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Requirement of Fingerling White Fish *Coregonus lavaretus maraena* for Dietary n-3 Fatty Acids*1

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A feeding experiment was conducted to determine the requirement of fingerling white fish *Coregonus lavaretus maraena* for dietary n-3 fatty acids. Growth, and feed efficiency together with survival rate were lowest in fish receiving a diet containing 5% 18: 1n-9 as a sole lipid source; all parameters were effectively improved by supplementation of linolenic acid (18: 3n-3) or n-3 highly unsaturated fatty acids (n-3 HUFA), and highest in fish fed a diet containing n-3 HUFA greater than 0.5%. The liver lipid content was highest in fish fed the 18: 1n-9 diet, and was effectively reduced by supplementation of n-3 HUFA by more than 1.0%, but not by 18: 3n-3 even at a level of 2%. Dietary 18: 3n-3 was converted to 22: 6n-3, but the percentage of 22: 6n-3 in the liver polar lipid of fish fed 1 and 2% 18: 3n-3 was much lower than that of fish fed 0.25% n-3 HUFA. The data obtained in the present study indicate that n-3 fatty acids are essential for white fish and that the EFA value of n-3 HUFA was much higher than that of 18: 3n-3. The requirement of fingerling white fish for dietary n-3 HUFA was found to be around 1%.

In the previous study,[1] a feeding experiment was conducted to determine the effect of dietary n-6 and n-3 fatty acids on growth, fatty acid composition and histological change of fingerling white fish *Coregonus lavaretus maraena*. Growth and feed efficiency together with survival rate were lowest in fish fed the diet containing methyl oleate (18: 1n-9) as a sole lipid source, and the liver of these fish showed abnormal conditions in histological examination. The growth performance and histological abnormalities were effectively improved by supplementation of n-3 fatty acids, such as methyl linolenate (18: 3n-3) and n-3 highly unsaturated fatty acids (n-3 HUFA), but not by methyl linoleate (18: 2n-6).

Following the previous experiment the current study was designed to determine the requirement of white fish for n-3 fatty acids.

This paper deals with these results.

Materials and Methods

The basal diet used for the present study was the same as that used in the previous study.[1] Namely, the diet contained the following ingredients: 52% casein; 15% α-starch; 15% dextrin; 4.07% cellulose; 5% mineral mix.; 1% vitamin mix.; 0.5% choline chloride; 0.06% α-tocopheryl acetate; 2.47% amino acid mix. and 5% methyl ester of oleic acid. This diet was already shown to induce EFA deficiencies in white fish in the previous study.[1] Dietary treatments in the present study are shown in Table 1. In the experimental diets, 1–2% of 18: 1n-9 in the basal diet was replaced by the same amount of 18: 3n-3 (1 and 2%) and n-3 HUFA (0.25–1.5%). Diet 8 contained 4.5% methyl laurate (12: 0) in place of 18: 1n-9 in diet 5 and 0.5% n-3 HUFA to compare the difference between 18: 1n-9 and 12: 0 as a basal lipid. The purity of 12: 0 obtained from Tokyo Chemical Industry Co. Ltd. was 99%. The other fatty acid methyl esters were the same as those reported in the previous paper.[1]

Fingerlings of white fish *Coregonus lavaretus maraena* were fed the basal diet for two weeks before the initiation of the experiment in order to accustom the fish to casein diets. They were then divided into 8 lots of 30 fish each (1.8 g mean weight) and fed the experimental diets for 9 weeks at a water temperature of 14–18°C. Other details of fish care and feeding were all described previously.[2]

To study the relationship between the fatty acids in the diet and those deposited in fish liver, fatty

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Table 1. Composition of dietary lipids

Diet no.	Dietary lipid*
1	5.0% 18:1n-9
2	4.0% 18:1n-9+1.0% 18:3n-3
3	3.0% 18:1n-9+2.0% 18:3n-3
4	4.75% 18:1n-9+0.25% n-3 HUFA
5	4.5% 18:1n-9+0.5% n-3 HUFA
6	4.0% 18:1n-9+1.0% n-3 HUFA
7	3.5% 18:1n-9+1.5% n-3 HUFA
8	4.5% 12:0+0.5% n-3 HUFA

* Fatty acids used are all methyl ester forms.

acid compositions were determined in the livers from fish at the beginning and those at the end of the feeding experiment. The procedures for lipid analyses including GLC operating conditions for determination of fatty acids were all reported in the previous paper.[3]

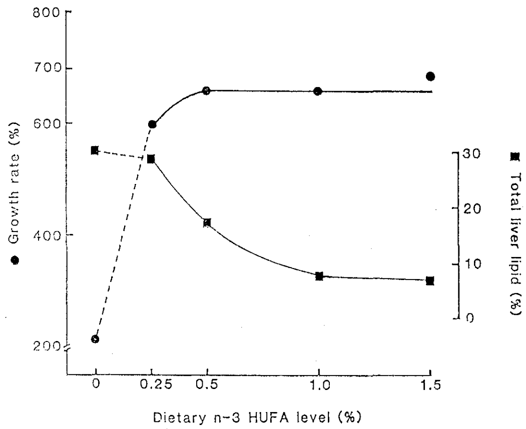

Fig. 1. Effect of dietray n-3 HUFA in the growth rate and total liver lipid of white fish.

Results and Discussion

Growth

Results of the 9 week feeding experiment are shown in Table 2 and Fig. 1. Effects of dietary fatty acids on growth of fish became marked from around the 6th week of feeding. The growth and feed efficiency were lowest in the fish fed the EFA-deficient diet containing 18:1n-9 as a sole lipid source and were effectively improved by supplementation of n-3 fatty acids. The growth of fish reached a plateau with n-3 HUFA supplementation at levels more than 0.5%. When growth was compared between fish receiving diets supplemented with 18:3n-3 and n-3 HUFA at the same level, it was higher in fish fed a diet with n-3 HUFA than those fed 18:3n-3, as observed in the previous experiment and in rainbow trout.[4,5] There was no difference in the growth between the fish fed a diet containing 18:1n-9 and 12:0 as a basal lipid source. Survival rate was also low in the fish fed the EFA-deficient diet and was effectively improved by addition of n-3 HUFA at a level more than 0.5%.

Lipid Classes in Liver

The effect of dietary fatty acids on total lipid (TL) content in the liver and their classes are shown in Table 3. The liver TL content was as high as about 40% in the initial trials with fish fed the basal diet (EFA-deficient) for two weeks before the experiments. The TL content at the end of the experiment was also highest in fish fed a diet containing 18:1n-9 as a sole lipid source, and was not reduced by feeding with 1% 18:3n-3 and 0.25% n-3 HUFA, but was effectively reduced by n-3 HUFA at more than the 1% level in the diet (Fig. 1). The polar lipid (PL) content in the liver was found to be relatively constant and the nonpolar lipid (NL) in terms of triglyceride (TG) reflected the TL content as usually observed in other species like rainbow trout,[6-8] carp,[9,10] *Tilapia*[11,12] and channel catfish.[13,14]

Table 2. Results of the 9-week feeding experiment

Diet no.	Av. body wt. (g) Initial	Av. body wt. (g) Final	Growth rate (%)	Feed* efficiency	Hepato-somatic index	Survival rate (%)
1	1.9	5.9	211	0.76	1.7	75
2	1.8	12.0	567	1.15	1.4	90
3	1.8	12.8	611	1.25	1.4	93
4	1.8	12.6	600	1.20	1.5	97
5	1.8	13.7	661	1.23	1.3	100
6	1.8	13.7	661	1.24	1.2	100
7	1.8	14.2	689	1.26	1.2	100
8	1.8	13.7	661	1.30	1.6	100

* g gain/g feed.

Table 3. Effect of dietary lipids on lipid content of the liver and their classes*

Diet no.	% in liver					
	TL	PL	NL	TG	FFA	Others
Initial	39.8	1.6	38.2	37.0	0.6	0.6
1	29.6	1.7	27.9	26.8	0.4	0.7
2	29.6	1.9	27.7	26.5	0.5	0.7
3	20.5	1.9	18.6	17.9	0.4	0.3
4	28.4	2.1	26.3	25.6	0.3	0.4
5	17.0	2.2	14.8	13.2	0.6	1.0
6	7.9	2.3	5.6	4.2	0.7	0.7
7	7.2	2.2	5.0	3.5	0.8	0.7
8	25.8	1.9	23.9	22.6	0.6	0.7

* Abbreviations: TL, total lipids; PL, polar lipids; NL, nonpolar lipids; TG, triglycerides; FFA, free fatty acids; Others, free sterols, diglycerides, monoglycerides.

Table 4. Fatty acid composition of nonpolar lipid fractions from the liver (Area %)

Fatty acid	Initial	Diet no.							
		1	2	3	4	5	6	7	8
16:0	15.8	9.1	12.0	13.0	12.4	15.1	13.1	15.2	15.4
16:1n-7	23.5	19.6	17.0	17.7	15.7	16.5	14.7	16.9	19.6
18:0	2.0	0.6	1.7	1.9	2.0	2.3	2.4	2.4	2.5
18:1n-(7+9)	49.0	57.8	60.2	57.8	61.2	58.1	59.2	54.0	54.1
18:2n-6	0.2	0.1	—	—	0.1	0.1	0.1	0.2	0.1
18:3n-3	—	—	0.3	0.7	—	—	—	—	—
18:4n-3	0.1	0.1	0.6	1.0	0.1	0.1	0.1	0.1	0.1
20:1n-9	2.1	1.7	1.8	1.7	2.3	1.8	1.7	1.5	1.7
20:3n-9	0.1	0.1	tr	—	—	tr	0.1	0.1	tr
20:4n-6	—	0.1	tr	—	—	—	0.2	0.3	tr
20:5n-3	0.1	—	0.1	0.2	tr	0.1	1.1	1.6	0.1
22:1n-(11+13)	0.2	0.3	0.3	0.3	0.3	0.2	0.2	0.1	0.2
22:5n-3	0.1	—	0.1	0.2	0.1	0.3	1.3	1.5	0.2
22:6n-3	0.1	0.2	0.3	0.3	0.2	0.1	0.2	0.1	0.1
Σn-3	0.4	0.3	1.4	2.4	0.4	0.6	2.7	3.3	0.5
Σn-6	0.2	0.2	—	—	0.1	0.1	0.3	0.5	0.1
Σn-3 HUFA	0.3	0.2	0.5	0.7	0.3	0.5	2.6	3.2	0.4

Fatty Acid Composition in Polar and Nonpolar Lipid Fractions in Liver

The fatty acids of nonpolar lipid (NL) fractions from the liver are shown in Table 4. Palmitic acid (16:0), palmitoleic acid (16:1n-7) and 18:1n-9 were the main components in NL fraction. The 16:1n-7 content decreased in all groups in comparison with that of the initial value and the 18:1n-9 content increased with feeding of the 18:1n-9 diet. However, the fatty acid profile of NL from the fish fed the 12:0 based diet, was not so different from the other groups fed 18:1n-9 and there was no accumulation of 12:0 in this group.

The effects of dietary n-3 fatty acids on fatty acid composition of the liver PL are shown in Table 5. The percentage of 20:3n-9 was higher in the fish fed the basal diet and the fish fed the EFA-deficient diet and was decreased by supplementation of n-3 fatty acids, but became lowest in fish fed a diet containing n-3 HUFA greater than 1%. Dietary 18:3n-3 was converted to 20:5n-3 and 22:6n-3. The content of 22:6n-3 was lowest in the fish on the 18:1n-9 diet and increased by supplementation of n-3 fatty acids to the diet. The 22:6n-3 content reached almost a plateau by adding n-3 HUFA at a level more than 0.5%. However, the concentration of 22:6n-3 in the fish fed the diet containing 1–2% 18:3n-3 was lower than that reported for other freshwater fish species fed similar diets.[6–10,13,14] The results of fatty acid analysis have suggested that the ability of chain elongation and desaturation from C 18 to C 20 to 22 in white fish is lower than that of the other freshwater fish.

Table 5. Fatty acid composition of polar lipid fractions from the liver (Area %)

Fatty acid	Initial	Diet no. 1	2	3	4	5	6	7	8
16:0	19.0	15.0	18.3	17.4	18.3	19.2	24.1	20.4	20.2
16:1n-7	10.3	8.9	6.5	8.4	8.3	5.8	5.7	5.0	7.8
18:0	2.5	1.5	1.6	1.7	1.4	1.7	2.0	2.0	2.0
18:1n-(7+9)	33.2	46.2	39.7	37.2	41.3	33.8	28.6	26.4	31.3
18:3n-3	tr	tr	1.4	2.2	tr	tr	tr	tr	—
18:4n-3	0.1	0.1	1.5	1.9	0.1	tr	tr	tr	—
20:1n-9	1.7	1.3	1.0	1.0	1.5	1.5	1.0	1.5	0.9
20:1n-7	0.5	0.1	0.1	0.2	0.4	0.2	tr	0.1	0.3
20:2n-9	0.9	1.7	1.0	0.5	1.2	0.8	0.3	0.2	0.9
20:2n-6	0.7	0.4	0.3	0.2	0.3	0.2	0.2	0.2	0.3
20:3n-9	5.5	7.0	1.3	0.7	1.9	1.0	0.3	0.2	1.3
20:3n-6	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
20:4n-6	0.6	0.4	0.2	0.1	0.4	0.7	0.9	1.0	0.5
20:4n-3	0.2	0.4	1.5	1.5	0.1	0.1	0.1	0.1	0.1
20:5n-3	3.2	1.1	5.5	7.4	1.8	4.4	7.6	9.4	2.3
22:1n-(11+13)	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.2	0.2
22:4n-6	0.4	0.3	0.1	0.1	0.3	0.3	0.1	0.3	0.3
22:5n-6	0.1	0.1	—	0.1	0.2	0.2	0.2	0.4	—
22:5n-3	0.9	0.2	1.1	1.4	0.4	0.9	1.3	1.9	0.7
22:6n-3	11.3	3.4	12.2	12.5	14.9	24.0	23.6	27.9	24.0
Σn-3 HUFA	15.6	5.1	20.4	23.0	17.3	29.5	32.6	39.3	27.1
20:3n-9 / 22:6n-3	0.5	2.1	0.1	0.1	0.1	tr	tr	tr	0.1

The ratio of 20:3n-9 to 22:6n-3 was highest in fish fed the 18:1n-9 diet, and was markedly reduced by supplementation of n-3 fatty acids. The ratio of 20:3n-9 to 22:6n-3 in liver PL is frequently used as an EFA index for freshwater species such as rainbow trout,[6-8] common carp,[10] channel catfish[13,14] and chum salmon.[15] However, it seemed very difficult to use the ratio as an EFA index for white fish, because the ratio became tremendously low in the fish showing good growth and a low liver TL content.

The results obtained in this experiment have shown that n-3 fatty acids enhance the growth of white fish. Growth rate, feed efficiency and survival of fish fed a diet containing 0.25% n-3 HUFA were better than those of the fish fed 1% 18:3n-3. Furthermore, the liver TL content of the fish fed 2% 18:3n-3 was higher than that of the fish fed 0.5% n-3 HUFA. Supplementation of n-3 HUFA above 1% resulted in a low lipid content, which is close to the lipid content in liver of wild white fish reported by Gunstone *et al.*[16] Supplementation of 2% 18:3n-3 could not reduce the liver lipid content to the level of the wild white fish. These data suggest that the EFA value of n-3 HUFA for white fish may be about four times higher than that of 18:3n-3, judging from the growth and the liver lipid content. In rainbow trout n-3 HUFA such as 20:5n-3 and 22:6n-3 are reported to have an EFA efficiency twice as high as 18:3n-3.[4]

Thus, white fish were found to require n-3 HUFA as EFA and the requirement is around 1% in diet. Fish oil, such as pollock liver oil and cuttlefish liver oil rich in n-3 HUFA, may be suitable as a lipid source for practical white fish feeds.

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