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Determination of Fatty Acids Content in Five Fish Species (*C. latticeps; D. rostratus; S. schall; S. mystus and H. bebe*) from River Niger in Edo State, Nigeria

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A B S T R A C T

Five West Africa freshwater fishes namely *Clarotes lapticeps; Distichodus rostratus; Synodontis schall; Schilbe mystus* and *Hyperopisus bebe* from the River Niger, Illushi, in Esan South East Local Government Area of Edo State were analysed to determine their fatty acids content. The fish specimens were purchased at the bank of the river, between June 2006 and January 2007. They were put in an iced box and immediately transported to the laboratory where routine body measurement and analysis were carried out. The result of the proximate compositions showed lipid had a value of 5.49; 6.81; 8.80; 5.06 and 6.27. For *C laticeps; D rostratus; S. schall; S. mystus* and *H. bebe* respectively. The fatty acid composition of the fish lipid as determined by GLC showed abundant stearic acid (47.45%; 47.26%; 45.21%; 47.20% and 45.26%) and palmitic acids (16.50%; 17.12%; 16.80%; 47.20% and 45.26%) for *C. laticeps; D. rostratus; S. schall; S. mystus and H. bebe* respectively. Polyunsaturated fatty acid (18:2 and 20:4) showed 21.74%; 21.72%; 21.52%; 23.11% and 22.12% for *C. laticeps; D. rostratus; S. schall; S. mystus and H. bebe* respectively. The short chain fatty acids were found to be in abundance in the freshwater fish lipid studied.

Introduction

Fish constitutes a major source of protein in our diet. Uboma; Fadayomoi; Ladipo; and Sagua (1981), reported that Nigerians obtain 40% of their animal protein from fish. A lot of calories are also obtained from fish, especially the fatty fishes. Fish apart from being important in human diet, it's fatty acids are currently under intense scientific investigation because of numerous health benefits attributed to them (Rahman; Huah; Hassan and David 1995 and Clucas and Sutcliffe, 1981).

Lipid is regarded as one of the most important food reserve contributing to the condition of the fish and this has led to the use of fat indices as a measure of relationship between percentage water and percentage fat (Sinclair and Duncan, 1972). Such estimates are used simply because the

measurement of water is easy and rapid. These relationships have been shown to exist in various fish species and have been extensively used for predictive estimates (Iles and Wood 1965; Brett; Shelbounsne; and Shoop 1969 and Salam; Ali; Chatta and Zuman 1993).

The aims of the present study includes, to determine the level of polyunsaturated Fatty Acid (PUFA) of the sampled fishes. To determine the type of fatty acid (long or short chain) that is present in the sampled fishes. And also to determine the importance of polyunsaturated acid (PUFA) in the sampled fishes.

The principal constituents of fish may be divided into five categories, namely; Protein, Lipid, Carbohydrate, Ash and Water. The biochemical analysis of these constituents may vary greatly from one species and one individual to another depending on age, sex, environment and season (Stansby, 1962 and Love, 1970). According to Kor (1995), the biochemical composition of fish is closely related to feed intake, migratory and sexual changes in connection with spawning. He stated that fish will have starvation periods for natural or physiological reasons such as during migration, spawning or because of external factors like as shortage of food. Usually spawning, whether occurring after long migration or not, calls for higher levels of energy. Therefore, fish having energy reserve in the form of lipids will rely on this. Species performing long migrations before they reach specific ground(s) or river(s) may utilize protein in addition to lipids for energy, thus depleting both the lipid and protein reserves, resulting in a general reduction of the biological condition of the fish (Kor, 1995). Most species in addition, do not usually ingest much food during spawning or migration and are therefore not able to supply energy through feeding.

The lipids present in teleost fish species may be divided into two major groups: the phospholipids and the triglycerides (Kor, 1995). According to him, the phosopholipids make up the integral structure of the unit membranes in the cells, thus they are often called structural lipids. The triglycerides are lipids used for storage of energy in form of fat, usually within special fat cells surrounded by a phospholipids' membrane and a rather weak collagen network.

The fat cells making up the lipid reserves in fatty species are typically located in the subcutaneous tissue, in the belly flap muscle and in the muscles moving the fins and tail (Kiessling; Aasgaard; Storebakken; Johansson and Kiessling, 1991). In some species which store extraordinary high amount of lipids, Kiessling *et al*. (1991) said the fat may also be deposited in the belly cavity. Depending on the amount of polyunsaturated fatty acids, most fish fats are more or less lipid at low temperature. Fat reserves are also found typically spread throughout the muscle structure. The concentration of fat cells appears to be highest close to the myocommata and in the region between the light and dark muscle (Kiessling *et al*. 1991). The dark muscle contains some triglycerides inside the muscle cells even in lean fish, as this muscle is able to metabolize lipids directly as energy. The corresponding light muscle cells are dependent on glycogen as a source of energy for the anaerobic metabolism. According to Kiessling *et al*. (1991), in dark muscle the energy reserve are completely catabolized to carbon (IV) oxide (CO_2) and water, whereas in light muscle, lactic acid is formed. The mobilization of energy is much faster in light muscle than in dark muscle, but the formation of lactic acid creates fatigue, leaving the muscle unable to work for long periods at maximum speed. Thus, the dark muscle is used for continuous swimming activities and the light muscle for

quick bursts such as when the fish is about to catch a prey or to escape a predator.

The polyunsaturated fatty acids (PUFAs) can be divided into two main groups; Omega -6 and Omega -3 (n-6 and n-3) which have different physiological functions and effects (Ian, 1995). Humans and animals cannot synthesis PUFAs from basic carbon sources. They require a dietary source. The main n-6 PUFAs are linoleic acid and its metabolites y-linolineic acid (GLA) occurring particularly in vegetable oils and arachidonic acid (AA) occurring in animal tissue. The main n-3 PUFAs are linolenic (ALA) and its metabolites eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA). EPA and DHA are found in fish particularly oily fish. They have a longer chain length in their molecular structure 20 and 22 carbon atoms compared with 18 in GLA and ALA. They are sometimes referred to as n-3 LC PUFAs. They are produced by phytoplankton; zooplankton and algae on which fish feed (Ian, 1999).

Researchers from Emory University School of Medicine have reviewed the data from randomized controlled clinical trails on n-3 PUFAs and coronary heart disease (Harper and Jacobson, 2005). The studies were divided into patients having Coronary Heart Disease (CHD) using plant based n-3 PUFAs (Alpha-linolenic acid –ALA), fish based n-3 PUFAs (eicosapentaenoic acid, (EPA), and docosahexanoic acid, -DHA) consumed in the diet. Fourteen randomized clinical trails were included in the review, six of which were of fish oil, including one large trial of 10,000 patients. The researchers reported a clear trend suggesting that there are important differences in Coronary Heart Disease (CHD) outcomes when using fish based EPA or DHA compared with plant based ALA. Most of the fish oil trails resulted in a significant reduction in total mortality and CHD, supporting the theory that fish based n-3 PUFAs may impact their cardiovascular protective effect by acting as an antiarrhythmic agent. They may do so by stabilizing the electrical activity of heart muscle cells or by decreasing the heart rate. Harper and Jacobson (2005), concluded that the evidence suggests a role for fish oil (EPA, DHA) or fish in secondary prevention, as clinical trial data demonstrate a significant reduction in total mortality and Coronary Heart Disease.

According to Kris-Etherton (2002), the American Heart Association has reviewed the benefits of regular consumption of fish and fish oils. It concludes that fish and fish oils help prevent cardiovascular disease including fatal and nonfatal heart attacks, strokes, sudden cardiac death and Coronary Artery Disease (angina). The authors believe that the mechanisms by which fish oils exert their protective effect include:- Reduction in susceptibility to ventricular arrhythmia; decrease in platelet aggregation; reduction in triglyceride levels; retardation of atherosclerosis; lowering of blood pressure; promotion of nitric oxide induced endothelial relaxation, anti-inflammatory effects.

The American Heart Association recommends that people increase their intake of long chain polyunsaturated omega-3-oils from fish or directly from fish oil supplements. Healthy people should consume oily fish at least twice a week. Patients with heart disease should eat enough oily fish on a daily basis to obtain about 1 gram per day of EPA and DHA combined or take a fish oil supplement providing 1 gram per day of EPA + DHA. Atherosclerosis increase the risk of stroke and heart disease because part of the atherosclerotic buildup (plague) on the inner

wall of arteries may dislodge and block smaller arteries in the brain and heart respectively and thus cut off the vital supply of oxygenated blood (Thies and Frank, 2003).

According to Iso and Hiroyasu (2001), a 1995 study showed men who ate fish five or more times per week had a 40 per cent lower risk of having a stroke than did men who ate fish less than once a week. Researchers at the Harvard Medical School and the Brigham Women's Hospital reported that the benefits of fish consumption are even more spectacular for women. Their study involves 79,839 female nurses who were between the ages of 34 and 59 years at the start of the study in 1980. After 14 years of follow-up a total of 574 strokes had occurred in the group. Most of the strokes (303) were ischemic, i.e. caused by a blood clot. There were also181hemorrhagic strokes, i.e. caused by a ruptured artery and 90 strokes of undetermined origin.

The two essential fatty acids, linoleic acids (n-6) and α linolenic acid (n-3) are transformed into longer chain PUFAs and their derivatives by enzymes (detaturases and elongases). These same enzymes metabolizes both n-6 and n-3 fatty acids. If the dietary intake of one is too great, metabolism of the other family can be impaired. This can lead to an imbalance in the production of prostaglandins, leukotrienes and thromboxanes (Vaughn and Reinhart 1994). These hormone-like compounds are involved in important physiological processes including the Central Nervous System, regulation of blood pressure and clothing time, inflammatory reactions and the immune system's defense mechanisms.

According to Stansby and Hall (1967), the percentage of polyunsaturated fatty acids with four, five or six double bonds is slightly lower in the polyunsaturated fatty acids' lipids from fresh water fish (approximately 7%) than in the corresponding lipids from marine fish (approximately 88%). However, the composition of the lipid is not completely fixed but can vary with the feed intake and seasons.

Seasonal Variation of Fatty Acid Content

The lipid fraction is the component showing the greatest variation. Often the variation within a certain species will display a characteristic seasonal curve with a minimum around the time of spawning (Ando *et al* 1985). According to Watts (1957), some tropical fish also show a marked seasonal variation in chemical composition. West African shad (*Ethmalosa dorsalis*) shows a range in fat content of 2.7% (wet weight) over the year with a maximum in July. Corvina (*Micropogon furnier*) and Pescada Foguele (*Marodon ancylodon*) captured off the Brazilian coast had a fat content range of 0.2 - 8.7% and 0.1 -5.4% respectively (Ito and Watanabe, 1968). They also observed that the oil content of these species varies with larger fish containing about 1% more oil than smaller ones. Watanabe, (1971) examined freshwater fish from Zambia and found a variation from 0.1 to 5% in oil content of four species including both pelagic and demersals.

The lipid stores are typically used for long spawning migrations and when building up gonads (Ando *et al.*, 1985). When the lipids are mobilized for these purposes, there are questions as to whether the different fatty acids present in the triglyceride are utilized selectively. According to Watts, (1957), this is apparently not the case in Salmon but in cod, a selective utilization of $C_{22:6}$ has been observed. The phospholipids may also be mobilized to a certain extent during

sustained migration, although this lipid fraction is considered to be conserved much more than the triglycerides (Love, 1970).

Materials and Methods

Sampling Site

Fresh fish samples of *C. latticeps; D. rostratus*; *S. schall*; *S. mystus* and *H. bebe* were purchased from commercial fish landing at the bank of the River Niger in Illushi on market days for an interval period of 7 months. Illushi is a town in Esan-South-East Local Government Area of Edo State. It lies between latitudes 6^{o} 33' 45N and longitude 6^{0} 33 30E (fig.1). The river Niger forms a boundary between Edo and Kogi states and the plains are always subjected to flooding annually whenever the river overflows it's banks during the raining season.

The fish samples were transported in an insulated iced container to the Zoology Lab, AAU, Ekpoma.

Laboratory Study of Samples

a. **Identification:** In the laboratory, the fish samples were identified using the taxonomy keys by Reed *et al*, (1967), Babatunde D.O. and Aminu R., (1998) and Elakhame, L.A. (2004). The identified samples were then labelled in triplicates.

Determination of Proximate Composition

Lipid Determination

14g of finely homogenized sample (i.e. flesh and bone) was weighed into a conical flask. To this was added chloroform and methanol in a ratio of 1:2. It was blended for 5 minutes and the mixture centrifuge for another five minutes. The supernatant was decanted using a filter paper. This process was repeated using the residue with 0.8ml water added. Chloroform was added to the supernatant to make a ratio of 1:1 with 0.8ml of water added. Using a separating funnel, the oil was separated from the solvent. Subsequently, at 40^{0}C using a water bath, the solvent was evaporated from the lipid and weigh.

Methylation

2ml of sample was placed in a clean completely dry 100ml round bottomed flask with 25ml of methanol and a few boiling chips added. 3ml by conc. H_2SO_4 was added to the solution and reflux at a gentle boiling rate for at least 30 minutes. The content of the flask was added to 50ml water (excluding the boiling chips) in a 250ml separating funnel. The water/methanol solution was extracted with three 20ml portion of diethylether. Moisture content was dried with 5g of anhydrous sodium sulphate.

Using a steam plate hood, sample was vaporized using a narrow glass stirring rod. The ether solution was then stirred until the methyl ester of the fatty acid remains (Gunstone, 1969).

Determination of Fatty Acids Composition in Lipids

50 milligram of various standards were compose into 50mls volumetric flask and made into dissolution solution with diethyl ether. The methyl ester of the fatty acid was diluted to 60mls with diethyl ether.

The column of the G.C (Hewlet Packed (hp) 5890 series II) was set at 190^{0C}. Detected temperature was set 240^{0C}. Nitrogen, the carried gas was set at a flow rate of 50ml per minute. 0.6ul of both standard and sample

were injected into system respectively and the peak determined.

Results and Discussion

Lipid Content

S. schall recorded the highest lipid value of 8.7980±1.10, followed by 6.8130±1.10, 6.2700±0.45 and 5.4860±0.28 for *D. rostratus, H. bebe* and *C. laticeps* respectively. The least lipid content was observed in *S. mystus* with a value of 5.0610±0.12 (Table 1). Only mean recorded comparism between *S. schall* with *C. laticeps, H. bebe*, and *S. mystus* recorded significant difference ($P<0.05$) in lipid content (Table 1).

Fatty Acid Composition of the Lipid

From the result obtained, it was observed that linolenic acid and palmitoleic acid appeared as trace, while stearic acid and palmitic acid were in abundance in all the fish sampled. Arachidic acid was observed as trace in *Hyperopisus bebe, Schilbe mystus* and *Clarotes laticeps,* while 2.10 ± 0.71% and 2.10 ± 0.21% in *Distichodus rostratus, Syndontis schall* respectively were observed (Table 2)

For the three catfishes, it was observed that *Schilbe mystus* had the highest percentage of arachidonic acid (12.846±0.18). Arachidic acid had a value of 2.100± 0.21 for *S schall,* while others appeared as trace. (Table 2).

Relative distribution of fatty acids in all the fish samples showed that *Schilbe mystus* was richest in Polyunsaturated Fatty Acid (PUFA) with a value of 23.11±0.52, followed by *H. bebe* with value 22.115±1.08. Comparable amount were also recorded in *C. laticeps, D. rostratus,* and *S. schall* with values 21.74±1.18, 21.722±0.82 and 21.522±1.64 respectively (Table 3).

Fat values obtained in this study are higher than figures obtained for marine fishes such as *Cyprinus carpio*, *Trigia sp*, *Clupea herengus* etc., with a range of 0.5-2.2% (Murray and Burt, 1969, and Turan; Sonmez; Gulsah and Kaya 2007). As the fat content rises, the water content falls and vice versa. As shown in the result, the sum of fat and moisture for any of the studied fishes approximates 80% (Murray and Burt,1969; Gunstone, 1969; and Poulter and Nicoiaides, 1985). This explains the difference between the result of this study (freshwater fishes) and that obtained from marine fishes. The value for fat in this study agrees with results obtained from the study of fish composition in South African Freshwater fishes (Eramus; Ewa; Mary-Jane; Luke; Hermogene and Hlanganani 2008). Fish are usually categorize as lean, moderately fat, and fat according to it's fat content, which is, less than 5 percent, from 5 to 10 percent and greater than 10 percent respectively (Dean, 1990). Thus the five fish species: *D. rostratus; C. laticeps; S schall; S.mystus* and *H. bebe* can be classified as moderately fat with it's fat content ranging between 5 to 10%. Lipids in fishes are important for energy reserve and is useful in consideration of fish wholesomeness when determined as fat index i.e relationship between percentage water or moisture and percentage fat. It serves as predictive estimates of fish condition (Iles and Wood, 1965; Brett *et al.*, 1969; Sinclair and Duncan, 1972 and Salem *et al.*, 1993). These values can be accepted as the condition of wholesome fish sourced in these locality. The values can serve as a quality index for trade purpose though more extensive investigation is still needed to confirm these indices.

Table.1 Interspecies Comparison of Lipid Content in all the Fish Sample

(1) SPECIES	(J) SPECIES	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval	
					Lower Bound	Upper Bound
C. laticep	*D. rostratus*	-1.3270	±1.0206	.223	-3.6011	.9471
	S. schall	-3.3120*	±1.0206	.009	-5.5861	-1.0379
	S. mystus	.4250	1.0206	.686	-1.8491	2.6991
	H. bebe	-7840	1.0206	.460	-3.0581	1.4901
D. rostratus	*C. laticeps*	1.3270	1.0206	.223	-.9471	3.6011
	S. schall	-1.9850	1.0206	.080	-4.2591	.2891
	S. mystus	1.7520	1.0206	.117	-.5221	4.0261
	H. bebe	.5430	1.0206	.606	-1.7311	2.8171
S. schall	*C. laticeps*	3.3120*	1.0206	.009	1.0379	5.5861
	D. rostratus	1.9850	1.0206	.080	-.2891	4.2591
	S. mystus	3.7370	1.0206	.004	1.4629	6.0111
	H. bebe	2.5280*	1.0206	.033	.2539	4.8021
S. mystus	*C. laticeps*	-4250	1.0206	.686	-2.6991	1.8491
	D. rostratus	-1.7520	1.0206	.117	-4.0261	.5221
	S. schall	-3.7370*	1.0206	.004	-6.0111	-1.4629
	H. bebe	-1.2090	1.0206	.264	-3.4831	1.0651
C. laticeps	*C. laticeps*	.7840	1.0206	.460	-1.4901	3.0581
	D. rostratus	-.5430	1.0206	.606	-2.8171	1.7311
	S. schall	-2.5280*	1.0206	.033	-4.8021	-0.2539
	S. mystus	1.2090	1.0206	.264	-1.0651	3.4831

*The mean difference is significant at the .05 levels

Table.2 Fatty Acid Composition of the Lipid of Five Fish Species

Fatty Acid	***H.bebe***	***S.schall***	***S.mystus***	***C.laticeps***	***D.rostratus***
Lauric (12.0)	2.596±0.72	2.475±0.38	2.817±0.41	1.984±0.34	2.649±0.67
Myristic (14.0)	3.379±0.50	3.564±0.67	3.339±0.50	3.521±0.37	3.947±0.40
Palmitic (16.0)	16.929±0.26	16.795±0.60	17.019±0.25	16.494±0.47	17.12±0.72
Palmitoleic (16.1)	T	T	T	T	T
Stearic (18.0)	45.257±0.32	45.214±0.25	47.196±0.57	47.452±0.58	47.259±0.50
Oleic (18.1)	6.001±0.65	6.099±0.76	5.992±0.78	6.422±0.17	6.822±0.21
Linoleic (18.2)	10.119±0.57	10.425±0.67	10.264±0.34	9.926±0.39	10.996±0.39
Linolenic (18.3)	T	T	T	T	T
Arachidic (20.0)	T	2.10±0.21	T	T	2.10±0.71
Arachidonic (20.4)	11.996±0.51	11.097±0.97	12.846±0.18	11.814±0.79	10.726±0.43

Result represents the mean ± SEM of three replicates, T = trace

Table.3 Relative Distribution of Fatty Acid in all the Fish Samples

Samples	TSFA%	TUFA%	MUFA%	PUFA%
Hyperopisus bebe	68.161±1.8	28.116±1.73	6.001±0.65	22.115±1.08
Synodontis schall	70.148±2.11	27.621±2.40	6.099±0.76	21.522±1.64
Schilbe mystus	70.371±1.73	29.102±1.30	5.992±0.78	23.11±0.52
Clarotes laticeps	69.451±1.76	28.162±1.35	6.422±0.17	21.74±1.18
Distichodus rostratus	73.075±3.00	28.544±1.03	6.822±0.21	21.722±0.82

Result represents the mean ±SEM of three replicates; TSFA - Total Saturated Fatty Acid
TUFA - Total Unsaturated Fatty Acid; MUFA -Monounsaturated Fatty Acid
PUFA - Polyunsaturated Fatty Acid

Available report show that freshwater fishes contain high level of omega 6, while, marine fishes are characterized with high concentration of omega 3 (Gunstone, 1969; Pearson, 1976; Mohen, 1985; Vileg and Body, 1988; Wang; Miller; Perren and Addis 1990; Ayinla; Idoruboye-Obu; Langholz; Omuame and Vehlow 1993; Hyvonen and Koivistoinen, 1994). The studied samples contained Omega 6 and lesser varieties of free fatty acid comparable to varieties in marine fishes (Turan *el al*., 2007). Fish feeds and habitat determines the nature/type of fatty acids in their oil (Clucas, 1985; Hearn *et al*., 1987 and Rahman *et al.*, 1995).

The only mono-unsaturated fatty acid (MUFA) present in this study is Oleic acid (Omega 9). It is used by the body to fight inflammation to reduce arthrosclerosis, to maintain blood sugar balance and to boost the immune system (Wendy, 2006).

The analysis of the result showed that from the free fatty acid profile, the amount of Linoleic and arachidonic acids were higher than that reported for oils sourced from cod, anchovy, herring, white fish and thornback Ray (Alister and Colin, 1992 and Turan *et al*., 2007). Linoleic acid get converted in the body to a substance which helps to regulate inflammation and blood pressure as well as heart, gastrointestinal and kidney functions (Wendy, 2006).

Also, excessive intake of linoleic acid in human leads to synthesis of alpha-linolenic (ALA) that is beneficial as eicosapentaenoic acid (EPA) and decosahaxaenoic acid (DHA). Arachidonic acid is a factor in prostaglandin and prostocycline activity associated with cardiac dysfunction (Vaughn *et al.*, 1994). According to Turan *et al*. (2007). All unsaturated fatty acids, regardless of their double bond number, position, or configuration, are considered equally effective in decreasing antherogenicity.

The total saturated fatty acid values of the studied fishes are similar with the values obtained from the whole fresh samples of *Labeo cubie* and *Laboe senegalensis* 71.82 ± 1.84 and 71.22 ± 2.38 respectively, and the total saturated fatty acid content of the liver were however lower 58.63 ± 1.07 and 59.52 ± 1.08 respectively (Ekpo and Elakhame, 1998 and 2004). Oil obtained from marine thornback ray (*Raja clavata*) contains total saturated fatty acid value of 48.28±0.99 that is significantly lower than that observed in the studied samples.

In marine fishes, palmitic acid was found to be the most abundant in different fish species (Alasalvar, Taylor, Subcoy, Alexis, 2002; Celik and Gokee, 2003; Sengor, Ozden, Erkan, Tuter, Aksoy 2003, Wheeler and Morrissey, 2003; De Silva, Gunasekera, Ingram 2004; Rossano, Caggiano,

Mastrangelo, DiLauro, Ungaro, Enorre, Riccio 2005; Bayir, Haliloglu, Sirkecioglu, Aras 2006; Senso *et al*., 2007; Turan *et al.*, 2007 and Erasmus *et al.*, 2008). Wikipedia, (2008), stated that palmitic acid has no hypercholesteroleamic effect if intake of linoleic acid is greater than 4.5%. This study revealed comparable abundance of stearic acid as in the works of Ekpo and Elakhame (1998 and 2004). Mary (2008), stated that stearic acid could promote iron utilization in the human body.

Myristic acid is a very important fatty acid that stabilizes many different proteins, including proteins used in the immune system and to fight tumors-myristoylation, while lauric acid has antimicrobial function and stabilizes certain protein having similar fashion as myristic acid and palmitic acid (Erasmus *et al.*, 2008 and Mary, 2008). Generally, lack of saturation fatty acid in the body is responsible for age related decline in white blood cells' function (Mary, 2008).

In conclusion, people are advised to consume fish and fish product as they are beneficial in human nutrition, prevent cardiovascular disease including fatal and nonfatal heart attacks, strokes, sudden cardiac death and Coronary Artery Disease (Simopoulos, 1991 and Ian, 1999).

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