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Increased docosahexaenoic acid levels in total and polar lipid of European sea bass (*Dicentrarchus labrax*) postlarvae fed vegetable or animal phospholipids

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Abstract A 6-week feeding trial was conducted with 44-d-old European sea bass (*Dicentrarchus labrax* L.) in order to examine the effect of various dietary phospholipid (PL) sources on the incorporation of *n*-3 highly unsaturated fatty acids (HUFA) in tissue lipids. From weaning onwards the fish received diets prepared by coating different lipid fractions (7.5% diet) on an extruded basal diet (92.5% diet). The two PL-free control diets contained 0.5 and 2% of an emulsifier blend, respectively. Seven other diets contained 2% PL, differing by their purity and origin (vegetable or animal). All diets were rendered isolipidic by the addition of hydrogenated coconut oil. Feeding the PL-supplemented diets, except the diet containing hydrolyzed soybean PL (lyso PL), resulted in a higher survival and a 10 to 30% better growth as compared to the PL-free diets. No difference according to the PL origin was observed. The sea bass final lipid content increased with increasing body weight. Also the lipid class composition of the fish was clearly correlated with the final weight gain. Total neutral lipid increased from 51% of total lipid (initial fish) to 76% for fish fed the PL-free diets, and up to 88% for fish fed the sunflower PL. Weaning the fish on the experimental diets induced important changes in their fatty acid profiles characterized by a decrease in 18:3*n*-3, 20:5*n*-3 and 20:4*n*-6 and an increase in saturated fatty acids and 22:6*n*-3 (DHA). According to the fatty acid composition of both total and polar lipid, the weaned fish could be divided into three groups reflecting the dietary fatty acids: a group fed the vegetable PL, a group fed the animal PL and a PL-deprived group. An effect of dietary PL on the incorporation of dietary *n*-3 HUFA, more particularly DHA, was noticed. For a similar supply of DHA through the neutral lipids in the diet, fish fed PL-supplemented diets (except for the lyso PL diet) had 10 to 25% higher DHA levels in total and polar lipid than PL-deprived fish. This PL effect was already clear at the end of the weaning and was not related to the presence of *n*-3 HUFA in the PL source, as suspected in a previous study when feeding egg yolk PL. A better incorporation of DHA was not obtained by replacing the PL by an emulsifier or by lyso PL with higher emulsifying properties. Present results confirm a role of dietary PL in the absorption of dietary neutral lipids, by a mechanism other than emulsification.

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Introduction

Dietary phospholipids (PL) are essential for good growth and survival of larval and juvenile fish (Kanazawa et al. 1981, 1985; Kanazawa 1993; Geurden et al. 1995, 1997a, b). Because most animals can synthesize PL, this requirement in fish, which is particularly pronounced during early development, is unusual and poorly understood (Sargent et al. 1993). Several hypotheses explaining the beneficial PL effect have been formulated (reviewed by Coutteau et al. 1997). These are related to physical roles [e.g. emulsifying (Koven et al. 1993) or antioxidative (McEvoy et al. 1995)], as well as to specific nutritional properties of the PL molecule (e.g. source of choline, inositol and of its constituent fatty acids). However, the latter explanations did not explain the beneficial PL effect seen in larval carp (Geurden et al. 1995, 1997a).

Recently, we observed better growth and a 50% increase of the *n*-3 HUFA (highly unsaturated fatty acid) level in turbot and sea bass lipid, when fish were fed a diet containing 2% PL from hen egg yolk compared to a PL-free diet with similar *n*-3 HUFA content (Geurden et al. 1997b). Though, it was not clear whether this was due (a) to a much better retention of the small amount of DHA contained in the PL source than of the DHA provided by neutral lipid (NL) or (b) to the aid of the dietary PL in absorbing the NL dietary fatty acids. In

the latter case, a PL effect could be expected either on the emulsification of lipid in the intestinal lumen or on the export of absorbed lipid from the enterocytes.

Because of the importance of *n*-3 HUFA in lipid metabolism of marine fish (Sargent et al. 1993), we decided to investigate the interaction between dietary PL and *n*-3 HUFA incorporation. We examined if vegetable PL sources (free of *n*-3 HUFA) were as efficient as animal PL sources (containing some *n*-3 HUFA) in enhancing the incorporation of dietary *n*-3 HUFA into fish lipids. Furthermore, we compared the effect of these PL with that of hydrolyzed soybean PL (lyso PL), selected for its higher emulsifying properties, and with that of a non-PL emulsifier blend.

Materials and methods

Experimental diets

Nine isolipidic diets were prepared as described previously (Geurden et al. 1997b) by coating a different lipid fraction (7.5% diet) on a basal extruded nucleus (92.5% diet). The nucleus contained 4.3% hydrogenated coconut oil and 69% of a protein mixture consisting mainly of defatted fish meal (Coutteau et al. 1996). The nucleus lipids (4.7% of dry diet) consisted for 3.8% of PL, resulting in less than 0.2% PL on dry diet basis.

The coated lipid fractions had an equal level of fish oil ethyl ester concentrate, providing approximately 2% *n*-3 HUFA on a dietary dry weight basis. In the two PL-free diets, EM0.5 and EM2.0, the PL were replaced by hydrogenated coconut oil plus, respectively, 0.5 and 2% of an emulsifier blend with the same hydrophilic–lipophilic balance value as soybean lecithin. Seven diets were formulated to contain 2% of various types of PL (Table 1). Diet Ly-PL was supplemented with partially hydrolyzed (phospholipase A_2) soybean PL which consisted for 1/3 of *sn*-1 lyso PL. Diets S-PL, S-PC and Sf-PL contained soybean PL, purified soybean phosphatidylcholine (95% PC), and sunflower PL, respectively. Animal PL were derived from hen egg yolk with a different PC content, i.e. E-PL (60% PC) and E-PC (94% PC), and from marine fish (M-PL). Details on the lipid class composition of the PL sources are given in Geurden et al. (1995, 1997a). Generally, the animal PL had a higher PC and a lower phosphatidylinositol (PI) content than the vegetable PL. All vegetable PL were de-oiled and had a PL content (% PL source) ranging from 51 to 97%. The animal PL sources in diets M-PL and E-PL were not completely de-oiled and consisted, respectively, of 19 and 27% of triglycerides. The level of hydrogenated coconut oil varied according to the purity of the PL source, rendering the diets isolipidic (Table 1).

The vegetable PL sources contained no fatty acids longer than 18 C, whereas the animal PL, particularly the marine PL, contained some *n*-6 and *n*-3 HUFA. Lyso PL had a higher ratio of saturated to *n*-6 fatty acids than the non-hydrolyzed soybean PL (Table 1).

The fatty acid analysis of the coated experimental diets showed similar amounts of *n*-3 HUFA (1.9 to 2.0% of dry diet) in all diets, except in diet M-PL (2.5%) supplemented with the marine PL. The higher content in linoleic acid distinguished the diets containing vegetable PL (0.8 to 1.1% of dry diet) from the other diets (0.2 to 0.5%). Diets with animal PL had relatively more 16:0 and 18:0. The total level of saturated fatty acids was influenced by the level of hydrogenated coconut fat, being the highest in diet EM0.5 (Table 1).

Experimental design

The 6-week feeding trial was conducted with European sea bass (*Dicentrarchus labrax* L.) obtained from the commercial hatchery Sepia International S.A. (Gravelines, France). During the 10-d acclimation period the larvae received on alternating days freshly hatched or 24-h enriched (Super Selco, INVE Aquaculture N.V., Belgium) *Artemia* nauplii (EG type, INVE Aquaculture N.V., Belgium). At the age of 44 d, the fish (3.54 mg dry wt ind^{-1}) were randomly stocked (15 ind l^{-1}) in 30-liter tanks of a recirculating rearing system. Weaning started the day of stocking and lasted for 11 d by gradually decreasing the daily number of *Artemia* nauplii and increasing the amount of experimental diet (150 to 300 µm, particle size) delivered by automatic feeders. For the subsequent 30 d, food supply (300 to 500 µm, particle size) was continuous (every 30 min) throughout the 12-h artificial light period. The daily food amount ranged between 3 and 4% of the tank biomass. Each of the nine diets was fed to triplicate groups of fish. Every day uneaten food, faeces and dead fish were removed from the tanks and ammonia-N, nitrite-N (0.2 ± 0.1 and 0.5 ± 0.1 mg l^{-1}, respectively) and temperature (21 ± 1 °C) were measured.

Parameters

The percentage of final mortality was based on daily recordings and corrected for the number of fish sacrificed in sampling. Fish were starved for 16 h prior to each sampling. For the determination of individual dry weight, samples of 30 to 40 fish from each replicate were taken at the start (age: 44 d) and at weekly intervals starting from the end of the weaning (55, 62, 69, 76 d) till the end of the experiment (84 d). The fish were anaestethized with phenoxy ethanol, rinsed with tap water, freeze-dried and weighed. Pooled freeze-dried fish were homogenized and stored under vacuum at −20 °C for further analysis.

Lipid analysis

Total lipid was extracted according to the method of Folch et al. (1957) as detailed in Coutteau and Sorgeloos (1995). After final drying under nitrogen, the lipid extracts were weighed, redissolved in chloroform/methanol (2:1) at a final concentration of 10 mg lipid ml^{-1} containing 0.05% butylated hydroxytoluene and divided into subsamples.

A first subsample was used for the determination of the lipid class composition of the initial and final fish lipids. Analyses were performed by Iatroscan thin-layer chromatography and flame-ionization detection (TLC-FID, Iatroscan Mark V) following Fraser et al. (1985). The FID operated at an air flow of 2 liters min^{-1} and a hydrogen flow of 160 ml min^{-1}. Approximately 20 µg of lipid was spotted in triplicate at the origin of the Chromarods (S-III type). After partial scanning of the neutral lipids, developed in a mixture of hexane/diethylether/formic acid (85:15:0.04), the remaining polar lipids were separated in chloroform/methanol/water (75:35:3.5) followed by a full scan of the rods. Identification and quantification were based on the retention time and response factors of standards (Avanti Polar Lipids, Inc., USA).

A second subsample, for determining the total fatty acids, was transesterified using a methanol/acetylchloride (20:1) mixture.

A third subsample, for determining the polar fatty acids, was applied on 20 × 20 cm glass plates coated with silica gel G (E. Merck). The separation of polar from neutral lipid classes was mainly as described in Tocher et al. (1985) using unidimensional TLC and a neutral development mixture consisting of hexane/diethylether/glacial acetic acid (80:20:2). After exposure to iodine vapour for visualizing the lipid classes, polar lipids were scraped off the plate and transesterified by an overnight acid-catalyzed transesterification (Christie 1982). An internal standard (20:2*n*-6) was added prior to the reaction. The resulting fatty acid methyl esters were analysed by injecting (on column) into a Chrompack CP9001 gas chromatograph operating with hydrogen (100 kPa) as carrier gas and flame ionization detection. It was equipped with a 2.5 m methyl deactivated precolumn connected to a 50 m polar capillary column (BPX70, SGE, Australia) of 0.32 mm internal diameter and a layer thickness of 0.25 µm. Temperature was programmed to rise from 85 to 180 °C. Peak identification was based on standard

Table 1 Major lipid characteristics of the experimental diets and PL sources used for weaning and further growth of European sea bass (*Dicentrarchus labrax*)

	EM0.5	EM2.0	Ly-PL	S-PL	S-PC	Sf-PL	E-PL	E-PC	M-PL
Variable part of coated lipid fraction[a] (% diet)									
PL source[b]	–	–	4.0	3.1	2.1	3.7	3.1	2.1	2.5
Emulsifier[c]	0.5	2.0	–	–	–	–	–	–	–
Coconut fat[d]	4.5	3.0	1.0	1.9	2.9	1.3	1.9	2.9	2.5
Fatty acid composition PL source (% fatty acids)									
14:0	–	–	tr	tr	0.1	tr	2.0	1.7	2.9
16:0	–	–	30.3	19.3	13.8	16.1	25.9	32.2	21.9
18:0	–	–	5.7	3.9	3.5	5.6	14.2	12.1	17.1
18:1*n*-9	–	–	10.3	8.7	8.4	10.5	29.0	27.4	9.0
18:2*n*-6	–	–	46.2	58.9	65.8	65.6	17.1	17.2	tr
18:3*n*-3	–	–	5.9	8.2	5.9	0.3	0.1	0.2	0.1
20:4*n*-6	–	–	–	–	–	–	4.7	3.1	4.7
20:5*n*-3	–	–	–	–	–	–	tr	tr	10.0
22:6*n*-3	–	–	–	–	–	–	3.5	2.5	10.5
Fatty acid composition experimental diets (mg g^{-1} dry diet)[e]									
14:0	14.8	11.1	9.6	8.9	10.9	8.9	9.1	10.7	9.8
16:0	11.4	9.8	11.8	9.5	9.3	8.8	11.6	11.9	11.2
18:0	11.1	7.1	5.9	6.4	6.2	6.1	8.1	7.8	9.2
16:1*n*-7	0.5	0.8	0.3	0.4	0.4	0.5	0.6	0.6	0.8
18:1*n*-9	7.3	10.0	5.0	5.2	4.5	5.0	8.1	7.1	5.5
18:1*n*-7	0.7	0.8	0.7	0.8	0.9	0.8	0.8	0.7	0.8
20:1*n*-9	0.4	0.5	0.5	0.6	0.4	0.5	0.3	0.4	1.1
18:2*n*-6	2.7	2.9	8.8	11.0	11.6	11.3	4.9	4.3	2.6
20:4*n*-6	0.2	0.3	0.2	0.3	0.3	0.2	0.8	0.5	0.6
22:5*n*-6	0.1	tr	0.1	tr	0.1	0.1	0.2	0.2	0.2
18:3*n*-3	0.3	0.3	1.0	1.3	1.1	0.3	0.3	0.3	0.4
20:5*n*-3	5.7	5.8	6.2	5.8	5.5	5.9	5.9	5.7	7.9
22:5*n*-3	1.3	1.1	1.3	1.4	1.3	1.5	1.5	1.3	1.6
22:6*n*-3	11.9	12.0	12.7	12.4	11.7	12.1	12.9	12.5	15.1
Saturates	37.3	31.2	27.3	24.8	25.7	23.8	28.6	29.7	29.5
Monoenoics	9.1	12.3	6.5	7.2	6.2	6.9	9.9	9.1	8.4
n-6 PUFA	3.0	3.2	9.1	11.4	12.1	11.6	5.9	5.0	3.4
n-6 HUFA	0.4	0.2	0.3	0.4	0.4	0.3	1.0	0.7	0.8
n-3 PUFA	19.2	19.2	21.2	20.9	19.6	19.8	20.6	19.8	25.0
n-3 HUFA	18.9	18.9	20.2	19.6	18.5	19.5	20.3	19.5	24.6
n-3/*n*-6 PUFA	6.4	6.0	2.3	1.8	1.6	1.7	3.5	4.0	7.3
DHA/EPA	2.1	2.1	2.0	2.1	2.1	2.1	2.2	2.2	1.9

[a] The constant coated lipid fraction contained 2.56% fish oil ethyl esters with approximately 50% *n*-3 HUFA (INVE Aquaculture N.V., Belgium), 0.02% α-tocopherol-acetate (Roche N.V., Belgium), 0.015% 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinolin (Sigma E-8260)
[b] Except for the PL-free diets, diet codes refer to the origin and purity of the PL-source: *Ly-PL*, *S-PL* and *Sf-PL*, respectively, hydrolysed soybean PL (lyso PL), soybean PL (Vamothin F1) and sunflower PL (Vandemoortele N.V., Belgium); *S-PC*, *E-PL* and *E-PC*: soybean PC (Epikuron 200), hen egg yolk PL (Ovothin 160) and hen egg yolk PC (Ovothin 200) (Lucas Meyer GmbH, Germany); *M-PL*: marine PL (CTPP, France)
[c] Glycerol mono-oleate/sorbitan monostearate,1/1 (Fina Chemicals N.V., Belgium)
[d] Hydrogenated coconut fat, cocos 32/34 (Vandemoortele N.V., Belgium)
[e] Sums include minor fatty acids not given in the table (*tr* trace amount, i.e. <0.1). Standard deviations are omitted for clarity. The coefficient of variation for the individual fatty acids was $<5\%$

reference mixtures (Nu-Check-Prep Inc., USA). Integration and calculations were done with the help of the software program Maestro (Chrompack, the Netherlands). The final fatty acid composition is expressed in milligrams per gram dry weight (diets) or as a percentage of total fatty acids (fish). It is based on single (total fatty acids) or duplicate (polar fatty acids) analyses performed on each of the duplicate total lipid extracts.

Statistical analyses

Final results were subjected to a one-way analysis of variance and Newman–Keuls multiple range test to determine differences in means at $P < 0.05$. Regressions and principal component analyses were performed with STATISTICA (Microsoft, StatSoft, Inc.).

Results

Survival and growth

Table 2 summarizes the rearing results for sea bass after the 41-d feeding period. Mortality occurred mainly during the 2 weeks following the end of weaning whereafter it stabilized, except in groups fed the diets EM0.5, EM2.0 and Ly-PL. This resulted in a significantly higher final mortality in the latter groups (12.5 to 13.7%) compared to groups fed the other diets (5.3 to 7.7%).

Table 2 *Dicentrarchus labrax*. Observed mortality, dry body weight (DW), theoretical biomass (DW × survival, S), total lipid content (TL) and lipid class composition of the initial sea bass (44 d old) and sea bass at the end (84 d old) of the feeding trial. Values sharing a common superscript in the same row are not significantly different ($P < 0.05$) (tr trace amount, i.e. <0.1)

	Initial	EM0.5	EM2.0	Ly-PL	S-PL	S-PC	Sf-PL	E-PL	E-PC	M-PL
Mortality (%)		13.2^{b}	12.5^{b}	13.7^{b}	7.7^{a}	6.1^{a}	7.3^{a}	7.7^{a}	5.3^{a}	6.8^{a}
Dry weight, DW (mg)	3.54	75^{c}	79^{bc}	76^{bc}	85^{abc}	92^{ab}	99^{a}	85^{abc}	90^{ab}	86^{abc}
DW × S (g)		6.5^{c}	6.9^{bc}	6.6^{bc}	8.0^{ab}	8.6^{a}	9.1^{a}	7.8^{ab}	8.6^{a}	8.0^{ab}
Total lipid, TL (%DW)	14.9	21.2^{d}	21.4^{d}	21.9^{cd}	23.6^{b}	24.1^{b}	24.7^{a}	22.5^{c}	23.9^{b}	22.8^{c}
Lipid class composition (%TL)										
Total neutral lipid	51.5	75.7^{e}	75.5^{e}	80.0^{d}	85.1^{b}	84.4^{b}	88.0^{a}	81.8^{c}	84.4^{b}	83.1^{bc}
Sterol ester	3.2	0.8	1.0	1.0	0.9	0.6	0.8	0.9	0.8	0.7
Triglyceride (TAG)	22.7	55.2^{d}	51.9^{e}	59.3^{cd}	62.5^{bc}	66.9^{a}	66.3^{a}	58.4^{cd}	64.4^{b}	60.7^{c}
Free fatty acids	0.3	4.1	5.4	5.2	6.6	3.7	6.4	7.3	5.1	7.2
Cholesterol	25.6	15.6^{b}	17.3^{a}	14.5^{bc}	15.0^{bc}	13.2^{d}	14.5^{bc}	15.1^{bc}	14.1^{c}	14.3^{c}
Total polar lipid	48.5	22.4^{a}	23.1^{a}	19.2^{b}	14.3^{c}	14.0^{c}	11.4^{e}	16.4^{c}	13.4^{cd}	15.0^{c}
Phosphatidylethanolamine (PE)/phosphatidic acid	13.1	3.8^{a}	4.0^{a}	2.7^{b}	2.4^{bc}	2.0^{c}	1.5^{c}	2.7^{b}	2.2^{bc}	2.7^{b}
Phosphatidylserine/ phosphatidylinositol	2.6	1.8^{ab}	2.5^{a}	2.6^{a}	1.6^{ab}	1.2^{ab}	0.5^{b}	2.0^{ab}	0.6^{b}	1.3^{ab}
Phosphatidylcholine (PC)	30.7	15.8^{a}	15.7^{a}	13.2^{b}	9.9^{c}	10.6c	9.9^{c}	11.3^{c}	10.2^{c}	10.5^{c}
Sphingomyelin	2.1	1.0	0.9	0.7	0.4	0.3	0.3	0.3	0.3	0.4
Lyso phosphatidylcholine	0.0	tr	tr	tr	tr	0.1	tr	0.2	0.1	0.2
TAG/PC + PE	0.52	2.8^{f}	2.6^{f}	3.7^{e}	5.1^{bc}	5.4^{b}	6.2^{a}	4.2^{ed}	5.2^{bc}	4.6^{cd}

Throughout the experiment the dry body weight of the sea bass increased from 3.5 to between 75 and 99 mg. Heterogeneity between replicates moderated the statistical significance for dietary-induced growth differences. Fish fed the PL-free diets (diet EM0.5 and EM2.0), but also the hydrolyzed soybean PL (diet Ly-PL), had a 10 to 30% lower final weight than those fed the PL-supplemented diets. Feeding diet Sf-PL yielded the highest growth. A PC enrichment of the egg yolk (diet E-PC) and soybean PL (diet S-PC) slightly enhanced growth as compared to the non-enriched PL in diets E-PL and S-PL, respectively. The final theoretical dry biomass was the highest for fish fed the PL-supplemented diets, irrespective the origin of the PL source (Sf-PL, S-PC and E-PC: 8.6 to 9.1 g), and the lowest for those fed the diets with the emulsifier or the lyso PL (EM0.5, EM2.0 and Ly-PL: 6.5 to 6.9 g).

Lipid content and lipid class composition of fish

The lipid content, expressed as a percentage of fish dry matter, increased from 14.9% prior to weaning to 21.2–24.7% at the end of the feeding experiment (Table 2). At the same time, the proportion of neutral lipid, as a percentage of total lipid, increased from 52% to 76–88% which was mainly due to the increasing TAG level (from 23 to 52–67%). As a compensation, proportions of cholesterol and polar lipid components decreased from 25 and 48% to 13–17 and 12–24%, respectively.

The final lipid content was clearly correlated to the fish dry weight ($r^2 = 0.78$). Fish with the lowest final weight (those fed the PL-free diets and Ly-PL diet) also had the lowest lipid content. The lipid of latter fish consisted of a significantly lower percentage of neutral lipid. Consequently, the ratio of TAG to PC + PE, reflecting the ratio of storage to membrane lipid, was also positively correlated ($r^2 = 0.89$) to final weight. When expressing the polar lipid classes as a percentage of total polar lipid (data not shown), no effect of the dietary PL composition on that of the fish was detected.

Fish fatty acids of total and polar lipid

In order to visualize the fatty acid composition of the diets and of fish total and polar lipid throughout the feeding trial, a first principal component analysis was performed. The analysis was based on specific individual fatty acids (FA) of the *n*-3 (18:3*n*-3, 20:5*n*-3 and 22:6*n*-3) and *n*-6 (18:2*n*-6 and 20:4*n*-6) series and on the sum of saturated and monoenoic fatty acids. Figure 1 represents the principal component plot of all the data, i.e. the nine experimental diets and the total and polar lipid of fish at six sampling times (fish ages in days): initial (44 d), weaned (55 d), intermediate (62, 69 and 76 d), and final (84 d) sampling. The successive samplings are represented in the plot as A, B, C, D and E, respectively. The first axis explained 38% of the variance and separated the sea bass into three groups according to sampling time. The first group consisted of the initial fish, the second of the weaned fish and the third overlapping group of the intermediate and the final fish. The second axis (25%) separated the diets from the fish. Within the diet group a distinction was clear according to the presence and origin of the PL source. For designating the dietary influence on fish lipid, a second principal component analysis was performed on fish sampled during the last 3 weeks (at the ages of 69, 76 and 84 d) of

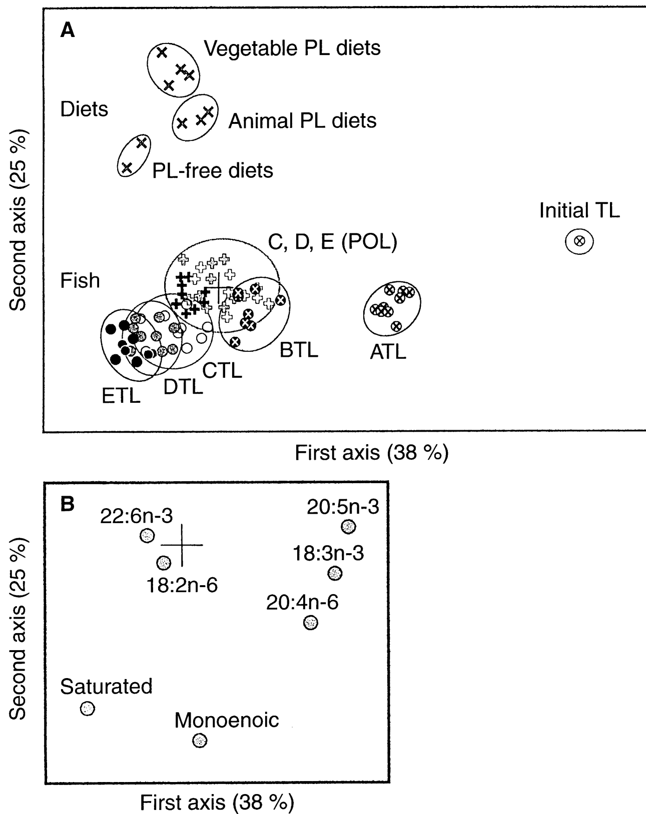

Fig. 1 *Dicentrarchus labrax*. Principal component analysis based on sum saturated fatty acids (FA), sum monoenoic FA, *n*-6 FA (18:2*n*-6, 20:4*n*-6) and *n*-3 FA (18:3*n*-3, 20:5*n*-3, 22:6*n*-3). **A** Diets are represented in the upper part and fish total lipid (*TL*) and polar lipid (*POL*) in the lower part. The plot shows the evolution (from right to left) of the successive samples: Initial, A, B, C, D and E, corresponding to ages 44, 55, 62, 69, 76 and 84 d post-hatch. **B** The corresponding factor loading plot shows which FA contributed most to the dispersion of the groups, i.e. along the first axis, the FA characteristic for the initial *Artemia*-fed fish (20:5*n*-3, 18:3*n*-3, 20:4*n*-6) and along the second axis the saturated and monoenoic FA

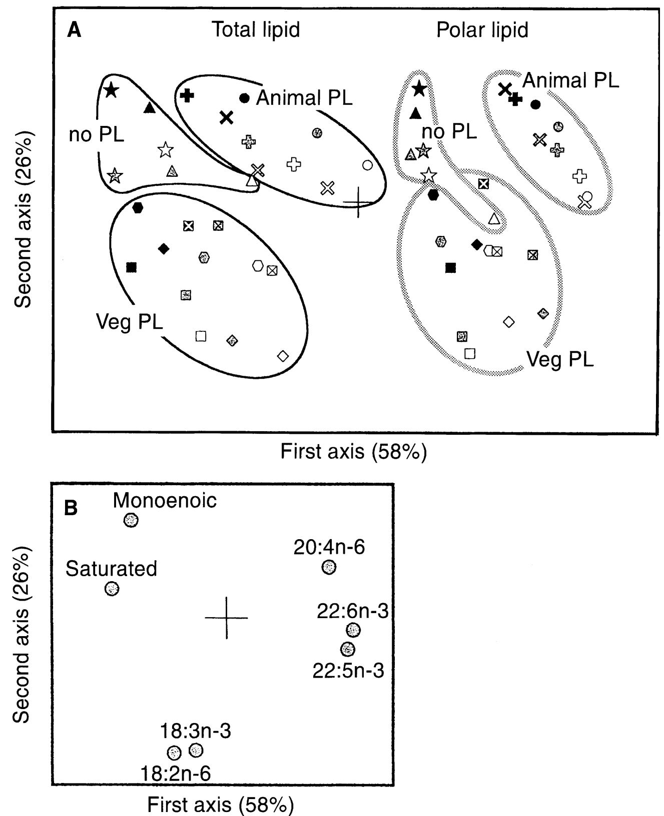

Fig. 2 *Dicentrarchus labrax*. Principal component analysis based on identical FA as in Fig. 1. **A** The plot shows different subgroups according to the lipid fraction (total and polar), the age at sampling: 69 d (*open symbols*), 76 d (*shaded*) and 84 d (*filled*), and the dietary treatments: animal PL diets: E-PL (✖), E-PC (✚), M-PL (●); vegetable PL diets: Sf-PL (☒), S-PL (◆), S-PC (■), Ly-PL (○) and PL-free diets EM0.5 (★), EM2.0 (▲). **B** The factor-loading plot illustrates the FA responsible for separation of total and polar lipids along the first axis and diets along the second axis

the experiment (Fig. 2). Along the first axis (58%) there was a marked separation of fish total lipid from polar lipid. The second axis (26%) divided both the fish total and polar lipid into three similar subgroups according to the type of PL supplementation. Within the latter subgroups a gradient was observed referring to sampling times.

Complete fatty acid analyses are shown for fish fed diets EM0.5, S-PC, E-PC and M-PL, representing four extreme diets at four of the six consecutive sampling times (fish ages in days): initial (44 d), weaned (55 d), 2 weeks after weaning (69 d) and final (84 d). Table 3 shows the changes due to the weaning. There was a strong decrease in 20:4*n*-6, 18:3*n*-3 and 20:5*n*-3, as also shown in Fig. 1, but also in other *n*-3 PUFA (polyunsaturated fatty acids), e.g. 18:4*n*-3, 20:3*n*-3 and 20:4*n*-3. Levels of saturated fatty acids, *n*-6 PUFA and DHA (docosahexaenoic acid) increased strongly by weaning the fish on the dry diets (Table 3). Two weeks after weaning, the proportion of saturated FA rose from 22% to approximately 40 and 35% of fish total and polar FA, respectively (Table 4). These levels remained unchanged during the rest of the experiment and were only slightly affected by dietary levels (Table 5).

Feeding the soybean PC (diet S-PC) resulted in an important increase in 18:2*n*-6, reducing the total lipid *n*-3/*n*-6 ratio from 4.5 to 1.5. Feeding the animal PL sources (diets E-PC and M-PL) increased the level of arachidonic acid in the fish polar lipids (Tables 4, 5). The DHA/EPA (eicosapentaenoic acid) ratio raised from 0.4 to 0.9–1.1 during weaning (Table 3) and reached final values of 2.4 to 2.7 in total lipid and 3.1 to 3.4 in polar lipid (Tables 4, 5). This was related to the continuous decrease of EPA, which dropped in total lipid from 14.7 to 4.2–5.0%, combined with an increase of DHA. Noteworthy is the higher DHA in fish fed the PL-supplemented diets as compared to PL-deprived fish, which was evidenced already at the end of weaning (Table 3). Total lipid of the latter fish contained a significantly higher proportion of monoenoic FA. Figure 3 shows that the proportion of DHA in total lipid of each dietary group decreased after a strong initial increase. This

Table 3 *Dicentrarchus labrax*. Fatty acid composition (% fatty acids) of total lipid of fish prior to weaning (initial fish, 44 d) and at the end of the weaning period (55 d) for fish fed diets EM0.5, S-PC, E-PC and M-PL. Sums include minor fatty acids not shown in table. The coefficient of variation for each individual fatty acid was always < 5%. Values sharing a common superscript in the same row are not significantly different ($P < 0.05$) (*tr* trace amount, i.e. < 0.1)

Fatty acid	Total lipid (44-d-old fish)	Total lipid (55-d-old fish)			
	Artemia	EM0.5	S-PC	E-PC	M-PL
14:0	0.5	4.7	4.5	4.6	4.7
16:0	12.9	17.7^{ab}	17.0^{b}	18.2^{a}	17.0^{b}
18:0	6.6	8.1	7.5	7.9	7.6
16:1*n*-7	2.0	2.2	2.0	2.2	2.4
18:1*n*-9	16.3	17.6^{a}	15.3^{c}	16.2^{b}	14.8^{c}
18:1*n*-7	6.7	4.6	4.2	4.3	4.6
20:1*n*-9	1.5	1.8	1.5	1.6	1.8
24:1*n*-9	0.2	0.5	0.3	0.4	0.5
18:2*n*-6	5.2	5.6^{c}	9.4^{a}	6.5^{b}	5.3^{c}
18:3*n*-6	0.5	0.1^{b}	0.5^{a}	0.4^{a}	0.2^{b}
20:3*n*-6	0.3	0.2	0.2	0.2	0.4
20:4*n*-6	2.5	2.1	2.0	2.2	2.1
22:4*n*-6	0.1	tr	tr	0.1	0.1
22:5*n*-6	0.4	0.2	0.3	0.5	0.2
18:3*n*-3	13.8	6.3^{a}	6.2^{a}	5.8^{b}	5.7^{b}
18:4*n*-3	2.0	1.1	0.9	1.1	1.2
20:3*n*-3	0.9	0.5	0.4	0.4	0.4
20:4*n*-3	1.2	0.4	0.6	0.6	0.5
20:5*n*-3	14.7	9.7^{c}	10.7^{b}	10.2^{bc}	11.6^{a}
22:5*n*-3	1.5	1.4	1.5	1.5	1.7
22:6*n*-3	6.4	9.0^{c}	10.2^{b}	10.6^{b}	12.2^{a}
Saturates	22.1	32.3^{a}	30.9^{b}	32.2^{a}	31.1^{ab}
Monoenoics	28.5	30.8^{a}	26.4^{c}	27.7^{b}	27.0^{bc}
n-6 PUFA	9.1	8.4^{c}	12.3^{a}	10.0^{b}	8.7^{c}
n-6 HUFA	3.3	2.4^{b}	2.4^{b}	3.1^{a}	2.9^{a}
n-3 PUFA	40.4	28.5^{c}	30.6^{b}	30.2^{b}	33.3^{a}
n-3 HUFA	24.6	21.1^{c}	23.4^{b}	23.4^{b}	26.4^{a}
n-3/*n*-6 PUFA	4.5	3.4^{a}	2.5^{b}	3.0^{ab}	3.8^{a}
DHA/EPA	0.4	0.9	0.9	1.1	1.1

change in DHA content was not observed in the polar lipid fraction where the DHA continued to accumulate, reaching a final level of twice that in total lipid (Tables 4, 5). Lipid of fish fed the marine PL (diet M-PL) had the highest DHA level, in accordance to the higher dietary level (Fig. 3). Despite the similar DHA content in the other diets, PL-deprived fish had accumulated less DHA than other fish (Fig. 3). Throughout the experiment, a positive correlation could be established between the DHA level in the diet and that in the fish, irrespective the origin of the PL-source (Fig. 4). However, this regression was not followed by the fish fed the PL-free diets and diet Ly-PL. The latter fish contained significantly less DHA than fish fed the PL-supplemented diets with comparable DHA content. The lower DHA retention in the EM0.5 fish as compared to, e.g., the E-PC fish was also demonstrated by a comparison of the *n*-3/*n*-6 ratios in diets and in fish.

Discussion

Present results showed that vegetable and animal PL are both equally effective in improving survival as compared to the PL-free diets. An exception here was the diet supplemented with the hydrolyzed PL (lyso PL) which did not perform as well as the other PL diets. Data on growth were less conclusive, due to the high variability between replicate tanks. However, in comparison to either EM0.5 or EM2.0 groups, the final weight of the fish was significantly increased by supplementing the diet with sunflower PL, which is free of *n*-3 and *n*-6 HUFA. Similar results have been observed in carp (*Cyprinus carpio*) larvae (Geurden et al. 1995). This confirms for sea bass, a marine fish, that dietary PL exert a beneficial effect on survival and growth without providing any supplementary essential fatty acids, as reported before for freshwater ayu (*Plecoglossus altivelis*) by Kanazawa et al. (1985) and later emphasized by Sargent et al. (1993). The levels of *n*-3 HUFA provided through ethyl esters in present diets were sufficient to meet the *n*-3 essential fatty acid requirement of postlarval sea bass (Coutteau et al. 1996). Therefore, a supplementary provision of these fatty acids was not expected to improve survival or growth. This was confirmed in the non-superiority of diet M-PL with the highest *n*-3 HUFA content. As noted before (Geurden et al. 1997a), the similarity in results between purified and less purified PL sources suggests that the observed PL effect was not due to the other lipids (mainly glycolipids) present in the less purified PL sources.

Although it seems now well established that a positive PL effect can be obtained without essential fatty acids in the PL-acyl groups, the nature of the latter is not always indifferent, possibly in relation to physical rather than nutritional properties. In this respect, the literature indicates two types of PL which do not enhance growth and survival in fish as efficiently as other PL sources, i.e. saturated PC (Kanazawa et al. 1985; Geurden et al. 1997a) and lyso PL (Geurden et al. 1995). The poor efficiency of lyso soybean PL was verified in the present study for *Dicentrarchus labrax*. In fish, there is a lack of information on intestinal extracellular phospholipase A_2 activity (Henderson and Tocher 1987), as well as on the absorption mechanisms of phospholipidic components. Nevertheless, when feeding di-^{14}C oleic PC to carp, Iijima et al. (1990) found that the radioactivity in the *sn*-1 position of plasma PC was twice that found in the *sn*-2 position, suggesting absorption of PL in the lyso *sn*-1 acyl form. Consequently, we hypothesized that, in analogy to mammals (Gurr and Harwood 1991), the uptake of the pre-hydrolyzed PL by the intestinal absorptive cells would be more quick and complete, resulting in a more pronounced PL effect. This was however not confirmed by a higher growth of the sea bass fed the lyso PL. On the other hand, Stafford and Dennis (1988) reported a toxic effect of lyso PL on the membrane system. In the present study, diets containing lyso PL did not appear

Table 4 *Dicentrarchus labrax*. Fatty acid composition (% fatty acids) of total and polar lipid of sea bass 2 weeks after the weaning period fed diets EM0.5, S-PC, E-PC and M-PL. Sums include minor fatty acids not shown in table. The coefficient of variation for each individual fatty acid was always < 5%. Values for total and polar lipid sharing a common superscript in the same row are not significantly different ($P < 0.05$) (*tr* trace amount, i.e. < 0.1)

Fatty acid	Total lipid (69-d-old fish)				Polar lipid (69-d-old fish)			
	EM0.5	S-PC	E-PC	M-PL	EM0.5	S-PC	E-PC	M-PL
14:0	8.6^{a}	8.1^{a}	7.4^{b}	7.6^{b}	3.6^{a}	3.4^{a}	2.7^{b}	3.0^{ab}
16:0	24.7	23.2	23.0	22.2	22.8^{a}	21.9^{bc}	21.4^{c}	22.2^{b}
18:0	6.9^{b}	7.5^{b}	8.3^{a}	7.9^{ab}	9.4	9.3	9.5	9.8
16:1*n*-7	5.5^{a}	3.2^{b}	3.3^{b}	3.1^{b}	4.7^{a}	2.6^{b}	2.7^{b}	2.5^{b}
18:1*n*-9	22.6^{a}	17.5^{c}	20.1^{b}	18.3^{bc}	17.7^{a}	12.9^{c}	14.3^{b}	14.7^{b}
18:1*n*-7	2.1	1.6	1.7	2.0	2.0	1.5	1.7	1.9
20:1*n*-9	1.6	1.1	1.4	1.7	1.3	0.9	1.1	1.1
22:1*n*-9	0.3	0.3	0.2	0.4	0.1	–	tr	0.1
24:1*n*-9	0.3	0.1	0.3	0.4	0.7	0.5	0.6	0.7
18:2*n*-6	6.1^{c}	12.1^{a}	7.0^{b}	5.7^{c}	5.1^{bc}	11.4^{a}	6.7^{b}	4.6^{c}
18:3*n*-6	0.3	0.3	0.3	0.1	0.3	0.4	0.2	0.2
20:3*n*-6	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
20:4*n*-6	0.6^{b}	0.6^{b}	1.4^{a}	1.3^{a}	1.3^{b}	1.0^{b}	2.4^{a}	1.9^{ab}
22:4*n*-6	tr	0.1	0.2	0.2	0.1	0.1	0.2	0.1
22:5*n*-6	0.2	0.1	0.4	0.3	0.4	0.4	0.7	0.6
18:3*n*-3	0.9^{b}	1.5^{a}	0.9^{b}	0.9^{b}	1.1	1.3	0.9	1.0
18:4*n*-3	0.3	0.3	0.4	0.3	0.2	0.2	0.2	0.2
20:4*n*-3	0.1	0.1	0.2	0.2	0.2	0.1	–	0.2
20:5*n*-3	5.2^{b}	5.4^{b}	5.7^{b}	6.8^{a}	7.1^{b}	7.4^{b}	7.5^{b}	8.6^{a}
22:5*n*-3	1.0^{b}	1.1^{b}	1.2^{b}	1.7^{a}	1.2^{b}	1.5^{ab}	1.7^{a}	1.7^{a}
22:6*n*-3	10.6^{c}	13.5^{b}	13.6^{b}	16.0^{a}	17.1^{c}	21.4^{b}	21.9^{ab}	23.0^{a}
Saturates	40.6^{a}	39.4^{b}	39.4^{b}	38.5^{c}	36.6^{a}	35.3^{b}	34.6^{c}	35.8^{b}
Monoenoics	33.5^{a}	25.1^{c}	28.7^{b}	27.7^{b}	28.5^{a}	19.7^{c}	22.7^{b}	22.4^{b}
n-6 PUFA	7.7^{c}	13.3^{a}	9.5^{b}	7.7^{c}	7.5^{c}	13.4^{a}	9.0^{b}	7.5^{c}
n-6 HUFA	0.9^{b}	0.9^{b}	2.1^{a}	2.0^{a}	2.0^{bc}	1.7^{c}	3.5^{a}	2.7^{b}
n-3 PUFA	18.2^{c}	22.2^{b}	22.3^{b}	26.1^{a}	27.2^{c}	31.9^{b}	32.3^{b}	34.7^{a}
n-3 HUFA	17.0^{c}	20.3^{b}	20.8^{b}	24.9^{a}	26.2^{c}	30.6^{b}	31.2^{b}	33.5^{a}
n-3/*n*-6 PUFA	2.4^{b}	1.7^{c}	2.4^{b}	3.4^{a}	3.6^{b}	2.4^{c}	3.6^{b}	4.7a
DHA/EPA	2.0	2.5	2.4	2.4	2.4	2.9	2.9	2.7

harmful as compared to the PL-free diets, excluding a toxic effect at least for the levels used. Also for early carp larvae lyso PL was not deleterious, as it yielded a growth intermediate to that of larvae fed the original soybean PL and no PL (Geurden et al. 1995).

The initial rationale for testing the lyso PL was related to its increased oil/water emulsifying properties. Koven et al. (1993) showed a higher incorporation of labeled oleic acid in 21-d-old seabream (*Sparus aurata*) due to supplementing soybean lecithin to the diet. The latter authors suggested that the lecithin stimulated the absorption of dietary neutral lipid through its function as emulsifier, complementary to bile secretion which would be limited at this stage. According to present results an increased emulsification is not sufficient to explain the enhanced lipid absorption observed with PL diets. This is related to the fact that lyso PL was inferior to the other PL and because the substitution of the phospholipids by an emulsifier blend did not replace or imitate the PL effect. Kanazawa et al. (1985) also showed that the addition of taurocholic acid to diets of 70-d-old ayu had no effect compared to a PL-free diet. Furthermore, the 40-d-old sea bass have a fully operational digestive system (Person Le Ruyet et al. 1993), a situation in which phospholipids should not be limiting for appropriate micelle formation in the gut lumen.

The lipid class composition of the initial sea bass resembled that given in Mourente et al. (1993) for 15-d-old seabream. At the end of the feeding period, both lipid content and lipid class profiles were affected by the final size of the fish, which in turn was affected by the dietary treatment. The ratio of adipose to membrane lipids, approximated by TAG/PC + PE, was positively correlated to the weight gain of the fish, in agreement with previous studies which used the lipid class composition as an indicator of the fish nutritional status (Fraser et al. 1987; Häkanson 1989). A final neutral lipid content of as much as 76 to 88% of total lipid, consisting mainly of triacylglycerol, is in accordance to values reported for juvenile seabream (Mourente and Tocher 1993) and adult European sea bass (McClelland et al. 1995). The latter fish species have similar life-styles and store their lipids principally in organs other than muscle, i.e. liver and adipose tissue (McClelland et al. 1995).

The increase of neutral lipid with respect to polar lipid during growth induced important changes in the fatty acid composition of the total lipid. This was illustrated for instance in the relative level of total lipid DHA

Table 5 *Dicentrarchus labrax*. Fatty acid composition (% fatty acids) of total and polar lipid of sea bass at the end of the experiment fed diets EM0.5, S-PC, E-PC and M-PL. Sums include minor fatty acids not shown in table. The coefficient of variation for each individual fatty acid was always <5%. Values for total and polar lipid sharing a common superscript in the same row are not significantly different ($P < 0.05$) (*tr* trace amount, i.e. <0.1)

Fatty acid	Total lipid (84-d-old fish)				Polar lipid (84-d-old fish)			
	EM0.5	S-PC	E-PC	M-PL	EM0.5	S-PC	E-PC	M-PL
14:0	8.2	8.3	7.7	7.5	3.8	3.6	3.5	3.4
16:0	24.9	24.1	24.9	24.5	22.8	22.3	22.6	22.3
18:0	6.8	7.3	7.4	7.8	9.0	9.4	9.4	9.4
16:1*n*-7	6.2^{a}	4.0^{b}	4.6^{b}	4.0^{b}	5.4^{a}	3.1^{b}	3.6^{b}	3.6^{b}
18:1*n*-9	24.0^{a}	1.3^{b}	23.1^{b}	22.7^{b}	19.1^{a}	15.2^{c}	16.8^{b}	16.6^{b}
18:1*n*-7	1.9^{a}	1.3^{b}	1.5^{b}	1.4^{b}	1.5	1.1	1.2	1.3
20:1*n*-9	1.6	1.0	1.3	1.3	0.8	0.6	0.8	0.9
22:1*n*-9	0.4	0.2	0.3	0.2	0.1	0.1	0.1	0.1
24:1*n*-9	0.4	0.1	0.2	0.3	1.3^{a}	0.8^{b}	0.9^{b}	1.3^{a}
18:2*n*-6	6.2^{c}	11.8^{a}	7.1^{b}	5.8^{c}	4.4^{b}	9.5^{a}	5.0^{b}	4.2^{b}
18:3*n*-6	0.3	0.4	0.4	0.3	0.2	0.3	0.2	0.1
20:3*n*-6	0.1	0.1	tr	0.1	0.1	0.1	0.2	–
20:4*n*-6	0.5^{b}	0.4^{b}	1.0^{a}	1.1^{a}	1.1^{b}	1.0^{b}	1.8^{a}	1.5^{ab}
22:4*n*-6	–	–	0.1	0.1	–	tr	0.3	0.1
22:5*n*-6	0.2	0.2	0.3	0.3	0.4	0.4	0.5	0.3
18:3*n*-3	0.6^{b}	1.0^{a}	0.5^{b}	0.6^{b}	0.5^{b}	0.8^{a}	0.4^{b}	0.5^{b}
20:4*n*-3	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.1
20:5*n*-3	4.2^{c}	4.6^{b}	4.5^{b}	5.0^{a}	6.2^{b}	6.6^{b}	6.6^{b}	7.1^{a}
22:5*n*-3	0.9	1.2	1.0	1.3	1.3	1.3	1.4	1.5
22:6*n*-3	9.8^{c}	11.4^{b}	11.3^{b}	13.2^{a}	18.8^{c}	21.7^{b}	22.0^{b}	24.0^{a}
Saturates	40.5	40.5	40.7	40.5	36.5	35.9	36.5	35.5
Monoenoics	36.2^{a}	27.9^{c}	32.6^{b}	31.2^{b}	29.6^{a}	22.1^{c}	24.4^{b}	24.9^{b}
n-6 PUFA	7.3^{c}	12.9^{a}	8.9^{b}	7.8^{c}	6.4^{c}	11.3^{a}	8.0^{b}	6.2^{c}
n-6 HUFA	0.8^{b}	0.7^{b}	1.4^{a}	1.6^{a}	1.6^{bc}	1.5^{c}	2.8^{a}	2.0^{b}
n-3 PUFA	15.9^{d}	18.8^{b}	17.8^{c}	20.4^{a}	27.1^{c}	30.7^{b}	31.0^{b}	33.3^{a}
n-3 HUFA	15.0^{c}	17.5^{b}	17.1^{b}	19.7^{a}	26.5^{c}	29.7^{b}	30.5^{b}	32.7^{a}
n-3/*n*-6 PUFA	2.2^{b}	1.5^{c}	2.0^{b}	2.6^{a}	4.2^{b}	2.7^{c}	3.9^{bc}	5.4^{a}
DHA/EPA	2.3	2.5	2.5	2.6	3.0	3.3	3.3	3.4

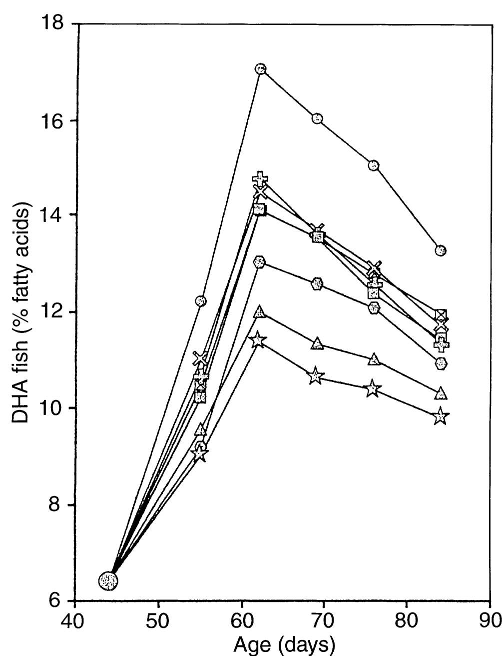

Fig. 3 *Dicentrarchus labrax*. Evolution of DHA level in total lipid as a function of fish age. Data for fish fed S-PL are not presented; symbols for dietary treatments are as in Fig. 2. The coefficient of variation for the individual levels was always <5%

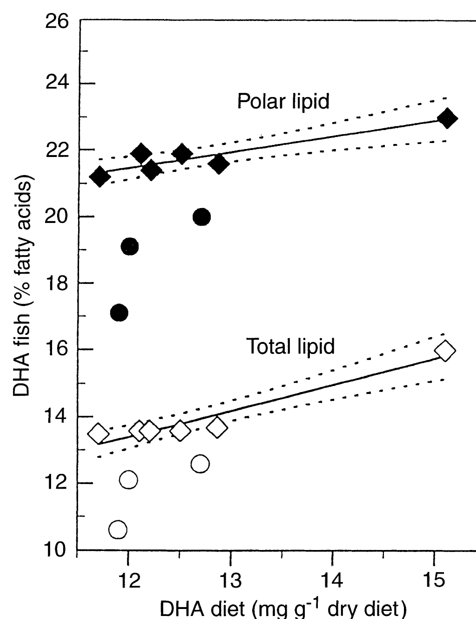

Fig. 4 *Dicentrarchus labrax*. Regression for the level of DHA in total lipid ($r^2 = 0.93$) and in polar lipid ($r^2 = 0.86$) of fish (69 d) fed the PL-supplemented diets and the DHA level in the respective diets (◆). PL-deprived fish and fish fed diet Ly-PL (●) are situated outside the 95% confidence limits

which started to decrease 1 week after weaning due to the diminishing proportion of DHA-rich lipid classes (e.g. PE) in total lipid.

The principal component analyses, based on the fatty acid composition of total and polar lipid of the fish, illustrated the evolution of the weaned postlarvae into different subgroups according to the fatty acid profile of their diets. Similarities between fatty acid profiles of fish lipid and dietary lipid have been reported repeatedly (Castell 1979; Watanabe 1982; Henderson and Tocher 1987), and principal component analysis is a useful tool for demonstrating that relation (Hove and Grahl-Nielsen 1991; Lie et al. 1993).

In marine fish, the enzymatic capacities of elongation and desaturation of shorter chain *n*-3 fatty acids to longer chain *n*-3 HUFA are very limited (Sargent et al. 1993). In wild populations of marine fish larvae, *n*-3 HUFA are provided by natural prey consumption. In commercial aquaculture, however, the most commonly used *Artemia* strains contain relatively low levels of DHA. Subsequently, weaning the fish onto a DHA-rich diet causes a drastic increase in DHA, as reported in turbot brain (Mourente et al. 1991). Also in weaned sea bass, tissue levels of DHA are well correlated with dietary DHA levels (Coutteau et al. 1996). In the present study such a correlation could be established only for groups fed the PL-supplemented diets, with the exception of the lyso PL diet (diet Ly-PL). It was clearly evidenced that for a similar dietary DHA level, the PL-free diets and diet Ly-PL resulted in much lower DHA in both the fish total and polar lipid fraction. The above finding confirms the beneficial role of dietary PL in neutral lipid absorption (Koven et al. 1993; Geurden et al. 1997b), but as discussed above, by a mechanism other than enhanced luminal emulsification. The small supplementary provision of DHA by the PL from egg yolk did not noticeably influence the tissue level as compared to the DHA-free vegetable PL. This was questioned in a previous experiment where, similarly, DHA levels in sea bass and turbot tissue increased through the addition of egg yolk PL (Geurden et al. 1997b). In the latter study it was evidenced that the lower DHA in PL-deprived compared to PL-fed fish was not due to a delay in accumulation related to the lower growth rate of the former. Hence, it can be speculated that the higher DHA level in fish fed the PL diets is due to an enhanced utilization of the DHA ethyl esters in these diets. An in vitro study conducted with rat cells showed a stimulation of the synthesis of intestinal lipoproteins by luminal PL (Field and Mathur 1995). Thus, an enhanced export of the absorbed neutral lipids from the enterocytes could subsequently lead to higher delivery of lipid and, thus, of energy and fatty acids, resulting in enhanced growth.

In contrast to the fatty acids of the *n*-3 series (2% of diet dry weight), only a minor quantity of *n*-6 fatty acids was provided by the neutral lipid fraction (0.3% of diet dry weight), which might explain the failure to detect an effect of PL-supplementation on the absorption and incorporation of the *n*-6 dietary neutral lipid fatty acids in the fish.

In conclusion, present results demonstrated a beneficial effect of dietary PL, derived from vegetable as well as animal origin, on essential fatty acid incorporation in postlarval sea bass. Because most of the tested PL were mixtures of several classes, it would be of further interest to examine the efficiency of the distinct PL classes for marine fish, parallel to recent data obtained for start-feeding carp larvae (Geurden et al. 1997a, c).

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