Hypocholesterolemic Effect of Fish Proteins and Fish Oils in Albino Rats

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Casein or fish protein (from *Nemipterus japonicus*), as the main source of protein and groundnut oil or sardine oil as the main source of fat, were used in four different combinations in diets of four groups of 5 week-old albino rats to see their effect on the cholesterol levels in the serum, liver and heart of the animals. Casein groundnut oil group (control) had the highest level of serum cholesterol and casein-fish oil group, the lowest. Fish protein-groundnut oil-fed animals also showed a significantly lower level of serum cholesterol, suggesting a hypocholesterolemic effect in the case of fish protein also. However, in the case of fish protein-fish oil group, the lowering of cholesterol was not as pronounced as would be expected of a combined hypocholesterolemic effect of fish oil and fish protein. The pattern of cholesterol levels was the same in liver and heart. Lowering of serum cholesterol did not result in an increase in liver cholesterol levels. Significance of these findings are discussed.

Elevated serum cholesterol level is known to be one of the risk factors indicating susceptibility to atherosclerotic heart disease. Serum cholesterol level is in equilibrium with the cholesterol pool in tissues. Its concentration is an index of metabolically important cholesterol in the body and it correlates strongly with the risk of pathological atherosclerosis (Truswell, 1977). It has been established that the diet has a very important role in determining the cholesterol levels of serum and tissues. Quality and quantity of dietary lipids, proteins, carbohydrates, fibre and various other components influence cholesterol metabolism in animals (Kritchevsky, 1978).

The nature of dietary fat has a prominent role in the regulation of blood cholesterol levels. Hypercholesterolemic properties of saturated fat in man and animals are known for a long time (Ahrens, 1957; Kritchevsky *et al.*, 1954). Also the evidences for the fact that dietary polyunsaturated fats have cholesterol lowering properties are longstanding (Kinsell *et al.*, 1952; Peifer *et al.*, 1960) and an inverse relationship between the levels of serum cholesterol and dietary polyunsaturated fatty acids had been reported (Feigenbaum *et al.*, 1961). Jones (1974) had found that a diet low in fat and cholesterol and high in polyunsaturated acids can reduce serum cholesterol concentration in man and this had helped to achieve a modest success in improving the morbidity due to atherosclerotic disease.

Cholesterol metabolism in animals is influenced by the quality of dietary proteins also. Diets containing casein is known to be hypercholesterolemic. Kritchevsky *et al.* (1959) reported that casein with cholesterol was considerably more cholesterolemic than soya protein with cholesterol in the diets. Replacement of casein by whole soya flour or hexane-extracted soya bean meal was found to be effective in preventing hypercholesterolemia and atherosclerosis in rabbits given semisynthetic diets. The fact that animal proteins, in general are more cholesterolemic than plant proteins had been established by a number of investigations. (Carrol & Hamilton, 1975; Hamilton & Carrol, 1976; Huff *et al.*, 1977; Forsythe *et al.*, 1980; Huff & Carrol, 1980a). The role of dietary proteins in controlling cholesterol metabolism is not yet clearly understood. Carrol & Hamilton (1975) have graded different protiens in respect of their cholesterolemic effects. According to them, fish protein is more effective than beef, egg etc. in lowering the serum cholesterol levels.

In a recent study reported from this laboratory, we have seen that whole fish (oil sardine), when incorporated into the diets of rats to provide 50% of the dietary supply of fat and protein, can reduce cholesterol levels significantly, even in presence of a known hypercholesterolemic agent such as coconut oil (Viswanathan Nair & Gopakumar, 1981). However, the extent to which the two components of the diet namely, protein and fat had contributed towards the observed lowering of cholesterol levels was not clear from this study. A detailed experiment to study the role of each on cholesterol levels in rats was, therefore, undertaken. Casein and fish protein as the source of protein and groundnut oil and fish oil as the source of fat were given to four groups of rats in four different combinations to study these effects. The effects of these diets on the fatty acid compositions

of the lipids of serum, liver and heart were also studied along with this.

Materials and Methods

Five week old male albino rats (Wistar strain) bred at the Nutrition Department of Kerala Agricultural University, Trichur, Kerala, with an average weight of 84.7 g were used in this study. After bringing to the laboratory they were put on a casein-ground nut oil diet (diet of group I, from which cholesterol was eliminated) for one week. Then they were divided into four groups of five rats each (of roughly equal weight) and put in four different cages. Each group was fed on a separate diet, the composition of which is presented in Table 1. The diets were so formulated that the protein and fat sources varied in all four possible combinations and they were identical in all other respects. Thus the protein source for group 1 and group 2 was casein and for the other two groups, fish protein concentrate prepared from Kilimeen *(Nemipterus japonicus)* according to the procedure of Ismail *et al.* (1968). Animals of groups 1 and 3 received groundnut oil as the dietary fat and those of group 2 and group 4 received fish oil, prepared from oil sardine *(Sardinella longiceps)*. Adequate quantities of vitamins and minerals were added to all diets. Addition of cholesterol, at 0.5% level, to the diets was to ensure a sufficiently high level of cholesterol in serum and tissues of the animals.

Table 1. *Composition of the diets given to the four groups of rats*

	Group 1 %	Group 2 %	Group 3 %	Group 4 %
Glucose	25.0	25.0	25.0	25.0
Sucrose	25.0	25.0	25.0	25.0
Wheat flour	24.3	24.3	24.3	24.3
Casein	16.0	16.0	—	—
Fish Protein concentrate	—	—	16.0	16.0
Groundnut oil	5.0	—	5.0	—
Sardine oil	—	5.0	—	5.0
Shark liver oil[1]	2.0	2.0	2.0	2.0
Salt mixture[2]	2.0	2.0	2.0	2.0
Vitamin mixture[3]	0.2	0.2	0.2	0.2
Cholesterol	0.5	0.5	0.5	0.5

1. Commercial preparation of shark liver oil diluted with groundnut oil, as a source of Vitamin
2. Hubbel *et al.* (1937)
3. Chapman *et al.* (1959)

Rats were fed for 90 days on these diets. Feeding was always *ad libitum*, as evidenced by the residual food found in the cages. Adequate quantities of drinking water was provided in the cages throughout. Weight gain of the animals was determined every week. At the end of the feeding period the rats were starved for 24 hours and then killed. Blood, liver and heart from each rat were collected separately. Tissues were frozen and kept in polyetheylene bags at −18°C pending analysis.

Serum was separated by centrifugation of the blood at 2500 *g* at 4°C for 10 minutes. Total and free cholesterol in serum were determined by precipitation as digitonide and development of colour by Liebermann-Burchard reaction (Hawk, 1976). Total lipids from tissues were extracted with chloroform-methanol (2:1 v/v) (Bligh & Dyer, 1959). Complex lipids were removed from these samples by eluting from silicic acid column with ether (Dittmer & Wells, 1969). The eluate was used for the determination of total and free cholesterol by the method of Sperry and Well as modified by Rodnight (Dittmer & Wells, 1969).

Serum from all the animals in a group was combined together and the lipid was extracted from it with chloroform-methonol (2:1) mixture and was used for the determination of fatty acid composition. In the case of liver and heart lipids, the lipid samples from all the animals in each group were pooled and aliquots from these were used for fatty acid analysis. Lipid samples were saponified to remove the usaponifiable matter and the free fatty acids isolated were esterified with boron trifluoride (14%) in methanol (AOAC, 1975). The methyl esters were analysed on a Toshniwal gas chromatograph, using a column of 10% Silar 5 cp on Gaschrom Q, 80–100 (Applied Science Laboratories, USA). Analysis was carried out as reported earlier (Viswanathan Nair &Gopakumar, 1981).

Results and Discussion

Data on the weight gain of the animals during this experiment did not show any statistically significant difference among the different groups. Cholesterol levels of serum, liver and heart at the end of the experiment are presented in Table 2. These results clearly show that changing dietary fat or protein significantly affect the cholesterol levels. The effect of fish oil on serum cholesterol level is evident in group 2. Substitution of fish oil in place of groundnut oil has lowered the serum total cholesterol level by about 70%. Similarly the levels of free cholesterol and esterified cholesterol were lower in this group. All these changes were found to be highly significant ($P = 0.01$). In liver there was a decrease in level of total cholesterol, but this was small and was not statistically significant. Concentration of free cholesterol increased from 166.3 mg% in the grountnut oil group to 238.7 mg% in the fish oil fed group. In heart lipids also there was a highly significant ($P=0.01$) lowering in the levels of total and free

Table 2. *Cholesterol levels (mean for animals) with standard deviation of serum liver and heart of the four groups of rats given different dietary fats and proteins*

	Serum mg/100 ml				Liver g/100 gm				Heart g/100 g			
Groups	TC**	FC**	EC**	EC/FC**	TC	FC**	EC	EC/FC**	TC**	FC**	EC	EC/FC
1	76.28±	18.56±	57.72±	3.26±	2.62±	0.1663±	2.446±	14.86±	0.1081±	0.088±	20.12±	0.226±
	14.06	5.89	9.36	0.67	0.48	0.0018	0.47	2.61	0.0272	0.019	12.58	0.121
2	27.33±	7.88±	19.45±	2.52±	2.14±	0.2387±	1.898±	8.05±	0.0495±	0.445±	4.95±	0.1275±
	2.72	1.55	1.51	0.36	0.38	0.032	0.37	1.72	0.0130	0.014	1.48	0.064
3	41.14±	11.2±	29.94±	2.69±	1.65±	0.2205±	1.43±	6.48±	0.110±	0.0988±	13.98±	0.1475±
	10.83	3.07	7.86	0.25	0.37	0.010	0.36	1.53	0.0084	0.011	6.97	0.083
4	36.84±	12.78±	24.06±	1.98±	2.13±	0.2535±	1.874±	7.33±	0.1349±	0.121±	13.9±	0.1175±
	7.03	3.71	4.94	0.54	0.59	0.016	0.58	1.99	0.0081	0.009	5.2	0.046

** Differences among the groups highly significant (p=0.01)

cholesterol, while the changes in the concentration of esterified cholesterol were not statistically significant. In all these cases the ratio of esterified to free cholesterol (EC/FC) was higher in the groundnut oil fed groups compared to the fish oil fed groups.

Many theories have been put forward in the past to explain the mechanism by which fish oils and other polyunsaturated fats reduce cholesterol levels in animals. Dietary fat influences to a great extent the absorption of cholesterol from the intestine (Lutton *et al.*, 1980). These authors have found that the absorption coefficient of dietary cholesterol was 71% with lard as dietary fat and this was not changed apparantly with triolein or tripalmitin. But absorption was drastically reduced in rats receiving trierucin or tristearin (to 45% and 34% respectively). It is not known whether fish oil influences the absorption process to any appreciable extent in rats. However, cholesterol levels in group 4 animals, which also received fish oil in the feed, were somewhat higher than that of group 2 animals. If the reduced cholesterol levels in the fish oil-fed group are assumed to be due to a lowered absorption of cholesterol from the diet, then this effect should have been reflected in the case of group 4 animals also to the same extent. But, in spite of the fact that cholesterol has a higher absorption coefficient in pressence of casein, the dietary protein for the group 2 animals, in this group the cholesterol levels were lower. Therefore, the reduction in cholesterol levels observed here may not be due to any differences in the absorption of dietary cholesterol.

The polyunsaturated acids are believed to be involved in the normal transport and oxidation of cholesterol (Goldestein & Brown, 1977). Paul & Ganguly (1976) had reported that the polyunsaturated acids induced increased bile flow and sterol excretion and thereby lowered cholesterol levels. Control of biosynthesis of cholesterol by the dietary fat appears to be another mechanism in maintaining the cholesterol levels. Sondergaard & Johansen (1979) had shown that increased levels of dietary linoleic acid, in the form of vegetable oils, had resulted in decreased levels of biosynthesis of cholesterol. They had also reported that serum cholesterol level was linearly related to the biosynthesis of cholesterol. But the dietary linoleic acid does not seem to be the deciding factor in the present experiment because in the diet of the fish oil group the proportion of this acid was only about 50% of that in the diet of the groundnut oil group (Table 3). Similarly the dietary level of arachidonic acid, the other major ω6 polyunsaturated acid, for this group was about one-fourth

Table 3. *Dietary fatty acid composition of the four groups of rats (in weight percentage)*

Fatty acids	Group 1	Group 2	Group 3	Group 4
8:0	0.3	0.1	0.3	0.2
10:0	0.4	0.4	0.2	0.3
12:0	1.6	0.4	0.8	1.0
13:0	1.5	0.7	1.4	—
14:0	3.2	7.4	1.5	6.7
14:1	0.3	0.6	—	0.5
15:0	0.5	1.1	0.3	1.0
15:1	—	0.2	—	0.2
16:0	15.2	13.2	15.0	11.8
16:1	2.7	7.7	1.9	6.8
16:2	0.6	2.0	0.5	3.1
17:0	0.4	1.8	0.3	2.8
18:0	8.7	5.3	8.0	5.9
18:1	22.3	11.4	23.6	12.6
18:2	20.3	11.9	22.2	11.0
18:4	—	6.5	—	6.2
20:1	4.7	0.5	4.5	0.6
20:2	3.2	1.8	3.0	1.4
20:3	—	0.8	0.4	0.4
20:4	7.8	1.5	7.9	2.8
20:5	1.2	11.2	2.3	10.5
22:1	—	2.6	—	1.6
22:3	—	0.5	—	0.7
22:4	3.0	1.0	3.1	1.7
22:6	2.0	9.1	2.8	10.2

of that for the fish oil group. At the same time there was a significant increase in the levels of ω3 unsaturated acids (18:3 ω3, 20:5 ω3 and 22:6 ω3). Thus the net effect of changing the dietary fat source from groundnut oil to fish oil on the levels of polyunsaturated fatty acids was a substantially lower dietary avaliability of ω6 acids and a correspondingly higher levels of ω3 acids for the fish oil-fed group. Since the two diets were identical in all respects other than the dietary fat, the lowering of cholesterol levels observed in the group which was given fish oil can only be due to the effects of dietary fat. From these results, we may conclude that among the polyunsaturated acids, it is the ω3 acids which are responsible for the cholesterol lowering properties of fish oils. Antithrombotic and platelet deaggregating properties of the ω3 acids, especially of C20:5 ω3 acid, are known (Whitaker *et al.,* 1979) and our results indicate that these acids may be responsible for the lowering of cholesterol levels. Other major differences between the fatty acid compositions of the diets of the two groups were in the lower levels of stearic and oleic acids and higher proportions of myristic and palmitoleic acids in group 2. It is not certain whether these differences play any major role in lowering cholestrol levels.

The fatty acid patterns of liver, serum and heart lipids were found to be influenced to some extent by the dietary fatty acids. This was most evident in liver lipids where there was a substantial increase in ω3 acids and a corresponding lowering of ω 6 acids. In serum the differences were significant only in the levels of 22:6 ω3 and 14:0 acids. Fish oil fed group had higher levels of 22:6 ω3 and lower levels of 14:0. At the same time it may be noted that lowering of cholesterol was most pronounced in serum lipids. It is interesting to note that the serum lipids in contrast to the liver lipids, are not incorporating the ω3 acids from the diet with any special affinity at the expense of other fatty acids. So it is not clear at what stage the cholesterol metabolism is influenced by ω3 polyunsaturated acids. It is possible that these polyunsaturated acids may be disappearing along with cholesterol during the catabolism of cholesterol esters.

The theory of re-dsitribution of cholesterol between serum and other tissues, as a mechanism for lowering of serum cholesterol (Kaunitz, 1975; Crocker *et al.,* 1979), does not hold good in the present case because the lowering of serum cholesterol was not accompanied by any increase in liver or heart cholesterol levels. These results are in agreement with our findings in a previous study (Viswanathan Nair & Gopakumar, 1981). Peifer *et al.* (1962) had shown that certain fractions of menhaden oil could mobilise cholesterol out of the liver of hypercholesterolemic rats. Sen *et al.* (1977) also did not observe any significant increase in tissue cholesterol levels subsequent to the lowering of serum cholesterol levels in rats. Our results show that feeding fish oil results in lower cholesterol levels in liver and heart tissues also and this is probably due to the eliminaton of cholesterol from the system rather than a more change in the site of its storage.

Another interesting point observed was that in all cases the proportion of esterified cholesterol was more in the groundnut oil fed groups. Dietary supply of linoleic acid for this group was higher than that for the group which was given fish oil and this is known to be a favourable condition for cholesterol esterification process in rats. The polyunsaturated fatty acids in the fish oil diet also are highly efficient in forming cholesterol esters. Spector *et al.* (1980) had reported that diets rich in polyunsaturated fatty acids activate lecithin-cholesterol acyl transferase to a greater extent than those poor in polyunsaturated fatty acids. Thus it appears that the different effects of fish oil and groundnut oil on cholesterol metabolism in rats are not due to their ability to form cholesterol esters. This is known to be a critical step in its metabolic process. The differences may be in the later stages of metabolic processes.

Another way in which the polyunsaturated fatty acids can affect the cholesterol levels is by inhibiting the biosynthesis of cholesterol. Ramesha *et al.* (1980) had suggested that the lowering of serum cholesterol, brought about by polyunsaturated fatty acids, was the result of decreased rate of biosynthesis of cholesterol accompanied by increased fecal loss of cholesterol and bile acids. From all these facts we may conclude that the reduction of cholesterol levels brought about by fish oil is probably due to the ω3 polyunsaturated fatty acids present in them. It appears that these acids eliminate cholesterol from the system and also suppress the biosynthesis of the cholesterol.

Protein source for the third group of rats was fish protein concentrate. Data of the cholesterol levels of this group show clearly that fish protein has got cholesterol-lowering properties. In most cases the effect was the same as that of fish oil qualitatively, though there were quantitative differences. In general fish oil was more effective than fish protein in bringing down the cholesterol levels in serum and tissues. The reduction in cholesterol levels caused by changing the dietary protein was found to be highly significant (in serum and liver). The efficiency of fish protein was about 2/3 that of fish oil. As in the case of fish oil, fish protein also caused an increase in the free cholesterol level in liver and a lowering of the proportion of esterified cholesterol in serum and tissues. Hypocholesterolemic properties of plant proteins vis-a-vis animal proteins have been investigated extensively and it is generally concluded that plant proteins are hypocholesterolemic compared to animal proteins. The status of fish proteins in this respect has not received much attention. Our results show that fish protein is an efficient cholesterol lowering agent and may be comparable to plant proteins in this respect. This effect is not due to any

polyunsaturated fatty acids remaining in the protein preparation, as can be seen from the fatty acid compositions of the feeds of groups 1 and 3.

The mode of action of proteins in cholesterol metabolism is still unclear. Huff & Carrol (1980b) had found that the turn over of plasma cholesterol and the excretion of neutral sterols and bile acids were increased in casein fed rabbits. Similar observations had been made by Nagata *et al.* (1981) also. This was accompanied by decreased levels of absorption and biosynthesis of cholesterol and the net result was a lowering of cholesterol levels in animals (Carrol, 1981).

Huff & Carrol (1980b) had reported that a mixture of amino acids can influence the concentration of plasma cholesterol in rabbits. Their experiments had shown that the difference between plant and animal proteins on plasma cholesterol was partially due to their different amino acid compositions. Interactions between essential and non essential amino acids may be playing an important role. However, they conclude that it is not clear how amino acids influence plasma cholesterol levels and whether the effect is at the level of absorption or at other stages of amino acid metabolism. It is also not clear whether amino acid mixtures affect plasma cholesterol levels in exactly the same way as the intact proteins. Enzyme hydrolysates of casein and soya protein were found to have hypocholesterolemic effects comparable to those of the intact proteins, but the results obtained with amino acid mixtures corresponding to the respective proteins were not so clear cut (Carrol & Hamilton, 1975). A hypothesis proposed to explain the difference between the effects of casein and soya protein is the lysine-arginine ratio (Kritchevsky, 1979) which is 2.0 for casein and 0.9 for soya protein. Lysine inhibits liver arginase activity and in casein fed animals more arginine will be available for incorporation into an arginine rich approprotein which is atherogenic for rabbits. Kritchevsky *et al.* (1978) added enough lysine to soya protein to approximate the lysine-arginine ratio of casein and found that the atherogenicity of the diet was enhanced. But the results obtained by adding arginine to casein to make the lysine-arginie ratio approximately the same as that of soya protein were equivocal. Thus it appears that there is an inherent constitutional difference between the two proteins.

Another possible factor in the control of cholesterol metabolism is the actual level of arginine or lysine, rather than the ratio of the two (Kritchevsky, 1979). An arginine rich lipoprotein, designated as HDLC, formed under pathological conditions in the plasma of swine, fed high cholesterol diets was found to bind with a high affinity to the LDL receptor of human fibroblast cells, thus playing a vital role in the control of cell-and plasma cholesterol through the LDL pathway (Goldstein & Brown, 1977). The availability of amino acids like arginine may also be a factor in the control of atherosclerosis. The effect of fish protein, in all probability, can be due to its characteristic amino acid composition. It is not known whether this property is common for fish proteins as a whole or proteins from different species have differences in their hypocholesterolemic properties.

The cholesterol levels of serum and tissues of the group 4 animals present a more interesting picture. In this case, both the dietary protein and fat, namely, fish protein and fish oil, were hypocholesterolemic agents, but the net result was not an additive one. Changes in cholesterol levels in this group was not to the same extent as those due to the fish oil, but comparable to those due to fish protein alone. Lofland *et al.* (1966) had reported that any single dietary component may be influenced by other dietary ingredients and that the effects are not always additive. Addition of corn oil to casein diet has been found to prevent rise in plasma cholesterol levels. At the same time, in the case of soya protein diet the effect was only small (Carrol & Hamilton, 1975). But Forsythe *et al.* (1980) had found that there was no interaction between dietary fat and protien. From our results it appears that there exists some degree of interaction between the dietary fat and protein.

The above interaction has influenced the fatty acid composition of the serum lipids also (Table 4). Proportions of the polyunsaturated acids, 18:2 ω6, 20:4 ω6, 20:5 ω3 and 22:6 ω3, were significantly lower in group 4 animals when compared with animals of group 2. Both these groups received fish oil as the dietary fat and the fatty acid compositions of the lipdis extracted from these two diets do not show any notable differences. Thus the differences observed in the fatty acid patterns of serum can be due to the protein fat interactions. It could not be established whether the lowered levels of polyunsaturated acids was due to any inhibition at the absorption stage or due to other influences in the further metabolic processes. However, in liver lipids these differences were not so pronounced and in heart lipids the differences had practically disappeared. As discussed earlier, the presence of appreciable quantities of the acids like C 20:5 ω3 and C 22:6 ω3 which are characteristic of fish oils, in the liver and heart tissues indicate that absorption of these acids from the diet was not completely blocked, but incorporation of these acids into serum lipids was inhibited at some stage by fish protein. In this context, it may be recalled that the extent of cholesterol lowering due to the fish protein fish oil diet was approximately the same as that due to the fish protein diet. This might have been due to the failure of the lipids to incorporate the polyunsaturated acids from the diet.

From the data of our present investigation we conclude that sardine oil is a good hypocholesterolemic agent, mainly due to the high content of ω3 polyunsaturated acids. Fish protein also has strong hypocholesterolemic properties, though not to the same extent as that of fish oil. This may primarily be due

Table 4. *Fatty acid composition of the lipids of serum, liver and heart of the rats fed different dietary fats and proteins (in weight percentage)*

	Serum				Liver				Heart			
Fatty acids	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4
8:0	0.7	0.5	0.3	0.5	—	—	—	—	0.8	0.3	—	0.2
10:0	0.4	0.2	0.1	0.4	—	—	—	—	0.2	0.4	—	0.2
12:0	0.8	0.7	0.4	0.6	—	—	—	—	0.6	0.5	0.3	0.2
13:0	0.7	—	—	0.7	—	—	—	—	—	0.4	—	—
14:0	4.1	2.2	1.1	1.8	1.0	0.4	0.8	0.9	2.8	2.2	2.2	3.1
14:1	0.3	1.0	0.5	1.2	—	—	—	—	0.2	0.4	0.1	0.3
15:0	0.8	0.6	0.5	1.4	0.4	0.4	0.2	0.3	0.3	1.0	0.3	0.5
15:1	0.3	0.8	0.3	1.1	0.2	0.2	0.2	0.2	0.4	1.1	0.3	0.5
16:0	19.0	19.7	17.3	20.6	17.5	15.0	17.8	19.5	16.7	15.9	19.6	12.8
16:1	7.8	7.1	5.1	8.3	5.0	5.5	4.9	6.6	6.4	6.1	6.2	7.8
16:2	3.5	1.5	1.0	3.3	0.4	0.9	0.6	1.2	0.7	1.6	0.6	1.5
17:0	3.0	2.3	1.2	3.8	0.8	1.2	1.2	1.4	1.4	2.3	1.1	2.0
18:0	7.9	9.0	8.6	12.2	10.3	12.8	10.5	13.5	14.5	15.9	11.5	15.2
18:1	20.3	22.2	26.1	24.4	27.9	20.4	25.2	21.3	27.3	17.8	29.2	19.9
18:2	15.0	14.6	23.7	8.2	22.2	16.0	23.7	14.1	19.2	14.1	21.0	14.2
18:3	—	—	—	2.9	—	0.6	—	—	—	—	—	0.7
20:1	1.5	0.6	0.6	1.7	0.3	1.1	0.4	0.6	0.6	1.7	1.0	1.3
20:2	1.5	1.2	2.0	2.9	1.6	1.5	1.9	2.1	1.8	2.0	2.4	2.1
20:3	—	—	—	—	0.3	—	0.6	—	0.2	1.2	—	—
20:4	4.9	5.4	7.7	0.7	7.7	7.5	8.0	8.4	3.9	7.1	3.1	8.2
20:5	4.0	4.8	2.1	0.9	0.8	7.0	1.5	5.3	0.5	1.7	—	2.1
22:1	2.5	—	—	0.8	0.8	—	—	—	—	—	—	—
22:3	—	—	—	—	—	—	—	—	—	0.5	—	—
22:4	—	—	—	0.8	—	0.1	—	—	0.4	0.7	—	0.6
22:5	—	—	—	—	—	1.2	—	—	—	—	—	1.5
22:6	1.0	5.8	1.3	0.9	2.8	8.0	2.5	4.7	1.1	5.0	1.1	4.7

to the characteristic amino acid composition of this protein. Fish oil and fish protein, combined together, do not show an additive effect, but rather resembles fish protein alone in their cholesterol-lowering action. It was also observed that incorporation of dietary fatty acids into serum lipids was inhibited by dietary fish protein.

The authors express their sincere gratitude to Shri M.R. Nair, Scientist-in-Charge, Biochemistry and Nutrition Division for the valuable guidance and to Dr. C. C. P. Rao, Director, Central Institute of Fisheries Technology, Cochin for the kind permission to publish this paper.

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