Asian Fisheries Science 11(1998):253-259
Asian Fisheries Society, Manila, Philippines

https://doi.org/10.33997/j.afs.1998.11.3-4.008

Dietary Essentiality of Phospholipids in Indian Major Carp Larvae[1]

B.N. PAUL, S. NANDI, S. SARKAR and P.K. MUKHOPADHYAY

Central Institute of Freshwater Aquaculture
Kausalyaganga, Bhubaneswar-751002
India

Abstract

The effectiveness of phospholipid (PL) addition to formulated diets for rohu, mrigal and catla larvae was investigated. The test diets in the first two experiments were supple-mented with 0, 2 and 4% of a purified phospholipid and 6, 4 and 2% of vegetable oil and cod liver oil mixture while, in the third experiment, two more levels of purified PL were incorporated, i.e. 0, 2, 4, 5 or 6% of purified PL and 6, 4, 2, 1 or 0% of vegetable oil and cod liver oil (1:1). All the diets were isolipidic and had similar protein and energy levels. Growth and survival was significantly higher in the 4% PL group in rohu larvae, in the 2 and 4% PL groups in mrigal larvae and in the 4, 5 and 6% PL groups in catla larvae. The lipid and PL content of five-day-old rohu were 16.5% and 42.30%, those in mrigal were 19.40% and 31.80%, and, in catla, 16.80% and 43.00%, respectively. Both lipid and phospho-lipid levels were lower in the final tissue sample of Indian major carp larvae with incorpo-rated graded levels of PL in the diet. Results suggest that the inclusion of dietary phospho-lipid at 4% in the larval diet is necessary for rapid growth and high survivability of Indian major carps.

Introduction

Although juvenile and adult carp can synthesize phospholipid (PL) (Sargent 1976), PL need to be added to diets for carp larvae reared in the nursery since carp larvae cannot synthesize PL de novo (Sargent *et al.* 1994; Geurden *et al.* 1995). Catla (*Catla catla* Ham), rohu (*Labeo rohita* L.) and mrigal (*Cirrhinus mrigala* L.) are freshwater carp species intensively cultured in ponds and lakes in India. However, there has been no published information on the beneficial response to dietary phospholipid of Indian major carp (IMC) larvae. This paper reports on the results of an experiment that evaluates the relevance of dietary phospholipid incorporation on growth and survival of IMC larvae.

Materials and Methods

Larvae of rohu (*Labeo rohita* L.), mrigal (*Cirrhinus mrigala* L.) and catla (*Catla catla* Ham.) were collected from the Central Institute of Freshwater Aquaculture's carp hatchery. The feeding trial run for 28 d in the first two experiments and 21 d in the third experiment. There were three treatment

[1]A preliminary report of this paper was presented at the National Seminar on Fish Biology held on 17-19 March 1997, at Santiniketan, Visva Bharati University, India.

groups of 150 larvae each in a flow-through system fitted with 30-l plastic troughs. Water flow was maintained at 1.0-1.5 l per hour, continuous aeration was provided by an air compressor and stored ground water was used for larval rearing. The length and weight of the fish were recorded at the start of the experiment. Total length of the larvae was measured weekly. Daily observations on behavioral response, i.e. abnormality, if any, were recorded. At the close of the experiment, the larvae were weighed using a precision balance. Five formulated diets (isonitrogenous and isocaloric) were prepared using finely powdered and sieved soyabean meal, (roasted) groundnut cake, fish meal, rice bran, vitamin-mineral mixture and different levels of phospholipid (Soya lecithin), vegetable oil and cod liver oil (Mukhopadhyay and Rout 1996). The ingredient and proximate composition of the diets are presented in Table 1. During the experimental period, water temperature, pH and dissolved oxygen ranged from 28-33°C, 7.4-7.8 $mg \cdot l^{-1}$, and 6-8 $mg \cdot l^{-1}$, respectively. The proximate composition of the different diets was analyzed using standard procedures (AOAC 1980). Lipids were extracted (Bligh and Dyer 1959) and total lipid was estimated gravimetrically. Subsamples of lipid were extracted for total phospholipid analysis. Triplicate samples were taken for analysis (Mukhopadhyay *et al.* 1984). The larval length and growth data were subjected to two-way analysis of variance to test the significance among the treatment means (Snedecor and Cochran 1967).

Results

The percent incorporation of different ingredients were similar in all the diet treatments. Zero, two and four percent PL in diet were tested in the first two experiments with rohu and mrigal larvae. In addition to these diet treatments, five and six percent PL in diet were tested in the third experiment

Table 1. Percent incorporation of different feed ingredients

Ingredients	PLO	PL2	PL4	PL5	PL6
Soybean meal	10	10	10	10	10
Ground nut cake	32	32	32	32	32
Fish meal	20	20	20	20	20
Rice bran	30	30	30	30	30
Vitamin & mineral Premixa	2	2	2	2	2
Phospholipid[b] (as soya lecithin)	-	2	4	5	6
Veg oil: Fish oil (1:1)	6	4	2	1	-
Proximate composition of feed treatments (% DM basis)					
Crude protein	35.30	35.10	35.20	35.15	35.25
Crude lipid	9.50	9.55	9.60	9.58	9.45
Ash	14.30	14.20	14.25	14.20	14.25
Energy (MJ/kg)	15.10	15.15	15.20	15.10	15.20
Phospholipid* (%)	2.10	22.45	42.65	52.25	63.40

*Expressed as percent of total lipid
[a]Supplevit M, Sarabhai Company Ltd. India
[b]Soya lecithin procured from HIMEDIA RM 637, India

with catla larvae. Crude protein, crude lipid, ash and total energy content were similar in all the diet treatments, but the phospholipid varied from two to six percent in the different treatment groups. Phospholipid incorporation in the diet of rohu and mrigal significantly improved growth and survival ($P<0.01$) (Table 2). The growth and survival of rohu larvae were significantly higher in the two and four percent PL diet groups. The length increment in rohu and mrigal larvae were also significantly ($P<0.01$) higher in the two and four percent PL enriched diets (Table 2). The effect of incorporation of phospholipid on growth and survival of catla larvae are presented in Table 3. The growth and survival of catla larvae were significantly ($P<0.01$) higher in the four, five and six percent PL enriched diets. The gain in length of catla larvae followed a similar pattern. The lipid and phospholipid composition of five-day-old and final larval tissues of rohu, catla and mrigal are depicted in Figs. 1, 2 and 3. Phospholipid content was expressed as percent of total lipid. It was observed that both lipid and phospholipid content of tissue samples were lower in the final samples. Percent phospholipid was markedly lower in the final samples of all

Table 2. Effect of incorporation of phospholipid on weight gain and survivability of rohu and mrigal larvae.

Phospholipid incorporation	PLO	PL2	PL4
Rohu larvae			
Initial weight (mg/larvae)	6.60±0.85	6.50±0.80	6.70±0.97
Final weight** (mg/spawn)	21.46±1.32^a	28.90±1.52^b	36.00±1.29^c
Survival (%)	89.78±0.95^a	96.10±1.05^b	94.77±0.89^c
Length increment (mm) in 28d	13.00±0.31^a	15.13±0.24a^b	15.93±0.85^b
Mrigal larvae			
Initial weight (mg/larvae)	5.20±0.25	5.00±0.65	5.50±0.51
Final weight** (mg/larvae)	20.59±0.13^a	25.28±0.97^b	32.81±0.23^c
Survival** (%)	86.12±0.55^a	93.77±0.91^b	94.80±0.79^c
Length increment (mm)* in 28d	11.72±0.05^a	16.61±0.20^b	16.89±0.11^b

Values bearing different superscripts in a row differ significantly *($P<0.05$), **($P<0.01$).

Table 3. Effect of incorporation of phospholipid on growth and survivability of catla larvae.

Phospholipid incorporation	PLO	PL2	PL4	PL5	PL6
Initial weight (mg/larvae)	3.00±0.11	3.10±0.14	3.40±0.19	3.20±0.20	3.50±0.10
Final weight** (mg/larvae)	4.75^a±0.10	5.20^b±0.11	6.70^c±0.23	6.77^c±0.19	6.82^c±0.21
Survival**	81.30^a±2.14	94.20^b±0.96	90.40^c±1.74	88.90^c±1.56	92.22^c±0.45
Length increment in 21 days**	9.16±0.17^a	9.67±0.33^b	10.17±0.17^b	10.50±0.30^b	10.80±0.45^b

Values bearing different superscripts in a row differ significantly.
**($P<0.01$).

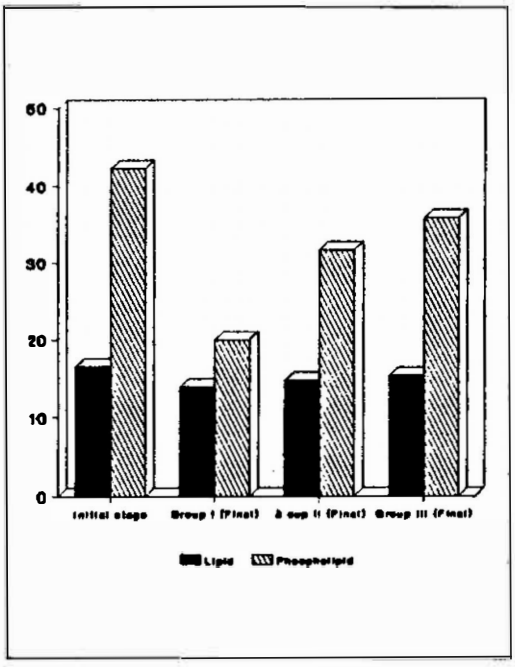

Fig. 1. Changes in lipid composition of initial and final tissue samples of rohu larvae.

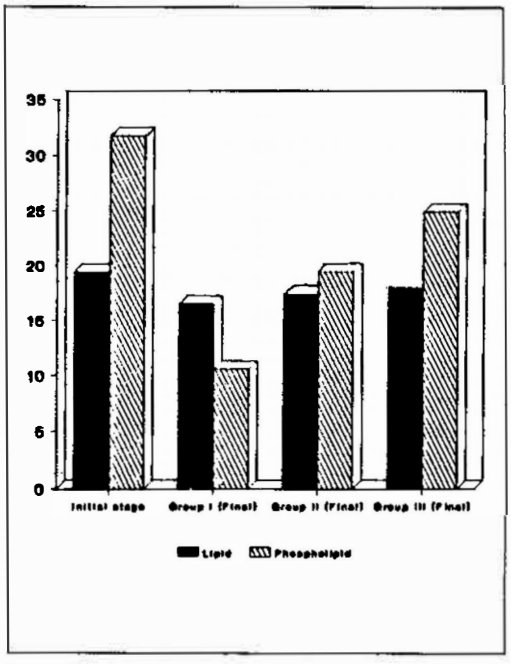

Fig. 2. Changes in lipid composition of initial and final tissue samples of mrigal larvae.

the species wherever phospholipid was not supplemented. However, the reduction was more pronounced in mrigal (Fig. 2). The percentage of phospholipid in the tissue in the phospholipid-supplemented group in all three IMC species increased.

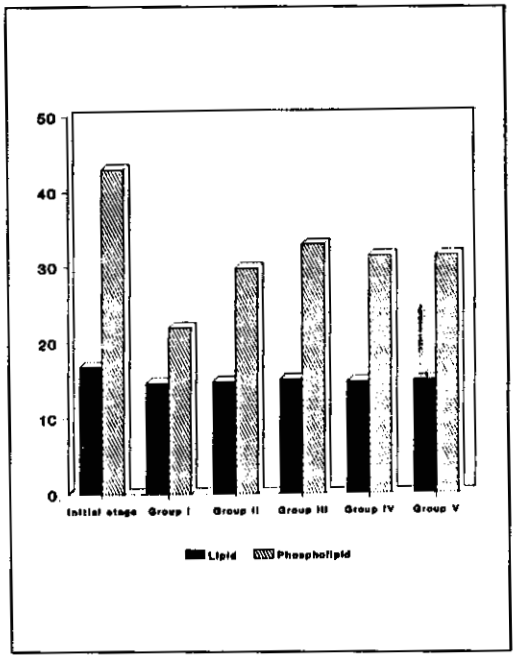

Fig. 3. Changes in lipid composition of initial and final tissue samples of catla larvae.

Discussion

The supplementation of four percent dietary phospholipid in rohu and mrigal, and four, five and six percent phospholipid in catla resulted in significantly higher growth, survivability and length increment in the IMC species. The total lipid present in the different diets was about 9.5%. Other studies have indicated that about 10% lipid is advantageous for nursery rearing of Indian major carps (Jena *et al.* 1996; Gangadhara *et al.* 1997). It has also been reported that 9% lipid is optimum for growth of *Labeo rohita*. The growth increment resulting from the incorporation of phospholipid in diets as reported in this paper are in agreement with earlier studies (Ketola 1976; Radunz-Neto *et al.* 1994; Geurden *et al.* 1995) that have suggested the beneficial effects of dietary phospholipids. The role of phospholipid seems to be crucial at the larval stage due to a need for efficient utilization of neutral lipids to meet the increased energy demand for growth. This is of particular relevance at the larval stage, when larvae cannot either synthesize phospholipid de novo or synthesizes at a rate not commensurate to its requirements (Sargent *et al.* 1994; Geurden *et al.* 1995).

Phospholipid incorporation is essential to fulfill the demand for building and renewing of cellular membrane during the early growth stages as well as for improving the efficacy of essential fatty acid utilization (Kontara *et al.* 1997). It may be speculated that formation of lipoprotein assemblages such as membranes and circulating lipoproteins have a high requirement for rapid phospholipid synthesis, which could possibly exceed the larval capabilities in the absence of a dietary supply of phospholipid (Geurden *et al.* 1995). In this study, soya lecithin was used as source of phospholipid, since it is known to be very effective in promoting growth and survival of fish and crustacean larvae

(Coutteau *et al.* 1997). After hatching and during larval development, phospholipid, particularly lecithin, was shown to be catabolized by the larvae of halibut, plaice and cod as a source of metabolic energy (Rainuzzo *et al.* 1992). These results are in agreement with those found for cod, where lecithin was utilized after hatching, affirming that lecithin has nutritional, biochemical and physiological functionalities.

Dietary phospholipid incorporation study in Indian major carp has shown that tissue phospholipid level can be enhanced in all three species to maximize survival and growth; at the early feeding stage, a provision of four percent phospholipid seems to be the optimal inclusion level in the larval diet. However, the biochemical basis for the effectiveness of phospholipid incorporation, specially during larval stage of growth, needs further investigation.

Acknowledgments

The authors express their sincere thanks and gratitude to Dr. S. Ayyappan, Director, Central Institute of Freshwater Aquaculture, for his encouraging words and for providing the necessary facilities for this study.

References

AOAC 1980. Official methods of analysis, 14th ed. Association of Official Analytical Chemists, Washington D.C. 1094 p.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911-917.

Coutteau, P., I. Geurden, M.R. Camara, P. Bergot and P. Sorgeloos. 1997 Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture 155:149-164.

Gangadhara, B., M.C. Nandeesha, T.J. Varghese and P. Keshavanath. 1997. Effect of varying protein and lipid levels on the growth of rohu *Labeo rohita*. Asian Fisheries Science 10(2):146-154.

Geurden, I., J. Radunz-Neto and P. Bergot. 1995. Essentiality of dietary phospholipids for carp (*Cyprinus carpio* L.) larvae. Aquaculture 131:303-314.

Jena, J.K., P.K. Mukhopadhyay, S. Sarkar, P.K. Aravindakshan and H.K. Muduli. 1996. Evaluation of a formulated diet for nursery rearing of Indian major carp under field condition. Journal of Aquaculture in the Tropics 11:299-305.

Ketola, H.G. 1976. Choline metabolism and nutritional requirement of lake trout (*Salvelinus namaycush*). Journal of Animal Science 43:474-477.

Kontara, E.K.M., P. Coutteau and P. Sorgeloos. 1997. Effect of dietary phospholipid on requirements for and incorporation of n-3 highly unsaturated fatty acids in post larval *Penaeus japonicus* Bate. Aquaculture 158:305-320.

Mukhopadhyay, P.K., A.P. Mukherjee and P.V. Dehadrai. 1984. Phospholipids and fatty acids in the liver of catfish *Clarias batrachus* exposed to sublethal malathion. Journal of Environmental Biology 5(4):221- 229.

Mukhopadhyay, P.K. and S.K. Rout. 1996. Effects of different lipids on growth and tissue fatty acid changes in fry of carp *Catla catla* (Hamilton). Aquaculture Research 27:623-630.

Radunz-Neto, J., G. Corraze, N. Charlon and P. Bergot. 1994. Lipid supplementation of casein based purified diets for carp (*Cyprinus carpio* L.) larvae. Aquaculture 128:153-161.

Rainuzzo, J.R., K.I. Reitan and L. Jorgensen. 1992. Comparative study on the fatty acid and lipid composition of four marine fish larvae. Comparative Biochemistry and Physiology 103:(B) 21-26.

Sargent, J.R. 1976. The structure, metabolism and function of lipids in marine organisms. Biochemistry Biophysics Perspective of Marine Biology 3:149-212.

Sargent, J.R., J.G. Bell, M.V. Bell, R.J. Henderson and D.R. Tocher. 1994. The metabolism of phospholipids and polyunsaturated fatty acids in fish. In: Coastal and estuarine studies. Aquaculture: fundamental and applied research (ed. B. Lahlou and P. Vitiello), pp. 103-124. American Geophysical Union, Washington, DC.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical methods, 6th ed. Oxford & IBH Publication Company, New Delhi.

Manuscript received 14 May 1997; Accepted 15 July 1998.