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Environmental and dietary effects on the composition and metabolism of lipids in fish Practical effects of research

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By
Hossam Mohamed Kotkat
B.Sc. Vet. Med.
M.Sc. Vet. Med. Biochemistry

Under the supervision of
Prof. Dr. Nemcsok Janos
D.Sc.
Department of Biochemistry
Jozsef Attila University

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CONTENTS

ABBREVIATIONS

AA	**Arachidonic acid**
BHT	**Butylated hydroxytoluene**
CL	**Cardiolipin**
Chl	**Cholesterol**
DHA	**Docosahexaenoic acid**
EPA	**Ecosapentaenoic acid**
HDL	**High-density lipoprotein**
HPLC	**High-performance liquid chromatography**
LDL	**Low-density lipoprotein**
MUFAs	**Monounsaturated fatty acids**
PA	**Phosphatidic acid**
PC	**Phosphatidylcholine**
PE	**Phosphatidylethanolamine**
PG	**Phosphatidylglycerol**
PI	**Phosphatidylinositol**
Pls	**Polar lipids**
PL	**Phospholipid**
PS	**Phosphatidylserine**
PUFAs	**Polyunsaturated fatty acids**
SFAs	**Saturated fatty acids**
Sn-1	**Stereospecific numbering at the no.1 carbon atom in the glycerol backbone**

Sn-2	**Stereospecific numbering at the no.2 carbon atom in the glycerol backbone**
TG	**Triacylglycerol**
TLC	**Thin-layer chromatography**
UFAs	**Unsaturated fatty acids**
UI	**Unsaturation index = ∑ (number of double bonds % of total fatty acids)**
VLDL	**Very-low density lipoprotein**
Polyene index A	**∑% 18:3 + 20:5 + 22:6 per 14:0 + 16:0 + 18:0**
Polyene index B	**∑% unsaturated fatty acids per 14:0 + 16:0 + 18:0**
∑SFA	**Sum of 14:0, 16:0 and 18:0**
∑MUFA	**Sum of 16:1 n-7, 18:1 n-9 and 20:1 n-9**
∑n-3 PUFA	**Sum of 18:3, 20:5, 22:5 and 22:6**
∑n-6 PUFA	**Sum of18:0, 20:2, 20:3, 20:4, 22:4 and 22:5**

INTRODUCTION

For years we have been told that cholesterol (Chl) is a major killer and that we should avoid foods high in Chl, such as eggs. This has been extremely damaging to the egg industry. Many national heart foundations have recognized their serious error and are now allowing 4 eggs to be consumed each week per person. Today, we know that dietary Chl is not an important risk factor in heart disease for 98% of the population. There are many other factors that are more important, such as the amount and nature of the fat in the diet. The Greenland Eskimos consume almost twice as much Chl in their diet as do their neighbors the Danes, but the incidence of deaths from heart disease is almost 7 times higher among the Danes. The answer lies, in part, in the source and nature of the fat consumed. If we examine the diets of large populations, we find that those who consume significant amounts of fish on a regular basis, throughout their life, generally have a reduced incidence of deaths from heart disease.

Nutritional scientists completely overlooked the fact that there was another family of polyunsaturated fatty acids (PUFAs) that were equally important. These are the omega-3 (n-3) PUFAs found largely in fish, but also in some vegetable oils, such as rapeseed, soybean and in large quantities in linseed oil. The precursor (alpha-linolenic acid), which can be used to build the important omega-3 PUFA, is found in a number of vegetable oils.

In summary, dietary n-3 PUFAs reduce the (n-6) PUFAs levels of cells, with the concurrent replacement with eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), thereby decreasing the availability of arachidonic acid (AA) for eicosanoid synthesis. Both EPA and DHA are effective inhibitors of cyclooxygenase and lipooxygenase, and when released from the phospholipid (PL) pools they effectively inhibit the amounts of eicosanoid produced.

The extensive use of herbicides and pesticides has become very wide spread in recent decades and the incidence of environmental contamination and pollution has therefore increased. It is washed by the rain into the soil and natural waters, where it is rapidly accumulated in the different organisms (Gluth et al., 1985). The direct effect of these pollutants may result, for example, in the death of fish or provide the superoxide radical (O_2^-) which has destructive effects on the animal tissues and influences the reoxidation of reductase enzymes (Mezes et al., 1997).

The aims of the present study:

1- To compare the n-3 fatty acid compositions of poikilothermic and homothermic animals.

2- To explore the useness of fish oil as a source of EPA (20:5 n-3), to assess the safety of long-term fish oil consumption for the prevention and treatment of human disease.

3- To examine the dietary incorporation of n-3 fatty acids into egg yolk to produce an omega-3 fortified egg.

4- To obtain an insight into the mechanisms of action of an insecticide (deltamethrin), a herbicide (atrazine) and a petroleum derivative (phenol) on the omega-3 fatty acids of the fish erythrocyte plasma membrane.

LITERATURE SURVEY

2.1. Epidemiological study on the function of omega-3 PUFAs:

Epidemiological examinations of the health effect of n-3 PUFAs began with studies conducted in a coastalsettlement of Greenland Eskimos in the 1970s. It was observed that the intake of long-chain, marine n-3 PUFAs was about 7.0 g/day among these people, compared with less than 0.06 g/day in most western diets (Bang and Dyerberg, 1985). This group of about 2,400 Eskimos had an incidence of ischemic heart disease that was estimated to about 8% of that seen in a comparable population in Denmark. Plasma lipid and lipoprotein levels were measured in a representative group of these Eskimos and compared with those in a matched group of Danes. The results showed that the Chl level was 21% lower, the triacylglycerol (TG) level was 63% lower, the very low density lipoprotein (VLDL) level was 76% the low density lipoprotein (LDL) level was 12% lower, and the high density lipoprotein (HDL) level was 50% higher in the Eskimos as compared to the Danes.

The beneficial effects of fish oil are mediated by its high contents of long-chain omega-3 fatty acids, mainly EPA (20:5 n-3) and DHA (22:6 n-3). n-3 PUFAs compete with AA (20:4 n-6) for the C-2 position of PLs, possibly reducing the formation of vasoconstrictor, prothrombotic and pro-inflammatory metabolites. Fatty acids of the n-3 family are also known to inhibit the desaturation of n-6 fatty acids (Huang et al., 1992). Subsequent studies have shown that a considerable lowering of plasma lipids, particularly TG, by inhibiting the synthesis and secretion of TG-rich lipoprotein particles by the liver, an increased beta-oxidation, a decreased activity of esterifying enzymes and changes in the ratios of fatty acid esters formed are all mechanisms by which fish oil may inhibit TG and subsequently VLDL production (March et al., 1987). Decreased hepatic phosphatidate phosphohydrolase activity has been suggested as a possible site of the fish oil effect (March et al., 1987). The de novo synthesis of phosphatidylcholine (PC) is required for VLDL secretion and changes in

cytidylytransferase activity could account for a decreased VLDL output (Halminski et al., 1991). It has recently been recognized (Farrell, 1993 and 1996) that the intake of n-3 fatty acids is significantly ($P \leq 0.01$) increased in HDL and significantly ($P \leq 0.01$) declined in LDL.

2.2.Dietary supplementation of marine fish oil rich in n-3 PUFAs to:

2.2.1. Freshwater fish:

The lipid metabolism of fish has been investigated for many years and the experiments showed no substantial differences from that of mammals. According to the literature, the long-chain PUFAs of fish lipids are derived from the diet, and they are excreted from the body in a short time if the diet does not contain these fatty acids. It was also found that fish were able to form these fatty acids from linoleic and linolenic acids in the same biochemical pathway as for mammals, and the formation of these fatty acids was relatively slow. Furthermore, a definite amount of linolenic acid in the diet is essential for fish; otherwise, the animals grow more slowly and they are more sensitive to diseases.

The dynamics of membrane structure and function point to the complex role of lipids in environmental adaptation. The interrelationships between dietary fatty acids, membrane integrity and metabolic pathway are particularly evident in poikilothermies since the lipid state in these animals, by definition, is in constant flux (Green and Selivonchick, 1987). However, it has been established that the liver is the major site of fatty acid synthesis in fish and, in contrast with mammals, very little, if any, synthesis takes place in the adipose tissue. Radiolabelling studies have proven the elongation and desaturation pathways of dietary n-9, n-6 and n-3 fatty acids in fish, which appear to differ from those in rats only in the significant production of dead-end elongation products (Green and Selivonchick, 1987).

Several nutritional studies have shown that fish do not possess the Δ_{12} and Δ_{15} desaturases necessary to produce the PUFAs 18:2 (n-6) and 18:3 (n-3), respectively, from endogenously synthesized 18:1 (n-9). Consequently, both series are likely to be essential for normal growth and survival, although the role of the n-6 series is generally less certain (Henderson and Tocher, 1987).

The requirement for C_{18} PUFAs is associated with the ability of fish to convert them by desaturation and elongation to longer-chain (C_{20} and C_{22}) PUFAs. Marine fish seem to lack one or more of the desaturases necessary to accomplish this process (Sargent et al., 1989). The total rate of elongation and desaturation of dietary shorter-chain PUFAs, however, is likely to be regulated by a number of dietary and environmental factors.

The dietary influence on the rate of the metabolism of C_{18} and C_{22} PUFAs in freshwater fish is less well described. As in mammals, the desaturation and elongation activity of 18:3 (n-3) in fish is higher than that of 18:2 n-6. Long-chain PUFAs, especially those of the n-3 series, may also exert feed-back inhibition on the desaturation of C_{18} PUFAs (Olsen et al., 1990). Feeding with a PUFA deficient diet reduces the Δ_5 desaturation as compared to that in fish maintained on a PUFA-supplemented diet, and the Δ_6 desaturation was reduced only in fish fed C_{18} PUFAs (Olsen and Ringo, 1992).

2.2.2. Rats:

When EPA (20:5 n-3) is fed to rats, the 20:5 n-3 and DHA (22:6 n-3) levels are increased in the membrane PLs. It not clear whether 22:6 n-3 increases in the membrane solely as a consequence of the in vivo conversion of 20:5 n-3, to 22:6 n-3, or because most sources of 20:5 n-3 also contain some 22:6 n-3 (Venkatraman et al., 1992). Recent experiments have used various dietary levels of 18:3 n-3 to discern a dietary threshold level of 0.4 to 2.4% of energy to produce significant changes in membrane 22:6 n-3 and 22:5 n-6 levels. A low

intake of dietary n-3 fatty acids has been associated with learning deficits in rats, and with visual abnormalities in both rats and monkeys (Bazan, 1990). Harris (1989) showed that EPA deficiency led to a greater accumulation of liver triacylglycerols (TG) and cholesterol esters (CE) and to greater changes in the fatty acid pattern of liver lipids. Rats maintained on diets rich in carbohydrates and poor in fats, displayed an elevated rate of fatty biosynthesis and accumulated besides 20:3 n-9 high amounts of oleate in their lipids (Demeyer et al., 1974).

2.2.3. Laying hens:

The egg is a much maligned food, despite its excellent concentration of essential nutrients and balance of amino acids. The concern over the consumption of animal fat and the incidence of heart disease in humans has focused on the negative aspects of the egg. In fact, the PUFA/SFA ratio (P/S) in egg lipid is almost 0.6, and well above that recommended for a diet. However, there are contrary views. A typical 60 g hen's egg contains about 6 g of fat. Of this, 1.2 g is PUFAs, 2.7 g is monounsaturated fatty acids (MUFAs) and only 2.1 g is SFAs (Farrell, 1993). It has been recognized only recently that the PUFAs consist of two distinct families, the omega (n)-3 and the omega (n)-6, which are found in vegetable oils. It is therefore important for human health to maintain a dietary balance between the n-6 and n-3 PUFAs.

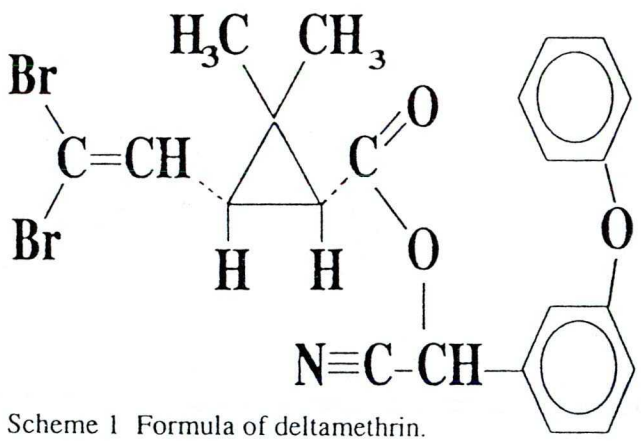
A typical Western diet has a ratio of > 25:1; the ideal ratio is about 5:1. An imbalance is of great concern and suggests that the consumption of fish, our main dietary source of n-3 PUFAs, is declining, and with this some aspects of human health. It is well known that the PUFAs content of the hen's egg can be manipulated, and there is therefore an opportunity to enrich the hen's egg with n-3 PUFAs. The results of the experiments of Farrell (1996) demonstrated that it is possible to introduce into the hen's egg substantial concentrations of the n-3 fatty acids without altering its acceptability.

It is generally accepted that HDL is a beneficial form of Chl, since it has the capacity to transfer Chl from the cells to the liver, where it is metabolized. The increase ($P \leq 0.05$) in HDL over time is a positive attribute of eggs. Furthermore, the consumption of only one of these eggs per day can significantly reduce the ratio of n-3/n-6 and not change the plasma Chl level (Harris, 1989; Fernandez et al., 1992).

2.3. Effects of some environmental pollutants on the PL metabolism in fish:

2.3.1. Effects of the insecticide deltamethrin:

Deltamethrin [or decamethrin, DM; type II pyrethroid; (s)-alpha-cyano-3-phenoxybenzyl (1R,3R)-cis-2,2-dimethyl-3-(2,2-dibromovinyl) cyclopropane carboxylate] ($C_{22}H_{19}Br_2NO_3$; Scheme 1) is a powerful pyrethroid insecticide which exhibits very toxicity to aquatic organisms.



Scheme 1 Formula of deltamethrin.

DM was synthesized in 1974, and first marketed in 1977. The consumption of DM in the world was about 250 tons in 1987. It is mostly used on cotton, on crops such as coffee, maize, cereals, fruit and vegetables and on stored products. DM is also used in animal health, in vector control and in public health. In Egypt is widely used for plant protection and for the control of ectoparasites in farm animals (WHO, 1990).

During the metabolism of DM in fish, in contrast with other animals the ester cleavage of this compound is very slow; the main metabolic pathway is hydroxylation to give the 4-hydroxyphenoxy derivative, which is excreted in the bile as glucuronide (Edwards and Millburn, 1985). The presence of the derivatives is generally connected with the increased activity of enzymes, whose main role is to eliminate the multiplied radicals or derivatives during metabolism of similar chemicals.

The clinical signs of DM toxicity are tremor and salivation in animals (Ray and Cremer, 1979). In fish (especially at higher dosages of DM), sings of toxicity are irregular movement of the operculum and disturbances the coordination of swimming (Szegletes et al., 1995).

In field experiments, the insecticide Glossinex-cocktail (endosulfan / deltamethrin) proved to be highly toxic to freshwater fish, affected on fatty acid concentration in liver and on acetylcholinesterase (AchE) activity in brain, muscle and liver of juvenil tilapia rendalli rendalli (Pisces Cichlidac) (Knauer et al., 1993). Ghosh and Banerjee (1992) found that catfish (*Heteropneustes fossilis*) exposed to lethal and prolonged sublethal concentrations of DM exhibited striking alteration in haemopoietic tissue smears and blood parameters.

The in vitro and in vivo effects of the insecticides DM on acetylcholinesterase and some biological blood parameters, such as the levels of glutamicoxaloacetic transaminase (GOT), lactate dehydrogenase (LDH) and glucose in different tissues were examined in adult carp (*Cyprinus carpio L.*). The kinetic results showed that the inhibition of brain acetylcholinesterase, while the GOT and LDH activities increased 2.5 fold and 1.5 fold as compared with those of the control carp. Also, the blood glucose level was 30% higher than that of the control fish (Balint et al., 1995).

Recently a few studies have been published demonstrating the most significant change caused by DM pollutant was the prolonged elevation of the level of phosphorylation on a number of key synpatic proteins (Calcium-calmodulin dependent protein kinase and synapsin I) beyond the normal time of their recovery to the dephosphorylated state (Matsumura et al., 1989). Also demonstrating that the immunosuppresive effect of DM, Ashry and El-Ghareib (1997) who found that the insecticide DM suppressed both cell mediated immunity and antibody forming ability of lymphocytes.

2.3.2. Effects of the herbicide atrazine:

In 1952, Geigy in Basle, Switzerland, started investigations with triazin derivatives as potential herbicides. Their working hypothesis was based on the fact that the triazine molecule inserted into relatively complex compounds yields compounds with similar properties. In plants, the herbicide atrazine (2,4-D methoxychloro-carbonyl) has been shown to inhibit growth and chlorophyll formation (Abou-Wally et al., 1991a). The effects of the herbicide on the lipid metabolism has not been as extensively studied. It has been observed that atrazine inhibits the accumulation of all cellular phospho- and glycolipids blocks de novo fatty acid biosynthesis through the blocking of acetyl-CoA carboxylase (Burgastahler and Lichtenthaler, 1984), and has a preferential oxidative effect on the unsaturated fatty acids (USFAs) (El-Sheekh et al., 1994). Freshwater fish are normally exposed to a great variety of pollutants in the surface water in industrial areas. The uptake and degradation of herbicides by organs of fish have been investigated in several ways (Hanke et al., 1983; Gluth et al., 1985).

Gluth et al. (1985) determined the internal bioaccumulation factor (Bf) of atrazine in fish: it was 3-4 in the liver, kidney and intestine, but 1 in the blood, muscle and gills. This could be explained by the increase of the lipophilic nature, and accumulation within the muscle tissues is always lower than that in the liver.

Several other biochemical changes in response to external pollution with atrazine are described by Hanke et al. (1983), such as atrazine-induced changes in the cortisol and glucose levels in the serum, variations in the amount of liver glycogen and the liver function, and changes in the activities of gill ATP-ase and acetylcholinesterase in the brain and serum of carp.

2.3.3. Sublethal effects of phenol:

Recently, the rapid development of the chemical industry has resulted in the contamination of the environment, and particularly of the aquatic system. Phenolic compounds are among the important chemical pollutants present in industrial drains. These compounds arise from oil refineries, chemical plants, livestock dips, human and animal wastes and the use of herbicides (Nemcsok and Benedesky, 1990). They are the main causes of river pollution in several countries (Nemcsok and Benedesky, 1990). Phenolic wastes consist largely of pure phenol, and affect fish by their direct toxicity to aquatic life. Being mainly oxidizable, they can also contribute to depletion of the dissolved oxygen concentration of polluted waters and the introduction of a bad taste and odour to the edible flesh of fish. Exposure to sublethal levels of phenolic wastes has been noted to evoke a variety of lesions, such as gill necrosis, degenerative changes in the muscle and also various inflammatory degenerative and necrotic changes in the heart, liver and spleen (El-Manakhly and Soliman, 1993).

MATERIALS AND METHODS

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3. Materials and Methods

3.1. Materials

Experimental animals:

The following animals were used in this study:

Table 1 .

Animal species	Common name	Scientific name	Type of diet	Origin
Freshwater fish	**Tilapia spp.**			River Nile at Behera Edfina
	Nile tilapia	Oreochromis miloticus	Benthic algae	
		Oreochromis aureus	Benthic feeder on algae and detritus	
	Cyprineda			
	Common carp	Cyprinus carpio L.	Benthic feeder	
	Silver carp	Hypophthalmichythys nolitrix	Phytoplankton	
	Grass carp	Chenopharyngodon idellus	Omnivorous, but mostly a predator	
	Sharptooth			
	Cat-fish	Clarias gariepinus	Predator carnivorous	
	Bagrus bayad	Clarias lazera	Predator carnivorous	
	Eel (Hamash)	Anguilla anguilla	Small fish	
	Pike	Esox lucius L.		
	Denies	Sparus aurata	Small crustaceans and molluscs	
Marine fish	**Gilt - Sardine**	Sardinalla aurita	Benthic on algae and diatoms	Coastal line of Mediterr-anean Sea
	Pilchard	Sardina pilchardus		
	Sea bream	Dicentrachus	Small fish	
	Sea bass	Labrax	Crustaceans and mulluscs	
	Sole (Mousa)	Solea solea	Diatoms	
	Grey mullets	Mugil cephalus	Algae	
Poultry	**Laying hens (pullets)**	Fayoumi	Commercial poultry meal (finisher rations)	
Lab. animals	**Rats**	Male albino rats	Lab. pellets	

1. Collection of fish:

In Egypt, there are three main regions for the collection of marine fish; all are located along the coastal line of the Mediterranean Sea, around or near lake mouths or river mouths. These regions are Mex, Rosetta and Damietta. Freshwater fish were collected from their natural habitats and nearest farms. The three pelagic sardine spp. were caught by purse-serine. The water temperature varied according to the season.

2. Dietary supplementation of fish oil to:

2.1. Freshwater fish

A group of young tilapia (Oreochromeous niloticus), mean weight 5 g, caught in autumn, were fed a semisynthetic fat-free diet for 3 months and were then divided into four groups in twelve circular tanks each measuring 38.5 cm in diameter and 41 cm high). One group was kept on a fat-free diet, while the others received fatty acid-supplemented diets (cat-fish feed, marine oil or active EPA-30) for the next 2 months, as shown in Table 2.

Table 2.

Percentage compositions of the experimental diets of Tilapia

Ingredients g/100 g	Fat-free diet	Cat-fish pellets	Marine oil	Active EPA-30
Starch	68.0	65.0	65.0	65.0
Fish meal	25.0	25.0	25.0	25.0
Salt mix	5.0	5.0	5.0	5.0
Glycine	1.6	1.6	1.6	1.6
Choline	0.3	0.3	0.3	0.3
Inositol	1.0	1.0	1.0	1.0
Vitamin mix	0.2	0.2	0.2	0.2
Cotton seed oil	--	3.0	--	--
Cod liver oil	--	--	3.0	--
Active EPA-30	--	--	--	3.0

In the case of the fat-free diet, all the diet contents were acetone-extracted by the Soxhlet method.

Vit.mix mg/g diet, thiamine: 6, Ca-pantothenate: 25, pyridoxine: 8, niacine: 7, folic acid: 25, β-aminobenzoic acid: 3, vit. B_{12}:6, α-tocopherol: 1.0, menadione: 1.5, vit. A: 33 and vit. D_3: 0.4.

At the end of the experiment, the fish were killed, edible parts from each group were extracted and lipid analyses were performed.

2.2. Rats:

Experimental I:

Sixty healthy (3-month old) young male albino rats weighing 110-160 g were housed in separate cages (22x36x15 cm) throughout the experimental period (2 months) and were allowed food and water ad libitum. The rats were randomly assigned into three groups.

One group was fed a semi-purified diet containing 5% pig fat. The second group received a modified diet in which 50% of the pig fat was replaced by active EPA-30, while the group fed on lab pellets (Table 3) acted as control animals. The fatty acid composition of the diets is presented in Table 4.

Experimental II

The same experiments were repated using (18-months old) old male albino rats, weighing 250-300 g.

Table 3.

Percentage compositions of experimental diets of rats

Ingredients g/100 g	Lab pellets	Pig fat	Active EPA-30
Casein	20.0	20.0	20.0
L-Methionine	0.30	0.30	0.30
Vit. mix	1.00	1.00	1.00
Salt mix	3.50	3.50	3.50
Choline bitartrate	0.20	0.20	0.20
Inositol	0.025	0.025	0.025
Cellulose	5.00	5.00	5.00
Sucrose	25.00	25.0	25.0
Corn-starch	40.00	39.5	42.5
Soybean	5.00	--	--
Pig-fat	--	5.50	--
Active EPA-30	--	--	2.50

(a) The vit. mix provided per kilogram final feed:

Vit. A: 4,000,000 IU, vit. D_3: 1,000,000 IU, vit. E: 300,000 IU, vit. K: 0.5 mg, niacine: 20 mg, riboflavin: 3 mg, thiamine: 4 mg, vit. B_{12}: 0.05 mg, choline chloride: 1800 mg.

(b) The mineral mix provide (% / kg premix):

Na: 30.6, Cu: 0.13, Mn: 0.65, Cl: 47.3, Co: 0.002, Zn: 0.003, Phosph: 3.6, Fe: 0.58, Ca: 6.8 (Alexandria Pharm. Chem. Ind. Co.).

Table 4.

Fatty acid compositions of experimental diets (% w/w)

Fatty acids %	Control diet (soy oil)	Deficient diet (pig fat)	Repletion diet Active EPA-30
14:0	0.65	5.71	5.2
16:0	15.69	22.97	15.7
16:1 n-7	0.8	0.57	7.81
18:0	4.05	2.6	2.5
18:1 n-9	21.79	10.21	15.8
18:2 n-6	45.41	59.51	1.4
18:3 n-6	----	---	2.0
18:3 n-3	4.37	0.3	8.5
20:0	0.18	0.27	----
20:2 n-6	0.28	0.24	0.28
20:3 n-6	0.58	0.04	0.60
20:4 n-6	0.62	0.4	1.45
20:4 n-3	----	----	2.71
20:5 n-3	----	----	20.0
22:4 n-6	0.12	----	0.62
22:5 n-6	0.32	----	1.77
22:5 n-3	----	----	2.19
22:6 n-3	----	----	10.83
Total n-3	4.37	0.3	44.11
Total n-6	47.33	60.15	8.0
$\sum$n-3/$\sum$n-6	0.09	0.005	5.51
Sat/Unsat	0.20	0.46	0.30

2.3. Laying hens:

A total of 120 pullets from Fayioumy (17 weeks old) were housed in a conventional three-tier laying battery (3 birds/cage of 40x40x44 cm) throughout the experimental period (2 months). They were divided into three groups (three replicates of 10 birds for each group) and fed on semi-purified diets (Table 4) for

at least 2 months. For the control group, the fat source was soybean oil which contained certain amounts of both linoleic and linolenic acids. For the supplemented animals, the fat sources were EPA (20:5 n-3) and DHA (22:6 n-3). The first supplemented group was switched to a fish oil replacing 50% of the soybean oil, and the second supplemented group to a fish oil replacing 100% of the soybean oil. The fatty acid composiotion of the experimental oils and diets are represented in Table 5.

Table 5.

Composition (%) and chemical analysis of the experimental diets of pullets

Item ingredients g/100 g	Control basal diet	Chemical analysis (%)	
White corn, ground	63.90	Me (kcal/kg)	2855
Soybean meal	20.44	Moisture	9.86
Wheat bran	5.00	CP	18.0
Fish meal	5.00	EE	3.85
Bone meal	1.43	CF	3.52
Cotton seed oil	3.00	NFE	59.7
Active EPA-30	---	Ash	5.04
Limestone, ground	0.48	Ca	1.00
Common salt	0.50	Lysine	1.02
Premix	0.25	Methionine	0.36

Premix (supplied per kilogram final feed)

Vit. A: 10,000 IU, vit. D_3: 2,500 IU, vit. E: 11 mg, vit. K: 1 mg, vit. B_2: 5 mg, vit. B_6: 3 mg, choline chloride: 500 mg, pantothenic acid: 11 mg, folic acid: 1 mg, niacine: 30 mg, vit. B_{12}: 10 mg, biotin: 75 mg, Mn: 60 mg, Zn: 45 mg, Fe: 60 mg, Cu: 5 mg, Se: 0.1 mg (Fizer premix).

Table 6.

Fatty acid compositions of experimental oils and diets (percent of total fatty acids)

Fatty acid % w/w	Experimental oils		Experimental diets		
	Fish oil (Active EPA-30)	Cotton seed oil	Basal diet	Repletion diets (Active EPA-30)	
				diet 1	diet 2
14:0	5.20	7.70	4.40	4.36	4.33
16:0	15.4	10.7	14.4	14.47	14.54
16:1 n-7	7.70	----	----	0.11	0.23
18:0	2.50	4.20	2.10	2.07	2.04
18:1 n-9	15.1	23.7	25.6	25.47	25.34
18:2 n-6	1.40	53.4	51.6	50.82	50.34
18:3 n-3	8.50	0.30	1.90	1.98	2.06
20:4 n-6	1.40	----	----	0.02	0.04
20:4 n-3	2.70	----	----	0.04	0.08
20:5 n-3	20.0	----	----	0.30	0.60
22:5 n-6	0.60	----	----	0.01	0.02
22:5 n-3	1.70	----	----	0.02	0.05
22:6 n-3	10.8	----	----	0.16	0.32
Total n-3	42.7	0.30	1.90	2.50	3.11

Basal diet, mixture of 3% of cotton seed oil and 0.85% other oils

Repletion diet (1), mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% other oils.

Repletion diet (2), mixture of 3% active EPA-30 + 0.85% other oils.

3.1. Fish and environmental pollution:

The carp (*Cyprinous carpio L.*) which were used in our experiments (150-200 g) were purchased from the Alexandria Governorate fish farm and acclimatized to laboratory conditions for 2 weeks in large tanks at about 18 °C with filtered tap-water and fed daily on commercial pellets (Table 6). They were brought into the test aquaria (100 L) 2 weeks before the experiment started. Each aquarium contained 9 fish. The fish were not fed for 2 days before or during the experimental period.

Table 7.

Composition (%) and chemical analysis of carp diet

Feed ingredients (%)		Chemical analysis (%)	
Fish meal	27.0	Crude protein	34.00
Soybean meal	40.0	Crude fat	6.00
Wheat milling by-product	29.0	Crude fiber	3.90
Oil	3.0	Ash	13.68
Vitamin mix	0.5	Nitrogen-free extract	42.40
Mineral mix	0.5		

Vit.mix: each kg contains vit. A, 2,000,000 IU; vit. D, 200,000 IU; vit. E, 10,000 IU; vit. K, 0.5 g; vit. B_1 0.89 g; vit B_2, 3.6 g; vit. B_6, 3 g; vit. B_{12}, 0.02 g; panathonic acid, 10 g, nicotinic acid, 27 g; folic acid 0.55 g; biotin, 0.15 g, choline 130 g.

Mineral mix: each kg contains Mn 60 g; Fe,80 g; Cu, 59 g; Zn, 40 g; Se, 0.15 g and I, 0.35 g.

The pollutants used in these experiments were of the purest chemical grades commercially available. The durations of treatment were 48 and 96 hr for all pollutiontants.

The pesticide deltamethrin [or decamethrin, DM; type II pyrethroid; (s) -α-cyano-3 phenoxybenyl- (1R)-cis-2,2-dimethyl -3- (2,2-dibromovinyl) cyclopropane-carboxylic acid] ($C_{22}H_{19}$ Br_2 NO_3) was used at concentrations of 0, 0.5, 1 and 2 μg/L.

The herbicide atrazine (triazine derivatives) was used at concentration of 0, 10, 100 and 1000 μg/L.

Phenol (oil refineries) was used at concentrations of 0, 5, 10 and 20 μg/L.

3.2. Methods:

1. **Collection and preparation of samples**: The animals were killed by vertebral rupture and their organs muscle or livers were rapidly removed, cleaned from accessory connective and adipose tissues and washed with Tris-sucrose solution to remove blood. The net weights of the tissues were measured.
2. **Isolation of erythrocyte plasma membrane**: This was carried out according to Sorensen (1990). Following an overnight fast, blood samples were collected from polluted fish tail vein, into vacutainer tubes containing acid citrate dextrose as a preservative (Becton Dickinson, Rutherford, N.J.), and stored at 4°C for 1-3 h. The blood was washed with phosphate-buffered saline (mM), NaCl (145); Kcl (5); Na_2PO_4 (5); pH 7.4 and filtered through cotton wool to remove platelets and leukocytes followed by gentle centrifugation for 10 min. The sample was washed and centrifuged twice more and any remaining buff coat aspirated with the supernatant. The erythrocytes were gently pelleted and the supernatant discarded. The pellet was washed twice more and 4 ml of phosphate buffer, pH 7.4, was added and the mixture was wortexed for 5 minutes, followed by sonication in bath type sonicator for 20 minutes and recenterfuged and the supernatant discarding and the depost was extracted according to Folch et al., (1957).

3. Lipid analysis:

Extraction of total lipids: These were extracted according to Folch et al. (1957). The tissues was homogenized with a 2:1 v/v chloroform-methanol mixture to a final value 20 times the weight of the wet tissue sample.

Separations of polar lipids (Pls): The phospholipid (PL) pools were purified by silicic acid column chromatography, using chloroform to elute neutral lipids and methanol for PL.

Separation of PL subfractions: These were subfractionated by TLC on precoated G-60 silica gel TLC plates (F254, Merck, Darmstdt, Germany). The spots were identified by using authentic standards (Serdary Res. Lab., Bellefonte, Cal., USA). The spots were detected under UV at 254 nm and removed for quantitative determination of phosphorus according to Rouser et al. (1970).

Determination of fatty acid composition: The fatty acid compositions of total lipids or PL subfractions were transesterfied in the presence of dry methanol containing 5% HCl at 80°C under CO_2 or N_2 within 2-3 hr. The methyl esters were separated using a Hewlett- Packard 5890 II gas chromatography, equipped with a capillary column coated with SP2330 of 0.25m thickness (0.25mm I.D. x 30mi CPS-Li Quadrex, New Haven, CT, U.S.A.). High- purity nitrogen was applied as carrier gas with a pressure of 230 KPa. Hydrogen was used at 100 KPa and 280 KPa. The dual column system was programmed from 160°C to 200°C to give partial separation of 18:3 n-3 and 20:1 n-9 at a flow rate of 1°C/min. The detector temperature and injector temperature were 250°C and 230°C, respectively. The peaks were identified by means of primary and secondary standards and by plotting log relative elution temperature versus the number of carbon atoms (Schmit and Wynner, 1966). The percentage composition was calculated as weight percentage (%w/w) using a Hewlett-Packard 3396A integrator. All peaks between myristic acid (14:0) and DHA (22:6 n-3) were included in the calculations.

Separation of molecular species and quantitation by HPLC:

The PE was hydrolysed with phospholipase from Bacillus cereus (Sigma Chemicals, St. Louis, MO) by a modification of the method of Takamura et al. (1986). Dinitrobenzol derivatives was prepared and separated by HPLC (water associated model 440) on a Nucleosyl C-18 column (5 μm, 4 mm i.d x 250 mm), using acetonitrile - propanol (80:20 v/v) of HLPC grade. Carlo Erba, Milano, Italy) as mobil phase with a flow rate of 1.0 ml/min, pressure 54 kg/cm^2, temp. 250°C).

4. Other biochemical measurements:

Plasma total lipids: These were determined calorimetrically by using the sulphophosvanillin reaction as described by Frings et al. (1972).

Plasma TG: This was determined with the TG kits of Sclavo, by the methods of Young et al. (1975).

Plasma total Chl: This was measured by using the Chl kits of Sclavo, according to Watson (1960).

Plasma PLs: These were measured by the method of Connerty et al. (1961).

HDL: This was estimated by using the kits of Sclavo, according to Lopes-Virella et al. (1977).

VLDL: This can be calculated in mg/dL:

$$= \frac{\text{plasma TG}}{5}$$

LDL: This can be calculated in mg/dL:

= total Chl (VL + HDL).

Statistical analysis:

The data obtained were subjected to Statistical Analysis System (SAS, 1994 microcomputer version). The least significant difference test was applied to compare means among the treatments.

RESULTS AND DISCUSSION

4. Results and Discussion

4.1. Comparative study of the fatty acid compositions of poikilothermic and homothermic animals:

4.1.1. Fatty acid compositions of poikilothermic Pls:

The fatty acid compositions of the liver Pls in the freshwater and marine fish are listed in (Table 8). There are considerable differences between the fish species, reflecting the differences in their diets and temperature environments. This is especially true for linoleic acid (18:2 n-6), AA (20:4 n-6), EPA, (20:5 n-3) and DHA (22:6 n-3). The share of the monoene oleic acid (18:1 n-9) was elevated in freshwater fish, suggesting their ability to introduce the n-9 double bond [Δ9 desaturase in stearic acid (18:0)], or this might be ascribed to a more intensive conversion of 18:0 to 18:1 n-9 (Blond et al., 1981). The total concentration of n-6 PUFAs was significantly higher ($P \geq 0.05$), but the total n-3 PUFA level and the n-3/n-6 ratio were significantly lower ($P \geq 0.05$) in the freshwater fish as compared with the marine fish (Borlongan and Benitez, 1992; Saito et al., 1997). The low level of n-3 PUFAs in the freshwater fish might be explained by the absence or scarcity of 18:3 n-3 in their diets, this acting as a precursor of those acids (Olsen et al., 1990).

4.1.2. Fatty acid compositions of homothermic Pls:

The fatty acid compositions of homothermic (laying hens and rats) livers are shown in (Table 8). In hens, the highest proportion consisted largely of MUFAs (45%). The proportion of omega (n)-3 PUFAs was generally lower than those in freshwater and marine fish.

It is clear from the same Table that, in rats the SFAs 16:0 and 18:0 were the predominant fatty acids, comprising about 39% of the total fatty acids. Linoleic acid (18:2 n-6) and AA (20:4 n-6) were found to be major constituents in rats, with concentrations of 17% and 27%, respectively.

Table 8.

Fatty acid compositions of Pls of the most important poikilothermic and homothermic livers (means ± S.E.)

Fatty acids w/w %	Poikilothermic		Homothermic	
	Freshwater fish	Marine fish	Pullets	Rats
14:0	2.89 ± 0.38	2.35 ± 0.52	0.31 ± 0.04	1.61 ± 0.30
16:0	17.60 ± 1.50	15.96 ± 1.64	26.04 ± 0.33	20.31 ± 1.71
16:1 n-7	8.25 ± 0.99	5.98 ± 1.02	2.82 ± 0.30	2.33 ± 0.76
18:0	6.59 ± 0.91	5.61 ± 1.05	17.22 ± 0.27	17.06 ± 0.11
18:1 n-9	23.11 ± 2.98	15.35 ± 1.37	32.38 ± 1.90	7.55 ± 2.42
18:2 n-6	6.86 ± 1.26	1.80 ± 0.22	11.87 ± 0.59	17.07 ± 1.78
18:3 n-3	0.60 ± 0.01	3.08 ± 0.36	0.12 ± 0.01	0.31 ± 0.17
20:4 n-6	7.42 ± 1.50	4.30 ± 0.96	8.70 ± 0.32	27.01 ± 2.90
20:5 n-3	6.11 ± 0.96	13.59 ± 1.28	0.17 ± 0.08	0.73 ± 0.25
22:5 n-6	1.62 ± 0.25	0.89 ± 0.22	0.24 ± 0.03	0.60 ± 0.20
22:5 n-3	3.80 ± 0.50	2.27 ± 0.56	0.16 ± 0.01	0.10 ± 0.12
22:6 n-3	9.63 ± 1.28	25.67 ± 2.48	2.83 ± 0.28	6.94 ± 0.73
n-3/n-6	1.31 ± 0.16	10.83 ± 1.97	0.15 ± 0.28	0.18 ± 0.10

The data for freshwater fish are the mean values for common carp, grass carp, silver carp, tilapia nilotica, tilapia zilli, eel, pike, cat-fish, bagrus-bayad and denies.

The data for marine fish are the mean values for gilt-sardine, pilchard, sea bream, sea bass, sole and grey mullets.

The given values are average of 9 speciements(±S.E). Only the most common fatty acids are listed.

4.2. Dietary supplementation with fish oil rich in n-3 PUFAs:

4.2.1. Effect of dietary supplementation of n-3 PUFAs on freshwater fish PL metabolism:

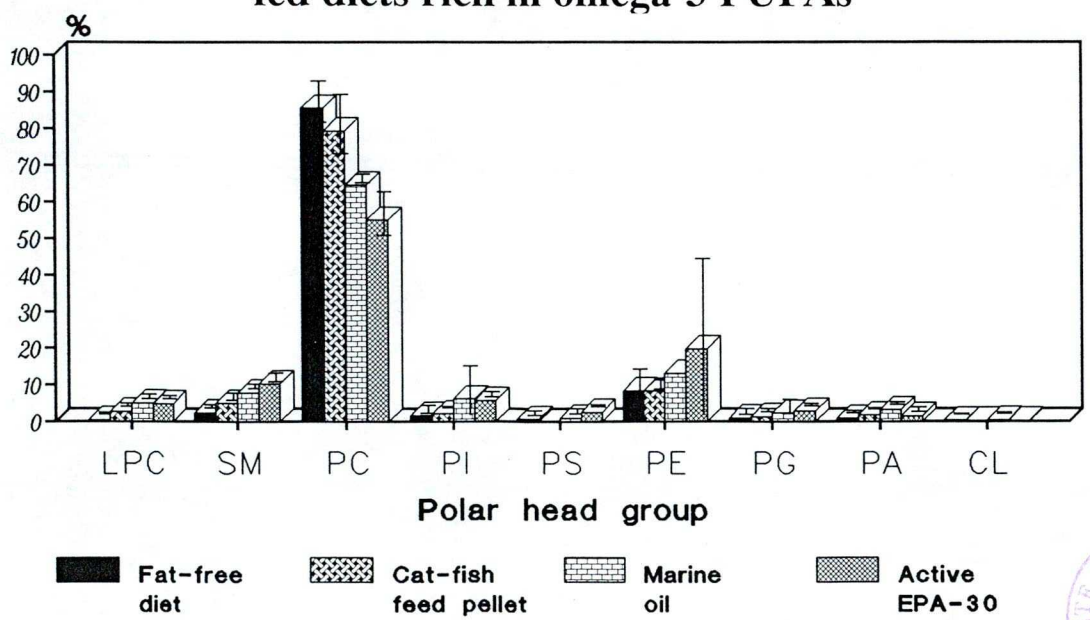
I Polar head group composition of fish flesh:

The PL compositions of edible parts of tilapia fed on diets containing different levels of n-3 PUFA show some general patterns (Figure 1). It may be seen from this Figure that PC and PE are the major Pl subclass, and there are significantly higher percentages of PE, SM and PI in the fish fed on fish oil and active EPA-30 as compared with the other diets (fat-free diet and cat-fish pellets). It has been proposed that for the stability of the bilayer, the level of wedge-shaped PL (PE) in this case is required to incorporate more UFAs into the membranes (Dey et al., 1993; Kotkat et al., 1993).

Figure 1.

Pl class contents of tilapia

fed diets rich in omega-3 PUFAs



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II Fatty acid compositions of fish flesh Pl:

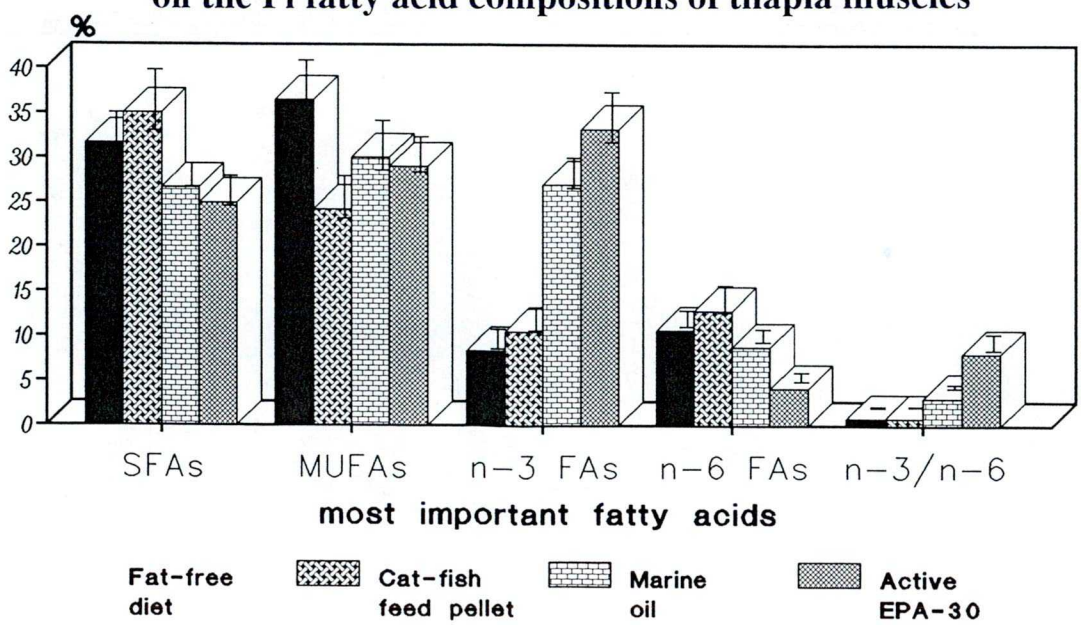
The fatty acid compositions of the edible parts (dorsal muscle) of fish fed on diets containing different levels of n-3 PUFA are listed in (Figure 2). As expected, the dietary fatty acid composition greatly affects the composition of the fish flesh. There were some changes of considerable interest. The fat-free diet group showed relative to the cat-fish feed pellet group less 16:0 and 18:0, but more 18:1 n-9. The significantly higher levels of 18:1 n-9 and 18:2 n-6 in the control group (cat-fish pellets) fish muscle apparently resulted from their abundance in the diet.

The effectiveness of the fat-free diet feeding regime in depleting long-chain (n-3) PUFA from muscle lipids was evident from the large decrease in the n-3/n-6 PUFA ratio in PLs.

The considerably higher level of linolenic acid in the fish oil diet resulted not only in a generally high level of 18:3 n-3, but also in higher levels of long-chain n-3 fatty acids (20::5 n-3 and 22::6 n--3). This indicates that dietary 18:3 n-3 had been extensively elongated and desaturated into long-chain n-3 PUFA by tilapia (Olsen and Ringo, 1992; Craig et al., 1995). N-3 PUFA enriched diets may also enhance osmotic resistance, general performance and disease resistance (Mourente and Rodriguez, 1997).

Figure 2.

Influence of the dietary supplementation of n-3 PUFAs on the Pl fatty acid compositions of tilapia muscles



III Molecular species composition of fish flesh PL (PE):

The molecular species of ethanolamine glycerophospholipids in the fish flesh fed diet containing different levels of n-3 PUFAs are shown in (Table 9). A total of 22 molecular species were identified and quantified. There were 5 dipolyunsaturated species in the n-3 series (i.e. containing an n-3 fatty acid in the Sn-2 position), 5 species in the n-9 series and only one containing SFAs in both Sn-1 and Sn-2 positions 16:0/18:0. In the fish feeding on fish oil, the proportion of species containing n--3 PUFAs was increased from 4.9% to 34.6% and they synthesized a new molecular species not found in the n-3 PUFA-deficient diet (fat-free and cat-fish pellets) fed fish: 18:0/20:5 n-3. The total species of the n-6 series decreased to very low levels. Fish oil also significantly increased the proportion of 18:1/20:4 and total species containing Sn-1 18:1 at the expense of 16:0. Substitution of 16:0 by 18:1 in the Sn-1 position further accentuates the coincidental of PE; the introduced cis double bond of these fatty acids may be regarded as a factor affecting membrane behavior (Fodor et al., 1995). Didocosahexaenoyl species were major components of PE from fish muscle fed n-3 PUFA enriched diets (marine oil and active EPA-30) accounting for 9.2 and 13.37 of total respectively. The present analytical results agree with the findings of Chen and Claeys (1996); Bell et al., (1997) who reported that 22:6/22:6 was the major components of cod and rainbow trout organs. Also, determination of the molecular species composition of PE revealed a 2 - fold and 6 - fold increase in the level of 18:1/22:6 and 18:1/20:5 species, respectively. It is concluded that it is not the gross amount of long-chain PUFAs in PL, but rather their specific combination with cis delta 9 monounsaturated fatty acids in the position Ssn-1, especially in PE, that is important in determining the physical properties of biomembranes (Dey et al., 1993; Fodor et al., 1995; Yeo et al., 1997).

Table 9.

Molecular species compositions of PE from tilapia muscle fed on n-3 PUFAs

Fatty acids %	Fat-free diet	Cat-fish feed pellet	Marine oil	Active EPA-30
20:5/20:5	---	---	1.6	1.5
20:5/22:6	1.87	1.1	8.5	9.2
22:6/22:6	1.6	2.0	9.2	13.3
22:5/22:6	1.3	1.9	2.9	3.6
22:5/22:5	0.14	2.3	1.1	0.8
Total PUFA/PUFA	4.92	7.3	23.3	34.6
16:0/20:5	1.4	5.0	8.1	4.2
16:0/22:6	19.62	16.0	12.7	8.0
16:0/20:4	2.45	0.6	2.1	1.9
16:0/22:5	2.6	---	1.5	1.1
18:0/20:5	---	---	0.95	1.5
18:0/22:6	29.10	31.3	4.8	3.0
18:0/20:4	3.19	2.2	0.8	0.5
18:0/22:5	2.89	2.9	3.5	0.7
Total SFA/PUFA	62.70	56.8	36.5	20.9
18:1/20:5	1.3	1.8	6.5	8.0
18:1/22:6	17.32	15.9	26.2	30.6
18:1/20:4	2.74	2.3	3.7	1.9
18:1/22:5	5.48	4.4	1.6	1.0
Total MUFA/PUFA	26.84	27.0	36.0	43.5
16:0/16:1	---	0.7	1.1	0.4
16:0/18:0	---	1.7	0.5	0.1
18:0/18:1	1.87	3.3	0.5	---
Total SFA/MUFA	1.87	5.7	2.1	0.5
16:1/16:1	3.61	2.2	1.2	---
18:1/18:1	---	---	0.7	0.5
Total MUFA/MUFA	3.61	2.1	1.9	0.5
18:1/22:5/18:0/22:5	1.9	1.52	0.46	0.27
18:1/22:6/18:0/22:6	0.55	0.35	5.87	10.02

4.2.2. Effect of dietary supplementation of n-3 PUFAs on homothermic (rat) lipid metabolism:

I Polar head group composition of rat liver:

The data presented in Table 10 show that in the liver of young rats fed a diet supplemented with active EPA-30, the choline moiety (LPC, SM and PC) and PS are lower, while the PE and PI levels are higher than in the pig fat fed rats. In contrast, the liver of old rats, has larger amounts of SM and CL, but lower amounts of lysophosphatidylcholine (LPC) and PA in active EPA-30 supplemented rats compared to the pig fat fed rats.

Hargreaves and Clandinin (1988) reported that feeding of a fish oil diet to rats resulted in an increased microsomal membrane content of PE species of six double bonds, and rendered them more ordered to molecular interaction in the polar head region; they displayed great metabolic importance in the membrane structure and function, with subsequently increased growth in rates, and also led to a decreased level of lecithin cholesterol acyltransferase.

There is a fundamental difference between PE and PC in their molecular shapes: the former are conical, while the latter are cylindrical. As concerns the accumulation of PE in fish oil fed rats, it has been proposed that an elevated level of conical shaped molecules is required to maintain the functional integrity of biomembranes.

Table 10.

PL class contents (% wt) in liver of rats fed diets containing different levels of essential fatty acids (omega-3 fatty acids) compositions

	Old rats			Young rats		
	Control diet (soy oil)	Deficient diet (pig fat)	Repletion diet (Active-EPA-30)	Control diet (soy oil)	Deficient diet (pig fat)	Repletion diet (Active-EPA-30)
LPC	2.13 ± 0.5^{A}	1.53 ± 0.3^{B}	0.43 ± 0.1^{C}	2.08 ± 0.2^{A}	1.28 ± 0.3^{B}	1.01 ± 0.1^{C}
SM	2.22 ± 0.6^{A}	3.22 ± 0.65^{B}	5.98 ± 1.0^{C}	2.24 ± 0.3^{A}	1.33 ± 0.3^{B}	2.83 ± 0.3^{B}
PC	36.87 ± 2.8^{A}	45.05 ± 6.17^{B}	36.41 ± 2.35^{A}	30.6 ± 4.2^{A}	34.06 ± 3.45^{A}	25.55 ± 0.3^{B}
PI	9.28 ± 1.6^{A}	11.36 ± 1.2^{B}	8.28 ± 2.09^{A}	16.86 ± 2.0^{A}	12.54 ± 2.3^{B}	19.22 ± 1.3^{C}
PS	2.69 ± 0.9^{A}	1.57 ± 0.3^{B}	2.24 ± 0.4^{A}	3.56 ± 0.4^{A}	2.44 ± 0.2^{B}	5.52 ± 0.91^{C}
PE	32.21 ± 5.1^{A}	33.56 ± 2.4^{A}	39.5 ± 5.15^{A}	29.34 ± 2.5^{A}	24.68 ± 2.7^{B}	33.87 ± 3.2^{C}
PG	1.34 ± 0.3^{A}	0.18 ± 0.0^{B}	1.03 ± 0.1^{A}	8.18 ± 1.0^{A}	4.41 ± 1.3^{B}	9.19 ± 1.7^{A}
PA	1.09 ± 0.2^{A}	0.71 ± 0.3^{B}	0.31 ± 0.1^{C}	5.56 ± 0.75^{A}	13.43 ± 2.04^{B}	1.63 ± 0.1^{C}
CL	1.14 ± 0.2^{A}	2.56 ± 0.52^{B}	5.34 ± 0.8^{C}	1.67 ± 0.00^{A}	3.96 ± 0.35^{B}	1.14 ± 0.2^{C}

The given values are average of 6 specimens (±S.E). Means within the same row between the different treatments {control (soy oil), pig fat and active EPA-30} with the same superscript are not significantly different at ($P \leq 0.05$).

II PL fatty acid composition of rat livers:

1- Fatty acid composition of Pls from liver of young rat:

The changes in major n-3 and n-6 fatty acids in the liver lipids (total lipid and total and major individual phospholipid) in response to changes in the n-3 fatty acid in the diet of young rats are shown in (Table 11). In rats fed on a n-3 PUFA-deficient diet (pigfat), there was a drastic decrease in the amount of omega-3 fatty acids especially EPA (20:5 n-3) and DHA (22:6 n-3), and compensatory increases in SFAs (16:0) and n-6 PUFAs, AA (20:4 n-6). In contrast, in rats fed on a diet rich in n-3 PUFAs (active EPA-30) there was a complementary enrichment with n-3 PUFA (20:5 n-3 and 22:6 n-6). It was observed that the reduction of 20:4 n-6 in the rats fed a diet rich in n-3 PUFA may reflect a reduced conversion from 18:2 n-6, and an increasing dietary intake of 20:5 n-3 and 22:6 n-3 was shown to inhibit the activity of Δ6 and Δ5 desaturase (Venkatraman et al., 1992). The level of 22:6 n-3 reached a maximum very rapidly when only a small amount of n-3 fatty acids was included in the diet (Huang et al., 1992).

The finding that rats fed a diet (pigfat) containing relatively low amounts of both 20:5 n-3 and 22:6 n-3 can be interpreted in that long-term pig fat nutrition can produce some metabolic imbalance, and also suggests a very low capacity of the liver to convert 18:3 n-3 to 22:6 n-3.

Table 11.

Fatty acid composition (% w/w) of total lipids, total phospholipids, phosphatidylcholine and phosphatidylethanolamine of liver of young rats fed diets containing different levels of omega-3 fatty acids

Fatty acids	Total lipids			Total phospholipids			Phosphatidylcholine			Phosphatidylethanolamine		
	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)
16:0	22.07 ± 2.0^{A}	25.53± 2.10^{B}	18.60± 0.31^{C}	20.31± 1.71^{A}	24.01 ± 3.1^{B}	17.76± 0.88^{C}	19.49± 3.72^{A}	21.9 ± 3.10^{A}	19.75± 1.12^{A}	21.06± 2.46^{A}	24.10 ± 3.2^{A}	18.34± 0.81^{B}
16:1 n-7	3.77 ± 1.40^{A}	4.27 ± 1.10^{A}	1.46 ± 0.82^{B}	2.33 ± 0.76^{A}	4.23 ± 0.80^{B}	0.72 ± 0.19^{C}	3.17 ± 1.22^{A}	3.75 ± 1.62^{A}	1.28 ± 0.38^{B}	1.47 ± 0.78^{A}	2.80 ± 1.1^{B}	0.6 ± 0.23^{C}
18:0	10.3 ± 1.96^{A}	13.38 ± 2.7^{B}	9.24 ± 1.20^{A}	17.06± 0.11^{A}	8.40 ± 2.11^{B}	17.75± 0.84^{A}	11.13± 0.11^{A}	9.10 ± 1.26^{B}	13.36± 1.79^{A}	15.71± 1.18^{A}	17.11 ± 1.6^{A}	17.9±2.08^{BA}
18:1 n-9	9.74 ± 0.60^{A}	8.11 ± 0.91^{B}	8.78 ± 0.87^{AB}	7.55 ± 2.42^{A}	9.40 ± 1.65^{A}	5.32 ± 0.77^{A}	8.16 ± 0.74^{A}	9.30 ± 0.59^{B}	6.89 ± 0.90^{C}	6.31 ± 2.11^{A}	6.00 ± 0.71^{A}	5.03 ± 0.58^{A}
18:2 n-6	21.74± 2.43^{A}	17.34 ± 1.3^{B}	23.02 ± 3.1^{A}	17.07± 1.78^{A}	23.15 ± 3.3^{B}	16.50± 1.52^{A}	25.11± 4.31^{A}	27.07± 4.13^{A}	20.85 ± 1.8^{B}	11.66± 1.68^{A}	13.60 ± 2.0^{A}	11.81± 1.61^{A}
18:3 n-6	0.14 ± 0.10^{A}	------	trace	trace	------	trace	trace	------	trace	0.51 ± 0.07^{A}	------	trace
18:3 n-3	0.27 ± 0.12^{A}	------	1.21 ± 0.10^{B}	0.31 ± 0.17^{A}	------	0.93 ± 0.15^{B}	0.50 ± 0.00^{A}	------	0.93 ± 0.12^{B}	0.35 ± 0.12^{A}	------	0.85 ± 0.00^{B}
20:4 n-6	24.63± 1.41^{A}	25.63 ± 2.3^{A}	11.67± 2.46^{B}	27.01 ± 2.9^{A}	28.13 ± 4.1^{A}	15.90 ± 2.1^{B}	25.26± 5.12^{A}	25.42± 3.00^{A}	15.20± 1.06^{B}	29.83± 1.03^{A}	30.58± 4.01^{A}	13.24± 2.44^{B}
20:4 n-3	------	------	------	------	------	------	------	------	------	------	------	------
20:5 n-3	0.42 ± 0.18^{A}	------	9.13 ± 0.48^{B}	0.73 ± 0.25^{A}	------	8.81 ± 1.92B	0.52 ± 0.44^{A}	------	8.55 ± 0.89B	0.54 ± 0.28^{A}	------	9.33 ± 3.66B
22:4 n-6	0.40 ± 0.10^{A}	0.68 ± 0.30^{A}	0.30 ± 0.1^{AB}	0.30 ± 0.23^{A}	0.35 ± 0.00^{A}	0..30 ± 0.20^{A}	0.40 ± 0.14^{A}	0.50 ± 0.20^{A}	0.42 ± 0.20^{A}	0.38 ± 0.20^{A}	0.45 ± 0.20^{A}	0.28 ± 0.00^{A}
22:5 n-6	0.50 ± 0.20^{A}	1.01 ± 0.30^{B}	0.71 ± 0.3^{BA}	0.60 ± 0.20^{A}	0.22 ± 0.10^{B}	------	0.31 ± 0.20^{A}	0.20 ± 0.20^{A}	trace	0.46 ± 0.20^{A}	0.74 ± 0.10A	0.17 ± 0.10B
22:5 n-3	0.20 ± 0.10^{A}	1.35 ± 0.20^{B}	2.75 ± 0.60^{C}	0.10 ± 0.12^{A}	------	2.26 ± 0.57B	------	------	1.52 ± 0.78	1.16 ± 0.30^{A}	------	3.00 ± 0.62B
22:6 n-3	5.82 ± 0.54^{A}	2.32 ± 0.30^{B}	13.14± 0.62C	6.94 ± 0.73^{A}	2.11 ± 0.30^{B}	13.75± 1.08^{C}	5.92 ± 0.39^{A}	2.76 ± 0.63^{B}	10.91± 0.19^{C}	10.56± 1.42^{A}	4.62 ± 1.42^{B}	19.4 ± 0.84^{C}
n-3/n-6	0.14 ± 0.00	0.08 ± 0.02	0.45 ± 0.01	0.18 ± 0.00	0.04 ± 0.01	0.79 ± 0.03	0.14 ± 0.00	0.05 ± 0.01	0.60 ± 0.02	0.29 ± 0.01	0.10 ± 0.00	1.28 ± 0.25
sat/unsat	0.48 ± 0.48	0.64 ± 0.30	0.39 ± 0.01	0.59 ± 0.10	0.48 ± 0.13	0.55 ± 0.10	0.44 ± 0.02	0.45 ± 0.02	0.49 ± 0.02	0.58 ± 0.04	0.70 ± 0.05	0.57 ± 0.05
UI	199.1±24.2^{A}	178.0±22.5^{A}	267.2±25.9^{B}	200.6±16.4^{A}	187.6±19.6^{A}	245.4±30.5^{B}	207.8± 18.3^{A}	188.4±20.1^{A}	231.9±30.5^{A}	229.0±21.3^{A}	191.5±20.2^{B}	265.6±31.7^{C}

Values are the average of 6 specimens. Means within the same row between the different treatments {control (soy oil), pig-fat and active EPA-30} with the sar superscript are not significantly different at (P≥0.05).

2- *Fatty acid composition of Pls from liver of old rats:*

Total lipids and total and individual phospholipid fatty acid composiitions, in livers of the old rats fed a diet with low and high n-3 fatty acids content (pig fat and active EPA-30 respectively), are shown in (Table 12). The major changes, in liver lipid fatty acid composition, were a slight reduction in the content of 20:5 n-3 and 22:6 n-3 as compared to the rats fed active EPA-30. The decrease in the 22:6 n-3 or 20:5 n-3 was compensated for by an increase in the n-6 fatty acids, 20:4 n-6 and to a lesser extend to 18:2 n-6 (Lin et al., 1990). The total content of PUFAs in liver lipids was similar for both diet (low and supplemented EPA-30).

However, in active EPA-30 deficient diet, there was a slight increase in the total PUFA (n-6). The levels of AA were slightly lower in pig fat fed rats than in the active EPA, but there were no significant differences between them, indicating that the liver content of this fatty acid in old rats is relatively resistant to large changes in the n-3 fatty acids in the diets.

Table 12 shows also, that n-3/n-6 ratio in the liver lipids of aged rats did not respond to the increase in the n-3 fatty acids content of the diet. This might be due to that the accumulation of n-3 fatty acids may depend on the age as well as the activity of desaturation which tends to decrease with aging (Kotkat et al., 1997, 1999).

A significant changes of the ratio of 20:4 n-6/18:2 n-6 in liver PLs indicates that there was no further inhibition on the conversion of 18:2 n-6 to 20:4 n-6 in these dietary groups as a results of inactivation of phospholipid synthesis acyltransferase in old rats (Phillips et al., 1969; Mourente and Tocher, 1992).

Table 12.

Fatty acid composition (% w/w) of total lipids, total phospholipids, phosphatidylcholine and phosphatidylethanolamine of liver of old rats fed diets containing different levels of omega-3 fatty acids

Fatty acids	Total lipids			Total phospholipids			Phosphatidylcholine			Phosphatidylethanolamine		
	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)	Control diet (soy oil)	Deficient diet (Pig-fat)	Repletion diet (Active EPA-30)
16:0	25.66± 2.74^{A}	24.10 ± 2.5 ^{A}	25.07± 1.98^{A}	23.30± 1.31^{A}	24.10 ± 2.4 ^{A}	21.94± 1.43^{A}	25.01± 1.84^{A}	26.17 ± 3.4^{A}	25.01± 3.02^{A}	23.48± 2.09^{A}	25.21 ± 2.4^{A}	21.35±1.36^{AB}
16:1 n-7	1.29 ± 1.49^{A}	2.12 ± 1.0 ^{B}	0.84 ± 0.75 ^{C}	0.56 ± 0.76 ^{A}	1.21 ± 0.30 ^{B}	0.37 ± 0.28 ^{A}	0.94 ± 1.27 ^{A}	1.15 ± 0.30 ^{A}	0.65 ± 0.35 ^{A}	0.71 ± 0.49 ^{A}	2.11 ± 0.20 ^{B}	0.51 ± 0.46 ^{A}
18:0	10.32± 1.61^{A}	13.75± 1.40^{B}	12.23± 1.53^{A}	18.75± 1.19^{A}	21.22± 1.90^{B}	17.58± 0.82^{A}	11.63± 2.19^{A}	16.01± 2.01^{B}	11.11± 2.57^{A}	16.03± 2.44^{A}	19.41 ± 2.9^{A}	16.2 ± 1.66^{A}
18:1 n-9	7.75 ± 1.46 ^{A}	4.75 ± 1.20 ^{B}	6.20 ± 0.94 ^{A}	3.02 ± 0.33 ^{A}	2.85 ± 0.61 ^{A}	3.57 ± 0.75 ^{A}	3.45 ± 0.83 ^{A}	4.00 ± 0.50 ^{A}	5.18 ± 2.16 ^{A}	2.54 ± 0.21 ^{A}	2.60 ± 0.30 ^{A}	3.23 ± 0.54 ^{A}
18:2 n-6	25.28± 6.11^{A}	26.02± 4.41^{A}	22.21± 3.39^{A}	13.33± 0.52^{A}	13.3 ± 1.76 ^{A}	15.02± 0.72^{B}	15.95± 1.43^{A}	15.10 ± 2.0^{A}	16.01 ± 0.8^{A}	9.56 ± 0.84 ^{A}	10.70 ± 1.2^{A}	10.48± 0.64^{A}
18:3 n-6	0.27 ± 0.10 ^{A}	------	0.57 ± 0.49 ^{B}	0.15 ± 0.10 ^{A}	------	0.42 ± 0.40 ^{A}	0.22 ± 0.15 ^{A}	------	0.75 ± 0.72 ^{B}	0.07 ± 0.01 ^{A}	------	0.29 ± 0.25 ^{B}
18:3 n-3	trace	------	------	-----	------	------	------	------	------	------	------	------
20:4 n-6	20.37 ± 3.5 ^{A}	21.42 ± 2.1 ^{A}	25.16± 3.25^{B}	29.79± 1.72^{A}	29.19 ± 4.2 ^{A}	31.11± 2.75^{A}	31.08± 1.56^{A}	30.11± 4.61^{A}	31.76± 6.06^{A}	30.35 ± 2.7^{A}	29.05± 3.17^{A}	33.01± 1.42^{A}
20:4 n-3	trace	------	0.10 ± 0.10 ^{A}	------	trace	------	0.49 ± 0.10 ^{A}	------	------	------	------	------
20:5 n-3	0.43 ± 0.46 ^{A}	------	0.32 ± 0.18 ^{A}	0.37 ± 0.34 ^{A}	------	0.34 ± 0.23 ^{A}	0.47 ± 0.39 ^{A}	------	0.49 ± 0.27 ^{A}	0.38 ± 0.33 ^{A}	------	0.39 ± 0.24 ^{A}
22:4 n-6	0.51 ± 0.10 ^{A}	0.61 ± 0.00 ^{A}	-----	------	0.59 ± 0.10	------	0.50 ± 0.20 ^{A}	0.51 ± 0.20 ^{A}	0.50 ± 0.10 ^{A}	0.48 ± 0.20 ^{A}	0.50 ± 0.10 ^{A}	------
22:5 n-6	0.30 ± 0.20 ^{A}	0.30 ± 0.20 ^{A}	-----	trace	0.41 ± 0.20	------	0.20 ± 0.00 ^{A}	0.35 ± 0.10 ^{B}	0.41 ± 0.1^{BC}	0.31 ± 0.00 ^{A}	0.11 ± 0.00 ^{B}	------
22:5 n-3	0.21 ± 0.10 ^{A}	------	032 ± 0.10 ^{A}	-------	------	trace	------	------	------	------	------	------
22:6 n-3	7.61 ± 1.37 ^{A}	6.93 ± 0.67 ^{A}	7.08 ± 0.64 ^{A}	11.73± 2.52^{A}	7.13 ± 0.91 ^{B}	9.65 ± 0.42 ^{A}	10.05± 2.19^{A}	6.30 ± 1.01 ^{B}	8.13 ± 0.53 ^{A}	16.08± 3.67^{A}	10.31 ± 1.1^{B}	14.54± 0.65^{A}
n-3/n-6	0.18 ± 0.00	0.14 ± 0.01	0.61 ± 0.01	0.28 ± 0.01	0.16 ± 0.00	0.21 ± 0.00	0.23 ± 0.02	0.14 ± 0.00	0.17 ± 0.01	0.40 ± 0.01	0.26 ± 0.01	0.34 ± 0.00
sat/unsat	0.56 ± 0.02	0.61 ± 0.03	0.60 ± 0.00	0.27 ± 0.20	0.83 ± 0.12	0.65 ± 0.03	0.58 ± 0.04	0.73 ± 0.13	0.57 ± 0.20	0.65 ± 0.05	0.81 ± 0.20	0.60 ± 0.05
UI	194.29 ± 14^{A}	199.81 ± 21^{A}	199.81 ± 24^{A}	222.08 ± 30^{A}	194.61 ± 18^{A}	218.94 ± 27^{A}	228.88 ± 25^{A}	197.38 ± 20^{B}	222.42 ± 24^{A}	245.83 ± 32^{A}	206.72 ± 17^{B}	246.80 ± 34^{A}

Values are the average of 6 specimens. Means within the same row between the different treatments {control (soy oil), pig-fat and active EPA-30} with the sar superscript are not significantly different at (P≥0.05).

III Distribution of molecular species in membrane PE from liver of young rats:

The changes in the distribution of all the molecular species of the diacylethanolamine glycerophospholipid in the liver of rats fed the three different diets are shown in (Figure 3). When rats were fed the n-3 fatty acids deficient diet (pig fat), the species containing n-3 fatty acids decreased to 5.1% of total, compared to 15.1% in the control (soy oil) rats. The amount of decreases was similar for all species containing an n-3 fatty acid. Conversely, the n-6 series incresed to 68.7%, with the species containing 20:4 n-6, 22:4 n-6 and 22:5 n-6 responsible for all of the increase.

Fish oil (active EPA-30) feeding to the n-3 deficient rats increased the proportion of species containing an n-3 fatty acid from 4.5% to 34%. This n-3 enrichment of the liver was much higher than in control rats (15.1%).

Furthermore, the results in Figure 3 show that 16:0/20:4 and 18:0/20:4 were the most abundant molecules (26% and 16%, respectively) and there was no altered pairing of these acids. In the active EPA-30 fed rats, the molar amounts of these species was declined, while the ratios of 16:0/22:6 to 18:0/22:6 and 16:0/20:5 to 18:0/20:5 were significantly increased. In addition, the levels of 18:1/22:6, 18:1/20:5 and 18:1/20:4 species were increased 2-fold, 4-fold and 2-fold respectively.

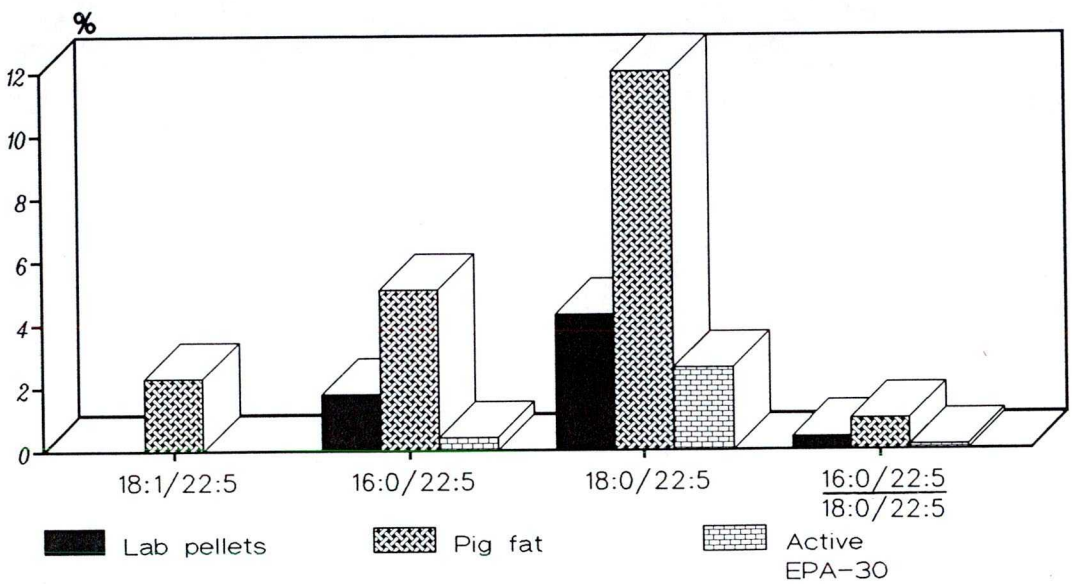
Phospholipids are an important of the lipid bilayer of the cell membrane. Different PL molecular species would be expected to have different metabolic and physical properties, and the distribution of molecular species was found to be closely associated with cell membrane fluidity, function and the activity of membrane bound enzymes. (Salem et al., 1986). The substantial diet induced changes in the phospholipid molecular species composition seen in the present study indicate a significant turnover of rats Pls. This turn over must have resulted from either degradation and resynthesis or deacylation reacylation of Pls (Dawson, 1985).

Attempting to ascertain the metabolic pathway responsible for the observed changes, we calculated the ratios of 18:0/22:6 to 16:0/22:6 and 18:0/22:5 to 16:0/22:5 for the various diets. From this calculation, it seems possible that the decrease of the 22:6 n-3 species and the corresponding increase of the 22:5 n-6 species seem in animals on the deficient diet may be due to simple replacement of 22:6 with 22:5 through deacylation-reacylation.

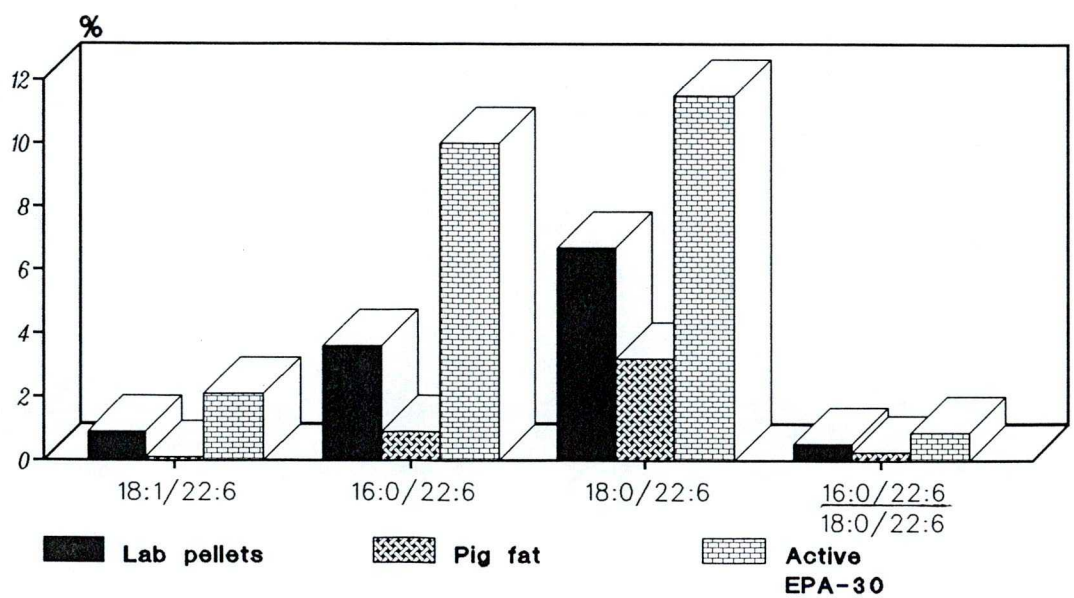
Figure 3.

Diet induced changes in the proportion of diacyl ethanolamin glycerophospholipid molecular species containing (A) 22:5 n-6 and (B) 22:6 n-3

(A) 22:5 n-6



(B) 22:6 n-3



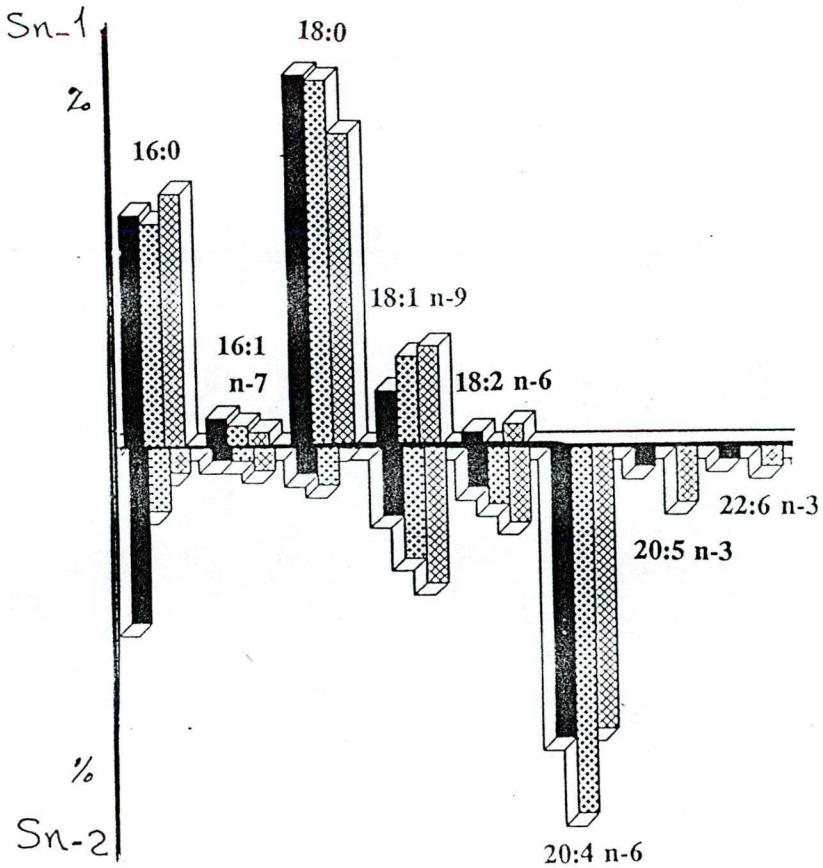
IV Positional distribution of fatty acids of PE from the liver of young rats:

The data in Figure 4 reveal that about 50% of the molecular species of this fraction contain AA (20:4 n-6) at the Sn-2 (β) position. In both groups (control and treated) of animals, SFAs 16:0 and 18:0 are predominant in the Sn-1 (α) position, while PUFAs are accumulated in the Sn-2 position. It is striking that the dietary supplementation of active EPA-30 enhanced oleic acid (18:1 n-9) in the Sn-1 position at the expense of stearic acid, and the most drastic effect was the absence of stearic acid. The PUFAs show a clear preference for the β-position. These acids (20:5 n-3 and 22:6 n-3) are typical of fish oil. They are of linolenic type; they cannot be synthesized by animals, but are derived from the diet.

Explicitly, the linkage of essential fatty acids to the β-position of glycerol would play the role of a reservoir for the PUFAs and protect them from oxidation and maintain the membrane integrity. Finally, the positions of the double bonds of these acids may be considered to be a factor affecting membrane behavior (Dey et al., 1993 and Foder et al., 1995).

Figure 4 .

Fatty acid distribution in Sn-1 (α) and Sn-2 (β) positions of PE (mol %) for rats fed different levels of omega-3 fatty acids



4.2.3. Effect of dietary supplementation with n-3 PUFA on lipid metabolism of laying hens (pullets):

I Lipoprotein metabolism:

The effects of fish oil (active EPA-30) on the plasma lipids and lipoprotein levels of laying Fayuomi pullets receiving a basal diet as control group or two different active EPA-30 concentrations (1.5 and 3.0%) (as treated groups) are summarized in (Table 13). As shown in this Table, the total plasma lipids, Chl, LDL, VLDL and TG were significantly ($P \leq 0.01$) decreased in pullets feeding on fish oil rich in n-3 PUFAs as compared to the control group, while the total PLs and HDL were significantly ($P \leq 0.01$) increased. Such decreases and increases in these parameters agree with the results of El-Shazly et al. (1995) and Farrell (1996), who found that the linolenic acid (18:3 n-3) of linseed oil has the same effectiveness in reducing blood lipids and lipoprotein levels.

Table 13.

Effect of active EPA-30 supplementation on plasma lipids and lipoproteins in Fayoumi pullets.

mg/dl	Control	Repletion diets (Active EPA-30)	
	Basal diet	Diet (1)	Diet (2)
	Mean ± S.E.	Mean ± S.E	Mean ± S.E
Total lipid	558.52 ± 9.19^{A}	507.22 ± 4.48^{B}	522.67 ± 6.20^{C}
Phospholipids	142.82 ± 2.12^{C}	171.26 ± 2.44^{B}	183.81 ± 4.32^{A}
Triacylglycerol	171.43 ± 2.30^{A}	137.58 ± 2.06^{B}	100.78 ± 3.68^{C}
Cholesterol	243.82 ± 8.27^{A}	198.38 ± 4.61^{B}	195.88 ± 2.36^{B}
HDL	73.24 ± 2.08^{C}	99.44 ± 3.27^{B}	138.48 ± 3.20^{A}
(LDL	136.39 ± 6.70^{A}	68.37 ± 4.47^{B}	38.10 ± 0.75^{C}
VLDL	34.29 ± 0.46^{A}	27.52 ± 0.41^{B}	20.20 ± 0.75^{C}

The given values are average of 6 speciemens (±S.E). Means within the same row with the same superscript are not significantly different at ($P \leq 0.01$).

The reduction in hen's plasma TG by dietary active EPA-30 is suggested mainly from the inhibition of hepatic VLDL production (Popp-Snijders et al., 1987), and reduction of hepatic synthesis and secretion of TG by decreasing the activity of acyl-coenzyme A and 1,2-diaclglycerolacyl transferase as well as increasing proximal beta oxidation activity (Yamazaki et al., 1987).

The highly significant ($P \leq 0.01$) decrease in plasma Chl, LDL and VLDL caused by supplementation of active EPA-30 was more clearly apparent with the use of the higher concentration (3%) than with the lower ones (1.5%) (Table 13). This appears to be in close agreement with the findings of Fernandez et al., (1992) who concluded that, the diet containing PUFA lowered the LDL-cholesterol concentration in normal subjects. They attributed these findings to the increase in the expression of hepatic receptor for LDL, increased LDL apo-B fraction catabolic rate and decreased LDL-apo-B production. Harris, (1989) reported that, the VLDL-Cholesterol significantly reduced after supplementation with salmon oil (rich in omega-3 fatty acids) in normal subjects due to the increased conversion of TG rich apo-B to LDL-apo-B. This is due to enhancement of degradation of TG rich lipoprotein or to the activation of lipoprotein lipase system which led to decrease of B-lipoprotein (lecithin cholesterol acyltransferase) that mediated the incorporation of cholesterol into HDL and elevation of cholesterol turnover rate which in turn decreased LDL-cholesterol (Erden, 1985).

The hypocholesterolemic effect of active EPA-30 may be attributed to the oxidation of cholesterol to bile acids, interfering with cholesterol absorption through the formation of insoluble mixed crystals and decrease cholesterol synthesis via decreasing 7-α-hydroxylas activity (Barbara et al., 1977).

II PL fatty acid composition of pullet liver:

The fatty acid compositions of the experimental diets and the liver tissues of laying hens fed experimental diets are listed in (Table 6 and 14) respectively. As expected, dietary fatty acid composition greatly affected the composition of hen's liver fatty acids. Significantly higher levels of oleic (18:1 n-9) (29.6±3.8) and linoleic (18:2 n-6) (11.1±3.2) in the liver of the pullets fed the basal diet (3% cotton seed oil) apparently resulted from their abundance in the diet 25.6 and 51.6 respectively. The considerably higher level of linolenic (18:3 n-3) (8.5) in the active EPA-30 supplemented diets resulted in not only generally high levels of 18:3 n-3 but also in higher levels of long chain n-3 fatty acids (7.3±0.5) especially EPA (20:5 n-3) and DHA (22:6 n-3) acids in the liver of active EPA fed hens.

The total n-3 fatty acids for hen's liver fed 1.5% and 3% active EPA-30 supplemented diets was 2-fold (6.1±0.6) and 3-fold (7.3±0.5) respectively higher than that of hens fed basal diet (2.9±0.6). It has been shown that a dietary intake of 2-3% of total n-3 fatty acids is sufficient to result in the maximum incorporation of 22:6 n-3 into membrane lipids and for the AA (20:4 n-6) in the membrane to reach the minimum (Cai and Curtis, 1989).

Regardless the great differences in the 18:2 n-6 levels in the diets which was almost the only exogenous source of n-6 fatty acids for these pullets, the total n-6 fatty acids level in hens fed 1.5% supplemented active EPA-30 was very similar (around 23%). Although hens fed 3% supplemented active EPA-30 had significantly lower Δ6 desaturation (20.2±1.9) compared to those fed cotton seed oil (24.9±3.6). These results suggested that the lower total n-6 fatty acids levels in hens fed active EPA-30 was due to the shortage of 18:2 n-6 in the diet, not from the influence of high levels of n-3 fatty acids in the diet. Furthermore,

levels of individual long chain n-6 fatty acids namely AA (20:4 n-6) for hens fed basal diet was even higher (13.8±1.2) than that for hens fed active EPA-30 (8.9±1.4) which was rich in linolenate. This was an indication that fish oil selectively deposit dietary n-6 fatty acids in hen's liver and that the elongation and desaturation of 18:2 n-6 appeared not or at least seriously unaffected by the presence of high levels of 18:3 n-3 into diets (Huang et al., 1992; Venkatraman et al., 1992).

Table 14.

Effects of active EPA-30 supplementation on PL fatty acid composition in Fayoumi pullet liver

Fatty acids % w/w	Control Basal diet Mean + S.E	Repletion diets (Active EPA-30)	
		Diet (1) Mean ± S.E	Diet (2) Mean ± S.E
16: 0	25.65 ± 1.05^{B}	23.62 ± 1.12^{B}	30.61 ± 0.68^{A}
18:0	13.33 ± 1.74^{B}	17.43 ± 1.12^{A}	13.42 ± 0.87^{B}
16:1 n-7	2.28 ± 0.54^{A}	0.95 ± 0.27^{B}	0.06 ± 0.02^{B}
18:1 n-9	29.59 ± 1.58^{A}	25.70 ± 1.24^{A}	27.36 ± 1.11^{A}
18:2 n-6	11.09 ± 1.34^{A}	10.35 ± 0.89^{A}	11.23 ± 0.68^{B}
18:3 n-3	0.17 ± 0.05^{A}	0.45 ± 0.04^{A}	0.35 ± 0.05^{B}
20:4 n-6	13.78 ± 0.51^{A}	13.55 ± 1.19^{A}	8.96 ± 0.59^{B}
20:5 n-3	0.58 ± 0.14^{B}	1.31 ± 0.12^{A}	1.17 ± 0.12^{A}
22:6 n-3	2.13 ± 0.24^{C}	4.33 ± 0.22^{B}	5.74 ± 0.19^{A}
n-3/n-6	0.12 ± 0.01	0.25 ± 0.004	0.34 ± 0.01
Sat/Unsat	0.652 ± 0.04	0.725 ± 0.02	0.808 ± 0.01

The main values are average of 6 speciemens (±S.E). Means within the same row with the same superscript are not significantly different at (P≤0.01). Only the most common fatty acids are listed.

III Pl fatty acid composition of hen's egg yolk:

The values for the individual fatty acids in hen's egg yolk are given in (Table 15). From these, it is clear that the increase in the n-3/n--6 ratio was due to an increase in the n-3 fatty acids, with a reciprocal decrease in n-6, and particularly of 20:4 n-6, thereby reflecting the similar n-3/n-6 reciprocally in the diet (Wainwright et al., 1992).

The levels of EPA (20:5 n-3) and DHA (22:6 n-3) were increased by 3-fold and 4-fold, respectively, as the dietary n-3 fatty acids increased, whereas the levels of monoenoic 16:1 n-7 and 18:1 n-9 were significantly decreased with an increasing intake of n-3 PUFA. Interestingly, the levels of 20:4 n-6 were decreased in both pullet liver and egg yolk by partial replacement of dietary 18:2 n-6 with 18:3 n-3. There are two possible mechanisms which may account for this, both related to desaturase activity, which reduces the formation of 20:4 n-6 from 18:2 n-6 and the presence of a large amount of 18:2 n-6 may result in substrate inhibition of Δ6 desaturase (Garg et al., 1988). In both instances the provision of 18:3 n-3 would provide the effects of a decline in Δ6 desaturase activity.

Table 15.

Effects of active EPA-30 supplementation on PL fatty acid composition in hen's egg yolk

Fatty acid patterns (w/w%)	Control Basal diet	Repletion diets (Active EPA-30)	
		Diet (1) Mean ± S.E	Diet (2) Mean ± S.E
16:0	25.25 ± 0.83^{B}	26.96 ± 0.65^{B}	32.06 ± 0.99^{A}
18:0	9.99 ± 0.67^{A}	9.11 ± 0.43^{A}	9.70 ± 0.57^{A}
16:1 n-7	4.11 ± 0.50^{A}	4.50 ± 0.26^{A}	2.48 ± 0.31^{B}
18:1 n-9	43.16 ± 1.50^{A}	40.16 ± 0.51^{A}	32.78 ± 1.06^{B}
18:2 n-6	10.78 ± 0.46^{B}	10.72 ± 0.42^{B}	12.61 ± 0.54^{A}
18:3 n-3	0.21 ± 0.03^{B}	0.36 ± 0.05^{A}	0.26 ± 0.03^{AB}
20:4 n-6	3.96 ± 0.31^{A}	2.61 ± 0.14^{B}	3.03 ± 0.20^{B}
20:5 n-3	0.22 ± 0.04^{B}	0.36 ± 0.03^{A}	0.62 ± 0.05^{A}
22:6 n-3	1.76 ± 0.12^{C}	4.67 ± 0.23^{B}	6.60 ± 0.19^{A}
n-3/n-6	0.15 ± 0.01	0.43 ± 0.02	0.48 ± 0.01
Sat/Unsat	0.538 ± 0.03	0.570 ± 0.02	0.722 ± 0.02

The given values are average of 10 speciemens (±S.E). Means within the same row with the same superscript are not significantly different at (P≤0.01). Only the most common fatty acids are listed.

4.3. Environmental effects on the composition and metabolism of lipids in fish:

4.3.1. Effects of the pesticide deltamethrin on:

I- Polar head group of carp erythrocyte plasma membrane:

The results presented in Table 16 provide a quantitative PL picture of carp erythrocyte plasma membrane in untreated animals (control) and treated ones with different doses of DM (0.5, 1.0 and 2.0 ppm) for short and long-term exposure times (48 and 96 hrs), respectively. The choline moiety (LPC, SM and PC) dominated the PL fraction (64.7 ± 7.0%) followed by PE (21.2 ± 3.2%) and PS (6.1 ± 0.52%). There are small amounts of PI, with traces of phosphglycerides (PG, PA and CL). The major differences between control and polluted samples were significantly ($P \leq 0.01$) decreased in PE, PS and phosphoglycerides. The amounts of marmoset erythrocyte PC were not altered by the DM concentrations in the long-term exposure, whereas, in the short-term exposure it significantly ($P \leq 0.05$) increased with an increasing of DM concentrations.

These Pls and their fatty acid composition are the major factors controlling membrane fluidity and linked functions (Lin et al., 1990). The decrease in the PE during the experiments might result in more rigid membranes. In fact, rigidification of these structures has been observed in several systems, but this might also be attributed to increased sterol to PL ratios. Moreover, the decrease in Pls fractions with increasing DM concentrations may be due to the increased catabolic rate or reduced activity of the phospholipase (Boudet et al., 1988) or may be due to impairment of feed back inhibition mechanism of PL synthesis (Olsen et al., 1990).

Table 16.

Influence of different concentrations (ppm) of deltamethrin on polar head compositions in carp erythrocyte plasma membrane

	48 hours				96 hours			
	0 ppm	0.5 ppm	1.0 ppm	2.0 ppm	0 ppm	0.5 pmm	1.0 pmm	2.0 pmm
LPC	0.94 ± 0.1^{A}	2.06 ± 0.15^{B}	3.00 ± 0.5^{B}	4.50 ± 0.4^{C}	1.21 ± 0.1^{A}	2.00 ± 0.1^{B}	1.30 ± 0.1^{A}	2.50 ± 0.2^{B}
SM	7.70 ± 0.51^{A}	8.10 ± 2.16^{A}	8.90 ± 1.7^{A}	10.3 ± 2.12^{B}	8.05 ± 1.05^{A}	7.50 ± 1.2^{A}	8.90 ± 1.4^{A}	11.4 ± 0.95^{B}
PC	56.1 ± 7.0^{A}	55.3 ± 5.35^{A}	58.8 ± 8.2^{A}	61.7 ± 7.4^{A}	54.11 ± 8.2^{A}	56.0 ± 6.7^{A}	59.8 ± 5.4^{A}	65.4 ± 6.7^{B}
PI	2.70 ± 0.25^{A}	3.16 ± 0.27^{A}	2.80 ± 0.45^{A}	3.36 ± 0.1^{B}	3.05 ± 0.25^{A}	3.40 ± 0.31^{A}	5.00 ± 0.33^{B}	4.60 ± 0.4^{B}
PS	6.12 ± 1.2^{A}	5.80 ± 0.87^{A}	5.20 ± 0.65^{A}	4.01 ± 0.5^{B}	7.18 ± 0.4^{A}	7.50 0.71^{A}	5.11 ± 0.5^{B}	5.00 ± 0.6^{B}
PE	21.16 ± 3.2^{A}	20.7 ± 2.4^{A}	18.2 ± 3.08^{B}	15.0 ± 4.59^{C}	21.0 ± 2.4^{A}	19.0 ± 1.8^{A}	16.12 ± 1.5^{B}	9.00 ± 1.3^{C}
PG	1.45 ± 0.2^{A}	1.30 ± 0.1^{A}	0.72 ± 0.1^{B}	0.04 ± 0.0^{C}	0.80 ± 0.1^{A}	1.00 ± 0.1^{A}	0.80 ± 0.1^{A}	0.40 ± 0.1^{C}
PA	1.40 ± 0.0^{A}	1.50 ± 0.1^{A}	0.93 ± 0.3^{B}	0.16 ± 0.0^{C}	1.990 ± 0.2^{A}	1.87 ± 0.2^{A}	1.40 ± 0.3^{B}	1.00 ± 0.11^{B}
CL	2.30 ± 0.21^{A}	1.21 ± 0.3^{B}	1.10 ± 0.6^{B}	0.86 ± 0.4^{C}	2.46 ± 0.3^{A}	1.50 ± 0.21^{B}	1.05 ± 0.4^{C}	0.80 ± 0.34^{C}

The given values are averages for 6 fish (±SE). Means within the same row with the same superscript are not significantly at (P≤0.05).

II Pl fatty acid composition of carp erythrocyte plasma membrane:

DM pollutant brought about marked differences in the fatty acid patterns of the Pls from erythrocyte plasma membrane (Table 17). Increase of the DM concentration led to an increase in the level of SFAs (16:0 and 18:0) as well as in the level of AA (20:4 n-6). The levels of MUFAs (16:1 n-7 and 18:1 n-9) were significantly decreased. At the same time, the levels of some PUFAs (20:5 n-3 and 22:6 n-3) were significantly decreased.

The results inTable 17. demonstrate the effects of DM on the acyl chain indices (Polyene index A and Polyene index B) of the Pl fatty acids. These indices had significantly higher values in control fish than in treated ones. The differences due to the different DM concentrations were more significant in the case of Polyene index A than for Polyene index B. Also, the high doses of DM led to a decrease in the ratios of the linolenic acid to linoleic acid family ratios $\sum$ n-3 / $\sum$ n-6.

DM was chosen for use in the experimentals, as a representative and wide spread pollutant which gave promising results during preliminary tests. There is a general consensus that DM can inhibit several key enzymes and metabolic processes (Akhtar et al., 1985).

Our results demonstrated the capacity of DM to react with plasma membrane sulphhydryl groups to form disulphides and to cause the production of superoxide radicals. As a result of dismutation, this reaction would be a source of hydrogen peroxide, and it also possible that the DM participates in the breakdown of peroxide to yield peroxidation- initiating radicals, and direct by participates in an initiating reaction by electron abstraction from unsaturated fatty acids (Frankel, 1985). It is evident from the results obtained here that an increase in the DM concentrations is accompained by an increase in the levels of palmitic acid and stearic acid. The formation of lipid peroxides in itself may be sufficient cause the distripution of membrane and haemolysis (Hai DQ et al., 1997). So the membrane fluidity changes can act a short time to protect the enzymes.

The ratio of linoleic acid to the linolenic acid family decreases in the presence of a low concentrations of DM, but increases at a higher concentrations. This observation can be explained by the relative increases in the turnover rate of the (n-6) fatty acids, or by a lowering of the turnover rate of the (n-3) fatty acids (Kotkat et al., 1999).

The ratio of saturated to unsaturated fatty acids elevated in the treated tissues, indicating that DM at high concentrations may have a deleterious effect on the internal constituents of tissues which provide protections against fluidity changes due to an increased level of SFAs.

Table 17.

Effects of different concentrations (ppm) of deltamethrin on PL fatty acid (%w/w) compositions in carp erythrocyte plasma membrane

Fatty acids	48 hours				96 hours			
	0 ppm	0.5 ppm	1.0 ppm	2.0 ppm	0 ppm	0.5 pmm	1.0 pmm	2.0 pmm
T. SFAs	33.3 ± 3.1^{A}	35.8 ± 2.7^{A}	38.6 ± 3.2^{A}	47.8 ± 3.8^{B}	35.3 ± 2.8^{A}	39.6 ± 2.1^{A}	44.47 ± 3.2^{B}	55.3 ± 4.1^{C}
T. MUFAs	16.0 ± 1.7^{A}	14.8 ± 1.6^{A}	14.5 ± 1.3^{A}	11.8 ± 1.2^{B}	15.5 ± 1.3^{A}	17.0 ± 1.6^{A}	15.8 ± 1.5^{A}	12.6 ± 1.2^{B}
T. n-6 PUFAs	17.5 ± 1.3^{A}	18.8 ± 2.0^{A}	19.8 ± 2.3^{A}	20.1 ± 3.3^{A}	18.5 ± 1.2^{A}	20.6 ± 1.4^{A}	20.2 ± 2.2^{A}	22.6 ± 3.1^{A}
T. n-3 PUFAs	30.6 ± 2.6^{A}	29.7 ± 2.1^{A}	26.9 ± 2.8^{A}	18.8 ± 1.9^{B}	27.4 ± 2.0^{A}	22.02 ± 2.4^{B}	18.38 ± 1.6^{C}	11.3 ± 0.4^{D}
Polyene index A	0.92 ±0.04^{A}	0.83 ± 0.05^{B}	0.70 ± 0.08^{C}	0.39 ± 0.05^{D}	0.78 ± 0.09^{A}	0.56 ± 0.05^{B}	0.41 ± 0.03^{C}	0.20 ± 0.01^{D}
Polyene index B	2.0 ± 0.3^{A}	1.8 ± 0.2^{A}	1.6 ± 0.2^{B}	1.4 ± 0.1^{B}	1.8 ± 0.1^{A}	1.5 ± 0.1^{B}	1.6 ± 0.1^{B}	1.2 ± 0.01^{C}

The given values are averages for 6 fish (±SE). Means within the same row with the same superscript are not significantly at (P≤0.05).

4.3.2. Effects of herbicide atrazine on :

I Polar head group of carp erythrocyte plasma membrane:

The results in Table 18 show that the choline moiety (LPC, SM and PC) contents are relatively very close in the erythrocyte plasma membrane of carp, decreasing with increasing atrazine concentration and exposure period. On the other hand, the proportions of PE increased at lower atrazine concentration as a way to enhance membrane unsaturation and fluidity. In contrast, at higher concentrations, the levels of PE and phosphoglycerides (PG & PA) were significantly ($P \leq 0.01$) decreased.

Table 18.

Effects of different concentrations (ppm) of atrazine on polar head compositions of carp erythrocyte plasma membrane

	48 hours				96 hours			
	0	10	100	1000	0	10	100	1000
LPC	0.95 ± 0.11^{A}	0.67 ± 0.24^{B}	0.61 ± 0.21^{B}	0.36 ± 0.41^{C}	1.10 ± 0.00^{A}	1.48 ± 0.45^{A}	1.80 ± 0.41^{A}	0.48 ± 0.03^{B}
SM	7.58 ± 1.3^{A}	8.14 ± 0.91^{A}	8.32 ± 1.04^{A}	9.36 ± 1.75^{A}	6.90 ± 0.72^{A}	6.53 ± 0.90^{A}	6.18 ± 1.82^{A}	2.72 ± 2.05^{D}
PC	56.1 ± 6.75^{A}	52.4 ± 4.75^{B}	52.01 ± 6.17^{B}	50.21 ± 7.01^{B}	54.20 ± 5.12^{A}	45.92 ± 6.73^{B}	38.18 ± 4.12^{C}	36.9 ± 3.51^{C}
PI	2.80 ± 0.21^{A}	3.40 ± 0.24^{A}	5.69 ± 0.70^{B}	8.93 ± 0.81^{C}	4.01 ± 0.30^{A}	7.31 ± 0.64^{B}	16.82 ± 0.39^{D}	17.00 ± 0.21^{E}
PS	6.31 ± 0.75^{A}	5.20 ± 0.55^{A}	6.39 ± 0.52^{A}	6.57 ± 0.21^{A}	6.35 ± 0.21^{A}	7.09 0.63^{A}	13.2 ± 1.75^{C}	17.24 ± 1.52^{D}
PE	21.71 ± 0.25^{A}	25.08 ± 3.10^{A}	23.29 ± 2.0^{A}	15.14 ± 1.19^{B}	22.75 ± 2.41^{A}	28.51 ± 3.01^{B}	20.17 ± 0.95^{B}	14.37 ± 0.82^{C}
PG	1.00 ± 0.06^{A}	0.70 ± 0.11^{B}	0.16 ± 2.51^{C}	----	1.05 ± 0.00^{A}	1.03 ± 0.00^{A}	----	----
PA	1.30 ± 0.10^{A}	1.12 ± 0.12^{A}	0.79 ± 0.21^{B}	0.57 ± 0.21^{B}	1.50 ± 0.10^{A}	1.00 ± 0.10^{A}	0.69 ± 0.52^{C}	----
CL	2.25 ± 0.10^{A}	3.20 ± 0.42^{A}	3.34 ± 0.01^{A}	5.86 ± 0.40^{B}	1.92 ± 0.53^{A}	1.11 ± 0.10^{A}	6.90 ± 0.55^{C}	9.43 ± 1.09^{D}

The given values are averages for 6 fish (±SE). Means within the same row with the same superscript are not significantly at ($P \leq 0.05$).

II Pl fatty acid composition of carp erythrocyte plasma membrane:

The data presented in Table 19 illustrate the quantitative changes in Pl fatty acid patterns in polluted fish.

The SFAs (16:0) increased with increasing atrazine concentrations, while the levels of MUFAs (16:1 n-7 and 18:1 n-9) varied with the concentration of pollutant and exposure time. The long-chain MUFAs (20:1 and 22:1) were present in only trace amounts, but disappeared with increasing atrazine concentrations. The n-6 fatty acids linoleic acid (18:2 n-6) and AA (20:4 n-6) constituted a major proportion of the total fatty acids of polluted fish, more especially at higher concentrations than at lower ones. The n-3 fatty acids (20:5 n-3 and 22:6 n-3) constituted a major proportion of the total fatty acids of the control animals.

Many references are to be found in this literature that atrazine exerts its herbicide effect under aerobic conditions it act as electron donor, transformers molecular oxygen to active radical a like e.g. the superoxide radical (O_2), hydroxy radical (OH) and hydrogen peroxide (H_2O_2). These radicals are responsible for the destructive lipid peroxidation of the membrane . Whereas at higher concentrations it act as an electron acceptor, and extracts electrons from the O_2 anion and the H_2O. (Rady and Korshom 1995).

In general, the n-3 PUFAs are significantly decreased in the polluted fish relative to the controls. As established earlier, acetyl-CoA synthases and acetyl-CoA carboxlase responded rapidly to the action of external agents (Lichtenthaler, 1990): their activity decreased, and was then inhibited, causing

blocked de novo fatty acid biosynthesis. As a result of the inhibition of de novo fatty acid biosynthesis, there is a shortage of fatty acids for glycerolipid biosynthesis in the cell and the biosynthesis of Pls (PG, PC and PE) is also blocked by increasing atrazine concentration. The lack of fatty acids and the block of glycerolipid formation results in an inhibition of the formation of all cellular biomembranes, and finally necrosis and cell death develop (Burgastahler and Lichtenthaler 1984).

Table 19.

Effects of different concentrations (ppm) of atrazine on Pl fatty acid compositions in carp erythrocyte plasma membrane

Fatty acids	48 hr				96 hr			
	0 ppm	10 ppm	100 ppm	1000 ppm	0 ppm	10 pmm	100 pmm	1000 pmm
Sat/unsat	0.61 ± 0.04	0.63 ± 0.05	0.73 ± 0.04	0.92 ± 0.01	0.58 ± 0.03	0.76 ± 0.04	1.06 ± 0.1	1.25 ± 0.09
n-3/n-6	1.60 ± 0.1	1.23 ± 0.07	0.59 ± 0.04	0.49 ± 0.05	1.71 ± 0.1	1.13 ± 0.1	1.03 ± 0.09	0.45 ± 0.03
Unsat. Index	269.23 ± 25.7^{A}	256.32 ± 24.4^{B}	234.84 ± 31.35^{B}	218.3 ± 10.96^{C}	283.5 ± 31.2^{A}	245.9 ± 20.4^{B}	218.62 ± 24.3^{C}	195.67 21.1^{D}

Values are means ±S.E of six specimens. Means within the same row with the same superscript are not significantly different at (P≤0.0.5).

4.3.3. Effects of different phenol concentrations on:

I Polar head group of carp erythrocyte plasma membrane:

The results presented in Table 20. provide a quantitative PL picture of carp erythrocyte plasma membrane in untreated animals (controls) and those treated with different sublethal doses of phenol (5, 10 and 20 ppm) for short and long-term exposure (48 and 96 hr). PC dominated the PL fraction followed by PE and SM. Marmoset erythrocyte PC were not altered by the phenol concentrations in the short-term exposure time, whereas in the long-term exposure PC significantly ($P \leq 0.05$) increased with increasing phenol concentration. The proportions of LPC progressively increased from 1.2 to 8.5% in response to increase in phenol concentration. In contrast, PE significantly ($P \leq 0.01$) decreased from 24 to 7.8% during the experimental period. However, there were no changes in the distribution of SM; it remained at about 10% (mean values) of the total erythrocyte Pls.

The proportions of erythrocyte PI and PS significantly ($P \leq 0.05$ and $P \leq 0.01$) decreased respectively in response to increase in the phenol concentration in the short-term exposure, while these were eliminated in the long-term exposure. The higher concentrations (10 and 20 ppm) of phenol also eliminated PG and CL and leds to the formation of about 2-4 and 5-9% of an unknown PL (X) appearing in the short- and long-term exposure experiments, respectively, whereas the low dose (5 ppm) caused no significant changes.

Extensive data are available on the toxicity of phenolic compounds to fish under laboratory conditions, but only few field observations have been recorded. This is due primarily to difficulties of the analytical techniques. The clinical signs of phenol toxicosis encountered ranged from excitation to depression, which agrees with previous reports (Lukanenko 1967). The most severe lesions (congestion, edema, leukocytic infiltration, striking necrosis, hypertrophic changes) were encountered in gill tissue (Post, 1987) and other organs (liver, muscle, spleen, kidneys) suffered degeneration and necrosis (Post, 1987; Nemcsok and Benedeszky, 1990; El-Manakhly and Soliman, 1993).

The depletion of dissolved oxygen concentration of waters polluted with phenolic wastes (Phipps et al., 1981) leads to formation of free radicals, especially superoxide ($\overline{O_2}$), which acts by oxidizing various cellular substrates, especially unsaturated fatty acids which are very susceptible to free radical damage.

It is evident from the results obtained here that the polar lipid composition, with the predominance of PC is in general agreement with that of other fresh-water fish analyzed to date (Henderson and Tocher 1987; Olsen et al., 1990). The high levels of PC in blood lipids is consistent with HDL and LDL being major lipoproteins in the blood of *Cyprinious carpio* as in rainbow trout (Fremont et al., 1981) and Tilapia nilotica (Olsen et al., 1990). Also, the decreasing level of PE in response to increasing phenol concentrations enhanced aging of the cell membrane (Fillerup and Mead, 1966; Philips et al., 1969).

Table 20.

Effects of different concentrations (ppm) of phenol on polar head composition of carp erythrocyte plasma membrane

	48 Hr				96 Hr			
	0 ppm	5 ppm	10 ppm	20 ppm	0 ppm	5 ppm	10 ppm	20 ppm
LPC	1.20 ± 0.11^{A}	3.80 ± 0.10^{B}	8.75 ± 0.80^{C}	8.12 ± 0.65^{C}	1.23 ± 0.04^{A}	2.40 ± 0.10^{B}	5.72 ± 0.6^{C}	6.00 ± 0.50^{C}
SM	11.5 ± 0.19^{A}	10.70 ± 0.90^{A}	11.14 ± 0.50^{A}	10.35 ± 0.50^{A}	10.02 ± 0.54^{A}	9.30 ± 0.45^{A}	9.26 ± 0.64^{A}	10.75 ± 0.32^{A}
PC	54.2 ± 2.73^{A}	55.0 ± 2.15^{A}	59.0 ± 2.95^{A}	63.11 ± 3.50^{A}	53.4 ± 4.35^{A}	58.9 ± 2.62^{A}	64.2 ± 2.42^{B}	65.53 ± 3.4^{B}
PI	2.70 ± 0.61^{A}	2.69 ± 0.53^{A}	1.08 ± 0.10^{B}	0.19 ± 0.51^{C}	2.20 ± 0.17^{A}	2.10 ± 0.12^{A}	---	---
PS	4.10 ± 0.49^{A}	3.90 ± 0.20^{A}	1.18 ± 0.00^{B}	0.50 ± 0.04^{C}	4.30 ± 0.30^{A}	3.98 0.47^{A}	---	---
PE	20.70 ± 3.10^{A}	18.61 ± 1.04^{A}	12.30 ± 0.75^{B}	11.76 ± 0.14^{B}	23.20 ± 1.75^{A}	20.04 ± 1.14^{B}	12.44 ± 0.55^{C}	7.82 ± 0.39^{D}
PG	1.50 ± 0.10^{A}	1.60 ± 0.15^{A}	0.90 ± 0.04^{B}	0.77 ± 0.04^{B}	2.00 ± 0.04^{A}	2.30 ± 0.15^{B}	2.60 ± 0.09^{C}	---
PA	1.70 ± 0.04^{A}	1.10 ± 0.0^{B}	1.26 ± 0.09^{B}	----	1.20 ± 0.04^{A}	1.07 ± 0.07^{A}	---	---
CL	2.30 ± 0.40^{A}	1.60 ± 0.13	1.0 ± 0.09^{B}	---	2.45 ± 0.15^{A}	---	---	---
X	---	---	2.39 ± 0.35	4.20 ± 0.50	---	---	5.78 ± 0.46	6.30 ± 0.64

Values are means ± S.E. of six specimens. Means within the same row with the same superscript are not significantly different at (P≤0.01).

II PL fatty acid composition of carp erythrocyte plasma membrane:

The PL fatty acid composition of control and polluted carp erythrocyte plasma membrane is summarized in Table 21. The quantities of SFAs increased considerably in response to the higher doses (10 and 20 ppm) of phenol, whereas the lower dose of phenol (5 ppm) led to a slight increase in SFAs. Despite the changes in individual SFAs, stearic acid (18:0) increased to a higher degree from 4.5 - 15% and from 4.5 - 17% than palmitic (16:0) and myristic (14:0) acids in both short and long-term exposures, respectively. MUFAs increased uniformly

in response to phenol concentration. $\sum$ n-3 C_{18} - C_{22} PUFAs were greatly reduced in response to phenol, since EPA (20:5 n-3) was decreased frm 7.5 - 1.3%, docosapentaenoic acid (22:5 n-6) from 3.0 - 0.7%, and DHA (22:6 n-3) acid from 17- 1.5%. At the same time, the proportions of $\sum$ n-6 C_{18} - C_{22} PUFAs significantly ($P \leq 0.05$) increased as a function of the higher doses (10 and 20 ppm) of phenol during the experimental period. Despite the changes in the individual C_{18} - C_{22} n-3 and C_{18} - C_{22} n-6 PUFAs, the unsaturation index (UI) and $\sum$ C_{18} - C_{22} PUFA within the structural PL of the erythrocyte membrane were similar among the groups (Table 21); they significantly ($P \leq 0.05$) decreased in response to the higher doses of phenol in the short-term exposure, whereas the long-term exposure led to a highly significant ($P \leq 0.01$) decrease in these fatty acids.

The results from the present study are consistent with the well-established observation that the fatty acid composition of polar lipids in carp erythrocyte plasma membrane is influenced by the toxic action of phenol. This was particularly notable here for the long-chain SFAs and MUFAs. The higher proportions of SFAs and MUFAs in the PL of polluted fish in comparison with control fish suggests that long-term exposure to higher phenol (10 and 20 ppm) concentrations leads to decrease desaturase activity, which inhibited the conversion of linoleic (18:2 n-6) and linolenic (18:3 n-3) acids to longer-chain PUFAs.

Furthermore, the low levels of n-3 PUFAs in the erythrocyte plasma membrane of polluted fish in comparison with those of control fish are in keeping with the formation of oxidizable fatty acids (Kotkat et al., 1999). However, the fatty acids compositions of PL clearly shown that the elongation and desaturation of eicosapentaenoic (20:5 n-3) to docosahexaenoic (22:6 n-3) is depleting under effectiveness of the high phenol concentrations whereas, the enzymes involved in this conversion inhibited by the accumulation of large amount of phenol.

It is well know that the 22:6 n-3 in fish tissues is responsible for the biophysical properties of the membrane and modulation of lipid-protein interactions and membrane-bound enzymes, as well as being a precursor for functionally important lipooxygenase products (Bazan, 1990). Therefore, the decrease in this fatty acid and the double bond index / saturation of fatty acid, which is taken as indicative of membrane fluidity, led to changes in allosteric transitions, and could indicate that the condensing effect of the SFAs of the membrane increases the strength of interactions between the allosteric effector sites (Bloj et al., 1973). Also, the lake of unsaturated fatty acids and the block of glyerolipid formation result in an inhibition of the formation of cell membrane and cause necrosis and cell death (Kotkat et al.,1996). Meanwhile, the effects of low doses became mild at long exposure and even disappeared. This could be attributed to the time required to develop a state of acclimation to phenol either through its detoxification by formation of conjugation products with glycine, glucuronides and sulphates or through enzyme-related eliminations (Qwen and Rosso, 1981).

Table 21

Effects of different concentrations (ppm) of phenol on PL fatty acid (%w/w) composition of carp erythrocyte plasma membrane

Fatty acids	48 hr				96 hr			
	0 ppm	5 ppm	10 ppm	20 ppm	0 ppm	5 pmm	10 pmm	20 pmm
T. SFAs	34.4 ± 1.3^{A}	37.0 ± 1.75^{A}	42.9 ± 1.25^{B}	47.7 ± 1.10^{B}	34.0 ± 1.75^{A}	37.0 ± 1.0^{A}	43.2 ± 1.50^{B}	56.2 ± 2.79^{C}
T. MUFAs	16.9 ± 0.5^{A}	18.6 ± 0.69^{A}	20.5 ± 0.79^{B}	19.3 ± 0.87^{B}	16.2 ± 0.67^{A}	16.9 ± 0.83^{A}	20.6 ± 0.21^{B}	18.6 ± 0.54^{B}
PUFAs	50.8 ± 2.04^{A}	45.8 ± 1.58^{A}	37.5 ± 0.87^{B}	33.4 ± 1.42^{B}	51.1 ± 2.17^{A}	47.5 ± 2.25^{A}	38.3 ±1.21^{B}	31.55 ± 0.3^{C}
n-3 PUFAs	22.3 ± 1.29^{A}	20.9 ± 0.83^{A}	23.6 ± 0.71^{A}	27.0 ± 0.62^{B}	21.7 ± 0.79^{A}	23.3 ± 0.54^{A}	27.8 ± 0.67^{B}	27.3 ± 1.0^{B}
n-6 PUFAs	28.5 ± 1.54^{A}	25.0 ± 1.42^{A}	13.9 ± 1.04^{B}	6.40 ± 0.42^{C}	29.4 ± 1.12^{A}	24.2 ± 0.67^{B}	10.5 ± 0.50^{C}	4.2 ± 0.4^{D}
UI	221.2 ± 10.4^{A}	190.8 ± 8.75^{A}	179.7 ± 8.08^{B}	146.5 ± 6.37^{C}	248.5 ± 14.5^{A}	227.2 ± 11.79^{A}	175.5 ± 7.87^{B}	116.1 ± 5.75^{C}

Values are means ± S.E. of six specimens. Means within the same row with the same superscript are not significantly different at ($P \leq 0.01$).

SUMMARY AND CONCLUSION

1- Our project started by examining omega-3 PUFAs for the Pl composition in important animals such as the poikilothermic freshwater fish and marine fish, and the homothermic laying hens and laboratory animals. In these species, important differences were discovered in the quantitative compositions of omega-3 PUFAs.

It was established that more abundant amounts of omega-3 PUFAs are found mainly in the poikilothermic animals, and especially marine fish, as compared to the other species.

2- We next set out to determine the amounts and duration of omega-3 PUFA supplementation required to explore the usefulness of fish oil as the main source of PUFAs {EPA (20:5 n-3) and DHA (22:6 n-3)}.

The results showed that:

In freshwater fish (poikilothermic animals):

Fish fed on an omega-3 PUFA-deficient diet displayed a drastic decrease of n-3 fatty acids and a compensatory increase of n-6 fatty acids in the Pls of their flesh. The feeding on a fish oil and active EPA-30 diet led to an increase in the proportion of 18:1/20:4, whereas 16:0/20:4 and 18:0/20:4 decreased. Thus, the total species containing Sn-1 (18:1) increased at the expense of Sn-1 (18:0) in the active EPA-30 fed animals.

In rats (homothermic animals):

The results showed that young rats fed on an n-3 fatty acid-deficient diet exhibited a drastic decrease in the amount of PE and omega-3 fatty acids (EPA and DHA) and compensatory increases in PC, SFAs and n-6 PUFAs. In contrast, the young rats fed on active EPA-30 contained large amounts of PE and a complementary enrichment with n-3 fatty acids. At the same time, dietary supplementation of n-3 fatty acids did not effects on the phospholipid metabolism of livers of old rats.

The molecular species containing long-chain PUFAs are in general altered by feeding on active EPA-30, while those that contain C_{16} or C_{18} acids are resistant to changes.

The PUFAs showed clear perferences for the ß-position. These acids (20:5 n-3 and 22:6 n-3) are typical of fish oil, both types cannot be synthesized by animals, but are derived from the diet.

Introduction of active EPA-30 in laying hen's diet:

The addition of active EPA-30 to the laying hen's diet particularly lowers the plasma TG, total Chl and LDL.

There are large increases in the three different omega-3 PUFAs (18:3 n-3,20:5 n-3 and 22:6 n-3) in hen's liver and egg yolk, giving similar values after 2 months of feeding on these diets.

Omega-3 PUFAs and pollutants:

The pesticide deltamethrin, the herbicide atrazine and phenol pollutants caused oxidation enhancement in the carp tissues and influenced the Pl composition of carp erythrocyte plasma membrane.

The pesticide deltamethrin: A high concentration of it eliminated phosphoglycerides and CL and led to increased levels of palmitic (16:0) and stearic (18:0) acids as well as of AA acid. At the same time, the levels of omega-3 PUFAs are significantly ($P \leq 0.01$) decreased.

The herbicide atrazine: The amount of the choline moity (LPC + SM + PC) increased in parallel with the atrazine concentration; in contrast, PE decreased. According to the quantitative changes in the Pl fatty acids, the

long-chain MUFAs 20:1 and 22:1 disappeared at high concentrations of atrazine. The fatty acids linolenic acid (18:2 n-6) and AA (20:4 n-6) constituted a major proportion of the total fatty acids. The n-3 fatty acids (20:5 n-3 and 22:6 n-3) were significantly ($P \leq 0.01$) decreased in polluted fish relative to the controls.

Phenolic compound: The high concentrations of phenol pollutant led to an increase of PC and eliminated PA. AA (20:4 n-6) was present in greatest amounts at both low and high concentrations. The n-3 fatty acids displayed a fairly varied picture after exposure to phenol pollutant. Long-term exposure to higher phenol concentration led to elimination of these acids and significantly ($P \leq 0.01$) decreased the n-3/n-6 ratio.

Significance of the results:

1- Marine fish and fish oil are the main sources of omega-3 PUFAs. The amounts and duration of supplementation with these acids had profound qualitative and quantitative effect on the Pl molecular species of the freshwater fish and rat tissues, and these changes have implications for possible functional changes.

2- The linkage of essential fatty acids to the ß-position in general would play the role of a reservoir for PUFAs and protect them from oxidation.

3- It was also established that the inclusion of active EPA-30 in the laying hen's diet enriched their eggs with omega-3 PUFAs as compared to the control eggs.

4- Increases in the concentration of water pollutants led to decreased amounts of omega-3 PUFAs and caused marked differences in the polar head group, which makes the membrane more rigid and less permeable.

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