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Effect of Dietary Manipulation of Lipids on Mice, *Mus Musculus*

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Abstract: Dietary fatty acids play important role in various physiological processes and maintaining health. The beneficial effect of dietary fatty acids is hypothesized to depend upon its type, dose and duration of intake. Objective of this study was to find a particular dose and duration of intake of dietary fat which has the most beneficial effect. Experimental animal (*Mus musculus*) were administered fish oil and meat oil respectively in the form of 5, 10 and 20% doses blended with the pellet diet. Mice were sacrificed at the end of 30 and 60 days feeding period to collect blood, liver, kidney and heart tissues. Dietary intake of 10% fish oil for a period of 30 days maintained favourable tissue biochemical composition, hematological parameters, serum lipid and fatty acid profiles along with liver function tests when compared to that of meat oil. GC profiles showed more accumulation of eicosapentaenoic acid and docosahexaenoic acid in the tissues in contrast to that of meat oil where arachidonic acid was found to accumulate. Prolong intake of fish oil or higher doses of lipid might have negative effect. Results of our present study reveal a more beneficial effect of fish oil than meat oil in *Mus musculus*.

Key words: Dietary lipids · SFA · MUFA · PUFA · Health · Fish oil · Rodents

INTRODUCTION

Fat is an important dietary component affecting health and growth. Among various components of fat, fatty acids are ubiquitous biological molecules that are mainly used as metabolic fuels, as covalent regulators of signaling molecules and also as essential components of cellular membranes. Fat from animals is the only source of long chain (beyond 20 carbon) polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFA) are very essential for growth and development, regulation of cellular functions and maintaining the physiological homeostasis [1]. The two series of long chain PUFA viz. η-3 and η-6 have antagonistic functions [2]. Eicosanoids produced from η-6 long chain PUFA are pro-inflammatory, pro-aggregating, vaso- constricting and immunosuppressive, while those of η-3 long chain PUFA are anti- inflammatory, anti-aggregating, vasodilating, anti-arrhythmic and immunomodulatory [3]. Eicosanoids derived from arachidonic acid and eicosapentaenoic acids have opposing metabolic properties making it important to ensure a balanced dietary intake of η-6 and η-3 PUFA [4].

A large number of studies have shown positive health benefits associated with the consumption of η-3 PUFA on infant development, cancer, cardiovascular diseases and various mental illnesses such as depression, attention deficit hyperactivity disorder and dementia [5]. On the other hand, a few studies have also suggested that the total dietary fat intake is linked to an increased risk of obesity and diabetes [6]. Diets high in saturated fat are correlated with an increased incidence of atherosclerosis and coronary diseases [7]. Thus, some of the most common medical disorders today like cardiovascular disease, hyperlipidemia, obesity, cancer *etc.* are characterized by the altered levels of fatty acids or their metabolites. Effects of changing the total fat intake and saturated /unsaturated ratios are still controversial [8]. The cosmopolitan distribution of η-6 PUFA in nature makes it a predominant PUFA in our diet, thereby making the amount of η-3 consumed very less. Fish oil and fish meal have been used as the most effective means of correcting the balance between η-6/η-3 in terms of improvements seen in health. Animal studies have shown that increasing the availability of η-3 PUFA in our diet results in a decreased proportion of arachidonic acid and increased incorporation of η-3 PUFA [9].

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Table 1: Fatty acid profiles of Fish and Meat oil. Fish oil was supplied by E. Merck. Meat oil was extracted from *Capra aegagrus*

Type of fatty acid	Fish oil	Meat oil
14:00	11.62	8.97
16:00	23.27	32.88
18:00	5.76	26.31
20:00	0.86	1.57
18:01	11.9	1.52
20:01	10.69	12.55
22:01	0.12	0.35
18:2n6	3.13	9.25
20:2n6	0.16	0.32
20:4n6	1.73	4.17
18:3n3	0.21	0.1
20:5n3	17.86	0.52
22:5n3	1.83	0.12
22:6n3	10.86	1.37
Sat/Unsat ratio	0.709	2.304
n-3 /n-6	6.127	0.154

Dietary lipids derived from animal sources always have complex composition including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA of both ω-3 and ω-6 series and each of them having its own physiological importance. Therefore, the relative composition in the animal lipid can be considered to be playing significancant role in animal as well as human nutrition. The beneficial effect of dietary lipid is hypothesized to depend upon its type, dose and duration of intake. There is a lack of clear information regarding the effect of prolonged intake of dietary PUFA. Hence, to test this hypothesis, we selected two sources of dietary lipids namely, fish oil and meat oil (Table 1) at various doses (5, 10 and 20%). We tested the effect of intake of these dietary fatty acids for 30 and 60 days durations on various health parameters of mice, *Mus musculus*.

MATERIALS AND METHODS

Experimental Procedure: After the initial procurement of experimental animal *Mus musculus,* they were acclimatized for a period of one month and maintained according to the guidelines of animal ethics committee of Goa University, Goa, India. Animals weighing 22±0.5 g of both the sexes were selected for our present study and divided into seven different groups of 24 animals, with a male to female ratio of 1:1 in each group. One group was fed *ad libitum* with the commercial standard pellet diet and water and served as a control group. The other groups received same pellet diet blended with various doses (5, 10 and 20%) of either fish or meat oil for 60days. The experimental mice were sacrificed in batches of 6 animals each, at an interval of 30 days to collect various tissues like liver, kidney and heart. Blood was collected with moderate suction to avoid any haemolysis and was transferred into heparinized and non heparinized appendorf tubes for hematological and enzyme activity analysis.

Hematology: Total counts of blood cells like RBC, WBC and platelets were done according to the method of Sood [10] using Neubauer chamber. Hemoglobin level was measured by using Sahli's hemometer.

Biochemical Parameters: Total protein was assayed by using Lowry's reagent [11], urea by using diacetyl monoxime reagent [12], ascorbic acid by using dinitrophenyl hydrazine reagent [13] in various tissues like liver, kidney and heart. Triglyceride was assayed by using chromotropic acid reagent and cholesterol by using ferric chloride reagent according to the method of Kates [14] in the above mentioned tissues.

Fatty Acid Analysis: The extracted lipids were subjected to saponification with 1ml of 5N NaOH along with internal standard (C17:0) heptadecanoic acid at 80-90°C for 2 hrs followed by acidification and extraction with pet-ether (40-60°C). Fatty acids thus obtained were methylated using 2% H_2SO_4 in methanol at 70°C for 4 hrs. Methyl esters thus obtained were analysed on Agilent 6890 sereis GLC system equipped with FID detector, using supleco SP2330 fused silica capillary column using temperature programme. The column was initially maintained at 100°C for 5 in, increased by 30°C /min to 160°C and next by 5°C /min to 220°C and there it was kept for 10 min. Injector and detector ports were maintained at 220 °C and carrier gas (nitrogen) pressure was maintained at 18psi. [15]. Fatty acids were identified by frequent comparison with authentic standards obtained from M/s., Sigma-Aldrich, USA. The concentrations were expressed as nmole%.

Serum Lipid Profiles: The serum lipid profiles were monitored by using the diagnostic kit manufactured by Crest Biosystems, Coral Clinical System, Goa and by following the method of Trinder [16]. Serum triglycerides, total cholesterol and HDL cholesterol were assayed and from the values thus obtained, concentrations of LDL and VLDL were calculated as follows: VLDL=TG/5; LDL= [Cholesterol- (HDL+VLDL)].

Liver Function Enzymes: Activities of ALT (E.C. 2.6.1.2), AST (E.C. 2.6.1.1) by using dinitrophenyl hydrazine [17] and ALP (E.C. 3.1.3.1) by using p-nitro phenol reagent [18] were studied in both liver and serum samples. ALT and AST activities were expressed as μg/min/mg protein. ALP activities were expressed in IU/mg protein.

Statistical Analysis: Data were represented in the form of Mean±SE. The mean values were of six individual experimental animals. Experimental data of each group was compared with that of control group by using student't' test. F test was also conducted among fish oil and meat oil administered groups to find out the significance of dose dependent variations. These tests were conducted by using Microsoft Excel. Both the "t" and 'F' values were checked in the probability table. A value of $P \leq 0.05$ was considered as the criterion of significance.

RESULT AND DISCUSSION

This study investigated the effects of feeding two diets differing in their fatty acid compositions in *Mus musculus*. Fish oil and meat oil were used as models of oils from animal sources which are rich in unsaturated and saturated fatty acids respectively and also have different MUFA and PUFA composition. Dietary lipid administration was found to affect various hematological parameters significantly (Figure 1). While about 10% decrease ($P<0.001$) in total count of RBCs was observed on 60 days supplementation of 20% meat oil (M3group of mice) similar dose of fish oil showed 50% decrease ($P<0.001$) in the counts which was noticed from 30 days onwards. Dietary lipid manipulation except in the F3 group of mice showed an elevation in WBC counts, which reached a maximum on 30 days and then declined to normal on 60 days or remained steady. In contrast, supplementation of 20% fish oil brought significant decrease in WBC counts. Dietary supplementation of 10 and 20% doses of fish oil brought 30-40% decrease ($P<0.001$) in platelet counts from the beginning, similar doses of meat oil brought only 10-20% decrease ($P<0.05$) as seen in figure 1. Varying degrees of augmentation in the leukocyte counts may indicate enhanced immuno function upon supplementation of PUFA as reported earlier by Harbige [19]. An increase in the total leukocyte counts due to PUFA supplementation was also reported by Klinger *et al.* [20] and Roy *et al.* [21]. When fish oil is provided in human diet, the proportion of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in immune cells are significantly elevated, probably in a dose dependent manner [22]. Similar effects occur in neutrophils, monocytes, T lymphocytes and B lymphocytes [23]. About 10-40% decrease in platelet counts indicates that PUFA supplementation may affect platelet aggregating factor as reported earlier [24]. Fish oil supplementation for 30 days led to 20% elevation in blood hemoglobin concentration i.e. from 11.92±0.24 to 14.47±0.44 g/dl which was reduced upon prolonged

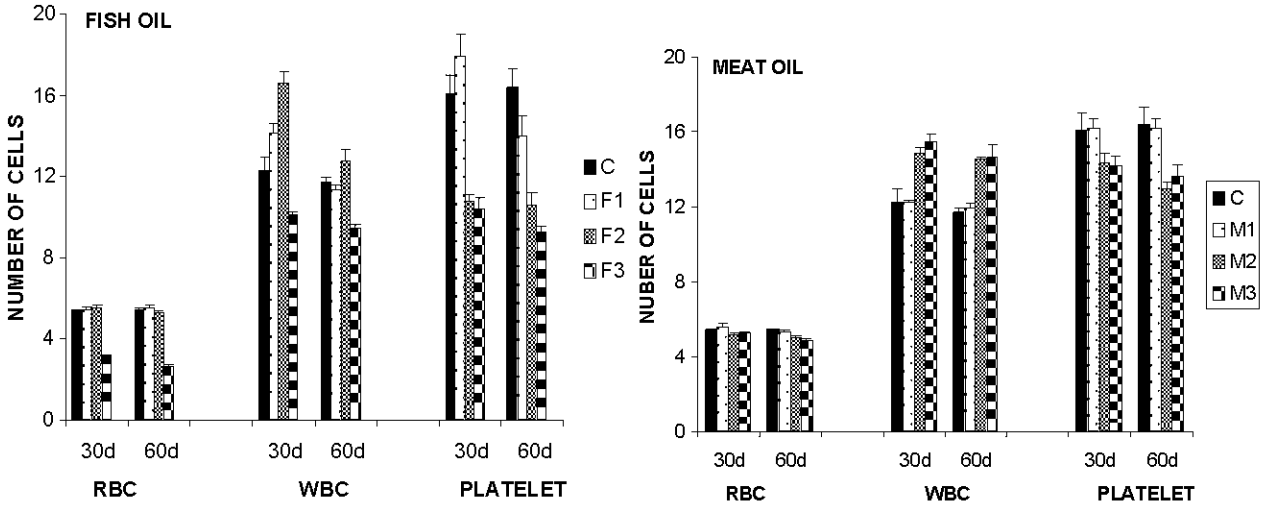

Fig. 1: Effect of dietary lipid manipulation for a period of 30 or 60 days on various hematological parameters of mice, *Mus musculus*.Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

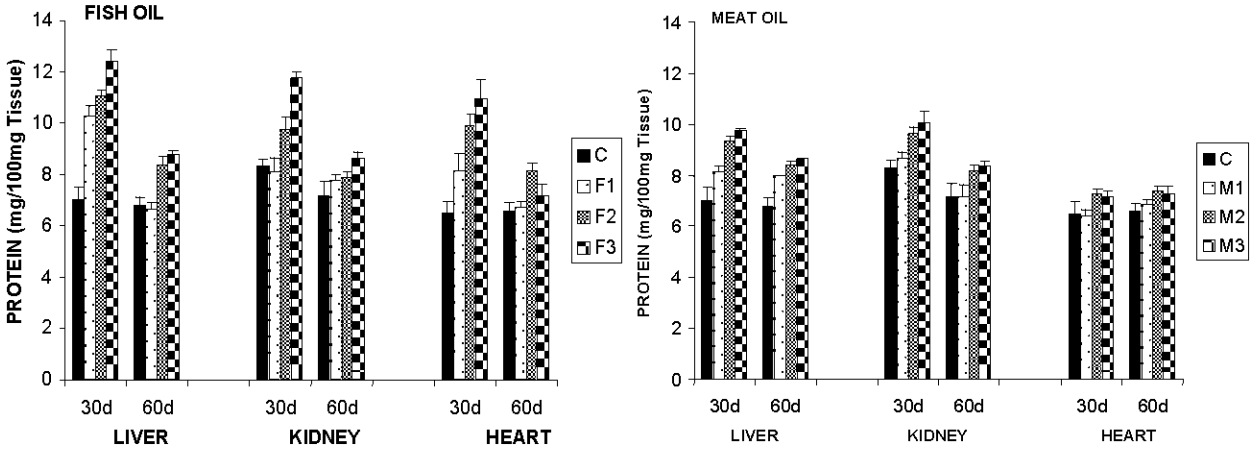

Fig. 2: Effect of dietary lipid manipulation for a period of 30 or 60 days on protein concentration (mg/100 mg tissue) of various tissues of mice, *Mus musculus*. Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

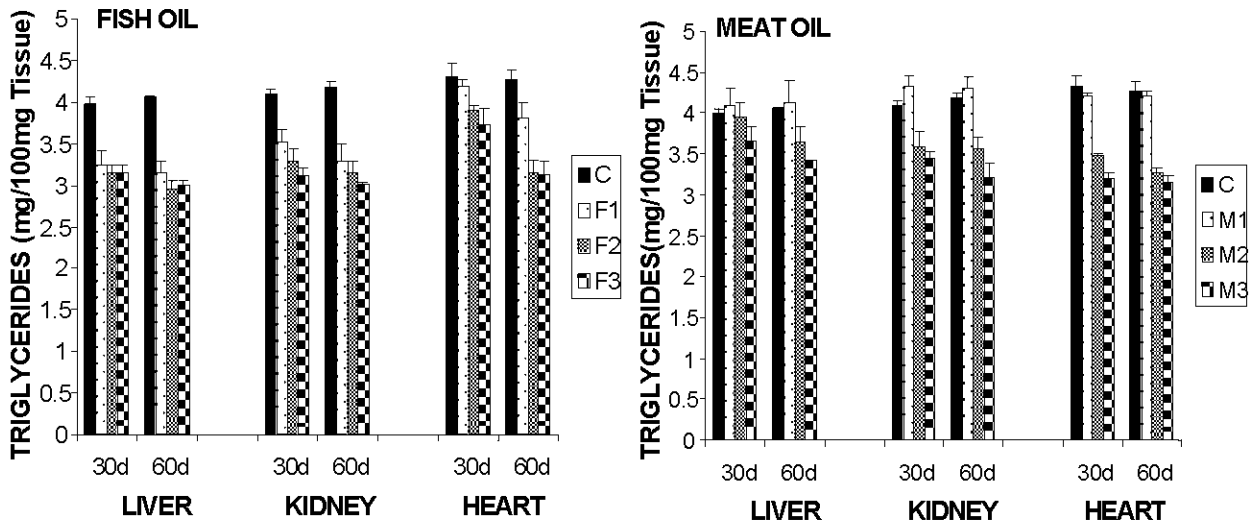

Fig. 3: Effect of dietary lipid manipulation for a period of 30 or 60 days on triglyceride concentration (mg/100mg tissue) of various tissues of mice, *Mus musculus*. Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

supplementation. Meat oil supplementation also brought about a similar augmentation, however, remained in an elevated state up to 60 days. Thus, we found that the dietary lipid manipulation in the form of 10% doses maintained haematological parameters favourably. However, the higher doses may exert adverse effects.

Higher doses of fish and meat oil were found to enhance total protein level by 40-80% in all tissues (Figure 2) under our observation namely liver, kidney and heart along with 20-30% reduction in total fat (Figure 3). This may be due to the fact that proteins were spared from energy yielding processes as the diets were enriched with PUFA. The PUFA composition of a diet influences the utilization of fat for energy yielding processes [25]. 10-80% reduction of urea in liver and heart (Figure 5) upon supplementation of oil once again supported the sparing of protein from energy yielding processes. Upon dietary supplementation of 10 and 20%

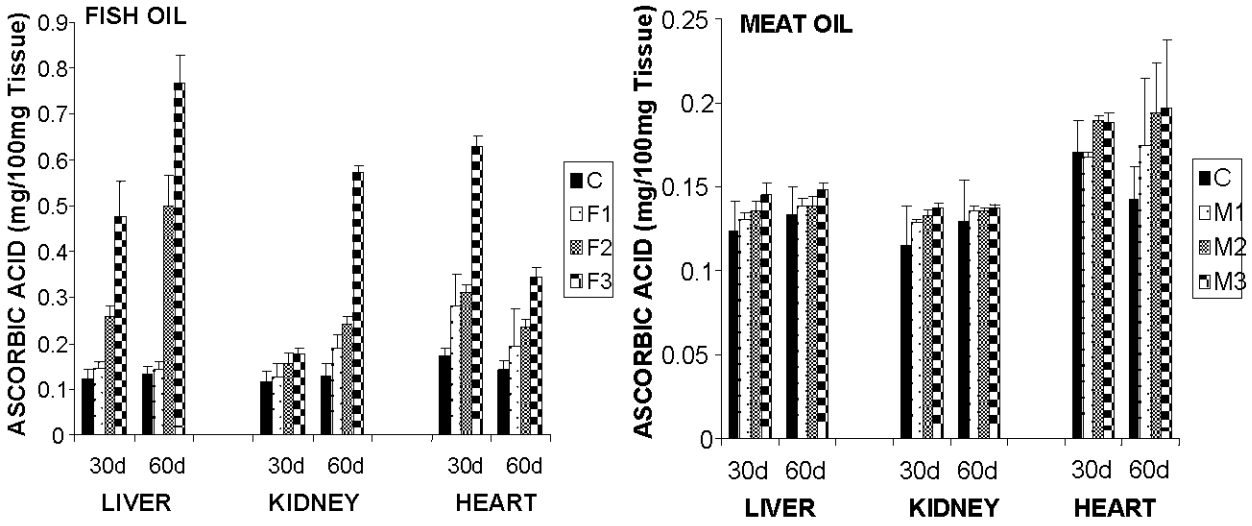

Fig. 4: Effect of dietary lipid manipulation for a period of 30 or 60 days on ascorbic acid concentration (mg/100mg tissue) of various tissues of mice, *Mus musculus*. Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

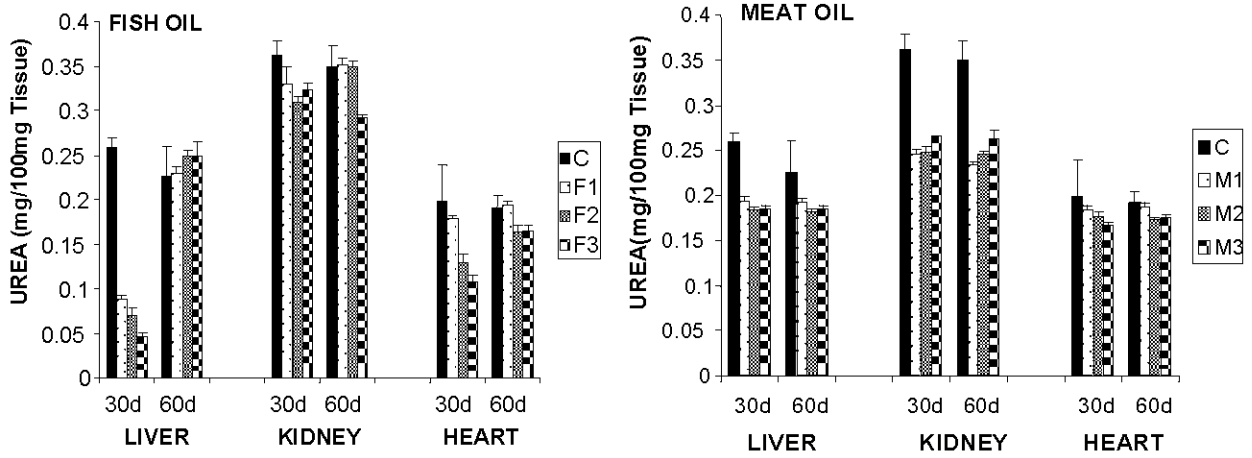

Fig. 5: Effect of dietary lipid manipulation for a period of 30 or 60 days on urea concentration (mg/100mg tissue) of various tissues of mice, *Mus musculus*. Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

fish oil a tremendous augmentation (up to 4 fold) in the level of ascorbic acid was noticed in liver, heart and kidney of *Mus musculus* (Figure 4). These observations were in accordance with earlier findings in *Gallus domesticus* [21]. The dietary supplementation of fish oil augmented the level of docosahexaenoic (C22:6) and eicosapentaenoic (C20:5) acids at the expense of palmitoleic (C16:1) and oleic (C18:1) acids (in liver and kidney tissues) or arachidonic (C20:4) acid (in heart and spleen). In contrast, meat oil supplementation enhanced the level of arachidonic acid in all the tissues (Table 2). Accumulation of η-3 PUFA in various tissues upon dietary supplementation of fish oil might have induced some free radical scavenging enzymes and there by spared the utilization of ascorbic acid to fight oxidative stress [26] maintaining the higher levels of ascorbic acid. We have found enhancement in Superoxide Dismutase and Catalase activities upon fish oil supplementation which was not observed with meat oil supplementation. (data is not shown here).

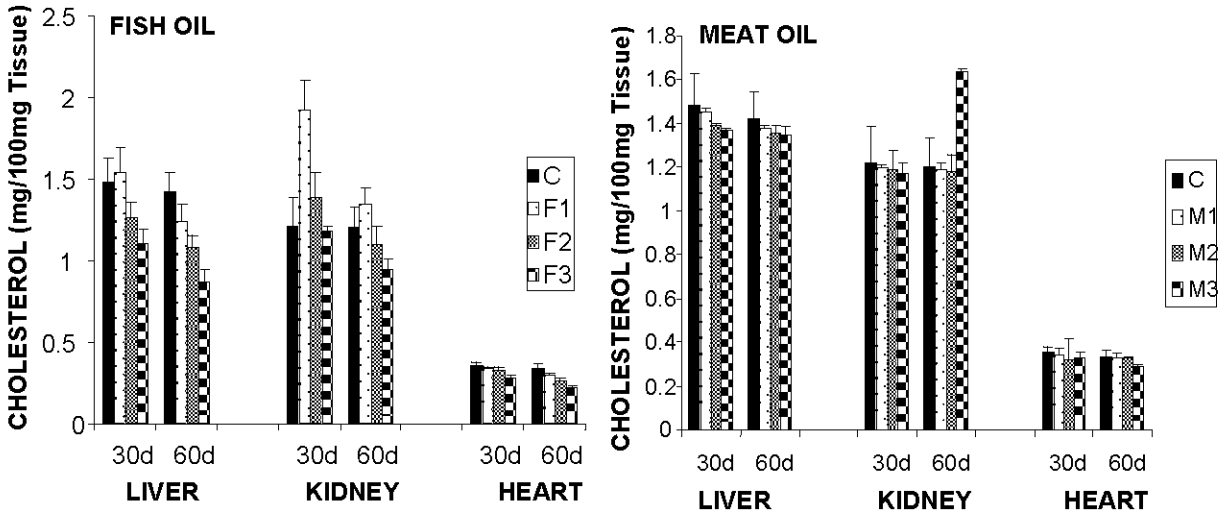

Fig. 6: Effect of dietary lipid manipulation for a period of 30 or 60 days on cholesterol concentration (mg/100mg tissue) of various tissues of mice, *Mus musculus*. Data is represented as a mean of six values and their standard errors. Fish oil (F) and Meat oil (M) group of mice were again subdivided into three different groups and each sub group was fed with standard pellet diet blended with 5, 10 and 20% of the respective oil.

Table 2: Effect of dietary supplementation of 10% PUFA for 30 days on the fatty acid profile (nmole%) of various tissues (liver, kidney and heart) in mice, *Mus musculus*

	LIVER			*KIDNEY*			*HEART*		
Type of fatty acids	C	F	M	C	F	M	C	F	M
C14:0	0.72	0.42*	0.79	1.54	1.56	1.75*	1.77	1.09*	1.25*
C16:0	25.73	31.30	27.10	21.77	23.33	25.89*	25.67	23.32	26.05
C16:1	5.76	1.46*	4.67	8.62	4.34*	6.91	2.19	3.07*	3.37*
C18:0	7.68	19.16*	9.74*	7.35	12.32*	7.58	20.70	19.00	17.97*
C18:1	39.10	15.14*	30.13*	49.94	26.58*	33.03*	13.90	14.02	16.47*
$C18:2^{6}$	9.92	10.99	13.01*	9.51	13.41*	17.92*	11.97	10.93	13.98*
$C20:3^{6}$	0.88	1.31*	2.32*	0.23	1.59*	1.07*	2.47	2.17	2.56
C22:0	0.09	0.09	0.11	0.06	0.23*	0.13*	Tr	Tr	Tr
$C20:4^{6}$	6.23	6.13	7.88*	2.42	3.79	6.85*	9.63	4.18	7.55
$C20:5^{3}$	0.05	1.32*	0.17	0.06	2.13*	0.09	Tr	0.82	Tr
$C22:5^{3}$	0.48	0.71*	0.13*	0.05	0.61*	0.09	4.63	1.18	0.55
$C22:6^{3}$	2.17	11.33*	3.23*	0.94	6.72*	1.98	6.90	17.35*	8.83
Others	1.19	0.64	0.72	-	3.39	-	0.17	2.87	1.42

* values were found to be significant when compared with the respective control values

Table 3: Effect of dietary supplementation of 10% PUFA for 30 days on various liver function enzyme activities in mice *Mus musculus*

	Liver			Serum		
Enzymes	C	F	M	C	F	M
ALT(µg/min/mg protein)	9.278±0.304	7.956±0.156	8.634±0.384	13.770±0.680	12.640±0.440	12.570±0.368
AST(µg/min/mg protein)	102.200±0.968	82.470±1.380	81.237±0.973	117.250±6.120	90.470±2.870	80.351±0.614
ALP(IU/mg protein)	16.656±0.358	17.540±0.300	16.045±0.274	17.310±2.100	22.090±1.470	16.945±0.290

C- Control group, F- Fish oil supplemented group, M- Meat oil supplemented group

Table 4: Effect of dietary supplementation of PUFA for 30 days on the serum lipid profiles (mg/dl) in mice, *Mus musculus* (Mean value of four samples and their standard error)

	Control group	Fish oil group			Meat oil group		
Days	C	F1	F2	F3	M1	M2	M3
TG	162.793±3.09	167.200±8.37	135.200±6.8	108.480±7.15	166.013±0.497	145.731±0.476	147.355±0.575
CH	153.060±4.65	115.400±2.11	86.600±2.66	65.900±2.28	153.240±0.657	148.480±0.27	135.568±0.318
HDL	21.111±1.29	33.700±1.61	41.200±0.8	38.900±1.16	28.348±0.418	27.225±0.048	29.409±0.189
VLDL	32.566±0.61	33.440±1.67	27.040±1.36	21.696±1.43	32.771±0.259	29.350±0.21	29.172±0.162
LDL	99.383±1.32	48.260±1.2	18.360±1.53	5.300±1.28	92.120±0.77	91.906±0.226	76.988±0.521
CH: HDL	7.25	3.424	2.102	1.694	5.405	5.454	4.609
CH:TG	0.94	0.69	0.64	0.607	0.923	1.018	0.92

Note: Fish oil (F) and Meat oil (M) group of mice were again subdivided into three groups and each sub group of mice were fed *ad libitum* same commercial feed like control group of mice(C) but blended with 5, 10 and 20% of respective oil

Alanine transaminase and Aspartate transaminase enzymes are both present in liver, while ALT is cytosolic, AST is predominately mitochondrial. These are released into circulation after cell damage or destruction [27]. Dietary lipid manipulation seemed to preserve the structural integrity of hepatocellular membrane as evident from 15-20% reduction ($P<0.05$) in these enzyme activities (Table 3). Alkaline phosphatase is a membrane bound glycoprotein enzyme involved in the transport of metabolites across cell membranes, protein synthesis, synthesis of certain enzymes, secretory activities and glycogen metabolism [28]. In the present study meat oil supplementation did not alter ALP activity (Table 3). However, 5-30% rise (statistically equivocal) in ALP activity was noticed in mice supplemented with fish oilindicate a disturbance in the secretory activity or in the transport of metabolites or may be due to altered synthesis of some enzymes.

Hypolipidemic effects of PUFA, especially n-3 PUFA have been well documented. In our present findings, 5% dose of dietary lipid did not alter serum triglyceride and cholesterol. Higher doses of fish oil significantly brought down serum triglyceride level by 15-30% ($P<0.001$) and cholesterol level by 40-60% ($P< 0.001$) with concomitant rise (60-95%, $P<0.001$) in HDL cholesterol. Identical dose of meat oil brought down serum TG and cholesterol to a lesser extent (5-15 % $P<0.001$) with an augmentation (35-40%, $P<0.001$) in the HDL cholesterol levels (Table 4). This could be due to the inhibition of triacylglycerol and VLDL synthesis. It has been reported earlier that fish oil has a higher LDL oxidizing capacity than other oils [29]. Nordoy *et al.* [30] have also reported that the presence of dietary η -3 fatty acids in both high and low saturated fat diets significantly lowers plasma total cholesterol, VLDL cholesterol, total triacylglycerol and VLDL triacylglycerol. Dose and time dependent decrease in the cholesterol level observed in liver tissue upon supplementation of fish oil (Figure 6) might indicate the suppression of *de novo* biosynthesis of cholesterol which needs to be verified. η-3 PUFA enriched diet decreased the activity of hydroxy methyl glutaryl-CoA reductase activity in *Gallus* [21]. The hypolipidemic effects of fish oil diet can be attributed to the MUFA or PUFA content in them which need to be verified further.

From our findings it can be concluded that 10% fish oil is more effective in maintaining various health parameters when administered for a period of 30 days. The beneficial effects may be attributed to their PUFA or MUFA content. Prolong intake of fish oil or higher doses of lipid might have negative effect. Results of our present study reveal a more beneficial effect of fish oil than meat oil in *Mus musculus*.

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