

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

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

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1 **Abstract**

2 The aim of the present study was to study the effect of different dietary phospholipids
3 derived from Krill (KPL) and soybean lecithin (SBL) with different levels of
4 phosphatidylcholine (PC) and n-3 HUFA on the performance and histological changes
5 in intestine and liver of seabream larvae. Sea bream larvae (16 dph) were fed for one
6 month five microdiets formulated by using two different sources of phospholipids
7 (Control, 7KPL, 9KPL, 7SBL and 9SBL). The larvae fed dietary KPL rich in
8 phosphatidylcholine and n-3 PUFA showed better performance in terms of survival and
9 growth than those fed SBL. The inclusion of KPL up to 7% PL (3.8% PC and 7.7% n-
10 3HUFA) in diet was sufficient enough to significantly improve larval survival and
11 growth compared to the highest dietary 9% PL of SBL (2.5% PC and 5.6% n-3HUFA)
12 due to the higher content of PC and n-3 HUFA in dietary KPL. The larval performance
13 in terms of survival and total length was positively correlated to dietary PC and n-
14 3HUFA contents. Also, inclusion of SBL was associated with a higher relative
15 accumulation of lipid droplets in the supranuclear zone of the enterocytes (intestinal
16 steatosis) and in hepatocytes (hepatic steatosis) compared to larvae fed dietary KPL.
17 The intestinal and hepatic steatosis were negatively correlated to dietary PC content. In
18 conclusion, dietary 7 % KPL (3.8 % PC and 7.7% n-3 HUFA) improved significantly
19 the larval performance and decreased significantly the intestinal and hepatic steatosis
20 compared to SBL, denoting better dietary lipid absorption, transportation and energetic
21 utilization.

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24 **Introduction**

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26 Phospholipids can be an important source of energy (fatty acids) in fish, particularly
27 during embryonic and early larval development in species that produce phospholipid
28 rich eggs (Tocher, 1995, Salhi *et al.*, 1999; Saleh *et al.*, 2013a,b, Zhu *et al.*, 2019).
29 Furthermore, larval fish at first feeding may be predisposed to digestion and metabolism
30 of phospholipids as well as the use of fatty acids from phospholipids for energy
31 (Sargent, McEvoy & Bell, 1997, Tocher 2008) due to their high content of omega-3 (n-
32 3) long chain polyunsaturated fatty acids (LC-PUFA) such as eicosapentaenoic (20:5n-
33 3; EPA) and docosahexaenoic acid (22:6n-3; DHA) acids that are essential fatty acids

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

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

18 Histology has been used to describe tissue changes and pathology in the liver, pancreas
19 and intestine of fish fed different dietary phospholipids compositions (Olsen et al.,
20 1999; Crespo et al. 2001; Caballero et al. 2003; Caballero et al. 2004; Wold et al., 2008).
21 Caballero et al., (2004) and Gisbert et al., (2005) found that hepatocytes are sensitive
22 to dietary phospholipids deficiency in histological studies and such deficiencies may
23 result in steatosis. Furthermore, dietary phospholipids seem to promote lipid absorption
24 and transport, as well as decrease the accumulation of lipid droplets in the enterocytes
25 (Diaz et al., 1997; Izquierdo et al., 2000; Olsen et al., 2003) and hepatocytes (Wold et

1 al., 2009). Furthermore, it has been suggested that phospholipids contribute to the
2 intestinal absorption of lipids by their emulsifying properties and compensate for the
3 presumed insufficient biliary secretion of larvae (Kanazawa et al., 1985). Liu *et al.*,
4 (2002) demonstrated that dietary soybean lecithin markedly increases the appearance
5 of lipoproteins in larval gut, enhancing lipid transport that decrease the intestinal and
6 hepatic steatosis in gilthead sea bream.

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

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

15

16 **Materials and methods**

17 Gilthead seabream larvae were obtained from natural spawning (Grupo de
18 Investigación en Acuicultura (GIA), University of Las Palmas de Gran Canaria
19 (ULPGC). The larval initial total length was 5.4 ± 0.7 mm; dry body weight was 123 ± 30
20 μg . The seabream larvae previously fed from 4 days post hatching (dph) upon rotifers
21 (*Brachinous plicatilis*) enriched with DHA Protein Selco[®] (INVE, Dendermond,
22 Belgium) until they reached 16 dph, then randomly distributed in 15 experimental tanks
23 ((200 L) at a density of 2100 larvae tank⁻¹ and fed one of the experimental weaning
24 diets tested in triplicates for one month. Water was continuously aerated (125 ml min⁻¹
25 ¹) attaining 6.5 ± 1 ppm dissolved O₂. Average water temperature and pH along the trial
26 were 19.3 ± 2.0 °C and 7.85, respectively. Photoperiod was kept at 12h light: 12h dark,

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

3 Five experimental microdiets (pellet size 250-500 μm) with increasing phospholipid
4 contents of krill phospholipids (KPL; Qrill oil, Aker BioMarine, Fjordalléen, Norway)
5 and soybean lecithin (SBL; Agramar S.A., Spain) were formulated. Their formulation
6 and proximate analysis are shown in Table 1. The microdiets were prepared by mixing
7 squid powder and water-soluble components, then the lipids and fat-soluble vitamins
8 and, finally, gelatin dissolved in warm water. The paste was compressed and pelleted
9 (Severin, Suderm, Germany), dried in an oven at 38 °C for 24 h (Ako, Barcelona,
10 Spain). Pellets were ground (Braun, Kronberg, Germany) and sieved (Filtru, Barcelona,
11 Spain) to obtain a particle size between 250 to 500 μm . Diets were prepared and
12 analyzed for proximate composition at GIA laboratories. Moisture, protein (A.O.A.C.,
13 1995), and lipid (Folch, Lees & Stanley, 1957) contents of diets were analyzed.

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

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1 **Histopathological analysis**

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3 Thirty larvae per tank were collected at the end of the feeding trial and fixed in 4%
4 buffered formalin for one day, dehydrated through graded alcohols, then xylene and
5 finally embedded in paraffin wax. Six paraffin blocks containing 5 larvae per tank were
6 cut at 4µm on a microtome (Leica, RM2135, Leica Instruments, Nussloch, Germany),
7 the sections stained with hematoxylin and eosin (Martoja and Martoja-Pearson,
8 1970) for histopathological evaluation. All the sections were evaluated under light
9 microscopy using a binocular microscope Olympus CX41 (Olympus, Hamburg,
10 Germany) connected to a camera Olympus XC30 (Olympus, Hamburg, Germany),
11 which was connected to a computer using image capturing software (CellB®,
12 Olympus, Hamburg, Germany). All observations were performed in the liver and the
13 anterior and posterior part of the intestine. Tissue morphology was evaluated by two
14 scientists unaware of the dietary treatments following the incidence degree
15 classification in Table 2. The degree of intestinal and hepatic steatosis incidence were
16 measured as relative percentage when clear vacuoles were observed in the supranuclear
17 zone of the enterocytes and in hepatocytes, in addition, hepatocytes nuclei
18 displacement, hepatocyte morphology.

19 20 21 **Statistical analysis**

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23 All data were tested for normality and homogeneity of variances. Means and standard
24 deviations were calculated for each parameter measured. Data were submitted to a one-
25 way analysis of variance (ANOVA) and significant differences were considered when
26 $p < 0.05$. When F values were significant, individual means were compared using post
27 hoc Tukey tor Games-Howell test for multiple means comparison. Analyses were
28 performed using the SPSS Statistical Software System v21.0 (SPSS, Chicago, IL,USA).

1 **Results**

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3 The larval performance in terms of survival and growth was different between the
4 treatments. The supplementation of dietary KPL rich in n-3 HUFA and PC improved
5 significantly ($P < 0.05$) the larval survival and growth in terms of total length compared
6 to larvae fed SBL and control diets (Fig. 1&2). The correlation between dietary n-3
7 HUFA of KPL treatments and larval survival showed a high positive correlation ($y =$
8 $4.7124x - 2.5406$, $R^2 = 0.9479$) as shown by their significantly ($p < 0.05$) higher
9 survival (39 %) compared to the larvae fed dietary SBL (29% survival) with lower n-3
10 HUFA content which presented less positive correlation ($y = 7.0426x - 10.389$, $R^2 =$
11 0.7818).

12 Also, the correlation between dietary n-3 HUFA and larval total length showed a
13 positive correlation ($y = 0.6789x + 6.0118$, $R^2 = 0.9695$) where the larval fed 9.3 % n-
14 3 HUFA dietary KPL showed significantly ($p < 0.05$) better growth in terms of total
15 length compared to the larvae fed dietary SBL with 5.6 % n-3 HUFA. For the
16 correlation between phosphatidylcholine and each of survival and growth (Fig. 3)
17 showed that both were positively correlated to PC content ($y = 0.9327x + 0.9841$, $R^2 =$
18 0.8877 ; $y = 0.142x + 5.9878$, $R^2 = 0.817$, respectively), where the larvae fed higher
19 dietary PC (4%) of KPL treatments showed significantly ($p < 0.05$) better survival and
20 growth compared to the lower dietary PC (2.5 %) of SBL treatments.

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

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

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5 **Discussion**

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

1 and survival of fish larvae (Kanazawa et al., 1985, Caballero et al., 2003, Tocher et al.,
2 2008, Saleh et al., 2014).

3 The results of histological study in the present work demonstrated high accumulation
4 of numerous lipid droplets within the enterocytes and hepatocytes of larvae fed control
5 and SBL diets compared to larvae fed KPL diets. Kanazawa et al., (1985) explained
6 the nature of PL requirement and the role of these lipids during larval stages and their
7 important role in intestinal absorption of lipids. Thus, based on the morphological
8 aspects of intestinal lipid absorption, two categories were distinguished: on one hand,
9 larvae fed dietary KPL rich in PC and n-3 HUFA showed better absorption of lipids,
10 and, on the other hand, larvae fed control and SBL diets, which are PC-deficient diets
11 showed lipids droplets accumulation. Also, the intestinal and hepatic steatosis were
12 negatively correlated to the dietary PC content ($y = -1.5345x + 75.728$, $R^2 = 0.9745$; y
13 $= -1.6576x + 88.096$, $R^2 = 0.9779$, respectively). This confirm a specific effect of PC
14 for the synthesis and secretion of chylomicrons or VLDL as observed in vitro in a rat
15 intestinal preparation by Field & Mathur (1995), these authors reported a specific effect
16 of PC on the synthesis of apolipoprotein B and thus the prevention of epithelial
17 steatosis. The PC is the major constituent of the polar lipid moiety of all lipoproteins,
18 amounting for up to 95% in VLDL of Atlantic salmon (Lie et al., 1993). Numerous
19 studies have shown that larval diets deficient in PL lead to the accumulation of large
20 amounts of lipid vacuoles in the enterocytes, probably due to insufficient lipoprotein
21 synthesis (Diaz et al., 1997; Fontagnè et al., 1998; Olsen et al., 1999; Salhi et al., 1999;
22 Izquierdo et al., 2000). Liu *et al.*, 2002 concluded that inclusion of SBL in diets for
23 gilthead sea bream larvae increased the appearance of lipoproteins in larval gut,
24 enhancing lipid transport and promoting growth and survival, this is in agreement with
25 the present work where the inclusion of SBL up to 9% PL (2.5 % PC & 5.6 % n-3

1 HUFA) improved significantly larval performance compared to the 7% SBL treatment.
2 Research has shown that lecithin energy, as PL source, is effective in the larval stage
3 by providing choline and inositol for fish growth through increasing the absorption of
4 nutrients (Geurden et al., 1998).
5 Geurden et al., (1998) and Lu et al., (2008) demonstrated that inclusion of PL especially
6 PC in diets induced high number of goblet cells in the intestine of turbot (*S. maximus*)
7 and *Pelteobagrus fulvidraco* that indicates the early maturation of intestine as a result
8 of high dietary PC inclusion. Furthermore, it has been reported that dietary PL can
9 promote somatic growth by increasing the effectiveness of lipid use through
10 emulsification and digestion, increasing lipid transport, and providing
11 phosphatidylcholine with a growth promoting effect (Kasper and Brown 2003; Geurden
12 et al. 1998; Tocher et al. 2008; Zhu et al. 2019).
13 In conclusion, the present study concluded that dietary 7% KPL rich in PC (3.8 %) and
14 n-3 HUFA (7.7%) decreased significantly the intestinal and hepatic steatosis compared
15 to 9 %SBL (2.5 % PC & 5.6 % n-3HUFA) that in turn led to better larval performance
16 in terms of survival and total length denoting better dietary lipid absorption,
17 transportation and energetic utilization which may be in relation to a good availability
18 of krill nutrients (growth factors, minerals, PL and astaxanthin) and a higher bioactivity
19 of their LC-PUFA.

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33 *morhua*). *Journal of Fish Biology* **72**: 1637-1658.

1 **Table 1 Formulation and proximate composition of the experimental microdiets**
 2 **containing several levels of either krill phospholipids (KPL) or soybean**
 3 **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
<i>Proximate analysis (g kg⁻¹ diet)</i>					
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
<i>Lipid classes composition</i>					
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

- 4 a Rieber and Son, Bergen, Norway.
 5 b Krill oil, Aker BioMarine, Fjordalléen, Norway.
 6 c Agramar S.A., Spain.
 7 d Agramar S.A., Spain.
 8 e Merck KGaA, Darmstadt, Germany.
 9

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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
<i>Phosphatidylcholine</i>					
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
<i>Phosphatidylinositol</i>					
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
<i>Phosphatidylserine</i>					
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
<i>Phosphatidylethanolamine</i>					
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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1 Table 3. The intestinal and hepatic steatosis evaluation scale

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

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1 Table 4. Fatty acids (% total identified fatty acids) composition in polar lipids of larvae
 2 fed 5 dietary phospholipid levels. Values (mean \pm 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

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5

1 **Figure legends**

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

6
7 Figure 2. Correlation between dietary n-3 HUFA and total length of seabream larvae
8 (44 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL
9 sources (KPL and SBL). Values (mean \pm standard deviation) with the same letters
10 were not significantly different ($P>0.05$).

11
12 Figure 3. Correlation between dietary PC and each of survival % (A) and total length
13 (B) of seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9% total PL)
14 using two different PL sources (KPL and SBL). Values (mean \pm standard deviation)
15 with the same letters were not significantly different ($P>0.05$).

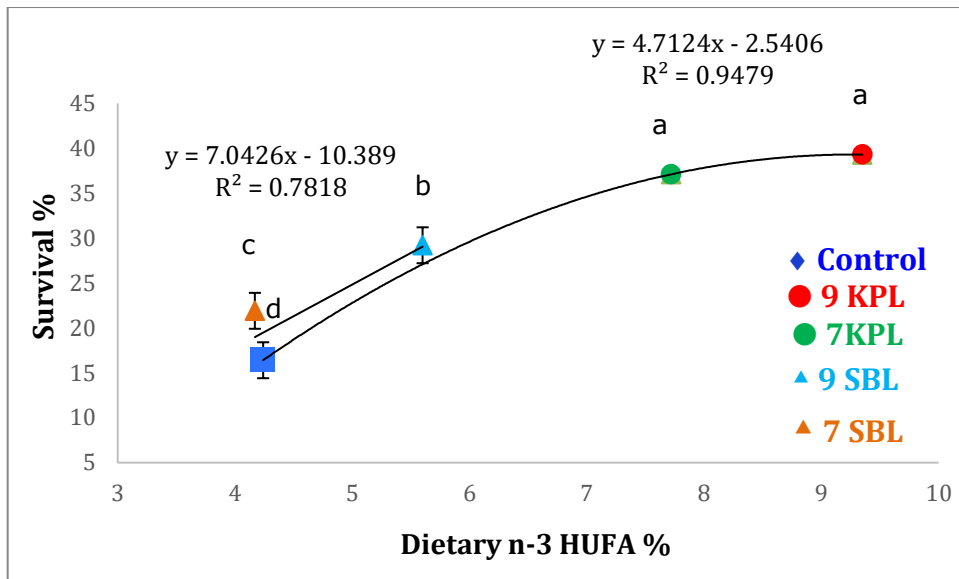
16
17 Figure 4. The relative percentage of (A) Intestinal steatosis and (B) Hepatic steatosis in
18 seabream larvae (44 dph) fed different dietary PL levels (5, 7 and 9%) using two
19 different PL sources (KPL and SBL). Values (mean \pm standard deviation) with the same
20 letters were not significantly different ($P>0.05$).

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22 Figure 5. Longitudinal sections (Haematoxylin and Eosin staining) of the intestine
23 showing intestinal steatosis in seabream larvae (44 dph) fed different dietary PL levels
24 (5, 7 and 9%) using two different PL sources (krill PL and soybean lecithin). (A)
25 Control, (B) KPL, (C) SBL.

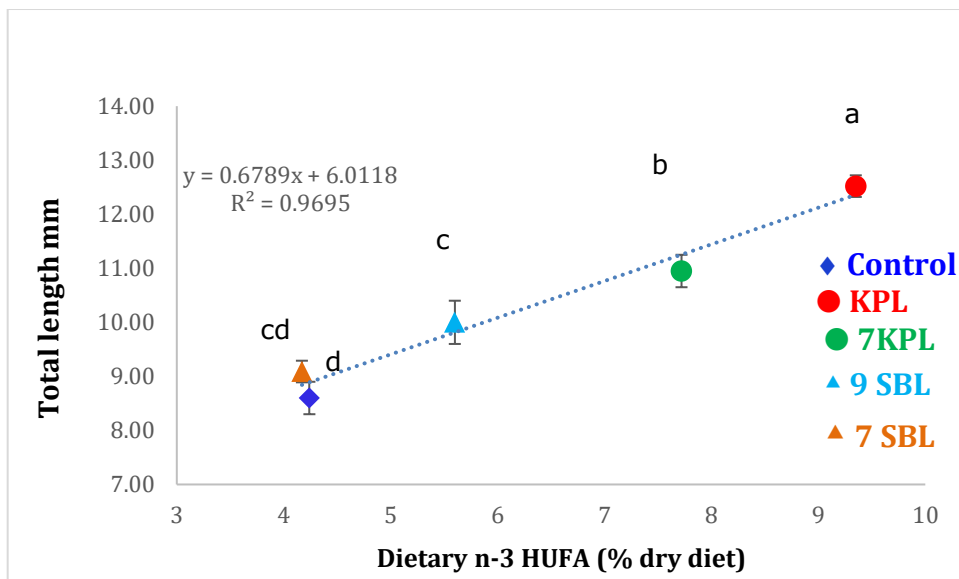
26
27 Figure 6. Longitudinal sections (Haematoxylin and Eosin staining) of the liver showing
28 hepatic steatosis in seabream larvae (44 dph) fed different dietary PL levels (5, 7 and
29 9%) using two different PL sources (Marine PL and soybean lecithin). (A) Control, (B)
30 KPL, (C) SBL.

31
32 Figure 7. Correlation between dietary PC and each of intestinal steatosis (A) and
33 hepatic steatosis (B) relative percentage in seabream larvae (44 dph) fed different
34 dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and
35 SBL). Values (mean \pm standard deviation) with the same letters were not significantly
36 different ($P>0.05$).

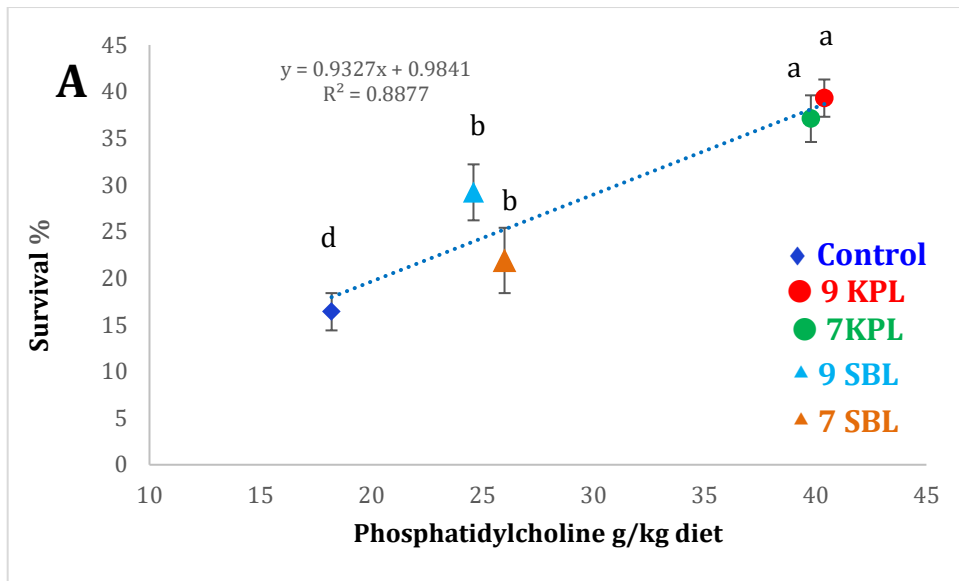
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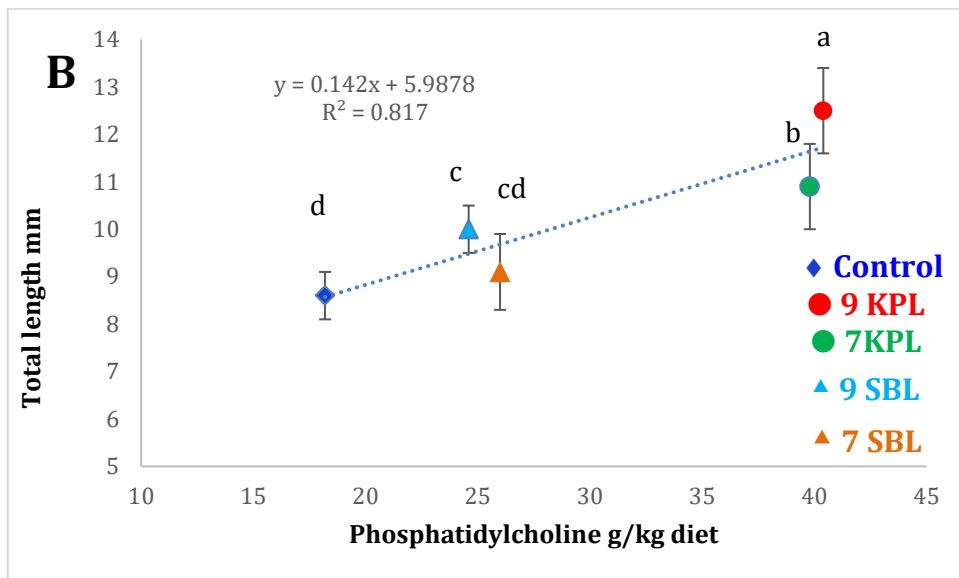
1
2 Figure 1. Correlation between dietary n-3 HUFA and survival % of seabream larvae
3 (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL
4 sources (KPL and SBL). Values (mean ± standard deviation) with the same letters were
5 not significantly different (P>0.05).
6
7



8
9 Figure 2. Correlation between dietary n-3 HUFA and total length of seabream larvae
10 (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL
11 sources (KPL and SBL). Values (mean ± standard deviation) with the same letters
12 were not significantly different (P>0.05).
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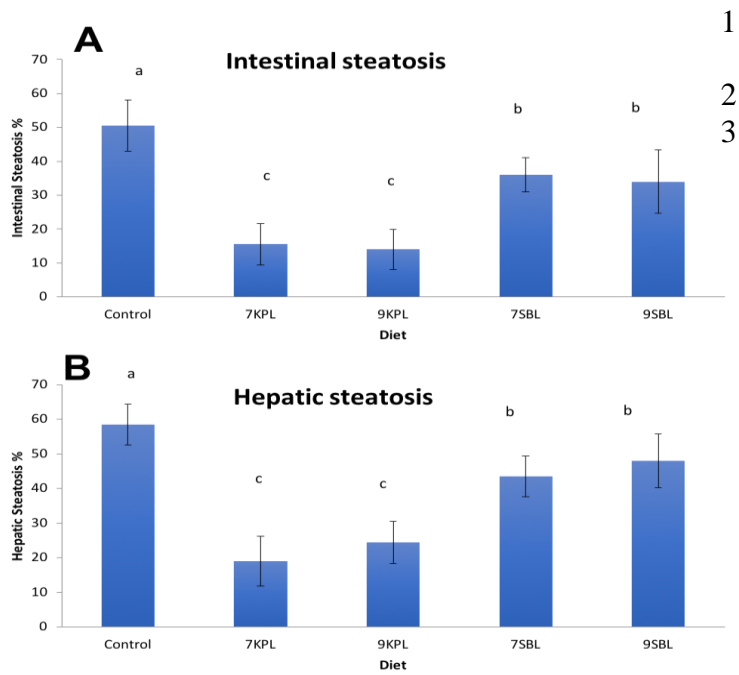


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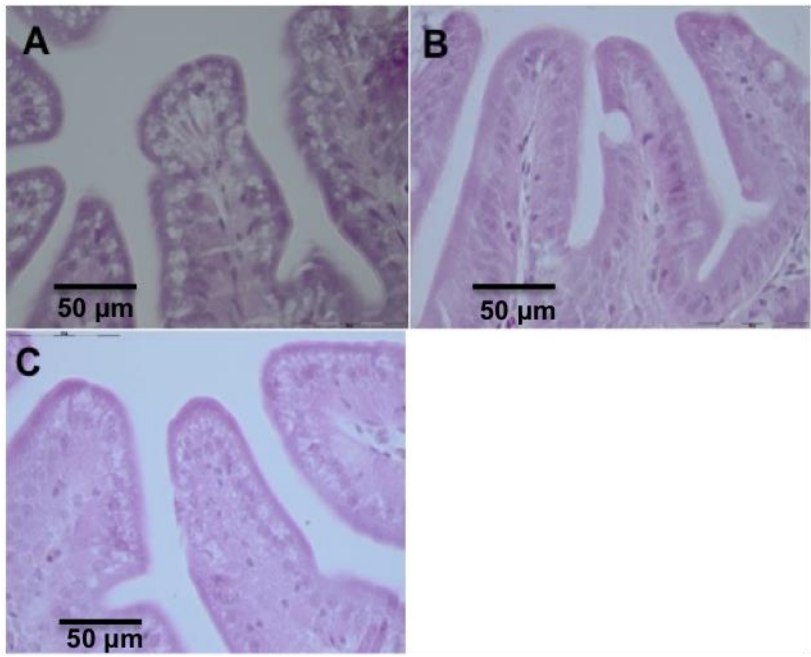
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Figure 3. Correlation between dietary phosphatidylcholine and each of survival % (A) and total length (B) of seabream larvae (45 dph) fed different dietary PL levels (5, 7 and 9% total PL) using two different PL sources (KPL and SBL). Values (mean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



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

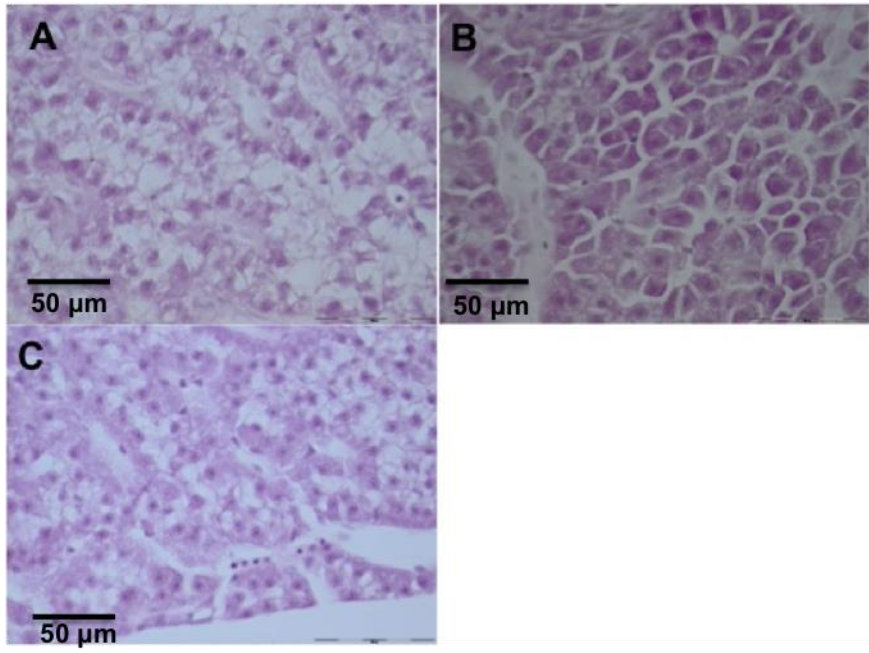
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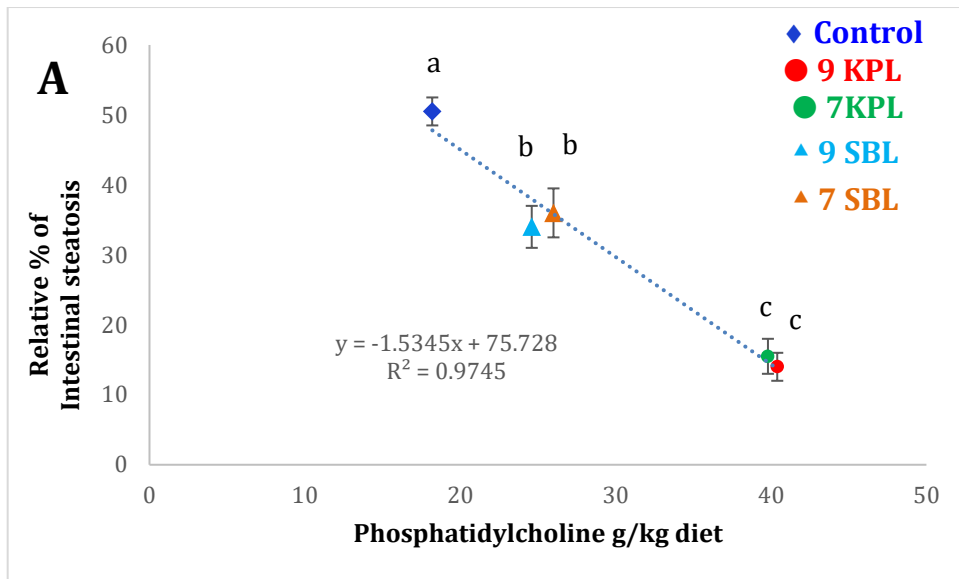
11 Figure 5. Longitudinal sections (Haematoxylin and Eosin staining) of the intestine
 12 showing intestinal steatosis in seabream larvae (45 dph) fed different dietary PL levels
 13 (5, 7 and 9%) using two different PL sources (krill PL and soybean lecithin). (A)
 14 Control, (B) KPL, (C) SBL.

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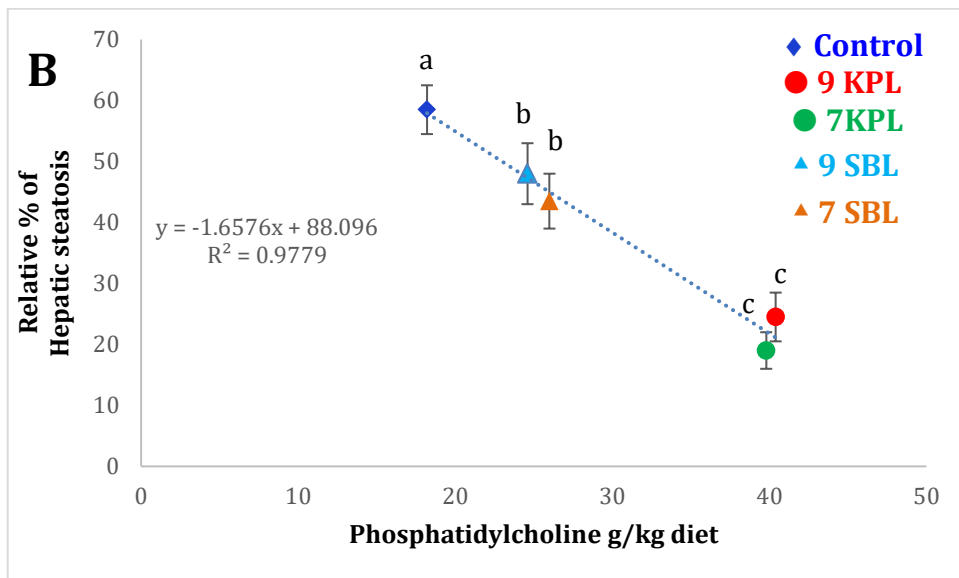


1
2 Figure 6. Longitudinal sections (Haematoxylin and Eosin staining) of the liver showing
3 hepatic steatosis in seabream larvae (45 dph) fed different dietary PL levels (5, 7 and
4 9%) using two different PL sources (Marine PL and soybean lecithin). (A) Control, (B)
5 KPL, (C) SBL.

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