Aquaculture 307 (2010) 75–82

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effects of dietary arachidonic acid on growth performance, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass, *Lateolabrax japonicus*

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ARTICLE INFO

Article history:
Received 31 March 2010
Received in revised form 22 June 2010
Accepted 4 July 2010

Keywords:
Japanese seabass
Juvenile
Arachidonic acid
Growth
Immune response
Fatty acid composition

ABSTRACT

A 12-week feeding experiment was conducted to investigate the effects of dietary arachidonic acid (ARA) on growth, survival, immune response and tissue fatty acid composition of juvenile Japanese seabass (*Lateolabrax japonicus*) (mean initial weight 9.48 ± 0.09 g) in seawater floating net cages (1.5 × 1.5 × 2.0 m). An ARA-enriched oil was supplemented into the basal diet to formulate six isonitrogenous and isoenergetic practical diets containing 0.08% (the control group), 0.22%, 0.36%, 0.56%, 1.33% and 2.12% ARA of dry weight, respectively. All diets had the same total n−3 long chain polyunsaturated fatty acid (LC-PUFA) level and 22:6n−3/20:5n−3 ratio. Triplicate groups of 30 fish were fed to apparent satiation twice daily for 12 weeks. The water temperature ranged from 22.5 to 31.5 °C, the salinity from 28‰ to 33‰ and the dissolved oxygen content was approximately 6 mg l^{-1} during the experimental period. The results showed that final weight (FW) and specific growth rate (SGR) significantly increased with increasing dietary ARA from 0.08% to 0.36% ($P<0.01$) and thereafter declined. The feed efficiency ratio (FER) had the similar pattern with SGR, and fish fed the diet with 0.36% ARA showed significantly higher FER than the control ($P<0.05$). No significant differences were found in survival rate among dietary treatments ($P>0.05$). The hepatosomatic index (HSI) decreased significantly with the increase of dietary ARA, and HSI in fish fed the diets with more than 0.22% ARA content were significantly lower than the control group ($P<0.01$). The serum lysozyme (LYZ), alternative complement pathway (ACP) and superoxide dismutase (SOD) activity were significantly enhanced by the supplementation of ARA, especially in moderate supplementation (0.36–0.56%). However, there were no differences in both respiratory burst activity of head kidney macrophage and serum catalase (CAT) activity among dietary treatments. The body composition analysis showed that whole-body protein first increased, then decreased with increasing dietary ARA, while whole-body lipid content followed the opposite pattern. The fatty acid composition of whole body and liver reflected closely those of the diets, while liver EPA levels were inversely related to dietary ARA. These results suggested that dietary ARA, especially moderate ARA level (0.22–0.56% d.w.), significantly enhanced growth and immune response and modified the chemical composition of whole body and liver of Japanese seabass juvenile.

1. Introduction

Marine fish species, which lack the ability to synthesize long chain polyunsaturated fatty acids (LC-PUFA) from their 18-carbon precursor fatty acids such as linolenic acid (18:3n−3) and linoleic acid (18:2n−6) (Tocher, 2003), require docosahexaenoic acid (DHA, 22:6n−3), eicosapentaenoic acid (EPA, 20:5n−3) and arachidonic acid (ARA, 20:4n−6) for their normal growth and development (Sargent et al., 1995). In the past decades, considerable studies have attempted to identify optimum dietary levels of EPA and DHA, the so-called n−3 LC-PUFA, and their physiological and biochemical functions in a range of marine fish species (Sargent et al., 1999; Lee, 2001), while the importance of ARA, the n−6 LC-PUFA, has been largely neglected since ARA is a minor component in fish cell membranes and required in relatively small quantities compared to DHA and EPA. Moreover, the contribution of ARA to growth and survival is easily masked if other essential fatty acid levels are sub-optimal. Only recently has this neglect been recognized and studies are now being performed to evaluate the importance of ARA in fish nutrition, with gradual elucidation of the physiological functions of the eicosanoids. It has been known that ARA can be metabolized into highly bioactive eicosanoids such as prostaglandins, thromboxanes and leukotrienes and these metabolites are very active even at low physiological concentrations, playing a critical role in the regulation of several biological processes (Piomelli, 1993; Tang et al., 1996).

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doi:10.1016/j.aquaculture.2010.07.001

The limited amount of work performed to date suggested that dietary ARA contributed to enhanced growth, survival and influenced tissue fatty acid profile in a variety of marine teleosts (Castell et al., 1994; Estévez et al., 1997; Bessonart et al., 1999). Moreover, ARA was also reported to play an important role in the regulation of a range of physiological processes in marine fish, such as reproduction (Bruce et al., 1999; Furuita et al., 2003), metamorphosis (Lund et al., 2008), pigmentation (Estévez et al., 1999; Copeman et al., 2002; Villalta et al., 2005; Lund et al., 2007, 2008) and resistance to various stressors (Koven et al., 2001, 2003; Van Anholt et al., 2004; Rezek et al., 2009). The effects of ARA and its metabolites on immune functions in culture cells (Surette et al., 1999; Peres et al., 2005), animal model and humans (Kelley et al., 1998; Harbige, 2003) have been widely demonstrated in previous studies and the evaluation of impact of ARA on fish immune response has also been reported, in pace with studies on effects of n−3 polyunsaturated fatty acid (PUFA) on fish immune functions (Wu et al., 2003). However, to date, no study on effects of dietary ARA on immune response of marine fish was reported. Several investigations in freshwater fish (Harel et al., 2001; Khozin-Goldberg et al., 2006) and oyster (Delaporte et al., 2006; Hurtado et al., 2009) showed that moderate level of dietary ARA was effective in modulating the hemocyte concentration and function, and the resistance to disease.

Japanese seabass (*Lateolabrax japonicus*) is a carnivorous species widely cultured in China because of its delicious meat and rapid growth. Few studies have been reported on the nutrition of this seabass (Ai et al., 2004, 2007a,b; Mai et al., 2006; Zhang et al., 2006) but almost no information is available on its lipid nutritional immunity. Meanwhile, considering that most of the previous studies on dietary ARA focused on marine larvae while investigations in juvenile marine fish are scarce, the present study was undertaken at juvenile stage of Japanese seabass to investigate the effects of varying dietary levels of ARA on the growth, survival, selected immunological parameters and tissue fatty acid composition.

2. Materials and methods

2.1. Experimental diets

The basal practical diet was formulated to contain approximately 44% crude protein and 14% lipid, which have been shown to be sufficient to support the optimal growth of Japanese seabass (Table 1). ARA-enriched oil (ARA content, 41% of total fatty acid; in the form of ARA-methylester; Hubei Youzhiyou Biotechnology Co., Ltd., China) was supplemented separately to the basal diet at the expense of soybean oil to obtain 0.00%, 0.14%, 0.28%, 0.56%, 1.12% and 2.24% (dry weight) ARA, respectively (Table 2). The corresponding levels of dietary ARA, analyzed by high-performance gas chromatography (GS, HP6890, USA), were 0.08%, 0.22%, 0.36%, 0.56%, 1.33% and 2.12% dry weight.

Ingredients were ground into fine powder through 320 μm mesh. All ingredients were thoroughly mixed with soybean oil and LC-PUFA-enriched oils (Table 1), and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill and dried for about 12 h in a ventilated oven at 45 °C. After drying, the diets were broken up and sieved into proper pellet size (1.5×5.0 mm, 2.5×5.0 mm), and were stored at −15 °C until used.

2.2. 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 the control diet for 2 weeks to acclimate to the experimental diet and conditions.

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

Ingredient	Arachidonic supplementation level (%)					
	0.08	0.22	0.36	0.56	1.33	2.12
Fish meal[a]	45.00	45.00	45.00	45.00	45.00	45.00
Soybean meal[a]	17.00	17.00	17.00	17.00	17.00	17.00
Wheat meal	24.10	24.10	24.10	24.10	24.10	24.10
Soybean oil	5.50	5.21	4.87	4.18	2.82	0.09
DHA-enriched oil[b]	1.50	1.50	1.50	1.50	1.50	1.50
EPA-enriched oil[c]	0.50	0.50	0.50	0.50	0.50	0.50
ARA-enriched oil	0.00	0.29	0.63	1.32	2.68	5.41
Attractant[d]	0.30	0.30	0.30	0.30	0.30	0.30
Mold inhibitor[e]	0.10	0.10	0.10	0.10	0.10	0.10
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[f]	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[g]	2.00	2.00	2.00	2.00	2.00	2.00
Proximate analysis						
Crude protein (%)	43.66	44.28	43.55	43.66	43.42	44.01
Crude lipid (%)	14.05	13.29	13.60	13.29	13.49	13.94
Ash (%)	12.21	12.17	12.17	12.11	11.95	11.90
ARA (%)	0.08	0.22	0.36	0.56	1.33	2.12

[a] Fish meal: crude protein 69.7% dry matter, crude lipid 7.1% dry matter; soybean meal, crude protein 53.3% dry matter, crude lipid 1.9% dry matter.
[b] DHA-enriched oil: DHA content, 36% of TFA; in the form of DHA-methylester; Hubei Youzhiyou Biotechnology Co., Ltd., China.
[c] EPA-enriched oil: EPA content, 52% of TFA; DHA content, 27% of TFA; both in the form of triglyceride; HEBEI HAIYUAN Health biological Science and Technology Co., Ltd., China.
[d] Attractant: glycine and betaine.
[e] Mold inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.
[f] Mineral premix (mg or g/kg 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; $Ca(H_2PO_3)_2 \cdot H_2O$, 3000 mg; zoelite, 15.55 g.
[g] Vitamin premix (mg or g/kg 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, wheat middling, 18.52 g.

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

Fatty acid	Arachidonic supplementation level (%)					
	0.08	0.22	0.36	0.56	1.33	2.12
C 14:0	2.03	2.06	2.22	2.34	2.33	2.31
C 16:0	14.13	13.89	14.18	14.23	13.53	13.19
C 18:0	3.70	3.92	4.05	4.74	6.73	7.97
C 20:0	4.23	4.26	4.30	4.41	4.60	4.51
∑SFA[b]	24.10	24.13	24.74	25.71	27.19	27.98
C 16:1n−7	2.14	2.18	2.25	2.50	2.59	2.44
C 18:1n−7	0.29	0.32	0.32	0.26	0.26	0.31
C 18:1n−9	17.59	17.00	16.81	16.51	15.85	15.29
∑MUFA[c]	20.01	19.50	19.38	19.27	18.70	18.03
C 18:2n−6	31.56	30.56	30.36	27.90	19.97	15.43
C 20:4n−6	0.46	1.57	2.59	4.91	12.10	15.96
∑n−6[d]	32.03	32.13	32.95	32.81	32.07	31.39
C 18:3n−3	3.72	3.54	3.52	3.16	2.01	2.36
C 20:5n−3	4.68	4.81	4.89	4.81	4.84	4.70
C 22:6n−3	7.66	7.80	7.82	7.60	7.95	7.92
∑n−3[e]	16.07	16.15	16.23	15.58	14.80	14.98
∑PUFA[f]	80.12	80.40	82.13	81.20	78.94	77.76
∑n−3LC-PUFA	12.34	12.61	12.71	12.41	12.79	12.61
∑n−3/∑n-6	0.50	0.50	0.49	0.47	0.46	0.48
DHA/EPA	1.64	1.62	1.60	1.58	1.64	1.69
EPA/ARA	10.11	3.07	1.89	0.98	0.40	0.29
C18:1n−9/∑n−3	1.42	1.35	1.32	1.33	1.24	1.21

[a] Some fatty acids, of which the contents are minor, trace amount or not detected, such as C22:0, C24:0, C14:1, C20:1n−9, C22:1n−11, C20:2n−6, C20:3n−6, C22:5n−3, were not listed in the table.
[b] SFA: saturated fatty acid.
[c] MUFA: monounsaturated fatty acid.
[d] n−6: n−6 unsaturated fatty acid.
[e] n−3: n−3 unsaturated fatty acid.
[f] PUFA: polyunsaturated fatty acid.