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Seasonal alteration in the muscle lipid fatty acid profiles of cultured Chinese carp species

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Abstract
Long chain polyunsaturated fatty acids (LC-PUFAs) represent approximately. 20% of brain dry matter and its deficit is critical for development and its function. Eicosapentaenoic (EPA, 20:5w3) and docosahexaenoic acid (DHA, 22:6w3) are the important omega-3 PUFAs. The Arachidonic acid (AA 20:4 w-6) and DHA are of special importance in the brain and blood vessels, and are considered essential for pre and post natal brain and retinal development. The present study was, therefore conducted to know the muscles lipid fatty acid content (MTLC) and fatty acid profiles of three carp species, *Cyprinus carpio* (Linnaeus), *Ctenopharyngodon idella* (Steindachner) and *Hypophthalmichthys molitrix* (Richardson). The MTLC varied from 1.10±0.02% in *C. idella* during spring to 3.03±0.05% in *C. carpio* during winter season. The total PUFAs content is maximum in *H. molitrix* in spring and minimum in *C. idella* in winter. The n-3/n-6 ratio was obtained in *C. idella* (5.36±0.63) during spring and minimum (0.49±0.16) in *C. carpio* during winter.

Keywords: Freshwater fishes, fatty acid profiles, polyunsaturated fatty acids, muscle lipid fatty acid content, n-3/n-6 ratio

1. Introduction
Fishes are considered as high quality human food and a rich source of proteins (15-25%), minerals (Ca, P, Fe) and vitamins (A, D, E, K) (Goodnight *et al* 1982; Kromkout *et al* 1985) [4, 12]. Recently, interest has grown in fish and fish products as sources of polyunsaturated fatty acids (PUFAs), mainly of the n-3 family (also known as omega-3 fatty acids). Fatty acids play an important role in the life and death of cardiac cells because they are essential fuels for mechanical and electrical activities of the heart (Honore *et al* 1994; Reiffel J A and McDonald A 2006; Landmark K and Alm C S 2006; Herbout 2006) [6, 15, 13, 5].

The health benefits of consuming increased levels of n-3 HUFA (omega-3) are now widely accepted and the range of human diseases and conditions that respond to n-3 HUFA continues to grow. Fish represent a virtually unique source of n-3 HUFA. Deficit in omega-3 fatty acids can result in neurological abnormalities and retardation in growth, weakness, reduced learning ability, disturbances in motor coordination, changes in behaviour, high level of triglycerides, high blood pressure, oedema, dry skin, mental retardation, immune disfunction etc. all symptoms of deficit can disappear with the return of omega-3 into nutrition (http://www.scitopics.com/relevance-of-essential-fatty-acid-metabolism-in-fish-tohuman nutririon.html)

Fishes vary in their ability to produce EPA and DHA endogenously. Habitat (freshwater/marine) appears to be the major determinant but the feeding habit (piscivorous/carnivorous/herbivorous/omnivorous) may be a confounding factor. Therefore, different fish species have different range of PUFAs and HUFAs qualitatively and quantitatively (http://topics.scirus.com). Simopoulos (1991) [16]. and Bimbo (1998) [3] have also supported this view and stated that fatty acid composition in general and EPA and DHA amounts in particular vary from species to species of fish and by seasonal and geographical parameters.

Thus, carps being the most commonly cultured fish species in India, including Punjab, need to be investigated for their fatty acid profiles. The present study was, therefore, conducted with a concept to generate information on fatty acid profiles of carp species being cultured in Punjab in order to make recommendations on their required intake by humans.

2. Materials and methods
Comparative studies on muscle total lipid content and fatty acid profiles of three Chinese carp fish species cultured in Punjab belonging to order Cypriniformes, family Cyprinidae were made. The fish species were the common carp, *Cyprinus carpio* (Linnaeus), grass carp, *Ctenopharyngodon idella* (Steindachner) and silver carp, *Hypophthalmichthys molitrix* (Richardson)

2.1 Collection of samples
Table-sized (> 500 g) fresh specimens of each of the three fish species were obtained from the local fish market in Ludhiana during each season of the year. Each fish was wrapped individually in labelled clean air tight ziplock polythene bags and embedded in sufficient crushed ice in the ice box. They were transported within minutes to the laboratory 30-45 and stored in a quick freezer, at -25 ^{0}C.

2.2 sample preparation
Each fish was scaled, finned, headed and gutted. The fish sample was then cleaned with tap water and 3 x 2.5 cm pieces of flesh were taken at random from three different parts of fish body *viz*. above lateral line from both the sides. Bones were removed and the flesh was thoroughly mixed together to form a composite or representative sample of edible portion of the fish. The whole procedure, which was done on ice, took about 10 minutes.

2.3 Estimation of Total Lipid Content
Total lipid (TL) content was determined by solvent extraction method as described by AOAC (2000) [1].

2.4 Determination of Fatty Acid Profiles
Fatty acid composition was determined by Gas Liquid Chromatography (GLC) on M/s Nucon Engineers AIMIL Gas Chromatograph (solid state) model Nucon series 5700/5765 equipped with flame ionization detector fitted with SS column 1/8" outer diameter x 2M length, packed with 15% D.E.G.S on CHROMOSORB W.H.P, 80-100 mesh size. Identification of peaks was done by comparison of their retention time with those of standard fatty acyl esters (M P Biomedicals Inc. USA). Relative concentration of fatty acid was calculated by use of an automatic integrator-Windows based AIMIL Ltd., DASTA 710 Gas Chromatograph Data station software, version WinAcds 7.1.

2.5 Statistical analysis
One way and Multifactor ANOVA was used to determine the inter-specific and inter-seasonal differences in the total lipids and fatty acid profiles of the three species of fish. The analyses were performed using Microsoft EXCEL and STATGRAPHICS statistical packages.

3. Results and discussion
3.1 Muscle total lipid content
A perusal of Table 1 shows that there were considerable variations in the muscle total lipid content (MTLC) with respect to the species of the fishes and different seasons of the year. The MTLC varied from 1.10±0.02% in *C. idella* during spring to 3.03±0.05% in *C. carpio* during winter season. These values are close to those observed by Aggelousis and Lazos (1991) [16] in the freshwater fishes of Greece (0.6-3.5%), Kaneniwa *et al* (2000) [9] in Chinese freshwater fishes (0.94 to 4.73%), Kminkova *et al* (2001) [11] in *Cyprinus carpio* (2.08-5.03%), Varjlin *et al* (2003) [17] in *D. vulgaris* and *C. conger* (1.3 to 3.7%). The minima in MTLC in all the presently studied three carp species were recorded during spring and maxima during winter except for *C. idella*, (which were recorded in autumn).

3.2 Comparison of Fatty Acid Profiles of Different Fish Species
Amongst n-3 fatty acids of *C. carpio*, len ranged between 0.30±0.16% in autumn and 2.79±1.04% in summer. The EPA was maximum (10.37±0.47%) in autumn and minimum (0.60±0.30%) in spring. The DPA was maximum (29.64±29.64%) in spring and minimum (0.73±0.34%) in winter. The Lin, amongst the n-6 fatty acids, ranged between 3.54±3.28% in autumn and 12.25±8.82% in summer. The AA showed minimum (0.44±0.22%) in summer and maximum (3.42±3.40%) in autumn (Table 2).

In *H. molitrix*, the Len ranged between 2.88±1.61% in winter and 8.93±3.92% in spring. Both EPA and DPA were maximum (5.65±3.23% and 6.88±3.89%, respectively) in spring and minimum (0.29±0.15% and 0.26±0.15%, respectively) in summer. Amongst n-6 fatty acids, Lin ranged between 4.05±0.96% in autumn and 9.38±1.59% in spring. AA was maximum (7.58±5.64%) in spring and minimum (2.11±0.25%) in summer. The EDA was also highest (0.99±0.80%) in spring and absent in summer (Table 3).

In case of *C. idella*, len ranged between zero in spring and 3.02±0.36% in summer. Both EPA and DHA were maximum (32.39±8.52% and 3.96±3.76%, respectively) in spring and minimum (0.06±0.06% and 0.54±0.30%, respectively) in winter. The DPA was highest (8.74±38.70%) in spring and lowest (1.62±0.72%) in autumn. Lin, amongst the n-6 fatty acids, ranged between 1.84±0.24% in winter and 8.15±3.04% in spring. The AA was maximum (10.17±7.91%) in summer and minimum (0.78±0.16%) in winter. The EDA was minimum (0.03±0.03%) in winter and maximum (0.31±0.31%) in autumn.

A comparison of fatty acid profiles of the three presently studied fish species revealed that amongst the n-3 fatty acids. The mean total n-3 PUFA values were recorded in the order of *C. idella* (17.84±4.17%)> *H. molitrix* (17.50±3.44%)> *C carpio* (15.96±7.60%). In case of *C carpio* the mean value of total n-3 PUFAs was 15.96±7.60%, in *H. molitrix* the mean value of total n-3 fatty acids was 17.50±3.44%, *C. idella* the mean value of total n-3 fatty acids was 17.83±4.17% (see Table 2, 3, 4) which is higher than the one reported by Rahman *et al* (1995) for (2.53%) the same fish species.

The fishes differed in respect of their n-6 PUFA composition also. *C. carpio* (7.06±2.55%), *C. idella* (5.55±1.17%), and *H. molitrix* (5.53±0.72%). The AA was highest in *H. molitrix* (5.00±1.57%), followed by *C. idella* (3.45±2.04%), and *C. carpio* (1.49±0.87%). The mean values of EDA also differed amongst different fish species *viz. H. molitrix* (0.36±0.21%), *C. idella* (0.21±0.11%), and *C. carpio* (0.07±0.06%). The mean total n-6 values were comparable, although highest values were observed in *H. molitrix* (10.90±0.16%), *C. carpio* (9.45±2.44%) and *C. idella* (9.21±1.7%) (Table 2, 3 & 4).

The n-3/n-6 ratio differed in different fishes. The highest n-3/n-6 (2.25±0.28) was found in *C. idella* and the lowest (0.13±0.03) in *C. Carpio.* Which is comparatibily same reported by Kaur M and Sehgal G.K (2013) [10] in other freshwater fishes. Thus, *C. idella* proved best in having highest total n-3 fatty acids and n-3/n-6 ratio. The only n-9 fatty acid, the eicosenoic acid was present in low amount in all the studied fishes. The maximum mean value of eicosenoic acid were recorded in *H. molitrix* (0.73±0.40%), *C. idella* (0.50±0.37%), and *C. carpio* (0.32±0.25%) as mentioned in tables 2, 3 & 4.

The order of presence of total PUFAs was *H. molitrix* (29.14±2.94%)> *C. idella* (27.46±5.86%)> *C. Carpio* (24.55±7.60%).

Table 1: Seasonal variations in muscle total lipid content (%) of different fish species.

Season (Month/Year)	Fish species		
	***Cyprinus carpio* (common carp)**	***Hypophthalmichthys molitrix* (silver carp)**	***Ctenopharyngodon idella* (grass carp)**
Spring (April,2009)	1.21±0.03[a12]	1.47±0.14[a12]	1.10±0.02[a1]
Summer (July,2009)	2.53±0.06[c4]	2.31±0.22[b2]	1.14±0.02[b1]
Autumn (October,2009)	2.30±0.04[b3]	2.28±0.13[b3]	2.07±0.02[c2]
Winter (January,2010)	3.03±0.05[d3]	2.33±0.03[b23]	2.00±0.01[c1]
Mean	2.28±0.02	2.10±0.06	1.57±0.01

Values with same alphabetic superscript in a column do not differ significantly ($p>0.05$).
Values with same numeric superscript in a row do not differ significantly ($p>0.05$).

Table 2: Seasonal differences in polyunsaturated fatty acids (PUFAs) of *Cyprinus carpio* (Linnaeus).

Fatty Acid	Season				Mean (of the four seasons)
	Spring (April, 2009)	**Summer (July, 2009)**	**Autumn (October, 2009)**	**Winter (January, 2010)**	
n-3 PUFAs					
Linolenic acid (C18:3 n-3)	0.57±0.57[a]	2.79±1.04[a]	0.30±0.16[a]	1.37±.035[a]	1.26±0.31
Eicosapentaenoic acid (C20:5 n-3)	0.29±0.29 [a]	1.00±0.38 [a]	10.37±0.47[b]	0.61±0.30 [a]	3.07±0.18
Docosapentaenoic acid (C22:5 n-3)	29.64±29.64[a]	1.93±1.23 [a]	6.74±0.58 [a]	0.74±0.34 [a]	9.76±7.42
Docosahexaenoic acid (C22:6 n-3)	1.23±1.08 [a]	0.60±0.21 [a]	4.51±1.56 [a]	1.15±0.65 [a]	1.87±0.50
Total n-3 PUFAs	31.73±30.30[a]	6.32±0.62 [a]	21.92±2.17 [a]	3.77±1.22 [a]	15.96±7.60
n-6 PUFAs					
Linoleic acid (C18:2 n-6)	6.06±3.93[a]	12.25±8.82[a]	3.54±3.28 [a]	6.40±0.10 [a]	7.06±2.55
Arachidonic acid (C20:4 n-6)	0.65±0.65 [a]	0.44±0.22 [a]	3.42±3.40 [a]	1.44±0.20 [a]	1.49±0.87
Eicosadienoic acid (C20:2 n-6)	0.22±0.22 [a]	__	0.03±0.03 [a]	0.03±0.03 [a]	0.07±0.06
Total n-6 PUFAs	6.92±3.88 [a]	12.69±8.94[a]	10.32±0.10[a]	7.87±0.14 [a]	9.45±2.44
n-3/n-6 Ratio	1.13±0.03 [a]	1.29±0.60[ab]	2.13±0.23 [b]	0.49±0.16 [a]	1.01±0.17
Eicosenoic acid (C20:1)	0.22±0.22 [a]	0.99±0.99 [a]	0.04±0.03 [a]	0.02±0.02 [a]	0.32±0.25
TOTAL PUFAs	34.18±29.21 [a]	20.00±8.04 [a]	32.25±2.12 [a]	11.76±1.08 [a]	24.55±7.60

Only major PUFAs have been included; Values are mean±S.E. and are presented as % age of total lipids; Values with same superscripts in a row do not differ significantly ($p>0.05$).

Table 3: Seasonal differences in polyunsaturated fatty acids (PUFAs) of *Hypophthalmichthys molitrix* (Richardson).

Fatty Acid	Season				Mean (of the four seasons)
	Spring (April, 2009)	**Summer (July, 2009)**	**Autumn (October, 2009)**	**Winter (January, 2010)**	
n-3 PUFAs					
Linolenic acid (C18:3 n-3)	8.93±3.92[a]	8.80±0.18[a]	3.77±1.84[a]	2.89±1.61[a]	6.10±1.16
Eicosapentaenoic acid (C20:5 n-3)	5.65±3.23 [a]	0.29±0.15 [a]	5.63±3.71 [a]	3.91±2.15 [a]	3.87±1.34
Docosapentaenoic acid (C22:5 n-3)	6.88±3.90 [a]	0.27±0.15 [a]	0.77±0.33 [a]	3.44±1.51 [a]	2.84±1.05
Docosahexaenoic acid (C22:6 n-3)	11.92±7.78 [a]	0.33±0.15 [a]	1.25±0.72 [a]	5.29±2.23 [a]	4.70±2.03
Total n-3 PUFAs	33.39±11.96[a]	9.68±0.18 [a]	11.43±2.87 [a]	15.52±6.15 [a]	17.50±3.44
n-6 PUFAs					
Linoleic acid (C18:2 n-6)	9.38±1.59 [a]	4.20±0.23 [a]	4.05±0.96 [a]	4.50±2.18 [a]	5.53±0.72
Arachidonic acid (C20:4 n-6)	7.59±5.64 [a]	2.11±0.25 [a]	5.83±1.26 [a]	4.47±2.40 [a]	5.00±1.57
Eicosadienoic acid (C20:2 n-6)	0.98±0.80 [a]	__	0.19±0.12 [a]	0.29±0.17 [a]	0.37±0.21
Total n-6 PUFAs	17.95±4.18 [b]	6.32±0.29[a]	10.07±1.69[ab]	9.26±1.06 [ab]	10.90±0.16
n-3/n-6 Ratio	2.38±1.11 [a]	1.54±0.10 [a]	1.27±0.42 [a]	1.68±0.62 [a]	1.72±0.34
Eicosenoic acid (C20:1)	1.46±1.46 [a]	0.02±0.01 [a]	0.06±0.06 [a]	1.40±0.62 [a]	0.73±0.40
Total Pufas	52.79±9.68[b]	16.02±0.20 [a]	21.55±1.52 [a]	26.19±6.44 [ab]	29.14±2.94

Only major PUFAs have been included; Values are mean±S.E. and are presented as % age of total lipids; Values with same superscripts in a row do not differ significantly ($p>0.05$).

Table 4: Seasonal differences in polyunsaturated fatty acids (PUFAs) of *Ctenopharyngodon idella* (Steindachner).

Fatty Acid	Season				Mean (of the four seasons)
	Spring (April, 2009)	Summer (July, 2009)	Autumn (October, 2009)	Winter (January, 2010)	
n-3 PUFAs					
Linolenic acid (C18:3 n-3)	__	3.02±0.36[a]	2.78±1.93[a]	1.15±0.33[a]	1.74±0.50
Eicosapentaenoic acid (C20:5 n-3)	32.39±8.52[b]	4.53±1.70 [a]	3.62±2.57 [a]	0.06±0.06 [a]	10.15±2.27
Docosapentaenoic acid (C22:5 n-3)	8.74±8.70 [a]	3.74±3.66 [a]	1.62±0.73 [a]	2.36±1.50 [a]	4.12±2.40
Docosahexaenoic acid (C22:6 n-3)	3.96±3.76 [a]	1.83±1.44 [a]	1.33±0.48 [a]	0.54±0.30 [a]	1.91±1.02
Total n-3 PUFAs	45.09±14.88[b]	13.12±7.07[ab]	9.35±2.09[ab]	3.77±1.51 [a]	17.83±4.17
n-6 PUFAs					
Linoleic acid (C18:2 n-6)	8.16±3.04 [a]	5.81±2.91 [a]	6.39±2.09 [a]	1.84±0.24 [a]	5.55±1.18
Arachidonic acid (C20:4 n-6)	0.84±0.73 [a]	10.17±7.91[a]	2.03±1.87 [a]	0.78±0.16 [a]	3.45±2.04
Eicosadienoic acid (C20:2 n-6)	0.28±0.24 [a]	0.20±0.20 [a]	0.32±0.31 [a]	0.32±0.31 [a]	0.21±0.11
Total n-6 PUFAs	9.27±3.66 [a]	16.18±4.91 [a]	8.74±2.94 [a]	2.65±0.43 [a]	9.21±1.7
n-3/n-6 Ratio	5.36±0.63[b]	0.71±0.17 [a]	1.28±0.33 [a]	1.66±0.87 [a]	2.25±0.28
Eicosenoic acid (C20:1)	0.03±0.03 [a]	1.70±1.46 [a]	0.26±0.20 [a]	__	0.50±0.37
Total Pufas	54.39±18.47 [a]	30.66±13.58 [a]	18.36±4.69 [a]	6.42±1.20 [a]	27.46±5.86

Only major PUFAs have been included; Values are mean±S.E. and are presented as % age of total lipids; Values with same superscripts in a row do not differ significantly (p>0.05).

4. Summery

It is concluded that the fatty acid profiles of the *presently* studied fish species varies according to the season. The total n-3 PUFAs was found to be maximum in *C. Idella* (45.09±14.88) in spring and minimum (3.77±1.22) in *C. Carpio* during winter. Total n-6 PUFAs was maximum in *H. molitrix* 17.95±4.18) in spring and minimum (2.65±0.43) in *C. idella* during winter. The n-3/n-6 was higher in *C. idella (5.36*±0.63) in spring and lower (0.13±0.03) in *C.carpio* again in spring season. Total PUFAs was maximum in *C. idella* (54.39±18.47) in spring and minimum (6.42±1.20) in *C. idella* during winter. The Overall polyunsaturated fatty acids content is significant in spring season in all fish species and showed their nutritional value for health benefits.

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