International Journal of Sciences & Applied Research

www.ijsar.in

Effects of Nutrition on the Fatty Acid Composition and ω-3/ω-6 Ratios of the Muscle Tissue of *Oncorhynchus mykiss*

Nükhet Akpinar and Mehmet Ali Akpinar*

Cumhuriyet University, Faculty of Science, Department of Biology, 58140 Sivas, Turkey.

Correspondence Address: *Mehmet Ali Akpinar, Cumhuriyet University, Faculty of Science, Department of Biology, 58140 Sivas, Turkey.

Abstract

In this study, it has been aimed to determine the fatty acid composition and ω-3/ω-6 ratios of the muscle tissue of rainbow trout (*Oncorhynchus mykiss*) fed and starved during 28 days. There were no qualitative differences in the fatty acid composition of the muscle of the fed and starved fish. However, it was determined that there were increases in the amounts of total ω-6 and ω-3 polyunsaturated fatty acids (PUFAs) of fed fish, while found decrease in total saturated fatty acids (SFAs). There were increases in the levels of C18:1 ω-9, C18:2 ω-6, C18:3 ω-3 and C22:6 ω-3 in fed fish. The ω-3/ω-6 ratios of the muscle tissue of starved and fed fish were found to be 1.27 and 1.49, respectively. From these results, during 28 days starvation period, it can be said that the fish can keep their saturated (SFA) and monounsaturated fatty acid (MUFA) metabolism although they showed a loss of weight. In conclusion, dietary ω-3 and ω-6 series fatty acids improved the muscle tissue quality of *Oncorhynchus mykiss.*

Keywords: Oncorhynchus mykiss, Fatty acids, Muscle, ω-3/ω-6 ratio, Starvation, Feeding

Introduction

The fatty acid compositions of fishes are rich in terms of ω-3 highly unsaturated fatty acids (HUFAs) and are beneficial in the human diet [1,2]. The beneficial ω-3 fatty acids found in fish tissues are in particular eicosapentaenoic acid (C20:5 ω-3: EPA) and docosahexaenoic acid (C22:6 ω-3: DHA) [3], which originate from phytoplankton and seaweed in the food chain [4]. In the light of recent studies, EPA and DHA are important in the prevention of cardiovascular diseases and neurological problems [5, 6].

The only sustainable alternatives to fish oils are vegetable oils which are rich in C18 polyunsaturated fatty acids (PUFA) such as linoleic (C18:2 ω-6) and linolenic (C18:3 ω-3) acids, but devoid of ω-3 HUFA [7]. The conversion of C18 PUFA to HUFA requires sequential steps of fatty acly chain desaturation and elongation [8].

Sargent *et al*. [9] indicated that these PUFAs are necessary to maintain membrane structure integrity and are precursors of eicosanoids. It is well established that fish require EPA, DHA and arachidonic acid (C20:4 ω-6: ARA) for normal growth, development, and reproduction [10,11]. High levels of EPA and DHA were reported by other studies in trout species and marine fish species [12,13]. Akpinar *et al*. [14] reported that the ω-3/ω-6 rations in male and

female fish. In this study, ω-3/ω-6 rations for livers were found to be 2.89 and 1.97, while in muscles of male and female *Salmo trutta macrostigma* the rations were as 2.59 and 2.26, respectively.

Dietary lipids play important roles in fish nutrition, both because of their role as energy providing molecules and as the source of essential fatty acids (EFA) [9,15]. Recent studies on the composition and significance of fatty acids in fish species have focused on ω-3 and ω-6 fatty acids [1,16,17]. Gelienau *et al*. [18], showed that long chain PUFAs are regular components of the examined fish tissues and, in the light of their known nutritional prostaglandinogenic and presumed structural roles, are thus of considerable biological significance. Many fish species are cultivated and harvested for human consumption. For that, *Oncorhynchus mykiss* is the most important cultured fish species. Therefore, the aim of the present work was to compare ω-3 and ω-6 fatty acids in the muscle tissue of starved and fed *O. mykiss.*

Materials and methods

Fish and feeding

The study was carried out in October 2013 at a commercial fish farm in Sivas (Gürün), Turkey. Mature (in the age of 1 year) *O. mykiss* (Walbaum, 1792) were selected and randomly divided into two groups of 20 fish. Each group was stocked in different two pools (60x80x190 cm) supplied with freshwater (temperature 12.0 ± 0.22^0C) and the groups contained both sexes. The oxygen content of water was maintained stable at around 8.06 ± 0.18 mg L^{-1} and pH was 8.6 ± 0.04. One of the groups of fish was fed by hand twice daily in the morning and afternoon (at 09:00 and 17:00 h for 28 days). The other group was starved for 28 days. In daily feeding fish, the amount of food (g) to be given was calculated as 2% of average weight per fish [19]. Commercial feeds used in the feeding experiment were purchased from a private feed manufacturer in Turkey. Feed formulation was crude protein 45%, crude fat 20%, crude ash 14%, total phosphorus 1.5% and vitamin premix (in unknown amounts). *Table 1* shows the lengths, weights and condition factors of the fish used in the study. Condition factor can be explained by the following equation [20].

$C = (W/L^3) . 10^5$

(C, condition factor. W, mean weight (g). L, mean fork length (mm)

At the end of the experiments, 3 fish from two pools stocks (a total of 9 fish) were selected for extraction studies. Samples of muscle tissue of all groups, 1 g each, were extracted for total lipid and fatty acids. 1g of muscle sample was directly taken from the area underneath of dorsal fin. Sample of commercial feed (1 g) was also taken for fatty acid analyses.

Lipid extraction and fatty acid analysis

A sample of muscle from each fish was homogenised in chloroform:methanol (2/1, v/v) using Ultra-Turrax T25 homogeniser. Autooxidation of PUFAs was minimised by adding 50 µl of butylated hydroxytoluene (2%, w/v in chloroform) to the extraction mixture. The lipids of muscle were extracted and purified according to the procedure described by Folch *et al*. [21]. The samples were stored at -20^0C until required. Extracts of muscle materials were saponified by refluxing with methanol (50%) containing 5% sodium hydroxide for 1 h at 80 ^{0}C. The saponifiable lipids were converted to their methyl esters for 20 min at 85^0C using the standard Boron triflouride-methanol (BF_3) method [22]. The resultant mixture of fatty acid methyl esters (FAMEs) in hexane:chloroform (4/1, v/v) was injected into Unicam-610 gas chromatograph equipped with flame ionization detector (FID),and capillary column (15m x 0.32mm) (70% Biscyanopropyl Polysilphenylene Silaxana). The carrier gas was nitrogen (2.5 ml min^{-1}) and column, injector port and

detector temperatures were 120, 240 and 280^0C, respectively. A small quantity of FAMEs solution (1 µl) was introduced onto the column. FAMEs were identified by comparison of their retention times with external standarts.

Statistical Analyses

All analytical determinations were performed in triplicate and the mean values were reported. The statistical analyses of percentages of fatty acid were tested by analysis of variance (ANOVA) and comparisons between means were performed with Tukey's test. Differences between means were evaluated as significant if $P \leq 0.05$.

Results

The fatty acid composition of commercial feed and muscle tissue of rainbow trout starved and fed are shown in *Table 2*. Nineteen fatty acids were identified from the muscle tissue while seventeen fatty acids were identified from feed. However, the percentages of the fatty acids were different among two fish groups and feed. But the fatty acid profiles in muscle of the starved and fed fish were quite similar.

There were variations in the levels of some fatty acids between the groups after 28 days. Palmitic (C16:0) and stearic (C18:0) acids were predominant fatty acids within the saturated fatty acids (SFAs) in muscle of fish and feed. The amount of C16:0 in muscle of starved fish was higher than in fed fish. There was no difference between C18:0 percentages in the groups. Lauric acid (C12:0), miristic acid (C14:0) and pentadecanoic acid (C15:0) contents in starved fish were higher than in the fed fish. Very significant difference was determined between SFA fraction of starved and fed fish muscle. Surprisingly, although starved fish contained excess of SFA, its value in fed fish was lower (37.28%) than in starved group (38.58%).

Oleic acid (C18:1 ω-9) was identified as major monounsaturated fatty acids (MUFAs) in muscle of the groups. Significantly higher contain of C18:1 ω-9 was observed in the fed fish than in the starved fish. C16:1 ω-9 (palmitoleic acid) and C14:1 (miristoleic acid) contents were lower amounts and decreased in the fed fish according to starved fish and feed. But, rates of MUFAs were very similar between the groups. This study revealed that in the PUFA ω-6 fraction, linoleic acid (C18:2 ω-6) was the major ω-6 acid with levels of 7.59%, 6.17% and 9.20% in starved fish, feed and fed fish, respectively. C18:2 ω-6 was significantly increased, whilst eicosadienoic acid (C20:2 ω-6) decreased in the fed fish according to starved fish. There were no differences in eicosatrienoic acid (C20:3 ω-6) and arachidonic acid (C20:4 ω-6: ARA) content between those groups.

Linolenic acid (C18:3 ω-3) content in the fed fish was higher (4.90%) than in the starved fish (3.02%) and feed (3.03%). However, EPA and docosapentaenoic acid (C22:5 ω-3: DPA) contents were definitely decreased in the fed fish according to starved fish. But, DHA was found to be at the highest level (8.37%) in the ω-3 PUFAs fraction and in the fed fish. C18:3 ω-3 was the fatty acid at the second high amount in ω-3 PUFAs of fed fish. ARA contents were similar in the starved (2.33%) and fed groups (3.06%). The percentages of total ω-3 PUFA and ω-6 PUFA in starved fish muscle were lower than in fed fish *(Table 2)*. Both in starved and fed fish, ω-3 fatty acids make up a larger fraction than ω-6 fatty acids. The ω-3/ ω-6 ratios in muscle were found to be 1.27 (starved fish) and 1.49 (fed fish). The higher ω-3/ ω-6 ratio in fed fish might a result of the feed acids.

Table 1: Lengths, weights and condition factors of the fish used in the study.

Groups	Length (cm)	Weight (gr)	Condition factor
Starved fish	20.12±0.46	105.18±8.36	1.29
Fed fish	21.17±0.98	138.08±6.12	1.56

Explanations: Each value represents the mean of three experiments. The values represent the data in the 28th day for starved and fed fish.

Table 2: Fatty acid composition in the muscles of the fed and starved *O. mykiss*[A].

Fatty Acids	Starved group 28th day Mean±S.E.	Feed acids Mean±S.E.	Fed Group 28th day Mean±S.E.
C12:0[B]	1.33±0.40[C]	---	0.94±0.12
C14:0	1.63±0.30a	4.34±0.66b	1.97±0.33a
C15:0	1.27±0.17	---	0.82±0.06
C16:0	30.18±1.55a	28.80±0.55b	29.27±0.62ab
C18:0	3.16±0.38a	3.74±.35a	3.22±0.38a
C20:0	1.01±0.30	---	1.06±0.14
ΣSFA	38.58±1.09a	36.88±0.73b	37.28±0.38b
C14:1 ω-5	3.36±0.57a	2.14±0.09b	1.87±0.42b
C16:1 ω-9	2.49±0.77a	5.16±1.01b	1.51±0.21c
C18:1 ω-9	23.20±0.07a	21.34±0.80b	24.51±0.47c
C20:1 ω-9	1.25±0.27a	2.10±032b	2.04±0.46b
ΣMUFA	30.30±0.54a	30.74±1.60a	29.93±0.52a
C18:2 ω-6	7.59±0.34a	6.17±0.16b	9.20±0.35c
C18:3 ω-6	---	1.04±0.41	---
C20:2 ω-6	1.92±0.03a	0.93±0.06b	0.91±0.29b
C20:3 ω-6	1.72±0.31a	4.78±0.37b	1.73±0.28a
C20:4 ω-6	1.46±0.24a	0.97±0.01a	1.27±0.25a
ΣPUFA ω-6	12.69±0.16a	13.89±0.83b	13.11±0.30b
C18:3 ω-3	3.02±0.54a	3.03±0.71a	4.90±0.44b
C20:4 ω-3	2.33±0.40a	1.27±0.08b	3.06±0.10a
C20:5 ω-3	3.24±0.57a	4.52±0.37b	2.08±0.30c
C22:5 ω-3	3.23±0.85a	0.86±0.03b	1.25±0.28b
C22:6 ω-3	4.36±0.36a	7.50±1.34b	8.37±0.16c
ΣPUFA ω-3	16.18±1.52a	17.18±1.30b	19.46±0.46c
ω-3/ ω-6	1.27±0.21a	1.24±0.10a	1.49±0.04a

[A]: Average of three lots analyzed. [B]: Values reported are means ± S.E. [C](a – b – c – d): Values for each sample with different superscript letters in the same fraction are significantly different at $P \leq 0.05$. Σ SFA: total saturated fatty acid; Σ MUFA: total monounsaturated fatty acid; Σ PUFA ω - 6: total ω - 6 polyunsaturated fatty acid; Σ PUFA ω - 3: total ω - 3 polyunsaturated fatty acid.

Discussion

The condition factor rainbow trout was found at the high level in fed fish (1.56) than starved fish (1.29) *(Table 1)*. The reason for this may be caused by the feeding twice a day. Increase in the fatty acid levels seems to support this idea *(Table 2)*.

It has been reported that C16:0 and C18:0 were the dominant SFAs in all fish species as in our study. A very important difference

was not detected between other SFAs (C12:0, C14:0 and C15:0) in starved and fed fish. This finding seems to agree with another study conducted on trout, including *O. mykiss* [4]. At the same time, the literature reports on SFA compositions of fish examined were similar to our results [13, 23,24].

Many fish tissues seem to contain excessive amounts of C18:1 ω-9 within the total MUFA [16,25]. The percentages of C16:0 and C18:1 ω-9 were higher in the fed fish and starved fish than in the feed in the present study. But, rates of MUFAs were very similar between fed fish and starved fish. This suggests that fish may have a high lipogenic capacity [26,27]. At the same time, body lipids can also be synthesized de novo from other carbon donors, such as carbohydrates or amino acids [28].

Essential fatty acids affect the fluidity, flexibility and permeability of membranes. At the same time, they are the precursors of eicosanoids. In general, lipids in marine fish contain excess amounts of ω-3 PUFA rather than ω-6 PUFA. ARA is a precursor for prostaglandins and thromboxane which will influence blood clot formation and its attachment to the endothelial tissue during wound healing [29]. However, these essential acids should be obtained by the diet. Depending on this data, in the present study, the highest ω-6 and ω-3 PUFA levels had been identified in the fed group. These results suggest that the fatty acid contents in the fed fish are related to feed lipid levels.

Many investigations have been carried out on the effect of fatty acid content of feed on fatty acid metabolism of cultured fish [13,17,30]. The profile of ω-3 PUFAs in the starved fish, fed fish and feed were similar in our study. It is well known that ω-3 PUFAs are the most important fatty acids of the fish and one of the pattern to determine the food quality of the fish. The percentages of these fatty acids in fed rainbow trout muscle were found the highest as 19.46% (*Table 2*). Researchers have also reported similar results in concentrations of ω-3 PUFAs in the muscles of some seawater, freshwater fish and other aquatic species. PUFAs are involved in a great variety of physiological functions, neural control and the functioning of the reproductive systems [16, 23, 31-33].

Güler *et al*. [34] emphasized that ω-3/ω-6 ratio is a very useful index for comparing nutritional value of fish oils. Our study showed that the ω-3/ω-6 fatty acid ratio was 1.27 in the starved fish, 1.24 in the feed and 1.49 in the fed fish. Therefore it could be said that *O. mykiss* was a freshwater fish species having a high nutritional value for human consumption due to its high ω-3/ω-6 ration. An increase in the human dietary ω-3/ω-6 fatty acid ratio is essential in the diet to help prevent coronary heart disease by reducing plasma lipids and to reduce cancer risk [35,36]**.** In conclusion, the nutritional fatty acids did affect much more on fatty acids of fed fish. Because, the use of a high fat diet depresses de novo fatty acid synthesis and increases lipid storage of dietary origin. Therefore, a high quality dietary lipid profile may impair the nutritional value of fish flesh as human food.

References

1. Fonseca-Madrigal, J., Karalazos, V., Campbell, P.J., Bell, J.G., Tocher, D.R. 2005. Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*). Aquacult. Nutr. 11, 241-250.
2. Bell, J.G., McEvoy, J., Tocher, D,R., McGhee, F., Campell, P.J., Sargent, J.R. 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (*Salmo salar*) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535-1543.
3. Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A.,

Smullen, R., Sargent, J.R. 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects tissue fatty acid compositions and hepatic fatty acid metabolism. J. Nutr. 132, 222-230.

4. Visentainer, J.V., Noffs, M.D'A., Carvalho, P.O., de Almeida, V.V., de Oliveira, C.C., de Souza, N.E . 2007. Lipid content and fatty acid composition of 15 marine fish species from the South coast of Brazil. J. Ame. Oil Chem. Soc. 84, 543-547.
5. Simopoulos, A.P. 1999. Essential fatty acids in health and chronic disease. American J. Clin. Nutr. 70, 560-569.
6. Lauritzen, I., Blondeau, N., Heurteaux, C., Widmann, C., Romey, G., Lazdunski, M. 2000. Polyunsaturated fatty acids are potent neuroprotectors. EMBO J. 19, 1784-1793.
7. Sargent, J.R., Tocher, D.R., Bell, J.G. 2002. The lipids. In: Fish Nutrition, 3rd edn (Halver JE and Hardy RW eds), 181-257, Academic Press, San Diego.
8. Cook, H.W. 1996. Fatty acid desaturation and chain elongation in eukaryotes. In: Biochemistry of Lipids, Lipoproteins and membranes (Vance DE and Vance JE eds), 129-152, Elsevier, Amsterdam.
9. Sargent, J.R., Bell, G., McEvoy, L., Tocher, D., Estevez, A. 1999. Recent developments in the essential fatty acid nutrition of fish. Aquacult. 177 (1), 191-199.
10. Leray, C., Pelletier, X. 1985. Fatty acid composition of trout phospholipids: Effect of (n-3) essential fatty acid deficiency. Aquacult. 50, 51-59.
11. Rodriguez, C., Acosta, C., Badia, P., Cejas, J.R., Santamaria, F.J., Lorenzo, A. 2004. Assessment of lipid and essential fatty acids requirements of black seabream (*Spondyliosoma cantharus*) by comparison of lipid composition in muscle and liver of wild and captive adult fish. Comp. Biochem. Physiol. 139 B, 619-629.
12. Bayır, A., Haliloğlu, H.I., Sirkecioğlu, A.N., Aras, N.M. 2006. Fatty acid composition in some selected marine fish species living in Turkish waters. J. Sci. Food Agricult. 86, 163-168.
13. Görgün, S., Akpinar, M.A. 2007. Liver and muscle fatty acid composition of mature and immature rainbow trout (*Oncorhynchus mykiss*) fed two different diets. Biologia. 62 (3),351-355.
14. Akpinar, M.A., Görgün, S., Akpinar, A.E. 2009. A comparative analysis of the fatty acid profiles in the liver and muscles of male and female *Salmo trutta macrostigma*.Food Chem.112,6-8.
15. Lee, S.M. 2001. Review of the lipid and essential fatty acid requirements of rockfish (*Sebastes schlegeli*). Aquacult. Res. 32 (1), 8-17.
16. Bulut, S., Konuk, M., Uysal, K., Mert, R., Cemek, M. 2010. Seasonal variation in the fatty acid composition and ω6/ω3 ratio of the pike (*Esox lucius*) muscle living in Karamik Lake, Turkey. Fresen. Environ. Bull. 19 (10), 2227-2231.
17. Akpinar, N., Akpinar, M.A., Gorgun, S., Akpinar, A.E. 2015. Fatty acid composition and ω-3/ω-6 ratios in the muscle of wild and reared *Oncorhynchus mykiss*. Chem. Natur. Comp. 51 (1), 22-25.
18. Gelienau, A., Corraze, G., Boujard, T., Larroquet, L., Kaushik, S. 2001. Relation between dietary lipid level and voluntary feed intake, growth, nutrition gain, lipid deposition and hepatic lipogenesis in rainbow trout. Reproduc. Nutr. Develop. 41, 487-503.
19. Çelikkale, S. 1988. İçsu Balıkları Yetiştiriciliği. Cilt I, Karadeniz Teknik Üniver. Basım, Trabzon-Türkiye.
20. Geldiay, G., Balık, S. 1988. Türkiye'nin Tatlısu Balıkları. Ege Üniver. Fen Fak. Kitap Ser. *97, 91-95,* Bornova, İzmir-Türkiye.

21. Folch, J., Less, M., Sloane, S.G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.
22. Moss, C.W., Lambert, M.A., Mervin, W.H. 1974. Comparison of rapid methods for analysis of bacterial fatty acids. Appl. Microbiol. 28 (1), 80-85.
23. Kalyoncu, L., Yaman, Y., Aktumsek, A. 2010. Determination of the seasonal changes on total fatty acid composition of rainbow trout, *Oncorhynchus mykiss* in Ivriz Dam Lake, Turkey. African J. Biotech. 9 (30), 4783-4787.
24. Görgün, S., Akpinar, N., Dirican, S. 2014. A comparative study on the fatty acid profiles of total lipid, neutral and polar lipids in the liver and muscle of *Capoeta sieboldii* (Steindacher, 1864) and *Capoeta baliki* (Turan, kottelat, Ekmekçi, İmamoğlu, 2006) from Tödürge lake (Sivas, Turkey). Acta Aliment. 43 (1), 170-181.
25. Vujkovic, G., Karlovic, D., Vujkovic, I., Vörösbaranyi, I., Jovanovi, B. 1999. Composition of muscle tissue lipids of silver carp and bighead carp. *J. Ame. Oil Chem. Soc.* 76 (4), 475-480.
26. Ghioni, C., Tocher, D.R., Sargent, J.R. 1997. The effect of culture on morphology, lipid and fatty acid composition and polyunsaturated fatty acid metabolism of rainbow trout (*Oncorhynchus mykiss*) skin cells. Fish Physiol. Biochem. 16, 499-513.
27. Ruyter, B., Rosjo, C., Masoval, K., Einen, O., Thomassen, M.S. 2000. Influence of dietary n-3 fatty acids on the desaturation and elongation of (1-^{14}C) 18:2 n-6(1-^{14}C) 18:3 n-3 in Atlantic salmon hepatocytes. Fish Physiol. Biochem. 23, 151-158.
28. Brauge, C., Corraze, G., Medale, F. 1994. Effects of dietary carbohydrate levels on growth, body composition and glycemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. Aquacult. 123, 109-120.
29. Steffens, W. 1997. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquacult. 151 (1-4), 97-119.
30. Reinitz, G.L., Yu, T.C. 1981. Effect of dietary lipids on growth and fatty acid composition of rainbow trout (*Salmo gairdneri*). Aquacult. 22, 359-366.
31. Wang, Y.J., Miller, L.A., Peren, M., Addis, P.B. 1990. Omega -3 fatty acids in lake superior fish. J. Food Sci. 55 (1), 71-73.
32. Harlıoğlu, M.M., Köprücü, K., Harlıoğlu, A.G., Yılmaz, Ö., Yonar, S.M., Aydın, S., Duran, T.Ç. 2015. Effects of dietary n-3 polyunsaturated fatty acids on the nutritional on the nutritional quality oh abdomen meat and hepatopancreas in a freshwater crayfish (*Astacus leptodactylus*). J. Food Comp. Analy. 41, 144-150.
33. Wen, J., Chen, D., Zeng, L., Wang, Y., Tian, L., Lai, J. 2015**.** Nutritional quality and safety of the important commercial cuttlefish *Sepia lycidas.* J. Aquatic. Food Prod. Techn. 24, 101-111.
34. Güler, G.O., Aktümsek, A., Citil, O.B., Arslan, A., Torlak, E. 2007. Seasonal variations on total fatty acid composition of fillets of zander (*Sander lucioperca*) in Beyşehir Lake (Turkey). Food Chem. 103 (4), 1241-1246.
35. Kinsella, J.E., Lokesh, B., Stone, R.A. 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular diease: possible mechanisms. Am. J. Clin. Nutr. 52 (1), 1-28.
36. Daviglus, M.L., Stamler, J., Orencia, A.J, Dyer, A.R., Liu, K., Greenland, P., Walsh, M.K., Morris, D., Shekelle, R.B. 1997. Fish consumption and the 30 year risk of fatal myocardial infarction. N. Engl. J. Med. 336, 1046-1053.