Different phosphatidylcholine and n-3 HUFA contents in microdiets for gilthead seabream (Sparus aurata) larvae: effects on histological changes in intestine and liver

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Suggested running title: Phosphatidylcholine and n-3 HUFA decrease intestinal and hepatic steatosis

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Abstract

The aim of the present study was to study the effect of different dietary phospholipids derived from Krill (KPL) and soybean lecithin (SBL) with different levels of phosphatidylcholine (PC) and n-3 HUFA on the performance and histological changes in intestine and liver of seabream larvae. Sea bream larvae (16 dph) were fed for one month five microdiets formulated by using two different sources of phospholipids (Control, 7KPL, 9KPL, 7SBL and 9SBL). The larvae fed dietary KPL rich in phosphatidylcholine and n-3 PUFA showed better performance in terms of survival and growth than those fed SBL. The inclusion of KPL up to 7% PL (3.8% PC and 7.7% n-3HUFA) in diet was sufficient enough to significantly improve larval survival and growth compared to the highest dietary 9% PL of SBL (2.5% PC and 5.6% n-3HUFA) due to the higher content of PC and n-3 HUFA in dietary KPL. The larval performance in terms of survival and total length was positively correlated to dietary PC and n-3HUFA contents. Also, inclusion of SBL was associated with a higher relative accumulation of lipid droplets in the supranuclear zone of the enterocytes (intestinal steatosis) and in hepatocytes (hepatic steatosis) compared to larvae fed dietary KPL. The intestinal and hepatic steatosis were negatively correlated to dietary PC content. In conclusion, dietary 7 % KPL (3.8 % PC and 7.7% n-3 HUFA) improved significantly the larval performance and decreased significantly the intestinal and hepatic steatosis compared to SBL, denoting better dietary lipid absorption, transportation and energetic utilization.

Introduction

Phospholipids can be an important source of energy (fatty acids) in fish, particularly during embryonic and early larval development in species that produce phospholipid rich eggs (Tocher, 1995, Salhi *et al*., 1999; Saleh *et al*., 2013a,b, Zhu et al., 2019). Furthermore, larval fish at first feeding may be predisposed to digestion and metabolism of phospholipids as well as the use of fatty acids from phospholipids for energy (Sargent, McEvoy & Bell, 1997, Tocher 2008) due to their high content of omega-3 (n-3) long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) acids that are essential fatty acids

(EFA) for marine fish development as they have limited capacity to synthesize them from de novo (Bell., McEvoy, Estevez, Shields & Sargent, 2003). Dietary phospholipids tend to be a richer source of EFA specially n-3 HUFA than neutral lipids such as triacylglycerols (Tocher, 1995). In addition, phospholipids are better than neutral lipids as a source of EFA in fish larvae due to their good digestibility (Sargent, McEvoy & Bell, 1997). In this regard Salhi et al., (1999) and Gisbert, et al., (2005) concluded that seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) larvae can utilize dietary essential PUFA contained in the phospholipids fraction better than those from the neutral lipid (NL) fraction, based on growth, survival, histological organization of the liver and intestine, as well as the digestive enzymes activity.

Phospholipids have been shown that have feed attractant properties (Harada, 1987; Koven et al., 2001), improve diet quality, mainly palatability (Tocher et al. 2008), have antioxidant prosperities (McEvoy et al., 1995; Saleh et al., 2014), have been suggested that they may help to reduce leaching of water-soluble micronutrients (minerals and vitamins) from semi-purified diets (Coutteau et al., 1997), and enhance feeding activity and diet ingestion rate (Koven et al., 1998, Saleh et al., 2012a,b)

Histology has been used to describe tissue changes and pathology in the liver, pancreas and intestine of fish fed different dietary phospholipids compositions (Olsen et al., 1999; Crespo et al. 2001; Caballero et al. 2003; Caballero et al. 2004; Wold et al., 2008). Caballero et al., (2004) and Gisbert et al., (2005) found that hepatocytes are sensitive to dietary phospholipids deficiency in histological studies and such deficiencies may result in steatosis. Furthermore, dietary phospholipids seem to promote lipid absorption and transport, as well as decrease the accumulation of lipid droplets in the enterocytes (Diaz et al., 1997; Izquierdo et al., 2000; Olsen et al., 2003) and hepatocytes (Wold et

al., 2009). Furthermore, it has been suggested that phospholipids contribute to the intestinal absorption of lipids by their emulsifying properties and compensate for the presumed insufficient biliary secretion of larvae (Kanazawa et al., 1985). Liu *et al*., (2002) demonstrated that dietary soybean lecithin markedly increases the appearance of lipoproteins in larval gut, enhancing lipid transport that decrease the intestinal and hepatic steatosis in gilthead sea bream.

Previous studies have shown that phospholipids are effective in the larval performance by providing inositol, choline that are important in increasing the absorption of nutrients (Geurden et al. 1998). Also, It has been reported that dietary phospholipids can promote body growth by providing phosphatidylcholine that improved the lipid transport with a growth promoting effect (Tocher et al., 2008; Saleh et al., 2014, Zhu et al., 2019).

The aim of the present study was to study the effects of different dietary phospholipids sources with different n-3 HUFA and PC contents on the seabream larval performance and the histological changes in intestine and liver.

Materials and methods

Gilthead seabream larvae were obtained from natural spawning (Grupo de Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria (ULPGC). The larval initial total length was 5.4 ±0.7 mm; dry body weight was 123±30 µg. The seabream larvae previously fed from 4 days post hatching (dph) upon rotifers (*Brachinous plicatilis*) enriched with DHA Protein Selco® (INVE, Dendermond, Belgium) until they reached 16 dph, then randomly distributed in 15 experimental tanks ((200 L) at a density of 2100 larvae $tank^{-1}$ and fed one of the experimental weaning diets tested in triplicates for one month. Water was continuously aerated (125 ml min^{-1}) attaining 6.5 ± 1 ppm dissolved O_2. Average water temperature and pH along the trial were 19.3 ± 2.0 °C and 7.85, respectively. Photoperiod was kept at 12h light: 12h dark,

by fluorescent daylights and the light intensity was kept at 1700 lux (digital Lux Tester YF-1065, Powertech Rentals, Western Australia, Australia).

Five experimental microdiets (pellet size 250-500 µm) with increasing phospholipid contents of krill phospholipids (KPL; Qrill oil, Aker BioMarine, Fjordalléen, Norway) and soybean lecithin (SBL; Agramar S.A., Spain) were formulated. Their formulation and proximate analysis are shown in Table 1. The microdiets were prepared by mixing squid powder and water-soluble components, then the lipids and fat-soluble vitamins and, finally, gelatin dissolved in warm water. The paste was compressed and pelleted (Severin, Suderm, Germany), dried in an oven at 38 ºC for 24 h (Ako, Barcelona, Spain). Pellets were ground (Braun, Kronberg, Germany) and sieved (Filtra, Barcelona, Spain) to obtain a particle size between 250 to 500 µm. Diets were prepared and analyzed for proximate composition at GIA laboratories. Moisture, protein (A.O.A.C., 1995), and lipid (Folch, Lees & Stanley, 1957) contents of diets were analyzed.

Diets were manually added fourteen times per day each 45 min from 9:00 to 19:00 for one month. Non-enriched rotifers were co-fed during days 16^{th} and 17^{th} (1 rotifer ml^{-1}). To assure feed availability, daily feed (pellet size 250 µm) supplied was maintained at 1.5 and 2.5 g per tank during the first and second week of feeding, then, was gradually increased to 4-5 g per tank with increasing in pellet size to 500µm, where an overlap using a mixture of both pellets sizes was conducted during the third and fourth week of feeding. Larvae were observed under the binocular microscope to determine feed acceptance.

Histopathological analysis

Thirty larvae per tank were collected at the end of the feeding trial and fixed in 4% buffered formalin for one day, dehydrated through graded alcohols, then xylene and finally embedded in paraffin wax. Six paraffin blocks containing 5 larvae per tank were cut at 4μm on a microtome (Leica, RM2135, Leica Instruments, Nussloch, Germany), the sections stained with hematoxylin and eosin (Martoja and Martoja-Pearson, 1970) for histopathological evaluation. All the sections were evaluated under light microscopy using a binocular microscope Olympus CX41 (Olympus, Hamburg, Germany) connected to a camera Olympus XC30 (Olympus, Hamburg, Germany), which was connected to a computer using image capturing software (CellB®, Olympus, Hamburg, Germany). All observations were performed in the liver and the anterior and posterior part of the intestine. Tissue morphology was evaluated by two scientists unaware of the dietary treatments following the incidence degree classification in Table 2. The degree of intestinal and hepatic steatosis incidence were measured as relative percentage when clear vacuoles were observed in the supranuclear zone of the enterocytes and in hepatocytes, in addition, hepatocytes nuclei displacement, hepatocyte morphology.

Statistical analysis

All data were tested for normality and homogeneity of variances. Means and standard deviations were calculated for each parameter measured. Data were submitted to a one-way analysis of variance (ANOVA) and significant differences were considered when $p < 0.05$. When F values were significant, individual means were compared using post hoc Tukey tor Games-Howell test for multiple means comparison. Analyses were performed using the SPSS Statistical Software System v21.0 (SPSS, Chicago, IL,USA).

Results

The larval performance in terms of survival and growth was different between the treatments. The supplementation of dietary KPL rich in n-3 HUFA and PC improved significantly ($P < 0.05$) the larval survival and growth in terms of total length compared to larvae fed SBL and control diets (Fig. 1&2). The correlation between dietary n-3 HUFA of KPL treatments and larval survival showed a high positive correlation ($y = 4.7124x - 2.5406$, $R^2 = 0.9479$) as shown by their significantly ($p < 0.05$) higher survival (39 %) compared to the larvae fed dietary SBL (29% survival) with lower n-3 HUFA content which presented less positive correlation ($y = 7.0426x - 10.389$, $R^2 = 0.7818$).

Also, the correlation between dietary n-3 HUFA and larval total length showed a positive correlation ($y = 0.6789x + 6.0118$, $R^2 = 0.9695$) where the larval fed 9.3 % n-3 HUFA dietary KPL showed significantly ($p < 0.05$) better growth in terms of total length compared to the larvae fed dietary SBL with 5.6 % n-3 HUFA. For the correlation between phosphatidylcholine and each of survival and growth (Fig. 3) showed that both were positively correlated to PC content ($y = 0.9327x + 0.9841$, $R^2 = 0.8877$; $y = 0.142x + 5.9878$, $R^2 = 0.817$, respectively), where the larvae fed higher dietary PC (4%) of KPL treatments showed significantly ($p < 0.05$) better survival and growth compared to the lower dietary PC (2.5 %) of SBL treatments.

Histological evaluation of the liver and intestine of 44 dph larvae revealed the presence of significantly ($P < 0.05$) high numerous of large vacuoles of lipid droplets within the enterocytes and hepatocytes of larvae fed both control and SBL diets compared to the larvae fed dietary KPL (Fig. 4) that displayed a denser appearance of both enterocytes and hepatocytes (Fig. 5 & 6). Also, the correlation between dietary PC and each of intestinal steatosis and hepatic steatosis relative percentage (Fig. 7) showed that the

intestinal steatosis and hepatic steatosis were negatively correlated to dietary PC content ($y = -1.5345x + 75.728$, $R^2 = 0.9745$; $y = -1.6576x + 88.096$, $R^2 = 0.9779$, respectively).

Discussion

In the present study, feeding gilthead sea bream larvae from 16 to 44 dah microdiets with different levels and sources of PL has shown higher effectiveness of dietary KPL rich in phosphatidylcholine and n-3 HUFA on larval performance in terms of survival and growth compared to dietary SBL, in addition to the preventing effect of intestinal and hepatic steatosis denoting the better utilization of dietary lipids by larvae fed KPL diets compared to larvae fed SBL and control diets. Similar results found by Betancor *et al.*, (2012) that showed the inclusion of marine PL improved larval performance due to the better dietary lipid utilization. In this sense, the dietary KPL is rich in phosphatidylcholine and n-3 HUFA which have a promoting effect on larval performance in terms of survival and growth because both of them were positively correlated ($R^2 = 0.8877$ and $R^2 = 0.817$, respectively) to the dietary PC and n-3 HUFA contents. They constitute important sources of essential fatty acids that have a crucial role in maintaining the structure and function of cellular membranes (Tocher, 2003). Phosphatidylcholine have an important role in dietary lipid absorption and transportation, Moreover, it stimulate lipoprotein synthesis in intestinal enterocytes (Fontagné et al., 1998; Geurden et al., 1998b; Liu et al., 2002) and play an important role in the transport and assimilation of dietary lipids (Izquierdo et al., 2001) which in turn decreases the accumulation of lipid droplets in the enterocytes and hepatocytes that lead to better utilization of dietary nutrients and consequently resulted in better growth

and survival of fish larvae (Kanazawa et al., 1985, Caballero et al., 2003, Tocher et al., 2008, Saleh et al., 2014).

The results of histological study in the present work demonstrated high accumulation of numerous lipid droplets within the enterocytes and hepatocytes of larvae fed control and SBL diets compared to larvae fed KPL diets. Kanazawa et al., (1985) explained the nature of PL requirement and the role of these lipids during larval stages and their important role in intestinal absorption of lipids. Thus, based on the morphological aspects of intestinal lipid absorption, two categories were distinguished: on one hand, larvae fed dietary KPL rich in PC and n-3 HUFA showed better absorption of lipids, and, on the other hand, larvae fed control and SBL diets, which are PC-deficient diets showed lipids droplets accumulation. Also, the intestinal and hepatic steatosis were negatively correlated to the dietary PC content ($y = -1.5345x + 75.728$, $R^2 = 0.9745$; $y = -1.6576x + 88.096$, $R^2 = 0.9779$, respectively). This confirm a specific effect of PC for the synthesis and secretion of chylomicrons or VLDL as observed in vitro in a rat intestinal preparation by Field & Mathur (1995), these authors reported a specific effect of PC on the synthesis of apolipoprotein B and thus the prevention of epithelial steatosis. The PC is the major constituent of the polar lipid moiety of all lipoproteins, amounting for up to 95% in VLDL of Atlantic salmon (Lie et al.,, 1993). Numerous studies have shown that larval diets deficient in PL lead to the accumulation of large amounts of lipid vacuoles in the enterocytes, probably due to insufficient lipoprotein synthesis (Diaz et al., 1997; Fontagnè et al., 1998; Olsen et al., 1999; Salhi et al., 1999; Izquierdo et al., 2000). Liu *et al.*, 2002 concluded that inclusion of SBL in diets for gilthead sea bream larvae increased the appearance of lipoproteins in larval gut, enhancing lipid transport and promoting growth and survival, this is in agreement with the present work where the inclusion of SBL up to 9% PL (2.5 % PC & 5.6 % n-3

HUFA) improved significantly larval performance compared to the 7% SBL treatment. Research has shown that lecithin energy, as PL source, is effective in the larval stage by providing choline and inositol for fish growth through increasing the absorption of nutrients (Geurden et al., 1998).

Geurden et al., (1998) and Lu et al., (2008) demonstrated that inclusion of PL especially PC in diets induced high number of goblet cells in the intestine of turbot (S. maximus) and Pelteobagrus fulvidraco that indicates the early maturation of intestine as a result of high dietary PC inclusion. Furthermore, it has been reported that dietary PL can promote somatic growth by increasing the effectiveness of lipid use through emulsification and digestion, increasing lipid transport, and providing phosphatidylcholine with a growth promoting effect (Kasper and Brown 2003; Geurden et al. 1998; Tocher et al. 2008; Zhu et al. 2019).

In conclusion, the present study concluded that dietary 7% KPL rich in PC (3.8 %) and n-3 HUFA (7.7%) decreased significantly the intestinal and hepatic steatosis compared to 9 %SBL (2.5 % PC & 5.6 % n-3HUFA) that in turn led to better larval performance in terms of survival and total length denoting better dietary lipid absorption, transportation and energetic utilization which may be in relation to a good availability of krill nutrients (growth factors, minerals, PL and astaxanthin) and a higher bioactivity of their LC-PUFA.

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Table 1 Formulation and proximate composition of the experimental microdiets containing several levels of either krill phospholipids (KPL) or soybean lecithin (SBL)

Ingredients (g kg^{-1} diet)	**Control**	**7 KPL**	**9 KPL**	**7 SBL**	**9 SBL**
Squid powder[a]	690	690	595	695	695
Krill PL[b]	0	130	210	0	0
Soybean lecithin[c]	0	0	0	45	85
Sardine oil[d]	55	0	0	0	0
Oleic acid[e]	90	15	30	95	55
Basal premix	165	165	165	165	165
Proximate analysis (g kg^{-1} diet)					
Lipid	194	201	221	212	222
Protein	612	589	610	607	615
Ash	72	75	74.3	73.0	73.9
Moisture	87	81	99	78	84
Lipid classes composition					
Phosphatidylethanolamine	16.8	16.8	25.7	23.2	25.9
Phosphatidylinositol	5.6	4.0	8.1	11.7	14.3
Phosphatidylserine	3.9	1.5	6.0	4.7	5.5
Phosphatidylcholine	18.2	39.8	40.4	26	24.6
Lysophosphatidylcholine	1.7	8.9	7.6	2.4	1.3
Total polar lipid	47.2	73.8	93.3	72.7	92.3

a Rieber and Son, Bergen, Norway.
b Qrill oil, Aker BioMarine, Fjordalléen, Norway.
c Agramar S.A., Spain.
d Agramar S.A., Spain.
e Merck KGaA, Darmstadi, Germany.

1 Table 2. Fatty acids (% dry weight) composition in lipid classes of diets containing
2 three dietary PL levels using two different PL sources.

	Control	7 MPL	9 MPL	7 SBL	9 SBL
Phosphatidylcholine					
Saturated	37.07	36.96	32.14	36.14	35.5
Monounsaturated	6.10	11.78	15.95	7.20	11.35
18:2n-6	0.19	1.27	2.44	7.96	19.48
Total n-3	55.58	48.97	48.12	47.77	62.53
ARA	0.38	0.53	0.78	0.35	0.42
EPA	6.76	18.30	29.50	6.08	7.51
DHA	47.86	40.92	52.11	27.14	14.29
Phosphatidylinositol					
Saturated	36.23	45.85	44.78	35.71	47.06
Monounsaturated	19.69	16.24	18.09	19.91	8.85
18:2n-6	20.80	0.82	0.25	20.94	31.93
Total n-3	20.95	34.20	33.50	21.08	11.27
ARA	0.69	1.42	1.77	0.70	0.32
EPA	8.69	17.03	17.61	8.79	3.48
DHA	7.81	15.81	14.46	7.87	3.84
Phosphatidylserine					
Saturated	30.22	34.34	31.01	42.00	34.20
Monounsaturated	18.40	20.72	21.70	17.52	18.53
18:2n-6	6.24	0.85	2.42	6.86	12.53
Total n-3	42.13	41.72	42.69	31.57	32.71
ARA	1.33	1.44	2.21	1.23	1.21
EPA	9.42	12.75	19.61	7.96	7.55
DHA	27.12	26.51	20.73	20.99	22.61
Phosphatidylethanolamine					
Saturated	25.34	23.42	22.23	28.61	24.73
Monounsaturated	13.93	12.89	17.26	11.77	13.84
18:2n-6	19.54	0.37	3.04	9.43	18.66
Total n-3	37.35	56.59	54.71	45.58	38.21
ARA	2.27	3.41	2.28	2.86	2.25
EPA	20.01	30.09	26.08	25.18	19.45
DHA	16.42	24.74	26.99	18.36	15.96

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Table 3. The intestinal and hepatic steatosis evaluation scale

Incidence Degree	Very low	Low	Medium	High
Intestinal Steatosis	+	+ +	+ + +	+ + + +
Hepatic Steatosis	+	+ +	+ + +	+ + + +

1 Table 4. Fatty acids (% total identified fatty acids) composition in polar lipids of larvae
2 fed 5 dietary phospholipid levels. Values (mean ± standard deviation) with the same
3 letters in the same row are not significantly different (P>0.05).

Fatty acids	Control	7 KPL	9 KPL	7 SBL	9 SBL
n-3	30.57^{c}	36.95^{b}	41.8^{a}	30.46^{c}	31.00^{c}
n-6	9.82^{b}	3.44^{d}	5.76^{c}	10.17^{b}	15.14^{a}
n-3HUFA	29.6^{c}	36.32^{b}	40.92^{a}	29.11^{c}	29.96^{c}
18:2n-6	7.03^{b}	0.98^{d}	3.65^{c}	7.72^{b}	12.85^{a}
EPA	4.75^{b}	9.01^{a}	9.19^{a}	5.10^{b}	5.84^{b}
DHA	23.98^{b}	26.84^{b}	29.97^{a}	23.05^{c}	23.64^{c}

4
5

Figure legends

Figure 1. Correlation between dietary n-3 HUFA and survival % of seabream larvae (44 dph) fed diffrent dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

Figure 2. Correlation between dietary n-3 HUFA and total length of seabream larvae (44 dph) fed diffrent dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

Figure 3. Correlation between dietary PC and each of survival % (A) and total length (B) of seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

Figure 4. The relative percentage of (A) Intestinal steatosis and (B) Hepatic steatosis in seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

Figure 5. Longitudinal sections (Haematoxilin and Eosin staining) of the intestine showing intestinal steatosis in seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (krill PL and soybean lecithin). (A) Control, (B) KPL, (C) SBL.

Figure 6. Longitudinal sections (Haematoxilin and Eosin staining) of the liver showing hepatic steatosis in seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (Marine PL and soybean lecithin). (A) Control, (B) KPL, (C) SBL.

Figure 7. Correlation between dietary PC and each of intestinal steatosis (A) and hepatic steatosis (B) relative percentage in seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

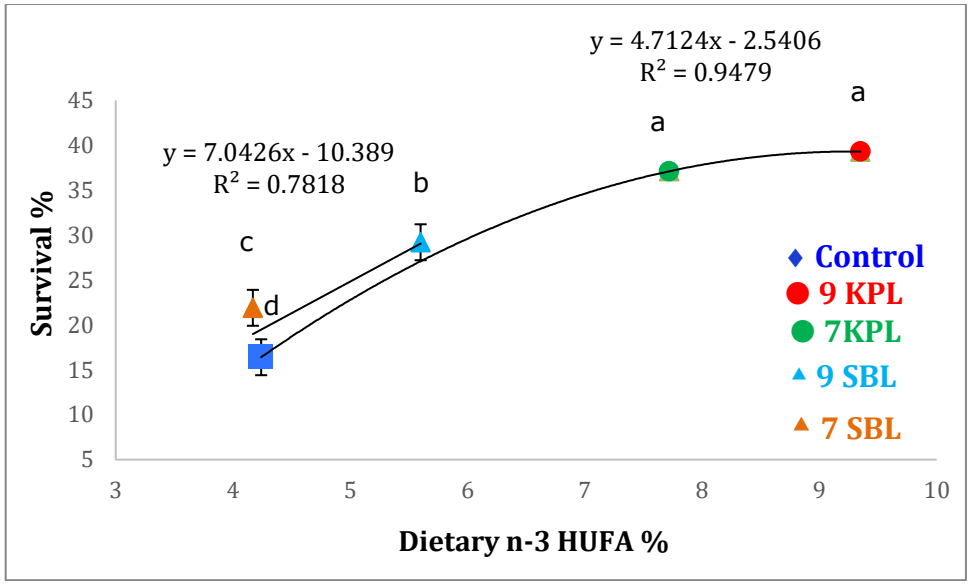


Figure 1. Correlation between dietary n-3 HUFA and survival % of seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

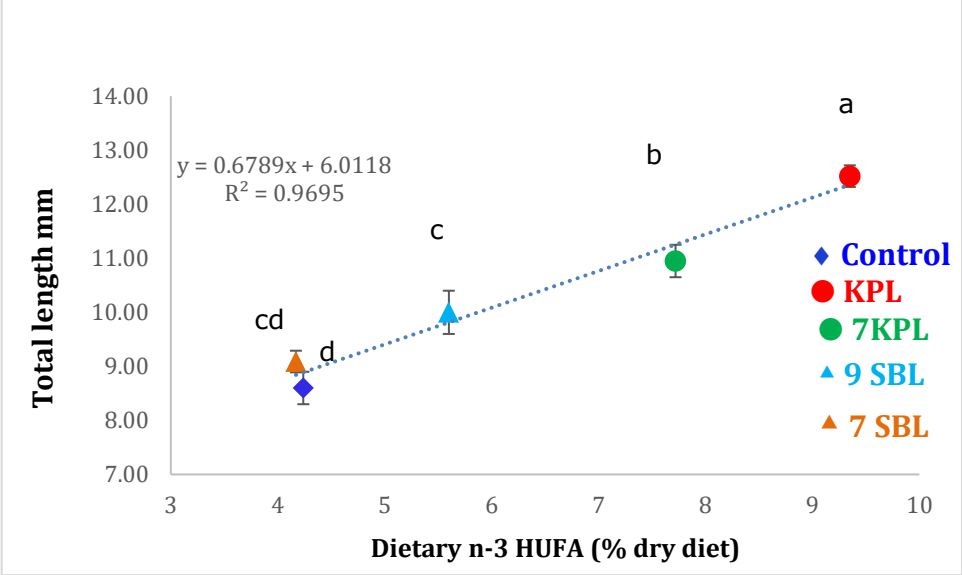
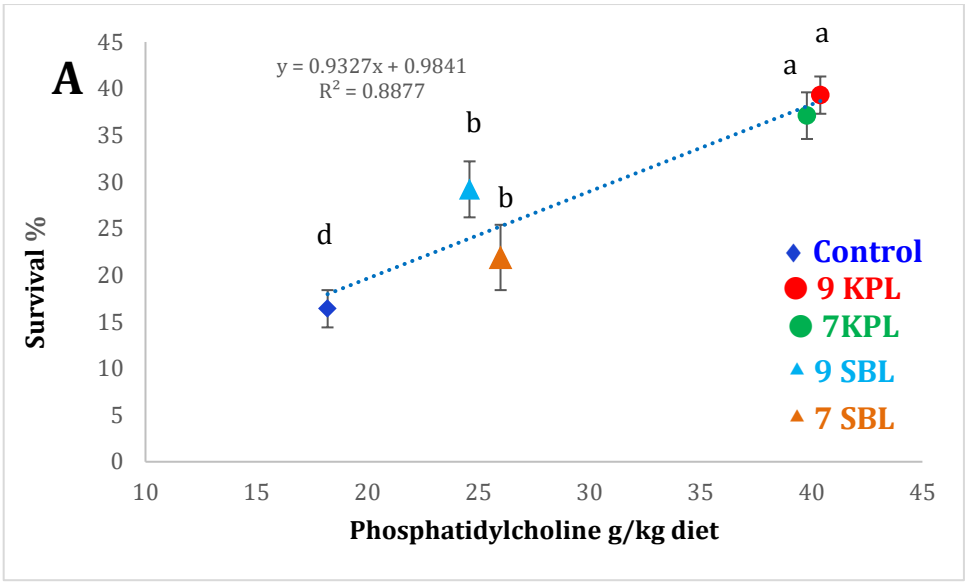


Figure 2. Correlation between dietary n-3 HUFA and total length of seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).



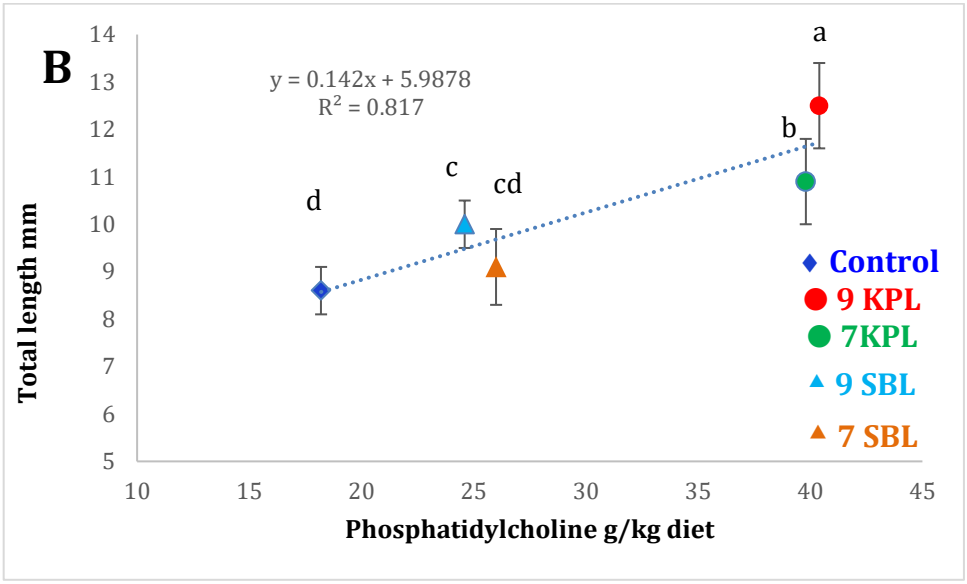


Figure 3. Correlation between dietary phosphatidylcholine and each of survival % (A) and total length (B) of seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

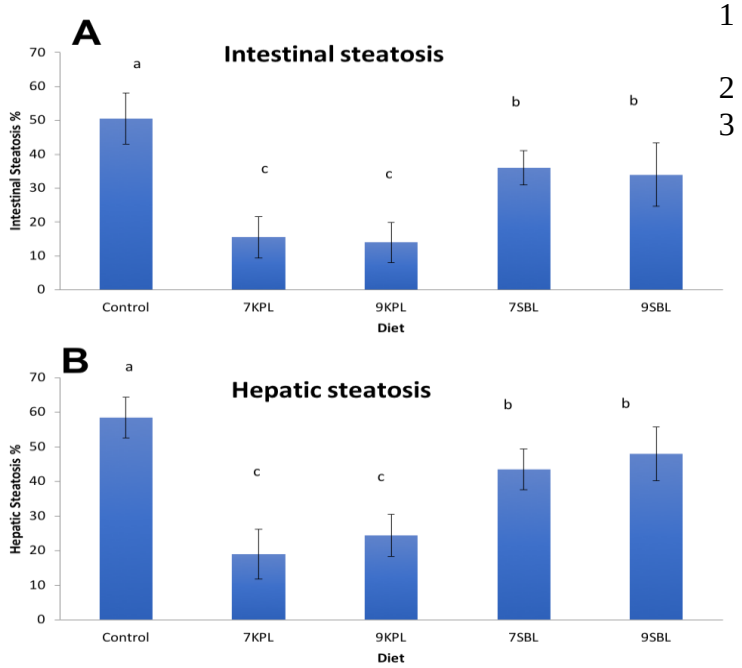


Figure 4. The relative percentage of (A) Intestinal steatosis and (B) Hepatic steatosis in seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).

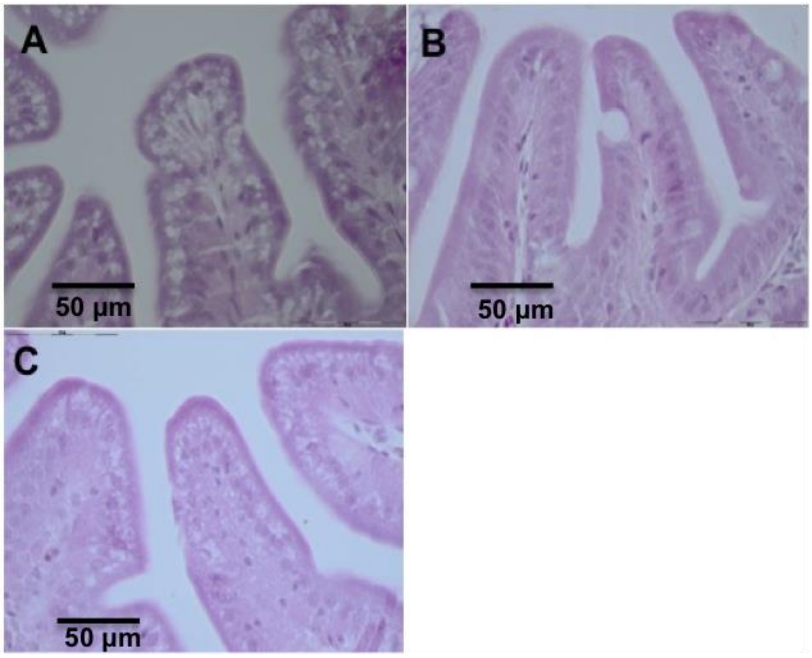


Figure 5. Longitudinal sections (Haematoxilin and Eosin staining) of the intestine showing intestinal steatosis in seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (krill PL and soybean lecithin). (A) Control, (B) KPL, (C) SBL.

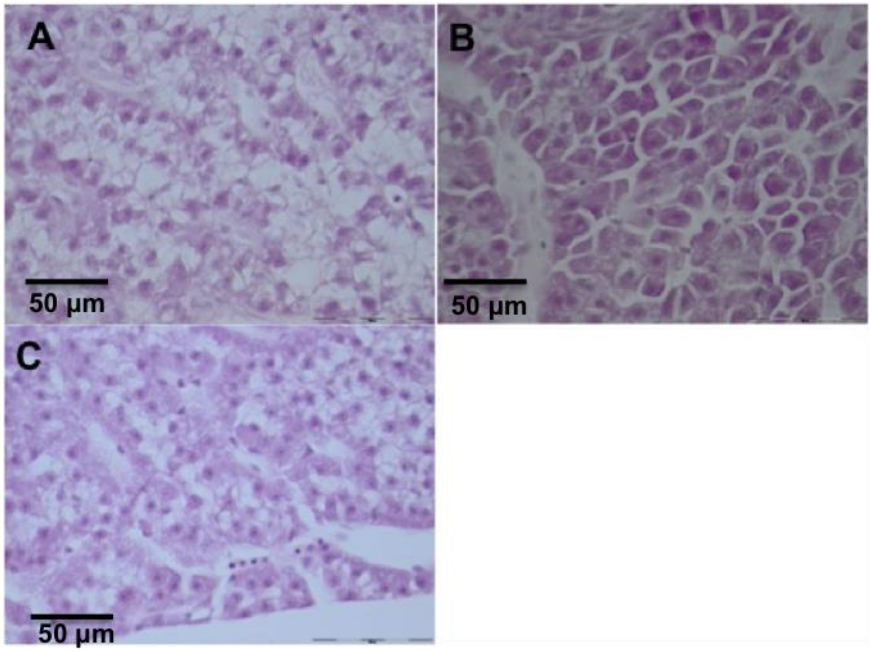
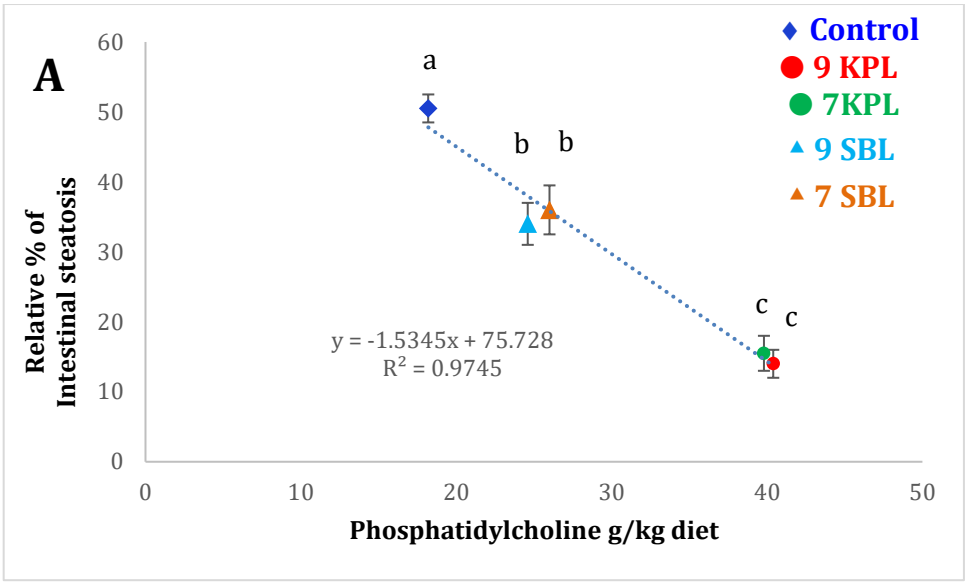


Figure 6. Longitudinal sections (Haematoxilin and Eosin staining) of the liver showing hepatic steatosis in seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9%) using two different PL sources (Marine PL and soybean lecithin). (A) Control, (B) KPL, (C) SBL.



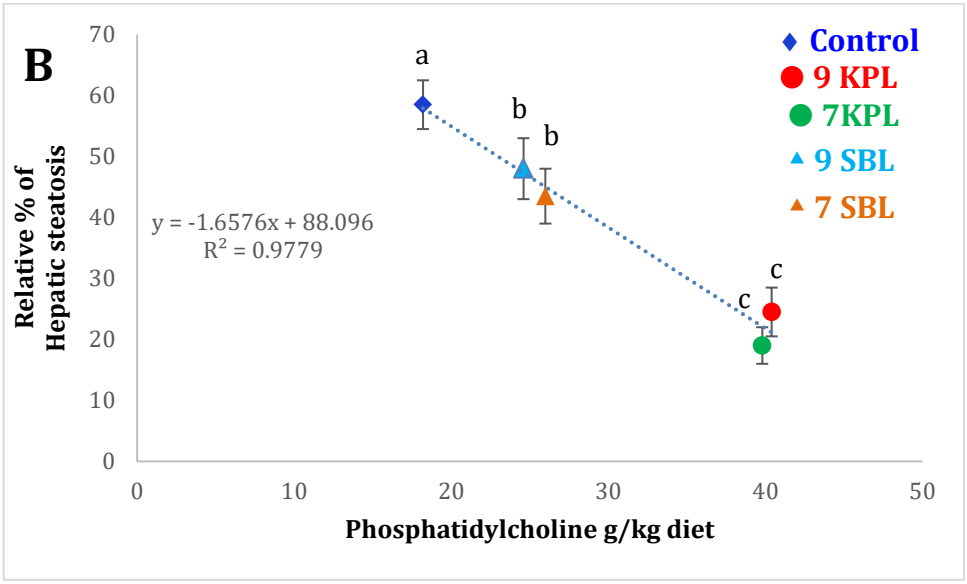


Figure 7. Correlation between dietary phosphatidylcholine and each of intestinal steatosis (A) and hepatic steatosis (B) relative percentage in seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were not significantly different (P>0.05).