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Effects of Dietary n-3 Highly Unsaturated Fatty Acids and Vitamin E Levels on the Growth and Fatty Acid Composition of Rockfish *Sebastes schlegeli*

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A feeding trial was conducted to investigate the effects of different levels of dietary n-3 highly unsaturated fatty acids (HUFA) (1.1-5.6%) and vitamin E (70 and 400 mg/kg) on the growth and body composition of juvenile rockfish. Six isonitrogenous (45% crude protein) and isolipidic (17% crude lipid) diets were formulated to contain graded levels of n-3 HUFA and vitamin E. Diets 1, 2 and 3 consist of 400 mg vitamin E/kg diet with graded levels of 1.1, 3.0, and 5.6% n-3 HUFA, respectively. Graded levels of n-3 HUFA (1.1, 3.0, and 4.0%) were added in diets 4, 5 and 6, respectively, containing 70 mg vitamin E/kg diet each. At the end of feeding trial, growth performance of rockfish was affected by neither dietary n-3 HUFA nor vitamin E levels. Feed efficiency and hepatosomatic index were slightly decreased ($P<0.05$) with increment of dietary n-3 HUFA at each dietary vitamin E level. Dietary vitamin E and n-3 HUFA levels did not affect proximate composition and vitamin E concentration in the dorsal muscle of rockfish. Liver moisture and crude protein contents positively related to dietary n-3 HUFA levels. Liver lipid content and hematocrit value were significantly decreased ($P<0.05$) by increasing dietary n-3 HUFA levels. Eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) concentrations in the dorsal muscle significantly correlated to dietary n-3 HUFA levels, except for fish fed the diet 6 containing 4% n-3 HUFA and 70 mg vitamin E/kg diet. EPA concentration in the dorsal muscle of fish fed the diet 6 was significantly lower than that of fish fed the diets 2, 3 and 5. The present findings suggest that feeding of diets containing excessive n-3 HUFA level with varying addition of vitamin E may alter fatty acid composition in the dorsal muscle, but do not affect growth of juvenile rockfish.

Key words: Rockfish, Fatty acids, n-3 HUFA, Vitamin E

Introduction

Marine fish species including Atlantic salmon, European sea bass, flounder, gilthead seabream, rockfish, starry flounder and yellowtail flounder require dietary n-3 highly unsaturated fatty acids (n-3 HUFA) as essential fatty acids (EFAs) for their normal growth and development (Fernández-Palacios et al., 1995; Rainuzzo et al., 1997; Rodriguez et al., 1998; Sargent et al., 1999a; Furuita et al., 2000; Copeman et al., 2002; Kim et al., 2002; Skalli and Robin, 2004). Lee (2001) reported that juvenile rockfish require 0.9% n-3 HUFA in diets for their optimal growth performance, and docosahexaenoic acid (DHA) is superior to eicosapentaenoic acid (EPA) at the same level. Studies (Ibeas et al., 1997; Rainuzzo et al., 1997; Sargent et al., 1999b, 2002) reported that the n-3 HUFA requirement of fish depends on several factors including fish species, environmental condition and dietary composition. Presence of some antioxidant vitamins such as vitamin E and C can modulate dietary n-3 HUFA requirement and affect growth performance, physiological changes and body compositions in fish (Cowey and Sargent, 1979; Mourente et al., 1999; Lee, 2001).

Rockfish (*Sebastes schelegi*) is a commercially important mariculture fish species in Korea. Rockfish aquaculture production in Korea has increased for the last decade and reached approximately 40,000 tons in 2007, and will increase rapidly in coming years.

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Several nutritional studies have been conducted to determine nutrient requirements and to develop proper feed formulations for this species (Lee et al., 1993, 1994; Bai and Lee, 1998; Lee, 2001, 2002; Lee et al., 2000, 2002; Wang et al., 2003). Bai and Lee (1998) revealed that dietary vitamin E requirement of this species was 45 mg/kg diet. However, growth and body composition of fish may be influenced by dietary levels of n-3 HUFA and other antioxidant vitamins, particularly vitamin E (Mourente et al., 1999, 2000; Lewis-McCrea and Lall, 2007). The aim of the present feeding trial was to investigate the effects of different dietary levels of n-3 HUFA and vitamin E on growth and fatty acid composition of juvenile rockfish.

Materials and Methods

Experimental diets

Six experimental diets were formulated to contain graded levels of dietary n-3 HUFA (1.1-5.6%) and vitamin E (70 and 400 mg/kg diet) (Table 1). White fish meal was used as the primarily dietary protein source. Dietary n-3 HUFA levels were adjusted by adding squid liver oil with soybean oil and concentrated n-3 HUFA. Major fatty acid composition of dietary lipid sources is presented in Table 2. All ingredients were thoroughly mixed with 40% distilled water, and pellets were prepared using a moist pelleting machine. The pellets were dried at room temperature for 24 h and stored at -30°C until used.

Experimental fish and feeding trial

Juvenile rockfish were obtained from the National Fisheries Research and Development Institute (Busan, Korea) and acclimated to experimental condition for 2 months by feeding a commercial diet containing 50% crude protein and 10% crude lipid. Three hundred and sixty fish (average weight, 33±0.3 g) were randomly distributed into 18 fiberglass reinforced plastic tanks (300 L water each) in a flow-through tank system. Each experimental diet was hand-fed triplicate groups of fish to visual satiety once a day for 16 weeks. Filtered seawater was supplied at a flow rate of 5 L/min to each tank. Water

Table 1. Ingredients and nutrient composition (% dry matter) of the experimental diets

	Diets					
	1	2	3	4	5	6
Ingredients						
White fish meal[1]	61	61	61	61	61	61
Dextrin	16	16	16	16	16	16
Brewer's yeast	3	3	3	3	3	3
Squid liver oil[2]	2	12	8	2	12	-
Soybean oil	10	-	-	10	-	8
n-3HUFA[3]	-	-	4	-	-	4
Vitamin premix (vitamin E free)[4]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[5]	4	4	4	4	4	4
α-cellulose	1	1	1	1	1	1
DL-α-tocopheryl acetate[6]	-	-	-	-	-	-
Choline salt (50%)	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient composition						
Crude protein	44.6	44.3	44.4	44.9	44.9	45.1
Crude lipid	16.7	16.6	16.6	16.4	16.8	16.7
n-3 HUFA	1.1	3.0	5.6	1.1	3.0	4.0
Vitamin E (mg/kg)	400	400	400	70	70	70

[1]Pollack fish meal was produced by steam dry method.
[2]Provided by E-wha Oil & Fat Ind. Co., Busan, Korea.
[3]Highly unsaturated fatty acids (C≥20) contained 82% n-3 HUFA (33% EPA + 49% DHA).
[4]Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 92.7; thiamin hydrochloride, 2.1; riboflavin, 7.0; pyridoxine hydrochloride, 1.4; niacin, 27.8; Ca-D-pantothenate, 9.7; myo-inositol, 139.1; D-biotin, 0.21; folic acid, 0.5; p-aminobenzoic acid, 13.9; menadione, 1.4; retinyl acetate, 0.6; cholecalciferol, 0.002; cyanocobalamin, 0.003.
[5]Mineral premix contained the following ingredients (g/kg premix): $MgSO_4 \cdot 7H_2O$, 80; $NaH_2PO_4 \cdot 2H_2O$, 370; KCl, 130; Ferric citrate, 40; $ZnSO_4 \cdot 7H_2O$, 20; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 1.
[6]Partially replaced cellulose to achieve graded levels of vitamin E.

Table 2. Major fatty acid composition (% of total fatty acids) of dietary lipid sources

Fatty acid	Lipid sources			
	Fish meal oil	n-3HUFA	Squid liver oil	Soybean oil
16:0	19.9	-	28.0	11.7
16:1	5.8	0.2	2.5	0.2
18:0	3.9	-	1.7	3.9
18:1	25.6	1.1	13.5	21.9
18:2n-6	1.0	1.2	1.5	53.7
18:3n-3	0.2	4.3	1.2	8.4
EPA	9.1	32.6	6.8	-
DHA	17.5	49.1	12.2	-
n-3 HUFA[1]	26.6	81.7	19.0	-

[1]Highly unsaturated fatty acids (C≥20).

temperature was maintained at 13.4±1.1°C (mean ± SD), and photoperiod was applied as the natural condition during the feeding period.

At the beginning and the end of feeding trial, fish in each tank were collectively weighed after being starved for 48 h and anesthetized in MS 222 (tricaine methanesulfonate, Sigma, USA) solution at a concentration of 100 mg/L.

Sample collection

Fifty fish at the beginning and 5 fish from each tank at the end of feeding trial were sampled and stored at -75°C for chemical analysis. Blood were taken from the caudal vein of 5 fish from each tank using heparinized syringes for determination of hematocrit and hemoglobin. Plasma from the blood samples was collected by centrifugation at 3,500 rpm for 5 min.

Chemical analysis

Proximate composition of the dorsal muscle and liver were analyzed according to standard methods (AOAC, 1995). Crude protein was determined by Kjeldahl method using Auto Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was analyzed with ether extraction in a soxhlet extractor (SER 148, VELP Scientifica, Milano, Italy), and moisture was determined using a dry oven at 105°C for 12 h. Ash content was determined after combustion at 550°C for 4 h in a muffle furnace.

Vitamin E (α- and β-tocopherol) contents in the dorsal muscle were analyzed using HPLC. The HPLC system consisted of a model RF-551 Spectro Flourometric detector, a Waters model 510 HPLC Pump, and a model LC 13 PVDF (Gelman Science) Aerodisc. The HPLC was operated by conditions of Lichropher 100RP-18 (5 μm) column, 95% methanol mobile phase, 1.4 mL/m flow rate, 40°C column temperature and 20 μL injection size.

Lipid for fatty acid analysis was extracted in a mixture of chloroform and methanol (2:1, v/v) according to the method described by Folch et al. (1957). Fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, MO, USA). Fatty acid composition was measured by a gas chromatography (HP-5890 II, Hewlett-Packard, Palo Alto, USA) with a flame ionization detector, equipped with HP-INNOWax capillary column (30 m×0.32 mm, i.d., film thickness 0.5 μm, Hewlett-Packard, USA). Injector and detector temperatures were 250 and 270°C, respectively. The column temperature was programmed from 170 to 225°C at a rate of 1°C/min. Helium was used as the carrier gas. Concentrations of fatty acids were determined by comparison with the known standards (Sigma, USA).

Hematocrit was determined with hematocrit capillary tubes after centrifugation at 4,000 rpm for 5 min. Hemoglobin was measured using cyanmethemoglobin procedure. Plasma total protein, glucose, triglyceride and phospholipids content were determined in three replications by an automatic analyzer (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis

Data were subjected to one- and two-way ANOVA in SPSS version 7.5 (SPSS Michigan Avenue, Chicago, IL, USA). Significant difference ($P<0.05$) among fish groups was determined by Duncan's multiple range test (Duncan, 1955). The data were checked for homogeneity of variances by the Bartlett test, if necessary the data were arc-sine transformed before the ANOVA analysis. The data are presented as mean±SE of three replications.

Results

Growth performance and proximate composition

Survival was 99-100% among the all treatments. Weight gain of juvenile rockfish was not affected by dietary n-3 HUFA and vitamin E levels (Table 3). At

each dietary vitamin E level, feed efficiency and hematosomatic index (HSI) negatively related to dietary n-3 HUFA. No interactions between dietary vitamin E and n-3 HUFA levels were observed in growth of fish.

Moisture, crude protein, crude lipid and ash contents in the dorsal muscle of fish were not different among the dietary treatments (Table 4). But liver lipid content was significantly decreased by increasing dietary n-3 HUFA level (Table 5). Vitamins E (α- and β-tocopherol) contents in the dorsal muscle of juvenile rockfish were affected by neither dietary n-3 HUFA nor vitamin E levels (Table 6).

Blood chemistry

Hematocrit of rockfish was significantly decreased with increasing dietary n-3 HUFA level. No significant differences were observed in plasma glucose, total protein, triglyceride, phospholipids and hemoglobin contents among fish groups fed the experimental diets (Table 7).

Fatty acid composition

Fatty acid composition in the dorsal muscle of rockfish was significantly reflected the fatty acid composition of diets (Table 8). Linoleic acid (18:2n-6) content in fish fed the diets 1, 4, and 6 containing soybean oil was significantly higher than that in fish fed other diets. Eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) contents in fish positively correlated to dietary n-3 HUFA content, except for fish fed the diet 6 containing 4% n-3 HUFA and 70 mg vitamin E/kg. EPA content of fish fed the diet 6 was significantly lower than that of fish fed the diet 2 (3% n-3 HUFA, 400 mg vitamin E/kg), diet 3 (5.6% n-3 HUFA, 400 mg vitamin E/kg) and diet 5 (3% n-3 HUFA, 70 mg vitamin E/kg). Total n-3 HUFA content was increased with increasing dietary n-3 HUFA level with 400 mg vitamin E/kg groups. The value of fish fed the diet 6 containing 4% n-3 HUFA with 70 mg vitamin E was

Table 3. Growth performance of rockfish fed the experimental diets for 16 weeks[1]

Diets	Dietary n-3 HUFA (%)	Dietary vitamin E (mg/kg)	Initial weight (g/fish)	Weight gain (%)[2]	Feed efficiency (%)[3]	HSI[4]
1	1.1	400	33.1±0.27	132±6.0	93.2±2.06^{bc}	4.01±0.074^{b}
2	3.0	400	33.0±0.22	132±4.0	90.9±1.43^{abc}	3.43±0.037^{a}
3	5.6	400	32.8±0.12	133±2.3	88.5±1.16^{a}	3.55±0.046^{a}
4	1.1	70	33.0±0.29	135±3.8	92.0±0.78^{abc}	3.76±0.169^{ab}
5	3.0	70	33.0±0.31	137±3.1	94.2±0.12^{c}	3.47±0.174^{a}
6	4.0	70	33.0±0.38	125±3.8	89.7±1.30^{ab}	3.42±0.074^{a}
Two-way ANOVA						
Dietary vitamin E				*P*<0.4	*P*<0.5	*P*<0.4
Dietary n-3HUFA				*P*<0.3	*P*<0.05	*P*<0.01
Interaction				*P*<0.9	*P*<0.1	*P*<0.3

[1]Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).
[2](Final body weight - initial body weight) × 100/initial body weight.
[3]Fish wet weight gain × 100/feed intake (dry matter).
[4]Hepatosomatic index = 100 × (liver weight/body weight).

Table 4. Proximate composition (% wet basis) in the dorsal muscle of rockfish fed the experimental diets for 16 weeks

Diets	Dietary n-3 HUFA (%)	Dietary vitamin E (mg/kg)	Moisture	Crude protein	Crude lipid	Ash
1	1.1	400	75.0±0.50	20.1±0.23	3.9±0.58	1.3±0.00
2	3.0	400	75.8±1.18	20.1±0.28	3.9±0.20	1.3±0.00
3	5.6	400	73.1±0.21	20.5±0.29	4.7±0.55	1.3±0.00
4	1.1	70	75.4±0.08	20.5±0.16	3.4±0.29	1.3±0.00
5	3.0	70	74.5±0.14	20.2±0.17	4.4±0.23	1.3±0.03
6	4.0	70	75.7±0.76	20.0±0.26	4.2±0.15	1.3±0.33
Two-way ANOVA						
Dietary vitamin E			*P*<0.6	*P*<0.4	*P*<0.9	*P*<0.5
Dietary n-3 HUFA			*P*<0.1	*P*<0.4	*P*<0.2	*P*<0.8
Interaction			*P*<0.2	*P*<0.6	*P*<0.3	*P*<0.5

decreased.

Table 5. Proximate composition (% wet basis) in the liver of rockfish fed the experimental diets for 16 weeks[1]

Diets	Dietary n-3 HUFA (%)	Dietary vitamin E (mg/kg)	Moisture	Crude protein	Crude lipid	Ash
1	1.1	400	51.2±1.12^{ab}	8.6±0.27^{a}	30.7±1.53^{cd}	0.8±0.03
2	3.0	400	53.9±0.50^{bc}	9.0±0.14^{a}	26.8±0.48^{ab}	0.8±0.03
3	5.6	400	55.0±0.77^{c}	10.1±0.18^{b}	25.4±1.04^{a}	0.9±0.03
4	1.1	70	50.6±1.14^{a}	8.6±0.12^{a}	31.6±1.58^{d}	0.7±0.03
5	3.0	70	53.4±1.00^{abc}	9.0±0.33^{a}	27.5±1.01^{abc}	0.8±0.00
6	4.0	70	52.5±0.46^{abc}	9.2±0.05^{a}	29.3±0.90^{bcd}	0.8±0.03
Two-way ANOVA						
Dietary vitamin E			$P<0.6$	$P<0.9$	$P<0.6$	$P<0.3$
Dietary n-3 HUFA			$P<0.05$	$P<0.001$	$P<0.01$	$P<0.06$
Interaction			$P<0.9$	$P<0.9$	$P<0.9$	$P<0.9$

[1]Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).

Table 6. Vitamin E content in the dorsal muscle of rockfish fed the experimental diets for 16 weeks

Diets	Dietary n-3 HUFA (%)	Dietary vitamin E (mg/kg)	α-tocopherol (mg/kg DM)	β-tocopherol (mg/kg DM)
1	1.1	400	85±14.6	8.1±3.32
2	3.0	400	122±37.6	11.7±0.90
3	5.6	400	208±64.1	12.1±1.34
4	1.1	70	134±56.0	12.9±0.93
5	3.0	70	198±60.5	11.1±0.68
6	4.0	70	77±22.8	10.4±1.75
Two-way ANOVA				
Dietary vitamin E			$P<0.2$	$P<0.3$
Dietary n-3 HUFA			$P<0.1$	$P<0.6$
Interaction			$P<0.8$	$P<0.2$

Table 7. Blood chemistry of rockfish fed the experimental diets for 16 weeks[1]

Diets	Dietary n-3 HUFA (%)	Dietary vitamin E (mg/kg)	Glucose (mg/100 mL)	Total protein (g/100 mL)	Triglyceride (mg/100 mL)	Phospholipid (mg/100 mL)	Hematocrit (%)	Hemoglobin (g/100 mL)
1	1.1	400	23.3±6.56	10.1±1.43	182±31.5	527± 36.3	52.6±0.66^{c}	10.7±1.79
2	3.0	400	26.5±8.64	10.2±0.55	217±14.7	567± 98.1	55.0±2.08^{bc}	9.6±1.11
3	5.6	400	18.9±3.65	9.9±0.90	208±51.6	781± 66.1	40.3±0.33^{a}	7.7±0.81
4	1.1	70	16.9±1.47	8.8±2.10	285±36.9	582±107.6	54.3±1.45^{bc}	11.3±0.02
5	3.0	70	16.2±1.41	9.1±0.24	144±26.3	545± 49.2	57.0±0.57^{c}	11.5±0.59
6	4.0	70	21.0±1.26	9.2±0.78	169±44.5	800±131.4	47.0±1.15^{b}	9.9±0.97
Two-way ANOVA								
Dietary vitamin E			$P<0.2$	$P<0.4$	$P<0.7$	$P<0.9$	$P<0.2$	$P<0.3$
Dietary n-3 HUFA			$P<0.7$	$P<0.9$	$P<0.4$	$P<0.1$	$P<0.001$	$P<0.3$
Interaction			$P<0.7$	$P<0.9$	$P<0.05$	$P<0.7$	$P<0.9$	$P<0.6$

[1]Values (mean±SE of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).

Discussion

No significant differences in growth performance of rockfish fed the experimental diets during feeding period indicate that dietary n-3 HUFA and vitamin E levels may be sufficient for growth performance of juvenile rockfish. Several studies reported that dietary inclusion of a proper ratio of polyunsaturated fatty acid (PUFA) and vitamin E did not affect growth of fish (Stephan et al., 1995; Mourente et al., 2000; Lewis-McCrea and Lall, 2007). No adverse effects were observed in growth or survival of juvenile gilthead sea bream fed the diets containing different

ratios of PUFA/vitamin E (Mourente et al., 2000). Similar findings were observed in turbot fed diets containing different n-6 or n-3 PUFA and vitamin E

Table 8 Major fatty acid composition (% of total fatty acids) in the dorsal muscle of rockfish fed the experimental diets for 16 weeks[1]

	Diets					
	1	2	3	4	5	6
16:0	14.9±0.17^{ab}	17.1±0.44^{c}	16.1±0.82^{bc}	15.1±0.30^{ab}	16.4±0.07^{c}	14.5±0.20^{a}
16:1n-7	4.6±0.12^{a}	7.2±0.38^{bc}	6.6±0.30^{b}	4.6±0.00^{a}	7.4±0.10^{c}	4.2±0.00^{a}
18:0	3.4±0.03^{b}	2.7±0.14^{a}	2.8±0.07^{a}	3.3±0.03^{b}	2.7±0.03^{a}	3.3±0.03^{b}
18:1n-(7+9)	26.8±0.55^{c}	24.1±0.87^{b}	22.6±0.18^{a}	26.8±0.34^{c}	24.6±0.27^{b}	24.9±0.09^{b}
18:2n-6	24.3±0.38^{c}	2.4±0.14^{a}	2.0±0.08^{a}	24.0±0.41^{c}	2.6±0.43^{a}	19.6±0.05^{b}
18:3n-3	1.8±0.02^{d}	0.9±0.01^{b}	0.7±0.02^{a}	1.7±0.04^{d}	0.9±0.02^{b}	1.0±0.00^{c}
20:5n-3	4.6±0.11^{a}	9.6±0.35^{c}	11.6±0.20^{d}	4.5±0.05^{a}	9.7±0.15^{c}	7.5±0.12^{b}
22:6n-3	8.5±0.09^{a}	13.8±0.53^{b}	18.1±0.41^{d}	8.6±0.26^{a}	14.0±0.40^{b}	15.3±0.03^{c}
Monoenes	31.4±0.65^{bc}	31.8±1.45^{bc}	29.6±0.29^{ab}	31.5±0.37^{bc}	32.6±0.35^{c}	29.1±0.11^{a}
n-3 PUFA[2]	15.9±0.29^{a}	29.4±1.12^{c}	35.9±0.70^{d}	15.9±0.29^{a}	29.6±0.55^{c}	26.4±0.03^{b}
n-6 PUFA[2]	26.3±0.38^{d}	7.7±0.59^{ab}	6.7±0.19^{a}	25.9±0.42^{d}	8.1±0.49^{b}	21.6±0.20^{c}
n-3 HUFA[3]	15.2±0.23^{a}	27.7±1.04^{c}	34.1±0.68^{d}	15.3±0.26^{a}	28.0±0.53^{c}	25.4±0.00^{b}

[1]Values (mean±SE of three replications) in each row not sharing a common superscript are significantly different ($P<0.05$).
[2]Poly unsaturated fatty acids (C≥18).
[3]Highly unsaturated fatty acids (C≥20).

levels (Stephan et al., 1995). Kiron et al. (2004) reported that growth of juvenile rainbow trout was more significantly affected by quality of lipid sources representing as n-3 HUFA content, rather than vitamin E levels.

Weight gain value in the present study was relatively lower than that in a previous study (Lee et al., 2000) in which juvenile rockfish (5.7 g/fish) were grown at 24°C, but comparable to that observed in other study (Lee et al., 2002) where 22 g rockfish were reared at 13°C. It is obvious that the differences in the weight gain value between the studies are due to different fish size and water temperature employed.

Lower feed efficiency and hepatosomatic index (HSI) of rockfish fed the diets containing higher dietary n-3 HUFA levels suggest that excessive dietary n-3 HUFA levels may reduce feed efficiency and HSI values of fish. Lower feed efficiency and HSI have been reported for gilthead seabream, starry flounder and olive flounder fed the diets containing excessive n-3 HUFA levels (Lee et al., 1993; Ibeas et al., 1996; Lee, 2001; Lee et al., 2003; Kim and Lee, 2004). Ibeas et al. (1996) reported that feed efficiency of gilthead sea bream (grown from 11 to 30 g) was decreased when dietary n-3 HUFA level increased from 1% to 1.5%.

In this study, liver moisture and crude protein contents significantly increased with increases of dietary n-3 HUFA level, regardless of vitamin E level, but an opposite trend was observed in liver lipid content. This indicates that higher dietary n-3 HUFA level may reduce the deposition of lipid in fish liver. Lee (2001) reported that liver lipid content of rockfish fed the n-3 HUFA deficient diets was higher than that of fish fed the n-3HUFA sufficient diets. Studies (Bell et al., 1998; Piedecause et al., 2007; Peng et al., 2008) have reported that the imbalance of n-3 and n-6 fatty acids in diets could lead to lipid deposition in liver. Similar findings were observed in fish fed the diets containing vegetable oils as fish oil replacements.

Hematocrit (Ht) was negatively influenced by the dietary n-3 HUFA levels. This is likely due the peroxidation of unsaturated fatty acids in the diets containing excessive n-3 HUFA levels with deficiency of vitamin E. Low Ht values have been reported for rockfish and channel catfish fed the diets without vitamin E supplementation (Murai and Andrews, 1974; Klinger et al., 1996; Bai and Lee, 1998). Bai and Lee (1998) reported that rockfish fed the vitamin E deficient diet had lower Ht value compared to that of fish fed diets containing 20-500 mg vitamin E/kg. Lower Ht value also was observed in channel catfish fed the diet containing high level of fish oil compared to that of the fish fed soybean oil, beef tallow and their mixture (Klinger et al., 1996). However, Kiron et al. (2004) did not find any differences in Ht value of rainbow trout when fed the diets containing high level of pollack oil with sufficient vitamin E supplementation. Mourente et al.

(1999) suggested that balance of EFA (n-3 HUFA) and adequate antioxidant level in fish diets must be specifically supplied to maintain optimal growth and health of fish species.

Generally, fatty acid composition in fish tissues significantly reflects the fatty acid composition in diets (Agradi et al., 1993, 1995; Ruping et al., 1993; Bell et al., 1998; Chaiyapechara et al., 2003; Nanton et al., 2003). In the present study, concentrations of EPA and DHA in the dorsal muscle of rockfish were positively increased with the increasing dietary n-3 HUFA level, except for EPA in the fish fed the diet 6 containing 70 mg vitamin E. This indicates that excessive dietary n-3 HUFA and insufficient vitamin E supplementation may result in faster peroxidation rate of n-3 HUFA, particularly EPA, and consequently reduce its content. Lower n-3 HUFA (EPA and DHA) concentrations were observed in halibut and gilthead sea bream fed the diets containing inadequate vitamin E or plant seed oils (Tocher et al., 2002; Menoyo et al., 2004). It is well established that vitamin E plays a role as an antioxidant to prevent HUFA from peroxidation, and an increase of dietary HUFA results in higher requirement of vitamin E (Cowey et al., 1981, 1983; Roem et al., 1990; Hamre and Lie, 1995; Mourente et al., 1999, 2000). Studies have reported that fish fed diets containing high HUFA and low vitamin E produced significantly higher values of lipid peroxidation products (Stephan et al., 1995; Mourente et al., 1999; Chaiyapechara et al., 2003). Stephan et al. (1995) reported that an increase of thiobarbituric acid reactive substances appeared to be accompanied by the loss of polyunsaturated fatty acids. Naturally, n-3 HUFA including EPA and DHA are susceptible to peroxidation which is identified as a main process *in vivo* lead to a decrease of the polyunsaturated fatty acids concentrations in fish tissues and their peroxidation rate depends on kinds of fatty acids, other antioxidants and fish species. Herzberg et al. (1996) reported that EPA was oxidized faster than DHA by muscle and liver homogenates. Lewis-McCrea and Lall (2007) reported lower concentrations of n-3 fatty acids in liver lipid of fish fed the diets without vitamin E. *In vitro* supplementation of vitamin E at sufficient concentration was reported to protect lipid peroxidation of mackerel oil (Zuta et al., 2007).

The findings in the present study suggest that feeding of diets containing excessive n-3 HUFA level with varying addition of vitamin E may alter the fatty acid composition in dorsal muscle, but do not affect growth of juvenile rockfish.

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References

Agradi E, Abrami G, Serrini G, McKenzie D, Bolis C and Bronzi P. 1993. The role of dietary n-3 fatty acid and vitamin E supplements in growth of sturgeon (*Acipenser naccarii*). Comp Biochem Physiol 105A, 187-195.

Agradi E, Bonomi L, Rigamonti E, Liguori M and Bronzi P. 1995. The effects of dietary lipids on tissue lipids and ammonia excretion in European eel (*Anguilla anguilla*). Comp Biochem Physiol 111A, 445-451.

AOAC. 1995. Official Methods of Analysis.16th Edn. Association of Official Analytical Chemists, Arlington, Virginia.

Bai SC and Lee KJ. 1998. Different levels of dietary DL-α-tocopheryl acetate affect the vitamin E status of juvenile Korean rockfish, *Sebastes schlegeli*. Aquaculture 161, 405-414.

Bell JG, Tocher DR, Farndale BM and Sargent JR. 1998. Growth, mortality, tissues histopathology and fatty acid compositions, eicosanoid production and response to stress, in juvenile turbot fed diets rich in γ-linolenic acid in combination with eicosapentaenoic acid or docosahexaenoic acid. Prostag Leukotr Ess 58, 353-364.

Chaiyapechara S, Casten MT, Hardy, RW and Dong FM. 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentration of lipid and vitamin E. Aquaculture 219, 715-738.

Copeman LA, Parrish CC, Brown JA and Harel M. 2002. Effects of docosahesaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. Aquaculture 210, 285-304.

Cowey CB and Sargent JR. 1979. Nutrition. In Fish Physiology (Vol. III). (Ed. Hoar, W. S., Randall, D. J. and J. R. Brett) Academic Press, Orlando, FL, pp1-69.

Cowey C, Adron J, Walton M, Murray J, Youngson A and Knox D. 1981. Tissue distribution, uptake and requirement for α-tocopherol of rainbow trout (*Salmo gairdneri*) fed diets with a minimal content of unsaturated fatty acids. J Nutr 111, 1556-1567.

Cowey C, Adron J and Youngson A. 1983. The vitamin E requirement of rainbow trout (*Salmo gairdneri*) given

diets containing polyunsaturated fatty acid derived from fish oil. Aquaculture 30, 85-93.

Duncan DB. 1955. Multiple range test and multiple *F*-tests. Biometrics 11, 1-42.

Fernández-Palacios H, Izquierdo MS, Robaina L, Valencia A, Salhi M and Vergara JM. 1995. Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). Aquaculture 132, 325-337.

Folch J, Lees M and Sloane-Stanley GH. 1957. A simple method for isolation and purification of total lipids from animal tissues. J Biol Chem 226, 406-509.

Furuita H, Tanaka H, Yamamoto T, Shiraishi M and Takeuchi T. 2000. Effects of n-3 HUFA levels in broodstock diet on the reproductive performance and egg and larval quality of the Japanese flounder, *Paralichthys olivaceus*. Aquaculture 187, 387-398.

Hamre K and Lie O. 1995. α-Tocorpherol levels in different organs of Atlantic salmon (*Salmon salar* L.)-Effects of smoltification, dietary levels of n-3 highly unsaturated fatty acids and vitamin E. Comp Biochem Physiol 111A, 547-554.

Herzberg GR, Craig Skinner RT and Levy R. 1996. Eicosapentaenoic acid is oxidized more rapidly than docosahexanenoic acid by muscle and liver. Nutr Res 16, 639-644.

Ibeas C, Cejas JR, Gomez T, Jerez S and Lorenzo A. 1996. Influence of dietary n-3 highly unsaturated fatty acids levels on juvenile gilthead seabream (*Sparus aurata*) growth and tissue fatty acid composition. Aquaculture 142, 221-235.

Ibeas C, Cejas JR, Fores R, Badia P, Gomez T and Lorenzo Hernendez A. 1997. Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 150, 91-102.

Kim KD and Lee SM. 2004. Requirement of dietary n-3 highly unsaturated fatty acids for juvenile flounder (*Paralichthys olivaceus*). Aquaculture 229, 315-323.

Kim KD, Lee SM, Park HG, Bai SC and Lee HY. 2002. Essentiality of dietary n-3 highly unsaturated fatty acids in juvenile Japanese flounder *Paralichthys olivaceus*. J World Aquacult Soc 33, 432-440

Kiron V, Puangkaew J, Ishizaka K, Satoh S and Watanabe T. 2004. Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. Aquaculture 234, 361-379.

Klinger RC, Blazer VS and Echevarria C. 1996. Effects of dietary lipid on the hematology of channel catfish, *Ictalurus punctatus*. Aquaculture 147, 225-233.

Lee SM. 2001. Review of the lipid and essential fatty acid requirements of rockfish (*Sebastes schlegeli*). Aquacult Res 32, 8-17.

Lee SM. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture 207, 79-95.

Lee SM, Lee JY, Kang YJ, Yoon HD and Hur SB. 1993. n-3 highly unsaturated fatty acids requirement of the Korean rockfish *Sebastes schlegeli*. J Kor Fish Soc 26, 477-492.

Lee SM, Lee JY and Hur SB. 1994. Essentiality of dietary eicosapentaenoic acid and docosahexaenoic acid in Korean rockfish, *Sebastes schlegeli*. J Kor Fish Sci 27, 721-726.

Lee SM, Hwang UG and Cho SH. 2000. Effects of feeding frequency and dietary moisture content on growth, body composition and gastric evacuation of juvenile Korean rockfish (*Sebastes schlegeli*). Aquaculture 187, 399-409.

Lee SM, Jeon IG and Lee JY. 2002. Effects of digestible protein and lipid levels in practical diets on growth, protein utilization and body composition of juvenile rockfish (*Sebastes schlegeli*). Aquaculture 211, 227-239.

Lee SM, Lee, JH and Kim KD. 2003. Effect of dietary essential fatty acids on growth, body composition and blood chemistry of juvenile starry flounder (*Platichthys stellatus*). Aquaculture 225, 269-281.

Lewis-McCrea LM and Lall SP. 2007. Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnor-malities in juvenile Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture 262, 142-155.

Menoyo D, Izquierdo MS, Robaina L, Gines R, Lopoz-Bote CJ and Bautista JM. 2004. Adaptation of lipid metabolism, tissue composition and flesh quality in gilthead sea bream (*Sparus aurata*) to the replacement of dietary fish oil by linseed and soyabean oils. Brit J Nutr 92, 41-52.

Mourente G, Toche, DR, Diaz-Salvago E, Grau A and Pastor E. 1999. Study of the n-3 highly fatty acids requirement and antioxidant status of *Dentex dentex* larvae at the Artemia feeding stage. Aquaculture 179, 291-307.

Mourente G, Diaz-Salvago E, Tocher DR and Bell JG. 2000. Effects of dietary polyunsaturated fatty acid/ vitamin E (PUFA/tocopherol ratio) on antioxidant defence mechanisms of juvenile gilthead sea bream (*Sparus aurata* L., Osteichthyes, Sparidae). Fish Physiol Biochem 23, 337-351.

Murai T and Andrews JW. 1974. Interactions of dietary α-tocopherol, oxidized menhaden oil and ethoxyquin on channel catfish (*Italurus punctatus*). J Nutr 104, 1416-1431.

Nanton DA, Lall SP, Ross NW and McNiven MA. 2003. Effect of dietary lipid level on fatty acid β-oxidation

and lipid composition in various tissues of haddock, *Melanogrammus aeglefinus* L. Comp Biochem Physiol 135B, 95-108.

Peng SM, Chen LQ, Qin JG, Hou JL, Yu N, Long ZQ, Ye JY and Sun XJ. 2008. Effects of replacement of dietary fish oil by soybean oil on growth performance and liver biochemical composition in juvenile black seabream, *Sebastes schelegi*. Aquaculture 276, 154-161.

Piedecause MA, Mazon MJ, Garcia BG and Hernandez MD. 2007. Effects of total replacement of fish oil by vegetable oils in the diets of sharpsnout seabream (*Diplodus puntazzo*). Aquaculture 263, 211-219.

Rainuzzo JR, Reitan KI and Olsen Y. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103-115.

Rodriguez C, Perez JA, Badia P, Izquierdo MS, Fernandez-Palacios H and Lorenzo Hernandez A. 1998. The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in the diet. Aquaculture 169, 9-23.

Roem A, Kohler C and Stickney R. 1990. Vitamin E requirement of the blue tilapia, *Oreochromis aureus* (Steindachner), in relation to dietary lipid level. Aquaculture 87, 155-164.

Ruping XU, Hung SSO and German BJ. 1993. White sturgeon tissue fatty acid compositions are affected by dietary lipids. J Nutr 123, 1685-1692.

Sargent J, Bell G, McEvoy L, Tocher D and Estevez A. 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 179, 191-199.

Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J and Tocher D. 1999b. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217-229.

Sargent JR, Tocher DR and Bell JG. 2002. The Lipids. In: Fish Nutrition (Ed. Halver J. E. and R. W. Hardy), Academic Press, NY. 182-246.

Skalli A and Robin JH. 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquaculture 240, 399-415.

Stephan G, Guillaume J and Lamour F. 1995. Lipid peroxidation in turbot (*Scophthalmus maximus*) tissue: effect of dietary vitamin E and dietary n-6 or n-3 polyunsaturated fatty acids. Aquaculture 130, 251-268.

Tocher DR, Mourente G, Van Der Eecken A, Evjemo JO, Diaz E, Bell JG, Geurden I, Lavens P and Olsen Y. 2002. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquacult Nutr 8, 195-207.

Wang XJ, Kim KW and Bai SC. 2003. Comparison of L-ascorbyl-2-monophosphate-Ca with L-ascorbyl-2-monophosphate-Na/Ca on growth and tissue ascorbic acid concentrations in Korean rockfish (*Sebastes schlegeli*). Aquaculture 225, 387-395.

Zuta PC, Simpson BK, Zhao X and Leclerc L. 2007. The effects of α-tocopherol on the oxidation of markerel oil. Food Chem 100, 800-807.

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