海水魚とニジマスにおけるリノレン酸からω3高度不飽和酸への転換

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Conversion of Linolenic Acid to ω3-highly Unsaturated Fatty Acids in Marine Fishes and Rainbow Trout*1

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[1-^{14}C] 18: 3 ω3 was administered to red sea bream, black sea bream, opaleye, stripped mullet, and rainbow trout, after 3 months of pre-feeding with a diet containing pollack liver oil. On the 6th day after administration, the distribution of radioactivity in the tissue fatty acids was examined. The radioactivity of 22: 6 ω3 fraction exhibited 14.5% and 3.6% of total activity in rainbow trout, but 4.9%~0.9% in marine fishes.

From this finding, it is supposed that the slow conversion of 18: 3 ω3 to 22: 6 ω3 which is an essential fatty acid, in marine fishes is a cause of the difference observed in the essential role of 18: 3 ω3 between marine fishes and rainbow trout.

Linolenic acid (18: 3 ω3) is essential in nutrition of freshwater fishes such as rainbow trout, *Salmo gairdneri*[1–6], carp, *Cyprinus carpio*[7], and eel, *Anguilla japonica*[8]. On the other hand, YONE *et al.* demonstrated that 18: 3 ω3 is not so much important *per se* for the nutrition of marine fishes such as red sea bream, *Chrysophrys major*[9–12], black sea bream, *Mylio macrocephalus*[13], opaleye, *Girella nigricans*[13], and yellowtail, *Seriola quinqueradiata*[13] as for that of freshwater fishes, and that ω3-highly unsaturated fatty acids with more than 20 carbon atoms (HUFA) play an essential role in nutrition of these marine fishes.

A reason for the difference observed in the essential role of 18: 3 ω3 between marine and freshwater fishes can be presumed from changes in fatty acid composition noted during a long period feeding with a 18: 3 ω3 supplemented diet. In rainbow trout and carp, the percentage of HUFA in body lipid increased by the feeding of 18:3 ω3, but did not in plaice[14], *Pleuronectes platessa,* and red sea bream[12]. From these findings, FUJII *et al.*[12] presumed that marine fishes possess lower abilities to convert 18: 3 ω3 to HUFA when compared with freshwater fishes.

Recently, this presumption was confirmed by OWEN *et al.*[15]. ^{14}C-labelled 18: 3 ω3 was orally administered to turbot, *Scophthalmus maximus,* and rainbow trout, after pre-feeding with a fat-free diet for 16 and 6 weeks respectively. Six days after the administration, it was found that about 83% of 18: 3 ω3 was converted in rainbow trout to longer chain fatty acids, mostly to docosahexaenoic acid (22: 6 ω3), as compared to only 15% in turbot.

In the present paper, therefore, the conversion of 18: 3 ω3 to HUFA in red sea bream, black sea bream, opaleye, and striped mullet, *Mugil cephalus,* were compared with that of rainbow trout, using the same method employed by OWEN *et al.*[15], except pre-feeding was with a diet containing pollack liver oil. The duplicate experiments were carried out at the different temperatures.

Materials and Methods

Hatchery reared rainbow trout and black sea bream were obtained from a trout farm and Aquaculture Research Laboratory, Nagasaki Prefectural Institute of Fisheries, respectively. Wild red sea bream, opaleye, and striped mullet were also used. Five fish having comparable weight were maintained in an aquarium (40×40×40 cm) in respective species. The initial average body weight, fork length, and lipid content of each fish group are shown in Table 1. Water filtered through sand bed was poured into each aquarium at a rate of 90 *l* per hour and air was supplied sufficiently.

After 3 months pre-feeding with a compounded feed containing pollack liver oil at a 5% level, a

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Table 1. Body weight, fork length, and lipid content of test fish at beginning of experiment

	Species	Body weight (g)	Fork length (cm)	Lipid content(%)
Exp.–1	Black sea bream	6.9±1.3	6.9±0.4	3.4±1.3
	Opaleye	6.0±0.8	6.7±0.2	5.7±1.3
	Striped mullet	5.3±0.3	7.4±0.2	3.0±0.8
	Rainbow trout	13.6	10.1	6.7
Exp.–2	Red sea bream	11.9±1.6	8.0±0.4	3.8±0.8
	Black sea brean	5.8±0.6	6.5±0.2	4.3±4.2
	Opaleye	6.7	6.9	5.4
	Striped mullet	5.1±1.5	7.2±0.4	3.2±1.6
	Rainbow trout	13.9±6.1	10.1±0.4	4.5±1.1

compounded feed containing about 40 μCi [1-^{14}C] linolenic acid (specific activity, 60 mCi/mmol: from the Radiochemical Center, Amersham, England) per gram was fed at 25 mg-feed per gram body weight once to each fish group.

The radioactive feed was prepared by soaking a compounded feed in the benzene solution of [1-^{14}C] linolenic acid, followed by evaporating the solvent under reduced pressure of nitrogen stream. After administration of the radioactive feed, fish were fed on the same diet as used in the pre-feeding for 6 days, and then the total lipid of whole body was extracted by the method of BLIGH and DYER[16]. Aliquots of the total lipid extracted from each fish, which have the same radioactivity, were mixed thoroughly in respective species, and the mixed lipid was transesterified with a boron-trifluoride in anhydrous methanol.

The radioactivity of ^{14}C incorporated into fatty acids was measured by the method devised by HAMMARSTAND *et al.*[17]. The separation of fatty acids was carried out by gas-liquid chromatography under the condition shown in Table 2. The column effluent was divided 1: 10 by a splitter and led to the flame ionization detector (FID) and to the outlet adaptor with a hypodermic needle, upon which a siliconized cigarette filter rod was impaled, respectively. The filter rod was replaced with a new one when a peak disappeared on the FID chromatogram, and then was placed in scintillation solution (0.5% 2,5-diphenyloxazole and 0.05% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene). The radioactivity was counted with an Intertechnique SL-32 Liquid Scintillation Spectrometer. Peaks on the chart were identified by comparison with the retention times of known standards.

Table 2. Condition of gas-liquid chromatography

Apparatus:	Shimazu gas chromatograph GC-4BMPF equipped with a hydrogen flame ionization detector and a needle collector	
Column:	Glass tube 2.0 m×3 mmϕ	
Packing:	10% DEGS on 80/100 mesh Chromosorb W(AW)	
Carrier gas:	Nitrogen	
Flow rate:	Nitrogen FID side	5.0 ml/min;
	Collector side	50 ml/min
	Hydrogen	0.65 kg/cm^2
	Air	0.9 kg/cm^2
Temperature:	Detector	220°C
	Column	185°C

Results and Discussion

In all species, a small proportion of radioactivity was found in shorter chain length and lower unsaturated fatty acids (14: 0, 16: 0, 16: 1, 18: 0, 18: 1) than linolenic acid, as shown in Tables 3 and 4. These fatty acids might be biosynthesized through acetyl-CoA resulting from β-oxidation of 18: 3 ω3. In rainbow trout, 39~60% of the total radioactivity counted was found

Table 3. Distribution of ^{14}C into fatty acids in total lipid of marine fishes and rainbow trout fed [1-^{14}C] linolenic acid (Experiment 1).

Values are expressed as percent of the total radioactivity of tissue lipid.

Fatty acid	Black sea bream	Opaleye	Striped mullet	Rainbow trout
14: 0	0.2	0.1	0.5	3.0
16: 0 16: 1	0.5	0.4	0.5	1.4
18: 0 18: 1	0.2	0.4	0.2	3.4
18: 3ω3	82.2	79.9	83.4	38.8
18: 4ω3*1	8.3	7.7	6.9	3.1
20: 3ω3*2	3.0	0.9	2.4	10.6
20: 4ω3	0.4	tr	0.3	4.3
20: 5ω3	0.1	0.7	1.1	2.6
22: 5ω3*3	tr	0.7	0.2	7.9
22: 6ω3	1.1	4.9	1.6	14.5

*1 18: 3 ω3 may be contained, *2 20: 4 ω3 may be contained, *3 22: 6 ω3 may be contained.

Water temperature: sea water 23.3–27.0°C, fresh water 24.4–26.1°C.

Table 4. Distribution of ^{14}C into fatty acids in total lipid of marine fishes and rainbow trout fed [1-^{14}C] linolenic acid (Experiment 2).

Values are expressed as percent of the total radioactivity of tissue lipid.

Fatty acid	Red sea bream	Black sea bream	Opal-eye	Striped mullet	Rainbow trout
14: 0	0.5	0.9	0.5	0.7	1.0
16: 0 + 16: 1	0.7	0.4	0.5	1.0	1.0
18: 0 + 18: 1	0.3	0.1	0.2	0.3	3.0
18: 3ω3	88.6	85.2	91.2	82.4	60.9
18: 4ω3*1	1.9	1.4	1.8	2.6	8.5
20: 3ω3*2	5.4	5.3	3.2	8.4	8.2
20: 4ω3	0.2	0.2	0.1	0.2	2.7
20: 5ω3	0.8	1.6	0.5	1.2	5.4
22: 5ω3*3	tr	0.4	0.1	0.3	4.2
22: 6ω3	0.9	3.0	1.5	1.2	3.6

*1 18: 3 ω3 may be contained, *2 20: 4 ω3 may be contained, *3 22: 6 ω3 may be contained.

Water temperature: sea water 24.4–25.6°C, fresh water 12.5–13.6°C.

in 18: 3 ω3 fraction, but 80~90% in marine fishes. The radioactivity of 22: 6 ω3 fraction exhibited 14.5 and 3.6% of total activity in rainbow trout, but 4.9~0.9% in marine fishes. OWEN *et al.*[15], however, demonstrated that when [1-^{14}C]linolenic acid was fed to rainbow trout about 70% of radioactivity was found in 22: 6 ω3 fraction, but in turbot it was scarcely converted to 22: 6 ω3. On the other hand, BRENNER[18] supposed that 22:6 ω3 inhibits the further desaturation of 18: 3 ω3 in terrestrial animals. It appears that BRENNER's supposition is available in explanation of the lower conversion of 18: 3 ω3 to 22: 6 ω3 in this experiment than that reported by OWEN *et al.*[15]. OWEN *et al.* used fish in which HUFA was perhaps scarcely present after a long period of pre-feeding with a fat-free diet, whereas we employed fish rich in HUFA resulting from the pre-feeding with pollack liver oil. Therefore, it is presumed that the 22: 6 ω3 fraction showed lower radioactivity in rainbow trout than that obtained by OWEN *et al.*

OWEN *et al.*[15] also demonstrated that turbot elongated 18: 3 ω3 by addition of 2 carbon atoms, but did not desaturate it. In our experiment, however, some desaturation also occurred in the test marine fishes.

From these findings, it is presumed that a conversion pathway from 18: 3 ω3 to HUFA is present in marine fishes such as red sea bream, black sea bream, opaleye, and striped mullet, but the conversion is slow in these fish as compared with that in rainbow trout, and the requirement of 22: 6 ω3 which is an essential fatty acid for marine fishes may not be satisfied.

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