have limited value in replacement pullet diets. The final feeding value of the products must be determined by feeding trials to determine the effects of the feedstuffs on palatability and productivity of the resulting diet.

Registry No. Ca, 7440-70-2; Mg, 7439-95-4; P, 7723-14-0; Al, 7429-90-5; Cu, 7440-50-8; Fe, 7439-89-6; Mn, 7439-96-5; Zn, 7440-66-6; Na, 7440-23-5; K, 7440-09-7; γ-chaconine, 511-36-4; β-chaconine, 472-51-5; β_2-chaconine, 469-14-7; α-chaconine, 20562-03-2; α-solanine, 20562-02-1; vitamin B, 12001-76-2; niacin, 59-67-6; riboflavin, 83-88-5; thiamin, 59-43-8.

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Influence of Commercial Dietary Fatty Acids on Polyunsaturated Fatty Acids of Cultured Freshwater Fish and Comparison with Those of Wild Fish of the Same Species

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Polyunsaturated fatty acids (PUFA) of the dorsal muscle lipids of cultured and wild freshwater fishes, and of the artificial diet lipids used in culture, were analyzed. The muscle lipids of cultured carp and rainbow trout contained higher percentages of linoleic acid (18:2ω6) than those of the wild fish and eels. The amount of 18:2ω6 in the fishes depended upon the diet lipids. High percentages of arachidonic and eicosapentaenoic acids (20:4ω6 and 20:5ω3) in wild carp, of linolenic acid (18:3ω3) in wild rainbow trout, and of docosahexaenoic acid (22:6ω3) in cultured rainbow trout were observed. The ratio of ω3 to ω6 PUFA for dorsal muscle lipids was in the following order: wild rainbow trout > cultured eel and rainbow trout > wild eel > wild carp > cultured carp.

INTRODUCTION

The lipids of marine fishes have beneficial effects on the cardiovascular disease. It is believed that the effects are caused by ω3 PUFA (especially, 20:5ω3) present in the fish lipids (Dyerberg et al., 1978; Dyerberg and Bang, 1979). The mortality rate of the cardiovascular disease in Japan is lower than that in the United States and Europe. The significance of freshwater fish lipids to the disease in Japan cannot be disregarded because of a comparatively high consumption of these fishes. Both cultured and wild freshwater fishes are available in the food market. Comparative studies of the fatty acid composition between lipids of cultured and wild ayu (Ohshima et al., 1982; Hirano and Suyama, 1983) and of eels (Otwell and Rickards, 1981/1982) have been carried out. However, there is not enough basic data necessary to estimate the contribution of freshwater fishes in the prevention of cardiovascular disease.

We have examined the difference between lipids of cultured and wild freshwater fishes in terms of the fatty acid composition of the dorsal muscle lipids. Carp and rainbow trout fatty acids were modified more by diet than were those of eel.

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MATERIALS AND METHODS

Samples. Cultured carp (*Cyprinus carpio*, 2 years old), rainbow trout (*Salmo gairdneri*, 3 years old), and eels (*Anguilla japonica*, 1–2 years old) were supplied by the

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Table I. Fatty Acid Composition[a,b] of the Diet Lipids of Some Cultured Freshwater Fishes

	carp		rainbow trout		eel	
fatty acid[c]	mean	SD[d]	mean	SD	mean	SD
14:0	3.9	0.2	3.3	0.3	3.7	0.1
16:0	23.0	0.1	24.9	0.9	22.2	0.4
16:1ω7	4.1	0.1	5.2	0.3	5.1	0.2
18:0	4.1	0.1	4.3	0.2	3.9	0.1
18:1ω9	23.0	0.6	23.7	0.6	18.7	0.4
18:2ω6	24.2	0.2	21.6	0.4	1.5**	0.2
18:3ω3	1.6	0.1	2.0	0.1	tr[e]	
20:1ω9	1.6	0.1	2.1	0.1	6.0**	0.1
20:4ω6	0.4	0.1	0.5	0.1	1.1	0.1
20:5ω3	4.6	0.1	4.5	0.3	13.1**	0.2
22:6ω3	5.2	0.1	4.3	0.3	15.2**	0.5

[a] Percent by weight. [b] Each value represents the mean and standard deviation for triplicate analyses. [c] For the nomenclature, see Materials and Methods. [d] SD = standard deviation. [e] Less than 0.1% in this sample. Key: **, $P < 0.01$ compared to the carp and rainbow trout diets.

Fresh Water Fisheries Experiment Station Ibaraki Prefecture (Lake Kasumigaura), the National Research Institute of Aquaculture (Nikko, Tochigi), and the Shizuoka Prefectural Fisheries Experiment Station (Lake Hamana-ko), respectively. Samples of the artificial diets used in rearing were obtained at the same time (June 1984). These were similar to the diets normally employed in commercial culture fisheries. Market-sized wild carp (*C. carpio*, 2–3 years old), rainbow trout (*S. gairdneri*, 3–4 years old), and eels (*A. japonica*, 2–3 years old), never fed artificial diets, were harvested in June (1984) from the Kokai river (Ibaraki), the Yukawa river (Tochigi), and lake Kitaura (Ibaraki), respectively. These samples were stored at −25 °C, and the fatty acid composition was analyzed within 2 months.

Fatty Acid Analysis. Lipids of the dorsal muscle of freshwater fishes (5 g) and the diets (5 g) were extracted by the method of Bligh and Dyer (1959). The extracted lipids were saponified in KOH–ethanol with heat (90 °C) for 1 h. The resulting mixtures of fatty acids were recovered and the isolated fatty acids transesterified. The fatty acid methyl esters were analyzed by using gas chromatography with the same condition as that used for a previous paper (Suzuki et al., 1985). The designation used for fatty acid identification was as follows: the number before the colon gives the number of carbon atoms in the fatty acid chain; the number following the colon and the ω number give the number of double bonds and carbons from methyl end to first double bond counting from that end, respectively. The ratio of ω3 to ω6 PUFA was calculated by dividing the total amounts of 18:3ω3, 20:5ω3, and 22:6ω3 by those of 18:2ω6 and 20:4ω6. All results are reported as the mean plus/minus standard deviation, and significant differences between cultured and wild fishes were determined by Student's t-test (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The fatty acid composition of the lipids of artificial diets is shown in Table I. No significant differences in the fatty acid composition between carp and rainbow trout diets were observed. The amount of eicosenoic acid (20:1ω9), 20:5ω3 and 22:6ω3 of the eel diet lipids was greater than that of the carp and rainbow trout diet lipids, while the amount of 18:2ω6 of the eel diet was less than that of the other diets. The fatty acid composition of carp and rainbow trout diets is basically derived from the lipids of wheat, soybean, and fish meal used in the raw materials, and that of eel diets is modified by the acids of fish liver oil.

Table II shows the fatty acid composition of the lipids of dorsal muscle of cultured and wild freshwater fishes. The lipids of both cultured carp and rainbow trout contained higher percentages of 18:2ω6 than those of the wild fish and eels ($p < 0.01$). This result indicates that the amount of 18:2ω6 of dorsal muscle lipids in the freshwater fishes depends on the diet lipids. High percentages of 20:4ω6 and 20:5ω3 in wild carp, of 22:6ω3 in cultured rainbow trout, and of palmitoleic acid (16:1ω7) and 18:3ω3 in wild rainbow trout were observed. No marked difference in the fatty acid composition between cultured and wild eels was seen. Despite the greater amount of 20:5ω3 and 22:6ω3 in the eel diet (Table I), the percentages of the fatty acids of dorsal muscle lipids in cultured eel were similar to those in cultured carp. The PUFA patterns of the muscle lipids of cultured eel (18 months) have been shown to be approximately the same as those of wild eels (Otwell and Rickards, 1981/1982). These data suggest that it may be difficult to change the amount of ω3 PUFA of the eel muscle lipids by modifying the diet.

Ayu is a freshwater fish that is relatively common as a food in Japan. The amount of 18:2ω6 in cultured ayu is higher than that in wild fish, while the amount of 18:3ω3 and 20:5ω3 in cultured ayu is less than that in wild ayu (Ohshima et al., 1982; Hirano and Suyama, 1983). These findings are similar to our observation on carp.

The ratio of ω3 to ω6 PUFA of dorsal muscle lipids of freshwater fishes is shown in Table III. The ratios for wild carp and rainbow trout were higher than those for the cultured fishes, whereas the ratio for wild eel was lower than that for the cultured eel. The ratios may be ranked in the following order: wild rainbow trout > cultured eel

Table II. Fatty Acid Composition[a] of the Lipids of Dorsal Muscle of Some Cultured and Wild Freshwater Fishes

	carp				rainbow trout				eel			
	cultured[b]		wild[b]		cultured[b]		wild[b]		cultured[b]		wild[b]	
fatty acid	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
14:0	2.0	0.3	1.4	0.2	1.4	0.2	3.8	0.4	3.8	0.4	4.3	0.4
16:0	22.2	0.7	19.5	1.6	24.8	0.9	22.8	1.8	22.9	1.1	20.1	1.0
16:1ω7	7.4	1.8	8.1	2.5	3.7	0.3	9.5**	0.8	8.1	0.2	12.5	0.4
18:0	4.7	1.0	7.0*	1.5	5.2	0.1	4.3	0.1	3.8	0.6	3.9	0.4
18:1ω9	32.8	5.4	22.9	6.8	16.6	0.6	21.6	2.8	46.1	0.9	39.8	2.2
18:2ω6	15.2	1.0	6.0**	0.3	10.1	0.3	4.9**	1.3	2.0	0.5	3.1	0.5
18:3ω3	1.1	0.1	1.8	0.5	0.9	0.1	6.9**	1.6	0.3	0.1	1.4	0.1
20:1ω9	2.6	0.2	1.6	0.6	1.0	0.1	0.8	0.3	3.5	0.5	1.0*	0.1
20:4ω6	0.9	0.4	7.5**	2.0	1.5	0.1	1.7	0.3	0.3	0.1	1.8*	0.2
20:5ω3	2.5	0.8	7.0**	2.7	5.2	0.5	5.3	1.1	2.3	0.1	2.9	0.4
22:6ω3	6.0	2.8	7.0	2.1	25.8	1.9	11.7**	1.9	4.0	0.2	2.7*	0.4

[a] Percent by weight. [b] Determined by three samples. Key: **, $P < 0.01$; *, $P < 0.05$ compared to the cultured fish.

Table III. Ratio of ω3 to ω6 Polyunsaturated Fatty Acids[a] of the Dorsal Muscle Lipids of Some Cultured and Wild Freshwater Fishes

fish	cultured mean	cultured SD	wild mean	wild SD
carp	0.59	0.16	1.18**	0.13
rainbow trout	2.77	0.16	3.68**	0.62
eel	2.96	0.79	1.43**	0.12

[a] For the ratio of ω3 to ω6 polyunsaturated fatty acids, see Materials and Methods. Key: **, $P < 0.01$ compared to the cultured fish.

and rainbow trout > wild eel > wild carp > cultured carp. It may be inferred from these results that the beneficial effects of freshwater fishes on cardiovascular disease would be similar in order.

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Phosphorus-31 Nuclear Magnetic Resonance Spectroscopic Determination of Phytate in Foods

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A direct quantitative method for the determination of phytate in foods, using phosphorus-31 Fourier transform nuclear magnetic resonance spectroscopy, has been substantially modified to improve its convenience and accuracy and eliminate interference from paramagnetic ions. An ion chromatographic method for phytate has also been employed, and good agreement was obtained between the two procedures.

Phytate (*myo*-inositol, hexakis(dihydrogen phosphate); Figure 1) is a naturally occurring organic substance that binds nutrient mineral cations, making them unavailable for nourishing the body (Oberleas et al., , 1966). It is found in most fruits, vegetables, and grains. Thus, the measurement of phytate is especially important in vegetarian diets (Harland and Peterson, 1978), in diets selected for their high content of plant fiber, and in diets that may be marginally deficient in minerals. There is increasing need for a precise method of determining phytate as Americans change their diets to incorporate more foods of plant origin. When dietary phytate levels are high and dietary mineral intakes are low, mineral status in animals and humans may be compromised. A tool that may be employed for estimating mineral bioavailability in phytate-containing diets is the phytate:mineral molar ratio. For a description of the calculation of this ratio, as well as a comprehensive list of phytate-containing foods, see Oberleas (1983b) and Oberleas and Harland (1981).

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A detailed account of the history of phytate methodology development may be found in a review by Oberleas (1983a). Heubner and Stradler (1914) developed the first method for quantifying phytate, based on the fact that, in the presence of excess ferric ion, phytate is insoluble in dilute acid. Various modifications in the acid extraction of foods and feeds, in the purification of phytate, and in the quantification of phytate have been used through the years.

Many attempts have been made to simplify and shorten the analysis of foods for phytate, but because analytical methods have been laborious and imprecise, understanding the role of phytate in the bioavailability of minerals from plant food has been delayed. Averill and King (1926), Young (1936), and Latta and Eskin (1980) modified the iron precipitation procedure, which shortened analytical time. Harland and Oberleas (1977) developed a method using step gradient elution with anion-exchange column chromatography. Ellis and Morris (1982) compared the anion-exchange method to an iron precipitation method. However, the ability of these methods to discriminate against lower inositol phosphates (pentaphosphates, tetraphosphates, etc.), which have been detected in certain processed foods (deLange et al., 1961; O'Neill et al., 1980; Phillippy and Johnston, 1985), has not been demonstrated.

This lack of specificity prompted O'Neill and co-workers (1980) to develop a ^{31}P Fourier transform nuclear magnetic resonance (^{31}P FT NMR) spectroscopic technique for a more precise analysis of foods for phytate. NMR is well suited for this task because the ^{31}P NMR spectrum of