Comparison Between Eicosapentaenoic and Docosahexaenoic Acids in Terms of Essential Fatty Acid Efficacy in Larval Red Seabream^{*1}

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Efficacy of EPA and DHA as the EFA for larval red seabream was evaluated by feeding them rotifers enriched with respectively methyl oleate, EPA, DHA and n-3 HUFA mixture for 8 days in 100 l tanks.

The larvae fed on rotifers containing a low percentage of n-3 HUFA showed low growth and survival rates together with a high incidence of the so-called hydrops. These larvae also showed poor vitality and all of them died in the both vitality tests. The growth and survival rates were effectively improved by the incorporation of EPA and DHA or n-3 HUFA mixture into the rotifers. However, the incidence of hydrops was not completely prevented by EPA and the n-3 HUFA mixture, but was almost totally prevented by DHA. The growth and the survival rate after vitality test were also highest in the larvae fed the DHA-rotifer.

The fatty acid analysis on the total lipids of larvae has shown that the level of assimilation of DHA was much higher than EPA, although EPA may partly be converted to 22:5n-3 and that no retroconversion of DHA may be available. Thus DHA is found to be superior to EPA as EFA for larval red seabream.

Recent investigations^{1,2)} on essential fatty acids (EFA) of fish have demonstrated that marine species of finfish require n-3 highly unsaturated fatty acids (n-3 HUFA) such as eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) as EFA for their normal growth. The requirements of n-3 HUFA is about 0.5% for both juvenile³⁾ and larval⁴⁾ red seabream, 2.0% for juvenile yellowtail,⁵⁾ 0.8% for turbot,⁶⁾ about 2.0% for flounder,⁷⁾ and around 1.8% for juvenile striped jack.⁸⁾ However, these requirements were all determined using lipids or methyl esters containing both EPA and DHA as n-3 HUFA, providing no information as to which fatty acid, EPA or DHA, is more important in various physiological functions. In rainbow trout, there is no difference in EFA efficiency between EPA and DHA, but growth and feed efficiency are enhanced by combining both fatty acids.⁹⁾ In juvenile red seabream, the EFA value of EPA is reported to be almost the same as a n-3 HUFA mixture con-

taining both EPA and DHA.¹⁰⁾ In freshwater species in which linolenic acid (18:3n-3) is converted to DHA via EPA, EPA may not vary so much from DHA in EFA efficacy but may vary in marine fish species in which the conversion for EPA to DHA is very limited.¹¹⁾ This is also supported by the fact that DHA which is usually high in the eggs of marine fish species is quickly reduced during larval development,¹¹⁾ although it is unknown whether or not DHA is utilized as energy during development or converted to other physiologically important substances such as prostaglandin.

Following the previous experiment⁴⁾ in which the requirement of larval red seabream for n-3 HUFA was determined by feeding them rotifers containing different levels of n-3 HUFA, this study was conducted to compare the dietary value in larval red seabream of EPA with DHA using rotifers enriched with EPA or DHA.

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Materials and Methods

Preparation of Emulsified Lipids

Four types of lipids were prepared by using methyl esters of oleic acid (18:1n-9), EPA, DHA and n-3 HUFA mixture, as shown in Table 1. Namely, 88% 18:1n-9 (Tank no. 1), 67% EPA by mixing 18:1n-9 and EPA (Tank no. 2), 67% DHA by mixing 18:1n-9 and DHA (Tank no. 3), and 85% n-3 HUFA containing 37% EPA and 42% DHA (Tank no. 4). The purity of 18:1n-9 and DHA purchased from Sigma Chemical Co., Ltd. was 88% and 90%, respectively. EPA was prepared from cuttlefish oil using vacuum distillation,⁹⁾ its purity was 85%. Each lipid was homogenized with soybean lecithin (5% in lipid) for 2–3 min by a cooled juice mixer to obtain an emulsion.¹²⁾ Emulsions were prepared each time just before enrichment of rotifers. The homogeneous condition of the emulsion was checked by a microscope.

Enrichment of Rotifers

Rotifers *Brachionus plicatilis* containing different types of fatty acids were prepared by the direct method¹²⁾ using the above mentioned four types of lipids. Rotifers which had been fed on baker's yeast *Saccharomyces cerevisiae* for 7 days in one-ton polycarbonate tanks were transferred to 5 l tanks (with 4 l water) at an initial density of 200 indiv./ml, and 5 ml of each emulsified lipid were added to each tank for enrichment. Enrichment was conducted for 12 h at a water temperature of 19.5–20.0°C with vigorous aeration. Rotifers were collected once before the initiation of feeding, and twice during 8 days of feeding for analysis of lipid classes and fatty acid composition. They were filtered, and washed with running tapwater and kept at –20°C until analysis.¹³⁾

Larval Rearing

Eggs of red seabream *Pagrus major*, obtained from the broodstock which had been fed a commercial red seabream diet and fresh raw fish were

hatched and fed on rotifers cultured with baker's yeast for 3 days at the Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries. The larvae were then randomly divided into 4 groups of 2,000 fish each and fed on one of the varieties of enriched rotifers for 8 days in 100 l tanks. The initial total length of the larvae was 3.91 ± 0.20 mm. Water temperature ranged from 19.0 to 21.5°C during the experimental period. The rates of aeration and sea water supply were 100 and 120 ml/min, respectively. The number of rotifers given to each larval tank was 2×10^6 indiv./day (given twice per day, each time 1×10^6 rotifers).

At the end of the feeding trial, the so-called vitality test was conducted twice to check the fish's response to the stress. In the first and second tests 50 larvae were taken out of the water and held in the air by a scoop net for 5 and 10 s, respectively, and moved to 30 l tanks to check their survival after 24 h. The total body length of 30 individual fish larvae was measured at the initiation and termination of the experiment. The remainder of the fish were sampled and analysed for lipid classes and fatty acid composition. The details of fish care, feeding and analytical procedures have all been described in previous papers.^{4,8)}

Results and Discussion

Lipid Classes and Fatty Acid Compositions of Enriched Rotifers

Lipid classes of rotifers enriched with the various emulsions containing respectively 18:1, EPA, DHA and n-3 HUFA mixtures are shown in Table 2. The lipid content was the highest in the rotifers enriched with the n-3 HUFA mixture, being reflected by the elevated levels of each lipid class. The EPA-deficient rotifers enriched with 18:1 were lower in proportion of polar lipids and free fatty acids.

The fatty acid composition of the total lipids from rotifers fed on various lipids is listed in Table 3. The values in the table are the averages

Table 1. Composition of the experimental oils used for preparation of emulsified lipids to feed rotifers

	Tank no.			
	1	2	3	4
Type of oil*	OAME	EPA+OAME	DHA+OAME	ME 85
HUFA in oil (%)	0	67	67	85

* Abbreviations: OAME; methyl oleate, EPA; methyl eicosapentaenoate, DHA; methyl docosahexaenoate, ME 85; methyl esters n-3 HUFA.

Table 2. Lipid classes in total lipids of rotifers (%)

Tank no.	Moisture	Crude lipid		in Rotifers*				
		wet basis	dry basis	CE	TG	FFA	DG	PL
1	88.8	2.0	17.8	0.43	6.68	0.46	0.27	9.95
2	87.7	2.5	19.8	0.56	4.39	2.62	0.36	11.93
3	89.6	1.9	17.8	0.46	4.04	1.19	0.28	11.80
4	88.0	2.8	23.7	0.64	5.56	4.31	0.47	12.66

* Abbreviations: CE; cholesterol esters, TG; triglycerides, FFA; free fatty acids, DG; diglycerides, PL; polar lipids.

three determinations on the rotifers collected before and during the feeding experiment as described above. There was no marked difference between the three determinations, thus the standard deviation was deleted from each value for simplification. Thus, the larvae of each group were found to have received rotifers with almost the same fatty acid composition during the feeding period of 8 days.

The rotifers enriched with 18:1n-9 were significantly higher in the percentage of monoethylenic fatty acids, especially high in 18:1 directly affected by the methyl oleate added to the culture medium. Therefore, they were exactly the EFA-deficient rotifers containing little n-3 HUFA. The proportion of monoethylenic fatty acids was low in rotifers fed on EPA, DHA and the n-3 HUFA mixture. The rotifers enriched with the emulsion containing 67% of EPA contained 22.3% EPA and 2.3% DHA in fatty acid composition (0.48% EPA and 0.05% DHA on a wet basis of rotifers). While those enriched with the same concentration of DHA contained 3.9% EPA and 21.8% DHA (0.06% EPA and 0.45% DHA on a wet basis). It was therefore found that two types of rotifers containing either EPA or DHA at almost the same level were successfully cultured during the period of feeding. On the other hand, both the fatty acids, EPA and DHA, were high in the rotifers fed on the emulsion containing the n-3 HUFA mixture. The percentages of EPA and DHA were 11.4% and 20.5% (0.28% EPA and 0.51% DHA on a wet basis), and the total amount of n-3 HUFA was the highest in these rotifers, as shown in Table 2. The concentration of n-3 HUFA in each group of rotifers except those fed on methyl oleate was enough to satisfy the EFA requirement of larval red seabream.⁴⁾ A relatively high percentage of 18:2n-6 in each rotifer must be due to the preliminary feeding of the rich in this fatty acid and 18:2n-6 contained in methyl oleate as the contamination.

Table 3. Fatty acid composition of total lipids in rotifers enriched with various lipids (area %)

Fatty acid	Tank no.			
	1	2	3	4
14:0	2.2	0.9	1.3	1.1
14:1	1.1	0.9	1.2	0.8
15:0	0.3	0.2	0.3	0.3
16:0	5.0	3.6	4.5	3.9
16:1n-7	10.5	7.4	8.8	7.2
16:1n-5	0.4	0.2	0.3	0.3
17:0	0.7	0.7	0.5	0.4
16:3n-6	1.2	0.7	0.9	0.7
16:4n-3	0.5	2.0	2.5	0.5
18:0	2.1	1.2	1.2	1.9
18:1n-9	51.0	15.1	25.7	21.8
18:1n-7	7.0	2.7	4.1	3.7
18:1n-5	1.2	0.5	0.3	0.2
18:2n-6	6.7	5.9	5.1	4.7
18:3n-6	0.7	0.6	0.4	0.4
19:0	0.2	0.4	0.2	ND
18:3n-3	0.6	1.6	1.5	0.8
18:4n-3	0.2	2.0	0.2	0.9
20:0	0.1	0.0	0.1	0.0
20:1n-11	0.5	0.1	ND	0.5
20:1n-9	1.3	0.8	1.5	1.2
20:2n-9	0.3	0.3	0.4	0.3
20:2n-6	0.4	0.3	0.3	0.4
20:3n-6	0.2	0.8	0.3	0.3
20:4n-6	0.3	6.6	0.9	1.1
20:3n-3	0.1	0.2	0.1	0.1
20:4n-3	0.1	2.2	0.5	1.1
20:5n-3	0.5	22.3	3.9	11.4
22:0	ND	0.7	0.4	0.2
22:1n-(13+11)	1.8	3.7	0.4	1.2
22:5n-6	0.3	0.1	0.1	0.3
22:4n-3	0.4	ND	0.4	ND
22:5n-3	0.2	0.7	0.1	2.2
22:6n-3	0.9	2.3	21.8	20.5
Σ n-3 HUFA	2.1	27.7	26.7	35.2
n-3 HUFA in rotifers (dry basis)	0.32	4.65	4.02	7.05

Table 4. Growth, vitality test, survival and incidence of hydrops in larval red seabream after 8 days of feeding

	Tank no.			
	1	2	3	4
Type of oil*	OAME	EPA+OAME	DHA+OAME	ME 85
n-3 HUFA in rotifers (dry basis)	0.32	4.65	4.02	7.05
Total length (mm)				
Average	4.40	4.69	4.98	4.71
SD	0.27	0.32	0.61	0.33
Survival after vitality test (%)				
Test I (5 s)	0	6	63	7
Test II (10 s)	0	17	52	29
Survival rate (%)	32	59	57	53
Hydrops incidence (%)	47	21	7	20

* See the footnote of Table 1.

Growth of Larvae Fed Enriched Rotifers

Results of feeding of larvae on rotifers with various treatments are shown in Table 4 and Fig. 1. As usually observed in larvae fed on yeast-fed rotifers,¹⁴⁾ feeding the EFA-deficient rotifers to larvae resulted in the lowest growth and survival together with a high percentage of fish with the so-called hydrops which was firstly described in larval red seabream by Yamashita.¹⁵⁾ These EFA-deficient larvae were also poor in vitality and all of them died in the both vitality tests. The growth and survival rates were effectively improved by incorporation of EPA and DHA or n-3 HUFA mixture, together with a reduction of the hydrops incidence. However, the occurrence of hydrops was not completely prevented by incorporation of EPA or n-3 HUFA mixture, but almost totally prevented by DHA. The growth and survival rates in the vitality test were also the highest in the larvae fed the DHA-rotifers. The dietary value for larval red seabream of rotifers containing DHA as a main n-3 HUFA was therefore found to be higher than those containing EPA as the main n-3 HUFA. Rotifers fed on n-3 HUFA mixture containing both EPA and DHA contained almost the same level of DHA as the DHA-rotifers, but their dietary value was poorer than that of the DHA-rotifers. This may partly be due to the ill effect of the n-3 HUFA methyl esters on both rotifers and larval red seabream.* The proportion of DHA to EPA may also be important for larval red seabream.

The result that DHA is superior to EPA as EFA for larval red seabream suggests that enrichment

Fig. 1. Relationship between the 18:1n-9/n-3 HUFA ratio in total lipids and growth, survival and incidence of hydrops in larval red seabream.

* M. S. Izquierdo, T. Takeuchi, T. Arakawa, C. Kitajima, and T. Watanabe: Abstracts of the annual meeting of the Japan. Soc. Sci. Fish., Tokyo, April, 1988, p. 250.

Table 5. Lipid classes of total lipids in larval red seabream fed the various enriched rotifers (wet basis) (%)

Tank no.	Crude lipid	in Fish*				
		CE	TG	FFA	FS*	PL
Initial	2.1	0.01	0.03	0.10	0.37	1.57
1	2.4	ND	0.11	0.15	0.13	1.99
2	2.4	ND	0.05	0.07	0.30	1.99
3	2.5	ND	0.05	0.17	0.26	2.10
4	3.5	0.05	0.10	0.31	0.49	2.57

* See the footnote of Table 2. FS; free sterols.

Table 6. Fatty acid composition of total lipids in larval red seabream (area %)

Fatty acid	Initial	Tank no.			
		1	2	3	4
14:0	1.3	2.2	1.8	1.6	4.0
14:1	0.5	0.6	0.4	0.4	0.9
15:0	0.6	0.8	1.1	1.0	2.1
16:0	16.4	13.2	15.3	15.5	17.7
16:1n-7	9.3	8.7	8.0	5.9	7.9
16:1n-5	0.3	0.2	1.6	0.7	0.2
17:0	1.3	1.4	1.9	1.5	1.7
16:3n-6	1.0	1.2	1.0	1.0	0.8
16:4n-3	0.5	0.7	0.8	0.3	0.8
18:0	9.3	5.5	9.5	9.4	7.6
18:1n-9	20.3	30.7	18.9	20.1	17.0
18:1n-7	0.1	ND	ND	ND	ND
18:2n-9	1.7	ND	ND	0.8	ND
18:2n-6	4.8	10.0	6.5	5.2	3.8
18:3n-6	0.6	0.2	0.1	0.2	0.2
18:3n-3	0.2	0.3	0.5	0.4	0.2
18:4n-3	0.1	0.1	0.3	ND	0.2
20:0	0.2	0.1	1.1	0.1	0.2
20:1n-(11+9)	2.1	1.3	1.7	0.1	1.5
20:2n-9	0.5	0.4	0.1	0.2	0.1
20:2n-6	0.4	0.8	0.3	0.6	0.3
20:3n-9	0.3	0.4	0.2	0.3	0.2
20:4n-6	2.7	2.0	5.5	2.0	1.6
20:3n-3	0.2	0.6	0.3	0.2	0.2
20:4n-3	0.4	0.3	1.0	0.3	0.5
20:5n-3	5.2	2.9	9.1	3.1	4.4
22:0	ND	ND	0.1	ND	0.1
22:1n-(13+11)	0.2	ND	1.4	ND	0.1
22:5n-6	0.1	ND	ND	ND	ND
22:4n-3	ND	0.2	ND	ND	ND
22:5n-3	4.0	1.5	2.2	0.9	1.6
22:6n-3	11.0	6.8	5.5	20.4	11.5
Σ n-3 HUFA	20.8	12.2	18.0	25.0	18.2
18:1n-9	0.98	2.52	1.05	0.80	0.93
n-3 HUFA in fish (wet basis)	0.38	0.26	0.39	0.56	0.58

of rotifers with fish oil rich in DHA is very effective for improvement of the dietary value of the *Chlorella*-fed rotifers which are usually high in EPA, but very low in DHA.

Lipid Classes and Fatty Acid Compositions of Larvae

Lipid classes of larvae fed rotifers with various treatments are shown in Table 5. The lipid content of larvae was the highest in those fed the n-3 HUFA-rotifers and the lowest in those fed the EPA-deficient rotifers, clearly reflected by the lipid content of rotifers. The content of polar lipids was also the highest in the former larvae and the lowest in the latter.

The fatty acid distribution of the total lipids of larvae is shown in Table 6. In the larvae fed the EPA-deficient rotifers the percentage of 18:1n-9 was elevated to 30.7% from the initial value of 20.3% during 8 days of feeding and that of EPA and DHA decreased to 2.9% and 6.8% from 5.2% and 11.0%, respectively. The 22:5n-3 level was also reduced from 4.0% to 1.5% in these fish. Consequently, the ratio between 18:1n-9 and n-3 HUFA (one of the EPA indices for juvenile red seabream proposed by Fujii *et al.*³¹⁾) increased to 2.52 from the original value of 0.98. In the fish fed the EPA-rotifers the proportion of EPA increased, but that of DHA decreased to almost one half the initial concentration. The percentage of 22:5n-3 was the highest in the larvae fed on EPA, suggesting the conversion of EPA to 22:5n-3. Feeding the DHA-rotifers resulted in an increase of DHA which was almost double the initial value. The EPA index was the lowest in this group. The level of assimilation of DHA was higher than EPA, although EPA may partly be converted to 22:5n-3 and no retroconversion of DHA may be available. This difference in tissue accumulation between EPA and DHA may partly be responsible for the difference in dietary value between these fatty acids.

This may also be supported by the results obtained in the larvae fed the n-3 HUFA-rotifers. In these larvae the percentage of EPA and DHA was 4.4% and 11.5%, almost the same levels as the original values, in spite of the fact that the rotifers fed to the larvae contained almost the same level of DHA as the DHA-rotifers. The low level of assimilation of EPA and/or DHA in the n-3 HUFA mixture may be attributable to the difference in growth and survival of larval red seabream.

The similar results suggesting that DHA is superior to EPA as EFA has also been reported in juvenile red seabream*¹ and juvenile striped jack.*² The concentration of DHA is usually high in eggs of both species and is quickly decreased during larval development, suggesting that DHA is selectively utilized as energy for tissue development or converted to physiologically important derivatives such as prostaglandins. Further experiments will be necessary to clarify these points and the differences in physiological functions between EPA and DHA in various fish species.

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