Effect of Dietary Linolenic Acid and Docosahexaenoic Acid on Growth and Fatty Acid Composition of Rainbow Trout *(Salmo gairdneri)*[1,2]

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ABSTRACT

Methyl linolenate 18:3ω3 and docosahexaenoate 22:6ω3 were incorporated in semipurified diets at several levels and fed to trout previously maintained on a fat-free diet. After 14 weeks, the weight gain and feed conversion of the fish on each diet were determined. The fatty acid composition of the lipid from each group of fish was analyzed by gas liquid chromatography. Both 18:3ω3 and 22:6ω3 fed at the 1% level supported maximum growth of the fish. The control group, which were fed no ω3 fatty acids, exhibited a shock syndrome, poor appetite and a very slow growth rate. Tissue fatty acid analysis revealed eicosatrienoic acid 20:3ω9 accumulated in the phospholipid fraction of this group. The 20:3ω9 level was lowered when either 18:3ω3 or 22:6ω3 was included in the diet. Analysis showed that the dietary 18:3ω3 was rapidly converted by the fish into 22:6ω3 with a high concentration in the phospholipid. However 22:6ω3 fed to the fish remained unchanged and little or no retroconversion of this fatty acid was observed.

[1]Presented in part at the AOCS Meeting, Atlantic City, October 1971.

[2]Technical paper no. 3247, Oregon Agricultural Experiment Station.

TABLE I

Composition of Trout Diets

Component	Weight %
Casein	53.9
Gelatin	9.7
Dextrin	17.1
Lipid[a]	2.0
Mineral mix[b]	4.0
Carboxymethylcellulose	1.4
Alpha-cellulose (Alphacel-NBC)	8.7
Vitamin mix[c]	2.0
Choline chloride	1.0
Vitamin E concentrate (330 IU/g as dl-α-tocopheryl acetate)	0.2

[a]Lipid composition listed in Table II.

[b]Bernhart-Tomerelli Salt Mix, modified by adding NaF and $CaCl_2$ at 0.002 and 0.02%, respectively.

[c]Supplied vitamins at following levels: (mg/kg) thiamine, 64; riboflavin, 144; niacinamide, 512; biotin, 1.6; Ca D-pantothenate, 288; pyridoxine, 48; folic acid, 19.2; menadione, 16; cobalamine, 0.159; i-inositol (meso-), 2500; ascorbic, 1200; and *p*-aminobenzoic acid, 400. Vitamins A and D were added at 25,000 and 4000 IU/kg, respectively.

INTRODUCTION

Recent studies have demonstrated that fatty acids of the linolenic family (ω3) are essential for rainbow trout, whereas acids of the linoleic family (ω6) do not seem to be required. Lee and coworkers (1) reported that corn oil in a semipurified diet led to poor growth and high mortality of rainbow trout. Replacing the corn oil with fish oil greatly stimulated growth and prevented further mortalities. Castell and coworkers (2,3) demonstrated that trout grew poorly on a diet containing 1% linoleic acid as

TABLE II

Effect of Dietary Lipids on Feed Efficiency and Mortality of Rainbow Trout

Diet no.	Lipid composition, % 12:0	18:3ω3	22:6ω3	Feed efficiency,[a] gain/feed	Accumulated mortality
1	2.00	0	0	0.32	17
2	1.75	0	0.25	0.80	0
3	1.50	0	0.50	0.88	1
4	1.00	0	1.00	0.91	1
5	1.50	0.50	0	0.84	3
6	1.00	1.00	0	0.90	1

[a]Feed efficiency is defined as units of weight gained per unit of dry feed consumed.

the only unsaturated fatty acid. The fish developed a shock syndrome, excessive liver mitochondrial swelling and other symptoms. On the other hand, Castell reported excellent growth of the fish with a diet containing 1% linolenic acid and recommended this level be included in diets for rainbow trout. Their results supported the findings of Richardson et al. (4), Brockerhoff and Hoyle (5), Brenner et al. (6) and Higashi et al. (7) that linolenic acid was nutritionally more important for fish than linoleic acid.

So far only linolenic acid has been used to study the $\omega 3$ fatty acid requirements of fish. The objective of this experiment was to isolate the long chain highly unsaturated docosahexaenoic acid 22:6$\omega 3$ from fish oil and to compare the effect of this fatty acid with that of linolenic acid on growth and fatty acid composition of rainbow trout.

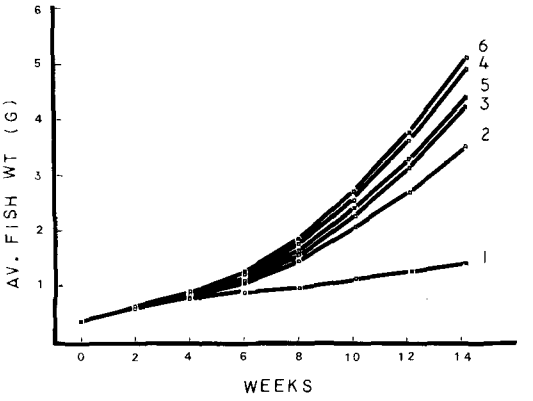

FIG. 1. Effect of dietary fatty acids on growth rate of rainbow trout. Curve 1, control trout diet containing no $\omega 3$ fatty acid. Curves 2, 3 and 4, diets containing 0.25, 0.5 and 1.0% 22:6$\omega 3$, respectively. Curves 5 and 6, diets containing 0.5 and 1.0% linolenic acid, respectively.

MATERIALS AND METHODS

Methyl linolenate 18:3$\omega 3$ and methyl laurate 12:0, both 99% pure, were obtained from the Hormel Institute. The source of docosahexaenoic acid 22:6$\omega 3$ was Pacific Coho salmon roe which had been freeze dried and the oil extracted with chloroform-methanol 2:1. The egg oil was converted to fatty acid methyl esters by use of sodium methoxide (8). The resulting esters were dissolved in acetone and subjected to low temperature crystallization at -80 C. After removal of the precipitated esters, the 22:6$\omega 3$ content increased from 21.0% to 30.6% as estimated by gas liquid chromatography (GLC). The 22:6$\omega 3$ was isolated from the ester mixture by argentation chromatography using a column packed with silicic acid containing 20% $AgNO_3$ (9). The elution solvents were similar to those described by Stein and Slawson (10) with the addition of equal parts of the antioxidants, butylated hydroxyanisole and butylated hydroxytoluene (0.001%) to prevent autoxidation of the unsaturated esters. The saturated and monounsaturated esters were eluted with diethyl ether-pentane 1:1 and more highly unsaturated esters were eluted with diethyl ether-pentane-cyclohexane 5:3:2. Finally the ester of 22:6$\omega 3$ was eluted with diethyl ether-cyclohexane 1:1. Fractions containing high percentages of 22:6$\omega 3$ were pooled. The final product, as analyzed by GLC, contained 97.0% 22:6$\omega 3$; 2.5% 20:5$\omega 3$, and was free from 18:3$\omega 3$ and $\omega 6$ fatty acids. The isolated 22:6$\omega 3$ was finally put through a silicic acid column to remove the antioxidants (11).

Rainbow trout *(Salmo gairdneri)* was used as the experimental animal. Eggs from the brood stock of this laboratory were hatched and the fry fed a fat-free diet for one month and then randomly divided into lots of 50 fish. Each experimental diet was fed to duplicate lots of fish. The composition of the diets is shown in Table I. The casein, gelatin, dextrin and cellulose used in the diet had been extracted with warm isopropanol to remove trace lipids. The

TABLE III

Effect of Dietary Lipids on Growth of Rainbow Trout

	Average fish wt (g) in weeks[a]							
Diet	0	2	4	6	8	10	12	14
1	0.53	0.76	0.97	0.97	1.03	1.21	1.35	1.56
2	0.53	0.85	0.90	1.23	1.56	2.15	2.73	3.58
3	0.50	0.78	0.92	1.19	1.63	2.39	3.23	4.32
4	0.51	0.76	0.98	1.37	1.84	2.62	3.70	5.00
5	0.53	0.79	0.94	1.28	1.75	2.51	3.37	4.47
6	0.52	0.84	1.00	1.39	1.93	2.84	3.88	5.20

[a]Average of duplicate tanks.

TABLE IV

Percentage Fatty Acid Composition of Phospholipids[a]

Fatty acids	Diet						
	Fat-free, 1 month	1	2	3	4	5	6
12:0	—	0.5	0.5	0.6	0.4	0.6	0.5
14:0	0.9	2.4	2.2	1.8	1.7	2.2	2.0
16:0	14.2	16.1	15.9	16.8	18.1	11.7	16.6
16:1ω7	9.7	14.1	12.7	11.9	10.5	12.1	10.5
18:0	7.2	5.1	4.6	5.3	5.7	4.8	5.0
18:1ω9	30.8	35.8	32.7	29.1	27.2	31.9	29.2
18:2ω6[b]	3.2	4.3	3.2	2.7	2.3	2.8	2.1
18:3ω3	—	—	—	—	—	1.6	3.8
20:1ω11[b]	3.5	2.9	2.6	2.5	1.8	2.3	2.0
18:4ω3	—	—	—	—	—	1.1	2.3
20:2ω9	2.4	2.5	2.5	2.3	2.1	2.4	1.4
20:3ω9	4.2	8.6	5.9	4.8	3.0	3.6	1.5
20:4ω6	1.1	1.2	0.6	0.5	Trace	0.8	0.6
20:4ω3	—	—	—	—	—	—	0.6
20:5ω3	Trace	—	Trace	0.9	0.8	1.7	1.5
22:5ω6	Trace	0.6	—	—	—	—	—
22:5ω3	Trace	—	—	—	—	Trace	Trace
22:6ω3	23.0	5.7	16.8	21.0	26.7	16.1	20.9

[a]Average of analysis of duplicate tanks.
[b]Other isomers may be present.

quantity of the lipids in the diets was adjusted to 2% by varying the amount of the added 12:0 and all diets were isocaloric. The lipids added to each diet are listed in Table II.

The fish were held in 75 liter fiberglass tanks. The water temperature was 11.5 C, and flow rate was ca. 8 liters/min. The fish were fed three times daily. Food was offered only as long as the fish continued to feed.

Feed consumption and mortality were recorded. The fish were weighed every 2 weeks and the experiment was terminated at the end of 14 weeks.

The fatty acid composition of the pooled samples of each lot was determined. The lipid was extracted from the fish by the method of Folch et al. (12) and further separated into phospholipid and neutral lipid fractions as described previously (1). Methyl esters were prepared from these lipids by transesterification with boron-trifluoride in anhydrous methanol (13). Separation and identification of the component fatty acids was carried out as described by Lee et al. (1).

RESULTS AND DISCUSSION

The growth rate was extremely slow for the group of fish receiving diet 1 (see Table I) which was devoid of ω3 fatty acids. The fish also exhibited poor appetite and a shock syndrome as described by Sinnhuber (14). Addition of ω3 fatty acids, even at a low level (diet 2), to the diet vastly improved the condition of the fish and increased the growth rate (Fig. 1). None of the fish on supplemental diets exhibited the shock syndrome.

The accumulated mortality was high in the group of fish on diet 1, without ω3 acids. Incorporation of ω3 fatty acids in the diets effectively lowered the mortality (Table II). The feed efficiency also increased with increasing levels of ω3 fatty acids as shown in Table II. These results indicated that trout responded equally well to dietary 18:3ω3 and 22:6ω3.

The growth of fish on diet 6, containing 1.0% 18:3ω3, was similar to that reported by Castell et al. (2,3). Their results showed that the growth rate of trout approached a maximum when 1% 18:3ω3 was added in the diet. The growth rate was considerably lower if only 0.5% of either 22:6 or 18:3 was incorporated in the diet (Table III).

The fatty acid composition of phospholipids and of neutral lipids is shown in Tables IV and V. The lipid extracted from the whole fish, after receiving a fat-free diet for one month was 1.14% and showed a high percentage of 22:6ω3 (23.0%) in the phospholipid fraction. The neutral lipid fraction contained ca. 10.0% 22:6ω3. These values represent the carry-over from the egg. Calculation of the total amount of 22:6ω3 remaining in the fish after 14 weeks on diet 1 showed that fish had conserved ca. 70% of the original 22:6ω3.

TABLE V

Percentage Fatty Acid Composition of Neutral Lipids[a]

Fatty acids	Diet						
	Fat-free, 1 month	1	2	3	4	5	6
12:0	—	4.9	9.4	9.1	5.2	7.5	5.5
14:0	2.6	4.8	4.1	3.3	2.9	3.4	3.0
16:0	15.8	14.7	14.6	15.9	16.5	16.0	17.5
16:1ω7	11.5	13.6	12.7	13.2	14.5	13.4	12.6
18:0	4.6	4.9	4.6	6.3	4.2	5.0	4.2
18:1ω9	42.6	48.6	43.5	38.0	41.0	40.7	37.4
18:2ω6[b]	3.6	2.1	1.8	2.9	2.3	1.8	1.6
18:3ω3	—	—	—	—	Trace	2.5	8.5
20:1ω11[b]	3.3	3.8	3.8	3.3	2.5	2.9	2.8
18:4ω3	—	—	—	—	—	0.7	1.5
20:2ω9	1.2	1.8	1.8	2.0	2.1	1.5	1.4
20:3ω9	1.8	1.5	1.2	1.2	1.0	0.8	1.2
20:4ω6	1.4	—	—	—	—	—	—
20:5ω3	1.9	—	—	Trace	1.0	—	—
22:5ω3	Trace	—	—	—	—	—	—
22:6ω3	10.0	—	2.9	5.2	7.1	4.0	3.9

[a]Average of analysis of duplicate tanks.
[b]Other isomers may be present.

The acid 20:3ω9 was biosynthesized and found at high concentration in the phospholipids of fish which received only lauric acid (diet 1). The concentration of 20:3ω9 was lowered when dietary 18:3ω3 or 22:6ω3 increased (Table IV). Linolenic acid appeared to be somewhat more efficient than 22:6ω3 in reducing the level of 20:3ω9. This is in agreement with the experiment of Brenner and Jose (15) which showed that the 20:3ω9 in liver and heart tissues of the fat deficient rats was lowered when 18:3ω3 or 22:6ω3 was included in their diet. They suggested that 22:6ω3 inhibited the incorporation of 20:3ω9 into the B position of the glycero phospholipids. As a result, further formation of the 20:3ω9 was inhibited. 18:3ω3 competitively inhibited the enzymatic desaturation and chain elongation reaction of 18:1ω9.

The formation of 18:4ω3, 20:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3 was observed in the phospholipids of the fish receiving dietary 18:3ω3 (diets 5 and 6). The concentration of 22:6ω3 was especially high. These results are quite similar to the conversion of linolenic acid into docosahexaenoic acid in kelp bass as reported by Kayama et al. (16). In the groups of fish placed on diets containing 22:6ω3 (diets 2-4), there was a very high concentration of 22:6ω3 in the phospholipids; while only a low concentration of 20:5ω3 was observed.

We believe the origin of the 20:5ω3 is from the small amount (2.5%) which is present in the 22:6ω3 supplement and is not the result of retroconversion. A positive answer will require further experimentation. No formation of 20:4ω3, 18:4ω3 and 18:3ω3 was detected in the lipids of the fish. Rat experiments conducted by Verdino and coworkers (17) and Schlenk et al. (18) showed that a large quantity of 20:4ω6 (essential to rats) was formed by retroconversion by fat deficient animals when placed on diets containing 22:5ω6. Only a trace of 18:2ω6 was formed. Furthermore the conversion of arachidonic acid to 18:2ω6 in rats was insignificant. It seems that 22:6ω3 in trout has a behavior similar to 20:4ω6 in rats.

Fish oils are generally low in 18:3ω3 but rich in other ω3 fatty acids: namely, 18:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3. The total ω3 fatty acid content in fish oil is ca. 20-30%. This experiment reaffirmed the ability of fish to synthesize 22:6ω3 from lower ω3 fatty acid and the capability of both 18:3ω3 and 22:6ω3 to alleviate essential fatty acid deficiency symptoms, increase feed efficiency and promote rapid growth.

This experiment stressed the importance of ω3 fatty acids in trout nutrition but did not rule out a possible requirement for ω6 fatty acids. A long term feeding experiment is in progress to determine whether trout can survive and reproduce without dietary ω6 fatty acids.

ACKNOWLEDGMENTS

T. Will and R. Foster gave technical assistance. This investigation was supported in part by the National Oceanic and Atmospheric Administration Institutional Sea Grant GH 97.

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[Received January 19, 1972]