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Review on the dietary effects of phospholipids in fish and crustacean larviculture

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Abstract

A beneficial effect of dietary phospholipid (PL) supplementation in purified diets in terms of survival, growth, resistance to stress tests, and occurrence of deformities has been demonstrated in larval and juvenile stages of various species of fish and crustaceans. The exact determination of PL requirements in larvae is complicated due to the difficulty to bio-encapsulate PL in live prey. Furthermore, the great variety in purity and composition of the PL sources, and the experimental conditions (such as diet formulation and extent of co/prefeeding with live food) makes it difficult to compare requirements determined with artificial diets. Larval stages are extremely sensitive to a dietary PL deficiency and require higher levels of dietary PL than juveniles. For most of the fish and crustacean species examined, the estimated PL requirement of larvae are in the range of 1–3% phosphatidylcholine + phosphatidylinositol (PC + PI) of diet dry weight. The absence of a PL

Abbreviations: Choline CDP: Choline cytidine-5'-diphosphate; DHA: Docosahexaenoic acid (22:6$n-3$); EFA: Essential fatty acids; EL: Hen-egg lecithin; EPA: Eicosapentaenoic acid (20:5$n-3$); EPC: Hen-egg phosphatidylcholine; FS: Free sterol; HUFA: Highly unsaturated fatty acids; NL: Neutral lipid; LPC: 1-Acyl lyso phosphatidylcholine; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; PL: Phospholipid; POL: Polar lipid; PS: Phosphatidylserine; PUFA: Polyunsaturated fatty acids; SE: Sterol esters; SL: Soybean lecithin; SM: Sphingomyelin; SPC: Soybean phosphatidylcholine; SPE: Soybean phosphatidylethanolamine; SPI: Soybean phosphatidylinositol; Lecithin: Although the term 'lecithin' is the trivial name for 'phosphatidylcholine', lecithin is defined in the food sector as a mixture of polar and neutral lipids with a polar lipid content of at least 60% (Hertrampf, 1992). In the present manuscript, the latter definition of 'lecithin' (e.g., EL, SL) was followed, whereas 'phosphatidylcholine' is used for the specific PL having choline as the headgroup (e.g., SPC, EPC).

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requirement in the freshwater prawn *Macrobrachium rosenbergii* exemplifies the important species differences. The few studies evaluating single PL demonstrate that PC and PI are the most efficient in most species. The presence of an unsaturated fatty acid in $sn-2$ position of the PL molecule seems to be essential for the functionality of PL. Some studies in crustaceans reported a relation between PL requirements and the protein source in the diet. Various hypotheses have been formulated to explain the effect of PL. The PL effect is not related to the provision of choline, inositol or essential fatty acids (EFA). However, PL may be superior to neutral lipids for larvae as a source of EFA and energy due to their better digestibility. PL may improve the performance of the diet by improving the water stability of food particles, or by their action as antioxidant or feed attractant. The effect of dietary PL appears not to be explained by their emulsifying ability. However, there are proofs that dietary PL interfere with lipid transport, especially cholesterol transport in crustaceans, and with retention of fatty acids provided by dietary triacylglycerol. Although the origin of the requirement is still unclear, dietary PL supplementation has potential importance for the formulation of practical larval diets. © 1997 Elsevier Science B.V.

Keywords: Phospholipid; Fish; Crustaceans; Larva; Juvenile

1. Introduction

Beneficial effects on culture performance due to the inclusion of phospholipids in the diet were first reported in the late seventies for lobster (Conklin et al., 1977) and larval fish and shrimp (Kanazawa et al., 1979, 1981). These findings were surprising, as de novo synthesis of phospholipids has been shown in various species of fish, where it is generally believed to follow the mammalian pathways of esterification (Henderson and Tocher, 1987; Sargent et al., 1993), as well as in crustaceans (Shieh, 1969; Chapelle et al., 1985; Teshima et al., 1986c; Kanazawa and Koshio, 1994). Despite substantial research efforts encouraged by this apparent discrepancy, the mechanisms behind the PL requirement are still not fully understood. This paper reviews the present experimental evidence of PL requirements and the various hypotheses formulated concerning their role in larval and early juvenile nutrition.

2. Phospholipid requirements

2.1. Particularities and constraints in studies on PL requirements in larvae

Due to the limited acceptance of artificial diets at start-feeding in many species of larval fish and shrimp, most studies determining larval nutritional requirements for essential fatty acids and vitamins have been carried out using enrichment techniques to manipulate the composition of live feeds such as rotifers (*Brachionus plicatilis*: Rainuzzo et al., 1994a) and brine shrimp (*Artemia* sp.: Léger et al., 1986; Sorgeloos et al., 1997). Initial studies on larval phospholipid requirements attempting to bio-encapsulate phospholipids in *Artemia* failed due to the general conservative nature of polar lipid class composition in live organisms (Tackaert et al., 1991). The latter was confirmed recently by Rainuzzo et al. (1994b) who found only minor shifts in the lipid class

composition of *Artemia* and rotifers enriched with an emulsion based on either halibut roe or ethyl esters (containing 71.2% and 0.8% of PL, respectively). Although it may not be possible to obtain live feed containing ranges of phospholipid levels suitable for nutritional studies, further research is needed to evaluate the effect of minor modifications in the lipid class composition such as the PC:PE ratio in *Artemia* (Rainuzzo et al., 1994b) on its nutritional value.

The exclusive use of artificial diets from start-feeding onwards is restricted to only few species, e.g., the goldfish *Carrassius auratus* (Szlaminska et al., 1993) and common carp *Cyprinus carpio* (Radünz-Neto et al., 1994; Geurden et al., 1995c), which readily accept semipurified diets upon first opening of the mouth. By contrast, most studies have been carried out with larvae and juveniles initially fed on live feed, which is relatively rich in PL (Teshima et al., 1987). The live feeding period varies from a short prefeeding on micro-algae (e.g., feeding diatoms till zoea I/II in larval *Penaeus japonicus*; Kanazawa et al., 1985a) or rotifers (e.g., till 10 days after hatching in larval ayu *Plecoglossus altivelis*; Kanazawa et al., 1981) to a complete larval rearing on live diets and consequent weaning onto the experimental diets (European sea bass *Dicentrarchus labrax*: Geurden et al., 1995b; *Penaeus* sp.: Camara, 1994; Coutteau et al., 1996; lobster *Homarus americanus*: Conklin et al., 1980).

Another difficulty is the nonstandardized composition of the experimental PL sources that differ in their content of neutral lipids (e.g., $< 1\%$ of total lipid in de-oiled SL versus 46.5% in raw SL) as well as the proportion of different PL classes (e.g., 23% PC versus 42.1% PC in two types of de-oiled SL) (data reported by Camara (1994) and Kanazawa (1993), respectively). Furthermore, variation of the level of dietary phospholipids has been compensated using cellulose (e.g., Kanazawa et al., 1983b; Hilton et al., 1984; Teshima et al., 1986a) or neutral lipids (Kean et al., 1985; Camara, 1994; Geurden et al., 1995c; Coutteau et al., 1996). The latter results in isolipidic diets and has been preferred in most recent studies. In addition to being isolipidic, diets designed to compare the nutritional value of different PL sources have been formulated to contain similar levels of phospholipids (Kanazawa et al., 1981; Geurden et al., 1995a,b,c), PC + PI (Kanazawa, 1993) or PC (Camara, 1994; Coutteau et al., 1996).

2.2. *Influence of stage and species*

Table 1 summarizes the PL levels reported to be optimal for several larval and juvenile crustaceans and fish, and the relevant experimental conditions. The criteria used to evaluate the nutritional value of PL consist mainly of survival and growth, sometimes completed with data on either skeletal anomalies, stress sensitivity, body composition or lipid analyses of the animals. Larval stages are very sensitive to PL deficiency. Zoea I/II stages of *P. japonicus* suffered 100% mortality before reaching the mysis stage when fed a PL-deficient diet (Kanazawa et al., 1985a; Fig. 1). For first-feeding carp larvae, the supplementation of 2% PL to the diet highly improved both survival and final weight (by a factor 2.0–2.5) compared to that of carp fed PL-free diets (Radünz-Neto et al., 1994; Geurden et al., 1995a,c). Similar drastic effects on survival were shown for larvae of goldfish (Szlaminska et al., 1993) and red seabream (Kanazawa et al., 1983a). The survival of juvenile fish appears to be less affected by the dietary PL supply than

Table 1
Phospholipid requirements of larval and juvenile stages of fish and crustaceans

Species	Initial size or stage [a]	Test period	PL source (purity) [b]	Basal diet	PL level (% diet) [c]		Author
					Optimal	Other tested	
Larval crustaceans							
Penaeus japonicus	Zoea I/II	6 days	SL (23.6% PC)	Casein	3.5–6 (G,S)	0; 1; 3; 7.5; 9	Kanazawa et al. (1985a)
Penaeus japonicus	Zoea I/II	8 days	SL (35% PC, 18.5% PI of POL)	Casein	3 (S)	0; 1; 5; 10	Teshima et al. (1986e)
Juvenile crustaceans							
Homarus americanus	Stage IV	90 days	SL (95% PL)	Casein	7.5 (S)	5; 10; 20; 35	Conklin et al. (1980)
Homarus americanus	Stage VI 120 mg WW	14 weeks	SL	Purified crab protein/gelatin	0 (S,G)	3; 6	Kean et al. (1985)
Macrobrachium rosenbergii	80 mg WW	12 weeks	SL	Casein/gelatin	0 (G,S)	2; 4; 6; 8; 10	Hilton et al. (1984)
Macrobrachium rosenbergii	121 mg WW	40 days	SL (35% PC)	Casein/albumin	0 (G,S)	5	Briggs et al. (1988)
Macrobrachium rosenbergii	750 mg WW	40 days	SL	Casein or purified crab protein	0 (G,S)	1; 2	Kanazawa (1993)
Penaeus chinensis	710 mg WW	40 days	SL (42% PC of POL)	Casein	2 (G)	0; 0.5; 1; 3	Kanazawa (1993)
Penaeus japonicus	1 g WW	30 days	SL (35% PC, 18.5% PI of POL)	Casein	3 (G,S)	0	Teshima et al. (1986a)
Penaeus japonicus	5 mg WW	44 days	SPC (95% PC)	Casein	1.5 (G,R)	0; 3	Camara (1994)
			SL (86% PL)		6.5 (G,S,R)	–	
Penaeus monodon	90 mg WW	8 weeks	SL (63% PL)	Practical	2 (+1) [d] (G)	0; 1 (+1) [d]	Piedad-Pascual (1986)
Penaeus monodon	450 mg WW	4 weeks	SPC (80% PC, 20% LPC)	Casein	1.25 (G)	0; 2.5; 5	Chen (1993)
Penaeus penicillatus	1 g WW	4 weeks	SPC (80% PC, 20% LPC)	Casein	1.25 (G)	0; 2.5; 5	Chen and Jenn (1991)
Penaeus vannamei	2 mg WW	41 days	SPC (95% PC)	Casein	1.5 (G)	0; 0.5; 3	Coutteau et al. (1996)
			SL (86% PL)		6.5 (G)	1.5	
Larval fish							
Chrysophrys major	4.8 mm TL	20 days	SL	Casein/gelatin	5 (G,S)	0	Kanazawa et al. (1983a)
Cyprinus carpio	2 mg WW	25 days	EL (98% PL)	Casein	2 (G,S)	0; 4	Geurden et al. (1995c)
		21 days	various PL [e]		2 (G,S) [e]	0	
Oplegnathus fasciatus	6.0 mm TL	22 days	SL	Mixture of various proteins	7.4 (G,S)	0; 2.5; 5	Kanazawa et al. (1983a)
Oplegnathus fasciatus	26 mg WW	28 days	SL (53% POL)	Mixture of various proteins	5 (G,S,R)	0; 3; 7	Kanazawa (1993)

Paralichthys olivaceus	4.6 mm TL	30 days	SL (53% POL)	Casein	7 (G,S)	0; 3; 5	Kanazawa (1993)
Plecoglossus altivelis	2.4 mg WW	20 days	EL or SL	Casein	3 (G,S,M)	0	Kanazawa et al. (1981)
Plecoglossus altivelis	9.6 mm TL	50 days	bonito egg PL	Casein	3 (G,S,M)	0	Kanazawa et al. (1983b)
			EL		3 (G,S,M)	0	
			SL		5 (G,S), 3 (M)	0; 1; 3	
Juvenile fish							
Acipenser transmontanus	5–10 g WW	6 weeks	SL (75% PL)	Casein	0	8	Hung and Lutes (1988)
Dicentrarchus labrax	3.54 mg DW	40 days	SL (65% PL)	Mixture of various	3 (G)	0	Geurden et al. (1995b)
			EPC (95% PC)	proteins	2 (G)	0	
			SPC (95% PC)		2 (G)	0	
Oncorhynchus mykiss	100–120 mg WW	20 weeks	SL (16% PC)	Soy protein/herring meal	4 (G)	0; 2; 8	Poston (1990a)
Oplegnathus fasciatus	11.6 g WW	60 days	SL (53% POL)	Casein	3 (G)	0; 5; 7	Kanazawa (1993)
Paralichthys olivaceus	2.94 g WW	30 days	SL (53% POL)	Casein	7 (G)	0; 3; 5	Kanazawa (1993)
Plecoglossus altivelis	87.2 mg WW	33 days	SL	Casein	3 (G)	0	Kanazawa et al. (1981)
			bonito egg PL		3 (G)	0	
			EL		3 (G)	0; 1; 5	
Pseudocaranx dentex	750 mg WW	6 weeks	SPC (98% PC)	Defatted fish metal	1.5 (G,S,R)	0; 0.5; 1.0; 2.0	Takeuchi et al. (1992)
			SPE (99% PE)		1.5 (G)	0	
Salmo salar	0.18 g WW	16 weeks	SL (65% PL)	Soy protein	4 (G)	0	Poston (1990b)
	1.0 g WW	12 weeks			4 (G)	0	
	1.7 g WW	12 weeks			4 (G)	0	
	7.5 g WW	12 weeks			0	4	
Salmo salar	180 mg WW	14 weeks	Corn PL/SL	Soy protein	6 (G)	0; 2; 4; 8	Poston (1991)

[a] Initial dry (DW) or wet (WW) weight, or total length (TL).
[b] Phosphatidylcholine (PC), total phospholipids (PL), and total polar lipids (POL) expressed as % of total lipids of source unless stated otherwise.
[c] Presumed requirement based on comparison of one PL level with PL deficient diet demonstrating beneficial effect on growth (G), survival (S), stress resistance (R) or malformations (M).
[d] Endogenous PL in the practical diet.
[e] Various PL sources formulated to contain 2% dietary PL: EL (69% PL); SL (65% PL); sunflower PL (55% PL); rapeseed PL (59% PL); marine PL (81% PL).

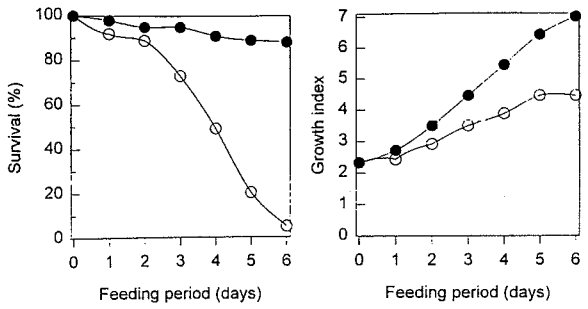

Fig. 1. Growth and survival of shrimp larvae (*P. japonicus*) fed a diet containing 0% (○) or 3.5% (●) of soybean lecithin (from Kanazawa et al., 1985a).

that of larvae as shown in rockbream *Oplegnathus fasciatus* (Kanazawa, 1993) and sea bass (Geurden et al., 1995b). Besides their effects on survival and growth, dietary PL were found to influence the occurrence of skeletal anomalies and stress sensitivity. Kanazawa et al. (1981, 1983b) indicated that larval ayu may require phospholipids in their diet to prevent the incidence of malformations, especially scoliosis and jaw deformities. Also, first-feeding carp fed either on a PL deficient or PC enriched diet exhibited a higher incidence of spinal deformities than larvae fed a PI enriched diet (Geurden et al., 1995a). The vitality index of larval rockbream fed 5% SL was three times higher compared to that of the group fed the PL-free diets (Kanazawa, 1993). Postlarval *P. japonicus* fed a PC-supplemented diet exhibited a higher resistance to a salinity stress than animals fed a PL-deficient diet (Camara, 1994). This PC effect on stress sensitivity could not be confirmed for *P. vannamei*, suggesting that this criterion may not be suitable for evaluating the nutritional status of euryhaline species (Coutteau et al., 1996).

The PL requirement generally decreases with age or developmental stage, as shown for rockbream where required levels decreased from 5% to 3% of soybean lecithin (equivalent with 1.6% to 1.0% PC + PI) from larva to juvenile (Kanazawa, 1993). There is some confusion over whether excessive levels of dietary phospholipids can be detrimental to fish and crustaceans. Increasing the dietary PL level beyond the required level did not affect survival or growth in various studies (Conklin et al., 1980; Kanazawa, 1993; Geurden et al., 1995c). However, survival of larval *P. japonicus* decreased when more than 3% soybean lecithin was added to the diet (Teshima et al., 1986e), and Coutteau et al. (1996) (Fig. 2) found lower growth of postlarval *P. vannamei* fed 3% pure PC compared to the shrimp fed 1.5%.

From Table 1, it is clear that PL requirements markedly differ between species. For instance, for juveniles of different penaeid shrimp, the requirements are mostly within the range of 1.2–1.5% of active PL (PI + PC) (Table 1: Chen and Jenn, 1991; Chen, 1993; Kanazawa, 1993; Camara, 1994; Coutteau et al., 1996). For juvenile flounder (*Paralichthys olivaceus*) and rockbream, these levels were 2.2% and 1% PC + PI, respectively (Kanazawa, 1993). In contrast, for the freshwater prawn (*Macrobrachium*

rosenbergii), no significant effect was found of dietary lecithin supplementation on growth nor survival (Hilton et al., 1984; Briggs et al., 1988; Kanazawa, 1993).

2.3. Influence of the lipid class composition of the PL source

A typical oil-free soybean lecithin contains 84% of PL, including 22% PC, 23% PE, 20% PI and 2% PS (Hertrampf, 1992). Nevertheless, a wide variety of PL sources has been used in nutritional studies (see 2.1) and only few investigators have determined PL requirements using highly purified PL sources, such as 80% (Chen and Jenn, 1991; Chen, 1993) or even 95% pure PC (Takeuchi et al., 1992; Camara, 1994; Coutteau et al., 1996). The few studies evaluating purified PL fractions suggest that PC and PI are the main growth-promoting fraction in lecithin. Comparing various PL fractions derived from bonito egg, Kanazawa et al. (1985b) found that PC (purity: 42% of total PL) or PC plus PI (52% + 40% of PL) gave good growth and survival rates in larval ayu, whereas PE (63% of PL) was less effective. As 1% dietary supplements to pollack liver oil (7% of diet), soybean PI and soybean PC were more effective in improving growth and survival of larval *P. japonicus* than bovine-brain PS, bonito-egg PE and bovine-brain PE, whereas a bovine-brain SM supplemented diet did not perform any better than the PL-deficient diet (Kanazawa et al., 1985a). In a similar study, soybean PE (95% PE of total PL) was inferior to soybean PI (60% of PL) and PC (68% of PL) as far as survival of larval *P. japonicus* was concerned (Teshima et al., 1986e). However, the relative importance of PC and PI is not necessarily the same for different species. The active component in lecithin for lobster nutrition was identified as phosphatidylcholine, since ovine PE and soybean PI could not substitute for the effect of soybean PC to reduce mortality in juveniles (D'Abramo et al., 1981). Similarly, Kanazawa (1993) reported a growth-promoting effect for Japanese flounder larvae only when soybean PC was supplemented, whereas soybean PI clearly did not improve growth compared to the PL-free control diet. By contrast, soybean PI provided a higher weight gain for 70 days old ayu than soybean or bonito egg PC (Kanazawa et al., 1985b). First-feeding carp fed

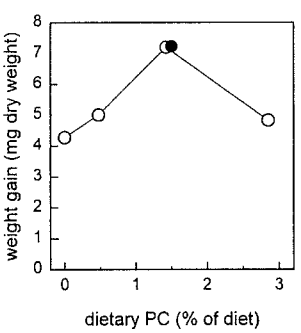

Fig. 2. Growth of shrimp postlarvae (*P. vannamei*) as a function of the level of dietary PC, presented as purified soybean PC (95% PC, ○) or de-oiled soybean lecithin (23% PC, ●). Data represent dry weight gain after 41 days of culture from an initial weight of 3 mg (from Coutteau et al., 1996).

a soybean PC-enriched diet exhibited a higher initial growth rate but a higher mortality rate, due to the appearance of severe lordosis, than larvae fed a soybean PI-enriched diet (Geurden et al., 1995a). The different effects of dietary PC and PI in the latter case of feeding fish larvae exclusively from first-feeding on artificial diets merits further investigation.

2.4. Influence of the fatty acid composition of the PL source

Chicken egg PC was not effective in improving growth and survival of larval *P. japonicus*, whereas bonito-egg PC and soybean PC were effective (Kanazawa et al., 1985a). Similarly, lecithin and PC derived from soybean or bonito egg were more effective in promoting growth of larval ayu *P. altivelis* than chicken egg lecithin (Kanazawa et al., 1981, 1985b). However, all these PL sources drastically improved growth and survival of the larvae compared to the PL-deficient control, whereas a saturated species of PC, dipalmitoyl PC, was completely ineffective (Kanazawa et al., 1985b). Similarly, D'Abramo et al. (1981) found that the effectiveness of PC-containing PL to prevent mortality in juvenile lobsters was dependent on the constituent fatty acids with soybean PC being more effective than egg lecithin and dipalmitoyl PC. In addition, 1-acyl lyso soybean PL in diets for carp resulted in low growth and survival, although superior to that of the PL-free diet (Geurden et al., 1995c). The latter finding questions the hypothesis that, in carp larvae, PL are absorbed after preferential hydrolysis at the $sn-2$ position as suggested in adult carp by Iijima et al. (1990). In this way, carp larvae would require intact phospholipid molecules containing $sn-2$ unsaturated fatty acids with either inositol or choline headgroups, which confirms the presumption of Kanazawa et al. (1981, 1985b) for larval ayu.

2.5. Influence of the protein source in experimental diets

Special attention should be paid to the poorly understood interaction between phospholipid requirements and other dietary ingredients such as the protein source. Juvenile American lobsters fed semi-purified diets based on casein showed high levels of mortality due to incomplete ecdysis (molt death syndrome, MDS), and this could be alleviated by the supplementation of 7.5% of dietary soybean lecithin (Conklin et al., 1980). However, no PL requirement was found for lobsters when purified crab protein ($< 0.02\%$ total lipid), rather than casein, was used as the primary protein source (Kean et al., 1985). More recently it was shown that further purification of the crab protein concentrate by using 0.06M EDTA in a 50:50 isopropanol:water solvent mixture resulted in a reappearance of a MDS for which soybean lecithin was ineffective (Castell et al., 1991; Conklin et al., 1991). Although some evidence was found by Castell et al. (1991) that B vitamins and manganese may play a role in MDS induction, the physiological factors responsible for molt death remain to be elucidated. Similarly, supplementary lecithin in the diet was not needed for larval *P. japonicus* when a mixture of casein and soybean protein was used instead of casein only (Teshima et al., 1993). Interestingly, the addition of the lipids extracted from the soybean protein source, consisting of PC (37%), LPC (23%), and partial glycerides (19%), were effective at

concentrations as low as 0.43% of the diet in improving growth and survival of the larvae fed the defatted soybean protein.

The possible interactions between the selection of the protein source and PL requirements may have important implications for the role of lecithin in the formulation of practical diets. Furthermore, the use of practical diets for confirming PL requirements detected with semipurified diets is poorly documented in literature. In this way, *P. monodon* juveniles fed a practical diet containing approximately 1% of endogenous PL, increased weight gain significantly when 2% of soybean lecithin was added to the diet (Piedad-Pascual, 1986). Comparative studies using similar PL sources added to both casein-based experimental diets and practical diets based on shrimp and/or fish meal would allow a better interpretation of the present knowledge of PL requirements.

3. Functionality of dietary phospholipids

3.1. Source of membrane phospholipids

Several authors have suggested that larval stages are not capable of synthesizing PL at a rate sufficient to meet the requirements for the formation of new cell components during the initially short period of rapid larval growth (Kanazawa, 1993; Geurden et al., 1995c). The difference in the activity among PL, with PC generally being the most active compound, may then be explained by the specific need for incorporation into membranes, which consist mainly of PC. The present understanding of the metabolism of phospholipids in fish and shrimp is mainly based on extrapolations from the knowledge of mammals (Sargent et al., 1993). It remains unclear whether EFA, which are preferentially esterified to the $sn-2$ position in PL, are assimilated as a single entity or separately from the PL molecule. The latter may have important implications for the possible direct effect of the fatty acid composition of dietary PL on the composition and function of tissue PL. Chen and Jenn (1991) found decreased $n-3$ HUFA levels in the polar fraction of the muscle lipids of *P. penicillatus* with increasing dietary soybean PC, reflecting the high 18:2(n-6) level of the PL source. The latter authors suggested the presence of a dynamic metabolism in the muscle PL pool in which the PL are replaced by PL of dietary origin. In particular for marine larvae, the discrepancy between the fatty acid composition of PL present in formulated diets, which are often from soybean origin, and that of their natural food deserves further research.

3.2. Provision of choline, inositol, EFA and energy

Preferential catabolism of PC during embryonic and yolk sac stages has been observed in various species of marine fish with the PC presumed to be mainly a source of inorganic phosphate and choline (herring: Tocher et al., 1985), DHA (cod: Fraser et al., 1988) or metabolic energy (cod, halibut, and plaice: Rainuzzo et al., 1992). Similarly, it has been postulated that dietary PL may serve as a direct source of nutrients for early feeding stages of fish and crustacea.

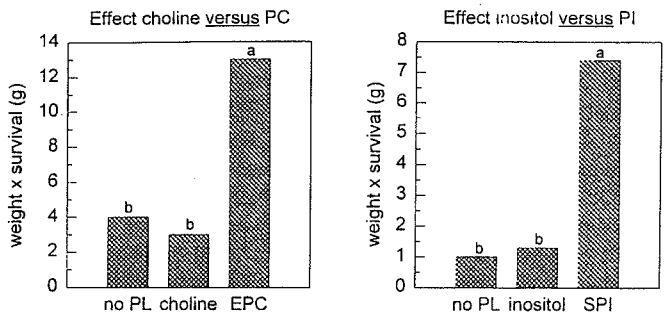

Fig. 3. Theoretical biomass (weight × survival) of larval carp (*C. carpio*) fed dietary supplements of choline or inositol versus PC or PI for 25 days after start-feeding. Results are also shown for a PL-free control diet (no PL). Significant differences ($P < 0.05$) are indicated with a different letter (from Geurden et al., 1995a,c).

Various forms and concentrations of dietary choline were not as effective as lecithin in reducing molt death syndrome in juvenile lobsters fed casein-based diets (Conklin et al., 1980). Similarly, the beneficial effect of dietary PC and PI on growth and survival of first-feeding carp larvae could not be replicated by the supplementation of choline or inositol, respectively (Geurden et al., 1995a,c; Fig. 3), and the addition of choline CDP could not correct the PL deficiency in the diet of larval ayu (Kanazawa et al., 1985b). Nonetheless, this does not exclude the possibility that PC can prevent a choline deficiency as shown by Hung (1991) in a study with juvenile white sturgeon *Acipenser transmontanus*.

In one of their earlier works, Kanazawa et al. (1979) showed that the growth-promoting effects of 1% dietary clam phospholipids on juvenile *P. japonicus* could not be replicated by including an equal level of the fatty acids derived from this PL source in the diet. Similarly, the beneficial effects of dietary soybean lecithin on growth and survival of larval ayu could not be attributed to the content of EFA, which was low in soybean PL, whereas EFA were supplied in sufficient amounts in the diet (Kanazawa et al., 1981). The latter authors concluded that the growth-promoting effect of PL was due to certain effects of the molecular form of the PL rather than to the EFA provided by them. For carp, sunflower lecithin, very poor in $n-3$ PUFA, provided better rearing results than did rapeseed lecithin or even a marine PL extract (Geurden et al., 1995c). The fact that experimental diets used to demonstrate PL requirements are formulated to contain sufficient amounts of EFA in the neutral lipid fraction (Kanazawa et al., 1981, 1985a,b; Camara, 1994; Geurden et al., 1995a,b,c; Coutteau et al., 1996) does not, however, exclude the possibility that phospholipids may be superior to neutral lipids as EFA source for larval stages, whose digestive capacity may not be fully developed. PL are more polar and may be more easily emulsified and thus less susceptible to a hypothetical bile salt limitation for their assimilation (Koven et al., 1993; Sargent et al., 1993). In this way, Olsen et al. (1991) suggested that larval and early juvenile cod may have an absolute need for polar lipids, both for the supply of energy and for essential fatty acids, because of the limited digestibility of neutral lipids.

3.3. Improvement of diet properties

One hypothesis for the effectiveness of dietary supplementation of soybean lecithin in preventing molt death in lobsters fed casein-based diets is related to the reduction of the leaching of water soluble nutrients, in particular manganese and B vitamins (Castell et al., 1991). The limited water stability of many semipurified diets that have been used to study phospholipid requirements in larval and postlarval stages should inspire further exploration of this hypothesis in other species that have been reported to require PL. The loss of total dry matter in casein-based microbound diets used for studies with postlarval penaeid shrimp amounted to 40% after 10 min of exposure to water, and was not influenced by the addition of PL (Camara, 1994). Nevertheless, more detailed studies are needed to evaluate the effect of PL on the leaching rate of micronutrients.

Phospholipids have been shown to exert antioxidant (King et al., 1992; McEvoy et al., 1995) and feed-attractant properties (Harada, 1987), and it remains to be investigated whether these may account to some extent for the beneficial effects of PL supplementation to larval and juvenile diets.

3.4. PL emulsifying properties

Several authors have suggested that lecithin may be required as a surfactant for efficient lipid emulsification and digestion in early stages of crustaceans and fish larvae (Kanazawa et al., 1979; Conklin et al., 1980; Koven et al., 1993). In this way, Koven et al. (1993) reported a 7-fold increase of ^{14}C-oleic acid in lipids of 22-day-old *Sparus aurata* when fed a lecithin supplemented diet compared to a PL-free diet. They attributed this increased lipid uptake to the emulsifying function of the lecithin. However, various lines of evidence indicate that dietary PL improve growth and survival by other effects than the enhancement of emulsification and absorption of dietary lipid in the digestive tract. The addition of taurocholic acid could not substitute for the beneficial effect of soybean PC either in larval *P. japonicus* (Kanazawa et al., 1985a), larval ayu *P. altivelis* (Kanazawa et al., 1985b), or in juvenile *H. americanus* (D'Abramo

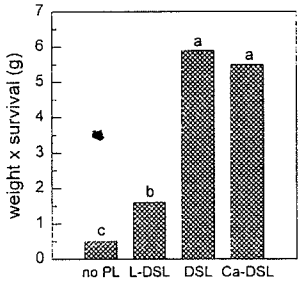

Fig. 4. Theoretical biomass (weight × survival) of larval carp (*C. carpio*) fed dietary supplements of de-oiled soybean lecithin (DSL) with varying emulsifying properties for 21 days after start-feeding. 1-acyl lyso DSL (L-DSL) and Ca^{2+}-enriched DSL (Ca-DSL) have, respectively, higher and lower emulsifying capability in vitro than the untreated DSL. See also Fig. 3 for explanation (from Geurden et al., 1995c).

et al., 1981). Besides, juvenile *P. japonicus* were capable of assimilating tripalmitin and cholesterol efficiently without dietary PL (Teshima et al., 1986c,d). In a recent study with start-feeding carp larvae, it was shown that PL added to a casein-based diet as the sole lipid source enhanced growth in the same way as when added to the same diet supplemented with 4% neutral lipids (Geurden et al., 1995c). In the same study, it was shown that the in vivo emulsifying properties of dietary soybean lecithin could not explain the fish performance: Ca^{2+} enrichment of the soybean lecithin, which decreases its emulsifying properties, did not affect its positive effect on growth and survival, whereas 1-acyl lyso lecithin, being a better oil/water emulsifier, gave poorer culture results than untreated soybean lecithin (Fig. 4).

3.5. Role in lipid transport

Various findings support the hypothesis that dietary PL enhance the transport of lipids in crustaceans, i.e., the export of absorbed lipids from the gut epithelium into the haemolymph, and the mobility of lipids between the various tissues and organs. The lack of dietary lecithin in casein diets for juvenile lobster was associated with lower haemolymph levels of phospholipids and cholesterol (D'Abramo et al., 1982). Using ^{3}H-cholesterol, D'Abramo et al. (1985) found relatively higher levels of cholesterol in the midgut gland and lower haemolymph levels in lobsters fed diets without lecithin, presumably due to a decrease of the transport rate of cholesterol out of the midgut gland into the hemolymph. Lobsters fed lecithin-supplemented diets exhibited higher levels of serum and lipoprotein cholesterol than those fed lecithin-deficient diets regardless of whether crab protein or casein was used as protein source (Baum et al., 1990). The latter finding showed that the impairment of cholesterol transport due to a shortage of lipoprotein–phospholipid–cholesterol complexes was not a possible cause of molt death syndrome observed in juvenile lobsters fed casein diets, but confirmed the role of lecithin in cholesterol transport. Using radioactively labelled tripalmitin and cholesterol, Teshima et al. (1986c,d) showed that dietary PL improve the mobilization of cholesterol, and to a lesser extent, of triglycerides from the gut to the hepatopancreas, hemolymph and muscle in *P. japonicus*. The latter authors hypothesized that the dietary PL, especially PC, may act as an acyl donor for the lecithin:cholesterol acyltransferase which converts free cholesterol into sterol ester (Teshima et al., 1986d). In contrast to the above suggested enhancement of cholesterol availability by dietary PL, several researchers failed to demonstrate an interaction between lecithin and cholesterol requirements (*P. japonicus*: Teshima et al., 1982; *P. penicillatus*: Chen and Jenn, 1991; *M. rosenbergii*: Briggs et al., 1988). In larval fish, little is known about the intervention of PL in lipid transport.

Teshima et al. (1986c) assumed that dietary PL may provide specific lipid classes as substrate for the formation of lipoproteins which are the main mediators of lipid transport in the hemolymph of shrimp, and contain polar lipids as the main lipid component (Teshima and Kanazawa, 1980). Lipoproteins also play an essential role in lipid transport in the circulatory system of fish, and PC is the predominant polar lipid class in fish lipoproteins (Henderson and Tocher, 1987; Sheridan, 1988). Nevertheless, it remains to be shown to what extent dietary PL supply may influence the pathways for the synthesis of lipoproteins responsible for the transport of absorbed lipids.

3.6. Effect of PL on body lipid composition

The inclusion of PL in the diet affected lipid deposition, resulting in increased lipid retention and levels in the animal (Teshima et al., 1986a; Chen and Jenn, 1991, Takeuchi et al., 1992). Some controversy remains regarding the influence of the level of dietary PL on the lipid class composition of the animal. Larval *P. japonicus* feeding on a diet containing 3% soybean lecithin showed higher tissue levels (% of wet weight) of SE, FS, PC and PI compared to larvae fed on a PL deficient diet (Teshima et al., 1986e). In a similar study with juvenile *P. japonicus*, Teshima et al. (1986a) found increased body levels of PL, in particular PC, and cholesterol due to the supplementation of 3% soybean lecithin in the diet. Increased total lipid levels in hepatopancreas and hemolymph in juvenile *P. japonicus* due to PL supplementation were found to be due to an increase of both neutral lipids, i.e., triglycerides and cholesterol, and polar lipids, mainly PC (Teshima et al., 1986b). In total muscle lipid of *P. penicillatus*, Chen and Jenn (1991) did not observe a preferential increase of the proportion of PC in total lipids due to the supplementation of purified PC up to 5% of the diet.

An interaction between the incorporation efficiency of dietary EFA and PL supplementation has been documented in various species. The proportions of n-3 HUFA in larval *P. japonicus* varied with the type of phospholipid supplemented to the diet, with the highest and lowest proportion occurring in the larvae receiving soybean PC and soybean PI, respectively (Teshima et al., 1986e). Also, a higher proportion of EPA and DHA was observed in juvenile *P. japonicus* due to the addition of 3% of soybean lecithin in the diet (Teshima et al., 1986a). The better efficiency of dietary EFA due to PL supplementation is likely the basis for the interaction between EFA and PL requirements, as was demonstrated by Kanazawa et al. (1985a) for larval *P. japonicus*, and deserves further investigation regarding its relevance in practical diets.

4. Conclusions

Various experimental results have shown the beneficial effects of dietary PL for larval and juvenile stages of several species of fish and crustacea. Nevertheless, further research using purified diets and PL sources is still needed to identify the physiological basis of the requirement, and define the optimal dietary PL supply in terms of PL classes and PL fatty acid composition. Furthermore, the interaction of PL requirements with other nutrients such as the dietary protein source and essential fatty acids deserves more investigation.

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Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures

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SUMMARY

1. A wide range of species that are cultivated in commercial mariculture are planktonic during at least part of their life cycle; for example, the larval stages of shellfish (shrimp and molluscs) and the live feeds (rotifers, brine shrimp, copepods) used in the larviculture of marine fish and shellfish. Over the last decades various techniques have been developed to deliver nutrients to these zooplanktonic organisms either through artificial diets or by manipulating the composition of the live prey fed to the carnivorous stages. This paper reviews the methodology that has allowed aquaculturists to gain knowledge of nutritional requirements and may offer interesting opportunities for ecologists to verify the importance of key nutrients in the natural food chain of marine as well as freshwater ecosystems.

2. Live micro-algae can be replaced partially or completely in the diet of filter-feeders such as rotifers, *Artemia*, shrimp larvae and bivalves, by various types of preserved algae, micro-encapsulated diets and yeast-based diets, whereas lipid emulsions and liposomes may be utilized to supplement specific lipid-and water-soluble nutrients, respectively. Microbound and micro-encapsulated diets have been designed to supplement live feed in the culture of micro-predators such as fish and shrimp larvae.

3. Live prey organisms, in particular rotifers and *Artemia*, can be 'bio-encapsulated' with a variety of enrichment diets to manipulate their content in certain nutrients, including ω3 highly unsaturated fatty acids (FA) and the vitamins C, A and E. Nevertheless, the enrichment techniques are not applicable for all nutrients and prey organisms. Phospholipid composition is difficult to manipulate through the diet of live feed and the enrichment of the essential FA docosahexaenoic acid (DHA) is hampered in most *Artemia* species due to the catabolism of this FA following enrichment.

Introduction

Commercial mariculture comprises a wide range of species that are planktonic during at least part of their life cycle, i.e. the larval stages of cultivated species of fish and shellfish, and a few selected species of zooplankton (copepods, rotifers, brine shrimp) that are used as live feed for the former. The natural diet of these planktonic organisms, feeding either as filter-feeders or micropredators, consists of a wide diversity of bacteria, detritus, phytoplankton and/or smaller zooplankton (Omori & Ikeda, 1994). This high diversity of food organisms of different sizes and biochemical composition provides good chances for meeting all the nutritional requirements of the larvae. Larviculture nutrition, more particularly for start-feeding early larval stages, appears to be one of the major bottlenecks for the industrial upscaling of the culture of many species of marine fish and shellfish. Collecting and feeding natural plankton is not practical on an industrial scale and natural food organisms are not consistently available. Over the past decades, trial and error approaches have resulted in the adoption of selected larviculture diets, various species of