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Effect of a fat-free diet and of different dietary fatty acids (palmitate, oleate, and linoleate) on the fatty acid composition of fresh-water fish lipids*

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SUMMARY

When fresh-water fish *Pimelodus maculatus* were fed a fat-deficient diet, the concentration of linoleic and arachidonic acids in glycerides and phospholipids decreased, and the concentration of palmitic, palmitoleic, oleic, and eicosatrienoic acids increased. When fat-deficient, fresh-water fish *Parapimelodus valenciennesi* were fed a diet containing methyl palmitate, methyl oleate, and methyl linoleate, palmitic and oleic acid seemed to be stored mainly in the glycerides, whereas linoleic acid was deposited in both glycerides and phospholipids. Some linoleic acid was transformed into arachidonic. The concentration of palmitic acid in fish lipids was very well regulated by the animal.

In previous experiments (1) on the effect of the composition of the natural diet on the depot fat of *Pimelodus marculatus* (Bagre manchado), some aspects of the fatty acid metabolism of this Río de la Plata fresh-water fish were considered. In that work, it was demonstrated that the depot fat did not retain composition of the natural diet, which is rich in palmitic, stearic, and oleic acids. Instead, the relative concentrations of palmitoleic and oleic acids were significantly increased in the depots. Desaturation seemed to be one of the main reactions taking place. The contents of linoleic and arachidonic acids in both the diet and the depot triglycerides were very low. When the fish were maintained on a semisynthetic, fat-free diet for a period of four months, the level of palmitic acid in triglycerides was somewhat higher than in fish fed a natural diet, whereas the levels of linoleic and arachidonic acids were not reduced, as would be expected for essential fatty acids. Considering these results, new experiments were designed to throw more light on the influence of palmitic, oleic, and linoleic acids on the fatty acid composition of fresh-water fish. In the first experiment, the composition of the lipids of *Pimelodus maculatus* was studied in animals that had received a fat-deficient diet for a period of 15 months. In the second experiment, the lipids of a related fish, *Parapimelodus valenciennesi* (Portenito), were studied after administration of diets containing palmitic, oleic, and linoleic acids.

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EXPERIMENTAL METHODS

Experiment I. A group of young *Pimelodus maculatus* (mean weight 15 g) caught in autumn, were fed a semisynthetic, fat-free diet of the following composition in g/100 g: acetone-extracted starch, 68; acetone-extracted fish meal (Vio Bin Corp., Monticello, Ill.), 25; McCollum-Davis salt mixture No. 4, 5; glycine, 1.6; choline, 0.3; inositol, 1; and vitamins. The diet contained the following vitamins in mg/kg: thiamine, 6; Ca pantothenate, 25; pyridoxine, 8; niacin, 70; folic acid, 25; *p*-aminobenzoic acid, 3; vitamin B_{12}, 6; α-tocopherol, 1; menadione, 1.5; vitamin A, 33; vitamin D_3, 0.4.

The fish were maintained in aerated tap water at 20°. After 7 and 15 months, respectively, two fish were killed and beheaded, and the total lipids of the carcass were extracted and purified by the procedure of Folch, Lees, and Sloane Stanley (2). The lipids

were dissolved in chloroform and the phospholipids fractionated by adsorption on activated silicic acid (3), followed by elution with methanol. Glycerides remaining in the chloroform solution were separated from the cholesterol esters on a silicic acid column by the procedure of Hirsch and Ahrens (4). Cholesterol esters, eluted by 230 ml of 1% ethyl ether in petroleum ether, were discarded; the fraction eluted by 190 ml of ethyl ether, containing the glycerides, was collected. The fatty acid composition of the phospholipids and glycerides was ascertained by gas-liquid chromatography (GLC) of the methyl esters prepared by the procedure of Stoffel, Chu, and Ahrens (5). A Pye apparatus with ionization detector was used. Argon was the gas. The esters were run on 15% polyethyleneglycol-adipate on Celite (80–120 mesh) at 180° and 200°, in a column 4 ft long and 4 mm in diameter. Acids were identified as described in previous reports (6, 7). The original samples were analyzed before and after hydrogenation and bromination. The molar composition was calculated by triangulation of the peaks.

Experiment II. A related species, *Parapimelodus valenciannesi*, was chosen for the second experiment because large numbers of the young of this species were easily obtained. The fatty acid composition of unfractionated lipids of these fish, which had been eating natural plankton, was determined as in the first experiment.

Two hundred fish, approximately six months old or younger (5.0–8.0 cm long, 1.9 g average weight) were fed for 55 days on the semisynthetic, fat-free diet described above. They were then divided into four groups. One was kept on a fat-free diet; the others were fed for 15 days on diets supplemented respectively with 10% methyl palmitate (99.0% pure), 10% methyl oleate (95% pure containing 2.9% methyl linoleate), and 10% methyl linoleate (93% pure containing 7% oleate). Methyl linoleate was obtained from sunflower seed oil by separation of the urea adduct (8) and vacuum distillation.

The mortality was very high. The surviving fish were fasted for 24 hr before being killed and beheaded. The bodies of the animals in each group were pooled and the lipids extracted by the procedure of Folch et al. (4) with chloroform–methanol 2:1. The total lipid content varied between 2.5 and 1.2%. Phospholipids were separated on silicic acid as previously described. The percentage of phospholipids found varied between 39.0 and 42.4% of the total lipids. The fatty acid composition of unfractionated lipids and phospholipids was determined by GLC as detailed before, and is graphically shown in Figs. 1, 2, and 3.

RESULTS AND DISCUSSION

In our previous experiment (1) on the effect of a fat-deficient diet, administered for four months, on the fatty acid composition of *Pimelodus maculatus* lipids, octadecadienoic (linoleic) and eicosatetraenoic acids were not lowered, in contrast to what might have been expected for indispensable fatty acids. Similar results have been found by Kelly, Reiser, and Hood (9) studying the effect of low-fat diets on the content of polyunsaturated fatty acids of the fresh-water fish *Ameiurus natilis natilis* (yellow bullhead), *Lepomis macrochirus macrochirus* (common bluegill), *Ambloplites rupestris ariommus* (rockbass), *Ictiobus bubalos* (smallmouth buffalo), and the *Salmo gairdnerii irideus* (rainbow trout).

In our present experiment I, *Pimelodus maculatus* were kept on the fat-free diet for longer periods (7–15 months). A decrease in the already low amounts of octadecadienoic and eicosatetraenoic acids was evident. This effect appeared both in glycerides and phospholipids (Table 1). It agrees with the findings of Klenk and Kremer (10) that trout livers in vitro are unable to synthesize linoleic acid from labeled acetate.

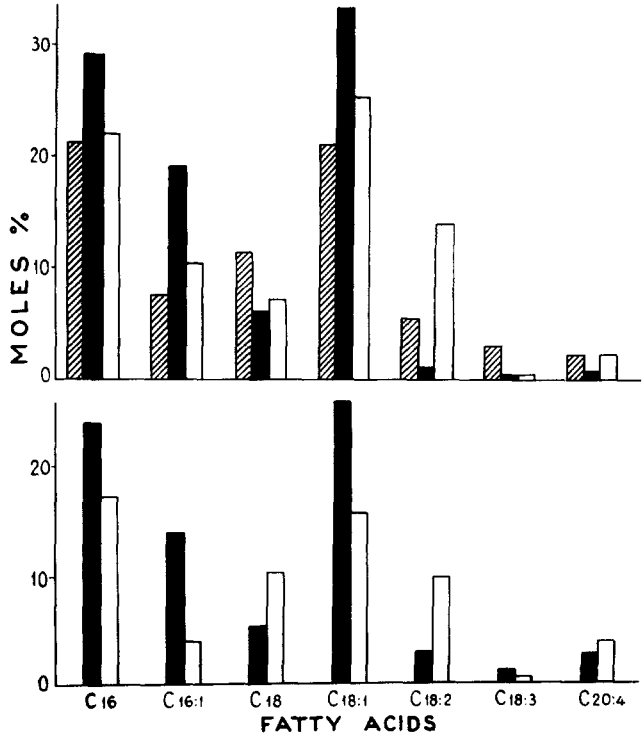

FIG 1. Influence of dietary methyl linoleate on unfractionated lipid and phospholipid fatty acid composition of *Parapimelodus valenciennesi*. Top: unfractionated lipids; bottom: phospholipids. Striped bars correspond to natural diet; solid bars, to fat-free diet; open bars, to fat-free diet supplemented with methyl linoleate.

TABLE 1. COMPOSITION OF THE MAJOR FATTY ACIDS OF THE GLYCERIDES AND PHOSPHOLIPIDS OF *Pimelodus maculatus* ON NATURAL AND FAT-FREE DIETS

Fatty Acid	Glycerides				Phospholipids		
	Natural Diet	Fat-Free Diet			Natural Diet	Fat-Free Diet	
		4 months*	7 months	15 months		7 months	15 months
	mole %	*mole %*	*mole %*	*mole %*	*mole %*	*mole %*	*mole %*
16:0	15.4	20.2	22.3	27.0	23.6	25.1	20.3
16:1	7.3	5.1	11.1	11.2	3.3	5.1	4.1
18:0	10.7	11.8	6.8	4.7	13.6	12.9	14.1
18:1	51.0	35.6	43.5	48.4	12.8	22.3	19.9
18:2	3.0	3.7	1.7	0.5	2.3	2.4	1.2
20:3	0.2	1.0	0.9	0.8	1.1	4.5	5.8
20:4	0.3	0.6	0.1	0.1	6.2	1.7	3.3
22:6	0.2	0.4	0.3	0.1	15.0	3.3	6.2

* Feeding period.

On the fat-free diet, the concentrations of palmitoleic and oleic acids in glycerides and phospholipids increased. These increases were more apparent in the phospholipids than in the glycerides (Table 1), because of the already high proportion of dietary oleic acid stored in glycerides of the fish eating a natural diet. In terrestrial animals, an increase in palmitoleic and oleic acids is associated with fat deficiency in the diet. This effect is generally attributed to the deficiency of fatty acids of the linoleic family (11). However, fatty acids of the linolenic type also seem to reduce the levels of oleic and palmitoleic acids (12–14). Eicosatrienoic acid, a typical acid produced in rats by a diet deficient in essential fatty acid, was also increased in the fish, mainly in the phospholipids (Table 1). However, fatty acids of both the linoleic (14) and linolenic family reduce eicosatrienoic levels in fat-deficient rats. Therefore, the increase in palmitoleic, oleic, and eicosatrienoic acids in fish cannot be attributed to the lack of acids of the linoleic acid family only since the diet was also deprived of acids of the linolenic family.

These considerations, and the small amounts of fatty acids of the linoleic family (linoleic and arachidonic acids) compared to the fatty acids of the linolenic type (docosahexenoic acid) found in the phospholipids, suggest that linoleic-type acids may be nonessential, or may be needed in very small quantities by fish. However, they are not synthesized de novo by the fish. The fatty acids of the linolenic family probably can replace them in many or all their functions. Richardson, Tappel, and Gruger (15) found low amounts of essential fatty acids in mitochondria of salmon liver and heart, and catfish and carp livers. Thus they suggested that the linoleic group of fatty acids is not necessary for the metabolism of fish mitochondria. Linolenic-type fatty acids probably replace them in mitochondrial functions.

In terrestrial animals, the heart is very rich in linoleic and arachidonic acids and is one of the organs most sensitive to variations of essential fatty acids in the

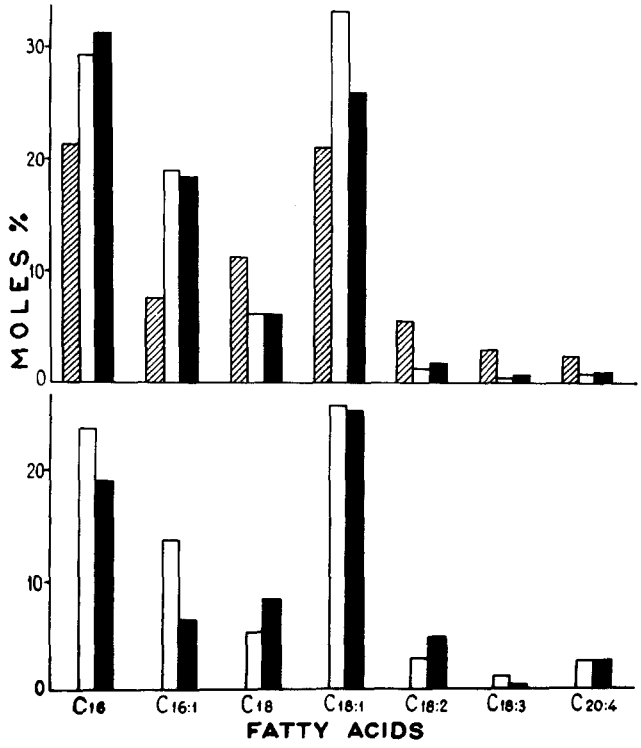

FIG 2. Influence of dietary methyl palmitate on unfractionated lipid and phospholipid fatty acid composition of *Parapimelodus valenciennesi*. Top: unfractionated lipids; bottom: phospholipids. Striped bars correspond to natural diet; open bars, to fat-free diet; solid bars, to fat-free diet supplemented with methyl palmitate.

diet. The heart is relatively poor in fatty acids of the linolenic type. In *Pimelodus maculatus* heart, low molar percentages of linoleic (2.5%) and arachidonic acids (7.6%) have been found (16), and still lower amounts were found in hearts of the marine fish *Miobatis zoodei* (linoleic 0.4%, arachidonic 7.4%), *Micropogon opercularis* (linoleic 2.9%, arachidonic 6.6%), and *Umbrinus canosai* (linoleic 1.6%, arachidonic 5.4%) (16). Relatively higher amounts of eicosapentenoic and docosahexenoic fatty acids were detected. These results are compatible with our suggestion that, though fish heart is able to concentrate fatty acids of the linoleic acid type and use them in its functions, linolenic-type acids can at least partially replace them.

The long period needed to decrease linoleic and arachidonic acid levels in the fish studied may be partially due to the age of the fish. When very young fish (*Parapimelodus valenciennesi*) were studied in experiment II, the content of linoleic and arachidonic acids in the total lipids (Figs. 1, 2, and 3, Table 2) decreased significantly after only two months of feeding

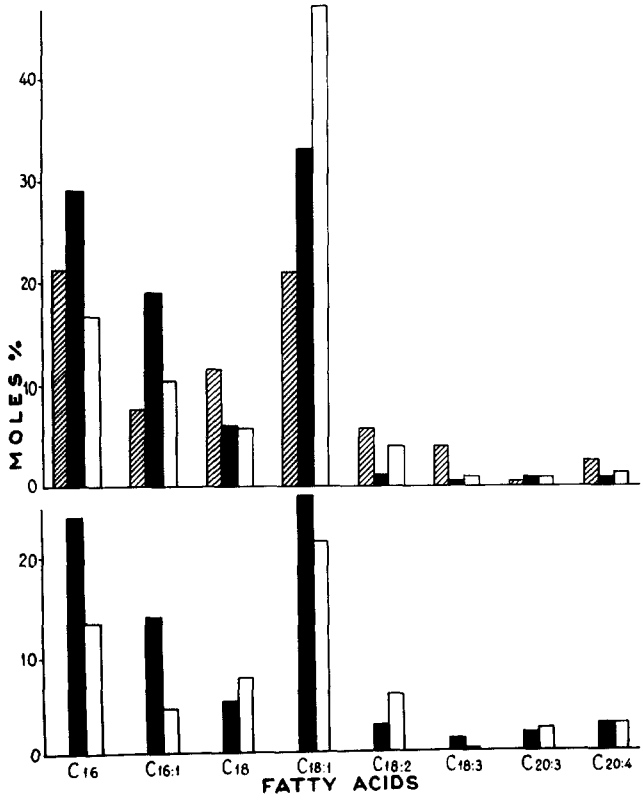

FIG. 3. Influence of dietary methyl oleate on unfractionated lipid and phospholipid fatty acid composition of *Parapimelodus valenciennesi*. Top: unfractionated lipids; bottom: phospholipids. Striped bars correspond to natural diet; solid bars, to fat-free diet; open bars, to fat-free diet supplemented with methyl oleate.

TABLE 2. FATTY ACID COMPOSITION OF THE TOTAL LIPIDS *of Parapimelodus valenciennesi* ON NATURAL DIET

Fatty Acid	mole %	Fatty Acid	mole %
11 to 14 br*	3.7	18:1	21.1
14:0	3.2	18:2	5.6
14:1	0.5	19	tr
15 br	0.7	18:3	3.0
15 + 15:1 (?)	4.4	20:1	0.7
16 br	1.1	20:3	0.4
1:60	21.4	20:(?) and 21:(?)	tr
16:1	7.6	20:4	2.4
17 br	0.6	20:5	1.1
17:0	0.8	22.4(?)	0.3
17:1 + 16:3(?)	2.7	22:5(?)	0.5
18 br	1.8	22:5′	0.8
18:0	11.5	22:6	2.1

* br = branched.

a fat-free diet. This effect was associated with a large increase in palmitic, palmitoleic, and oleic acids, and a small increase in eicosatrienoic acid. The addition of 10% methyl linoleate to the diet produced not only a high deposition of this acid in phospholipids and total lipids (Fig. 1), but also increased the level of arachidonic acid, especially in phospholipids. This increase would indicate a rapid synthesis of arachidonic acid from dietary linoleic acid. However, the levels of linoleic and arachidonic acids found in the fish are much lower than the levels found in the rat under similar conditions (17). As happens also in mammals, the addition of linoleic acid to the diet reduced the increased levels of palmitoleic and oleic acids produced by a diet deficient in essential fatty acid. Moreover linoleic acid also decreased the palmitic acid to normal levels.

In the glycerides of both *Pimelodus maculatus* (Table 1) and *Parapimelodus valenciennesi* (Fig. 2), a significant increase of palmitic acid and a decrease of stearic acid was noticed when fish received the fat-deficient diet. This effect was not seen in the phospholipids (Table 1). The addition of methyl palmitate to the fat-deficient diet did not modify the fatty acid composition of the total lipids (Fig. 2). It did not even increase the concentration of palmitic acid. Palmitic acid is synthesized in toto by animals, and this mechanism probably produced the high level of the acid in the fish fed on the fat-free diet. This level seems to be very well regulated by the fish because addition of palmitic acid to the diet did not alter it although palmitic acid was absorbed from the intestine.[1] This regulation seems to be exercised mainly through inhibition of palmitic acid synthesis[2] and total oxidation,

[1] N. R. Bottino and R. R. Brenner, unpublished observation.
[2] N. R. Bottino and R. R. Brenner, work in progress.

because neither palmitoleic and oleic acids nor longer chain fatty acids were increased when palmitate was added to the diet. We have shown,[2] using labeled acetate, that feeding palmitate to fat-deficient *Pimelodus maculatus* enormously reduces incorporation of acetate into fatty acids. Palmitic and stearic acids seemed to be mainly stored in the glycerides.

The addition of methyl oleate to the fat-deficient diet increased the amount of oleic acid in the total lipids of the fish to even higher levels (Fig. 3) than in those fish fed the fat-deficient diet, but it did not modify the percentage of oleate in phospholipids. It seems then that dietary oleic acid is stored in triglycerides but not in phospholipids. The proportions of palmitic and palmitoleic acids were reduced in both total lipids and phospholipids by addition of oleic acid to the diet, while the other acids, including the eicosatrienoic acid, were not affected. The small increase in linoleic acid found in the fish was undoubtedly due to the 2.9% of linoleic acid contaminating the dietary oleic acid.

One can conclude, therefore, that fish are not greatly different from mammals in the metabolism of palmitic, oleic, and linoleic acids, though the degree and rates at which this metabolism takes place seem to be different. This difference could be due to the fact that fish are poikilothermic while mammals are homeothermic. There is some evidence suggesting that fatty acids of the linoleic type are of less importance for fish than for mammals and probably acids of the linolenic type replace them in many of their functions.

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