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Research Article

Analysis of major lipid classes and their fatty acid composition of an Indian minor carp *Puntius sophore* in order to evaluate its nutritional aspects

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Abstract

The present analysis investigated the major lipids and their fatty acids in a small sized edible Indian minor carp *Puntius sophore*, by thin layer chromatography (TLC) and gas liquid chromatography. In *P. sophore* muscle lipid is <1%, for which fish can be regard as very low fat fish. Among the lipid classes, Phospholipids (PL) is the maximum and the fatty acid (FA) symphony consists of altogether 36 members which are quantified by open gas liquid chromatography. In *P. sophore* the DHA is 2.5 times higher than EPA and ω3/ω6 ratio is ranging from 3.69 to 5.57 in the major lipid fractions. *P. sophore* can be considered as an ideal diet as it is found to be extraordinarily loaded with ω-3 FAs. The A.I. and T.I. are investigated which are within the range considered for standard human health. The result clearly illustrates that the significance of lipids and their FAs is imperative in maintaining their own physiology as well as human nutrition for reducing incidence of wide range of common degenerative diseases, as well as the alleviation of clinical symptoms. Therefore additional awareness should be given to the management legislation and policies for this *P. sophore* culture, because of its substantial nutritional capability.

Keywords: Fresh water fish, human health, phospholipids, ω-3fatty acids, TLC.

Introduction

In the world, aquaculture has been identified as the highest promising division of animal food production sector since 1970 (FAO, 2008). The nutritional properties of fish and fish products formulate them valuable foodstuffs that are important for human health as it contains mainly biologically active protein, easy digestible fatty acid compositions and fat-soluble vitamins (Whalen, 2009). In the world inland fish production, India ranks second, next to China (Kohinoor *et al.,* 2005). Generally in Indian freshwater aquaculture system; carps are the spinal column comprising ~85% of the total freshwater creation (Mohanta *et al.,* 2008). India reported to have 2319 fin fish species out of which 838 are from fresh water. Among them, total 450 fish species could be categorised as small indigenous carp species (SIS) out of 765 native freshwater species, and 239 of them are from West Bengal (Barman, 2007). After the trigger of Blue revolution, the production of this freshwater SIS is stagnating for years due to the overlooked attitude towards minor carps, lack of interest about species diversification, poor strategies of replenishment and stock enhancement (Roos *et al.,* 2006; Lakra *et al.,* 2011). Recent few initiatives such as the CGIAR Research Programmes; USAID funded Feed the Future; Scaling Up Nutrition (SUN) Framework and Roadmap provides new possibilities to focus on management and culture of SIS for linking between agriculture, human nutrition and health. At present in Cambodia and Kenya the WINFOOD project being conducted, complementary foods for new-born and young children with powdered, nutrient-rich small fish species have been developed, and effectiveness studies are being carried out (Roos *et al.,* 2010).

A number of studies incorporated certain features regarding the nutritional assessment of worldwide freshwater fishes (Nair and Gopakumar, 1977, 1978; Sen *et al*., 1976 a, b, 1977; Chetty *et al*., 1989; Olsen *et al*., 1990; Aggelousis and Lazos, 1991; Ayala *et al*., 1993; Gopakumar, 1993; Ackman, 1995; Andrade *et al*., 1995; Rahanam *et al*., 1995; Lilabati and Viswanath, 1996; Zenebe *et al*., 1998; Ackmen *et al*., 2002; Rasoarahona *et al.,* 2004; Zuraini *et al*., 2006; Swapna *et al*., 2010; Jakahr *et al*., 2012; Dey *et al.,* 2015). However, comprehensive study of lipid classes and fatty acids of SIS species from India are not available except *Labeo bata* (Ackmen *et al*., 2002) and *Amblypharyngodon mola* (Dey *et al.,* 2015).

In view of this aforesaid context, appropriate nutrient profile documentation of these small indigenous groups is needful. *Puntius sophore* is one of the small indigenous polyphyletic groups (Kullander, 2008). It is selected for present study since of their elevated customer requirement for its taste as well as cost-effective importance. The current study is aimed to evaluate bioavailability of the major lipids focusing mainly on the fatty acid classes in the muscle (as a dietary component) of *Puntius sophore*. The hypothesis of the present work is that the fish muscle would be containing high nutritional constituents for their lipids and fatty acids from human nutritional standpoint. This is additionally hypothesized that the lipid and fatty acids of this fish have precise position in their own physiology. This information may also afford a quite constructive justification for conservation of this species for assistance of human being.

Materials and Methods

Adult *Puntius sophore* (Hamilton 1822,order- Cypriniformes, family- Cyprinidae) of large varity measuring about 4.3-4.8 cm (weight approximately 10-12 g), had been collected in July-August, 2013 for lipid analysis from ponds in and around South 24 Parganas, West Bengal (Latitude N 22°34', Longitude E 88°43') through local fisherman and transported to laboratory in ice. Fishes were identified in the laboratory by consulting taxonomic book.

Collection of materials

A total of 800g of flesh of respective fish were pooled from 4kg of fish samples (approximately from 500 individuals) for lipid analysis, stored in 4°C and were bought to laboratory.

The following steps of the present study are shown in Figure 1.

Extraction and fractionation of lipids

The total lipids (TL) were extracted from the sample, following the method of Bligh and Dyer (1959) using methanol-chloroform (2:1, v/v), methanol-chloroform-water (2:1:0.8, v/v/v), and then again with the first solvent system. Sample was ground with the solvent, filtered and residue was extracted with the next solvent system. The process was repeated. Finally, the three extracts were pooled, diluted with water and layer was allowed to separate in a separatory funnel. The Chloroform layer at the bottom was withdrawn and dried over anhydrous sodium sulphate. The chloroform solution of lipid was evaporated under vacuum, weighed and redissolved in distilled n-hexane and kept at -20˚C for future use. BHT (butylated hydroxyl toluene) was added at a level of 100mg/L^{-1} to the solvent as antioxidant.

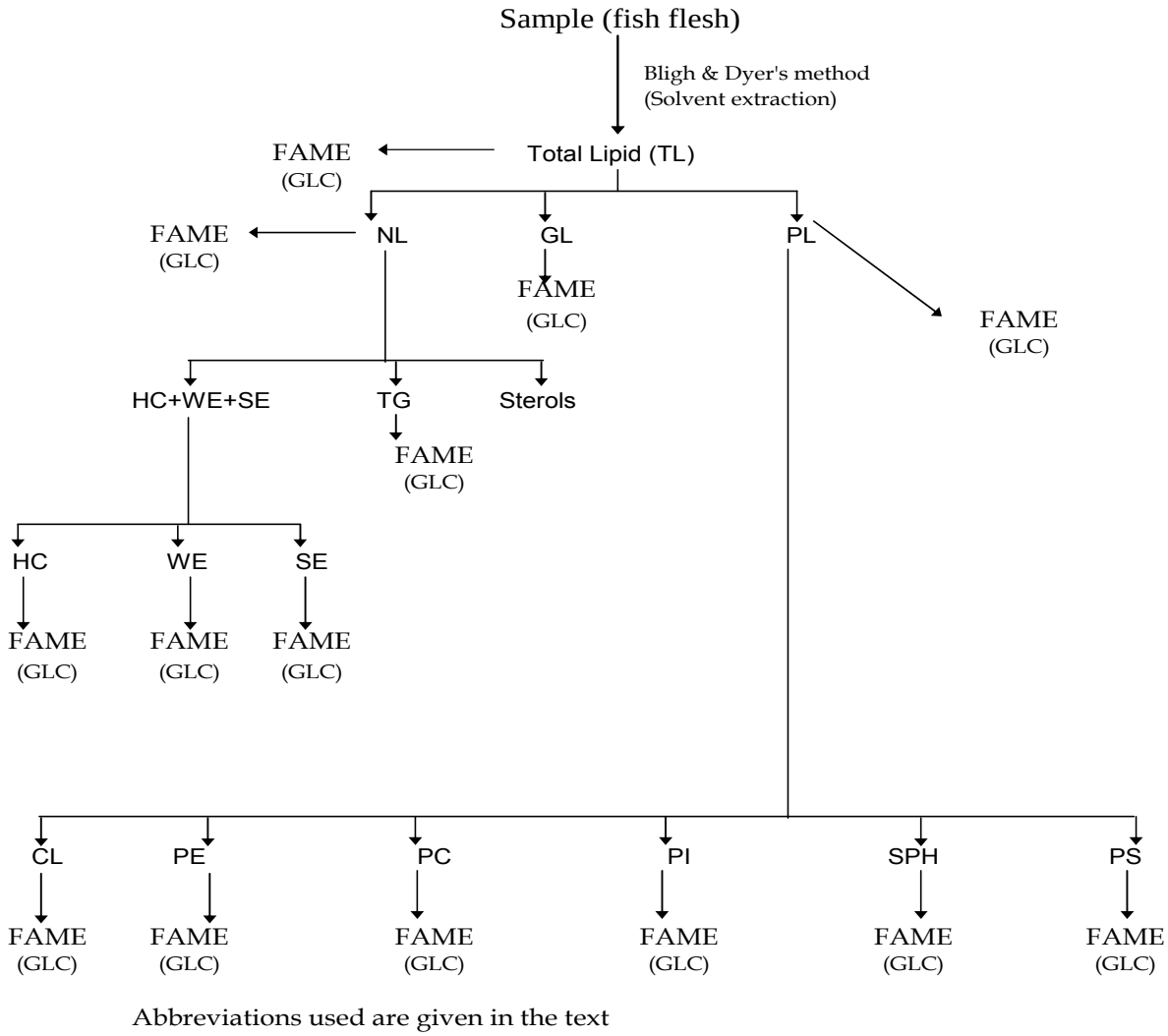
Fractionation of Total lipid by Column chromatography

According to Rouser *et al.* (1976) a portion of the TL was subjected to column chromatography to obtain Lipid classes. Each class of lipids was estimated by weighing in a microbalance.

Separation of Neutral lipids and Phospholipids component by Thin Layer Chromatography

Thin-layer chromatography was performed on 20x20 chromatoplates covered with silica gel G (0.50 mm thickness, Sigma Chemical., U.S.A). The neutral lipid samples were fractionated by preparative TLC using light petroleum ether (40°C-60°C) - diethyl ether-acetic acid (80:20:1, v/v/v) (Mangold, 1969).The three components, hydrocarbon, wax esters and sterylesters were separated into individual components and were analyzed by TLC using n-hexane: diethyl ether (49:1,v/v) as solvent system (Misra and Ghosh,1991).Phospholipids were separated into different classes, i.e. cardiolipins(CL), phosphatidylethanolamine(PE),phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SPH) , using a solvent system of methanol: chloroform: water (65:25:4, v/v/v) (Rouser *et al*.,1976).

Figure 1. Flow chart of the presented work on the lipids and fatty acids of the flesh of *P. sophore*.



Preparation and purification of Methyl Esters of fatty acids of various samples

Fatty acid methyl esters (FAME) of triacylglycerol (TG),wax esters (WE), sterol esters (SE), TL, neutral lipids (NL), glycolipids (GL), phospholipids (PL) and PL components, viz., CL, PC,PE,PI,SPH and PS were prepared by transmethylation according to Christie (2003).

Purification of FAMEs of by Thin Layer Chromatography

According to the method of Mangold(1969) the crude methyl esters thus prepared were purified by thin layer chromatography (TLC) on Silica Gel, using a solvent system of n-hexane: diethyl ether (90:10, v/v).

Gas Liquid Chromatography (GLC)

Gas liquid chromatography (GLC) of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). Quantification was done by computer using specific Clarity Lite software (Data Apex Ltd, Praha, Czech Republic).

Analysis of Fatty Acid Methyl Esters (FAME):

GLC of FAME was done on a BPX-70 megabore capillary column of 30 ml length and 0.53 mm i.d. obtained from SGE, Australia.

Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final temperatures were kept isothermal for 1 minutes and 20 minutes, respectively. Injection port and detector temperatures were 250°C and 300°C, respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.20ml/min. Identification of fatty acids was done by comparing their retention times with those of standards, chromatographed under identical operational conditions of GLC. A secondary standard of cod liver oil fatty acid methyl ester was also analyzed under the same GLC conditions, as those for the sample methyl esters as suggested by Ackman and Burgher (1965).

Analysis of hydrocarbons

Hydrocarbons were analyzed by directly injecting the samples into the GLC machine, without derivatization. Identifications were made by comparison of retention times of the peaks with those of standards, according to Misra and Ghosh (1991).

Results

The percent composition of TL and other three lipid classes (NL, GL and PL) of *P. sophore* were presented in table 1.The TL content of the flesh was only 0.60% to that of the wet weight of the tissue. Among the lipid classes, PL found to be the major component in comparison to its NL (31.15%) and GL (8.6%). In NL fraction, the combination of WE+HC+SE (41.4%) was appeared to present in equivalent quantity to that of the sterols (ST) 41.8%. However, the fraction of triacylglycerol (TAG) was found to be significantly lower (16.9%) in comparison to this combination. Hydrocarbons (HC) were estimated in highest amount (76.05%) among wax esters (WE, 11.3%) and steryl ester (SE, 12.6%). On the other hand, among PL, phosphatidylinositol (PI) (29.03% w/w of phospholipids) and sphingomyelin (SPH) were estimated as the major and minor components respectively (Fig.2).

Table-1: Composition of various classes of lipids obtained from Body Flesh samples of Puti (*Puntius sophore)*

	Different Classes of lipids	Puti/ Body Flesh
MAIN CLASSES	Total Lipids (TL) [a]	0.60
	Neutral Lipids (NL) [b]	31.15
	Glycolipids (GL) [b]	8.6
	Phospholipids (PL) [b]	60.25
NEUTRAL LIPIDS	(HC+WE+SE) [c]	41.35
	Triacylglycerol(TG)[c]	16.88
	Total Sterol (ST) [c]	41.77
	Hydrocarbon (HC) [d]	76.05
	Wax ester (WE) [d]	11.34
	Steryl ester (SE) [d]	12.6
PHOSPHO LIPIDS	Cardiolipin (CL)[e]	21.03
	Phosphatidylethanolamine (PE)[e]	18.54
	Phosphatidylcholine (PC)[e]	19.39
	Phosphatidylinositol (PI)[e]	29.03
	Sphingomyelin (SPH)[e]	5.64
	Phosphatidylserine (PS)[e]	5.94

Abbreviations

[a]Expressed as % w/w of wet tissue.

[b]Expressed as % w/w of total lipids.

[c]Expressed as % w/w of neutral lipids.

[d]Expressed as % w/w of (HC+WE+SE).

[e] Expressed as % w/w of Phospholipids (PL).

Figure 2. Comparative distribution of phospholipids fractions in the flesh of *P. sophore*.

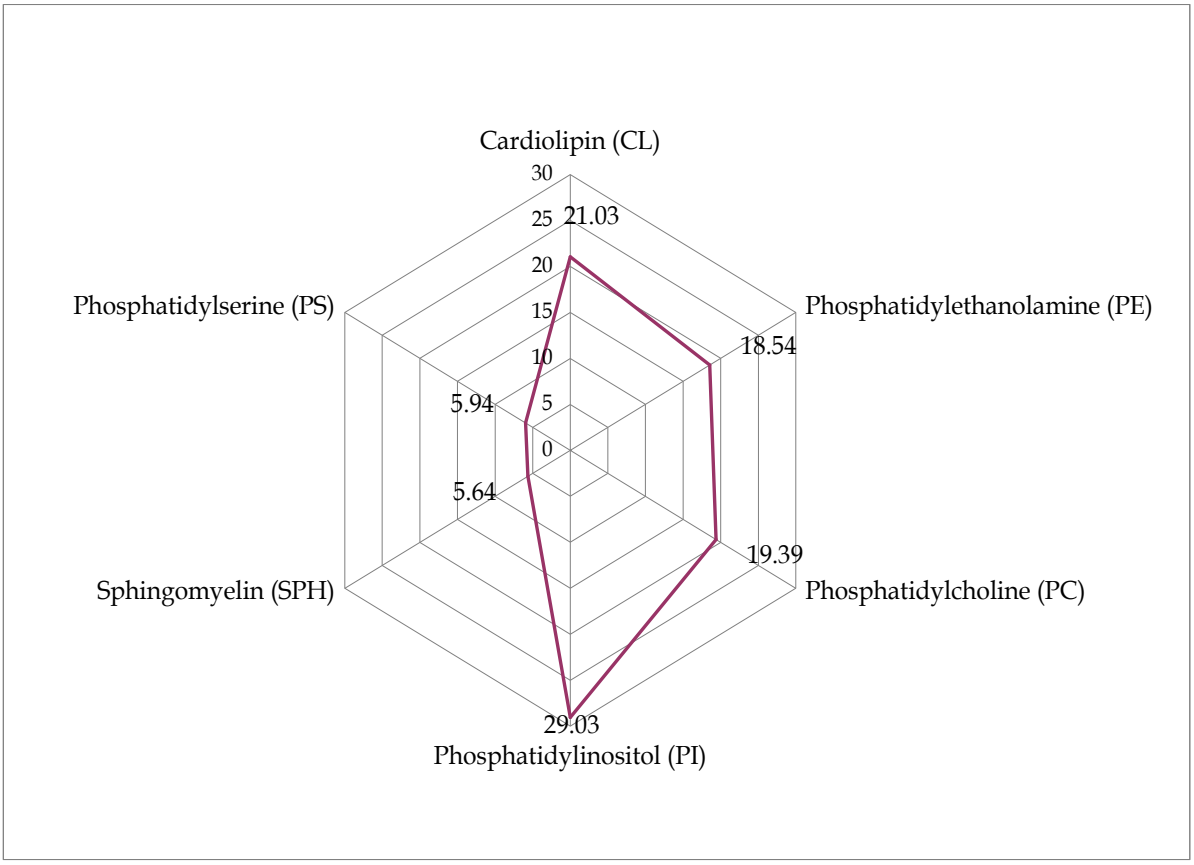


Table 2 was recorded almost in all lipid fractions, saturates were in maximum amount followed by monoenes, polyenes and dienes (SFA > MUFA > PUFA > DUFA). SFA was estimated to be major in amount in GL (59.41%), whereas MUFA (28.2%) and DUFA (7.6%) were maximum in NL (Fig.3). Similarly, PUFA ω-3 (20.7%) and ω-6 (2.6%) were found in highest extent in TL and NL respectively. SFAs and MUFAs mainly had carbon chain lengths varied from C14- C28, while for PUFAs it lied mostly in the range of C18- C22. Among SFA, palmitic acid (C16:0) was the highest (36%) in the GL, stearic acids (C18:0) in PL (12.9%) and behenic acids (C22:0) in TL (15.2%). Comparison of C-18 total and C-16 total fatty acids classes in all lipid fractions (TL, NL, GL, and PL) showed that in all cases C16-fatty acid is present in higher amount than that of C18-type (Fig.4). Distribution of all essential fatty acids (EFAs) with eicosapentaenoic acid (EPA) (20:5ω-3) and docosahexaenoic (DHA) (22:6ω-3) among these classes was presented in Fig.5. Ratio of ω-3/ω-6 was also found to be highest in TL (~9.88 fold) (Fig.6). Among the NL, GL and PL, the ω3/ω6 ratio is the lowest in NL (3.69) and maximum in PL (5.57). In PUFAs, ω-3 type fatty acids were present in higher amount than ω-6 types. On the other hand, Atherogenic index (A.I.) and Thrombogenic index (T.I.) were calculated as highest in GL in comparison with NL and TL (Fig.7).

In NL fractions, highest percentage of SFA (58.6%), DUFA (8.2%) and PUFA (7.7%) were found in WE whereas MUFA (44.2%) was in TAG (Table 3). Among SFAs, palmitic acid (16:0) was present in maximum amount in all cases (WE had highest amount 33.1%). Similarly, Oleic acid; 18:1ω9 (ω-9 fatty acid) was found to be present in highest amount in all fractions in MUFAs. Among HCs, n-alkanes were the major component (83.3%); whereas the branched chain alkanes, viz. iso- and anteiso-components were present only as 7.1 and 7.8% respectively in NL (Fig.8). Chain lengths of the n-alkanes were mostly varied from C-14 to C-31, whereas for iso- and anteiso- components the chain lengths were from C15-C29 and from C-16 to C-29 respectively. Shorter-chain n- alkanes between C-16

Table 2. Fatty acid compositions of Total Lipids (TL), Neutral Lipids (NL), Glycolipids (GL) & Phospholipids (PL) from Body Flesh samples of Puti (*Puntius sophore*) as determined by GLC of methylesters. (% w/w of each component in total fatty acids).

	Components[a]	TL	NL	GL	PL
SATURATES	14:0(Myristic a.)	0.6	4.8	1.9	0.7
	15:0(pentadecanoic)	1.0	5.0	2.0	1.6
	16:0(palmitic)	23.1	20.8	36	28.8
	17:0(margaric)	1.7	3.7	2.5	2.2
	18:0(stearic)	8.2	8.5	11.5	12.9
	20:0(arachidic)	0.02	0.4	0.1	
	22:0(behenic)	15.2	7.2	4.5	11.9
	24:0(lignoceric)	1.0	0.6	0.7	0.7
	26:0(cerotic)	0.2	0.1	0.01	0.02
	27:0(heptacosylic)		0.2		
	28:0(Montanic)	0.5	1.1	0.2	0.1
	∑	51.520	52.400	59.410	58.920
MONOENES	14:1(myristoleic)	0.1		0.2	
	15:1(pentadecenoic)			0.1	0.2
	16:1(palmitoleic)	1.5	2.4	3. 0	2.3
	17:1(heptadecenoic)	0.3		0.6	0.6
	18:1ω9(oleic)	12.3	14.3	15.1	16.4
	20:1ω9(gondoic)	0.7	0.7	0.9	0.6
	22:1ω11(cetoleic)	0.3	0.4	0.4	0.2
	24:1(nervonic)	2.1	0.3	0.7	1.3
	26:1(hexacosenoic)	0.2	0.2	0.1	0.04
	27:1(heptacosenoic)		0.1		
	28:1(octacosenoic)	2.7	9.8	1.3	0.5
	∑	20.2	28.2	22.4	22.14
DIENES	18:2ω6(linoleic)	6.5	7.3	6.6	5.8
	20:2(eicosadienoic)			0.3	
	26:2(hexacosadienoic)	0.1	0.3	0.4	0.2
	∑	6.6	7.6	7.3	6
POLYENES	18:3ω3(linolenic)	1.4	1.4	1.1	1.1
	18:3ω6(gamma-linolenic)	1.4	1.4	1.1	1.1
	20:3ω3(eicosatrienoic)	1.5	1.1	1.1	0.9
	20:3ω6(homogamma linoleic)				
	20:4ω3(eicosatetraenoic)		0.4	0.1	
	20:4ω6(arachidonic)(ARA)	0.6	0.7	0.8	0.8
	20:5ω3(eicosapentaenoic)EPA	4.6	3. 0	1. 0	2.8
	21:5ω3(Heneicosapentaenoic acid (HPA)		0.2	0.2	
	22:5ω3(docosapentaenoic)DPA	1.3	1. 0	0.8	0.8
	22:5ω6(Osbond acid)	0.2	0.5	0.2	0.2
	22:6ω3(docosahexaenoic)DHA	11.9	2.5	5.3	6.1
	∑PUFA	22.9	12.2	11.7	13.8
	∑ω3	20.7	9.6	9.6	11.7
	∑ω6	2.2	2.6	2.1	2.1
	SUM Total	101.220	100.400	100.810	100.860

First and second figures represent carbon chain length: number of double bonds.The ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end.

Table 3. Fatty acid compositions of Triacylglycerol (TG), Wax ester (WE) and Steryl ester (SE) of Puti (*Puntius sophore*)/Body Flesh samples as determined by GLC of methylesters. (% w/w of each component in total fatty acids).

Components		***Puntius sophore* /TAG**	***Puntius sophore* /WE**	***Puntius sophore* /SE**
SATURATES	14:0	5.7	4.7	1.3
	15:0	4.3	0.6	1. 0
	16:0	17.6	33.1	30.3
	17:0	0.7	0.8	1.8
	18:0	3.4	10.9	13. 0
	20:0		0.4	0.5
	22:0	1.4	3.2	0.3
	24:0	0.2	0.2	0.2
	26:0		0.1	0.1
	27:0		0.1	1 .0
	28:0	5.7	4.5	0.1
	∑	39. 0	58.6	49.6
MONOENES	14:1			
	15:1			0.7
	16:1	4. 0		1.5
	17:1	1.7		
	18:1ω9	17.7	8.2	13.5
	20:1ω9	2.5	0.3	0.6
	22:1ω11	0.2	0.7	0.6
	24:1	0.4	0.5	
	26:1	0.3	0.1	
	27:1			
	28:1	17.4	13.5	19.1
	∑	44.2	23.3	36. 0
DIENES	18:2ω6	7.6	7.6	7.6
	26:2	0.4	0.6	0.2
	∑	8. 0	8.2	7.8
POLYENES (OMEGA-3 Fatty Acids)	18:3ω3	0.9		
	20:3ω3	0.6	0.7	0.2
	20:4ω3		0.2	0.2
	20:5ω3	0.5	0.7	1.2
	21:5ω3	0.5	0.7	1.2
	22:5ω3	0.4	0.6	0.2
	22:6ω3	2. 0	2.3	0.02
	∑	4.9	5.2	3.02
POLYENES (OMEGA-6 Fatty Acids)	18:2ω6			
	18:3ω6	0.9		
	20:3ω6			1.2
	20:4ω6	0.4	2.1	0.2
	22:5ω6	0.1	0.4	0.05
	∑	1.4	2.5	1.45
	ω3/ω6	3.5	2.08	2.082
	∑PUFA	6.3	7.7	4.47
	Sum Total	97.5	97.8	97.87

and C-27 predominated, with C-22 being the major component (9.9%) (Table 4).In the PL fractions, amount of SFAs were dominant in PE (73.4%), MUFA in PS (47.4%), and PUFA in PC (19.28%). The MUFA fraction (56.3%) of PS was exceptionally high than SFAs (31.4%) (Table 5).

Table 4. Fatty acid composition of hydrocarbon of neutral lipid of Puti (*Puntius sophore*)/Body Flesh samples

SATURATES		ISO		ANTEISO	
COMPONENTS	***Puntius sophore* /Hydrocarbons**	**COMPONENTS**	***Puntius sophore* /Hydrocarbons**	**COMPONENTS**	***Puntius sophore* /Hydrocarbons**
14:0	0.3	15:iso	0.2	16:anteiso	0.1
15:0	0.3	16:iso	0.1	17:anteiso	0.2
16:0	3.8	17:iso	0.3	18:anteiso	0.2
17:0	4.2	18:iso	0.4	19:anteiso	0.5
18:0	4.4	19:iso	0.8	20:anteiso	0.5
19:0	6.7	20:iso	0.5	21:anteiso	0.7
20:0	9.2	21:iso	0.8	22:anteiso	1.4
21:0	9.7	22:iso	0.5	23:anteiso	1.2
22:0	9.9	23:iso	0.8	24:anteiso	0.7
23:0	9.5	24:iso	0.4	25:anteiso	0.9
24:0	8.5	25:iso	0.9	26:anteiso	0.5
25:0	5.7	26:iso	0.7	27:anteiso	0.4
26:0	4.3	27:iso	0.4	28:anteiso	0.4
27:0	2.8	28:iso	0.2	29:anteiso	0.1
28:0	1.7	29::iso	0.1		
29:0	1. 0				
30:0	0.6				
31:0	1.7				
∑	83.3	∑	7.1	∑	7.8

Figure 3. Composition of fatty acid classes of lipid fractions in the flesh of *P. sophore*.

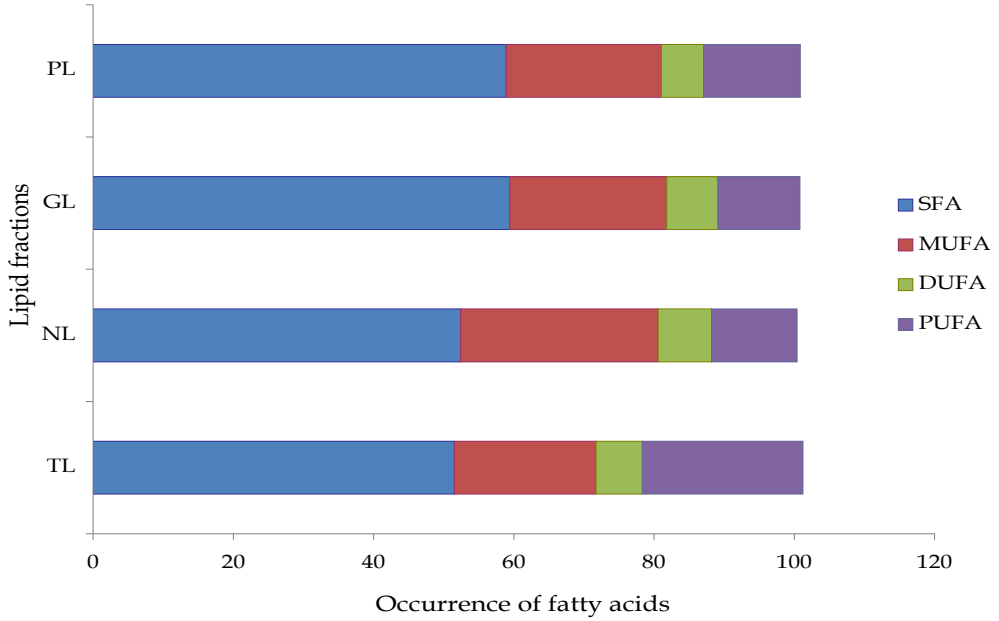


Figure 4. Comparison of C16 total and C 18 total in the major lipid fraction in the flesh of *P. sophore*.

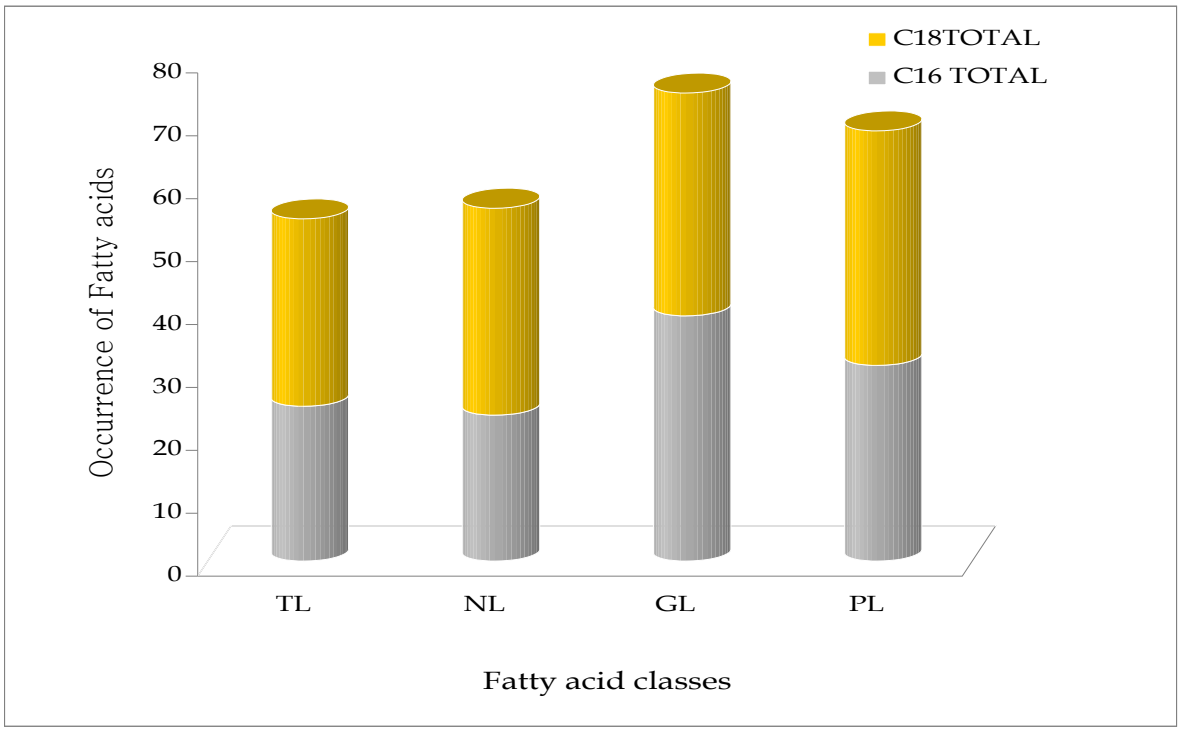


Figure 5. Comparative occurrence of EPA, DHA, ARA, LA and LNA in major lipid fractions in the flesh of *P. sophore.*

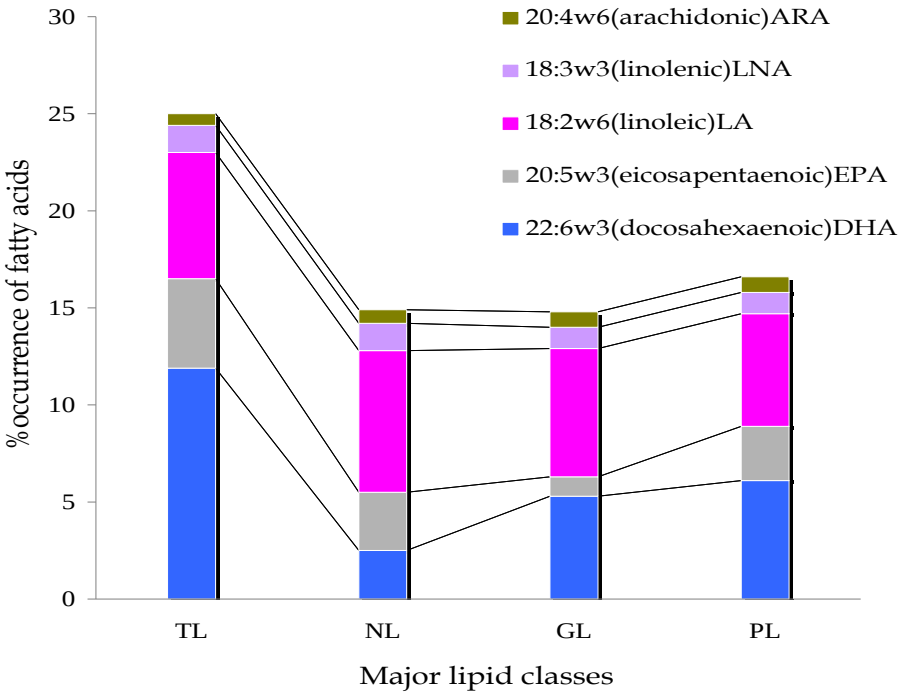


Figure 6. Comparison of ratio of ω3/ ω6 of major lipid classes from the flesh of *P. sophore*

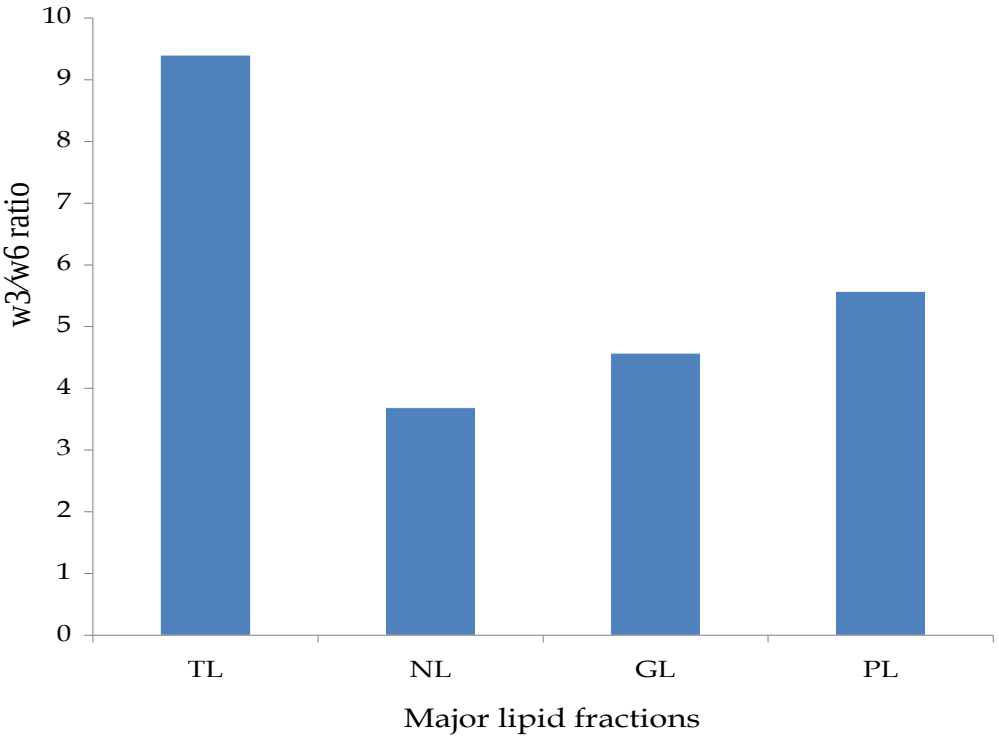


Figure 7. Comparative account of AI and TI of the major lipid classes of the flesh of *P. sophore.*

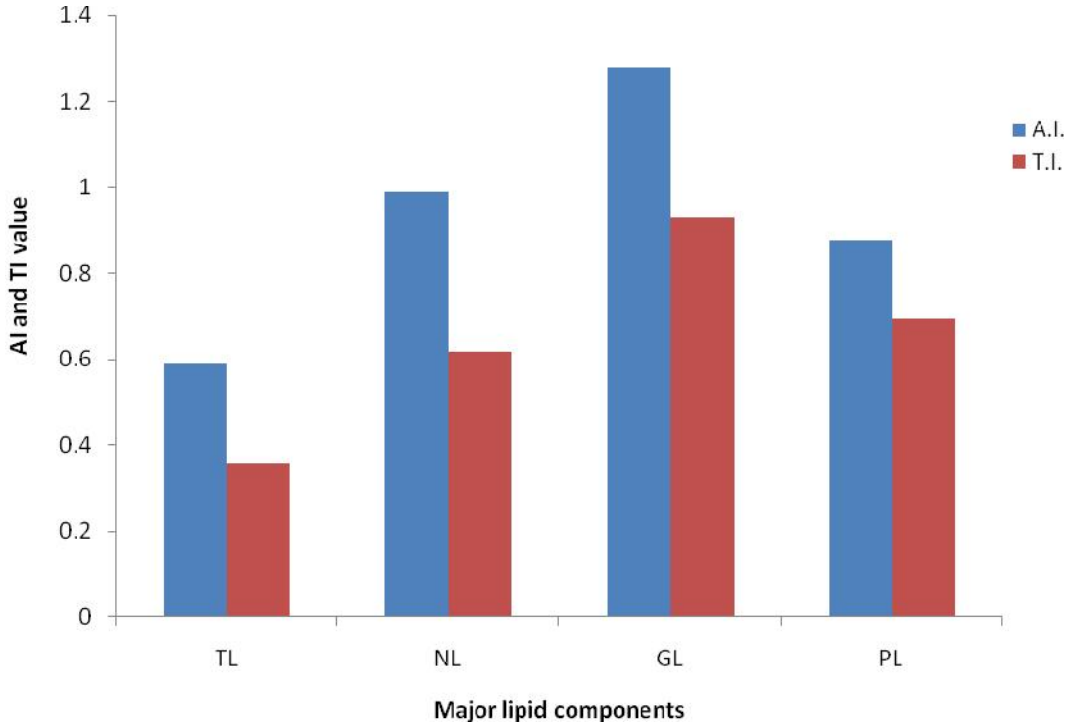


Table 5. Fatty Acid compositions of phospholipids components of Puti (*Puntius sophore*)/Body Flesh, as determined by GLC of the methyl esters.

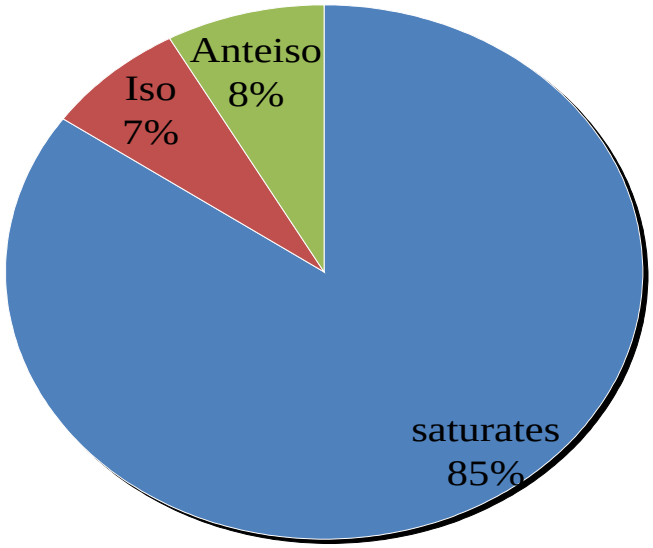
	Components	CL	PE	PC	PI	SPH	PS
SATURATES	14:0	3. 0	0.3	0.2	0.1	0.5	0.1
	15:0	5.9	1.3	0.7	0.1	2.3	1.4
	16:0	14.3	7.1	16.1	29.1	21.9	11.1
	17:0	1. 0	1.6	1.8	1.3	3.1	1.6
	18:0	8. 0	10.4	14.3	7.6	15.1	5.3
	20:0	0.04	0.03		0.7	0.2	
	22:0	4.2	6.8	17.7	12.4	5	5.8
	24:0	0.4	0.6	1. 0	0.6	1.6	0.2
	26:0	0.3		0.1	0.1	0.4	
	27:0	0.2	45		0.1	0.3	
	28:0	3.5	0.3	2. 0	0.6	2.9	5.9
	∑SFA	40.84	73.43	53.9	52.7	53.3	31.4
MONOENES	14:1				0.3		0.7
	15:1		0.2	0.1	0.5	0.2	
	16:1		0.5	1.1	1.4	1. 0	
	17:1	0.6	0.2	0.3	0.7	0.4	
	18:1ω9	12.2	4. 0	9.4	16.9	8.8	7.1
	20:1ω9	1.1	0.3	0.6	1.4	0.1	0.2
	22:1ω11	0.4	0.1	0.2	0.3	0.1	0.1
	24:1	1.4	1.8	1.9	1.3	0.2	0.7
	26:1			0.1	0.1		
	27:1	0.2	0.03	0.05	0.2	0.1	0.1
	28:1	18.2	5.4	8.3	5.7	17.7	47.4
	∑MUFA	34.1	12.53	22.05	28.8	28.6	56.3
DIENES	18:2ω6	10.7	2.5	4.2	5.3	8.1	3.4
	20:2	0.1	0.04				
	26:2	0.7	0.5	0.5	0.6	1.9	0.6
	∑DUFA	11.5	3.04	4.7	5.9	10. 0	4. 0
POLYENES (OMEGA-3 FATTY ACIDS)	18:3ω3	1.8	0.4	0.8	0.8	0.3	0.4
	20:3ω3		0.4	0.8	1.2	0.9	0.6
	20:4ω3	0.05				0.3	0.1
	20:5ω3	0.5	1.2	4.4	2.9	0.9	1.6
	21:5ω3			0.05		1	0.1
	22:5ω3	0.7	0.7	1.6	0.9	0.5	0.6
	22:6ω3	5.5	7.8	10.6	6.3	2.6	3.5
	∑ω3	8.55	10.5	18.25	12.1	6.5	6.9
POLYENES (OMEGA-6 FATTY ACIDS)	18:2ω6						
	18:3ω6	0.8	0.05	0.2	0.5		
	20:3ω6			0.03			0.01
	20:4ω6	1.8	0.4	0.6	0.5	0.2	0.3
	22:5ω6	0.4	0.1	0.2	0.1	0.1	0.2
	∑ω6	3. 0	0.55	1.03	1.1	0.3	0.51
	ω3/ω6	2.85	19.09	17.71	11. 0	55. 0	21.372
	∑PUFA	11.5	11.05	19.28	13.2	6.8	7.41
	Sum Total	97.99	100.05	99.93	100.6	108.7	103.11

CL :Cardiolipin; PE : Phosphatidylethanolamine; PC : Phosphatidylcholine; PI : Phosphatidylinositol; SPH : Sphingomyeline; PS : Phosphatidylserine

Table 6. Comparison of the different major lipid and FA classes' amount in between the Indian freshwater minor carp, *A.mola* (Dey *et al*., 2015) with the selected *P. sophore*.

Different Classes of lipids and Fatty acids	***Amblypharyngodon mola***	***Puntius sophore***
Glycolipids (GL)	24.78	8.6
Phospholipids (PL)	29.04	60.25
HC+WE+SE	23.29	41.35
Hydrocarbon(HC)	90.04	76.05
Triacylglycerol(TG)	34.6	16.88
Steryl ester (SE)	3.51	12.6
Wax ester (WE)	6.45	11.34
Cardiolipins (CL)	57.26	21.03
Phosphatidylinositol (PI)	6.96	29.03
Phosphatidylserine (PS)	1.63	5.94
∑SFA	53. 0	51.52
∑MUFA	25.5	20.2
∑PUFA	17.67	22.9
α-LNA	1.8	1.4
γ-LNA	0.3	1.4
LA	3.4	6.5
DHA	8.8	11.9
EPA	4.4	4.6
ARA	1. 0	0.6
∑ω-3	16.3	20.7
∑ω-6	1.37	2.2

Figure 8. Percent occurrence of Hydrocarbon classes in the body flesh of *P. sophore*.



Discussion

The TL content in *P. sophore* is 0.60% of w/w of wet tissue, which is almost similar to another minor carp, *A. mola* (Dey *et al.,* 2015). Among all the five Indian carps (Ackman *et al*., 2002) it is found that the TL content is the maximum in minor carp, *Labeo bata* (2.55%). Swapna *et al*. (2010) also revealed that the TL content is varied from 0.8–3.8% in the flesh of five commercially important fresh water fishes from Indian waters. In accordance with Kandemir and Polat (2007) this low TL content in the *P. sophore* can be corresponded with their commencement of breeding sequences. As per Ackman's data (1994 a,b) this fish can be judged as low fat lean fish and hence recommended as perfect count as low fat protein to the patients suffering from gastrointestinal difficulties and overweight.

PL constituted the main fraction of the TL and can be comparable to other freshwater fish quantitavely (Ghosh and Dua, 1997; Swapna *et al*., 2010) and also with the PL content (61.18%) in the flesh of an edible freshwater crab *Varuna litterata* (Das *et al.,* 2015). In most of the Australian fishes, mollusks and crustaceans, PLs are the chief lipid class (Nicholas *et al*., 1998) as also found in *P. sophore*. In *P. sophore,* the maximum amount of PLs may be linked with crystalline yolk formation, permeability of cell membranes, and transport of bioactive molecules into and outside the atretic oocytes in the maturation stage during the time of breeding period (Gershanovich, 1991). Langer *et al.* (2013) described six reproductive cycles throughout the year in *Puntius sophore* with a peak of breeding period during the July-August. Among PL fractions, the most attractive point is PI which is estimated as major one (29.03% w/w). The key physiological role of the PI may be influencing the ontogeny of bones and cartilage synthesis by osteocalcin vit–K dependent protein and also mineralization in gill cover (Hochachka and Mommsen, 1995). Through the rapid rate of metabolism, PI can altered into second messengers in signal transduction system such as DAGs and inositol phosphates, which are significant regulator in vital processes such as differentiation, proliferation, metabolism and apoptosis. Generally in the fish flesh, incorporation of 2% PI can induce high survival rate as found in *Cyprinus carpio* (Geurden *et al*., 1997). In addition, PI is also known to be the primary source of ARA used for synthesis of eicasanoids, a well-known anchor for connecting a variety of proteins to the external leaflet of the plasma membrane via a glycosyl bridge and cause the strongest inflammatory response in humans (Henderson, 1996). According to Sargent *et al.* (1995), the ratio of 18:1/22:6 PE is 1:2 in *P. sophore* which can be related with the possible mechanism for thermal adjustment in tropical pond water as found in *A. mola* (Dey *et al.*, 2015).

In *P.sophore*, molecular species ratio such as 18:1/22:6 and 18:1/20:5 can be play an imperative role in adapting the membrane physical state to temperature in agreement with Cevec (1991).

ST percent is highest among NL component, because this freshwater minor carp is omnivorous (Talwar and Jhingran, 1991). It can be assumed that high ST content may be providing energy for vitellogenesis as the fish go through breeding period (Ackman, 1995). This steroidal ingredient is perhaps thought to be a phytosterol relative which is center of modern awareness as it lowers the blood cholesterol intensity (Zamora, 2005). It was predicted that high level animal sterol restrains frequent pro-inflammatory and matrix degradation mediators typically connected in osteoarthritis-induced cartilage degradation (Gabay *et al.,* 2010). It is believed that fish sterols are participated an imperative role in the hanging up of the tumor progression and switch the appropriate cholesterol point in the blood (Genser *et al*., 2012). As this fish contains lower amount WE and SE than HC in the combination of HC + WE + SE, it can be deemed as low fat lean fish suitable for especially cardiac patients (Ackman, 1994 a, b).Glycolipids (GL) are fatty acid esters of sphingosine, carrying carbohydrate in addition, present in small quantity in the fish under study (Voet and Voet, 1995).

Fatty acids in fishes are derived from 2 main foundations, namely, diet and then biosynthesis (Kamler *et al*., 2001). Among the identified 36 fatty acids, principal fatty acids are palmitic acid (16:0), stearic acid (18:0), behenic acid (22:0), oleic acid (18:1ω9), linolenic acid (18:2ω6), linoleic acid (18:3ω3), EPA (20:5ω3), DHA (22:6ω3) and ARA (20:4ω6) etc. This distribution pattern corroborates with the result obtained from tropical freshwater fishes reported on in the above mentioned studies. Absence of eicosatrienoic acid (20:3ω-9) indicates there is no symptom of fatty acid deficiency in these fishes (Watanabe, 1982).

SFA are approximately 50 % of the total fatty acid content and MUFA is 28-30%. The higher content of SFA supplies energy stores to the fish muscle and also acts as components of cell bio-membranes (Tocher, 2003). For spawning migrations in the breeding season, SFAs function is an oxidative substrate as the *P. sophore* belongs to the peak time of breeding season (Shulman and Love, 1999). Among SFAs, palmitic acid (C16:0) is appeared to be highest in amount which is followed by stearic acid and behenic acid. This result is in agreement with the result by Csengeri and Farkas (1993) and Chauke *et al*. (2008). Ackman *et al*. (2000) remarked that the palmitic acid is the prime fatty acid at all evolutionary as well as tropic levels. In fish, the defense mechanism against microbial infections performed particularly by Palmitic acids through the pathogen-associated molecular patterns (PAMPs) and T-cell signaling (Bergsson, 2005). A possible explanation for the presence of unusual odd chain fatty acids in *P. sophore* like 15:0, 17:0 and their monoenes 15:1, 17:1 respectively is that cyclopropane fatty acids (C17 and C19) are found in browser freshwater gastropods (Misra *et al*., 2002). As there is no known metabolic role for C17 fatty acid in vertebrates this might have taken by the fish from phytoplankton.

According to Muhamad and Mohamed (2012) MUFAs appeared to be the major fatty acid class in freshwater fishes. Oleic acid is a very prevalent (40-62%) MUFA as it can be the primary precursor of the family of PUFA like gadoleic acid (20:1ω-9) and cetoleic acids (22:1ω-11) (Ugoala *et al*., 2009). The presence of these fatty acids indicate that they are derived directly from the oxidation of corresponding fatty alcohols ingested from wax ester rich copepods by studied fish (and hence long- chain PUFA rich).It can be assumed that, *P.sophore* exploiting these MUFA for heightening the physical movement and used the derived vitality for the journey for spawning (Henderson and Tocher, 1987). Generally fish having energy depots in the form of lipids, rely on the palmitoleic acid which is present in considerable amount in all major lipid fractions in *P.sophore*. In reproductive cycle, the combination of 16:0; 18:0; 16:1; 18:1 are favored as substrates for mitochondrial ß-oxidation and catabolised via the TCA cycle to generate the metabolic energy (Henderson *et al*., 1985).

Freshwater fish originally evolved in the ocean they returned to the terrestrial biosphere (freshwater) where conversion of 18:3ω-3 and 20:5ω-3 to 22:6ω-3 is necessary. Like all other vertebrates, fishes are deficient in the delta-12 and delta 15-desaturases and so cannot generate EFAs (Gurr and Harwood, 1991). So the input of plant resources of linoleic (LA) and linolenic acids (LNA) is clear in every lipid groups (Okuyama, 2000). Ackman (1994a) elucidated the pathways and relative sources of desaturation of 16:0 and 18:0 acids into MUFAs in both marine and freshwater fishes. ARA is the central product of the chain elongation and desaturation of γ-LNA mediated by the enzyme delta-6 desaturase. This PUFA is considered as the physiologically active factor in gonad maturation, egg quality (Izquierdo *et al.,* 2001), larval growth of fish (Tulli and Tibaldi, 1997), can increase flexibility of spermatozoa membrane in the regulation of cellular movement, gonodal metabolism of lipids in fusion capacity (Miliou *et al.*, 2006). Metabolism of ARA in fish produce the same range of eicasanoids as in mammals with the prostanoids, PGE, PGF and PGD, and TXB and 6-keto-PGF1α , the respective stable metabolites of TXA and PGI2 (Henderson, 1996). In fish as in mammals, eicasanoids actions are determined by the ratio of 20:4ω-6: 20:5ω-3 in cellular membranes, this in turn being established by the dietary intake of ω-6 and ω-3 PUFA (Bell *et al.*, 1994). According to March (1992) and Arts *et al*. (2001) this fish can renovate LA and LNA to EPA and then to DHA which is crucial for ability to capture prey at natural light and schooling behavior. In accordance with Pickova *et al.* (1997) and Bell and Sargent (2003) the present DHA: EPA ratio (2:1) may be positively correlated with egg superiority in breeding season. A positive correlation between dietary DHA/EPA and fish larval growth and survival has been reported by Pickova *et al.* (1997).Species that lead a more active life in motoric and social behavior is found to have increased content of DHA with elevated amount of MUFA as found in *P. sophore* (Velansky and Kostetsky, 2008). According to Takeuchi (1996) this carp possibly can be categorized as Type II class as par ω-6 and ω-3 both are concerned. Freshwater fish usually consist of more ω-6 polyunsaturated fatty acid, whereas the marine fishes are rich in ω-3.Such occurrences can be attributed to the fact that natural prey of many freshwater fish, particularly their invertebrate prey, is not rich in 22:6 ω -3, being rich instead in 18:2 ω- 6, 18:3 ω-3 and to a lesser extent 20:5 ω-3 (Wang *et al.,*

1990). This fact might be linked with species osmoregulatory adaptation or can be analyzed in view of first line innate immune defense mechanism (Li and Yamada, 1992).The ω3/ω6 ratio in *P. sophore* is ranging between 3.69-5.57 as they surfs on the natural foods full of detritus from the benthic as well as nektonic source always packed in rotten leaves and a mixture of planktons (Dhanapal *et al*., 2011). Ugoala *et al*. (2008) suggested that the branched chain iso-anteiso fatty acids of HC in this freshwater fish oils may be better fats after hydrogenation due to higher variety of branched chain fatty acid than marine species.

Analysis of the nutritional significance of *P. sophore* in respect to human benefit:

Numerous clinical and epidemiological studies on the Nunavik Inuit women and the Japanese societies as well as Danish research on Eskimo food in Greenland correlated the long term consumption of fish intake with a reduced incidence of wide range of common degenerative diseases such as acute myocardial infractions, ischemic heart diseases, atherosclerosis, thrombosis and cerebrovascular diseases (Dyerberg *et al.,* 1975; Blanchet *et al.,* 2000; Zuraini *et al*., 2006). Beneficial health effects of ω-3 PUFAs are well expressed in hypotriglyceridemic effect, autoimmune disorders, and cancer by altering the metabolism of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1) (Abe *et al.,* 1998). HUFAs are significant for normal neural and visual development in the human fetus and inversely related to the risk of impaired cognitive function (Lie, 2004). A recent study also proved that daily intake of crude fish oil supplements rather than fresh fish reduces the plasma concentration of vitamin E to below normal range by forming malondialdehyde (MDA) (Nair *et al.,* 1993).The biological significance of dietary DHA/EPA can be scrutinized in terms of competitive competition for the enzymes that esterifies fatty acids into the PLs structures (Sargent *et al.,* 1999). Furthermore, *P. sophore* can be necessitated throughout pregnancy, for the higher concentration of the DHA than EPA (Maes *et al*., 2000). A number of countries (Canada, Sweden, United Kingdom, Australia, and Japan) as well as the WHO and North Atlantic Treaty Organization have made formal population-based dietary recommendations for ω-3 PUFAs. Typical recommendations are 0.3 to 0.5 g/d of EPA +DHA and 0.8 to 1.1g of α-LA (Kris- Etherton *et al.,* 2002). These recommendations can easily be met by previous AHA Guidelines in 1996 to consume two servings of fatty fishes per week (Krauss, 2000).AHA also instructed to trim down the ω-6 PUFA intakes as because higher ω-6 PUFA can hinder the conversion of α-LA to EPA (Krauss, 2000). ARA and DHA play an important role in neonatal health and development, for this reason that both these fatty acids are recommended for inclusion in infant formula milks (Agostoni, 2008). In addition, patients with CHD (coronary heart disease) are recommended for reducing the risk of sudden death by consuming approximately 6 ounce (≅170 g) serving/week of fish richest in ω-3 PUFA (Strobel *et al*., 2012). This well-known machinery by which ω-3 PUFAs communicate through the diminution of nuclear factor-κB activation, increase secretion of adiponectin and increasing resolvins and protectin creation (Siriwardhana *et al.,* 2012).It is informed that very low amount of ARA(<1.0) is advantageous for consumer's cardiovascular health due to its antagonistic consequences to health benefits of the ω-3 fatty acids (Simopoulos, 2002).Still together with DHA, is crucial in pregnancy and infant nutrition (Ghebremeskel *et al.,* 2000). Incidence of breast cancer, metabolic syndrome like diabetes can be slowing down after a 5weeks administration of 4–8 capsules of fish oil corresponding to 1.26 to 2.5 g daily (Hedayatifard and Jamali, 2008). For these reasons MUFA rich diet like the *P. sophore* can be recommend as an element of prophylactic diet. Presence of considerable amount of Palmitoleic acid is also beneficial which increases insulin sensitivity by suppressing inflammation and inhibits the destruction of pancreatic β-cells which are known to secrete insulin (Dutta and Dutta, 2013).It is advantageous in this fish, that Erucic acid is absent in this minor carp, known to be an anti nutritional factor which could induce an increase in incidence of myocardial lipidosis in animal (Dutta and Dutta, 2013). Generally the combined content of MUFAs and PUFAs in fish oil with a relative content of SFAs remains the only animal fat in liquid form at room temperature. Index of atherogenicity and thrombogenicity points to the bond between the content of the main SFAs and that of main classes of UFAs (Higgs, 2000). The former being considered as proatherogenic while the latter shows the rapport with anti-atherogenic (Senso *et al.,* 2007). Interestingly, the *P. sophore* with low AI and TI values can be choose for lowering the TAG levels particularly in patients

with hypertriglyceridemia. This effect is not seen with ω3 PUFA of plant sources and milk-based products (Amerio *et al.,* 1996). In this point, *P. sophore* can be preferred more by the nutritionists for a balanced diet because of plenty of ω3content than ω6. Consequently the null hypothesis is established and this all interpretation signifies that this fish has a good oil value and is appropriate for applications in pharmaceutical and food industries. Present findings shows that *P.sophore* may have the prospective to be conserve by protecting their natural habitat, promote sustainable use in capture and culture fishery systems, enhancing appropriate policies and legislation in consideration the local socioeconomic context.

Comparison with other carp: *A. mola*

There are substantial differences in between the most commonly encountered minor carp species of India, *A. mola* (Dey *et al*., 2015) with *P. sophore*, is demonstrated in Table 6. *P. sophore* demonstrates discrepancy in their lipid and fatty acid composition with *A. mola* as because there is a correspondence between the fatty acid requirements of these two fishes with their situation in food web (Zenebe *et al.,* 1998). According to Ugoala *et al*. (2008) the existing interspecies inconsistency can be elucidated by the existence of a large number of external factors (like environment and tropic effects etc.) and internal factors (such as fish species, feeding régime, metabolism, life cycle stage, quantitative and qualitative characteristics of lipids TGs, PLs and their topographical origin in muscle tissue etc.).Recent findings substantiates with this. Further it can also be assumed that the variation among these two freshwater minor carps may be due to their foraging ecology and pond food web dynamics (Ugoala *et al.,* 2009).

Conclusion

It can be suggested from the above information that *P. sophore* can safely be used as curative diet for its high nutritional value. The lipids and fatty acids show that, it is a safe lean fish and muscles help as dietary substitute. For this reason, *P. sophore* could be considered as substitute against the IMCs. The fatty acids components of *P. sophore* indicate its importance in combating CHB, IBD, rheumatoid arthritis etc. in human body. Further, immediate attention is to be paid to conserve this fish and to sponsor economically feasible farming protocol for extensive farming of *P. sophore* in our country.

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