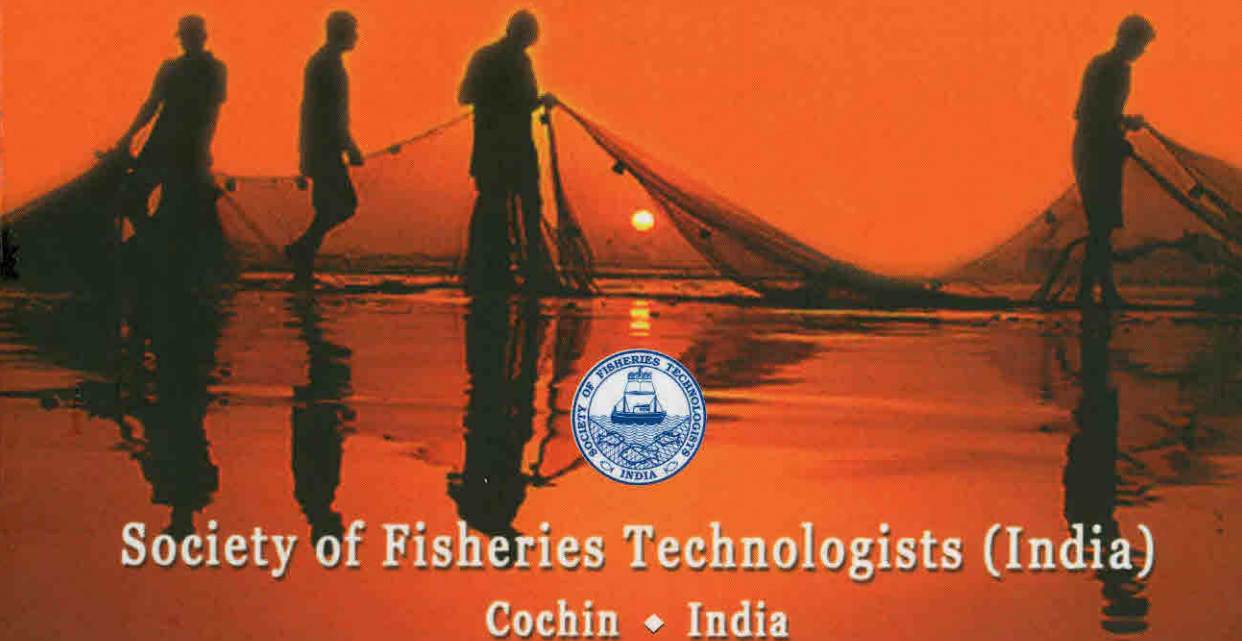
Coastal Fishery Resources of India

Conservation and Sustainable Utilisation



Society of Fisheries Technologists (India)

Cochin ◆ India

Coastal Fishery Resources of India: Conservation and Sustainable Utilisation

Proceedings of the National Seminar on Conservation and Sustainability of Coastal Living Resources of India, 1-3 December 2009, Cochin

Organised by

Society of Fisheries Technologists (India), Cochin
and
Centre for Ocean and Environmental Studies, New Delhi

In association with

Ministry of Earth Sciences (New Delhi)
Central Marine Fisheries Research Institute (Cochin)
National Institute of Oceanography (Goa) and
Central Institute of Fisheries Technology (Cochin)





ISBN: 978-81-901038-7-9

Published by

Society of Fisheries Technologists (India)
P.O. Matsyapuri, CIFT Junction, Cochin - 682 029, India

URL : www.fishtech.org
Phone : 91 (0)484-2666845
Fax : 91 (0)484-2668212
Telegram : FISHTECH / MATSYAOUDYOGIKI
E-mail : cift@ciftmail.org
enk_ciftaris@sancharnet.in

Citation:

Rao, G.S. (2010) Current status and prospects of fishery resources of the Indian continental shelf, In: Coastal Fishery Resources of India: Conservation and Sustainable Utilisation (Meenakumari, B., Boopendranath, M.R., Edwin, L., Sankar, T.V., Gopal, N. and Ninan, G., Eds.), p. 1-13, Society of Fisheries Technologists (India), Cochin

Cover design: Vineethkumar, P., CIFT, Cochin

Printed at PAICO, Cochin - 682 035, India

Coastal Fishery Resources of India

Conservation and Sustainable Utilisation

Editors

B. Meenakumari
M.R. Boopendranath
Leela Edwin
T.V. Sankar
Nikita Gopal
George Ninan

Society of Fisheries Technologists (India)
P.O. Matsyapuri, CIFT Junction, Cochin - 682 029, India

2010

Influence of Coconut Oil, Fish Oil and Polyunsaturated Fatty Acid enriched Diets on the Health Status of Albino Rats

Mathen Mathew* and Suseela Mathew
Central Institute of Fisheries Technology
P.O. Matsyapuri, CIFT Junction, Cochin - 682 029, Kerala, India
**E-mail: matdemo@gmail.com*

Introduction

Contrary to popular belief, fats and oils are an important part of nutrition in a healthy diet. Healthful sources of fat contribute to our energy stores and act as excellent lubricants, lubing up our digestive tract and providing protective insulation to our organs as well as allowing for the absorption of fat-soluble vitamin A, vitamin D, vitamin E, and vitamin K (He, 2009). Fish oils are rich in the fat soluble vitamins and they contain antioxidant hydrocarbons like squalene and immune boosting compounds like 1-O-alkyl glycerol ethers and are blessed with the long chain polyunsaturated fatty acids especially the n-3 series eg. EPA, DHA (Enas, 1996). These long chain n3 PUFAs have been shown to reduce cardiovascular risk factors and reduce symptoms in rheumatoid arthritis. Fish oil helps in reducing the risk of heart related disorders as it is a powerful anti-inflammatory and can help reduce the risk from the C-reactive proteins (Ciubotaru *et al.*, 2003). Secondly, the omega 3 fatty acids help to prevent clumping and stickiness of the blood. Thus, blood can flow more easily in the arteries reducing the probability of heart attacks or strokes. Fish oil also helps to prevent plaque build-up inside the arteries. The association between fish consumption and risk of cardiovascular disease (CVD) has been extensively studied (Virtanen *et al.,* 2008; Mozaffarian *et al.,* 2008; Leaf *et al.,* 2008; Rule *et al.,* 1996). Although the results are inconsistent, the majority of studies are in favour of the cardio protective effects of fish consumption. Dietary n3 PUFA of marine origin rich in EPA and DHA are reported to be more effective than vegetable oils in decreasing plasma triglyceride (TG) and cholesterol concentrations. The low incidence of coronary heart disease and inflammatory disorders among Greenland Eskimos has been attributed in

part to the production of eicosanoids from n-3 PUFA by platelets and blood vessel walls (Saremi and Arora, 2009; Huang and Fang, 2000). The beneficial effects of fish consumption on the risk of CVD are the synergistic effects of many nutrients in fish, and the integrative effects of fish consumption may reflect the interactions of nutrients in fish. But despite its multiple benefits to the living system most people hesitate to use fish oil as cooking oil, owing to its pungent taste and unpleasant aroma and hence limit their use in food preparations.

Most vegetable cooking oils are low in saturated fats and are "heart healthy" with the important exception of tropical oils, such as coconut and palm oil, which are very rich in saturated fats. Coconut oil forms a major part of the diet among the South Asians. And most of the food, and fish especially, are prepared in coconut oil to add flavour and increase overall acceptability. But coconut oil alone has its limitations. Coconut oil is rich in saturated fatty acids (SFA) namely lauric (C12:0) and myristic (C14:0) acids (Cox *et al.,* 1995). The hypercholesterolemic action of dietary saturated fatty acids is well established (Andrea *et al.,* 1992; Reiser *et al.,* 1985). Various authors have proposed that the C12-C18 saturated fatty acids are capable of raising serum cholesterol and triglyceride levels in the body (Lin and Maria, 1998). Saturated fats lead to deposition of fats in the blood vessels leading to atherosclerosis. Elevated blood cholesterol is the strongest risk factor for coronary artery disease, and dietary excess of saturated fats is its largest contributor. Though coconut oil contains no cholesterol, its cholesterol-raising potential is similar to or higher than most animal fats (Enas, 1996).

In view of the partial negative role played by coconut oil, the present study was taken up with an aim to replace coconut oil partially with a better substitute such as oil with more unsaturated fats, thereby causing less health havoc to the consumer. The fish oil could be given as a supplement in the form of an encapsulation and this could reduce the ill effects of coconut oil. Three isolipidemic diets – one comprising of coconut oil (CO) alone and the others comprising of fish oil (FO) and n3-polyunsaturated fatty acids (PUFA) the latter two made isolipidemic with coconut oil, were prepared and fed to male albino rats and the effects of these diets on the levels of diagnostic marker enzymes and lipid profile status in the blood, liver and heart tissues of the animals were studied.

Materials and Methods

All chemicals and reagents used were obtained from Merck (Darmstadt, Germany). The chemical standards used for the analyses

were purchased from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA). Commercially available coconut oil was used for the study. Fish oil was extracted following the method of Folch *et al.* (1957) from the liver of a *Centrophorus* sp. PUFA concentrate was prepared from sardine oil by removing the major part of saturated and monounsaturated fractions as urea inclusion complexes, using a modified procedure of Ackman *et al.* (1988). The fatty acid profile of the three lipid sources have been shown in Table 1. The fatty acid (Bakes and Nichols, 1995) composition was determined in a Gas Chromatograph (Thermo Electron Corporation, Milan, Italy) equipped with Perkin Elmer Elite 225 (Perkin Elmer Life and Analytical Services, Watham, MA) 50% cyanopropyl phenyl – 50% methyl capillary column (30 m × 0.25 mm i.d.) , an FID and a split/splitless injector. Peaks were quantified with Chromcard software by comparing retention time data with those obtained for authentic standards. The major fish oil constituents were determined using an Iatroscan – MK-6S (Mitsubishi Kagaku Latron Inc., Tokyo, Japan) to determine the abundance of individual lipid classes besides fatty acids, employing the procedure of Mathen *et al.* (2008).

Table 1: Fatty acid (as % total fatty acid) composition of lipid sources used in the study

Fatty acid	Coconut oil (CO)	Fish oil (FO)	Polyunsaturated fatty acid (PUFA) concentrate
8:0	8.86±0.66	ND	ND
10:0	6.17±0.04	0.53±0.06	ND
12:0	47.53±1.56	2.64±0.53	1.31±0.49
14:0	19.97±1.03	1.51±0.11	ND
16:0	7.84±0.35	10.82±0.92	5.86±0.68
16:1	ND	3.34±0.53	3.62±0.33
18:0	3.06±0.73	4.17±0.85	3.03±0.53
18:1n9	4.44±0.79	33.43±2.43	8.12±1.05
18:2n6	0.76±0.05	1.68±0.06	ND
20:0	0.10±0.34	0.31±0.12	0.43±0.72
20:1	ND	14.87±1.19	ND
20:4n6	ND	4.37±1.42	9.49±1.13
20:5n3	ND	9.46±0.95	27.53±2.76
22:6n3	ND	11.32±1.02	38.71±3.52
Others	1.27± 0.57	1.55±0.97	1.90±0.16

Wistar strain male albino rats (160-180 g) were used in the experimental study. They were housed individually in polypropylene cages under hygienic conditions and were provided food and water *ad libitum*. The animals were maintained on a 12:12 h light: dark photoperiod under standard conditions of temperature and ventilation. The experiments were performed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and with the approval of the Institutional Animal Ethics Committee (IAEC).

All animals were fed a basal diet enriched with either coconut oil (CO), fish oil (FO) or poly-unsaturated fatty acids (PUFA) for a period of 8 weeks. The composition of the basal diet was as follows: corn starch (60.6%), casein (18.3%), salt mixture (4%), vitamin mixture (1%), cellulose (5%), cholesterol (1%) and methionine (0.1%). Five days after acclimatization, the rats were divided into three groups of 6 animals each and were allowed free access to the experimental diets and water. The first group of animals (Group CO) was fed, in addition to the basal diet, coconut oil at 10% feed levels. The remaining groups were also fed with the basal diets and fish oil (Group FO) and polyunsaturated fatty acids (Group PUFA) at 5% and 1% feed levels respectively and made isolipidemic with coconut oil. Composition of experimental diets is given in Table 2.

Table 2: Diet composition

Diet components	CO diet, %	FO diet, %	PUFA diet, %
Corn starch	60.6	60.6	60.6
Casein	18.3	18.3	18.3
Salt Mixture	4.0	4.0	4.0
Vitamin mixture	1.0	1.0	1.0
Cellulose	5.0	5.0	5.0
Cholesterol	1.0	1.0	1.0
Methionine	0.1	0.1	0.1
Coconut oil	10.0	5.0	9.0
Fish oil	-	5.0	-
PUFA concentrate	-	-	1.0

Body weight and feed consumption were recorded every week. To avoid auto-oxidation of the fat, each diet was stored at -20°C and freshly prepared each day. At the end of the experimental period the animals were fasted overnight, thereafter, ether anesthetized and blood samples collected. They were then sacrificed; liver and heart tissues were excised, washed with chilled isotonic saline; a portion of the tissue homogenates were prepared in 0.1 M Tris-HCl buffer (pH 7.2) and subjected to further analyses.

The collected blood sera and heart tissue extracts from all groups of animals were analyzed for the level of diagnostic marker enzymes alanine aminotransferase (ALT) (Mohur and Cook, 1957), aspartate aminotransferase (AST) (Mohur and Cook, 1957) and lactate dehydrogenase (LDH) (King, 1965). Low and high density lipoproteins (LDL and HDL) in the blood sera were separated according to the method of Burstein and Scholnick (1972). Lipid extracts of the blood serum and heart tissues were prepared (Folch *et al.*, 1957) and analysed for the cholesterol (Parekh and Jung, 1970) contents, triglycerides (Rice, 1970), free fatty acids (Horn and Menahan, 1981), phospholipids (Fiske and Subbarow, 1925) and lipid peroxides (Ohkawa *et al.,* 1979). Aliquots of the heart tissue homogenates were also used for the determination of non-enzymatic and enzymatic antioxidants namely vitamin E (Baker *et al.,* 1980), reduced glutathione (GSH)(Ellman, 1959) and catalase (CAT) (Takahara *et al.,* 1960), superoxide dismutase (SOD) (Misra and Fridovich, 1972), glutathione peroxidase (GPx) (Paglia and Valentine, 1967) and glutathione-S-transferase (Habig *et al.,* 1974). The myocardial fatty acid composition of CO, FO and PUFA rats was determined using Gas Chromatograph (Thermo Electron Corporation, Milan, Italy).

The results are expressed as mean±SD and analyzed statistically by one-way analysis of variance using Duncan's test with a level of significance set at $P<0.05$. All data were analyzed with the aid of statistical software SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL). Pearson's correlation co-efficient test was performed between the various lipid components analyzed to determine the factor(s) responsible for the observed effects.

Results and Discussion

The major lipid components of the fish oil extracted from the livers of *Centrophorus* sp. was analysed (Table 3). The oil contained a major portion of the hydrocarbon squalene (47.81% total lipid) along with glycerol

ethers (12.31% total lipid). Squalene (Ko *et al.*, 2002) is an antioxidant with potent pharmaceutical values (Qureshi *et al.*, 1996). Glycerol ethers or 1-O-alkylglycerols (AKGs) constitute about 10-30% of the unsaponifiable matter of the fish oils (Hallgren and Larsson, 1962). It has been reported that naturally occurring AKGs have potent biological activities on various cells or systems (Devaraj and Jialal, 2000). The fat soluble vitamins (vitamin E and vitamin A) formed nearly 5% of the total lipids. The results are in close agreement to those reported by Peyronel *et al.* (1983) and Bordier *et al.* (1996).

Table 3: Major constituents of fish oil

Fish oil constituents	% of total lipids
Squalene	47.81±2.84
Cholesterol	4.72±1.24
Glycerol ethers	12.31±1.79
Vitamin E	3.65±1.13
Vitamin A	1.58±1.02

The oil from *Centrophorus* sp. and the polyunsaturated fatty acid concentrate contained 20 and 66% long chain n3 polyunsaturated fatty acid levels respectively (Table 1). It has been shown that these long chain n-3 polyunsaturated fatty acids (James *et al.*, 2003) lower the incidence of inflammatory diseases such as asthma and arthritis (Calder 2006), reduce the levels of arachidonic acid metabolites and lower the formation of proinflammatory compounds, like prostaglandins and leukotrienes, by blocking their activity (Olivera, 2004). Early studies reviewed by Stamp *et al.* (2005) and Calder (2006) attributed the anti-inflammatory effects of fish oils to competition with arachidonic acid for production of inflammatory eicosanoids. More recent investigations show that EPA and DHA produce novel anti-inflammatory lipids (i.e., resolvins and protectins) which appeared in a transgenic mouse model to have anti-inflammatory effects (Arita *et al.*, 2005; Lukiw *et al.*, 2005; Hudert *et al.*, 2006). EPA and DHA contained in fish oils also help to increase levels of digestive enzymes in the body thereby providing nutrients needed to build anti-inflammatory prostaglandin series 1 and 3, which helps in weight loss (Simopoulos, 1991).

In the present study, we compared the effects of three isolipidemic diets on the diagnostic marker enzyme levels, the lipid profile and the

antioxidant status in albino rats. Rats fed CO diets gained a significant increase ($P<0.05$) in body weight towards the end of the experimental period (from the 6th to the 8th week). However, there was no significant difference among rats fed either of the FO or PUFA diets (Table 4). Rats fed FO and PUFA diets showed a comparatively higher ($P<0.05$) feed conversion ratio than those on CO diets, from the sixth week of feeding Fig. 1. The results are in close agreement with the studies of fish oil supplementation in cattle diets by Nicholson *et al.* (1992) who reported an improved feed efficiency for cattle. Similarly, though rats fed on PUFA diets showed increase in both liver and heart weights, no significant differences were observed in their weights, in rats fed on any of the other experimental diets.

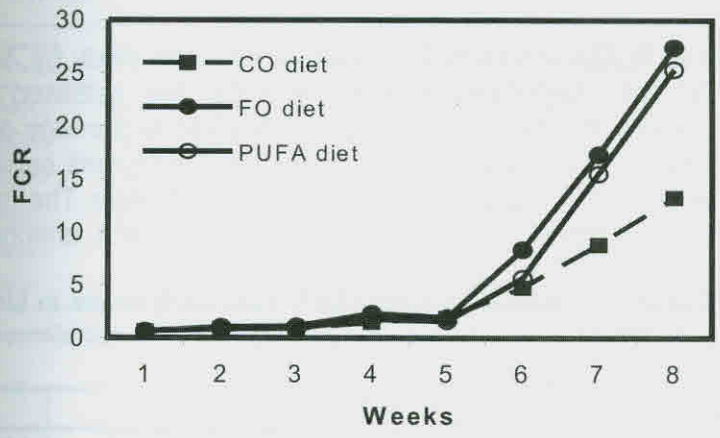


Fig. 1: Feed Conversion Ratio (FCR) of albino rats on CO, FO and PUFA supplemented diets

Rats fed with the high energy CO diets showed significantly high ($P<0.05$) levels of diagnostic marker enzymes (AST, ALT, LDH) in the blood serum and heart tissue extracts than those fed with FO or PUFA enriched diets (Table 5). This is in accordance with previously reported works which showed that supplementation of coconut oil to the diet induced cellular damage. (Gil-Villarino *et al.,* 1997) Moreover, diets rich in saturated fatty acids increase oxidative stress (Harvey *et al.,* 2009) and thereby improve the level of diagnostic marker enzymes in blood serum and heart tissues. (Nageswari *et al.,* 1999). This implies that consuming diets rich in saturated fats would lead to inflammatory disorders and CVDs (Fachinetto *et al.,* 2005). However, no significant differences in the enzyme levels were observed in the heart tissue extracts of rats fed with either FO or PUFA

Table 4: Effect of isolipidemic diets on body weights (g)*

Weeks	CO diet	FO diet	PUFA diet
1	197.6±2.4	196.0±2.7	196.0±2.2
2	219.7±3.1	220.3±3.6	218.5±3.5
3	236.3±2.3	235.5±4.7	233.6±2.9
4	249.7±2.6	247.3±2.9	247.8±3.5
5	256.8±2.4	252.8±3.3	255.7±3.1
6	275.3±2.7^{b}	264.8±3.1^{a}	267.8±2.6^{a}
7	283.7±2.4^{b}	274.8±2.9^{a}	275.8±3.6^{a}
8	284.5±2.8^{b}	275.6±2.4^{a}	277.2±3.1^{a}

Means within a row with different letters are significantly different ($P<0.05$)

diets. This is in agreement with an earlier reported study by Anandan *et al.* (2007). The diagnostic marker enzymes are released from the myocardium into the blood stream due to the tissue damage and due to the tissue specificity and catalytic activity. These enzymes serve as good markers of even the slightest myocardial tissue damage. The results also imply the cyto-protective activity of the FO and PUFA enriched diets.

Table 5: Diagnostic marker enzyme (AST, ALT, LDH) levels in blood serum and heart tissues of rats fed CO, FO and PUFA supplemented diets*

Diet type	Serum / tissue	AST	ALT	LDH
CO diet	Serum	109.12± 6.35^{c}	108.41± 2.39^{c}	152.03± 7.71^{b}
	Heart	164.59±4.80^{b}	105.53±6.89^{b}	195.19±4.80^{b}
FO diet	Serum	87.28±2.35^{b}	54.71±1.47^{a}	115.63±5.23^{a}
	Heart	65.04±4.23^{a}	33.65±7.55^{a}	85.48±6.25^{a}
PUFA diet	Serum	75.48±3.65^{a}	70.48±2.33^{b}	105.42±10.83^{a}
	Heart	58.18±5.40^{a}	46.21±5.08^{a}	79.77±4.14^{a}

* Serum AST, ALT, LDH in µmoles/h/L; Heart AST, ALT, LDH in nmoles/h/mg protein
Means within a column with different letters are significantly different ($P<0.05$)

Lipid composition of the blood serum and heart tissues of rats changed rapidly in response to the supplemented diets (Table 6). A significant increase ($P<0.05$) in the levels of cholesterol and triglyceride was observed in serum and heart tissues of CO rats compared to FO and PUFA fed rats, indicating the severity of the high saturated fat diet in CO rats. No significant differences were observed in cholesterol and

triglyceride contents in the sera and heart tissues of rats fed either FO or PUFA diets. However, a significant rise ($P<0.05$) in HDL contents and corresponding decline in LDL and VLDL contents were observed in the serum of FO and PUFA fed rats compared to CO rats (Fig.2). The elevated cholesterol level observed in the cardiac tissue of CO rats might be due to the increased uptake of LDL-cholesterol from the blood by the myocardial membranes. The results imply that substituting saturated fatty acids with polyunsaturated fatty acids results in a decrease in serum total and LDL cholesterol concentrations.

Table 6: Lipid composition (Triglycerides, cholesterol, phospholipids, free fatty acids, lipid peroxides) of the blood serum and heart tissues of rats, fed CO, FO and PUFA supplemented diets*

Diet type	Serum /Tissue	Triglycerides	Cholesterol	Phospho-lipids	Free fatty Acids	Lipid peroxides
CO diet	Serum	354.71±13.19 ^{b}	110.88± 10.01^{b}	1.88 ± 0.15^{a}	32.40 ±1.54^{b}	7.01± 0.25
	Heart	65.34±5.96^{b}	76.25±1.12^{c}	0.41± 0.15^{a}	11.11±1.75	1.67±0.33^{a}
FO diet	Serum	203.51±13.97^{a}	62.92±2.29^{a}	4.19± 0.42^{b}	14.41 ± 2.52^{a}	6.86 ± 0.10
	Heart	22.47±5.25^{a}	32.58±2.22^{a}	0.68±0.05^{b}	7.99 ± 1.32	2.34 ± 0.07^{b}
PUFA diet	Serum	221.42±13.76^{a}	69.35±6.70^{a}	4.14± 0.27^{b}	14.32±2.14^{a}	6.98±0.07
	Heart	19.83±0.87^{a}	37.95±2.44^{a,b}	0.98±0.21^{c}	9.47±0.89	3.18±0.19^{c}

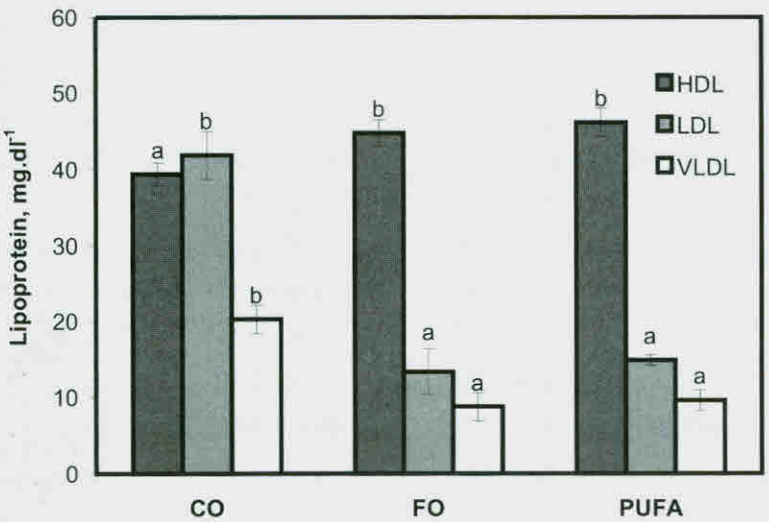


Fig. 2: Lipoproteins in the blood serum of CO, FO and PUFA fed rats (Means, for a particular lipoprotein, with different letters are significantly different)

In human beings, this is believed to be partly due to the effect of acyl-coenzyme A:cholesterol acyltransferase (ACAT), a key enzyme in cholesterol metabolism. This enzyme esterifies free cholesterol to cholesteryl ester within the cytoplasm of cardiocytes but it prefers unsaturated fatty acids, rather than saturated fatty acids, as the substrate for esterification. Diets high in unsaturated fats result in increased ACAT activity and decrease in the cardiac pool of free cholesterol. Low levels of free cytosolic cholesterol increases the transcription of the LDL receptor gene. The net result is an increase in the number of cardiac LDL receptors and a concomitant decrease in the serum LDL concentration.

A significant increase ($P<0.05$) was observed in the level of free fatty acids in the serum and heart tissues of CO rats compared to FO and PUFA fed rats (Table 7). The significant rise noticed in the levels of free

Table 7: Level of fatty acids (as % total fatty acids) in the heart tissues of Albino rats fed CO, FO and PUFA supplemented diets*

Fatty acid	CO	FO	PUFA
12:0	16.82 ± 0.20^{b}	1.53 ± 0.43^{a}	2.47 ± 0.54^{a}
14:0	11.66 ± 0.26^{c}	2.77 ± 0.34^{a}	4.20 ± 0.15^{b}
16:0	28.01 ± 1.89^{b}	23.02 ± 1.27^{a}	23.14 ± 1.18^{a}
18:0	12.52 ± 0.47^{c}	7.90 ± 0.65^{a}	9.25 ± 0.74^{b}
Σ saturated	69.01 ± 0.79^{c}	35.22 ± 0.41^{a}	38.86 ± 0.42^{b}
16:1	4.68 ± 0.35^{a}	8.97 ± 1.30^{b}	7.31 ± 1.22^{b}
18:1n9	7.37 ± 1.61^{a}	23.71 ± 2.19^{c}	18.45 ± 2.30^{b}
Σ mono-unsaturated	12.05 ± 0.18^{a}	31.68 ± 0.62^{c}	25.76 ± 0.76^{b}
18:2n6	5.12 ± 0.35^{a}	4.90 ± 0.72	5.36 ± 0.91
20:2	0.36 ± 0.10^{a}	7.32 ± 1.32^{b}	6.82 ± 0.94^{b}
20:4n6	10.91 ± 1.83^{b}	4.87 ± 0.33^{a}	3.42 ± 1.64^{a}
20:5n3	0.86 ± 0.04^{a}	6.92 ± 1.45^{b}	8.82 ± 1.62^{b}
20:6n3	1.46 ± 0.82^{a}	8.76 ± 0.78^{b}	10.57 ± 1.72^{c}
Σ poly-unsaturated	18.71 ± 0.73^{a}	32.77 ± 0.46^{b}	34.99 ± 0.48^{c}
Σ n6	16.03 ± 1.04^{c}	9.77 ± 0.27^{b}	8.78 ± 0.51^{a}
Σ n3	2.32 ± 0.55^{a}	15.68 ± 0.47^{b}	19.39 ± 0.07^{c}
Σ n6/n3	6.91 ± 0.35^{b}	0.62 ± 0.14^{a}	0.45 ± 0.31^{a}

* Means within a row with different letters are significantly different ($P< 0.05$)

fatty acids in serum and heart tissue of CO rats may be due to the enhanced breakdown of membrane phospholipids both in the adipose tissue and myocardium by the lipolytic action of phospholipase A2 which could likely be the reason for cell injury and ischemia in patients with cardiovascular disorders (Kobayashi *et al.,* 2009)

Phospholipid, measured as the levels of total inorganic phosphorus contents, varied significantly in the blood serum of CO, FO and PUFA rats. The levels were significantly higher ($P<0.05$) in the FO and PUFA fed groups compared to CO fed rats. FO and PUFA administration in the diets prevented the degradation of membrane phospholipids and hence improved membrane stability (Kabay *et al.,* 2009).

The lipid peroxide levels varied significantly ($P<0.05$) in heart tissues of rats fed with the different diets. Their levels were significantly higher in the ($P<0.05$) PUFA and FO rats than in the CO rats. When PUFA and FO are compared, significantly higher ($P<0.05$) contents of peroxides were noted in the PUFA compared to FO diet, which may be attributed to the high extent of unsaturation in the PUFA (Ganesan *et al.,* 2008).

Lipid peroxidation *in vivo* has been identified as one of the basic deteriorative reactions in the cellular mechanisms of aging (Sharma *et al.,* 2008). Lipid peroxidation of membranes is regulated by the availability of substrate in the form of polyunsaturated fatty acids, the availability of inducers such as free radicals and excited state molecules to initiate propagation, the antioxidant defence status of the environment and the physical status of the membrane lipids (Parvez *et al.,* 2006). In the present study, the polyunsaturated fatty acids in the biological membranes would have undergone oxidation causing impairment of membrane function, decrease in membrane fluidity, disruption of membrane structure and hence inactivation of membrane receptors and enzymes.

Antioxidants are well known to alleviate inflammatory processes mediated by allergic substances. The presence of natural antioxidants like vitamin E and squalene in the FO diets would have been responsible for the observed low levels of peroxides in the heart tissues of the respective animals. Numerous workers have proved the protective effects of vitamin E (Kabay *et al.,* 2009; Kobayashi *et al.,* 2009; Vittala *et al.,* 2004) and squalene (Sabeena *et al.,* 2007; Surendraraj *et al.,* 2009) against oxidative stress in animals.

Table 3 depicts the myocardial fatty acid composition of rats fed CO, FO and PUFA enriched diets. Significant changes were observed in the

fatty acid composition of the heart tissues of rats fed CO diets compared to those on FO and PUFA diets. Significant rise ($P<0.05$) in saturated fatty acids (12:0, 14:0, 16:0, 18:0) were observed in the heart tissues of rats on CO diets compared to those on FO or PUFA diets. The monounsaturated fatty acid composition of FO and PUFA ($P<0.05$) fed rats were significantly higher than CO rats, the former two groups being richer in oleic acid contents. Oleic acid is potential hypotensive agent and may hinder the progression of adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands (Rizzo *et al.,* 1986). Rats fed on FO and PUFA diets showed increased levels of polyunsaturated fatty acids in their heart tissues. There was a significant increase in the levels of arachidonic acid (20:4n6), in the CO fed group compared to FO and PUFA groups. EPA (20:5n3) and DHA (22:6n3) levels in FO and PUFA groups rose significantly ($P<0.05$) than in the CO group; EPA and DHA levels being higher in the PUFA group than in the FO group. The results are in close agreement to the reports of Castillo *et al.*(1999) who observed reduction in arachidonic acid levels and cholesterol in plasma of chicks fed menhaden oil.

Clinical studies (Okuyama, 2001; Griffin, 2008) have indicated that the ingested ratio of *n*"6 to *n*"3 (especially Linoleic vs Alpha Linolenic) fatty acids is important in maintaining cardiovascular health. Both *n*"3 and *n*"6 fatty acids are essential, i.e. humans must consume them in the diet. *n*"3 and *n*"6 compete for the same metabolic enzymes, thus the *n*"6:*n*"3 ratio will significantly influence the ratio of the ensuing eicosanoids (hormones), (e.g. prostaglandins, leukotrienes, thromboxanes etc.), and will alter the body's metabolic function (Tribole, 2006). Metabolites of *n*"6 are significantly more inflammatory (esp. arachidonic acid) than those of *n*"3. This necessitates that *n*"3 and *n*"6 be consumed in a *balanced proportion*; healthy ratios of *n*"6:*n*"3 range from 1:1 to 4:1 (Lands, 1992). In the present study n6:n3 ratio in CO animals was 6.9:1 as compared to 0.6:1 and 0.4:1 in FO and PUFA animals respectively. This implied that FO and PUFA diets provide balanced amounts of n6 and n3 fatty acids compared to CO diets.

Significant changes were observed in the activities of the antioxidant enzymes in the heart tissues of male albino rats fed on CO, FO and PUFA enriched diets. Administration of FO and PUFA diets had a significant increase ($P<0.05$) in the levels of glutathione dependent antioxidant enzymes, glutathione peroxidise (GPx) and glutathione-S-transferase (GST) and antiperoxidative enzymes, catalase (CAT) and superoxide dismutase (SOD), in the heart tissues of the animals (Table 8). These

results are in agreement with previously reported works (Erdogan *et al.*, 2004). Both CAT and GPx have complimentary roles in hydrogen peroxide detoxification Faria *et al.*, (2009). However no significant differences were observed in the vitamin E and reduced glutathione (GSH) contents between CO and PUFA fed rats, in the myocardial tissues. FO supplementation significantly elevated ($P<0.05$) the vitamin E and GSH contents in the respective rats. The glutathione antioxidant system plays a fundamental role in cellular defence against reactive free radicals and other oxidant species. The cellular tripeptide GSH thwarts peroxidative damage by neutralizing the free radicals. D'aquino *et al.*, (1991) observed a decrease in glutathione peroxidise activities in liver tissues of rats fed 15% coconut oil diets.

Table 8: Levels of reduced Glutathione (GSH) and vitamin E and activities of Glutathione peroxidise (Gpx), Glutathione–S-transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) in the heart tissues of rats fed isolipidemic diets*

Antioxidant parameters	CO	FO	PUFA
GSH	3.56 ± 0.32^{a}	4.83 ± 0.10^{b}	3.37 ± 0.28^{a}
Vitamin E	3.80 ± 1.12^{a}	9.72 ± 1.08^{b}	4.2 ± 0.47^{a}
GPx	118.32 ± 2.83^{a}	153.91 ± 3.22^{b}	157.2 ± 3.89^{b}
GST	475.62 ± 17.83^{a}	612.74 ± 24.85^{b}	711.42 ± 19.46^{c}
CAT	47.36 ± 1.14^{a}	50.29 ± 1.03^{b}	55.81 ± 3.89^{b}
SOD	2.09 ± 0.32^{a}	6.53 ± 1.86^{b}	5.87 ± 0.73^{b}

*GSH in nmoles/mg, vitamin E in mg/g tissue, GPx in nmol GST oxidised/min/mg protein, CAT nmol H_2O_2 decomposed/min/mg protein, SOD enzyme units

* Means within a row with different letters are significantly different ($P< 0.05$)

Pearson's correlation coefficient test (Table 9) was done to analyse the effect of feeding a fish oil diet on the various lipid and antioxidant components and peroxide levels in the blood serum of albino rats. Significant ($P<0.05$) and positive correlations were observed between saturated fatty acid and triglyceride/cholesterol and between lipid peroxides and n-3 PUFA whereas the correlations were found to be significant ($P<0.05$) and negative between n-3 PUFA and triglyceride/ cholesterol, lipid peroxides and vitamin E and between phospholipids and cholesterol. These results are in partial agreement with reports by other workers (Mohamed *et al.*, 2002; Nagyova *et al.*,2003; Leen *et al.*, 2005).

Table 9: Correlation between lipid components in animals fed with CS oil diets*

CS oil diet	TG	Cho	P	SFA	n-3 PUFA	Vit E	SOD	LPx
TG	1.00	0.50	-0.50	0.50*	-1.00**	-1.00**	-0.50*	0.50
Cho	0.50	1.00	-1.00**	0.50**	-1.00**	-0.50*	-0.50*	0.50
P	-0.50	-1.00**	1.00	-0.50	1.00	0.50	0.50	1.00
SFA	0.50*	0.50**	-0.50	1.00	-0.50	-0.50	-0.50	-1.00
n3PUFA	-1.00**	-1.00**	1.00	-0.50	1.00	1.00*	0.50	1.00**
Vit E	-1.00**	-0.50*	0.50	-0.50	1.00*	1.00	1.00**	-1.00**
SOD	-0.50*	-0.50*	0.50	-0.50	0.50	1.00**	1.00	-1.00**
LPx	0.50	0.50	1.00	-1.00	1.00**	-1.00**	-1.00**	1.00

*TG=triglyceride, Cho=cholesterol, P=phospholipids,SFA=saturated fatty acids, n3pufa= n3 polyunsaturated fatty acids,vit E= vitamin E, SOD=superoxidedismutase, LPx=lipid peroxides
n=6; * correlation significant at 0.05 level; **correlation significant at 0.001 level

Conclusion

The results of the present study indicate that consuming a coconut oil diet with a partial replacement up to 50% by fish oil can significantly lower the LDL or bad cholesterol and lipid peroxide contents and can significantly improve the antioxidant defence status in the blood and heart tissues of albino rats. The reduced levels of diagnostic marker enzymes, triglycerides, LDL cholesterol, lipid peroxides and enhanced levels of phospholipids, HDL cholesterol, antioxidants such as vitamin E and GSH, in the blood serum and heart tissues of albino rats upon consumption of FO diets could be attributed to the presence of anti-inflammatory LC-PUFAs and vitamin E in the fish oils. Moreover, the fish oil enriched diet is comparatively better than the coconut oil diet or the PUFA enriched diet. Alternately, fish oil may even be supplemented along with the diet as capsules. The protective effects of fish oil on the risk of CVD may be due to the synergistic effects of the nutrients in fish and not solely to the presence of LC-PUFAs. These results support the notion that fish oils may be effective dietary supplements in the management of various diseases in which oxidant or antioxidant defense mechanisms are decelerated.

The authors thank the Director of the Central Institute of Fisheries Technology, Cochin, Kerala for the facilities provided. The technical assistance rendered by Mr. B. Ganesan and Ms. Tessy Francis is acknowledged. This work was supported by grants from the Centre for Marine Living Resources and Ecology, Project, Ministry of Earth Sciences, New Delhi.

References

Ackman, R.G., Ratnayake, W.M.N and Olsson, B. (1988) The basic fatty acid composition of Atlantic fish oils: Potential similarities useful for enrichment of polyunsaturated fatty acids by urea complexation, J. Am. Oil Chem. Soc. **65:** 136–138

Anandan, R., Mathew, S., Sankar, T.V and Nair, P.G.V. (2007) Protective effect of n-3 polyunsaturated fatty acid concentrate on isoproterenol induced myocardial infarction in rats. Prostaglandins, Leukotrienes and Essential Fatty Acids. 76: 53-158

Andrea, B., Michael, B. and Scott, M. Grundy (1992) Metabolic effects of dietary stearic acid in mice: changes in the fatty acid composition of triglycerides and phospholipids in various tissues, Atherosclerosis 94(2-3): 19-127

Arita, M., Yoshida, M., Hong, S., Tjonahen, E., Glickman, J.N. and Petasis, N.A. (2005) Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis, Proc. Natl. Acad. Sci. USA 102: 7671-7676

Baker, H., Frank, O., De Angelis, B. and Feingold, S. (1980) Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol, Nutr. Rep. Int. 21: 531-536

Bakes, M.J. and Nichols, P.D. (1995). Lipid, fatty acid and squalene composition of liver oil from six species of deep-sea sharks collected in southern Australian waters, Comp. Biochem. Physiol. 110B: 267-275

Bordier, G., Sellier, N., Foucault, A.P. and Le Goffic, F. (1996) Purification and characterization of deep sea shark *Centrophorus squamosus* liver oil 1-O -aklylglycerol ether lipids, Lipids 31(5): 521-528

Burstein, M and Scholnick, H.R. (1972) Precipitaion of chylomicron and very low density lipoprotein from human serum with sodium lauryl sulphate, Life Sci. **11:** 177–184

Calder, P.C. (2006) n-3 polyunsaturated fatty acids, inflammation and inflammatory diseases, Am. J. Clin. Nutr. 83: 1505S–1519S

Castillo, M., Amalik, F., Linares, A. and García-Peregrín, E. (1999) Dietary fish oil reduces cholesterol and arachidonic acid levels in chick plasma and very low density lipoprotein, Mol. Cell Biochem. 200(1-2): 59-67

Ciubotaru, I., Lee Y.S. and Wander, R.C. (2003) Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT, J. Nutr. Biochem. 14(9): 513-21

Cox, C., Mann, J., Sutherland, W., Chiisholm, A. and Skeaff, M. (1995) Effects of coconut oil, butter, and safflower oil on lipids and lipoproteins in persons with moderately elevated cholesterol levels, J. Lipid Res. 36: 1787-1795

D'aquino, M., Benedetti, P.C., Di Felice, M., Gentili, V., Tomassi, G., Maiorino, M., Ursini, F. (1991) Effect of fish oil and coconut oil on antioxidant defence system and lipid peroxidation in rat liver, Free Rad. Res. 12(1): 147-152

Devaraj, S. and Jialal, I. (2000). Alpha tocopherol supplementation decreases serum C-reactive protein and monocyte interleukin-6 levels in normal volunteers and type 2 diabetic patients, Free Rad. Biol. Med. 29: 790-792

Ellman, G.L. (1959) Tissue sulfhydril groups, Arch. Biochem. Biophys. 82: 70–77

Enas, E.A. (1996) Cooking oils, cholesterol and CAD: facts and myths, Ind. Heart J. 48(4): 423-427

Erdogan, H., Fadillioglu, E., Ozgocmen, S., Sogut, S., Ozyurt, B., Akyol, O. and Ardicoglu, O. (2004) Effect of fish oil supplementation on plasma oxidant/ antioxidant status in rats, Prostaglandins, Leukotrienes and Essential Fatty Acids 71(3): 149-152

Fachinetto, R., Burger, M.E., Wagner, C., Wondracek, D.C., Brito, V.B., Nogueira, C.W., Ferreira, J. and Rocha, B.T. (2005) Pharmacolgy, biochemistry and behavior 81(3),pp 585-592.

Faria, M., Carrasco, L., Diez., S., Riva, M.C., Bayona, J.M. and Barata, C. (2009) Multi-biomarker responses in the freshwater mussel *Dreissena polymorpha* exposed to polychlorobiphenyls and metals, Comp. Biochem. Physiol. Part C: Toxicology and Pharmacology 149(3): 281-288

Fiske C.H. and Subbarow Y. (1925) The colorimetric determination of phosphorus, J. Biol. Chem. 66: 375–400

Folch, J., Lee, M. and Stanley, G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. 226: 497–509

Ganesan, B., Anandan, R. and Rajesh, R. (2008) Protective effect of betaine on changes in lipid profile, lipoproteins and fatty acid composition in experimentally induced myocardial infarction in Wistar rats, Int. J. Biomedi. Pharma. Sci. 2: 65-69

Gil-Villarino, A., Torres, M.I, Zafra, M.F. and Garcia-Peregrin, E. (1997) Supplementation of coconut oil from different sources to the diet induces cellular damage, Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrino. 117: 243-250

Griffin, B.A. (2008) How relevant is the ratio of dietary n"6 to n"3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study, Curr. Opin. Lipidol. 19 (1): 57–62

Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation, J. Biol. Chem. 249: 7130–7139

Hallgren, B. and Larsson, S. (1962) The glyceryl ethers in the liver oils of elasmobranch fish, J. Lipid Res. 3: 31-38

Harvey, K.A., Walker, C.L., Pavlina, T.M., Xu, Z., Zaloga, G.P. and Siddiqui, R.A. (2009) Long chain saturated fatty acids induce pro-inflammatory responses and impact endothelial cell growth, Clin. Nutr. 29(4):492-500

He, K. (2009) Fish, long-chain omega-3 polyunsaturated fatty acids and prevention of cardiovascular disease—eat fish or take fish oil supplement? Prog. Cardiovasc. Dis. 52(2): 95-114

Horn, W.T. and Menahan, L.A. (1981) A sensitive method for determination of free fatty acids in plasma, J. Lipid Res. 122: 377–381

Huang, X. and Fang, C. (2000) Dietary trans fatty acids increase hepatic acyl-CoA: Cholesterol acyltransferase activity in hamsters, Nutr. Res., 20(4): 547-558

Hudert, C.A., Weylandt, K.H., Lu, Y., Wang, J., Hong, S. and Dignass, A. (2006) Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis, Proc. Natl. Acad. Sci. USA 103:11276–11281

James, M.J., Proudman, S.M. and Cleland, L.G. (2003). Dietary n-3 fats as adjunctive therapy in a prototypic inflammatory disease: Issues and obstacles for use in rheumatoid arthritis, Prost. Leukot. Ess.. Fatty Acids 68: 399-405

Kabay, S.C., Ozden, H., Guven, G., Ustuner, M.C., Degirmenci, I., Olgun, E.G. and Unal, N. (2009) Protective effects of vitamin E on central nervous system in streptozotocin-induced diabetic rats, Clin Invest Med. 1:32(5): E314-E321

King, J. (1965), Lactate dehydrogenase, In: Practical Clinical Enzymology (Van, D., Ed.), Nostrand Co., London: 83-93

Ko, T.F., Weng, T.M. and Chiou, R.Y. (2002) Squalene content and antioxidant activity of Terminalia catappa leaves and seeds, J. Agri. Food Chem. 50: 5343-5348

Kobayashi, N., Machida, T., Takahashi, T., Takatsu, H., Shinkai, T., Abe, K. and Urano, S. (2009) Elevation by oxidative stress and aging of hypothalamic-pituitary-adrenal activity in rats and its prevention by vitamin E, J. Clin. Biochem. Nutr. 45(2): 207-13

Lands, W.E.M. (1992) Biochemistry and physiology of n–3 fatty acids, FASEB Journal (Federation of American Societies for Experimental Biology) 6 (8): 2530–2536

Leaf, A, Kang J.X. and Xiao, Y.F. (2008) Fish oil fatty acids as cardiovascular drugs, Rev. Curr Vasc Pharmacol. 6(1): 1-12

Leen, C.L., Holt, R.R., Oteiza, P.I., Fraga, C.G. and Schmitz, H.H. (2005) Cocoa antioxidants and cardiovascular health. Am. J. Clin. Nutr. 81(suppl): 298S-303S

Lin, H. and Maria, L.F. (1998) Saturated fat and simple carbohydrates elevate plasma LDL cholesterol concentrations by specific alterations on hepatic cholesterolmetabolism, Nutr. Res. 18(6): 1003-1015

Lukiw, W.J., Cui, J.G., Marcheselli, V.L., Bodker, M., Botkjaer, A. and Gotlinger, K. (2005) A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease, J. Clin. Invest. 115: 2774–2783

Mathen, M., Suseela, M., Kumar, K.A. and Anandan, R. (2008) Lipid profile of liver oil of the sickle-finned chimaera (*Neoharriotta pinnata*) of the Arabian Sea, Food: 176-178

Misra, H.P. and Fridovich, I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170–3175

Mohamed, A., Ahmed, S.H., Sam, J.B. and Hafez, Y.S. (2002) The effect of dietary menhaden, olive and coconut oil fed with three levels of vitamin E on plasma and liver lipids and plasma fatty acid composition in rats, J. Nutr. Biochem. 13(7): 435-441

Mohur, A. and Cook I.J.Y. (1957) Simple methods for measuring serum levels of glutamic-oxalo acetic and glutamic-pyruvic transaminase in routine laboratories, J. Clin. Pathol. 10: 394–399

Mozaffarian, D., Stein, P.K., Prineas, R.J. and Siscovick, D.S. (2008) Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults, Circulation 117: 1130–1137

Nageswari, K., Banerjee, R. and Menon, V.P. (1999) Effect of saturated, ù-3 and ù-6 polyunsaturated fatty acids on myocardial infarction, J. Nutr. Biochem. 10(6): 338-344

Nagyova, A., Haban, P., Klvanova, J. and Kadrabova, J. (2003) Effects of dietary extra virgin olive oil on serum lipid resistance to oxidation and fatty acid composition in elderly lipidemic patients, Bratisal Lek Listy 104(7-8): 218-221

Nicholson, J.W., Charmley, G.E. and Bush, R.S. (1992) The effect of supplemental protein source on ammonia levels in rumen fluid and blood and intake of alfalfa silage by beef cattle, Can. J. Anim. Sci. 72: 853–862

Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissue by thiobabituric acid reaction, Anal. Biochem. 95: 351–358

Okuyama, H. (2001) High n"6 to n"3 ratio of dietary fatty acids rather than serum cholesterol as a major risk factor for coronary heart disease, Eur. J. Lipid Sci. Technol. 103: 418-422

Olivera, M., O'reillya, G., Helen, M., Stephen, J.T., Philip, C.C., William, M.H. and Robert, F.G. (2004) Role of single nucleotide polymorphisms of proinflammatory cytokine genes in the relationship between serum lipids and

inflammatory parameters and the lipid-lowering effect of fish oil in healthy males, Clin. Nutr. 23: 1084-1095

Paglia D.E. and Valentine W.N. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70: 158-169

Parekh, A.C. and Jung, D.H. (1970) Cholesterol determination with ferric acetate-uranyl acetate and sulphuric acid-ferrous sulfate reagents, Anal. Chem. 42: 1423-1427

Parvez, S., Tabassum, H., Rehman, H., Banerjee, B.D., Athar, M., and Raisuddin, S. (2006) Catechin prevents tamoxifen-induced oxidative stress and biochemical perturbations in mice, Toxicol. 225(2-3): 109-118

Peyronel, D., Artaud, J., Iatrides ,M.C., Rancurel, P., and Chevalier, J.L. (1983) Fatty acid and squalene compositions of Mediterranean *Centrophorus* spp. egg and liver oils in relation to age, Lipids 19(9): 643-648

Qureshi, A.A., Lehmann, J.W. and Peterson, D.M. (1996). Amaranth and its oil inhibit cholesterol biosynthesis in six-week old female chickens, J. Nutr. 126: 1972-1978

Reiser, R., Probstfield, J.L. Silvers, A. Scott, L.W. Shorney, M.L. Wood, R.D. O'Brien, B.C., Gotto, A.M. and Insull, W. (1985) Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil, Am. J. Clan. Nutr. 42: 190-197

Rice, E.W. (1970) Triglycerides (neutral fat) in serum, In: Standard Methods of Clinical Chemistry Vol. 6 (Roederick, P. and MacDonald, R.P. Eds.), Academic Press, New York: 215 p.

Rizzo, W.B., Watkins, P.A., Phillips, M.W., Cranin, D., Campbell, B. and Avigan, J. (1986) Adrenoleukodystrophy: oleic acid lowers fibroblast saturated C22-26 fatty acids, 36(3): 357-361

Rule, D.C., Liebman, M. and Liang, Y.B. (1996) Impact of different dietary fatty acids on plasma and liver lipids is influenced by dietary cholesterol in rats, J. Nutr. Biochem. 7(3): 142-149

Sabeena, K.H.F., Kumar, H.S. Anandan, S., Mathew, S.R., Sankar, T.V. and Nair, P.G.V. (2007) Supplementation of squalene attenuates experimentally induced myocardial infarction in rats, Food Chem. 105 (4): 1390-1395

Saremi A. and Arora R. (2009) The utility of omega-3 fatty acids in cardiovascular disease, Am J Ther. 16(5): 421-36

Sharma, A., Kaur, P., Kumar, B., Prabhakar, S. and Gill, K. (2008) Plasma lipid peroxidation and antioxidant status of Parkinson and aposis disease patients in the Indian population, Parkinsonism and Related Disorders, 14(1): 52-57

Simopoulos, A.P. (1991) Omega-3 fatty acids in health and disease and in growth and development, Am. J. Clin. Nutr. 54: 438-463

Stamp, L.K., James, M.J. and Cleland, L.G. (2005) Diet and rheumatoid arthritis: A review of the literature. Arthritis Rheum. 35: 77–94

Surendraraj, A., Farvin, K.H.S. and Anandan, R. (2009) Synergestic effect of squalene and simvastatin on fecal cholesterol excretion in rats, Asian J. Clin Nutr. 1(2): 102-106

Takahara S., Hamilton B.H., Nell, J.V., Ogubra, T.Y. and Nishimura, E.T. (1960) Hypocatalasemia: a new genetic carrier state, J. Clin. Invest. 39: 610–619

Tribole, E.F. (2006) Excess omega-6 fats thwart health benefits from omega-3 fats, BMJ 332: 752–760

Virtanen, J.K., Mozaffarian, D., Chiuve, S.E. and Rimm, E.B. (2008) Fish consumption and risk of major chronic disease in men, Am. J. Clin. Nutr. 88(6): 1618-1625

Vittala, P. and Newhouse, I.J. (2004) Vitamin E supplementation, exercise and lipid peroxidation in human participants, Eur. J. Appl. Physiol. 93: 108-115