Research Article

Habitat related variations in omega series of unsaturated fatty acids in Indian major Carp *Cirrhinus mrigala*

Bilal Hussain^{1, 2*}, Tayyaba Sultana¹, Salma Sultana¹, Farhat Jabeen¹, Shahid Nadeem³ and Shahid Mahboob¹

1-Department of Zoology, Wildlife and Fisheries, Govt. College University, Faisalabad, Pakistan.

2-Department of Bioinformatics and Biotechnology, Govt. College University, Faisalabad, Pakistan.

3-Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan.

Corresponding author email: profbilal@yahoo.com

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Abstract

Atomic absorption spectrophotometry showed significant levels of contamination by Cd, Hg, Cu, Mn, Zn, Pb, Cr and Sn in fish habitats. Fatty acids from meat were detected by gas chromatography. C12:0, C20:0, C16:1(n-7), C16:1(n-9), C18:1(n-9), C20:1(n-9), C18:2(n-6), C18:4(n-3), C22:4(n-6), C20:5(n-3) and C22:4(n-6) fatty acids were found missing in fish obtained from area of higher pollution when compared to farmed fish. C16:1(n-7), C20:1(n-9), C20:2(n-6), C22:4(n-6) and C22:5(n-3) were found missing in the fish collected upstream to the areas of high intensity pollution. Loss of these important fatty acids indicates the sensitivity of the fish even in response to the lower intensity of pollution. Farmed fish showed optimal concentration of each fatty acid. Significant differences ($p < 0.05$) were observed in fatty acid profiles of different species when each was compared in relation to their own habitats. Habitat of even lower intensity pollution confiscates fatty acids of omega series and lower down the unsaturated fats for which fish is preferred on other animals.

Key words: Pollution load, fish, devastation, fatty acid profile.

Introduction

Fish culture is considered today as one of the most promising sources of animal proteins. During recent past, potential and prolific nature of fish culture has been directed towards its large scale adoption and promotion in Asian countries. Traditionally Indian major carps, viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* are commonly cultured in freshwater ponds. Fish are not only protein source but also contain considerable amount of unsaturated fatty acids. In recent years, fish lipids have also assumed a great nutritional significance owing to their protective role against the development of cardiovascular diseases and rheumatoid arthritis [1-3]. Coronary heart diseases have been identified as a leading cause of death in various parts of the world including Pakistan, with mortality rates increasing every year.

Fish lipids are rich in polyunsaturated fatty acids (PUFA), which are essential for fish health and growth. In comparison with terrestrial animal meat, fish lipids are beneficial to human health by reducing

the risk of cardiovascular and inflammatory disorders [2, 4, 5]. Fishes store lipids in muscles and liver in contrary to majority of mammals which store lipids in adipose tissues [6, 7]. With increasing demand, population of the fish species in different areas of the world has decreased drastically due to pollution and over fishing. Polluted water from industrial and urban areas of Faisalabad is disposed into the River Chenab which has led to the extinction and decrease in population of different species of the fishes in almost 190 Km length of the River Chenab. The present project is planned to evaluate the effect of pollution on the fatty acid profile of the fish in order to make it unambiguous that fish not only bioaccumulate toxicants in their body but also endow with reduced quality of the meat. There is awful need to reduce environmental pollution so that next generation may enjoy well quality fish meat with high quality lipid profile.

Materials and methods

Bottom feeder *Cirrhinus mrigala* (Indian major carps) collected from highly polluted areas, less

polluted areas and farmed fish were analyzed for the estimation of fatty acids profile.

Procurement of Fish

Fish species were harvested from the River Chenab through its 190 km of length in the District Jhang from Thatta Muhammad Shahh to Trimu Head. This is an area of high pollution because sewage and industrial wastes are disposed into the River Chenab through Chakbandi Drain at this point at latitude 31.570° and longitude 72.534°. Except rainy season only this polluted water flows through the River Chenab up to Trimu Head. Drag nets and gill nets were used to harvest fish from these highly polluted areas of the River. For control fish were harvested upstream from Tatta Muhammad Shah at the point Thalli comparatively less polluted areas of the River Chenab. To make the results more explicate these fishes were compared to farmed fish devoid of any type of pollution.

Preparation of Fish for Experiment

All the fish specimens were freshened out in running de-chlorinated tap water, thereby, facilitating the emptying and removal of stomach contents. Fish were then, slaughtered and dressed, which consisted of removing visceral organs, followed by meat filleting. Wild and farmed fish species were analyzed fresh each time for fatty acid profile.

Water Analysis

Water samples of the River Chenab were taken from areas of fish harvest and analyzed for selected heavy metals and other water quality parameters defined by Environmental protection Agency of Pakistan. Seven Surface, column and bottom water samples of about 1.5 liter (each) were collected in polypropylene bottles from each site and preserved in 5 ml of 55% HNO₃/L of water to prevent metal adsorption on the inner surface of the container and stored at 4 °C before their analyses defined by Boyed (1981) [8]. Metals analyzed were tin (Sb), chromium (Cr), lead (Pb), Zinc (Zn), manganese (Mn), copper (Cu), cadmium (Cd), and mercury (Hg). Concentration of each metal was detected by using heavy metal kits (Merck Germany) and by using Hitachi polarized Zeeman Atomic Absorption Spectrophotometer AAS, 2000 series.

Fatty Acid analysis

Lipids were extracted according to Folch et al. [9], as modified by Bell et al. [10] by further improvement of unit by execution of Soxhlet unit into Licon Nicolson concurrent distillation unit for rapid extraction. Seven fish specimen were used and fresh fish flesh tissues from each fish in replica of seven were oven dried and ground to made powder. Total lipids (oils and fats) were extracted by using soxhlet apparatus by n-Hexane heated at 65°C. Extracted lipids were converted to fatty acid (FA) methyl esters

by methanolic sulfuric acid through esterification process and after treating with ether samples were injected into the Gas Chromatograph. Chromatograms produced by each sample gave the retention time of each fatty acid (Fatty acid methyl esters (FAMES)) were compared to the chromatogram of standard (mixture of pure FAMES). Fatty acids were determined by gas-liquid chromatography, by using Flame Ionization detector according procedure defined by Kiessling et al. [6].

Statistical analysis

Different Procedure of Statistical Analysis System ANOVA by Minitab packages [11] was used to analyze the data. Results are presented as means and standard errors (SE) of physicochemical parameters and fatty acids in each category. Means of fatty acid profiles were segregated according to the Ryan-Einot-Gabriel-Welch Multiple Range Test (REGWq). Data subjected to other tests by using SPSS program version 10.0 to perform the analysis.

Results and discussion

Water analysis clearly showed significant change in the water quality of the River Chenab after receiving this drain. All water quality parameters were found far above than WHO permissible limits (Fig. 1; Table-1). The data shown for individual fatty acid of total lipids are limited to, fish flesh of two category habitats. One from highly polluted areas, where industrial and domestic wasters are disposed into the River Chenab through chakbandi drain and other from comparatively less polluted areas of the River Chenab upstream before the disposal of this drain to river. These two categories were compared to farmed fish. Comparison of above mentioned two categories provided clear evidence for domino effects that this environment has developed into very toxic habitat not suitable for the growth of fish to produce high quality meat. Higher concentrations of heavy metals in the water not only bioaccumulate in the fish body but also get in the way of metabolism hence reducing the quality of the fish [12].

Fish from highly polluted locale showed less number of fatty acids identified and more saturated fatty acids in their meat (Fig. 2) as compared to fish from less polluted areas. Here it is important to point out that *Cirrhinus mrigala* showed non-significant differences in the amount of saturated and unsaturated fatty acids from both wild categories indicating its higher sensitivity for pollution. This is due to the fact that pollution sediment with the passage of time [13] in the bottom and *Cirrhinus mrigala* is bottom feeder fish having maximum exposure to the pollution.

C12:0, C20:0, C16:1(n-7), C16:1(n-9), C18:1(n-9), C20:1(n-9), C18:2(n-6), C18:4(n-3), C22:4(n-6), C20:5(n-3), C22:4(n-6) and C22:5(n-3) fatty acids

Fig. 1. Comparison of heavy metals in fish environments. HPH: highly polluted habitat, LPH: less polluted habitat. Control: farmed.

Fig. 2. Total lipids and fatty acid profile of *Cirrhinus mrigala* from three different habitats. ΣSFA; saturated fatty acids, ΣMUFA; monounsaturated fatty acids, ΣPUFA; polyunsaturated fatty acids.

Table 1. Change in the water quality of the River Chenab due to pollution load.

Water Quality Parameters	Highly Polluted water	Less Polluted water (Upstream)	Control (Farmed)
pH	11.6 ± 0.23	8.0 ± 0.01	7.9 ± 0.00
BOD	76.70 ± 0.03	44.9 ± 0.03	33.50 ± 0.13
COD	192.0 ± 0.05	67.2 ± 0.05	60.0 ± 0.05
TDS	2399 ± 1.47	1295 ± 0.99	319 ± 0.47
TSS	301 ± 0.09	207 ± 0.22	179 ± 0.82
Salinity	1900 ± 0.07	401 ± 0.21	200 ± .032
Conductivity	3.214 ± 0.01	1.32 ± 0.03	0.29 ± 0.01
Phenols	2.22 ± 0.01	0.72 ± 0.01	0.14 ± 0.00
Sulphates	431 ± 0.06	318 ± 0.23	79 ± 0.55

Values mg/L (Mean ± SE) are average of five samples analyzed in triplicate. BOD: Biological oxygen demand, COD: Chemical oxygen demand. TDS: Total Dissolved solids, TSS: Total suspended solids

Table 2. Fatty acid profile % (Mean $\pm$ S.E) of *Cirrhinus mrigala* meat collected from three different habitats in pollution spectra.

Fatty acids	Highly Polluted habitat	Less polluted area (Upstream)	Non-polluted (Farmed)
Saturated fatty acids			
C10:0	07.7949 $\pm$ 0.88 b	01.3262 $\pm$ 0.04 ad	03.4125 $\pm$ 0.05 ac-f
C12:0	-----	00.6923 $\pm$ 0.05 abe	01.0547 $\pm$ 0.03 abc
C14:0	02.4699 $\pm$ 0.05 abd	00.4762 $\pm$ 0.01 b	04.2143 $\pm$ 0.09 be
C16:0	54.9414 $\pm$ 2.14 def	41.9244 $\pm$ 1.09 ab	12.0025 $\pm$ 0.23 abe
C18:0	10.1358 $\pm$ 0.15 gh	28.4393 $\pm$ 2.04 cde	07.0658 $\pm$ 0.10 ab
C20:0	-----	01.1707 $\pm$ 0.02 ah	00.2540 $\pm$ 0.10 abd
Σ SFA	75.3370 $\pm$ 0.80 abef	74.0291 $\pm$ 0.54 ab	28.0038 $\pm$ 0.07 ab
Monounsaturated fatty acids			
C16:1(n-7)	-----	-----	00.5073 $\pm$ 0.02 b
C16:1(n-9)	-----	00.3900 $\pm$ 0.01 abh	02.5471 $\pm$ 0.01 ace
C18:1(n-7)	00.4688 $\pm$ 0.08 af	00.3472 $\pm$ 0.03 bef	01.4587 $\pm$ 0.02 ace
C18:1(n-9)	-----	00.0742 $\pm$ 0.12 ab	11.8905 $\pm$ 0.07 abd
C20:1(n-9)	-----	-----	03.5412 $\pm$ 0.19 ad
C22:1(n-9)	05.2212 $\pm$ 0.10 bc	00.3526 $\pm$ 0.10 ab	00.6039 $\pm$ 0.03 ad
Σ MUFA	05.6900 $\pm$ 0.09 c-f	01.1640 $\pm$ 0.06 abc	20.5481 $\pm$ 0.05 abf
Polyunsaturated fatty acids			
C18:2(n-6)	-----	01.1625 $\pm$ 0.09 abe	00.0122 $\pm$ 0.02 abe
C18:3(n-3)	02.4446 $\pm$ 0.12 bc	04.0059 $\pm$ 0.02 abf	06.2521 $\pm$ 0.12 abe
C18:4(n-3)	-----	00.3921 $\pm$ 0.07 ade	00.5543 $\pm$ 0.01 ace
C20:2(n-6)	00.9421 $\pm$ 0.09 bdf	-----	00.6302 $\pm$ 0.01 ab
C20:4(n-6)	08.2893 $\pm$ 0.03 adef	00.4425 $\pm$ 0.04 abe	10.6500 $\pm$ 0.62 abc
C20:5(n-6)	03.0386 $\pm$ 0.10 ah	07.6222 $\pm$ 0.09 afe	00.1140 $\pm$ 0.12 bc
C20:5(n-3)	-----	00.1199 $\pm$ 0.13 abe	07.8503 $\pm$ 0.42 bce
C22:4(n-6)	-----	-----	00.4441 $\pm$ 0.01 abc
C22:5(n-6)	00.1366 $\pm$ 0.03 abg	04.9590 $\pm$ 0.06 ace	06.0210 $\pm$ 0.06 ab
C22:5(n-3)	03.3439 $\pm$ 0.04 acd	-----	02.0129 $\pm$ 0.02 ab
C22:6(n-3)	00.1151 $\pm$ 0.02 abc	02.2750 $\pm$ 0.03 ade	07.0014 $\pm$ 0.14 abd
Σ PUFA	18.3102 $\pm$ 0.06 ab	20.9791 $\pm$ 0.07 abe	49.5425 $\pm$ 0.14 ab

SFA; Saturated fatty acids, MUFA; Monounsaturated fatty acids, PUFA; Polyunsaturated fatty acids. Means sharing similar letter in a row or in a column are statistically non-significant ($P > 0.05$).

Table 3. Correlation coefficient matrix for unsaturated fatty acids and WQPs along with selected heavy metals.

Fatty Acids	BOD	COD	Phen	Cd	Cu	Mn	Zn	Pb	Cr	Sn	Hg
C16:1(n-7)	-0.548	0.562	0.866	0.701	1.000	-0.021	0.405	0.452	0.761	0.439	0.627
	0.631	0.036	0.000	0.000	0.016	0.944	0.068	0.040	0.000	0.047	0.002
C16:1(n-9)	0.999	0.808	0.893	0.743	0.039	-0.072	0.665	0.062	0.734	0.699	0.638
	0.027	0.000	0.000	0.000	0.894	0.681	0.001	0.791	0.000	0.000	0.002
C18:1(n-7)	-0.138	0.555	0.541	0.972	-0.113	0.883	0.754	0.812	0.947	0.671	0.445
	0.912	0.040	0.011	0.151	0.701	0.000	0.000	0.094	0.000	0.001	0.043
C18:1(n-9)	0.018	0.701	0.942	0.765	0.766	0.948	0.724	0.685	0.545	-0.724	0.774
	0.988	0.005	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.000
C20:1(n-9)	0.087	0.040	0.865	-0.007	0.312	0.642	-0.387	0.667	0.962	0.878	0.664
	0.945	0.891	0.000	0.980	0.278	0.000	0.172	0.001	0.000	0.000	0.001
C22:1(n-9)	0.984	0.522	0.711	0.542	-0.091	0.561	0.405	0.248	0.553	0.507	0.611
	0.114	0.056	0.000	0.011	0.758	0.008	0.068	0.279	0.009	0.019	0.003
C18:2(n-6)	0.989	0.522	0.879	0.932	0.002	0.891	0.496	0.699	0.212	0.959	0.505
	0.096	0.056	0.000	0.236	0.993	0.000	0.000	0.000	0.357	0.000	0.020
C18:3(n-3)	0.989	0.345	0.248	0.553	0.653	0.576	-0.741	0.494	0.640	0.921	0.333
	0.096	0.227	0.394	0.009	0.001	0.006	0.000	0.023	0.002	0.000	0.140
C18:4(n-3)	0.906	0.814	0.182	0.770	0.487	0.970	0.976	0.563	0.946	0.966	0.575
	0.279	0.000	0.534	0.000	0.025	0.158	0.139	0.008	0.000	0.000	0.006
C20:2(n-6)	-0.689	0.211	-0.224	0.159	0.554	0.863	0.882	0.494	0.545	0.519	0.576
	0.006	0.469	0.440	0.586	0.000	0.000	0.000	0.023	0.011	0.000	0.006
C20:4(n-6)	0.986	0.609	0.575	0.558	0.576	0.487	0.411	0.329	0.574	0.509	0.320
	0.107	0.003	0.006	0.009	0.006	0.025	0.064	0.145	0.007	0.018	0.157
C20:5(n-6)	0.976	0.782	0.277	0.334	0.775	0.450	0.786	-0.042	-0.295	0.161	0.817
	0.141	0.000	0.337	0.243	0.000	0.041	0.000	0.856	0.127	0.414	0.000
C20:5(n-3)	0.002	0.793	-0.821	-0.375	0.558	0.086	0.228	0.112	0.560	0.769	0.253
	0.588	0.000	0.000	0.027	0.009	0.710	0.321	0.630	0.008	0.000	0.193
C22:4(n-6)	-0.490	0.609	-0.071	0.086	0.154	0.544	-0.002	0.066	0.978	0.958	0.368
	0.674	0.003	0.955	0.945	0.902	0.011	0.994	0.778	0.000	0.184	0.101
C22:5(n-6)	0.786	0.575	0.224	0.078	0.561	0.800	0.750	0.257	0.690	0.447	0.624
	0.000	0.006	0.329	0.737	0.008	0.000	0.000	0.187	0.000	0.042	0.003
C22:5(n-3)	0.039	-0.028	0.941	0.966	0.018	0.295	0.530	-0.333	0.093	0.226	0.423
	0.842	0.886	0.000	0.000	0.926	0.127	0.004	0.083	0.637	0.326	0.056
C22:6(n-3)	-0.042	-0.403	0.145	-0.618	-0.535	0.126	0.024	-0.462	0.638	0.154	0.599
	0.887	0.154	0.620	0.018	0.049	0.668	0.936	0.096	0.014	0.599	0.004

Upper values indicate Pearson's correlation coefficient; Lower values indicate level of significance at 5% probability. Non-significant ($P>0.05$); Significant ($P<0.05$); Highly significant ($P<0.01$), WQPs; Water quality parameters, BOD; Biochemical Oxygen Demand, COD; Chemical Oxygen Demand, Phen; Phenols.

were missing in fish collected from the highly polluted habitat (Table-2). Loss of these beneficial

fatty acids could not be compensated when majority of the diet comprises of fish meat. *Cirrhinus mrigala*,

although widely cultured in the ponds but this fish species have least consumer preference in the vicinity of the River Chenab even from less polluted areas of the river due to the texture and aroma. Fish having high saturates accompanied with contaminants in the body requires more energy to perform daily activities especially during locomotion in struggle of food [7] as in our case *Cirrhinus mrigala* showed higher concentration of saturated fatty acids (Table-2; Fig. 2).

Fish is preferred as white meat only due to the presence of higher concentrations of polyunsaturated fatty acids. This study signifies that fish if grown in polluted waters would cause loss of beneficial fatty acids may be missing in the flesh of the fish reducing the quality of the fish meat as this study signified. High fat contents of *Cirrhinus mrigala* although help in the buoyancy as the density of the fat is lower than water [7, 14] but it deter the movement of the fish even in lower levels of the pollution. Perhaps this is among the major factors leading to the extinction of this fish species in polluted areas of the River Chenab. Fish from unpolluted areas clearly indicated higher levels of omega-3 polyunsaturated fatty acids and reasonable concentrations of the saturated fatty acids. Our previous study also verify that farmed fish is much better for consumption [14, 15] when fat is required for the energy and this study clearly indicated that River Chenab after receiving too much polluted water from variety of drains become unsuitable for fish growth to produce high quality meat. Previous study also reported the preference of wild fish when fish was consumed as source of white proteins [15]. Present study clearly revealed that before consuming wild fish for unsaturated fatty acids anybody must sure that fish is not approaching from the habitat of even lower intensity pollution because it may be deficit of essential fatty acids [7, 16, 17]. Heavy metals are present in the river water as a result of industrial and urban waste water discharge besides of natural sources. The major sources of Mn and Zn are the domestic wastes, municipal wastes followed by dumping [18]. Pb, Mn, Hg and Cu values in the water significantly higher than Cd may be explained by the fact that Cd sediment and is associated with the carbonate fraction and concentrates on the suspended matter having ability to mobilize from sediment to water. This argument was also supported by some studies [19-21]. Concentrations of metals may be more in the river water as many studies revealed that in higher pH rate of sedimentation of metals is higher [18, 21, 22] corroborating the findings of present study. Correlation coefficient matrix (significance at 5% probability) for unsaturated fatty acids of omega

series and some water quality parameters along with selected heavy metals is shown in Table-3.

It could be concluded from the present study that unhealthy habitats not only escort toxicant bioaccumulation in the fish tissues but also results in devastation of essential fatty acids. Prefer farmed fish or fish from entirely unpolluted environments when major concern is the need of unsaturated fatty acids of omega series. Reject fish even from mildly polluted areas with emphasis on conservative management of the water bodies. Peoples consuming fish for omega series of unsaturated fatty acids must ensure from where fish were harvested, as it may be deficient in these fatty acids.

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