Essentiality of Dietary Eicosapentaenoic Acid and Docosahexaenoic Acid in Korean Rockfish, *Sebastes schlegeli*

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Feeding trials were conducted to determine the essentiality of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and to compare the efficiency between EPA and DHA for juvenile Korean rockfish. Fish averaging 2.1 *g* were fed experimental diets containing different levels of EPA or DHA, and different combinations of EPA and DHA in two separate experiments. Graded levels (0.0~1.75%) of dietary EPA or DHA as ethyl esters were substituted for a part of the 8% beef tallow in the basal diet.

After 5 weeks of the experimental period, weight gain, chemical composition of whole body, protein and lipid retention efficiency, hepatosomatic index, and fatty acid composition of liver were measured. Daily growth rate and feed efficiency were the lowest in fish fed the diets without EPA and DHA. These responses were effectively improved by increasing EPA or DHA in the diets up to 1.0% levels, and then reached a plateau between 1.0 and 1.75% levels of either EPA or DHA. Protein and lipid retention efficiency were also improved with the high levels of dietary EPA or DHA. DHA was superior to EPA at the same level of each in weight gain, feed efficiency, and protein and lipid retention efficiency. Hepatosomatic index tended to decrease with increase of the dietary EPA or DHA levels. Lipid contents of whole body were increased with levels of EPA or DHA in the diets. Dietary EPA and/or DHA levels affected directly the fatty acid composition of liver polar lipid. EPA or DHA in the liver polar lipid were increased with levels of dietary EPA or DHA, respectively, whereas those in nonpolar lipid were not affected by the dietary levels of EPA and/or DHA.

These finding indicate that either of the EPA or DHA is essential for normal growth of Korean rockfish, and the essential fatty acid requirement is 1.0% of EPA and/or DHA in the diet. DHA is superior to EPA as essential fatty acid, and the dietary EPA/DHA ratio of less than 1.0 may be adequate for normal growth of Korean rockfish fed a diet enough n-3HUFA (EPA and DHA).

Introduction

Dietary lipids play an important role in fish nutrition for provision of both essential fatty acids (EFA) and energy (Sargent *et al.*, 1989). EFA requirements of fish are influenced by the temperature and/or salinity of water, and these differences are more complex than those of mammals (Castell, 1979; Cowey and Sargent, 1979). EFA and their requirements to fish have been reported to vary among species (NRC, 1983). Rainbow trout require 18:3n-3 rather than 18:2n-6 (Castell *et al.*, 1972a, b; Watanabe *et al.*, 1974a, b; Takeuchi and Watanabe, 1977a), and common carp and Japanese eel require a mixture of 18:3n-3 and 18:2n-6 (Takeuchi and Watanabe 1977b; Takeuchi *et al.*, 1980), whereas Tilapia require only 18:2n-6 (Kanazawa *et al.*, 1980; Takeuchi *et al.*, 1983a, b). Recent researches have indicated that the EFA of marine fish is n-3 highly unsaturated fatty acids (n-3HUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Red seabream (*Pagrus major*), striped jack (*Longirostris delicatissimus*), yellowtail (*Seriola quinqueradiata*), gilthead bream (*Sparus aurata*), and turbot (*Scophthalmus maximus*) have all shown optimal growth and feed efficiency when n-3HUFA levels are 0.5~2.0% in the diets (Fujii *et al.*, 1976; Deshimaru *et al.*, 1982a, b; Gatesoupe *et al.*, 1977; Leger *et al.*, 1979; Watanabe *et al.*, 1989a, b; Kalogeropoulos *et al.*, 1992). These fatty acids are thought to play an important role in permeability, enzyme activity, prostaglandin precursor, and other functions in polar lipid fraction of the biomembrane(Baud *et al.*, 1989; Broughton *et al.*, 1991; German *et al.*, 1987; Kaley *et al.*, 1985; Lokesh *et al.*, 1988; Lokesh *et al.*, 1989; Maroussem *et al.*, 1985; Stubbs and Smith, 1984; Swanson *et al.*, 1989; Thomson *et al.*, 1986).

Korean rockfish is a leading candidate for commercial culture in Korea. Fatty acid nutrition studies for this fish have been published by Lee *et al.* (1993c, d, e, f and 1994) and Lee (1994). These studies showed that Korean rockfish required n-3 HUFA as EFA like other marine fish, and the n-3 HUFA requirement of juvenile Korean rockfish was around 1.0% in the diet. Korean rockfish fed EFA-deficient diets exhibited poor growth performances, fatty liver and physiological disorders such as abnormalities of blood chemical values and membrane enzyme activity. Lee *et al.* (1993d) also suggested that DHA could be more important nutrient than EPA for enzyme activity in Korea rockfish. But there is no information available for the effect of EPA and/or DHA on growth and biochemical changes in Korean rockfish. Therefore, the objectives of the present study were to determine essentiality of dietary EPA and DHA, and to compare the effect of dietary EPA/DHA ratio on growth and body composition of Korean rockfish.

Materials and Methods

Experimental diet

The basal diet (Table 1) was prepared to meet the protein and energy requirement of Korean rockfish (Lee *et al.*, 1993a, b; Lee and Lee, 1994a, b). White fish meal was defatted four times with chloroform/methanol mixture (2:1, v/v) before incorporating into the experimental diets. Dextrin was used as dietary carbohydrate source. Beef tallow containing the high contents of saturated and monounsaturated fatty acids was used as lipid source of control diet. Dietary EPA and/or DHA level and ratio were adjusted by substituting the beef tallow with different proportions of ethyl ester of EPA and/or DHA at 8% dietary lipid level. Dietary levels of each EPA or DHA were 0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 1.75%, and EPA/DHA ratios were 1.0/0, 0.75/0.25, 0.5/0.5, 0.25/0.75, and 0/1.0 at 1.0% n-3HUFA (EPA+DHA) level. Control fish received a diet containing 8% beef tallow. The ethyl esters

Table 1. Composition of the basal diet

Ingredient	%
White fish meal[1]	58.0
Dextrin	21.0
Lipid premix[2]	8.0
Vitamin premix[3]	3.0
Mineral premix[4]	5.0
Sodium alginate	2.0
Alpha cellulose	3.0
Nutrient content in dry matter (%)	
Crude protein	49.4
Crude lipid	8.2
Crude ash	11.9

[1] Defatted with chloroform-methanol mixture (2: 1, v/v).
[2] Contained 0.015% BHA, see Table 3.
[3] Halver (1957).
[4] H-440 premix No. 5 (mineral) (NAS, 1973).

of EPA and DHA were purchased from KOHAP Ltd., Seoul, Korea. Fatty acid composition of the dietary lipid sources and lipid composition of the experimental diets are summarized in Table 2 and 3, respectively. Experimental diet was mechanically mixed with water (40 *g*/100 *g* dried diet mix.) and pressure-pelleted, and storage were followed by the method described previously (Lee *et al.*, 1993c).

Table 2. Fatty acid composition (area %) of dietary lipid sources

	Beef tallow	EPA	DHA
Fatty acids			
14:0	4.0	tr	tr
16:0	29.9	tr	tr
16:1n-7	2.4	tr	tr
18:0	17.0	tr	tr
18:1n-(7+9)	43.3	tr	tr
18:2n-6	2.7	0.5	tr
18:3n-3		tr	tr
18:4n-3		tr	tr
20:0		1.2	tr
20:1n-9		tr	tr
20:2n-6		tr	tr
20:4n-6		2.0	1.4
20:4n-3		1.4	3.5
20:5n-3		94.7	0.5
22:1n-9		tr	tr
22:5n-3		tr	tr
22:6n-3		tr	91.9
24:1n-9		tr	2.6
Σn-3HUFA[1]		96.1	95.9

tr: trace (< 0.04).

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

Table 3. Dietary lipid composition(% in diet)[1]

Lipid supplement	Dietary EPA or DHA levels
Control diet	
8.00% Beef tallow	
EPA diet	EPA
7.74% Beef tallow+0.26% EPA[2]	0.25
7.47% Beef tallow+0.53% EPA	0.50
7.21% Beef tallow+0.79% EPA	0.75
6.95% Beef tallow+1.05% EPA	1.00
6.68% Beef tallow+1.32% EPA	1.25
6.42% Beef tallow+1.58% EPA	1.50
6.18% Beef tallow+1.82% EPA	1.75
DHA diet	DHA
7.73% Beef tallow+0.27% DHA[2]	0.25
7.46% Beef tallow+0.54% DHA	0.50
7.18% Beef tallow+0.82% DHA	0.75
6.91% Beef tallow+1.09% DHA	1.00
6.66% Beef tallow+1.36% DHA	1.25
6.37% Beef tallow+1.63% DHA	1.50
6.10% Beef tallow+1.90% DHA	1.75
EPA/DHA diet	EPA:DHA
6.91% Beef tallow+1.09% DHA	0.00:1.00
6.92% Beef tallow+0.26% EPA+0.82% DHA	0.25:0.75
6.93% Beef tallow+0.53% EPA+0.54% DHA	0.50:0.50
6.94% Beef tallow+0.79% EPA+0.27% DHA	0.75:0.25
6.95% Beef tallow+1.05% EPA	1.00:0.00

[1] Contained 0.015% BHA as antioxidant.

[2] Purity of ethyl ester of EPA and DHA were 94.7% and 91.9%, respectively.

Fish and experimental conditions

Juvenile Korean rockfish (mean weight *ca* 0.8 *g*) were obtained from the Puan Hatchery NFRDA Branch (Puan, Korea) in June. Thereafter they were acclimated to our laboratory condition in a 2 ton FRP tank for 1 month. After that, in order to accustom fish to the experimental conditions, the fish were randomly distributed into eighteen 100 *l* tanks with 50 fish per tank and were fed a pre-feeding diet (n-3HUFA-free, control diet) for 10 days. After the conditioning period, the fish were weighed and redistributed into the same eighteen tanks with 30 fish (2.1 *g* initial mean weight) per tank. The fish were fed to satiety by hand three times per day (7 days a week) at 09:00, 13:00 and 17:00. The experiment lasted for 5 weeks. Filtered sea water was supplied to each tank at a flow rate of 2 *l*/min. The water was aerated continuously, and water temperature was maintained at 20.5 ± 0.8℃ (mean ± s.d.) using water temperature controller. During 5 weeks of the experimental period, specific gravity was 1.024 ± 0.002 and photoperiod was left at natural condition. Fish in each tank were weighed as a group on the days of initiation and termination after fish were anesthetized with MS222 at 100 ppm. Fish samples were randomly selected at the initiation (70 fish) and the termination (29~30 fish from each tank) of the feeding trial, and stored at -30℃ for analysis.

Analytical methods and statistical analysis

Protein, lipid, moisture and ash of diet and whole fish were determined according to AOAC methods (1984). Lipid in the liver was extracted (Folch *et al.*, 1957) and then nonpolar and polar lipid were separated on Sep-Pak silica cartridges (Waters Associates, Milford, MA) by successive elution with chloroform and methanol (Juaneda and Rocquelin, 1985). Fatty acid composition of polar and nonpolar lipid fractions were determined after methylation. Fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH for 30 min at 80 ℃. Fatty acid methyl esters were analyzed using a Varian Model 3400 gas-liquid chromatography equipped with an flame ionization detector. The column (30 *m*×0.25 *mm* ID., 0.25 *μm* DB-FFAP capillary column, J & W chromatography, Folson, CA) was operated isothermally at 200℃. Injector and detector temperatures were 220℃ and 250℃, respectively. Nitrogen served as carrier gas at a flow rate 30 *ml*/min. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (Sigma Chemical Co.) consisting of a mixture of saturated and unsaturated fatty acids and reference to other analyses. EPA and DHA requirement of Korean rockfish were determined by the broken line model (Robbins *et at.*, 1979).

Results and discussion

Growth performances

The changes of daily weight gain and feed efficiency of fish fed the diets containing various levels and ratios of EPA and/or DHA are presented in Fig. 1 and 2. The values of these parameters were the lowest in fish fed the n-3HUFA-free diet, and were effectively improved with the dietary levels of EPA or DHA up to 1.0%. And then there were no additional improvements above these levels. Lee *et al.* (1993e) indicated that Korean rockfish required n-3HUFA as EFA like other marine fish, and the n-3HUFA requirement was 0.9% in the diet. The present study also shows that each of the EPA and DHA requirement is 1.0% in the diet determined by broken line analysis (Fig. 3). On the other hand, Takeuchi *et al.* (1990) reported that EPA and DHA requirement of juvenile red seabream were 1% and 0.5%, respectively.

The daily growth rate and feed efficiency were higher in fish fed the DHA diet groups than in fish fed the EPA diet groups at the same dietary n-3 HUFA levels. In EPA/DHA diets containing different combinations of EPA and DHA at 1.0% n-3 HUFA (EPA+DHA) level, these parameters of

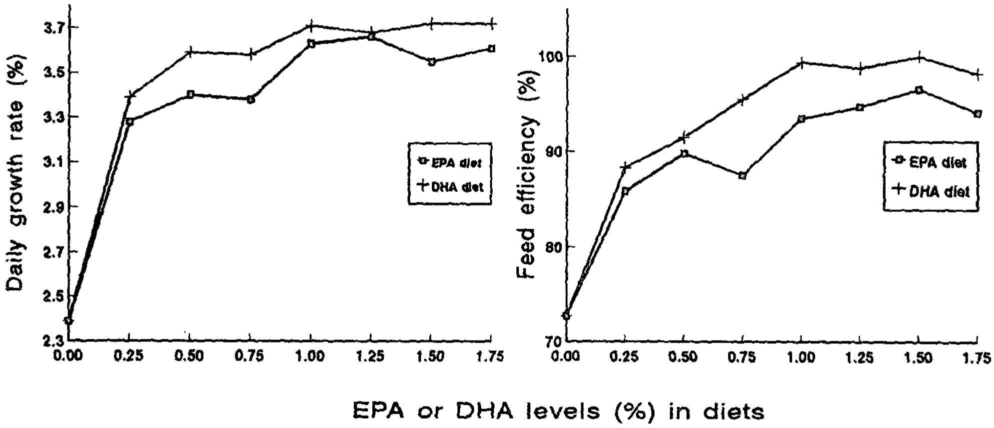

Fig. 1. Daily growth rate and feed efficiency of Korean rockfish fed the diets containing the different levels of EPA or DHA.

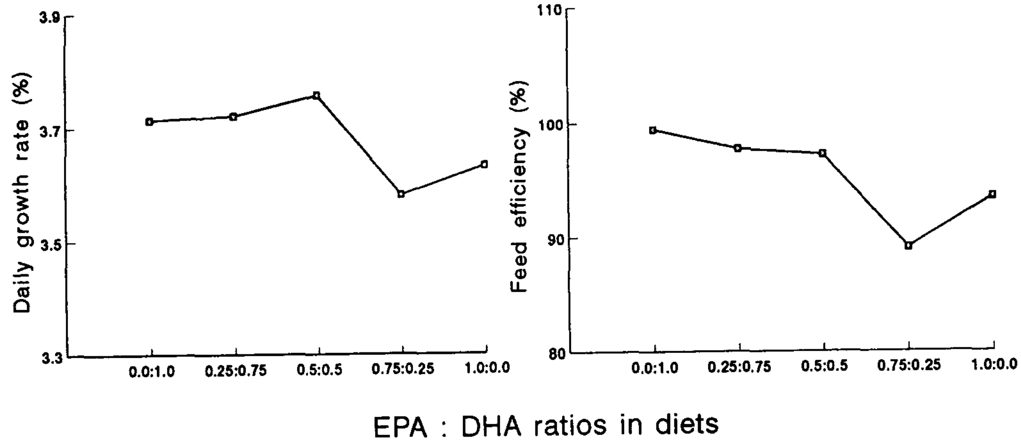

Fig. 2. Daily growth rate and feed efficiency of Korean rockfish fed the diets containing the different ratios of EPA/DHA.

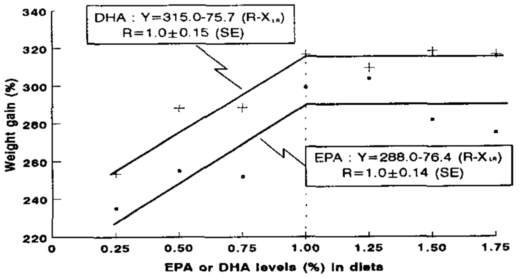

Fig. 3. Broken line model of weight gain for the dietary levels of EPA or DHA.

fish fed the diets containing 50%, 75% and 100% of DHA were better than others. Lee *et al.* (1993d) and Lee and Hur (1993) suggested that DHA had more important role in enzyme activity of cell membrane and in physiological balance during starvation. DHA is superior to EPA as EFA has also been reported in juvenile red seabream (Watanabe *et al.*, 1989b; Takeuchi *et al.*, 1990) and striped jack (Kanazawa, 1985; Watanabe *et al.*, 1989c). These results indicate that EPA and DHA may have different functions in biomembrane of marine fish.

Protein and lipid retention efficiency in res-

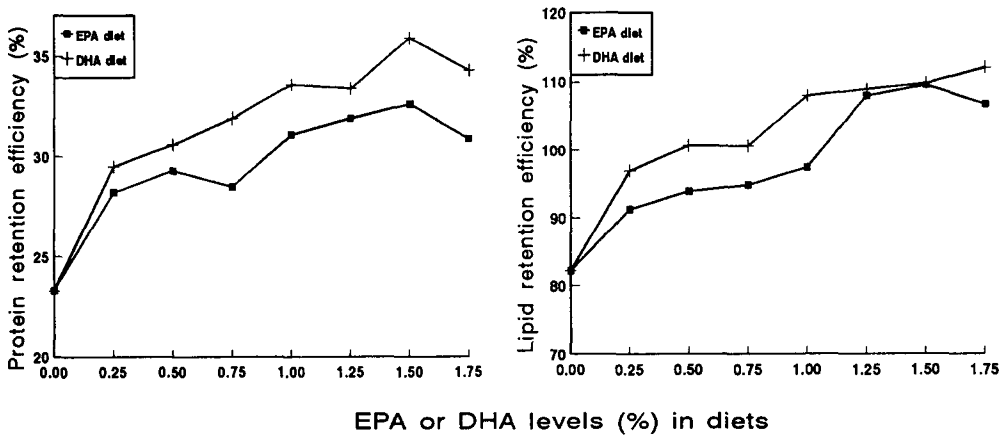

Fig. 4. Protein and lipid retention efficiency of Korean rockfish fed the diets containing the different levels of EPA or DHA.

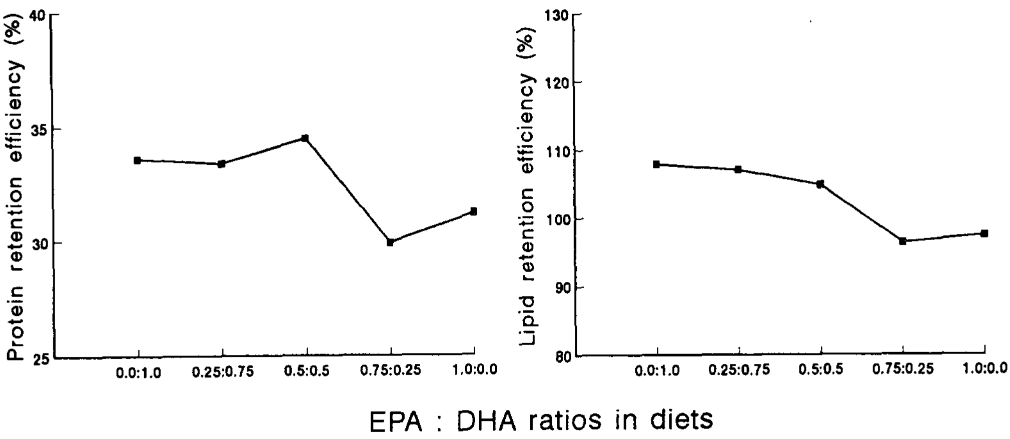

Fig. 5. Protein and lipid retention efficiency of Korean rockfish fed the diets containing the different ratios of EPA/DHA.

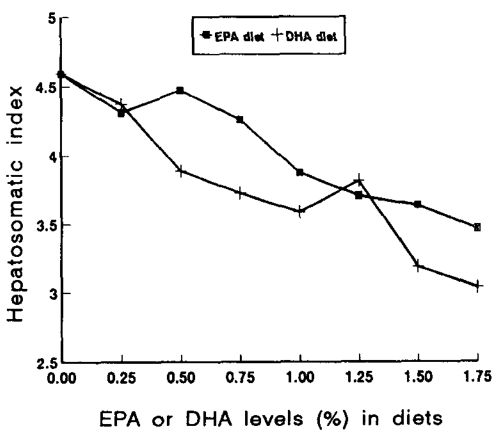

Fig. 6. Hepatosomatic index of Korean rockfish fed the diets containing the different levels of EPA or DHA.

ponse to EPA and DHA levels and EPA/DHA ratios are presented in Fig. 4 and 5. The values of these parameters were improved with the increase of dietary EPA or DHA levels, and with the decrease of dietary EPA/DHA ratios. Hepatosomatic index (HSI) was increased by decreasing EPA or DHA levels in diet (Fig. 6), which has been observed as a sign of EFA deficiency in other marine fish (Kalogeropoulos *et al.*, 1992 ; Takeuchi *et al.*, 1990) including Korean rockfish (Lee *et al.*, 1993c, e). DHA was more effective than EPA at the same dietary n-3HUFA levels to reduce HSI, which might indicate that DHA is superior to EPA as EFA.

As described previously, growth responses were improved according to increase of dietary EPA or DHA, and then reached a plateau between 1.0 and 1.75% EPA or DHA, without any additive or negative effects in the diet containing excessive these fatty acids such as 1.75% EPA or DHA diet. Lee *et al.* (1993e) have observed similar result for this species. In their study, juvenile Korean rockfish fed the diet containing 4.0% n-3HUFA was not observed any negative effects in growth performances and body chemical composition. On the other hand, negative effects of excessive EFA in diet, such as poor growth and feed efficiency, have been observed in rainbow trout (Takeuchi and Watanabe, 1979) and red seabream (Takeuchi *et al.*, 1992). This different response may be due to differences in species, feeding trial periods, optimum ranges of EFA requirement, or interactions with other nutrient such as vitamin E. Fish oils such as squid liver oil and cod liver oil contain high proportion of n-3HUFA (EPA+DHA) and adequate ratio of EPA /DHA (Lee *et al.*, 1993c ; Kalogeropoulos *et al.*, 1992) for marine fish, therefore we can use these oils as good dietary lipid sources to satisfy EPA and DHA. However, n-3HUFA in a diet is easily oxidized leading to increase TBA value, and increase vitamin E requirement (Watanabe *et al.*, 1981). Thus, it is not necessary to adding excessive n-3HUFA in a diet as EFA for normal growth and healthy condition of fish. Lee (1994) and Lee *et al.* (1994) studying with Korean rockfish have noted that growth performances were not influenced by different dietary lipid sources such as soybean oil and beef tallow when dietary n-3HUFA was satisfied.

During feeding period in this experiment, mortalities were low (0~1 fish per tank) and did not follow any specific trend.

Body composition

Lipid content of whole body was the lowest in fish fed the diet with 8% beef tallow, and

Table 4. Chemical composition (%) of whole body of Korean rockfish fed the diets containing various levels of EPA or DHA and ratios of EPA/DHA

Dietary EPA or DHA levels (%)	Moisture	Protein	Lipid	Ash
EPA				
0.00	73.5	16.0	5.3	4.5
0.25	72.4	16.2	6.2	4.4
0.50	71.6	16.2	6.2	4.2
0.75	72.0	16.1	6.4	4.1
1.00	71.6	16.4	6.4	3.7
1.25	72.5	16.6	7.1	4.6
1.50	71.8	16.6	6.9	3.8
1.75	71.4	16.2	6.9	4.0
DHA				
0.00	73.5	16.0	5.3	4.5
0.25	72.7	16.4	6.5	4.2
0.50	72.3	16.4	6.7	3.7
0.75	72.8	16.5	6.5	4.1
1.00	71.9	16.6	6.8	4.0
1.25	71.5	16.6	6.9	4.3
1.50	72.0	17.4	6.9	4.1
1.75	72.1	17.0	7.2	3.7
EPA:DHA				
0.00:1.00	71.9	16.6	6.8	4.0
0.25:0.75	71.8	16.7	6.9	3.7
0.50:0.50	71.2	17.2	6.8	4.0
0.75:0.25	71.5	16.5	6.6	3.9
1.00:0.00	71.6	16.4	6.4	3.7

increased with levels of EPA or DHA. Whereas protein contents of whole body were not affected by dietary EPA or DHA levels (Table 4). EFA deficiency has been reported to affect body composition, EFA-deficient fish having higher moisture and lower lipid levels (Castell *et al.*, 1972b; Watanabe *et al., 1974a; Kalogeropoulos et al.*, 1992). A tendency toward reduced lipid level and increased moisture level of fish fed the lower levels of dietary EPA or DHA is in accordance with this study, suggesting that these levels of dietary EPA or DHA are deficient in EFA. Higher value of HSI in fish fed the lower dietary EPA or DHA levels would be mainly due to accumulation of nonpolar lipid, estimated from change patterns of polar/nonpolar lipid ratios of liver in this study. Polar/nonpolar lipid ratios in the liver were increased by elevating EPA or DHA levels in diet(Fig. 7). Previous studies have shown that polar lipid content of liver is not easily influenced by different dietary n-3HUFA levels, while nonpolar lipid was increased in fish fed EFA deficient diets (Lee *et al.*, 1993c; Takeuchi *et al.*, 1990; Watanabe *et al.*, 1989).

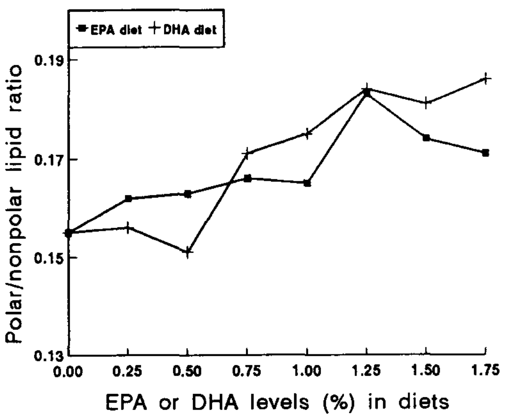

Fig. 7. Polar/nonpolar lipid ratio of liver of Korean rockfish fed the diets containing the different levels of EPA or DHA.

Fatty acid composition of liver

Fatty acid composition of liver polar lipid were influenced by dietary fatty acid composition (Table 5 and 7). As the result, fish fed the diet

Table 5. Fatty acid composition (area %) of polar lipid fraction from liver of Korean rockfish fed the diets containing various levels of EPA or DHA

Fatty acids	Initial	EPA or DHA levels (%) in diet							
		0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75
EPA diet									
14:0	1.8	2.0	3.2	5.8	3.9	4.2	2.9	2.6	3.6
16:0	14.7	11.2	15.9	14.7	15.5	15.1	15.7	14.7	14.6
16:1n-7	10.6	14.9	13.2	17.8	12.6	15.2	13.9	13.0	15.0
18:0	7.4	1.5	4.0	3.7	4.5	3.7	4.3	4.2	3.6
18:1n-(7+9)	50.0	56.9	59.3	54.4	51.8	47.0	53.0	53.6	54.5
18:2n-6	2.2	4.5	0.9	2.6	1.6	2.1	2.0	2.1	2.2
18:3n-3	0.3	0.2	0.1	0.3	2.3	2.7	0.1	0.1	0.1
18:4n-6	tr	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.2
18:4n-3	0.4	tr	tr	tr	tr	tr	tr	tr	tr
20:1n-9	1.8	0.9	0.5	0.8	0.1	0.9	0.4	0.9	0.8
20:2n-6	2.5	3.5	0.4	0.2	1.2	1.5	0.7	2.2	1.2
20:4n-6	0.5	0.2	tr	tr	tr	tr	0.1	0.1	0.1
20:4n-3	0.2	tr	tr	tr	tr	tr	tr	tr	tr
20:5n-3	2.2	0.4	1.2	1.2	3.9	3.9	3.0	4.3	3.0
22:1n-9	0.5	tr	tr	tr	tr	tr	tr	tr	tr
22:5n-3	tr	tr	tr	0.1	0.9	0.7	0.1	0.2	0.1
22:6n-3	3.6	1.0	0.3	0.2	0.5	0.8	0.3	0.6	0.2
24:0	0.3	tr	tr	tr	tr	0.4	0.2	0.3	0.2
Monoenes	62.9	72.7	73.0	73.0	64.5	63.1	67.3	67.5	70.3
Σn-6	5.2	8.5	1.4	2.9	2.9	3.7	2.9	4.6	3.7
Σn-3	6.7	1.6	2.6	1.7	6.7	7.4	3.4	5.0	3.3
Σn-3HUFA[1]	6.0	1.4	1.5	1.5	5.3	5.4	3.4	5.1	3.3
18:1n-(7+9)/n-3HUFA	8.3	40.6	39.5	36.3	9.8	8.7	15.6	10.5	16.5
DHA diet									
14:0	1.8	2.0	3.1	5.5	3.0	4.2	2.7	3.7	4.0
16:0	14.7	11.2	15.9	16.5	15.9	15.5	15.3	17.3	18.1
16:1n-7	10.6	14.9	14.8	15.0	16.9	13.1	19.5	14.1	18.4
18:0	7.4	1.5	3.8	4.7	3.5	4.5	3.0	4.3	3.4
18:1n-(7+9)	50.0	56.9	56.6	48.8	50.7	50.5	49.8	49.9	46.3
18:2n-6	2.2	4.5	0.9	0.9	0.6	1.7	1.0	1.6	0.9
18:3n-3	0.3	0.2	0.1	1.5	tr	tr	0.3	0.1	1.3
18:4n-6	tr	0.3	0.3	0.1	0.1	0.3	0.1	0.1	tr
18:4n-3	0.4	tr	tr	tr	tr	tr	tr	tr	tr
20:1n-9	1.8	0.9	0.5	1.4	0.3	0.4	0.5	0.6	0.4
20:2n-6	2.5	3.5	1.1	1.1	0.7	1.9	0.6	0.7	0.6
20:4n-6	0.5	0.2	tr	tr	0.1	tr	0.1	0.2	tr
20:4n-3	0.2	tr	tr	0.3	tr	tr	0.1	0.1	tr
20:5n-3	2.2	0.4	0.2	0.5	0.2	0.5	0.3	0.4	0.3
22:1n-9	0.5	tr	tr	tr	tr	tr	tr	tr	tr
22:5n-3	tr	tr	tr	tr	tr	tr	0.1	0.1	0.4
22:6n-3	3.6	1.0	1.8	2.5	3.8	6.4	5.4	5.7	4.7
24:0	0.3	tr	tr	tr	tr	tr	0.1	tr	tr
Monoenes	62.9	72.7	71.9	65.2	67.9	64.0	69.8	64.6	65.1
Σn-6	5.2	8.5	2.3	2.1	1.5	3.9	1.8	2.6	2.8
Σn-3	6.7	1.6	2.1	4.8	4.0	6.9	6.2	6.3	6.3
Σn-3HUFA	6.0	1.4	2.0	3.0	4.0	6.9	5.8	6.2	5.4
18:1n-(7+9)/n-3HUFA	8.3	40.6	28.3	16.3	12.7	7.3	8.6	8.0	8.6

tr: trace (< 0.04).

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

containing only beef tallow had high amounts of monoenes and n-6 series fatty acids and low amount of EPA and DHA in the liver polar lipid. In contrast, fish fed the diet containing n-3HUFA (EPA or DHA) had low amounts of monoenes and n-6 series fatty acids and high amount of EPA

Table 6. Fatty acid composition (area %) of nonpolar lipid fraction from liver of Korean rockfish fed the diets containing various levels of EPA or DHA

Fatty acids	Initial	EPA or DHA levels (%) in diet							
		0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75
EPA diet									
14:0	2.7	3.5	3.3	3.9	2.9	4.2	3.6	3.5	3.6
16:0	21.8	13.3	17.4	16.6	16.9	17.9	17.3	16.6	17.7
16:1n-7	8.2	13.0	11.6	15.1	13.7	14.2	14.5	13.1	12.9
18:0	5.1	3.1	4.4	3.8	3.5	3.9	3.7	4.0	3.7
18:1n-(7+9)	55.8	64.2	60.7	58.7	60.9	57.3	58.2	58.9	57.4
18:2n-6	1.3	0.4	0.3	0.1	0.1	0.2	0.2	0.4	1.7
18:3n-3	0.2	0.2	0.1	0.2	0.2	tr	tr	tr	tr
18:4n-6	0.2	tr	tr	tr	tr	tr	0.2	0.2	0.1
18:4n-3	0.3	tr	tr	tr	tr	0.2	0.1	0.1	0.2
20:1n-9	1.6	0.8	0.8	0.5	0.4	0.5	0.6	0.6	0.4
20:2n-6	0.4	0.3	0.1	0.1	0.3	0.2	0.1	0.2	0.3
20:5n-3	0.4	tr	0.1	0.1	0.2	0.3	0.5	0.8	1.0
22:1n-9	0.5	0.1	tr	tr	tr	tr	0.1	0.1	tr
22:6n-3	0.5	tr	tr	tr	tr	tr	tr	0.1	tr
Monoenes	66.1	78.1	73.1	74.3	75.0	72.0	73.4	72.7	70.7
Σn-6	1.9	0.7	0.5	0.2	0.4	0.4	0.5	0.8	2.1
Σn-3	1.4	0.2	0.2	0.3	0.4	0.4	0.6	1.0	1.2
Σn-3HUFA[1]	0.9	tr	0.1	0.1	0.2	0.3	0.5	0.9	1.0
DHA diet									
14:0	2.7	3.5	4.4	3.6	3.6	3.4	3.6	4.1	3.6
16:0	21.8	13.3	18.7	18.5	18.2	19.2	19.8	20.3	21.0
16:1n-7	8.2	13.0	14.1	14.6	16.5	15.0	15.7	16.4	13.6
18:0	5.1	3.1	3.9	2.9	3.4	3.8	3.5	3.5	3.9
18:1n-(7+9)	55.8	64.2	56.1	56.0	56.0	56.3	54.6	53.2	54.7
18:2n-6	1.3	0.4	0.3	0.4	0.2	0.2	0.1	0.1	0.2
18:3n-3	0.2	0.2	0.2	0.4	tr	tr	tr	tr	tr
18:4n-6	0.2	tr	0.2	0.4	0.1	0.1	0.3	0.1	0.2
18:4n-3	0.3	tr	0.1	0.4	0.1	0.1	0.1	0.1	0.1
20:1n-9	1.6	0.8	0.5	0.2	0.6	0.4	0.7	0.5	0.4
20:2n-6	0.4	0.3	0.2	0.1	0.2	0.2	0.3	0.1	0.6
20:5n-3	0.4	tr	0.1	tr	tr	tr	tr	tr	0.1
22:1n-9	0.5	0.1	tr	tr	tr	0.1	0.1	tr	tr
22:6n-3	0.5	tr	0.2	0.2	0.1	0.1	0.1	0.4	0.5
Monoenes	66.1	78.1	70.7	70.8	73.1	71.7	71.1	70.1	68.7
Σn-6	1.9	0.7	0.7	0.9	0.5	0.5	0.7	0.3	1.1
Σn-3	1.4	0.2	0.6	1.0	0.2	0.2	0.2	0.5	0.7
Σn-3HUFA	0.9	tr	0.3	0.2	0.1	0.1	0.1	0.4	0.6

tr: trace (< 0.04).

[1] Highly unsaturated fatty acids ($C \geq 20$).

Table 7. Fatty acid composition (area %) of polar and nonpolar lipid fraction from liver of Korean rockfish fed the diets containing various ratios of EPA/DHA

Fatty acids		EPA/DHA diet				
	EPA (%)	0.00	0.25	0.50	0.75	1.00
	DHA (%)	1.00	0.75	0.50	0.25	0.00
Polar lipid						
14:0		4.2	3.3	5.0	2.9	4.2
16:0		15.5	14.8	13.9	14.5	15.1
16:1n-7		13.1	13.6	15.7	13.7	15.2
18:0		4.5	3.6	3.6	4.5	3.7
18:1n-(7+9)		50.5	54.8	53.1	54.1	47.0
18:2n-6		1.7	1.0	1.4	0.8	2.1
18:3n-3		tr	tr	tr	tr	2.7
18:4n-6		0.3	tr	tr	tr	tr
18:4n-3		tr	0.5	tr	tr	tr
20:1n-9		0.4	0.1	0.4	0.5	0.9
20:2n-6		1.9	1.9	2.3	3.1	1.5
20:4n-6		tr	tr	tr	tr	tr
20:4n-3		tr	tr	tr	tr	tr
20:5n-3		0.5	1.3	2.0	2.8	3.9
22:1n-9		tr	tr	tr	tr	tr
22:5n-3		tr	tr	tr	tr	0.7
22:6n-3		6.4	4.3	2.4	2.0	0.8
24:0		tr	tr	tr	tr	0.4
Monoenes		64.0	68.5	69.2	70.9	63.1
Σn-6		3.9	2.9	3.7	3.9	3.6
Σn-3		6.9	6.1	4.4	4.8	8.1
Σn-3HUFA[1]		6.9	5.6	4.5	4.8	5.4
18:1n-(7+9)/n-3HUFA		7.3	9.8	12.1	11.3	8.7
Nonpolar lipid						
14:0		3.4	3.6	3.6	3.8	4.2
16:0		19.2	20.1	18.5	20.0	17.9
16:1n-7		15.0	14.2	17.8	14.3	14.2
18:0		3.8	4.0	6.3	4.2	3.9
18:1n-(7+9)		56.3	55.4	51.7	55.5	57.3
18:2n-6		0.2	0.2	0.2	0.2	0.2
18:3n-3		tr	tr	tr	tr	tr
18:4n-6		0.1	0.3	0.2	0.1	tr
18:4n-3		0.1	tr	tr	tr	0.2
20:1n-9		0.4	0.4	0.4	0.4	0.5
20:2n-6		0.2	0.2	0.1	0.2	0.2
20:5n-3		tr	0.2	0.1	0.2	0.3
22:1n-9		0.1	tr	tr	tr	tr
22:6n-3		0.1	0.2	tr	tr	tr
Monoenes		71.8	70.0	69.9	70.2	72.0
Σn-6		0.5	0.7	0.5	0.5	0.4
Σn-3		0.2	0.4	0.1	0.2	0.5
Σn-3HUFA		0.1	0.4	0.1	0.2	0.3

tr: trace (< 0.04).

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

or DHA. The EPA contents of liver polar lipid were increased with increasing EPA levels to 0.75% in the EPA diet, but the DHA were not changed by the dietary EPA levels. In contrast to EPA diet, the DHA contents of liver polar lipid were increased by elevating DHA levels to 1.0% level in the DHA diet, with no changes of the EPA content. In addition, the EPA of liver polar lipid were decreased and the DHA of liver polar lipid were increased according to replaced dietary EPA with DHA in the EPA/DHA diet. These fatty acids of polar lipid appear to respond directly to dietary fatty acid composition and level, because of possible lower capacity of the Korean rockfish to elongate and desaturate fatty acids. These results also suggest that EPA is not converted to DHA, and that DHA is not retroconverted to EPA. The capacity to elongate or desaturate fatty acids is species-dependent. Freshwater fish have a higher capacity than marine fish (Owen *et al.*, 1975; Yamada *et al.*, 1980). Korean rockfish may also have a limited capacity to elongation or desaturation as has already been pointed out in previous papers (Lee *et al.*, 1993c, e). Higher values for 18:1/n-3HUFA ratio and 18:1 content of liver polar lipid were observed in fish fed the EPA or DHA deficient diets. It has been proposed that the ratio of 18:1/n-3HUFA can serve as an index of EFA adequacy for red seabream (Fujii *et al.*, 1976) and gilthead bream (Kalogeropoulos *et al.*, 1992). From these studies, 18:1/n-3 HUFA ratios of less than 1 were found when dietary EFA were satisfied. However, in our studies for EFA nutrition of Korean rockfish (Lee *et al.*, 1993c, e) including this study, a much higher ratios in fish fed the diets containing sufficient n-3HUFA were found. These differences may be reason for species-dependent or fatty acid composition of dietary lipid sources used, and further studies are necessary to examine this aspect.

Except for higher levels of EPA (1.25, 1.5 and 1.75%) or DHA (1.5 and 1.75%) in diets, EPA or DHA in liver nonpolar lipid were not affected by dietary EPA and/or DHA levels, and maintained lower levels (Table 6 and 7). Similar results have also been described for this fish (Lee *et al.*, 1993e) and other species (Bird and Potter, 1983; Delgado *et al.*, 1994). EPA or DHA in liver nonpolar lipid were slightly increased in the higher levels of EPA (1.25, 1.5 and 1.75%) or DHA (1.5 and 1,75%) diets, respectively. These results may be due to excessive dietary EPA or DHA for Korean rockfish. In that respect, excessive dietary n-3HUFA in Korean rockfish may be stored in nonpolar lipid. These results are in agreement to previous study (Lee *et al.*, 1993e) in which it has been observed that n-3HUFA of the liver nonpolar lipid in juvenile Korean rockfish fed the diet containing 4.0% n-3 HUFA is significantly higher than those in the fish receiving 0~2.0% n-3HUFA diets.

Results of this study suggest that each of the EPA and DHA is required for normal growth of Korean rockfish and the requirements are both 1.0 % in diet, and that DHA is superior to EPA as EFA for Korean rockfish.

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조피볼락(*Sebastes schlegeli*) 사료의 EPA 및 DHA 필수성

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조피볼락 사료의 EPA 및 DHA 필수성과 적정 EPA/DHA 비를 구명하기 위해, 기본 지질을 우지로 하여 EPA와 DHA를 각각 0~1.75% 첨가한 사료와 EPA/DHA 비를 다르게 첨가한 사료 18종을 제조하여 조피볼락 치어(평균 체중 2.1 *g*)를 대상으로 5주간 사육 실험한 결과는 다음과 같다.

일간증중율(EPA 사료: 2.39~3.66%, DHA 사료: 2.39~3.72%)과 사료효율(EPA 사료: 73~97%, DHA 사료: 73~100%)은 EPA 및 DHA 사료 모두 사료중의 함량 1.0%까지만 증가하고 그 후에는 일정한 값을 나타내어, EPA 및 DHA 요구량이 모두 1.0%로 추정되었다. 단백질축적효율(EPA 사료: 23.3~32.6%, DHA 사료: 23.3~35.9%)과 지질축적효율(EPA 사료: 82.2~109.5%, DHA 사료: 82.2~112.1%)도 사료의 EPA 또는 DHA가 증가할수록 증가하였다. 이러한 결과들은 EPA 사료에서보다 DHA 사료에서 전반적으로 좋은 반응을 보였으며, EPA/DHA 사료에서도 EPA에 대한 DHA 비가 1.0 이상인 사료에서 양호한 결과를 보였다.

전어체의 단백질 함량은 큰 변화가 없었지만 지질 함량은 사료의 EPA 및 DHA가 증가할수록 약간씩 증가하는 경향을 나타내었다. 간중량비는 사료의 EPA 및 DHA 함량이 증가함에 따라 감소하였으며, DHA 사료가 EPA 사료보다 대체로 낮은 값을 보였다.

사료의 EPA가 증가함에 따라 간 극성지질의 EPA (0.4~4.3%) 함량은 증가하였으나 DHA (0.2~1.0%) 함량은 거의 증가하지 않았고, 사료의 DHA 증가와 더불어 간 극성지질의 DHA (1.0~6.4%) 함량은 증가하였으나 EPA (0.2~0.5%) 함량은 증가하지 않았다. 반면, 간 비극성지질의 지방산조성은 사료 지방산조성에 영향을 거의 받지 않아 변화 폭이 적었다.