	427.2	429.4	421.6	426.1	425.8	420.6
Crude lipid	119.7	123.0	130.2	127.0	132.1	125.5
Ash	115.2	115.3	116.1	118.0	118.7	119.3

*Fish meal: crude protein 697 g kg^{-1} dry matter, crude lipid 71 g kg^{-1} dry matter; soybean meal: crude protein 533 g kg^{-1} dry matter, crude lipid 19 g kg^{-1} dry matter.

†DHA-enriched oil: DHA content, 42% of TFA; in the form of triglyceride; Wuhan Bioco Sci. & Tech. Dev Co., Ltd., Wuhan, Hubei, China.

‡EPA-enriched oil: EPA content, 49% of TFA; DHA content, 25% of TFA; both in the form of triglyceride; Hebei Haiyuan Health biological Science and Technology Co., Ltd., Cangzhou, Hebei, China.

§ARA-enriched oil: ARA content, 41% of TFA; in form of triglyceride; Wuhan Bioco Sci. & Tech. Dev Co., Ltd., Wuhan, Hubei, China.

¶Attractant: glycine and betaine.

**Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.

††Mineral premix (kg^{-1} diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2{\cdot}6H_2O$ (1%), 50 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; $MnSO_4{\cdot}H_2O$, 60 mg; $MgSO_4{\cdot}7H_2O$, 1200 mg; $Ca(H_2PO_4)_2{\cdot}H_2O$, 3000 mg; and zoelite, 15.55 g.

‡‡Vitamin premix (kg^{-1} diet): thiamin 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin B_{12}, 0.1 mg; vitamin K_3, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; alpha-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin 150 mg and wheat middling, 18.52 g.

Table 2 Fatty acid composition of the experimental diets for Japanese seabass (*Lateolabrax japonicus*) (% total fatty acids)*

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio					
Fatty acid	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**
C 14: 0	1.51	1.70	2.16	2.50	2.59	2.76
C 16: 0	13.56	13.67	14.42	14.95	14.92	15.15
C 18: 0	3.66	3.71	3.40	3.37	3.47	3.34
∑SFA	18.73	19.08	19.98	20.81	20.98	21.24
C 16: 1n-7	1.85	2.00	2.34	2.73	2.57	2.75
C 18: 1n-7	0.22	0.42	0.30	0.26	0.33	0.27
C 18: 1n-9	16.86	16.44	16.12	13.66	13.43	12.79
C 20: 1n-9	4.38	4.30	4.08	3.84	4.19	4.15
C 22: 1n-11	1.38	1.39	1.21	1.08	1.37	1.36
∑MUFA	24.69	24.55	24.04	21.58	21.89	21.33
C 18: 2n-6	32.48	31.52	30.57	30.95	28.76	27.95
C 20: 4n-6	0.77	0.78	0.58	0.68	0.59	0.57
∑n-6	33.24	32.30	31.15	31.63	29.35	28.51
C 18: 3n-3	3.83	3.69	3.43	3.62	3.19	3.13
C 18: 4n-3	0.71	0.65	0.57	0.62	0.51	0.50
C 20: 5n-3	11.63	7.98	6.50	5.92	4.89	4.62
C 22: 5n-3	0.71	0.67	0.72	0.71	0.77	0.80
C 22: 6n-3	6.40	8.30	9.93	12.33	11.93	13.55
∑n-3	23.28	21.29	21.15	23.19	21.29	22.61
∑PUFA	56.53	53.59	52.29	54.82	50.64	51.12
∑n-3LC-PUFA	18.74	16.95	17.15	18.95	17.59	18.98
∑n-3/∑n-6	0.70	0.66	0.68	0.73	0.73	0.79
C18:1n-9/∑n-3	0.72	0.77	0.76	0.59	0.63	0.57
EPA/ARA	15.15	10.25	11.19	8.67	8.32	8.14
DHA/EPA	0.55	1.04	1.53	2.08	2.44	2.93

*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.

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..

mill and dried for approximately 12 h in a ventilated oven at 45°C. After drying, the diets were broken up and sieved into proper pellet size (1.5 × 3.0 mm, 2.5 × 5.0 mm). The pellets were stored at −15°C until used.

Experimental procedure

Japanese seabass (*Lateolabrax japonicus*) were obtained from a commercial farm in Ningbo, China. Prior to the start of the experiment, the juvenile seabass were reared in floating sea cages (3.0 × 3.0 × 3.0 m), and fed a low-lipid diet for 2 weeks to acclimate to the experimental diets and conditions.

At the onset of the feeding trial, the fish were fasted for 24 h and weighed after being anaesthetized with eugenol (1:10,000) (Shanghai Reagent, Shanghai, China). Fish of similar sizes (9.48 ± 0.09 g) were randomly distributed into 18 sea 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 (starting time, 05:00 and 17:00). The feeding trial lasted 10 weeks. During the experimental period, the temperature ranged from 22.5 to 31.5°C, salinity from 28‰ to 33‰ and the dissolved oxygen content was approximately 6 mg L^{-1}. At the end of the feeding trial, the fish were fasted for 24 h before harvest. Total number and body weight of fish in each cage were recorded.

Sample collection, functional immune assay and analysis of fatty acid composition

Following the feeding trial, after being fasted for 24 h, blood samples were collected from the caudal vein of five fish per cage with a 27-gauge needle and 1 mL syringe, and allowed to clot at room

temperature for 2 h and then for 4 to 6 h in the cold. The clot was removed and residual blood cells separated from the straw-coloured serum by centrifugation (836 *g*, 10 min, 4°C). The serum was frozen at −80°C until use. Head kidney macrophages from five fish in each cage were isolated as described by Secombes (1990) with some modifications. Briefly, the head kidney was excised, cut into small fragments and transferred to RPMI-1640 (Gibco, Carlsbad, CA, USA) medium supplemented with 10 IU mL^{-1} heparin (Sigma, Saint Louis, MO, USA), 100 IU mL^{-1} penicillin (Amresco, Solon, OH, USA), 100 IU mL^{-1} streptomycin (Amresco, Solon, OH, USA), and 2% foetal calf serum (FCS) (Gibco). Cell suspensions were prepared by forcing the head kidney through a 100 μm steel mesh. The resultant cell suspensions were enriched by centrifugation (836 *g* for 25 min at 4°C) on 34%/51% Percoll (Pharmacia, Cambridge, England) density gradient. The cells were collected at the 34–51% interface and washed twice. Cell viability was determined by the trypan blue exclusion method and the cell density was determined in a haemocytometer. Then additional RPMI 1640 medium was added to adjust the cell concentration (1×10^7 mL^{-1}) for analysis.

The activity of serum lysozyme was determined as described by Ellis (1990). Results were expressed in units of lysozyme mL^{-1} serum. One unit is defined as the amount of sample causing a decrease in absorbance of 0.001 min^{-1} at 530 nm compared to the control (*Micrococcus lysodeikticus* suspension without serum).

The activity of serum alternative complement pathway was assayed according to Yano (1992). Briefly, a series of volumes of the diluted serum ranging from 0.1 to 0.25 mL were dispensed into test tubes and the total volume made up to 0.25 mL with barbitone buffer in presence of ethyleneglycol-bis (2-aminoethoxy)-tetraacetic acid (EGTA) and Mg^{2+}, then 0.1 mL of rabbit red blood cells (RaRBC) was added to each tube. After incubation for 90 min at 22°C, 3.15 mL 0.9% NaCl was added. Following this, the sample was centrifuged at 836 *g* for 5 min at 4°C to eliminate unlysed RaRBC. The optical density of the supernatant was measured at 414 nm. The volume of serum producing 50% haemolysis (ACH_{50}) was determined and the number of ACH_{50} units mL^{-1} was obtained for each group.

Production of intracellular superoxide anion (O_2^-) (activity of respiratory burst of head kidney macrophages) was evaluated using nitroblue tetrazolium (NBT) (Sigma) reduction following the method of Secombes (1990) with some modifications. A 100 μL cell suspension was stained with 100 μL 0.3% NBT and 100 μL Phorbol 12-myristate 13-acetate (PMA) (Sigma) (1 mg mL^{-1}) for 40 min. Absolute methanol was added to terminate the staining. Each tube was washed three times with 70% methanol and air-dried. Then 120 μL 2 M KOH and 140 μL dimethyl sulfoxide (DMSO, Sigma) were added and the colour was subsequently measured at 630 nm with a spectrophotometer using KOH/DMSO as a blank.

The activity of serum superoxide dismutase was measured spectrophotochemically by the ferricytochrome C method using xanthine/xanthine oxidase as the source of superoxide radicals. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome C and 0.024 IU mL^{-1} xanthine oxidase. The reaction was triggered after the addition of the xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome C reduction rate measured at 550 nm (McCord & Fridovich 1969). Enzyme activity was expressed as units per millilitres serum (U mL^{-1}).

The activity of serum catalase was determined by measuring the decrease of H_2O_2 concentration at 240 nm according to Aebi (1984). The reaction mixture contained 50 mm potassium phosphate buffer (pH 7.0) and 10.6 mM H_2O_2 freshly prepared. One activity unit was defined as the amount of enzyme necessary to consume 1 μmol H_2O_2 during 1 s. Enzyme activity was expressed as units per millilitres serum (U mL^{-1}).

The fatty acid content of diets and fish tissues was determined as described by Mourente, Tocher, Diaz-Salvago, Grau and Pastor (1999) with minor modifications using gas chromatography (GS, HP6890, Santa Clara, CA, USA). Results are expressed as the percentage of each fatty acid with respect to total fatty acids.

Air exposure test

The tolerance to exposure to air of juveniles was examined according to the method of Yokoyama, Koshio, Takakura, Oshida, Ishikawa, Gallardo-Cigarroa, Catacutan and Teshima (2006) with

modification of exposure time, which was determined based on LD50 (50% of tested fish died). A black coloured net with small mesh was spread out on a plastic container, with a slight sag. Ten Japanese seabass from each sea cage were randomly captured and placed in the net for 65 min. Before exposure to air, water on the body surface of the juveniles was removed using a napkin. After 65 min exposure, fish were immediately returned to the sea cages under the same conditions as the feeding trial and the number of dead fish was recorded. Evidence of death of the test fish was judged by lacking of gill movement within 2 h after return to the sea cages.

Calculations and statistical methods

The following variables were calculated:

$$\text{Specific growth rate (SGR) (\% day}^{-1}) = (\text{Ln } W_t - \text{Ln } W_0) \times 100/t$$

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

$$\text{Percent survival (\%)} = N_t \times 100/N_0$$

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

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

$$\text{Condition factor} = 100 \times W_t/(L_t^3)$$

where W_t and W_0 was final and initial fish weight, respectively; N_t and N_0 was final and initial number of fish, respectively; t was duration of experimental days; L_t was final body length.

All data were subjected to one-way analysis of variance 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 ± standard error of the means. The optimum dietary DHA/EPA ratio based on SGR was estimated using second-order polynomial regression analysis. Two-tailed Pearson correlation analysis and linear regression analysis were used where necessary.

Results

Growth performance

The specific growth rate (SGR) increased significantly ($P < 0.01$) with dietary DHA/EPA ratio increasing from 0.55 to 2.08, and thereafter decreased significantly ($P < 0.01$) (Table 3). Fish fed D/E2.08 showed the highest SGR among experimental groups while fish fed D/E0.55 showed significantly lower SGR compared to other groups. The final weight followed the same pattern as SGR. The feed efficiency ratio (FER) in fish fed D/E2.08 was significantly higher ($P < 0.05$) compared to group D/E0.55. No significant difference ($P > 0.05$) in FER was observed either among groups with DHA/EPA of 0.55, 1.04, 1.53, 2.44 and 2.93 or among groups with DHA/EPA ratio greater than 0.55.

No significant difference ($P > 0.05$) was observed in per cent survival, hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor among dietary treatments.

Second-order polynomial regression analysis on SGR estimated the optimum dietary DHA/EPA ratio to be 2.05 for juvenile Japanese seabass under the present experimental conditions (Fig. 1).

Immune parameters

Fish fed diets with DHA/EPA greater than 1.04 showed significantly ($P < 0.01$) higher activity of serum lysozyme (LYZ) than fish fed D/E0.55 (Table 4). No significant difference ($P > 0.05$) was observed in activity of serum LYZ either among groups with DHA/EPA ratio greater than 0.55 or between group D/E0.55 and D/E1.04. The activity of serum alternative complement pathway (ACP) in fish fed D/E1.53 was significantly higher ($P < 0.05$) compared with group D/E2.93. No significant difference ($P > 0.05$) was observed in activity of serum ACP either among groups with DHA/EPA of 0.55, 1.04, 2.08, 2.44 and 2.93 or among groups with DHA/EPA ratio less than 2.93.

The activity of serum superoxide dismutase (SOD) in group D/E0.55 was significantly lower ($P < 0.01$) compared to other groups (Table 4). Fish fed D/E1.53 showed the highest activity of serum SOD among experimental groups, which was significantly higher ($P < 0.01$) compared with group D/E0.55 and D/E2.44. There

Table 3 Growth performance and survival of Japanese seabass (*Lateolabrax japonicus*) fed the diets with graded levels of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio (means ± standard error of means, $n = 3$)*

Growth response	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio						*F*-value	*P*-value
	D/E0.55	D/E1.04	D/E1.53	D/E2.08	D/E2.44	D/E2.93		
Initial weight (g)	9.48 ± 0.09	9.48 ± 0.09	9.48 ± 0.09	9.48 ± 0.09	9.48 ± 0.09	9.48 ± 0.09		
Final weight (g)	64.41 ± 0.42^{c}	78.61 ± 3.15^{b}	83.47 ± 0.60^{b}	97.39 ± 1.32^{a}	81.66 ± 1.70^{b}	82.06 ± 2.65^{b}	30.231	0.000
SGR (% d^{-1})	2.74 ± 0.01^{c}	3.02 ± 0.06^{b}	3.11 ± 0.01^{b}	3.33 ± 0.02^{a}	3.08 ± 0.03^{b}	3.08 ± 0.05^{b}	31.290	0.000
Per cent survival (%)	80.00 ± 5.09	90.00 ± 3.33	76.67 ± 1.93	73.33 ± 5.09	83.33 ± 5.09	72.22 ± 6.19	2.053	0.143
FER	0.82 ± 0.05^{b}	0.86 ± 0.03^{ab}	0.91 ± 0.06^{ab}	1.07 ± 0.06^{a}	1.07 ± 0.06^{ab}	0.84 ± 0.03^{ab}	4.758	0.013
HSI (%)	1.16 ± 0.03	1.04 ± 0.03	0.96 ± 0.04	0.92 ± 0.08	1.05 ± 0.04	0.99 ± 0.07	2.460	0.093
VSI (%)	7.35 ± 0.41	7.21 ± 0.07	8.37 ± 0.43	7.92 ± 0.13	7.70 ± 0.43	7.65 ± 0.41	1.397	0.293
Condition factor	1.59 ± 0.02	1.56 ± 0.01	1.61 ± 0.01	1.61 ± 0.02	1.60 ± 0.02	1.59 ± 0.02	1.351	0.309

*Means in the same row sharing a same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$).
SGR, specific growth rate; FER, feed efficiency ratio; HSI, hepatosomatic index; VSI, viscerosomatic index.

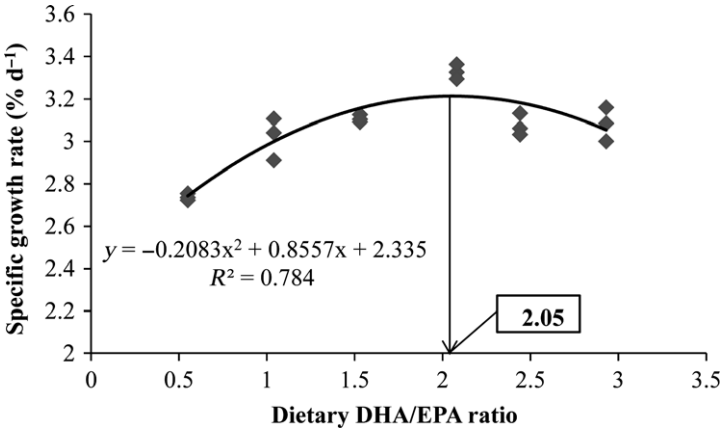

Figure 1 Optimum dietary DHA/EPA ratio based on specific growth rate of Japanese seabass, *Lateolabrax japonicus*. *F* and *P*-value was 27.222 and 0.000, respectively, for analysis of variance in regression analysis using SPSS.

was no significant difference ($P > 0.05$) in activity of serum SOD among fish fed diets with DHA/EPA ratio of 1.04, 1.53, 2.08 and 2.93.

No significant difference ($P > 0.05$) was observed in activities of both respiratory burst (RPB) of head kidney macrophage and serum catalase (CAT) among dietary treatments.

Survival after air exposure

The per cent survival after exposure to air in group D/E0.55 was the lowest among experimental groups and was significantly lower ($P < 0.01$) than that in groups with DHA/EPA of 1.53, 2.08 and 2.93 (Fig. 2). No significant difference ($P > 0.05$) was observed either among groups with DHA/EPA of 0.55, 1.04 and 2.44 or among groups with DHA/EPA greater than 0.55.

Fatty acids composition

The fatty acid composition of whole body, liver and muscle is presented in Tables 5–7 respectively. With the increase of dietary DHA/EPA ratio, the contents of C14:0, C16:1n-7 and DHA in whole body and liver, C18:1n-7 in muscle, and DHA/EPA ratio in whole body and analysed tissues significantly increased, while the contents of C18:1n-9 in whole body and muscle, C18:2n-6 and C22:5n-3 in liver and muscle, C18:3n-3 in

Table 4 Immune response of Japanese seabass (*Lateolabrax japonicus*) fed the experimental diets with graded levels of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio (means ± standard error of means, $n = 3$)*

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio							
Immune response	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**	***F*-value**	***P*-value**
Respiratory burst activity (OD630)	0.10 ± 0.03	0.12 ± 0.01	0.15 ± 0.04	0.16 ± 0.01	0.08 ± 0.02	0.13 ± 0.04	1.140	0.392
Lysozyme activity (units ml^{-1})	93.68 ± 8.49^{b}	109.22 ± 6.05^{ab}	135.74 ± 5.57^{a}	132.12 ± 10.61^{a}	130.15 ± 8.30^{a}	129.34 ± 1.92^{a}	5.088	0.010
ACP activity (ACH_{50} units ml^{-1})	162.53 ± 24.04^{ab}	179.32 ± 11.38^{ab}	216.12 ± 20.05^{a}	168.48 ± 21.37^{ab}	162.55 ± 11.86^{ab}	118.69 ± 13.25^{b}	3.146	0.048
SOD activity (units ml^{-1})	425.61 ± 14.11^{c}	570.31 ± 21.52^{ab}	584.2 ± 12.51^{a}	525.77 ± 14.98^{ab}	504.45 ± 15.73^{b}	518.13 ± 15.32^{ab}	12.459	0.000
Catalase activity (units ml^{-1})	2.98 ± 0.54	2.60 ± 0.52	2.83 ± 0.64	2.55 ± 1.33	1.73 ± 0.62	1.13 ± 0.37	1.336	0.325

*Means in the same row sharing a same superscript letter are not significantly different determined by Tukey's test ($P > 0.05$).
ACP, alternative complement pathway; ACH_{50}, 50% haemolysis; SOD, superoxide dismutase.

muscle, EPA in whole body and analysed tissues and EPA/ARA ratio in whole body and liver significantly decreased. N-3 fatty acids contents, n-3 LC-PUFAs contents, and the n-3/n-6 fatty acids ratio in whole body, but not in liver and muscle, significantly increased with the increase of dietary DHA/EPA ratio while n-6 fatty acids in liver and muscle significantly decreased meantime. Different from DHA contents in whole body and liver, the DHA content in muscle was not significantly influenced by dietary DHA/EPA ratio.

The DHA contents were much higher than EPA contents in whole body, liver and muscle (Fig. 3). DHA and EPA contents in muscle were much higher than those in whole body and liver respectively.

Discussion

Results of this study suggested that Japanese seabass need moderate dietary DHA/EPA ratio (1.53–2.08) to support normal growth performance and the optimal dietary DHA/EPA ratio in juvenile Japanese seabass was estimated to be 2.05 based on specific growth rate, indicating that juvenile Japanese seabass required more DHA than EPA in diets. This was in agreement with findings on other marine species such as larval gilthead seabream (*Sparus aurata*) (Rodríguez *et al.* 1997), juvenile grouper (*Epinephelus malabaricus*) (Wu *et al.* 2002) and juvenile cobia (*Rachycentron canadum*) (Trushenski *et al.* 2012), which obtained the highest growth rate when fed diets with DHA/EPA ratio of 1.4, 3.0 and 3.2 respectively. However, different results were observed in another study on gilthead seabream (Ibeas *et al.* 1997), which showed that juvenile gilthead seabream required more EPA (1.0%) than DHA (0.5%) in diets. Villalta and Estévez (2005) also observed that benthic fauna Senegal sole larvae *Solea senegalensis* required negligible amounts of dietary DHA but probably higher EPA content. These discrepancies suggested that the difference between effects of DHA and EPA on fish was species-specific. Rearing condition might be another factor determining the optimum dietary DHA/EPA ratio of fish. Dantagnan *et al.* (2010) reported that common galaxias larvae reared at higher salinities (10‰ and 15‰) had higher dietary requirement for DHA, whereas larvae reared at 0‰ salinity showed higher requirements for EPA. Moreover, other dietary nutrients could also

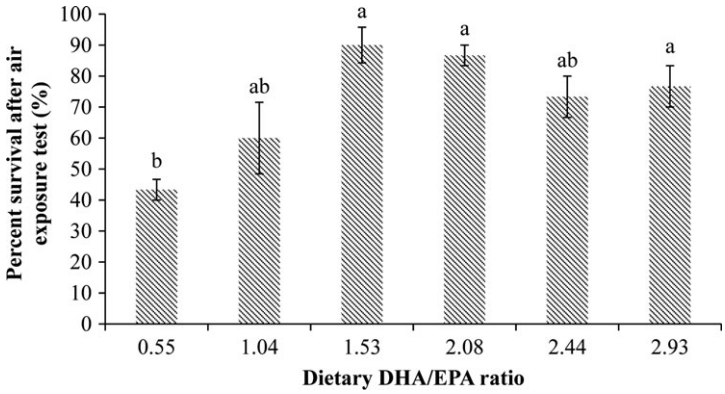

Figure 2 Survival of Japanese seabass after air exposure. Bars denoted by the same letter are not significantly different ($P > 0.05$) ($n = 3$).

Table 5 Whole-body fatty acid composition of Japanese seabass (*Lateolabrax japonicus*) fed the experimental diets with graded levels of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio (% total fatty acids) (means ± standard error of means, $n = 3$)*

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio						*F*-	*P*-
Fatty acid	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**	**value**	**value**
C 14: 0	1.65 ± 0.10^{b}	2.35 ± 0.16^{a}	2.50 ± 0.06^{a}	2.50 ± 0.09^{a}	2.62 ± 0.08^{a}	2.78 ± 0.03^{a}	13.997	0.000
C 16: 0	14.44 ± 0.35	14.98 ± 0.54	15.49 ± 0.57	14.81 ± 0.11	15.76 ± 0.20	15.48 ± 0.22	1.970	0.169
C 18: 0	4.19 ± 0.06	4.05 ± 0.09	4.14 ± 0.25	3.72 ± 0.13	3.79 ± 0.15	3.81 ± 0.19	2.189	0.137
∑SFA	20.28 ± 0.51	21.39 ± 0.69	22.13 ± 0.88	21.02 ± 0.10	22.18 ± 0.06	22.07 ± 0.44	2.482	0.104
C 16: 1n-7	2.94 ± 0.18^{b}	3.86 ± 0.32^{ab}	3.77 ± 0.03^{ab}	3.75 ± 0.24^{ab}	4.39 ± 0.31^{a}	4.14 ± 0.06^{ab}	4.129	0.027
C 18: 1n-7	0.32 ± 0.02	0.32 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.30 ± 0.01	0.001	1.829
C 18: 1n-9	20.63 ± 0.32^{ab}	20.82 ± 0.62^{a}	20.02 ± 0.13^{ab}	18.71 ± 0.25^{b}	19.18 ± 0.27^{ab}	19.14 ± 0.53^{ab}	5.062	0.014
C 20: 1n-9	4.20 ± 0.14	3.86 ± 0.04	3.86 ± 0.07	3.90 ± 0.12	3.72 ± 0.29	3.75 ± 0.06	1.148	0.397
C 22: 1n-11	2.61 ± 0.10	2.28 ± 0.02	2.34 ± 0.09	2.31 ± 0.16	2.13 ± 0.25	2.13 ± 0.20	1.272	0.348
∑MUFA	30.71 ± 0.74	31.13 ± 0.84	30.27 ± 0.27	28.96 ± 0.12	29.61 ± 0.28	29.46 ± 0.34	2.309	0.122
C 18: 2n-6	29.99 ± 1.00	26.69 ± 1.31	27.05 ± 0.55	25.51 ± 1.84	25.42 ± 0.42	23.88 ± 0.12	2.992	0.066
C 18: 3n-6	0.39 ± 0.04	0.31 ± 0.02	0.25 ± 0.03	0.32 ± 0.01	0.26 ± 0.01	0.32 ± 0.04	3.183	0.063
C 20: 4n-6	0.80 ± 0.03	1.01 ± 0.12	0.82 ± 0.08	0.72 ± 0.03	0.99 ± 0.10	0.88 ± 0.10	1.818	0.197
∑n-6	31.18 ± 0.94	28.01 ± 1.44	28.11 ± 0.67	26.55 ± 1.83	26.58 ± 0.38	25.08 ± 0.25	2.95	0.068
C 18: 3n-3	2.69 ± 0.13	2.60 ± 0.10	2.47 ± 0.20	2.49 ± 0.15	2.49 ± 0.03	2.29 ± 0.00	1.157	0.393
C 18: 4n-3	0.54 ± 0.01	0.57 ± 0.05	0.50 ± 0.06	0.55 ± 0.04	0.54 ± 0.02	0.48 ± 0.01	0.715	0.627
C 20: 5n-3	4.04 ± 0.19^{b}	3.25 ± 0.28^{ab}	3.22 ± 0.16^{ab}	3.20 ± 0.14^{ab}	2.95 ± 0.07^{a}	2.71 ± 0.02^{a}	6.181	0.007
C 22: 5n-3	0.98 ± 0.10	0.87 ± 0.01	0.96 ± 0.13	1.12 ± 0.07	1.07 ± 0.10	1.02 ± 0.04	1.177	0.385
C 22: 6n-3	4.32 ± 0.15^{d}	5.39 ± 0.19 ^{cd}	6.20 ± 0.43^{bc}	7.20 ± 0.41^{ab}	7.64 ± 0.45^{ab}	7.89 ± 0.12^{a}	18.505	0.000
∑n-3	12.57 ± 0.20^{b}	12.67 ± 0.27^{ab}	13.36 ± 0.97^{ab}	14.55 ± 0.24^{ab}	14.69 ± 0.53^{a}	14.40 ± 0.08^{ab}	5.738	0.009
∑PUFA	43.75 ± 1.08	43.75 ± 1.27	43.75 ± 0.31	43.75 ± 1.59	43.75 ± 0.48	43.75 ± 0.17	1.542	0.262
∑n-3LC-PUFA	9.34 ± 0.15^{b}	9.51 ± 0.27^{b}	10.39 ± 0.71^{ab}	11.52 ± 0.35^{a}	11.66 ± 0.49^{a}	11.62 ± 0.06^{a}	8.66	0.002
∑n-3/∑n-6	0.40 ± 0.01^{b}	0.46 ± 0.03^{ab}	0.48 ± 0.05^{ab}	0.55 ± 0.05^{ab}	0.55 ± 0.03^{ab}	0.57 ± 0.01^{a}	4.465	0.021
C 18: 1n-9/∑n-3	1.64 ± 0.04^{a}	1.64 ± 0.07^{a}	1.51 ± 0.12^{ab}	1.29 ± 0.02^{b}	1.31 ± 0.05^{b}	1.33 ± 0.04^{b}	8.865	0.002
EPA/ARA	5.06 ± 0.43^{a}	3.38 ± 0.61^{ab}	4.01 ± 0.58^{ab}	4.42 ± 0.04^{ab}	3.04 ± 0.28^{ab}	2.50 ± 0.66^{b}	4.085	0.024
DHA/EPA	1.08 ± 0.09^{d}	1.69 ± 0.19 ^{cd}	1.92 ± 0.04^{bc}	2.27 ± 0.21^{abc}	2.59 ± 0.10^{ab}	2.91 ± 0.06^{a}	19.513	0.000

*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.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; n-6, n-6 unsaturated fatty acid; n-3, n-3 unsaturated fatty acid.

influence the requirements of n-3 LC-PUFAs in fish. A study on cobia (Ding *et al.* 2009) showed that the growth of juvenile cobia was not greatly influenced by ratios of DHA to EPA since a high content of dietary fishmeal (67%) used in the study probably have met the requirement of n-3 LC-PUFAs in cobia and masked the effects of the additive DHA and EPA.

Table 6 Liver fatty acid composition of Japanese seabass (*Lateolabrax japonicus*) fed the experimental diets with graded levels of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio (% total fatty acids) (means ± standard error of means, $n = 3$)*

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio						*F*-	*P*-
Fatty acid	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**	**value**	**value**
C 14: 0	1.69 ± 0.05^{c}	2.37 ± 0.20^{bc}	2.91 ± 0.01^{ab}	2.95 ± 0.06^{ab}	2.91 ± 0.22^{ab}	3.34 ± 0.16^{a}	10.488	0.002
C 16: 0	18.69 ± 0.52	20.45 ± 1.88	23.24 ± 1.22	23.97 ± 0.69	23.32 ± 0.89	23.10 ± 1.27	2.176	0.147
C 18: 0	6.99 ± 0.07	6.93 ± 0.71	7.17 ± 0.63	7.99 ± 0.64	7.52 ± 0.47	6.73 ± 0.56	0.575	0.719
∑SFA	27.36 ± 0.40	29.75 ± 2.77	33.32 ± 1.86	34.91 ± 1.27	33.76 ± 1.26	33.17 ± 1.88	1.902	0.189
C 16: 1n-7	2.97 ± 0.03^{b}	3.99 ± 0.39^{ab}	4.45 ± 0.10^{a}	4.23 ± 0.18^{ab}	4.38 ± 0.34^{a}	4.81 ± 0.06^{a}	4.805	0.020
C 18: 1n-7	2.43 ± 0.38	2.79 ± 0.35	3.79 ± 0.24	3.90 ± 0.19	3.62 ± 0.35	4.04 ± 0.39	3.231	0.060
C 18: 1n-9	16.07 ± 0.01	16.61 ± 0.86	17.14 ± 0.55	16.80 ± 0.01	16.44 ± 0.34	16.29 ± 0.24	0.453	0.802
C 20: 1n-9	3.61 ± 0.18	3.57 ± 0.10	4.23 ± 0.07	4.01 ± 0.13	4.04 ± 0.12	3.41 ± 0.39	1.992	0.174
C 22: 1n-11	1.00 ± 0.15^{b}	1.02 ± 0.04^{b}	1.50 ± 0.06^{a}	1.10 ± 0.15^{ab}	1.39 ± 0.09^{ab}	1.45 ± 0.08^{ab}	5.995	0.010
∑MUFA	26.07 ± 0.08	27.98 ± 1.46	31.11 ± 0.19	30.03 ± 0.26	29.87 ± 0.80	30.01 ± 0.55	3.456	0.051
C 18: 2n-6	27.50 ± 0.09^{a}	22.93 ± 1.95^{ab}	19.52 ± 1.80^{ab}	19.07 ± 0.56^{b}	19.74 ± 1.47^{ab}	19.82 ± 1.64^{ab}	3.505	0.049
C 18: 3n-6	0.30 ± 0.01	0.26 ± 0.01	0.23 ± 0.01	0.25 ± 0.02	0.23 ± 0.01	0.26 ± 0.02	2.08	0.160
C 20: 4n-6	0.80 ± 0.06	0.59 ± 0.07	0.48 ± 0.00	0.55 ± 0.01	0.61 ± 0.03	0.51 ± 0.08	3.183	0.063
∑n-6	28.61 ± 0.04^{a}	23.78 ± 2.00^{ab}	20.23 ± 1.80^{ab}	19.87 ± 0.53^{b}	20.58 ± 1.51^{ab}	20.58 ± 1.65^{ab}	3.644	0.044
C 18: 3n-3	2.12 ± 0.06	1.53 ± 0.29	1.18 ± 0.16	1.17 ± 0.06	1.28 ± 0.12	1.15 ± 0.17	3.258	0.059
C 18: 4n-3	0.27 ± 0.01	0.24 ± 0.02	0.24 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.02	1.433	0.320
C 20: 5n-3	2.60 ± 0.17^{a}	1.75 ± 0.32^{ab}	1.19 ± 0.22^{b}	1.11 ± 0.25^{b}	0.93 ± 0.06^{b}	0.73 ± 0.14^{b}	9.354	0.002
C 22: 5n-3	1.15 ± 0.11^{a}	0.70 ± 0.20^{ab}	0.50 ± 0.09^{b}	0.42 ± 0.07^{b}	0.47 ± 0.03^{b}	0.36 ± 0.06^{b}	5.43	0.014
C 22: 6n-3	2.47 ± 0.10^{b}	2.93 ± 0.61^{ab}	3.51 ± 0.20^{ab}	3.59 ± 0.06^{ab}	3.78 ± 0.06^{ab}	4.12 ± 0.44^{a}	4.108	0.046
∑n-3	8.62 ± 0.02	6.17 ± 0.75	6.62 ± 0.66	6.49 ± 0.46	6.62 ± 0.19	6.78 ± 0.89	2.006	0.182
∑PUFA	37.23 ± 0.02	29.95 ± 2.42	26.85 ± 2.46	26.37 ± 0.99	27.20 ± 1.70	28.41 ± 3.11	3.181	0.071
∑n-3LC-PUFA	6.23 ± 0.05	4.40 ± 0.73	5.20 ± 0.50	5.11 ± 0.38	5.19 ± 0.01	5.40 ± 0.55	1.436	0.309
∑n-3/∑n-6	0.30 ± 0.00	0.26 ± 0.03	0.33 ± 0.00	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	2.208	0.153
C 18: 1n-9/ ∑n-3	1.86 ± 0.00	2.75 ± 0.27	2.61 ± 0.18	2.60 ± 0.18	2.49 ± 0.07	2.41 ± 0.30	2.138	0.162
EPA/ARA	3.24 ± 0.04^{b}	2.95 ± 0.24^{b}	2.51 ± 0.48^{ab}	2.01 ± 0.50^{ab}	1.53 ± 0.05^{a}	1.45 ± 0.23^{a}	7.771	0.004
DHA/EPA	0.96 ± 0.10^{c}	2.02 ± 0.24^{bc}	3.01 ± 0.39^{ab}	3.40 ± 0.73^{ab}	4.10 ± 0.31^{a}	4.76 ± 0.10^{a}	13.545	0.002

*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.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; n-6, n-6 unsaturated fatty acid; n-3, n-3 unsaturated fatty acid.

As in effects on growth performances, the difference between DHA and EPA in influence on immune functions of animals has also been reported though related information was not abundant. *In vivo* studies on human have shown that DHA-rich fish oil caused an increase in leucocyte functions, while this phenomenon was not observed in treatments with EPA-rich fish oil (Kew, Mesa, Tricon, Buckley, Minihane & Yaqoob 2004; Miles, Banerjee, Dooper, M'Rabet, Graus & Calder 2004; Gorjão, Verlengia, Lima, Soriano, Boaventura, Kanunfre, Peres, Sampaio, Otton, Folador, Martins, Curi, Portiolli, Newsholme & Curi 2006; Gorjão, Azevedo-Martins, Rodrigues, Abdulkader, Arcisio-Miranda, Procopio & Curi 2009). In contrast, a study on mice showed that solely supplementation of DHA had potentially adverse effects on host resistance to *Paracoccidioides brasiliensis* infection (Oarada, Tsuduki, Suzuki, Miyazawa, Nikawa & Hong-quan 2003). In fish, the study on immune modulation by dietary DHA/EPA ratio was even more limited. Wu *et al.* (2003) reported that compared with low DHA/EPA ratio (0.3–0.7), higher ratio (2.0–3.0) significantly enhanced the activities of phagocytosis and respiratory burst in grouper head kidney leucocytes, indicating that DHA was more optimal than EPA affecting the cellular defence responses in grouper juveniles. Another study conducted on large yellow croaker (Zuo *et al.* 2012a) showed that compared to low DHA/EPA ratio (0.61), moderate (2.17) or higher ratio (3.04) enhanced the nitro blue tetrazolium (NBT) positive leucocytes percentage of head kidney and the activity of serum lysozyme. In the present study, it was also observed that compared to low DHA/EPA ratio (0.55), moderate ratios (1.53–2.08) enhanced the innate immune responses in serum such as activi-

Table 7 Muscle fatty acid composition of Japanese seabass (*Lateolabrax japonicus*) fed the experimental diets with graded levels of docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio (% total fatty acids) (means ± standard error of means, $n = 3$)*

	Docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio							
Fatty acid	**D/E0.55**	**D/E1.04**	**D/E1.53**	**D/E2.08**	**D/E2.44**	**D/E2.93**	***F*-value**	***P*-value**
C 14: 0	1.01 ± 0.04	1.05 ± 0.11	1.28 ± 0.06	1.31 ± 0.17	1.25 ± 0.26	1.26 ± 0.26	0.669	0.655
C 16: 0	17.35 ± 0.20	17.85 ± 0.47	17.89 ± 0.07	18.31 ± 0.40	18.37 ± 0.70	18.50 ± 0.74	0.865	0.534
C 18: 0	6.94 ± 0.35	7.03 ± 0.35	6.79 ± 0.07	7.06 ± 0.35	6.93 ± 0.49	7.17 ± 0.37	0.126	0.984
∑SFA	25.30 ± 0.48	25.92 ± 0.49	25.95 ± 0.07	26.68 ± 0.54	26.56 ± 0.97	26.93 ± 0.84	0.948	0.488
C 16: 1n-7	2.00 ± 0.03	1.98 ± 0.27	2.20 ± 0.04	2.15 ± 0.13	2.08 ± 0.15	2.16 ± 0.18	0.329	0.885
C 18: 1n-7	2.90 ± 0.07^{b}	2.85 ± 0.15^{ab}	3.24 ± 0.01^{ab}	3.23 ± 0.09^{ab}	3.38 ± 0.19^{ab}	3.41 ± 0.18^{a}	3.523	0.038
C 18: 1n-9	15.35 ± 0.08^{a}	14.48 ± 0.50^{ab}	13.39 ± 0.04^{bc}	13.39 ± 0.42^{bc}	13.26 ± 0.56^{bc}	12.26 ± 0.16^{c}	7.434	0.003
C 20: 1n-9	2.75 ± 0.03	2.52 ± 0.06	2.73 ± 0.20	2.96 ± 0.30	3.04 ± 0.21	2.53 ± 0.37	1.037	0.443
C 22: 1n-11	0.84 ± 0.01	0.76 ± 0.05	0.87 ± 0.12	1.02 ± 0.21	1.15 ± 0.11	1.15 ± 0.16	1.695	0.216
∑MUFA	23.85 ± 0.19	22.60 ± 0.85	22.43 ± 0.33	22.75 ± 1.11	23.20 ± 1.05	21.53 ± 0.73	0.820	0.560
C 18: 2n-6	23.09 ± 0.61^{a}	21.31 ± 0.83^{ab}	19.37 ± 1.18^{ab}	19.00 ± 2.14^{ab}	17.03 ± 1.45^{ab}	15.97 ± 2.09^{b}	3.203	0.050
C 20: 4n-6	2.33 ± 0.24	2.31 ± 0.13	2.29 ± 0.19	2.23 ± 0.36	2.31 ± 0.21	2.55 ± 0.50	0.129	0.983
∑n-6	25.42 ± 0.36	23.61 ± 0.70	21.66 ± 0.99	21.23 ± 1.78	19.34 ± 1.34	18.52 ± 1.59	4.432	0.019
C 18: 3n-3	1.92 ± 0.09^{a}	1.67 ± 0.10^{ab}	1.60 ± 0.12^{ab}	1.76 ± 0.08^{ab}	1.26 ± 0.17^{b}	1.23 ± 0.23^{b}	4.602	0.016
C 20: 5n-3	5.23 ± 0.19^{a}	4.62 ± 0.26^{ab}	3.93 ± 0.11^{bc}	3.56 ± 0.17^{bc}	3.37 ± 0.30^{c}	3.15 ± 0.18^{c}	13.356	0.000
C 22: 5n-3	2.13 ± 0.01^{a}	1.82 ± 0.12^{ab}	1.56 ± 0.06^{bc}	1.44 ± 0.12^{bc}	1.36 ± 0.09^{c}	1.27 ± 0.08^{c}	11.881	0.000
C 22: 6n-3	14.78 ± 0.12	16.69 ± 1.17	18.36 ± 1.16	17.80 ± 2.28	19.07 ± 1.00	20.88 ± 2.48	1.868	0.180
∑n-3	24.06 ± 0.39	24.80 ± 1.10	25.46 ± 1.11	24.56 ± 2.57	25.05 ± 1.24	26.54 ± 2.52	0.247	0.933
∑PUFA	49.49 ± 0.75	48.41 ± 0.77	47.12 ± 0.24	45.79 ± 1.03	44.40 ± 2.30	45.06 ± 0.93	2.626	0.085
∑n-3LC-PUFA	22.14 ± 0.30	23.13 ± 1.19	23.85 ± 1.23	22.80 ± 2.54	23.80 ± 1.22	25.30 ± 2.74	0.392	0.844
∑n-3/∑n-6	0.95 ± 0.00	1.05 ± 0.07	1.18 ± 0.10	1.20 ± 0.23	1.30 ± 0.08	1.46 ± 0.26	1.513	0.263
C 18: 1n-9/∑n-3	0.64 ± 0.01	0.59 ± 0.04	0.53 ± 0.03	0.56 ± 0.07	0.53 ± 0.05	0.47 ± 0.05	1.411	0.294
EPA/ARA	2.30 ± 0.29	2.02 ± 0.19	1.74 ± 0.16	1.65 ± 0.17	1.49 ± 0.22	1.27 ± 0.18	2.856	0.068
DHA/EPA	2.83 ± 0.08^{d}	3.63 ± 0.31 ^{cd}	4.68 ± 0.32^{bc}	4.96 ± 0.41^{abc}	5.74 ± 0.54^{ab}	6.60 ± 0.42^{a}	12.553	0.000

*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.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; n-6, n-6 unsaturated fatty acid; n-3, n-3 unsaturated fatty acid.

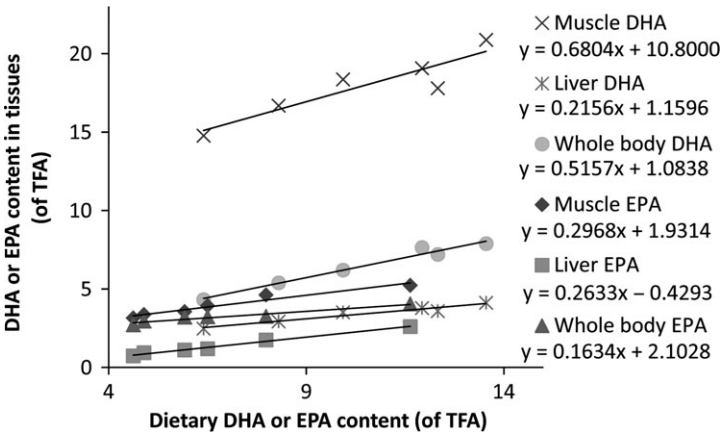

Figure 3 Regressions between the DHA or EPA content in tissues and its content in the diet. The (R, P) value for muscle DHA, Liver DHA, whole body DHA, muscle EPA, liver EPA and whole body EPA was (0.615, 0.009), (0.838, 0.000), (0.937, 0.000), (0.901, 0.000), (0.911, 0.000) and (0.840, 0.000) respectively.

ties of lysozyme and superoxide dismutase. These results indicated that high levels of dietary EPA or low levels of dietary DHA could inhibit the immune function. Bell, Tocher, MacDonald and Sargent (1995) even found that dietary oils with high EPA content could generate histopathological lesions. However, the highest dietary DHA/EPA ratio (2.93) in the present study significantly reduced the activity of serum alternative complement pathway compared to the moderate ratio

(1.53), suggesting that excess dietary DHA or deficiency of dietary EPA could also cause inhibition on fish immune functions. Considering the deposition ratio of DHA is higher than that of EPA in Japanese seabass, excess DHA more possibly account for the immune reduction in experiment fish fed diets with high DHA/EPA ratios.

Little information is available regarding the mechanisms involved in the difference between DHA and EPA in influencing fish immunity, though the relationship between dietary lipids or fatty acids and fish immune responses have been widely studied (Thompson *et al.* 1996; Farndale *et al.* 1999; Lauridsen *et al.* 2007; Berge *et al.* 2009). Fatty acids are known to modulate leucocyte functions by influencing the physical properties of cell membranes and activity of membrane-associated enzymes (Johnston 1985). The differences between DHA and EPA in affecting fish immunity could be related to that membrane properties of leucocytes such as fluidity and binding to transcription factors could be influenced by the carbon length and desaturation degree of fatty acids (Hatala, Rayburn & Rose 1993; Drevon, Nenseter, Brude, Finstad, Kolset & Rustan 1995; Logan 2003). The immune relevant enzymes or proteins such as lysozyme and alternative complement pathway influenced by dietary DHA/EPA ratio in the present study could be related to the alteration of membrane properties and subsequently to the alteration of signal transduction and cell membrane interactions (Weldon, Mullen, Loscher, Hurley & Roche 2007; Gorjão *et al.* 2009). Another mechanism involved in immune modulation by DHA/EPA ratio is that the alteration of EPA content could cause altered EPA/ARA ratio, and thus cause altered profiles of EPA- and ARA-derived immune active metabolites such as prostaglandins (PGs) and leucotrienes (Kinsella, Lokesh, Broughton & Whelan 1990; Calder, Bevan & Newsholme 1992; Rowley, Knight, Lloyd-Evans, Holland & Vickers 1995; Bell *et al.* 1996). Reduced immune responses by low (0.55) or relatively high (2.93) DHA/EPA ratio in this study could be partially due to that unbalanced profiles of eicosanoids derived from EPA and ARA such as PG/PGE_2 exerted immunosuppressive effects (Hwang 1989, 2000; Farndale *et al.* 1999; Furne, Holen, Araujo, Lie & Moren 2013). Moreover, it has been reported that fatty acids could also modulate fish immunity through regulating the redox environment (Calder 2001; Delaporte, Soudant, Moal, Giudicelli, Lambert, Séguineau & Samain 2006). In the present study, the activity of serum SOD was significantly influenced by DHA/EPA ratio, indicating the difference among n-3 LC-PUFAs in modulating the redox environment. However, the other two redox relevant immune parameters, activities of respiratory burst of macrophages and serum catalase, were not significantly influenced by dietary DHA/EPA in the present study. This might be due to that different immunological parameters respond differently to dietary treatments (Zuo *et al.* 2012a). The immunity fatigue due to long-term continuous administration of immunoregulatory nutrients could also partially account for the absence of significant differences in certain immune parameters in this study (Chang, Chen, Su & Liao 2000; Bai, Zhang, Mai, Wang, Xu & Ma 2010). The mechanisms involved in immune modulation by DHA/EPA ratio in fish were still poorly understood and need further research.

The difference between DHA and EPA in affecting stress resistance has been reported in several studies on fish larvae. Studies on Japanese flounder larvae showed that DHA was superior to EPA in enhancing the tolerance to stress factors such as changes in water temperature and salinity and exposure to low dissolved oxygen (Furuita *et al.* 1999; Tago *et al.* 1999). This was in accordance with the result of the present study, which showed that the tolerance to air exposure of juvenile Japanese seabass was significantly enhanced by higher dietary DHA/EPA ratio (1.53–2.93) compared to low ratio (0.55). However, the study on turbot juvenile (Bell *et al.* 1998) showed inconsistent results, which recorded that the recovery time from anaesthesia was not significantly different between fish fed diets containing DHA-enriched tuna orbital oil and fish fed EPA-enriched southern hemisphere fish oil Marinol, and the latter even performed slightly better. The study on gilthead sea bream larvae also showed that increased dietary EPA improved larval stress resistances to handling and temperature tests (Liu *et al.* 2002). The discrepancy among these studies could be related to the different stress types used in these studies because fatty acids mainly affect stress resistance through affecting steroidogenesis, which plays different roles in different stress tests (Van Anholt, Koven, Lutzky & Bonga 2004). It could also be related to different developmental stages which affected the sensitivity of fish to stress (Koven, Van

Anholt, Lutsky, Ben, Nixon, Ron & Tandler 2003; Bransden, Cobcroft, Battaglene, Dunstan, Nichols & Bell 2004).

Taking into account growth, immune responses and stress resistance, an interesting and noteworthy pattern regarding the correlations between these parameters was observed, which showed that significant positive correlations existed between growth, stress resistance and certain immune parameters, such as lysozyme and superoxide dismutase in serum, and respiratory burst of head kidney macrophages (Fig. 4). This suggested that dietary DHA/EPA probably influence growth, immunity and stress resistance of fish through the same mechanisms. Though fatty acids could affect growth and immune responses in various ways as stated above, eicosanoids could be the mediators involved in effects of dietary DHA/EPA on both growth and immunity. Alterations of the profile of prostaglandins (PGs) such as PGE, PGE_2 and PGF_{2a} not only modulate the immune functions but also affect the growth rate through influencing muscle fibre formation (Palmer 1990). PGs have also been shown to be involved in stress resistance-related metabolisms, such as control of osmoregulatory processes and regulation of stress-induced hypothalamus-pituitary-interrenal axis, which facilitates the release of cortisol, the main corticosteroid in teleost fish (Van Praag, Farber, Minkin & Primor 1987; Wales 1988). Besides eicosanoids, more fundamental processes like alterations in membrane properties and cellular signal transduction must also contribute to the consistent effects of dietary DHA/EPA on growth, stress resistance and certain immune responses. However, precise mechanisms are not well known and need to be elucidated by further studies.

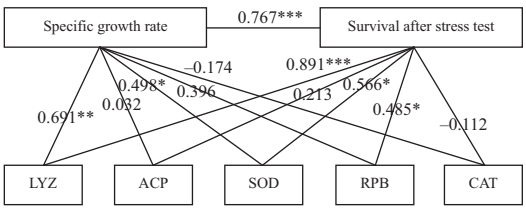

Figure 4 Pearson's correlation coefficients (r) and P-values between growth, immunity and stress resistance of Japanese seabass affected by dietary docosahexaenoic acid to eicosapentaenoic acid (DHA/EPA) ratio. SGR, specific growth rate; LYZ, lysozyme; ACP, alternative complement pathway; SOD, superoxide dismutase; RPB, respiratory burst; CAT, catalase. ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.

It has been well revealed in previous studies (Farndale *et al.* 1999; Bransden, Battaglene *et al.* 2005; Villalta *et al.* 2008; Hossain *et al.* 2012) that the fatty acid profiles of fish tissues closely reflect those of the diets, which was also observed in the present study for most fatty acids. However, an exception was observed in this study, which showed that the C22:5n-3 contents in tissues were not positively correlated with the dietary C22:5n-3 content (the correlation coefficient (r) was −0.616 ($P < 0.01$) and −0.478 ($P > 0.05$) in muscle and liver, respectively), whereas significant positive correlations were observed between tissue C22:5n-3 contents and the dietary EPA content (r was 0.906 and 0.845 in muscle and liver respectively; $P < 0.01$). This suggested that the C22:5n-3 contents in tissues of Japanese seabass might be mainly derived from the bio-conversion from EPA and thus Japanese seabass might have the capacity of biosynthesizing LC-PUFAs to some degree, as was observed in other marine fish such as Atlantic bluefin tuna, gilthead seabream and European sea bass (Morais, Mourente, Ortega, Tocher & Tocher 2011; Vagner & Santigosa 2011). If Japanese seabass indeed have the capacity of biosynthesizing LC-PUFAs to some degree, it might partially account for the much higher DHA content than EPA in tissues of experimental fish for that DHA could be synthesized from EPA through desaturation and elongation. As was observed in other species (Wu *et al.* 2002; Trushenski *et al.* 2012), in this study the preferentially accumulated DHA in tissues of Japanese seabass was in accordance with the predominance of DHA in nutritional requirement of this fish in terms of growth, immune response and stress resistance. In addition, unlike in liver and whole body, the DHA content in muscle of experimental fish was relatively constant among all dietary treatments, not significantly influenced by dietary DHA, and the content in muscle was much higher than that in whole body and liver. The selective accumulation of DHA in muscle must be related to the importance of DHA in muscle-involved physiological capacities of fish such as swimming and predation (Wagner, Balfry, Higgs, Lall & Farrell 2004; Trushenski, Schwarz, Lewis, Laporte, Delbos, Takeuchi & Sampaio 2011).

In conclusion, results of the present study suggested that at the same dietary contents of n-3 LC-PUFAs (abbr. 18% of TFA) and ARA (abbr. 0.7% of TFA), compared to low (0.55) or high

(2.93) ratios, moderate dietary DHA/EPA ratios of 1.53–2.08 significantly enhanced the growth performance, certain innate immune responses, and the stress tolerance of Japanese seabass, which was in accordance with the preferential incorporation of DHA into fish tissues.

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