JOURNAL OF OLEO SCIENCE

J. Oleo Sci., Vol. **52**, No. 8, 439-442 (2003)

JOS

NOTE

Lipid Profile and Fatty Acid Composition in Eggs of Common carp (*Cyprinus carpio*)

Tapas MUKHOPADHYAY and Santinath GHOSH*

Department of Chemical Technology, Oil Technology Division, University of Calcutta
(92, Acharyya Prafulla Chandra Road, Kolkata-700 009, West Bengal, INDIA)

Edited by H. Shimasaki, Teikyo Univ., and accepted April 21, 2003 (received for review February 28, 2003)

Abstract: The lipid class and fatty acid composition in eggs of Indian fresh water common carp *Cyprinus carpio* (Var. Cummunis Linnaeus) have been studied. The mean wet weight of mature eggs are 4.1% of total weight of fish, of which 9.8% (on dry basis) is lipid. The major portion of the lipid is phospholipid (PL), about 64.3%, of which phosphatidylcholine (PC) is predominant. The fatty acid composition of the total lipid, triacylglycerol (TAG) and phospholipids are observed. Palmitic acid (16:0) is the most abundant saturated fatty acids present in the total lipid (24.2%), TAG (33.2%) and PL (27.4%) fractions. Polyunsaturated fatty acids (PUFA) are more accumulated in the PL fraction than in the TAG. Arachidonic acid (20:4 n-6, AA) and docosahexaenoic acid (22:6 n-3, DHA) in the PL fraction are 13.0% and 15.2% and in TAG fraction 0.6% and 1.0%, respectively. The results of the study indicate that *C. carpio* eggs are rich in PUFAs which are very much essential in terms of fertilization success and larval development.

Key words: *Cyprinus carpio*, egg, fatty acid, lipid

1 Introduction

Cyprinus carpio is one of the most common carp in fresh water found all over the world. Its rapid growth, tasty flesh, good reproductive ability and modest food requirements have led to the carps becoming the stable fish of worm water fisheries. Its cage culture is also popular in commercial scale in Japan. It is well adopted for pond culture as it tolerates low oxygen and high CO_2 concentration. In India, a common practice to the fish farmer, is mixed fish culture including catla, roghu, silver carp and mrigale in scheduled ratios. These fishes are cultured in composite fish culture because of their noncompetable nature (1). The research work on Indian fresh water fish egg lipids and their biochemical and nutritional significance of essential fatty acids are scares, taking into consideration the impact of the fish brood on the quality of the eggs.

The egg quality of fresh water fishes are measured as hatchability and other parameters and are related to the content of fatty acids in eggs (2). For marine fish, high content of n-3 PUFAs in eggs and larvae are required for successful development of larvae (3). An essential role of AA (20:4 n-6) has also been postulated due to the unique and consistent fatty acid composition of phosphatidylinositol (PI) at the early stage of fish development (4).

Several researchers have worked on the lipid composition of fish eggs of marine and fresh water fishes and their probable correlation with the fertilization and embryonic development in fish (5-8). The lipid nutrition in fish suggest that certain larval stages require PL

*Correspondence to: Santinath GHOSH, Department of Chemical Technology, Oil Technology Division, University of Calcutta, 92, Acharyya Prafulla Chandra Road, Kolkata-700 009, West Bengal, INDIA
E-mail: santi@cucc.ernet.in

in their diets (9,10). The essentiality of PL for carp larvae (*C. carpio* L.) have also been studied (11,12). Carp Larvae can be fed exclusively on semi purified diets and strongly react to PL supplementation (11).

The present study aims at, investigating the lipid profile of *C. carpio* eggs of Indian origin to find out whether egg lipids contain any important fatty acids in significant proportions, their nutritional significance on the basis of essential fatty acids present in neutral and PL fractions.

2 Experimental

2·1 Sample Collection and Lipid Extraction

Matured unfertilized eggs of *C. carpio* of six different gravid females weighing about 0.85 kg were collected during spawning season from traders at Kolkata, West Bengal, India. The samples were stored in glass vials containing chloroform/methanol (2:1, v/v) and 0.01% (w/v) butylated hydroxytoluene (BHT) at −20℃ prior to extraction.

The total lipid was extracted by the method of Folch *et al*. (13). After extraction, chloroform was removed under the stream of nitrogen and finally the lipid was dried under vacuum and stored at −20℃ for analysis.

2·2 Lipid Analysis

The isolated lipid was mainly a mixture of PL, cholesterol and TAG, identified on the thin layer chromatographic (TLC) plate (silica gel G; solvent system, hexane/diethyl ether, 70:30 v/v) with phosphate stain, standard cholesterol and TAG. The PL and TAG were separated on a preparative TLC plate (20 cm × 20 cm) with the above solvent system, and the individual fractions were extracted with diethyl ether.

The total fatty acid composition of the lipid and of the isolated TAG and PL were determined by Gas-liquid chromatography (GLC) method after derivatization into methyl esters (14). The HP-5890A GLC (Hewlett Packard, Palo Alto, CA) was connected with a glass column (183 cm × 0.31 cm i.d.) packed with 10% diethylene glycol succinate (DEGS) supported on Chromosorb- WHP (100/200 mesh) of HP make. The oven, injector and detector block temperatures were maintained at 190, 230, and 240℃, respectively. IOLAR-2 nitrogen (BOC India Ltd, Kolkata, India) was used as the carrier gas (flow rate 45 ml per min). The fatty acid esters peaks were identified and calibrated with standard methyl esters supplied by Sigma Chemical Co. (ST. Louis, MO). Total PL content and individual PLs were measured by fractionation into two dimensional TLC followed by phosphorous measurement following Official Methods and Recommended Practice of A.O.C.S. (15).

Table 1 Total Lipid Content and Lipid Class Composition in Eggs of *Cyprinus carpio*.

Total weight of fish (wet weight in g)	857 ± 80
Weight of fish eggs (wet weight in g)	35.4 ± 3.9
Egg moisture content %	54.4 ± 2.5
Lipid content (% dry weight)	9.8 ± 0.5
Lipid class composition (% of total lipid)	
Phospholipid (PL)	64.3 ± 0.6
Cholesterol	5.2 ± 1.1
Triacylglycerol (TAG)	30.4 ± 1.9

Values are Mean ± SE, n=6

3 Results and Discussions

Six individual eggs of *C. carpio* were analysed and total lipid content and lipid class compositions are shown in **Table 1**. Mean wet weight of mature eggs are 4.1% of total weight of fish. The lipid content is about 9.8% on dry basis. The major portion of the lipid is composed of mainly PL, about 64.3%, TAG about 30.4% and cholesterol about 5.2%. Besides these components, trace amount of fatty acids, monoglycerides, and diglycerides are observed in thin layer chromatographic plate.

PLs present in the total lipid are fractionated to determine the individual components (**Table 2**). Among the PLs most abundant is phosphatidylcholine (PC), about 68.5%. The other components are phosphatidylethanolamine (PE, 21.6%) and phosphatidylinositol (PI, 10.2%).

PL is the major lipid fraction present in the eggs of

Table 2 Major Phospholipid Fractions in the Eggs of *Cyprinus carpio*.

Phosphatidyl choline (PC)	68.5 ± 5.2
Phosphatidyl inositol (PI)	10.2 ± 2.5
Phosphatidyl ethanolamine (PE)	21.6 ± 2.6

Values are Mean ± SE, n=6

Table 3 Fatty Acid Composition in Eggs of *Cyprinus carpio*.

	Fatty Acid Composition (% w/w) in		
Fatty acids	Total	TAG	PL
14:0	0.6 ± 0.1	2.7 ± 0.3	0.4 ± 0.1
16:0	24.2 ± 4.5	33.2 ± 3.7	27.4 ± 4.0
18:0	6.1 ± 0.4	1.9 ± 0.2	6.5 ± 0.3
20:0	2.1 ± 0.6	0.3 ± 0.2	2.2 ± 0.3
Total saturates	32.9 ± 4.2	38.2 ± 3.5	36.5 ± 1.9
16:1	5.9 ± 1.8	12.8 ± 2.7	4.1 ± 1.9
18:1	25.0 ± 1.1	38.8 ± 1.6	18.9 ± 1.1
20:1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Total monounsaturates	31.4 ± 2.5	52.1 ± 4.3	23.3 ± 2.3
18:2n-6	5.0 ± 0.8	6.5 ± 0.1	3.8 ± 0.7
20:2n-3	1.4 ± 0.4	0.3 ± 0.1	1.2 ± 0.2
20:3n-3	1.6 ± 0.3	0.4 ± 0.1	1.8 ± 0.4
20:4n-6	12.2 ± 1.8	0.6 ± 0.2	13.0 ± 0.5
20:5n-3	0.9 ± 0.3	0.3 ± 0.2	1.9 ± 0.5
22:4n-3	2.9 ± 0.6	0.2 ± 0.1	2.3 ± 0.5
22:5n-3	0.9 ± 0.1	0.1 ± 0.1	0.9 ± 0.1
22:6n-3	10.6 ± 1.2	1.1 ± 0.8	15.2 ± 0.9
Total polyunsaturates	35.4 ± 3.6	9.5 ± 1.3	40.3 ± 1.2
n-3	30.4 ± 3.6	3.0 ± 1.2	36.7 ± 1.6
n-6	5.0 ± 0.8	6.5 ± 1.0	3.8 ± 0.7
n-3/n-6	6.9 ± 1.6	0.5 ± 0.2	12.8 ± 4.5

Values are Mean ± SE, n=6

C. carpio of which PC is predominant. PC is long been known for its structural role in eggs. PC is also implicated as a very important metabolic precursor in the eggs of larvae of many marine species. PC is catabolised in halibut plaice (*Pleuronectes platessa*) and turbot during embryogenesis, prior to first feeding (16). Fresher *et al*. (9) showed that PC was a source of metabolic energy and a source of essential fatty acid (EFA), organic phosphorous in eggs and larvae of cod (*Godus morhua*). Evans *et al*. (7) also showed that PC was playing an important role after fertilization for Atlantic halibut (*Hippoglossus hippoglossus*). High amount of PC in the eggs of *C. carpio* will definitely play a significant role during embryogenesis.

Fatty acid composition of total lipid, TAG and PL fractions are shown in **Table 3**. Among the saturated fatty acids most abundant is palmitic acid (16:0), about 24.2, 33.2 and 27.4% in the total fatty acid, TAG and PL fractions, respectively. The monoenoic fatty acids are represented by two major components, palmitoleic acid (16:1) and oleic acid (18:1). AA (20:4 n-6) and DHA (22:6 n-3) in the PL fraction are 13.0% and 15.2% and in the TAG fraction 0.6% and 1.0%, respectively. So, polyunsaturated fatty acids are accumulated in the PL fraction than in the TAG. The n-3/n-6 ratio in the PL fraction is 12.7 which is very high than that in TAG (0.5).

The health benefits of fish oils containing n-3 fatty acids like eicosapentaenoic acid (EPA) and DHA, have been extensively investigated due to low incidence of coronary heart disease among populations who consume large amount of fish (17). Marine fish species are rich in n-3 PUFAs. Compared with marine fish, fresh water fish contain high level of C_{18} PUFA and also substantial amount of EPA and DHA (18). A significant amount of EPA and DHA (about 11.5%) is present in eggs of *C. carpio* also support their observations. Fresh water fishes are able to desaturase and elongate larger quantities of C_{18} n-6 and n-3 PUFAs to C_{20} and C_{22} desaturates (2,19,20). About 12.2% of total fatty acids is AA which is accumulated in eggs of *C. carpio* and a major portion in PL fraction, about 13.1%. So, *C. carpio* have a substantial amount of desaturation activity. This is very important in brood stock diets where AA is needed to produce the PLs particularly PC for incorporation into eggs. *C. carpio* along with its eggs may be used as a very good source of essential fatty acids from nutritional point of view.

DHA is essential for the development of brain and retina in marine fish and any deficient may show visual and neural abnormalities (21-23). Thus 22:6 n-3 are being supplemented to the first feeding larvae to achieve good growth and performance of the developing larvae (24). *C. carpio* is accumulating about 10.6% DHA in its eggs of which major portion is in PL fraction. Report regarding absolute requirement of DHA and other essential fatty acids during early larval development of *C. carpio* are not available. But the presence of DHA certainly dictate its requirement during early larval development.

4 Conclusion

The results show that *C. carpio* eggs are rich in PUFA of which a major portion is distributed in PL fraction. The information should help in aquaculture of

C. carpio and nutritional significance of the fish along with its eggs.

References

1. P.K. TALWAR and A.G. JHINGRAN, *Inland Fishes of India and Adjacent Countries*, Vol. **1**, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 184-186 (1991).
2. T. WATANABE, T. TAKUCHI and M. SAITO, Effect of Low Protein High Calorie or Essential Fatty Acids Deficiency Diet on Reproduction of Rainbow Trout, *Bull. Jpn. Soc. Fish*, Vol. **50**, 1207-1215 (1984).
3. D.R. TOCHER, A.J. FRASER, J.R. SARGENT and J.C. GAMBLE, Fatty Acid Composition of Phospholipids and Neutral Lipids during Embryonic and Early Larval Development in Atlantic Herring (*Clupea harengus* L), *Lipids*, Vol. **20**, 69-74 (1985).
4. D.R. TOCHER and J.R. SARGENT, Analysis of Lipids and Fatty Acids in Ripe Rose of Some North West European Marine Fish, *Lipids*, Vol. **19**, 1218-1228 (1984).
5. K.A. ULRUND and O.G. NEILSEN, Fatty Acid Composition in Eggs of Atlantic Cod (*Gadus morhua*), *Can. J. Fish Aquat. Sci*., Vol. **45**, 898-901 (1988).
6. M.H. REGINAL and L. CURRY WOODS Ⅲ, Comparative Fatty Acid Composition of Eggs from Domesticated and Wild Stripped Bass (*Morone saxotilis*), *Aquaculture*, Vol. **133**, 225-233 (1995).
7. R.P. EVANS, C.C. PARRISH, J.A. BROWN and P.J. DAVIS, Biochemical Composition of Eggs from Repeat and First-time Spawning Captive Atlantic Halibut (*Hippoglossus hippoglossus*), *Aquaculture*, Vol. **139**, 139-149 (1996).
8. J.R. RAINUZZO, K.I. REITAN and Y. OLSEN, The Significance of Lipids at Early Stages of Marine Fish: A Review, *Aquaculture*, Vol. **155**, 103-115 (1997).
9. A.J. FRASER, J.C. GAMBLE and J.R. SARGENT, Changes in Lipid Content, Lipid Class Composition and Fatty Acid Composition of Developing Eggs and Unfed Larvae of Cod (*Gadus morhua*), *Marine Biology*, Vol. **99**, 307-311 (1988).
10. J.R. SARGENT, J.G. BELL, M.V. BELL, R.J. HENDERSON and D.R. TOCHER, The Metabolism of Phospholipids and Polyunsaturated Fatty Acids in Fish, in *Costal and Estuarine Studies, Aquaculture; Fundamental and Applied Research* (B. LAHLOU and P. VITIELLO, eds), American Geophysical Union, Washington, DC, pp. 103-124 (1994).
11. J. RADUNZ-NETO, G. CORRAZE, N. CHARLON and P. BERGOT, Lipid Supplementation of Casein-based Purified Diets for Carp (*Cyprinus carpio* L.) Larvae, *Aquaculture*, Vol. **128**, 153-161 (1994).
12. I. GEURDEN, R. NETO and J. BERGOT, Essentiality of Dietary Phospholipids for Carp (*Cyprinus carpio* L.) Larvae, *Aquaculture*, Vol. **131**, 303-314 (1995).
13. J. FOLCH, M. LEES and G.S. STANLEY, A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues, *J. Biol. Chem*., Vol. **226**, 497-509 (1957).
14. L.D. METCALFE and A.A. SCHMITZ, The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis, *Anal. Chem*., Vol. **33**, 363-364 (1961).
15. Official Methods and Recommended Practice of American Oil Chemists' Society, Method No. Ja 7-86 (Total Phospholipid); Ca 12-55 (Phospholipid Fractions), 4th Edn. (D. FIRESTONE, ed.), AOCS, Washington DC, (1991).
16. J.R. RAINUZZO, K.I. REITAN and L. JORGENSEN, Comparative Study on the Fatty Acid and Lipid Composition of Four Marine Fish Larvae, *Comp. Biochem. Physiol*., Vol. **103B**, 21-26 (1992).
17. D. KRAMHOUT, N-3 Fatty Acids and Coronary Heart Disease: Epidemiology from Eskimos to Western Populations, *J. Inter. Med*., Vol. **225** (Suppl. 1), 47-51 (1989).
18. R.G. ACKMAN, Characteristics of the Fatty Acid Composition and Biochemistry of Some Fresh Water Fish Oils and Lipids in Comparison with Marine Oils and Lipids, *Comp. Biochem. Physiol*., Vol. **22**, 907-922 (1967).
19. T. TAKUCHI, T. WATANABE and T. NOSE, Requirement for Essential Fatty Acids of Chum Salmon (*Oncorhynchus keta*) in Freshwater Environment, *Bull. Jpn. Soc. Sci. Fish*, Vol. **45**, 1319-1323 (1979).
20. K. YAMADA, K. KOBAYASHI and Y. YONE, Conversion of Linolenic Acid to ω3-Highly Unsaturated Fatty Acids in Marine Fishes and Rainbow Trout, *Bull. Jpn. Soc. Fish*, Vol. **46**, 1231-1233 (1980).
21. M.V. BELL and J.R. DICK, Molecular Species Composition of the Major Diacylglycerol Phospholipids from Muscle, Liver, Retina and Brain of Cod (*Gadus morhua*), *Lipids*, Vol. **26**, 565-573 (1991).
22. G. MOURENTE and D.R. TOCHER, Effect of Weaning onto a Pelleted Diet on Docosahexaenoic Acid (22:6 n-3) Levels in Brain of Developing Turbut (*Scophthalmus maximus* L.), *Aquaculture*, Vol. **105**, 363-377 (1992).
23. J.R. SARGENT, M.V. BELL and D.R. TOCHER, Docosahexaenoic Acid and the Development of Brain and Retina in Marine Fish, in *Omega-3 Fatty Acids: Metabolism and Biological Effects* (C.A. DEVON, J. BAKSAAS and H.E. KROKAN, eds), Birkhauser Verlag, Basel, pp. 139-149 (1993).
24. R. VAZQUEZ, S. GONZALEZ, A. RODRIGUEZ and G. MOURENTE, Biochemical Composition and Fatty Acid Content of Fertilized Eggs, Yolk Sac Stage Larvae and First-feeding Larvae of the Senegal Sole (*Solea senegalensis kaup*), *Aquaculture*, Vol. **119**, 273-286 (1994).