ANALYSIS OF LIPID AND LIPID-FRACTIONS OF SOME FRESHWATER FISHES AND THEIR INTER-RELATIONSHIP

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ABSTRACT

Two fish species each from carnivorous (*Clarias batrachus, Channa punctatus*), omnivorous (*Cyprinus carpio, Cirrhinus reba*), and plankton feeder (*Catla catla, Labeo rohita*) were collected from freshwater sources under natural habitat to study their total lipid (TL) and lipid-fractions. Significant relationship between these parameters was also worked out. The variation of total lipid and lipid-fractions in tissues of freshwater fishes were not significantly different (P>0.05). But a higher trend of total lipid and glyceride (TGL) contents were found in carnivores followed by omnivores and least in plankton feeders. The trend was reverse for total phospholipid, cholesterol and free fatty acids. TGL content in all class of fishes was significantly related with TL (P<0.01), phospholipid (PL) (P<0.001), cholesterol (P<0.05), free fatty acids (P<0.05) and monoglycerides (P<0.001). Similarly total lipid was linearly related with total glycerides (TL = -3.02 + 0.10 TGL) and phospholipid (TL = 7.13 – 0.12 PL). From this study it is concluded that almost all lipid- fractions of freshwater fishes can be predicted easily from total lipid content of the tissue.

Keywords: - Freshwater fish, lipid, phospholipid, cholesterol, triglycerides, free fatty acids

INTRODUCTION

Lipid is the major nutrient in fish tissue next to protein. Moreover, the taste, texture and characteristic flavour of the fish depends mainly on lipid profiles of the tissues. The nature and quantity of these lipids in fish vary according to species and habitat (Acman and Eaton, 1966; Acman, 1967). There are reports on effects of different environmental factors such as season, temperature and stage of reproduction affecting the lipid content of the tissues of different species (Jezierska *et al.*, 1982; Lee *et al.*, 1993). Available literatures reveals that most of the work on lipid profile of fishes emphasizes on fatty acids only and the reports on lipid-

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fractions such as phospholipids, total glycerides, free fatty acids, sterols are scarce. In the present study a specific importance was given on analysis of total lipid and lipid-fractions of some freshwater fishes having different feeding habit and to find out any relation between them so that all the lipid-fractions can be predicted only from the total lipid content of the tissues. which may find some practical application.

MATERIALS AND METHODS

Marketable size freshwater fishes of different feeding habit such as *Clarias batrachus* (352 ± 16.88g) and *Channa punctatus* (178 ± 18.11g) of carnivorous group; *Cyprinus carpio* (980 ± 40.03g) and *Cirrhinus reba* (720 ± 30.41g) of omnivorous group; *Labeo rohita* (875 ± 32.37g) and *Catla catla* (810 ± 23.48g) of plankton feeder group were collected from natural sources during the month of March-April. Natural food, consisting mostly of planktonic organisms, filamentous algae and some crustaceans, was the main food available to them. Icing of the fish was done immediately after catch. The fishes were arranged in such a way that every fish was covered with ice. A ratio of one part of fish to two parts of ice was used. Muscle tissues in triplicate were taken from the white dorso-lateral epraxual muscle starting one centimeter behind the head and proceeding posteriorly. Care was taken to exclude bone, skin and red muscle.

Total lipid was extracted from the tissues by the method of Bligh and Dyer (1959). Ten gram homogenised tissue samples was blended with 20 volumes of chloroform:methanol (2: 1, v/v), filtered and volume was reduced in the rotary evaporator. A known volume was made by dissolving the lipid extract in chloroform. Butylated hydroxy toluene (0.01 %) was added as an anti-oxidant to prevent lipid oxidation. Extracts were kept at –18°C until further analysis. A suitable aliquot of lipid extract was pipetted into stainless steel planchets and the total lipid contents were estimated gravimetrically.

A suitable aliquot of lipid extract was taken in 5 ml of freshly neutralised alcohol. Fatty acids were titrated against 0.01 N NaOH using phenolphthalein as indicator (Koniecko, 1979). Palmitic acid was used as standard.

Procedure of Bartlett (1959) as modified by Marinetti (1962) was used for the estimation of phospholipid phosphorus in lipid extracts. The phospholipid content was calculated by multiplying the inorganic phosphorus content with the factor of 25.

Total cholesterol was determined as per the procedure of Henly (1957). Suitable aliquot was dried in water bath at 60-70°C and treated with ferric chloride-acetic acid reagent. Optical density was recorded at 560 nm.

Total glycerides were calculated indirectly by subtracting the sum of total phospholipid, total cholesterol and free fatty acids from total lipid.

Separation of phospholipid (PL) fractions was performed by thin layer chromatography (TLC). Clean glass plates (20 cm X 20 cm) were coated with silica gel-G (250 μm thickness) according to the method of Abramson and Blecher (1964). The plates were activated by heating at 110°C in hot air oven for 90 min and cooled before sample application. Aliquots of lipid extracts were applied on the TLC plates along with suitable standards. Separation of phospholipid fractions were performed by using solvent mixtures of chloroform : methanol : 7M ammonium hydroxide (v/v/v) : 115 : 45 : 7.5. Estimation of PL was carried out as per the procedures given by Bartlett (1959) and Marinetti (1962).

For fractionation of neutral lipids, plates were developed in a unidimensional three solvent system containing n-hexane, diethyl-ether and glacial acetic acid in the proportion of 60 : 40 : 1, 90 : 10 : 1 and 30 : 70 : 1, (v/v/v), respectively (Mishra, 1968). Neutral lipid spots were identified by comparing their Rf values with authentic standards co-chromatographed with each run. Cholesterol fractions (esterified and free cholesterol) were estimated as described by Henly (1957). Glyceride fractions (mono, di and triglycerides) were estimated as per the method of Van-Handel and Zilversmit (1957).

The different means were tested by one way ANOVA and the P value has been given as per the methods given by Snedecor and Cochran (1980). Regression analysis was done to determine the relationship between two parameters.

Results and Discussion

Total lipid (TL) contents of all class of fishes ranged from 2.05 to 3.83 % (Table 1). These fishes may be categorized as non-fatty fishes as Arroya (1974) reported that fatty fishes are those containing more than 4-5 % fat. Though insignificant ($P>0.05$) but higher lipid content was noted in carnivore fishes which may be attributed to the major sources of their food, rich in lipid (Love, 1974). Variation of the lipid content in other class fishes might be due to their different feeding habit as reported by Acman and Eaton (1966) and Acman (1967).

Triglycerides constituted the major lipid fraction followed by phospholipid in all class of fishes (Table 1). This confirms to the finding of Sampekalo *et al.* (1992) and that the depot fat of the body lipid consists largely of triglycerides followed by phospholipid classes. Triglycerides are the major energy reserve and the principal neutral derivatives of glycerol found in animal. Mono and diglycerides also exits, but are far less common than the triglycerides. In the present study a trend of higher TGL content ($P>0.05$) was found in carnivorous followed by omnivorous and plankton feeders. This

Table 1 : Total lipid (% wet weight basis) and lipid-fractions (% of total lipid) of different freshwater fishes

Parameters	CARNIVOROUS			OMNIVOROUS			PLANKTON FEEDER			P VALUE
	C. batrachus	*C. punctatus*	*Mean± SD*	*C.carpio*	*C.reba*	*Mean± SD*	*C.catla*	*L.rohita*	*Mean± SD*	
PL	21.42	34.73	28.07±9.41	28.69	36.43	32.56±5.47	44.63	44.44	44.53±0.13	0.16
CH	1.50	2.10	1.80±0.42	1.80	2.06	1.93±0.18	3.50	2.52	3.01±0.69	0.15
FFA	1.40	2.40	1.90±0.70	2.90	4.80	3.85±1.34	4.10	4.70	4.40±0.42	0.14
TGL	75.50	60.60	68.05±10.54	66.40	58.01	62.20±5.93	47.68	48.20	47.94±0.37	0.13
TL	5.04	2.62	3.83±1.21	3.38	2.47	2.92±0.46	1.77	2.34	2.05±0.20	0.38

All the values are the mean of three

PL Phospholipid; CH Cholesterol; FFA Free fatty acids; TGL Total glycerides; TL Total lipid

Table : 2 Phospholipid fractions (% of total lipid) of tissues of different freshwater fishes

Parameters	CARNIVOROUS			OMNIVOROUS			PLANKTON FEEDER			P VALUE
	C. batrachus	*C. punctatus*	*Mean± SD*	*C.carpio*	*C.reba*	*Mean± SD*	*C.catla*	*L.rohita*	*Mean± SD*	
PI	1.45	1.91	1.68±0.33	2.37	3.50	2.93±0.79	2.90	1.70	2.30±0.85	0.33
PS	3.05	3.11	3.08±0.04	4.22	1.83	3.02±1.68	3.09	2.70	2.89±0.27	0.98
LPC	4.96	2.31	3.63±1.87	2.31	2.58	2.44±0.19	2.93	3.60	3.26±0.47	0.61
LPE + SPH	10.25	11.06	10.65±0.57	10.04	13.44	11.74±2.4	12.48	9.20	10.84±2.31	0.85
PC	51.83	53.54	52.68±1.21	51.55	45.54	48.54±4.25	50.03	47.53	48.78±1.77	0.36
PE	25.09	22.43	23.76±1.88	24.00	27.31	28.50±3.67	25.91	31.10	28.50±3.67	0.35

PI- phosphatidyl inositol; PS- phosphatidyl serine; LPC - Lyso phosphatidyl choline;
LPE- Lyso phosphatidyl ethanolamine; SPH- sphingomyelin; PC- phosphatidyl choline;
PE- phosphatidyl ethanolamine

Table : 3 Cholesterol and glycerides fractions (% of total lipid) of freshwater fishes

Parameters	CARNIVOROUS			OMNIVOROUS			PLANKTON FEEDER			P VALUE
	C. batrachus	*C. punctatus*	*Mean± SD*	*C.carpio*	*C.reba*	*Mean± SD*	*C.catla*	*L.rohita*	*Mean± SD*	
EC	20.20	14.75	17.47±3.85	16.41	26.70	21.55±7.28	12.96	12.25	12.61±0.50	0.31
FC	79.70	85.24	82.07±3.85	83.58	73.29	78.43±7.28	87.03	87.74	87.38±0.50	0.31
MG	4.06	13.80	8.93±6.89	3.87	4.15	4.01±0.19	4.92	3.71	4.31±0.85	0.48
DG	7.07	10.30	8.68±2.28	3.20	3.66	3.436±0.32	3.83	3.52	3.67±0.22	0.048
TG	88.85	75.89	82.37±9.16	92.96	92.17	92.56±0.55	91.23	92.76	91.99±1.08	0.25

EC-esterified cholestrol; FC- free cholesterol; MG- monoglycerides;
DG- diglycerides; TG- triglycerides

may be correlated with the high-energy requirement of carnivores fishes followed by omnivores and plankton feeders, respectively (Halver, 1972). It was found that TGL content of tissue was significantly related with maximum parameters like total lipid ($P<0.01$), phospholipid ($P<0.001$), cholesterol ($P<0.05$), free fatty acids ($P<0.05$) and monoglycerides ($P<0.001$). This indicates that TGL content of the tissue is a key factor from which many other parameters can be predicted (Table 4).

Total phospholipid content of different fishes varied from 28.07 % (carnivorous) to 44.53 % (plankton feeders). In the present study an inverse relation ($R^2 = 0.88$) was found between total lipid and triglycerides content with phospholipid in all class of fishes. Phospholipid content was significantly related (Table 4) with total lipid ($P<0.01$), free fatty acids ($P<0.0.05$), cholesterol ($P<0.05$) and TGL ($P<0.001$). There was a concomitant increase of total phospholipid as TL and TGL contents decreased in the tissue. The increase in muscle phospholipid content with respect to total lipid may be caused by an increase in the amount of membranes rather than an accumulation of depot lipid as reported by Hazel and Prosser (1974) and Reinhardt and Van-Vleet (1985). Among the phospholipid fractions, phosphatidyl choline (PC) constituted the major component (Table 2) followed by phosphatidyl ethanolamine (PE) and others, which is in agreement with Sampekalo *et al.* (1992).

Table 4: Relation between different parameters

S.N.	Relation between parameters	Predicted equation Y = a + b X	R^2 value	Signifi cance
1	Total lipid (TL) and Phospholipid (PL)	TL = 7.13 – 0.12 PL	0.88	P<0.01
2	Total lipid and Total glycerides (TGL)	TL = -3.02 + 0.10 TGL	0.88	P<0.01
3	Phospholipid (PL) and Free fatty acids (FFA)	PL = 16.46 + 5.50 FFA	0.69	P<0.05
4	Phospholipid and Cholesterol (CH)	PL = 10.04 + 1113 CH	0.75	P<0.05
5	Phospholipid and Total glycerides	PL = 25.08 – 0.84 TGL	0.99	P<0.001
6	Cholesterol and Total glycerides	CH = 5.64 – 0.06 TGL	0.76	P<0.05
7	Free fattyacids and Total glycerides	FFA = 9.75 – 0.10 TGL	0.70	P<0.05
8	Free fatty acids and Phosphatidyl choline (PC)	FFA = 23.04 – 0.39 PC	0.73	P<0.05
9	Phosphatidyl inositol and Lyso-phosphatidyl ethanolamine + Sphingomyelin (LPE + SPH)	PI = - 2.39 + 0.72 LPE + SPH	0.76	P<0.05
10	Mono glycerides (MG) and Total glycerides	MG = 58.02 – 0.59 TGL	0.95	P<0.001
11	Esterified Cholesterol (EC) and Free cholesterol (FC)	EC = 99.82 – 0.99 FC	0.99	P<0.001
12	Monoglycerides and Diglycerides (DG)	MG = 0.57 + 1.2 DG	0.74	P<0.05
13	Monoglycerides and Triglycerides (TG)	MG = 58.02 – 0.59 TG	0.95	P<0.001
14	Diglycerides and Triglycerides	DG = 41.93 – 0.41 TG	0.91	P<0.01

* P<0.05; ** P<0.01; *** P<0.001

Total cholesterol content ranged from 1.80 % (carnivorous) to 3.01 % (plankton feeders). Out of total cholesterol free cholesterol content was more than the esterified cholesterol in all the species (Table 3) which is in agreement with the results of Lee *et al.* (1993). The cholesterol of muscle tissue was undoubtedly an integral part of membranes. Other biological membranes are constructed with appreciable amounts of free and esterified cholesterol (Cornwell and Horrocks, 1964). The free cholesterol content (Table 3) was more in plankton feeders followed by omnivorous and least in carnivorous. However, the variation was not statistically significant (P>0.05). The variation in cholesterol content may be due to variation in feeding habit as Love (1970) reported that cholesterol content in fish tissue

depends on sexual development, maturation and the dietary level.

Though insignificant (P>0.05) but a distinct variation was found in muscle free fatty acid contents of all class of fishes. Higher free fatty acids were recorded in plankton feeders followed by omnivorous and least in carnivorous fishes. The lower free fatty acid contents may be due to higher and quick oxidation rate in the tissue. The energy supplied to the animal by the breakdown of lipid reserves comes primarily from the oxidation of fatty acids. Continuous movement of fish decreases the muscular free fatty acids by oxidation to carbon dioxide and water to get the required energy for swimming. This conforms the low fatty acid contents of most active fishes (carnivorous) and more in sluggish fishes (plankton feeders) in the present study. Free fatty acid was linearly related with TGL (P<0.05), phosphatidyl choline (P<0.05) and phospholipid (P<0.05) content of the tissues.

From the above findings it may be concluded that fresh water fishes under natural feeding condition have low body fat. Higher deposition of total lipid and total glycerides were found in carnivorous followed by omnivorous and least in plankton feeders. The reverse trend was found for phospholipid, cholesterol and free fatty acids. Total glycerides followed by phospholipid constituted the major lipid class among all freshwater fishes and phosphatidylcholine was the major phospholipid class. Total glyceride content of the tissue was found to be the key factor from which total lipid, phospholipid, cholesterol, free fatty acids and monoglyceride can be predicted. Similarly, there was a significant relationship between total lipid with phospholipid and total glycerides.

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