COMPOSITION AND DIETARY EFFECTS OF THE FISH OIL FROM "MANDI" *(Pinelodus clarias)*

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SUMMARY

A study was carried out to determine the chemical composition and dietary effects of the oil from "mandi" *(Pimelodus clarias)*. Findings revealed that it had a low proportion of essential C18:2 fatty acids and a high percentage of oleic acid (20.93o/o), as well as polyunsaturated fatty acids with more than 18 carbon atoms. Of the long-chain fatty acids, C22:6 was present in the highest percentage (1.94o/o).

When rats were fed a diet the lipid source of which was the oil of mandi, they showed a reduced growth rate as compared to animals receiving the control diet (corn oil). The fatty acid composition of the liver and heart of rats from the experimental group was modified. The greatest variation occurred in the percentage of C22:6 in the heart muscle, wherein a five-fold increase was observed.

Reduction of growth and alteration in the polyunsaturated fatty acids levels may be due to a deficiency in C18:2-w6, or to a possible imbalance between C18:2-w6 and C18:3-w3.

INTRODUCTION

The chemical composition of most fish oils differs from the majority of other fats and oils with respect to the great variety of classes of lipids that fish oils possess. They differ principally in relation to fatty acids with chains longer than 18 carbons which have a high proportion of polyunsaturated fatty acids with more than four double bonds per molecule (1, 2). Even within the same species of fish, variations in the propor-

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Manuscrito modificado recibido: 24–4–86.

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tion of fatty acids occur, according to geographic variations, season of the year, and part of the fish from which oil was extracted (1, 3,4).

Because of the abundance of polyunsaturated triglycerides in fish oils, interest in studying the dietary effects of fish oil was stimulated. It has been observed that fish oil can cause the disappearance of essential fatty-acid deficiency syndromes in rats, even though the polyunsaturated fatty acids of fish oil belong more to the linolenic acid (W-3) than to the linoleic acid family (5, 6). The deficiency syndrome is related to the linoleic acid family, as opposed to the linolenic acid family.

On the other hand, it has also been observed that when animals are fed different types of fat, variations in the per cent composition of fatty acids in various organs and tissues occur. Dietary lipids promote a fatty acid profile in the organs which is similar to that contained in the diet (1, 7-11). The fatty acids composition in the fish oil may produce an inadequate composition of polyunsaturated fatty acids in the organs of the animals which ingest them.

The present study relates the analysis of the fatty acid composition of the lipid extract of mandi, the effect of this lipid extract on growing rats, and its effect on the fatty acid composition of the liver and heart of rats fed this extract. Mandi is a fresh-water fish extensively consumed in Brazil and easily found in any market.

MATERIALS AND METHODS

Materials

The solvents utilized were reagent grade.

Fish samples of both sexes and of various sizes, ages and weights were obtained commercially, covering the annual production of the fish which is currently sold in the market. They were cleaned and divided into sections of approximately 100 g each, discarding the viscera and fins. The fish samples were ground in an industrial-type meat grinder (Malpa, Maquinas Lo Puma, S. A.), homogenized and separated into portions of approximately 500 g each, and frozen. Before freezing, aliquots of 5 g were taken for determination of humidity and fat content. Each portion was lyophilized for 72 hr, and the combined samples were stored at -20^{o}C. Corn oil and lard were obtained commercially.

Methyl Ester Standards

The methyl esters of hexadecanoic, tetradecanoic, octadecanoic and eicosanoic acids were obtained from Analabs, Inc. (USA). Methyl arachidate, Methyl *cis*-eicosanoate, methyl arachidonate, methyl eicosapentanoate and methyl docosahexenoate were obtained from Applied Science Laboratories, Inc. (USA). The methyl esters of oleic, linoleic and linolenic acids were synthesized by treating the respective acids with BF_3/CH_3OH (12).

Lipid Extraction

A 50 g portion of the lyophilized sample was extracted with 250 ml of

$CHCl_3:CH_3OH$ (2:1) by shaking for three hours. The material was filtered and again shaken with 150 ml of solvent for 24 hr (Water Bath Shaker, Arthur Thomas Co. USA). The extracts were then combined and evaporated on a rotary evaporator, homogenized, and stored at -20°C. Analysis of the extracts by gas chromatography showed no traces of chloroform or methanol.

Determination of Humidity

Duplicate samples of 5.00 g of lyophilized samples were mixed with 2.00 g acid washed sand and dried in an oven at 60°C for 30 min, and then at 100°C to constant weight (12).

Fractionation

A 2.4546 g aliquot of fish lipid extract was refluxed for 6 hr with 25 ml isopropanol and 15 ml 0.42N KOH in methanol-isopropanol, and then extracted with 3 X 50 ml ether. The ether fractions were combined, washed three times with 20 ml water, three times alternately with 20 ml 0.5N KOH and 20 ml water and, finally, with water until the washings were neutral. The combined ether fractions were evaporated, 3 ml acetone were added to the residue and the residue was dried for 30 min at 100°C. The aqueous fractions were combined, acidified and extracted continuously with ether for 24 hr (12).

Methylation of the Lipid Samples

The saponifiable fraction of the fish lipid extract was methylated with BF_3/MeOH by the AOAC method cited above. Methylation was also performed on the fish oil, corn oil and lard without previous fractionation (12).

Gas Chromatography of Fish Oil, Corn Oil and Lard

The methyl esters of the fatty acids obtained from fish oil, corn oil and lard were chromatographed on a gas chromatograph Model CG 17 from Instrumentos Cientificos Ltda (Brazil), equipped with a flame ionization detector and using a 2 mm x 2 m glass column filled with 30% DEGS on 80 - 100 mesh Chromosorb W-HP-HMDS. The column temperatures used varied from 166 to 190°C; the injector temperature was 220°C and the detectors temperature, 240°C. The nitrogen flow rate was 25 ml/min.

Biological Assay

Holtzman rats from the colony of this Department were weaned at 21 days of age and distributed at random in individual cages. Three groups of eight rats each were formed. The three diets used differed only in the source of lipid and had the following composition: 8% fat, 18% casein, 1% cellulose, 15% sucrose, 5% minerals (12), 1% vitamins (12), 0.005% vitamin A and 0.300% choline. Water and food were offered *ad libitum* to the animals during four weeks. Control of the food intake and weight gain was performed weekly.

After four weeks, the animals were sacrificed by decapitation; and livers and hearts were removed, weighed and stored at -20°C.

Extraction of Lipids from the Rat Livers and Hearts

The livers were sectioned into small pieces and lyophilized for 24 hr; the dried livers were then placed in an Erlenmeyer flask with 50 ml $CHCl_3:CH_3OH$ (2:1). The flasks were shaken for three hours and the material was filtered and again extracted with 50 ml $CHCl_3:CH_3OH$ (2:1). The combined extracts were concentrated on a rotary evaporator at 40°C to a final volume of 10 ml, transferred to wide-mouth vials, evaporated to dryness with a stream of nitrogen and weighed. The hearts underwent the same process of extraction, combinations of two hearts being extracted with 20 ml $CHCl_3:CH_3OH$ (2:1) (13, 14).

Methylation of Lipid Extracts from Rat Livers and Hearts

Aliquots of 250 mg of rat liver lipid extract were refluxed for 30 min with 6.0 ml of 0.5N NaOH; then, 5.0 ml of BF_3/CH_3OH were added and the mixture was refluxed for 10 min. After the addition of 3.0 ml of cyclohexane, reflux was continued for an additional 10 min. After cooling, approximately 45 ml of saturated NaCl solution were added and the cyclohexane layer, removed. This solution was dried over Na_2SO_4 and stored at -20°C (12). The heart lipid extracts were methylated by the same procedure, except that the aliquots taken varied in weight. The NaOH solution contained 5 mg/100 ml of t-butylhydroquinone as antioxidant.

Gas Chromatrography of Fatty Acid Esters from Rat Liver and Heart Lipid Extract

The fatty acid methyl esters were chromatographed isothermally at various temperatures ranging between 120°C and 210°C and using a 2mm x 2m glass column containing 30°/o DEGS (stabilized) on 80 – 100 mesh Chromosorb W-HP-HMDS. The remaining conditions were identical to those previously detailed.

RESULTS

The moisture content of the fish was determined to be 70.65°/o, while the lipid content was 8.78°/o; 1.79 kg of lyophilized material were obtained from 6.1 kg of fresh fish. Extraction of the lipid fraction from this mixture yielded 535.5 g of total lipids as extracted by $CHCl_3$: MeOH. Table 1 exhibits the fatty acid composition of lard, corn oil and the saponifiable fraction of the oil. The non-saponifiable fraction corresponded to 6.04°/o of the fish lipid extract. The percentages of unsaturated fatty acids in the corn oil, fish oil and lard were 75.04°/o, 64.74°/o and 54.57°/o, respectively. The fish oil had a large range of fatty acids,

TABLE 1

PERCENTAGE COMPOSITION OF FATTY ACIDS IN CORN OIL, LARD AND FISH OIL

Fatty acids	Lipids		
	Corn oil o/o	Lard o/o	Fish oil o/o
C14:	2.88	5.92	2.56
a			1.12
b			1.64
c			0.59
C16:0	17.81	23.21	25.86
C16:1		3.99	14.24
d			2.82
e			2.92
C18:0	4.51	12.29	7.09
C18:1	21.45	41.66	29.93
C18:2	51.04	12.91	4.41
C18:3	2.58		3.45
C20:0			2.23
C20:4			1.84
f			0.19
g			1.51
h			0.72
i			0.16
C22:5			1.05
C22:6			1.94

The fatty acids a, b, c, d, e, f, g, h and i were not experimentally identified.

varying from the saturated to the highly unsaturated. The biggest difference detected occurred in relation to the presence of polyunsaturated fatty acids with more than 20 carbons, which were not found in corn oil nor in lard. Several fatty acids of low abundance were not identified. These probably included the C15:0, C17:0, C20:5, and 22:1 fatty acids, as well as isomers of the known fatty acids.

Feed intake and weight gain of the three groups of rats are presented in Table 2. As the figures indicate, those animals fed the fish oil diet showed lower weight gain, feed intake and feed efficiency than those fed either the control or the lard diets. The livers extracted from the rats which ingested fish oil diets had a lighter color and higher weight than those from the other two groups (Table 3). In contrast, the hearts from the fish-oil group had the lowest weight (Table 3). The lipids content in the liver paralleled the organ weight of each group, being higher in rats fed the fish oil diet (Table 3). On the other hand, as the same Table reveals, the amount of lipids in the heart was higher in the group fed corn oil than in the other two groups.

TABLE 2

RESULTS OF THE BIOLOGICAL ASSAY OF THE THREE DIETS CONTAINING CORN OIL, LARD AND FISH LIPID EXTRACT, FED TO RATS DURING A FOUR-WEEK PERIOD

	Initial weight (g)	Gain in weight (g)	Total food intake (g)	Food efficiency
Corn oil	44.21	113.80	319.73	0.36
Lard	46.06	110.53	312.94	0.35
Fish oil	43.94	78.25*	278.10*	0.28*

* Statistically different from the other groups in the same column, at the 5o/o level of significance.

TABLE 3

MEAN WEIGHTS OF LIVERS, HEARTS AND LIPID EXTRACTS FROM LIVERS AND HEARTS OF RATS FED THE THREE DIETS CONTAINING CORN OIL, LARD AND FISH LIPID EXTRACT

Group	Mean liver weight (g)	Mean heart weight (g)	Mean weight of liver extracts (g)	Mean weight of heart extracts (g)
Corn oil	6.7048	0.5532	0.5738	0.0306*
Lard	6.7016	0.5584	0.5303	0.0258
Fish oil	7.5639	0.4689*	0.7762*	0.0255

* Statistically different from the other groups in the same column,at the 5o/o level of significance.

The fatty acid composition of the lipid extracts from the livers and hearts of the three groups are shown in Tables 4 and 5, respectively. (Results for the percentage compositions of the acids in the liver and heart lipid extracts were statistically analyzed by analysis of variance, and the "t" test).

DISCUSSION

The fish samples contained 70.65o/o moisture. Therefore, in order to avoid difficulties in extracting the lipids from the aqueous fraction (15), the samples were first lyophilized. The binary system chloroform: methanol (2:1) could then be used to perform the extraction (13, 15, 16). The lipid extract used in the biological assay contained no trace of the solvents utilized in the extraction.

TABLE 4

FATTY ACID COMPOSITION OF LIVER LIPID EXTRACTS OF RATS FROM GROUPS FED THE CORN OIL, LARD AND FISH OIL DIETS

Fatty acids	Groups: Corn oil (o/o)	Lard (o/o)	Fish oil (o/o)
C14:0	1.02	0.86	0.86
C16:0	22.38	26.20	25.21
C16:1	3.89*	6.75	8.02
C18:0	14.69	13.49	7.21*
C18:1	19.18*	28.68*	37.74
C18:2	14.98*	7.46*	6.07
C18;3	Trace	Trace	0.68
C20:0	Trace	Trace	0.40
C20:4	15.90*	12.23*	5.05
f	Trace	0.12*	0.92
g	0.41*	0.12	Trace
h	1.49*	0.31*	0.59
i	3.11*	1.24*	0.42
C22:5	0.51*	0.19*	2.22
C22:6	2.11	1.96	4.60*

* Asterisks represent statistical difference from the other two means, in the same row, at the 5o/o level of significance.

The oils from the edible portions of 13 fish species were chromatographically analyzed by Khalid, Mizra and Khan (17). As these authors inform, fish oils originating from the coast of Karachimakran have fatty acids which vary from 10 to 24 carbons, with zero to six double bonds. The oil from mandi analyzed in the work herein presented, did not have fatty acids with less than 14 carbon atoms. Khalid, Mizra and Kahn (17) did not encounter a large quantity of the fatty acids C18:2 and C18:3, whereas in our research work, 4.41o/o C18:2 and 3.45o/o C18:3 were found. In the fish oils analyzed by the same authors (17), the acid C20:0 was encountered, whereas Gruger *et al.* (18) did not detect this acid. In the mandi oil, 2.23o/o of C20:0 were detected.

The most abundant monounsaturated fatty acid was C18:1 (29.93o/o) a finding which is in agreement with the values cited in the literature (18-24).

Peaks between C14:0 and C16:0, and two peaks between C16:1 and C18:0 were not identified. Data from the literature indicate that these peaks probably include C15:0 and C17:0.

Four peaks corresponding to fatty acids with more than 20 carbon atoms were not identified. Reference to the literature indicates that two of these peaks could be C20:5 and C22:1, while the others could be

TABLE 5

FATTY ACID COMPOSITION OF THE HEART LIPID EXTRACTS OF RATS FROM GROUPS FED THE CORN OIL, LARD AND FISH OIL DIETS

Fatty acids	Groups: Corn oil (o/o)	Lard (o/o)	Fish oil (o/o)
C14:0	0.58	0.68	0.56
C16:0	15.92	15.58	16.56
C16:1	2.34*	3.19	4.02
C18:0	20.71	22.62*	19.36
C18:1	13.33*	17.27	16.17
C18:2	20.14*	13.87*	8.01
C18:3	Trace	Trace	Trace
C20:0	Trace	Trace	Trace
C20:4	16.44	17.45	15.26
f	0.22	0.18	0.57*
g	Trace	Trace	Trace
h	1.80	1.28	0.58*
i	4.97*	3.35*	0.84
C22:5	1.16	1.01	2.28*
C22:6	2.46*	3.74*	13.81

* Asterisks represent statistical difference from the other two means, in the same row, at the 5o/o level of significance.

isomers of these acids (18, 20-24). The largest quantities of long-chain polyunsaturated fatty acids were encountered for the C20:4, C22:5 and C22:6 acids. The results obtained in our work are comparable to those encountered in the literature (18, 20-24).

Rats fed the diet based on fish oil were the ones that ingested the smallest amount of feed and gained the least weight. This fact may be due to the reduced palatability of the diet (1, 25). No manifestation characteristic of an essential fatty acid deficiency was observed in these animals (26, 27). Fish oil has an adverse effect on the growth of the liver; the livers studied were larger and had a higher fat content. On the other hand, heart weights and heart lipid extracts were lower than those of the control group. This effect on the heart weights may be related to the lower body growth of the animals, although this does not explain the effect observed on the livers. A possible hepatotoxic effect should be systematically studied, if a recomendation on the use of this fish oil as a food source for humans is to be considered.

The presence of polyunsaturated fatty acids, being easily oxidized, can cause serious nutritional problems due to the oxidation products (25, 28). Vitamin E was added to prevent oxidation, and no effects of oxidation products such as diarrhea or weight loss were observed (25). Although no new peak was observed upon chromatography, one cannot completely exclude the presence of a small amount of oxidation product in the diet.

Studies performed in various laboratories have shown that the fatty acid composition of the tissue lipids is markedly altered when rats are maintained on diets free of essential fatty acids (29-32). This alteration is manifested primarily by a lowering of the level of the C18:2 and C20:4 acids (29, 30). When the animal is maintained on a fat-free diet or on a diet deficient in essential fatty acids, this metabolic pathway is diminished or does not operate due to the low level or absence of fatty acids of the w-6 series.

If compositions of the lipid extracts from the livers and hearts of the rats receiving the three diets are compared, one can see that the rats on the lard and fish oil diets presented changes in the concentration of certain acids. There was an increase in the levels of C16:1 and C18:1 acids both in the livers and the hearts of the lard and fish oil groups, and a decrease of the C18:0, C18:2 and C20:4 acids in the livers of the fish-oil group, C18:2 and C20:4 in the livers of the lard group, and C18:2 and C20:4 in the hearts of the lard group. The C18:2 fatty acid composition of the hearts roughly paralleled its composition in the diet. This leads one to believe that, although the rats receiving fish oil had a lower weight gain than the lard group, they demonstrated a fatty acid composition of the liver and heart lipids which was approximately characteristic of an essential fatty acid deficiency. The fatty acid compositions of the liver and heart lipids of the fish oil group, however, were more characteristic of an essential fatty acid deficiency than the other two groups. This confirms the lower feed efficiency of the fish oil diet which leads to a reduction in the growth of the rats of this group. The reduced growth and alteration in the levels of the polyunsaturated fatty acids may be due to a deficiency of the C18:2-w6 or a possible imbalance between C18:2-w6 and C18:3-w3 (33).

The polyunsaturated fatty acids of the cardiac muscle are more sensitive to modifications in the fatty acid composition of the diet than any other organ (34, 35). The increase in availability of the C22:6-w3 results in an increase of this acid in the phospholipids and a smaller increase in the neutral lipids. The lipid extract from fish contains a large proportion of this fatty acid. Of the long chain fatty acids, C22:6 was the most abundant in the lipid extract of the rat hearts of the fish oil group. This increase of C22:6-w3 may have important consequences for cellular metabolism. There are many ways in which variations in lipid composition of a cellular membrane may influence the functions of a cell or an organ (34, 36).

RESUMEN

COMPOSICION Y EFECTOS DIETETICOS DEL ACEITE DE PESCADO "MANDI" *(Pimelodus clarias)*

Se llevó a cabo un estudio para determinar la composición química del aceite de "mandi" *(Pimelodus clarias)*. Los hallazgos revelaron que éste acusa una baja propor-

ción de ácidos grasos esenciales C18:2 y un alto porcentaje de ácido oleico (29.93o/o); también contiene ácidos grasos poli-insaturados con más de 18 átomos de carbono. De los ácidos grasos de cadena larga, C22:6 está presente en más alto porcentaje (1.94o/o). Al alimentar a ratas con una dieta cuya fuente de lípidos provenía de aceite de mandi, se encontró que éstas acusaban una reducción de crecimiento en comparación con el de animales que consumieron la dieta control (aceite de maíz). La composición de ácidos grasos del hígado y del corazón de las ratas del grupo experimental presentaban modificaciones. La mayor variación se constató en el porcentaje de C22:6 del músculo cardíaco, en el que se observó un incremento de cinco veces.

Puede ser que la reducción del crecimiento y la alteración en los niveles de los ácidos grasos poli-insaturados se deba a una posible deficiencia en C18:2-w6 o a una falta de equilibrio entre C18:2-w6 y C18:3-w3.

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