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Replacement of fish meal by Antarctic krill meal in diets for European sea bass *Dicentrarchus labrax*: Growth performance, feed utilization and liver lipid metabolism

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ABSTRACT

A sustainable growth of the aquaculture sector implies the use of sustainable novel raw materials as replacers of the traditional fishmeal (FM) and fish oil (FO) ingredients. This fact has led to the development of sustainable and functional diets as part of a management strategy to reduce the effects on fish growth performance and health derived from low FM/FO dietary contents. In this sense, Antarctic krill (*Euphausia superba*) is considered a potential candidate in dietary inclusions to potentiate fish growth and health status. In this study, European sea bass (*Dicentrarchus labrax*) were fed a practical diet with either a 15% fishmeal content (KM0; control diet) or the same diet substituted by 30% (KM5; 5 g KM/kg diet) or 50% (KM7.5; 7.5 g KM/kg diet) Antarctic krill meal (KM) for 12 weeks in triplicates. At the end of the feeding trial, growth performance, liver morphology, liver proximate composition, lipid classes and fatty acid profiles, as well as the expression of hepatic genes related with lipid metabolism were evaluated. Fish fed KM-based diets presented higher ($p < 0.05$) final weight, protein and lipid efficiency ratios, specific growth rate (SGR) and improved feed conversion ratio (FCR), irrespective of the KM dietary level. Whole body and muscle proximate composition and fatty acid profiles were similar among dietary groups. Livers of European sea bass fed the experimental diets presented similar ($p > 0.05$) biochemical composition and fatty acid profile. However, smaller hepatocellular area and lower grade of cytoplasm vacuolization as well as a better alignment around sinusoidal spaces were found. The analyses of liver lipid classes revealed a positive correlation between the level of dietary KM and the pigmented material such as astaxanthin and free fatty acid content, as well as a negative correlation with the cholesterol levels. The expression of hepatic genes studied demonstrated a downregulation of *3*-hydroxy-3-methylglutaryl-coenzyme A reductase *(hmgr)* and delta-6-desaturase (*fads2*) expression levels in fish fed KM-based diets. Besides, gene expression levels of fatty acid binding protein 7 (*fabp7*) and lipoprotein lipase (*lpl*) were significantly correlated with KM dietary levels. Altogether, these results profile KM as a potential promoter of growth and liver health in European sea bass fed low fish meal and oil diets.

1. Introduction

Aquaculture products represent the 60% of total fish consumption and its production is expected to continue rising (FAO, 2020). An adequate development of the aquaculture market implies the use of sustainable alternative protein and lipids sources to traditional marine

Abbreviations: Fishmeal, (FM); Fish oil, (FO); Krill meal, (KM); Specific growth rate, (SGR); Feed conversion ratio, (FCR); *3*-hydroxy-3-methylglutaryl-coenzyme A reductase, (HMGR); Delta-6-desaturase, (FADS2); Fatty acid binding protein 7, (FABP7); Lipoprotein lipase, (LPL); Gut associated lymphoid tissue, (GALT); Condition factor, (K); Angiopoietin-like 3, (ANGPTL3); Protein efficiency ratio, (PER); Lipid efficiency ratio, (LER); Triacylglycerols, (TAG); Free fatty acids, (FFA); Cholesterol, (Chol); Diacylglycerol, (DAG); Phosphatidylethanolamine, (PE); Phosphatidylinositol, (PI); Phosphatidylcholine, (PC); Lysophosphatidylcholine, (LPC); Polar Lipids, (PL); Neutral Lipids, (NL); Highly unsaturated fatty acid, (HUFA); Long chain polyunsaturated fatty acid, (LC-PUFA); Peroxisome proliferator-activated receptor alpha, (PPARα); Sterol regulatory element-binding protein 1, (SREBP-1).

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raw materials. Certainly, the inclusion levels of fish meal (FM) and fish oil (FO) in aquaculture feed formulations has significantly decreased in the last decade, in favor of more sustainable sources such as plant and single cell biomass, domestic animal by-products, insects and krill (Sørensen et al., 2011; Tacon et al., 2011; Naylor et al., 2021). FM can be partially replaced in the diets of many fish species, but in most cases, high or complete replacements by terrestrial ingredients have detrimental effects on fish performance and health (Hernandez et al., 2007; Xu et al., 2012; Conde-Sieira et al., 2018). This effect uses to be associated to imbalanced amino acid and micronutrients profiles, to the presence of anti-nutritional factors (Ng et al., 2019; Xu et al., 2017) or to a reduction in feed intake because of a decreased diet acceptability as the level of alternative vegetal protein sources increases (Chatzifotis et al., 2008; Kissil et al., 2000; Kubitza and Lovshin, 1997).

Particularly in European sea bass (*Dicentrarchus labrax*), a marine teleost widely farmed in the Mediterranean region (FAO, 2019), several studies showed that it is possible to replace up to 50% of FM by plant-based meals without compromising growth performance (Ballestrazzi et al., 1994; Kaushik et al., 2004; Tibaldi et al., 2006; Torrecillas et al., 2017a). Indeed, recent studies emphasized that it is feasible to reduce both FM and FO levels down to 10% and 3%, respectively in practical and commercially manufactured diets without affecting European sea bass juveniles' growth performance and feed utilization (Torrecillas et al., 2017a). However, further FM reduction down to 5% of total diet ingredients markedly reduces feed intake and fish growth (Torrecillas et al., 2017a). Inasmuch, high FM replacements by terrestrial meals may results in a disruption of gut homeostasis by increasing oxidative stress (Guerreiro et al., 2015), submucosa cellular gut associated lymphoid tissue (GALT) response (Torrecillas et al., 2017b; Azeredo et al., 2017), mucus production (Torrecillas et al., 2017b) and autochthonous gut microbiota (Torrecillas et al., 2017b).

Antarctic krill (*Euphausia superba*) is a small marine crustacean organism that has generated strong research interest during the past several years (Xie et al., 2019) as a sustainable source of protein and lipids in aquafeeds (Burri and Nunes, 2016). Based on its nutritional profile, krill meal (KM) contains protein, amino acids and ash levels comparable to that of FM (Hertrampf and Piedad-Pascual, 2000; Tou et al., 2007). Noteworthy is that it presents relatively high levels of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Köhler et al., 2015), phospholipids (PLs), vitamins, nucleotides, trimethylamine N-oxide, chitin and natural astaxanthin, which has been demonstrated to stimulate the feeding appetite of fish (Everson et al., 2018), and to efficiently facilitate the reduction of FM and FO in diets (Hatlen et al., 2016; Mørkøre et al., 2020; Saleh et al., 2018; Thakara et al., 2020).

Over the past years, several studies have been carried out to test the benefits of KM in diets for various fish species. Saleh et al. (2018) showed that FM can be reduced by 4% in diets for gilthead seabream (*Sparus aurata*) juveniles without negatively affecting growth performance, when KM is added between 3 and 9%. Indeed, inclusion of 9% KM in a 16% FM based diet given for 12 weeks promotes gilthead sea bream growth performance and improves feed conversion and trends to reduce hepatocytes cytoplasm vacuolization, promoting liver health status (Saleh et al., 2018). Moreover, Thakara et al. (2020) observed in olive flounder (*Paralichthys olivaceus*) juveniles increased growth performance and improved feed utilization efficiency in relation to a better dry matter and protein digestibility and higher feed intake after 12 weeks, when KM was added in a low FM diet (28% FM, g/100 g diet), where FM inclusion levels up to 56% are common. In salmonids, Mørkøre et al. (2020) demonstrated a better fish health status, as well as improved meat quality in adult Atlantic salmon (*Salmo salar*) fed KM at 12% (g/100 g diet) for 12 weeks in low fish meal diets (15%, g/100 g diet). These results were associated with upregulation of several hepatic immune and structural genes, including junctional complexes and of genes encoding myosin heavy chain proteins in skeletal muscle. Besides, Atlantic salmon fed KM meal diets presented an altered metabolism of n-3, n-6 and saturated fatty acids of skeletal muscle (Mørkøre et al., 2020).

Despite that KM supplementation to low FM based diets is a current industrial practice (Thomassen et al., 2007; Ytrestøyl et al., 2015), there is no information on the effect of dietary KM on European sea bass performance and lipidic metabolism in liver. The aim of the current investigation is therefore to evaluate the effect of partial replacement of FM with KM on European sea bass juvenile growth performance, feed utilization, liver morphology, tissue proximate and fatty acid composition, liver lipid classes and hepatic genes related with lipid metabolism.

2. Material and methods

2.1. Ethics statement

Animal manipulation during these experiments complied with the guidelines of the European Union Directive (2010/63/EU) and Spanish legislation (RD 53/2013) for animal experiments. The Bioethical Committee of the University of Las Palmas de Gran Canaria (Rec. code: 28/2019 CEBA ULPGC). Fish handling was performed under natural clove oil anesthesia (0.2 ml/l; Guinama S.L; Spain, Ref. Mg83168), and discomfort, stress and pain to the experimental animals was avoided, as much as possible, along the experiment. For sampling, fish were euthanized with an overdose of natural clove oil (5 ml/l; Guinama S.L; Spain, Ref. Mg83168).

2.2. Experimental diets

Three isoproteic and isolipidic experimental diets (pellet size of 2 and 3 mm) were formulated containing different KM levels: 0% (control

Table 1
Main ingredients and analyzed proximate composition of the experimental diets.

Ingredients (%)	Diets		
	KM0	KM5	KM7.5
Fishmeal LT70[a]	15	10	7.5
Krill meal[b]	0	5.0	7.5
Haemoblobin powder	2.0	2.0	2.0
Poultry meal 65	7.0	7.0	7.0
Soy protein concentrate	10	10	10
Wheat gluten	8.0	8.9	9.4
Corn gluten	8.0	8.0	8.0
Soybean meal 48	12.0	12.0	12.0
Rapeseed meal	5.0	5.0	5.0
Wheat meal	11.5	11.5	11.4
Whole peas	4.0	4.0	4.0
Fish oil (Sardine/Anchovy)	4.5	4.23	4.11
Rapeseed oil	10.5	9.9	9.6
Vit & Min Premix INVIVO 1%[c]	1.0	1.0	1.0
Antioxidant	0.2	0.2	0.2
Sodium propionate	0.1	0.1	0.1
MAP (Monoammonium phosphate)	0.8	0.8	0.8
DL-Methionine	0.4	0.4	0.4
Proximate composition (% of dry matter)			
Crude lipids	20.24	20.46	20.62
Crude protein	47.42	47.65	48.23
Moisture	6.71	6.35	5.97
Ash	7.08	6.79	6.35

[a] Fishmeal LT70, Norvik 70, Sopropêche, France.
[b] Qrill™ Aqua; Aker BioMarine Antarctic AS, Norway. Protein 56%, Moisture 6%, Ash 9%, lipids 29%.
[c] Premix INVIVONSA Portugal SA, Portugal: Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 500 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): copper sulphate, 9 mg; ferric sulphate, 20 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 27.5 mg; sodium chloride, 400 mg; excipient wheat middling's.

group), 5% and 7.5% KM (Qrill™ Aqua; Aker BioMarine Antarctic AS, Norway) (Table 1). Diets were manufactured by SPAROS Lda (Portugal). All powder ingredients were mixed accordingly to the target formulations in a double-paddle mixer (model RM90L, Mainca, Spain) and ground (below 250 μm) in a micropulverizer hammer mill (model SH1, Hosokawa-Alpine, Germany). Diets, with a pellet size of 2.0 and 3.0 mm, were manufactured with a twin-screw extruder (model BC45, Clextral, France) with a screw diameter of 55.5 mm. Extruded pellets were dried in a vibrating fluid bed dryer (model DR100, TGC Extrusion, France). Oils were added by vacuum coating (model PG-10VCLAB, Dinnissen, The Netherlands). Immediately after coating, diets were packed in sealed plastic buckets and shipped to the research site. Diets covered all known nutritional requirements for sea bass and were produced by Sparos Lda (Olhão, Portugal). The proximate analysis and fatty acid composition of the experimental diets are listed in Table 2.

Table 2
Fatty acid composition (% of total identified fatty acids) of the experimental diets.

Fatty acids (% total fatty acids)	Diets		
	KM0	KM5	KM7.5
14:0	1.78	0.88	1.37
14:1n-7	0.03	0.01	0.02
14:1n-5	0.06	0.03	0.03
15:0	0.14	0.08	0.11
15:1n-5	0.02	0.01	0.02
16:0ISO	0.03	0.02	0.03
16:0	9.27	7.52	8.71
16:1n-7	2.30	1.82	2.24
16:1n-5	0.08	0.07	0.08
16:2n-4	0.28	0.23	0.28
17:0	0.35	0.25	0.34
16:3n-4	0.09	0.08	0.07
16:3n-3	0.04	0.03	0.04
16:3n-1	0.04	0.02	0.03
16:4n-3	0.43	0.40	0.50
18:0	2.69	2.74	2.58
18:1n-9	40.86	40.91	39.64
18:1n-7	3.07	3.30	3.26
18.1n-5	0.08	0.08	0.07
18:2n-9	0.04	0.05	0.05
18.2n-6	17.94	18.02	18.20
18:2n-4	0.11	0.10	0.11
18:3n-6	0.21	0.11	0.10
18:3n-4	0.07	0.09	0.09
18:3n-3	5.99	5.96	6.03
18.3n-1	0.17	0.19	0.18
18:4n-3	0.62	0.65	0.76
18:4n-1	0.06	0.07	0.06
20:0	0.54	0.63	0.54
20:1n-9	0.15	0.14	0.13
20:1n-7	1.75	1.88	1.54
20.1n-5	0.13	0.17	0.13
20:2n-9	0.06	0.08	0.08
20:2n-6	0.13	0.17	0.13
20:3n-9	0.06	0.07	0.08
20:3n-6	0.04	0.06	0.07
20:4n-6	0.35	0.38	0.39
20:3n-3	0.04	0.07	0.05
20:4n-3	0.20	0.26	0.24
20:5n-3	4.48	5.92	6.18
22:1n-11	0.93	0.86	0.60
22:1n-9	0.27	0.36	0.35
22:4n-6	0.08	0.11	0.10
22:5n-6	0.08	0.10	0.10
22:5n-3	0.57	0.70	0.62
22:6n-3	3.31	4.30	3.70
Saturated	14.77	12.10	13.67
Monoens	49.72	49.64	48.10
Σn-3	15.68	18.29	18.11
Σn-6	18.83	18.95	19.08
Σn-9	41.37	41.62	40.32
Σn-3 HUFA	8.60	11.25	10.78
Σn-6 HUFA	0.54	0.65	0.66
Σn-3/Σn-6	0.83	0.97	0.95

2.3. Feeding trial

Three hundred and eighty-four European sea bass juveniles produced at the Grupo de Investigación en Acuicultura (GIA) facilities were maintained in stocking tanks and fed with a commercial diet until being fully adapted to the environmental conditions. Afterwards, fish were randomly distributed in 12 indoor cylindroconical 500 l fiberglass tanks (3 tanks/diet) at an initial stocking density of 1.5 kg·m^{-3} (32 fish per tank). Fish average initial weight and length were 22.54 ± 0.30 g and 11.4 ± 0.1 cm, respectively (mean ± SD). Tanks were supplied in a flow-through system with filtered sea water and natural photoperiod (12 L:12D). Fish were manually fed until apparent satiation with one of the three experimental diets for 90 days (3 times a day, 9:00, 12:30, 16:00; 6 days a week). Feed intake was calculated, and survival monitored daily, and growth performance observed monthly. After 90 days of supplementation, the whole fish population was sampled for final weight, final length, condition factor (K), specific growth rate (SGR) and food conversion ratio (FCR). Livers, dorsal muscle and whole-body samples from 5 fish per tank were sampled and pooled for chemical composition, fatty acid and lipid classes analyses. Livers of 3 fish per tank were collected for morphological and gene expression analyses.

2.4. Biochemical composition and lipid classes analyses

The proximate composition of the feed and samples were analyzed according to standard procedures (AOAC, 2000). Dry matter content was determined after drying in an oven (110 °C) to constant weight and ash content was determined by combustion in a muffle furnace (600 °C, 12 h). The crude lipids were extracted as described by Folch et al. (1957) and crude protein content (Nx6.25) by following the Kjeldahl method. Fatty acid methyl esters were obtained by transmethylation with 1% sulfuric acid in methanol as described by Christie (1989) and separated by gas chromatography (GC-14A, Shimadzu, Japan). A GC Supercolovax-10-fused silica capillary column (Supelco, Bellefonte, USA) was used for the separation with helium as a carrier gas, applying the conditions described by Izquierdo et al. (1992). Fatty acid methyl esters were quantified by flame ionizator detector and identified by comparing them with external and well-characterized fish oils standards (EPA 28, Nippai, Ltd. Tokyo, Japan).

Lipid classes were separated by double-development, high performance thin-layer chromatography (HPTLC) using 10 × 10 cm plates (VWR, Lutterworth, UK) according to common procedures described elsewhere (Tocher and Harvie, 1988; Olsen and Henderson, 1989). Total lipid samples (1–2 μg) were applied as 3 mm origins and the plates developed in methyl acetate/isopropanol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.) to 5.2 cm. Excess solvent was evaporated via air drying and vacuum desiccation and plates developed to 9.5 cm using a solvent mixture containing isohexane/diethyl ether/acetic acid (80:20:1, by vol.) before termination and drying as above. Lipid classes were visualized by spraying with 3% (*w*/*v*) aqueous cupric acetate containing 8% (*v*/v) phosphoric acid and charring plates at 160 °C for 20 min. Lipid classes were quantified by densitometry using a CAMAG-3 TLC Scanner (version Firmware 1.14.16; CAMAG, Muttenz, Switzerland) with winCATS software (Planar Chromatography Manager, version 1.2.3).

2.5. Morphological studies

Liver samples ($n = 9$ fish per diet) were fixed in 4% neutral-buffered formalin, divided in three sagittal sections (right, mid and left), embedded in paraffin, cut in sections (5 μm-thick) in a Leica 2055-Autocut microtome (Leica Instruments GmbH, Nussloch, Germany) and stained with H&E (hematoxylin and eosin) (Martoja and Martoja-Pierson, 1970). Digital images of the slides for examination were

obtained using Olympus VS120 digital scanner at 20× and 40× (Optic system BX61VS, Tokyo, Japan) equipped with VC50 and VS-XM10 cameras and were processed with Olympus VS software (VS-NIS-SQL-V2.6, Tokyo, Japan). The morphological pattern of hepatocytes, level of vacuolization and nuclei alignment around sinusoidal lines, as well as the liver steatosis status were evaluated by two independent scientists, unaware of the experimental treatments. In case of the steatosis status the evaluation was accordingly to a previously established scoring scale ranging from 1 to 4, where 1 refers to very low incidence and 4 to high incidence. The parameters evaluated were: nuclei displacement, nuclei alignment along sinusoidal lines, hepatocyte size, hepatocyte morphology, presence or cytoplasmatic microvacuoles.

2.6. Gene expression analysis

Total RNA was extracted from the liver samples using an automatic system (Maxwell116 Instrument, Promega) and a total RNA purification kit (Maxwell116 LEV simply RNA Tissue) according to the manufacturer's instructions. The RNA was quantified by using NanoDrop™ spectrophotometer (Thermo Scientific, Italy) and reverse transcribed into cDNA following the protocol of the SuperScript III Reverse Transcriptase kit (Invitrogen, Milan, Italy). Relative gene expression of the genes *fatty acyl desaturase* (*fads2*), *3-hydroxy-3-methylglutaryl coenzyme A reductase* (*hmgr*), *angiopoietin-like 3* (*angptl3*), *fatty acid binding protein 7* (*fabp7*) and *lipoprotein lipase* (*lpl*) were determined by Real-Time PCR (RT-PCR) in an iQ5 Multicolour Real-Time PCR detection system (Bio-Rad) using *ef-1* as housekeeping gene, with the following conditions: a first step of 3 min 30 s at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, 1 min at 95 °C, and a final 81 cycles of 10s from 55 °C to 95 °C. Primers sequences are shown in Table 3. All PCR reactions were carried out in in a final volume of 20 μl, with 7.5 μl of Brillant SYBR Green QPCR Master Mix (Bio-Rad Hercules, CA, USA), 0.6 μl of each primer (10 mM), 5 μl of cDNA (1:10 dilution) and 1.3 μl of MiliQ water. MiliQ water also replaced cDNA in blank control reactions. Each run was ended with an analysis of melting curve leading to a melting peak specific for the amplified target DNA. Relative expression level was calculated by using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) in relation to house-keeping reference genes and normalized using the corresponding control diet data.

2.7. Statistical analysis

Statistical analyses were performed following the followed methods described by Sokal and Rolf (1995). All data presented were tested for normality and homogeneity of variance. Means and standard deviations (SD) were calculated for each parameter measured. If necessary, arc sin square root transformation of the data was performed, particularly when data was expressed as % (Fowler et al., 1998). 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. When applicable, data were subjected to the best fit correlations, which were checked for significance at $p < 0.05$, and Pearson's coefficients were determined. Analyses were performed using the SPSS Statistical Software System v21.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Growth performance

Fish accepted well the experimental diets, which had no effect on fish survival neither condition factor (Table 4). After 88 days of feeding, fish fed KM5 and KM7.5 diets presented higher final weight, improved SGR than fish fed control diet ($p < 0.05$) (Fig. 1). FCR was improved from 60 days of feeding onwards when diets were supplemented with KM (Fig. 1). Despite that total feed intake remained unaffected ($p > 0.05$), fish fed supplemented krill diets presented a tendency ($p = 0.1$), regardless of the KM level fed, to higher feed intake (6–8%) than non-supplemented fish. Furthermore, protein efficiency ratio (PER) and lipid efficiency ratio (LER) were significant higher ($p < 0.05$) in fish fed KM-supplemented diets (KM5 and KM7.5) than in those fed KM0 (Table 4).

3.2. Biochemical and fatty acid composition of fish tissues

At the end of the trial, whole-body, muscle and liver proximate composition were not affected by the experimental diets (Table 5). Even though, a tendency to present higher lipids, both in whole-body and liver, were noted in fish fed KM5 and KM7.5 compared with those fed the control diet KM0 (Table 5).

Regarding tissue fatty acid composition, whole-body and muscle seemed to be more affected by diet composition than liver (Tables 6, 7 and 8). In whole-body, all saturated fatty acids, except 15:0 and 16:0, as well as the total content of saturated, were the highest in sea bass fed KM7.5, particularly when compared with those fed KM0 ($p < 0.05$; Table 6). Similarly, Σn-9 fatty acids was also the highest in whole-body of fish fed KM7.5 ($p < 0.05$; Table 6). In contrast, Σn-3 fatty acids were lower in fish fed KM7.5 than in those fed the KM5 and KM0 diet ($p < 0.05$; Table 6). However, fish fed KM5 presented the highest 18:3n-3 ($p < 0.05$; Table 6). Interestingly, despite the lowest dietary EPA contents in KM0, fish fed this diet showed similar EPA contents in whole-body than those fed supplemented KM diets ($p < 0.05$; Table 6). Also, these fish showed the highest 18:2n-9 content despite the similar levels of this

Table 4
Survival, nutrient efficiencies and condition factor of sea bass juveniles fed the experimental diets for 12 weeks.

	Diets		
	KM0	KM5	KM7.5
Survival (%)	99.63 ± 0.64	98.89 ± 1.11	99.26 ± 0.64
PER	1.33 ± 0.07^{b}	1.51 ± 0.02^{a}	1.50 ± 0.01^{a}
LER	2.90 ± 0.16^{b}	3.29 ± 0.05^{a}	3.31 ± 0.04^{a}
K	1.47 ± 0.01	1.51 ± 0.01	1.51 ± 0.03

Values expressed in mean ± SD. Different superscript letters in the same row are significantly different ($P < 0.05$) based on Tukey's multiple-range test (one-way ANOVA). PER (protein efficiency ratio) = weight gained/weight of protein consumed; LER (lipid efficiency ratio) = weight gained/weight of lipid consumed; K (condition factor) = (weight/length^{-3}) x100. KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

Table 3
Primer sequences used for running RT-PCR analysis in sea bass livers fed the experimental diets.

Gene	Forward sequence	Reverse sequence	Accession number
fads2	CCTTCACTGCTCTTCATCCCAA	CCCAGGTGGAGGCAGAAGAA	EU439924
hmgr	CCAGCTTCGTATTCAGCACA	GCTTTGGAGAGGTCGATGAG	AY424801
angptl3	CAACATCTTGCAGGAGCGTA	CTCTCCGACAGTCCCTTCAG	FM023639
fabp7	GAAGGCACTTGGTGTTGGTT	CAGGGTTTTCACCACCACTT	FM000669
lpl	GAAGGCACTTGGTGTTGGTT	CAGGGTTTTCACCACCACTT	AM411614
ef-1	F: GCTTCGAGGAAATCACCAA	CAACCTTCCATCCCTTGAAC	AJ866727

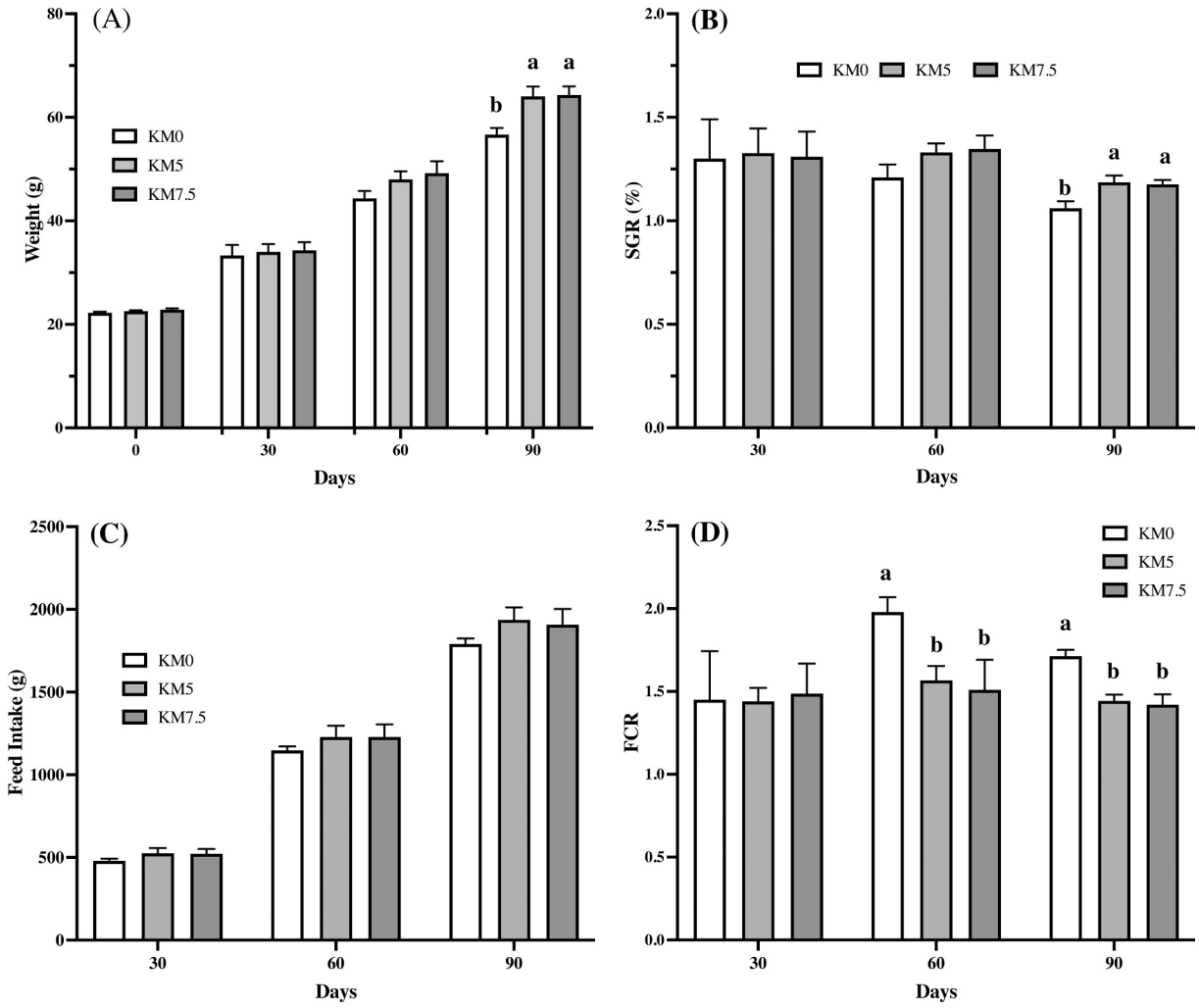

Fig. 1. Weight, growth performance and feed utilization of European sea bass (*Dicentrarchus labrax)* juveniles fed diets with graded KM levels along the feeding trial. Values expressed in mean ± SD. Different superscript letters are significantly different ($P < 0.05$) based on Tukey's multiple-range test (one-way ANOVA). Specific growth rate (SGR) = [(ln final weight - ln initial weight)/number of days] x 100; Food conversion ratio (FCR) = (total feed fed/total weight gained). KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

Table 5
Whole-body, muscle and liver biochemical composition (% wet weight) of European sea bass juveniles fed the experimental diets for 88 days.

	Diets		
	KM0	KM5	KM7.5
Whole-body			
Crude Protein	14.45 ± 0.21	14.71 ± 0.57	15.00 ± 0.56
Crude Lipids	16.14 ± 3.22	18.72 ± 1.18	19.02 ± 0.77
Ash	1.43 ± 0.21	1.44 ± 0.19	1.36 ± 0.02
Moisture	66.03 ± 3.42	63.48 ± 0.93	63.04 ± 0.62
Muscle			
Crude Protein	19.38 ± 0.53	19.26 ± 0.08	19.08 ± 0.08
Crude Lipids	10.10 ± 3.44	11.69 ± 0.55	11.43 ± 1.08
Ash	1.27 ± 0.06	1.23 ± 0.08	1.29 ± 0.03
Moisture	68.88 ± 2.88	67.53 ± 0.67	67.44 ± 0.66
Liver			
Crude Protein	8.29 ± 0.56	8.12 ± 0.63	8.12 ± 0.49
Crude Lipids	29.52 ± 3.39	31.77 ± 3.87	32.74 ± 3.06
Ash	0.84 ± 0.27	0.45 ± 0.21	0.58 ± 0.14
Moisture	69.01 ± 3.56	67.66 ± 4.46	67.32 ± 2.24

Values expressed in mean ± SD. KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

fatty acid among all the diets ($p < 0.05$; Table 6).

In fish muscle, similar tendency as whole-body were observed for saturated fatty acids, being the highest in sea bass fed KM diets ($p < 0.05$; Table 7). In contrast, muscle total monoens were not affected by the increase in dietary KM content, but it significantly decreased muscle 20:4n-6 and Σn-6 HUFA, contrary to the dietary pattern. Fish fed KM5 diet, showed a slight decrease in Σn-3, but no differences were observed for any specific fatty acids of this family ($p < 0.05$; Table 7).

Only Σn-6 HUFA as well as 22:4n-6 contents were slightly affected in liver composition among fish fed the experimental diets, being the higher value found in livers of fish fed KM5 diet ($p < 0.05$; Table 8).

Despite no significant differences were observed for liver lipid classes among fish fed the different experimental diets, a tendency to present lower levels of cholesterol could be noted in those fed KM5 or KM7.5 diets when compared to those fed KM0 (Table 9). Interestingly, cholesterol levels were negatively correlated with dietary EPA content ($r = -0.99$, $p = 0.03$). In addition, a significant correlation was also observed between hepatic free fatty acids (FFA) and liver lipid content ($r = 0.99$, $p = 0.04$), both being also significantly correlated with the dietary content of KM ($r = 0.99$, $p = 0.005$ for liver lipids, and $r = 1$, $p = 0.03$ for liver FFA). A similar tendency was also noted between liver PL and dietary DHA content ($r = 0.99$, $p = 0.03$). Besides. KM dietary content was positively correlated with the pigmented material content

Table 6
Whole-body fatty acid composition (% of total fatty acids) of sea bass juveniles fed the experimental diets for 12 weeks.

Fatty acids (% total fatty acids)	Diets		
	KM0	KM5	KM7.5
14:0	1.54 ± 0.29^{b}	1.68 ± 0.01^{ab}	1.99 ± 0.09^{a}
14:1–7	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
14:1n-5	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.05 ± 0.00^{a}
15:0	0.14 ± 0.00	0.14 ± 0.00	0.16 ± 0.01
15:1n-5	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
16:0ISO	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
16:0	13.92 ± 1.42	14.68 ± 0.33	15.67 ± 0.10
16:1n-7	3.50 ± 0.46	3.62 ± 0.00	3.71 ± 0.07
16:1n-5	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.00
16:2n-4	0.17 ± 0.01^{b}	0.20 ± 0.00^{a}	0.21 ± 0.01^{a}
17:0	0.17 ± 0.01^{b}	0.19 ± 0.01^{a}	0.18 ± 0.01^{ab}
16:3n-4	0.17 ± 0.01^{a}	0.14 ± 0.01^{b}	0.16 ± 0.00^{ab}
16:3n-3	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.00
16:3n-1	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.01
16:4n-3	0.13 ± 0.01^{b}	0.17 ± 0.00^{a}	0.15 ± 0.02^{ab}
18:0	3.68 ± 0.13^{b}	3.77 ± 0.04^{b}	4.18 ± 0.12^{a}
18:1n-9	39.16 ± 0.67	39.76 ± 0.61	39.81 ± 0.12
18:1n-7	2.91 ± 0.22	2.78 ± 0.07	2.87 ± 0.14
18:1n-5	0.11 ± 0.00	0.10 ± 0.01	0.11 ± 0.02
18:2n-9	0.68 ± 0.04^{a}	0.59 ± 0.02^{b}	0.55 ± 0.02^{b}
18:2n-6	12.48 ± 0.45	12.69 ± 0.02	12.08 ± 0.22
18:2n-4	0.11 ± 0.01	0.10 ± 0.00	0.11 ± 0.00
18:3n-6	0.31 ± 0.05^{a}	0.25 ± 0.01^{ab}	0.24 ± 0.01^{b}
18:3n-4	0.08 ± 0.02	0.08 ± 0.00	0.07 ± 0.00
18:3n-3	3.60 ± 0.09^{b}	3.92 ± 0.05^{a}	3.46 ± 0.05^{b}
18:3n-1	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
18:4n-3	0.59 ± 0.05	0.60 ± 0.02	0.55 ± 0.01
18:4n-1	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.00
20:0	0.28 ± 0.01^{b}	0.29 ± 0.00^{b}	0.30 ± 0.00^{a}
20:1n-9	0.25 ± 0.07^{b}	0.16 ± 0.02^{b}	2.47 ± 0.02^{a}
20:1n-7	2.86 ± 0.32^{a}	2.32 ± 0.11^{b}	0.19 ± 0.01^{c}
20.1n-5	0.17 ± 0.08	0.11 ± 0.00	0.12 ± 0.01
20:2n-9	0.10 ± 0.04	0.06 ± 0.00	0.07 ± 0.01
20:2n-6	0.62 ± 0.04	0.55 ± 0.03	0.56 ± 0.01
20:3n-9	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
20:3n-6	0.11 ± 0.03	0.08 ± 0.01	0.08 ± 0.00
20:4n-6	0.36 ± 0.05	0.32 ± 0.01	0.30 ± 0.00
20:3n-3	0.12 ± 0.02	0.09 ± 0.01	0.10 ± 0.01
20:4n-3	0.30 ± 0.05	0.27 ± 0.02	0.25 ± 0.00
20:5n-3	3.61 ± 0.28^{ab}	3.98 ± 0.11^{a}	3.45 ± 0.10^{b}
22:1n-11	1.45 ± 0.36^{a}	0.89 ± 0.13^{b}	1.04 ± 0.04^{ab}
22:1n-9	0.41 ± 0.07	0.31 ± 0.03	0.33 ± 0.00
22:4n-6	0.08 ± 0.01	0.07 ± 0.00	0.08 ± 0.02
22:5n-6	0.12 ± 0.03	0.09 ± 0.01	0.09 ± 0.01
22:5n-3	0.78 ± 0.11^{a}	0.67 ± 0.04^{ab}	0.58 ± 0.01^{b}
22:6n-3	4.49 ± 0.77	3.84 ± 0.30	3.28 ± 0.14
Saturated	19.72 ± 1.84^{b}	20.75 ± 0.32^{ab}	22.48 ± 0.07^{a}
Monoens	50.98 ± 0.04^{a}	50.21 ± 0.24^{b}	50.84 ± 0.22^{a}
Σn-3	13.66 ± 1.19^{a}	13.58 ± 0.47^{ab}	11.87 ± 0.20^{b}
Σn-6	14.08 ± 0.65	14.04 ± 0.07	13.43 ± 0.23
Σn-9	40.63 ± 0.54^{b}	40.90 ± 0.54^{b}	43.24 ± 0.15^{a}
Σn-3 HUFA	9.30 ± 1.23	8.85 ± 0.46	7.66 ± 0.23
Σn-6 HUFA	0.67 ± 0.12	0.56 ± 0.03	0.54 ± 0.03
Σn-3/Σn-6	0.97 ± 0.04	0.97 ± 0.03	0.88 ± 0.03

Values expressed in mean ± SD. Different superscript letters within a row are significantly different (P < 0.05) based on Tukey's multiple-range test (one-way ANOVA). HUFA, highly unsaturated fatty acids.

Table 7
Muscle fatty acid composition (% of total fatty acids) of sea bass juveniles fed the experimental diets for 12 weeks.

Fatty acids (% total fatty acids)	Diets		
	KM0	KM5	KM7.5
14:0	1.68 ± 0.08	1.76 ± 0.03	1.77 ± 0.01
14:1–7	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
14:1n-5	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
15:0	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01
15:1n-5	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
16:0ISO	0.02 ± 0.00	0.09 ± 0.12	0.02 ± 0.00
16:0	13.84 ± 0.42^{b}	14.79 ± 0.33^{a}	15.11 ± 0.17^{a}
16:1n-7	3.38 ± 0.21	3.51 ± 0.11	3.49 ± 0.10
16:1n-5	0.07 ± 0.00	0.08 ± 0.00	0.07 ± 0.00
16:2n-4	0.19 ± 0.01	0.21 ± 0.00	0.22 ± 0.00
17:0	0.19 ± 0.02	0.19 ± 0.00	0.20 ± 0.00
16:3n-4	0.14 ± 0.01	0.14 ± 0.00	0.13 ± 0.01
16:3n-3	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
16:3n-1	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00
16:4n-3	0.16 ± 0.02	0.16 ± 0.00	0.18 ± 0.01
18:0	3.70 ± 0.08^{b}	3.92 ± 0.10^{a}	4.08 ± 0.07^{a}
18:1n-9	37.99 ± 0.38	38.74 ± 0.19	38.72 ± 0.70
18:1n-7	2.77 ± 0.07	2.70 ± 0.08	2.83 ± 0.05
18:1n-5	0.10 ± 0.00	0.10 ± 0.01	0.09 ± 0.00
18:2n-9	0.56 ± 0.01	0.51 ± 0.06	0.51 ± 0.04
18:2n-6	13.07 ± 0.62	12.53 ± 0.04	12.73 ± 0.06
18:2n-4	0.11 ± 0.01	0.10 ± 0.00	0.10 ± 0.00
18:3n-6	0.29 ± 0.02	0.25 ± 0.01	0.24 ± 0.03
18:3n-4	0.08 ± 0.01	0.33 ± 0.45	0.07 ± 0.01
18:3n-3	4.06 ± 0.21	3.84 ± 0.06	3.84 ± 0.05
18:3n-1	0.10 ± 0.01	0.09 ± 0.00	0.09 ± 0.01
18:4n-3	0.57 ± 0.01	0.57 ± 0.01	0.58 ± 0.02
18:4n-1	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.00
20:0	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.01
20:1n-9	0.18 ± 0.02^{a}	0.15 ± 0.00^{b}	0.14 ± 0.01^{b}
20:1n-7	2.37 ± 0.24	2.30 ± 0.07	2.13 ± 0.04
20.1n-5	0.11 ± 0.01	0.11 ± 0.00	0.11 ± 0.00
20:2n-9	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01
20:2n-6	0.58 ± 0.06	0.58 ± 0.04	0.53 ± 0.01
20:3n-9	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
20:3n-6	0.10 ± 0.02	0.08 ± 0.00	0.08 ± 0.01
20:4n-6	0.44 ± 0.02^{a}	0.36 ± 0.00^{b}	0.35 ± 0.02^{b}
20:3n-3	0.11 ± 0.01	0.10 ± 0.00	0.09 ± 0.01
20:4n-3	0.28 ± 0.01	0.26 ± 0.01	0.25 ± 0.02
20:5n-3	4.41 ± 0.07	4.31 ± 0.14	4.43 ± 0.11
22:1n-11	0.92 ± 0.19	0.81 ± 0.07	0.72 ± 0.06
22:1n-9	0.31 ± 0.04	0.32 ± 0.05	0.29 ± 0.01
22:4n-6	0.08 ± 0.01^{a}	0.06 ± 0.00^{b}	0.06 ± 0.00^{b}
22:5n-6	0.13 ± 0.02^{a}	0.11 ± 0.00^{ab}	0.10 ± 0.01^{b}
22:5n-3	0.82 ± 0.08	0.72 ± 0.05	0.67 ± 0.06
22:6n-3	5.33 ± 0.66	4.69 ± 0.24	4.23 ± 0.36
Saturated	19.84 ± 0.41^{b}	21.09 ± 0.46^{a}	21.59 ± 0.20^{a}
Monoens	48.30 ± 0.23	48.92 ± 0.17	48.67 ± 0.60
Σn-3	15.77 ± 0.60^{a}	14.69 ± 0.51^{b}	14.31 ± 0.57^{ab}
Σn-6	14.69 ± 0.58	13.97 ± 0.08	14.09 ± 0.12
Σn-9	39.14 ± 0.31	39.83 ± 0.25	39.75 ± 0.67
Σn-3 HUFA	10.94 ± 0.82	10.07 ± 0.44	9.67 ± 0.56
Σn-6 HUFA	0.74 ± 0.04^{a}	0.61 ± 0.01^{b}	0.59 ± 0.04^{b}
Σn-3/Σn-6	1.08 ± 0.08	1.05 ± 0.03	1.02 ± 0.03

Values expressed in mean ± SD. Different superscript letters within a row are significantly different (P < 0.05) based on Tukey's multiple-range test (one-way ANOVA). HUFA, highly unsaturated fatty acids.

($r = 0.84$, $p = 0.01$).

3.3. Liver morphology

After 88 days of feeding, fish fed KM diets (Fig. 2B, C) presented smaller hepatocytes area with a more regular-shaped morphology around sinusoidal spaces compared to fish fed the control diet (Fig. 2A). This effect was particularly noticeable in fish fed KM7.5, pointing to a dose-dependent effect of KM on these parameters. Moreover, a lower level of cytoplasm vacuolization can be observed in fish fed supplemented diets in relation to hepatocytes of fish fed the control diet (Fig. 2).

3.4. Gene expression

After 12 weeks of feeding, sea bass fed KM5 and KM7.5 diets showed decreased *mRNA* levels of *fads2* ($p < 0.1$) and *hmgr* ($p < 0.05$) (Fig. 3). Furthermore, a strong correlation was observed between these two genes (Table S1; Pearson correlation = 1, $p = 0.005$), as well as with *fabp7* expression (Pearson correlation = 0.99, $p = 0.03$ and Pearson correlation = 0.99, $P = 0.04$, respectively). Despite that no significance differences in *fabp7* relative expression were detected among sea bass

Table 8
Liver fatty acid composition (% of total fatty acids) of sea bass juveniles fed the experimental diets for 12 weeks.

Fatty acids (% total fatty acids)	Diets		
	KM0	KM5	KM7.5
14:0	1.23 ± 0.03	1.09 ± 0.25	1.14 ± 0.23
14:1–7	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
14:1n-5	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
15:0	0.07 ± 0.01	0.08 ± 0.02	0.06 ± 0.01
15:1n-5	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
16:0ISO	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
16:0	17.34 ± 1.20	16.34 ± 1.03	18.51 ± 0.85
16:1n-7	4.13 ± 0.39	3.62 ± 0.37	3.87 ± 0.51
16:1n-5	0.09 ± 0.01	0.07 ± 0.00	0.09 ± 0.02
16:2n-4	0.06 ± 0.02	0.08 ± 0.03	0.08 ± 0.02
17:0	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.01
16:3n-4	0.18 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
16:3n-3	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
16:3n-1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
16:4n-3	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01
18:0	4.70 ± 0.16	4.95 ± 0.49	5.46 ± 0.56
18:1n-9	49.53 ± 1.44	49.89 ± 1.20	50.02 ± 1.50
18:1n-7	3.18 ± 0.23	3.40 ± 0.04	3.25 ± 0.35
18:1n-5	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.02
18:2n-9	1.51 ± 0.08	1.31 ± 0.31	1.38 ± 0.18
18:2n-6	7.78 ± 0.84	8.04 ± 1.17	6.93 ± 0.45
18:2n-4	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00
18:3n-6	0.47 ± 0.08	0.38 ± 0.03	0.35 ± 0.04
18:3n-4	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
18:3n-3	1.89 ± 0.27	1.93 ± 0.33	1.60 ± 0.20
18:3n-1	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
18:4n-3	0.29 ± 0.06	0.29 ± 0.05	0.25 ± 0.06
18:4n-1	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.00
20:0	0.21 ± 0.01	0.25 ± 0.06	0.22 ± 0.02
20:1n-9	0.14 ± 0.01	0.16 ± 0.01	0.62 ± 0.90
20:1n-7	1.98 ± 0.21	2.22 ± 0.07	1.91 ± 0.01
20.1n-5	0.09 ± 0.02	0.11 ± 0.03	0.09 ± 0.01
20:2n-9	0.09 ± 0.02	0.09 ± 0.03	0.08 ± 0.01
20:2n-6	0.38 ± 0.05	0.47 ± 0.04	0.38 ± 0.04
20:3n-9	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
20:3n-6	0.06 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
20:4n-6	0.19 ± 0.04	0.18 ± 0.02	0.15 ± 0.01
20:3n-3	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
20:4n-3	0.09 ± 0.00	0.10 ± 0.02	0.08 ± 0.02
20:5n-3	1.19 ± 0.28	1.31 ± 0.25	1.13 ± 0.40
22:1n-11	0.34 ± 0.05	0.43 ± 0.15	0.22 ± 0.02
22:1n-9	0.25 ± 0.03	0.30 ± 0.05	0.24 ± 0.02
22:4n-6	0.05 ± 0.01^{ab}	0.05 ± 0.01^{a}	0.04 ± 0.01^{b}
22:5n-6	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.01
22:5n-3	0.23 ± 0.05	0.25 ± 0.06	0.23 ± 0.09
22:6n-3	1.52 ± 0.32	1.61 ± 0.34	1.34 ± 0.40
Saturated	23.64 ± 1.25	22.79 ± 1.30	25.45 ± 1.01
Monoens	59.95 ± 1.52	60.41 ± 0.62	60.52 ± 0.46
Σn-3	5.34 ± 0.97	5.62 ± 0.77	4.75 ± 1.12
Σn-6	8.97 ± 0.99	9.23 ± 1.14	7.92 ± 0.53
Σn-9	51.54 ± 1.39	51.76 ± 1.35	52.35 ± 0.82
Σn-3 HUFA	3.09 ± 0.66	3.34 ± 0.57	2.84 ± 0.89
Σn-6 HUFA	0.34 ± 0.05^{ab}	0.35 ± 0.02^{a}	0.26 ± 0.02^{b}
Σn-3/Σn-6	0.60 ± 0.09	0.61 ± 0.09	0.60 ± 0.15

Values expressed in mean ± SD. Different superscript letters within a row are significantly different ($P < 0.05$) based on Tukey's multiple-range test (one-way ANOVA). HUFA, highly unsaturated fatty acids.

Table 9
Lipid classes (% total lipids) of livers of sea bass juveniles fed the experimental diets for 12 weeks.

Lipid class	Diets		
	KM0	KM5	KM7.5
Wax/Sterol esters	0.27 ± 0.25	0.57 ± 0.47	0.60 ± 0.26
Triacylglycerols	62.73 ± 1.42	62.6 ± 2.30	63.3 ± 0.4
Free fatty acids	18.90 ± 6.35	19.93 ± 3.80	20.43 ± 1.70
Cholesterol	6.53 ± 3.93	4.57 ± 1.27	4.33 ± 0.46
Diacylglycerol	8.30 ± 3.19	7.73 ± 0.50	7.37 ± 0.81
Total Neutral lipids	96.1 ± 0.36	95.4 ± 1.10	96.03 ± 0.21
Phosphatidylethanolamine	0.70 ± 0.34	0.60 ± 0.30	0.47 ± 0.06
Phosphatidylinositol	0.73 ± 0.35	0.70 ± 0.50	0.40 ± 0.20
Phosphatidylcholine	1.27 ± 0.25	1.13 ± 0.25	0.87 ± 0.15
Sphingomyelin	0.17 ± 0.15	0.23 ± 0.06	0.23 ± 0.06
Lysophosphatidylcholine	0.27 ± 0.23	0.43 ± 0.23	0.37 ± 0.06
Pigmented material	0.50 ± 0.44^{b}	1.50 ± 0.26^{a}	1.63 ± 0.35^{a}
Total Polar lipids	3.63 ± 0.81	4.60 ± 1.10	3.97 ± 0.21

Values expressed in mean ± SD. Different superscript letters within a row are significantly different ($P < 0.05$) based on Tukey's multiple-range test (one-way ANOVA). KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

fed the different dietary KM contents, fish fed KM5 and KM7.5 diets expressed slight lower *mRNA* levels (Fig. 3), which were significantly correlated with liver cholesterol (Pearson correlation = 0.99, $p = 0.04$). In addition, *lpl* mRNA levels were linearly correlated with liver lipids (Pearson correlation = 0.99, p = 0.03).

4. Discussion

The present study aimed to investigate the potential of Antarctic KM as FM partial alternative in practical European sea bass diets. After 3 months of feeding European sea bass with the experimental diets, no effect was observed in survival or condition factor of fish, suggesting that KM-supplemented diets did not cause major alterations in fish health or condition for this fish species and time of supplementation.

Despite the partial replacement of FM (33% and 50%, respectively) with the two levels of KM (5 and 7.5%KM), which did not alter the protein and lipid contents of the diets, European sea bass fed KM-based diets presented a higher growth performance after 12 weeks of feeding, regardless of the level of KM fed. A similar improvement of fish growth performance has been reported in previous studies in gilthead sea bream fed for 12 weeks a 9% KM-based diet as a partial replacement to FM (20% of total FM; Saleh et al., 2018). Atlantic salmon fed KM as replacer of FM (20–40% of total FM) presented improved SGR after feeding for 71 days KM diets, however this effect points to be time dependent and disappeared when fed for longer periods of time (Olsen et al., 2006). However, other studies reported a successful partial replacement of FM by KM but without a positive effect on fish growth performance (Storebakken, 1988; Anderson, 1997; Julshamn et al., 2004; Yoshitomi et al., 2006, 2007; Mørkøre et al., 2020), indicating the influence of the fish species and starting size studied, as well as the level of KM supplementation used and time of supplementation.

In relation with the improved fish growth performance observed in the present study, fish fed KM-based diets presented an improved FCR and a tendency to increased FI, suggesting the potential of KM being a palatability enhancer. Palatability often is affected when high plant meal-based diets are fed. Similarly, gilthead sea bream juveniles presented improved FCR fed a 9% KM-based diet for 12 weeks (Saleh et al., 2018). On the contrary, KM included at higher levels (60% and onwards of total dietary protein content) in Atlantic salmon diets did not affect FCR or FI when fed for longer periods of time (Olsen et al., 2006). This effect may be related with KM chitin content and its effects on fish gut health, by decreasing lipid absorption and increasing water content in feces when fed in considerable levels (Olsen et al., 2006). The optimized FCR found in the present study was accompanied by increased PER and LER, and by a slight and not significant tendency to higher tissue lipid content, 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. In this sense, Mørkøre et al. (2020), attributed to Atlantic salmon fed KM enhanced lipid transport and higher FFA in skeletal muscle, indicating an effect of KM on the bioconversion of n-3 and n-6 fatty acid series and pointing to a KM-derived n-3 HUFAs higher bioactivity compared to those from FO origin.

In particular, the lipid fraction of KM, which is a source of essential n-3 HUFAs for marine fish, is predominantly in the phospholipid (PL) form (mainly in phosphatidylcholine (PC)) compared to the triacylglycerol

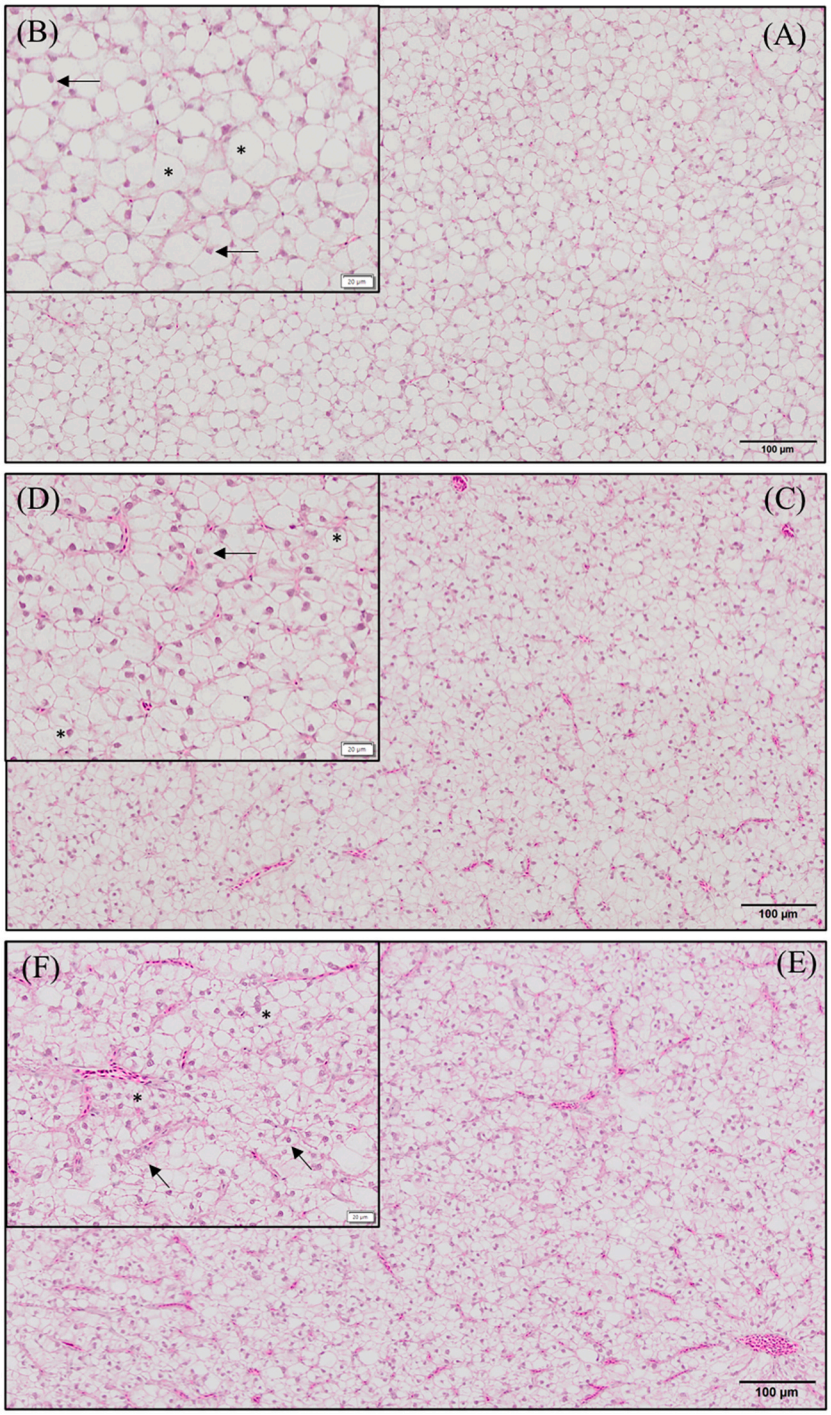

Fig. 2. Liver morphology of European sea bass (*Dicentrarchus labrax*) juveniles fed diets with graded krill meal (KM) levels for 88 days stained with Hematoxylin and Eosin (H&E). (A, B) KM0, (C, D) KM5 and (E, F) KM7.5. Observe the general healthier morphological pattern on fish fed KM5 and KM7.5 diets (C, E) compared to fish KM0 diet (A). Scale bar 100 μm. Observe on the detailed micrographs (B, D, F) the smaller hepatocellular area (*) and the reduced cytoplasm vacuolization (→) of fish fed with KM-supplemented diets as well as the better alignment around sinusoidal spaces of their nuclei. Scale bar 20 μm KM = Krill meal. KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

(TAG) form of FO. On one hand, the higher bioavailability of KM PLs (mainly phosphatidylethanolamine (PE) and PC) could be influencing the synthesis and deposition in liver of the first resultant compounds of PLs synthesis (PC and PE) but also by modulating the glycerol-3-phosphate pathway affecting diacylglycerol (DAG) synthesis and thus, PC and PE levels. Indeed, dietary PLs levels upregulate the mRNA expression levels of 1, 2-diacylglycerol choline phosphotransferase in livers of yellow croaker (*Larimichthys crocea*) (Cai et al., 2016). Furthermore, the slight decrease of PC in livers of fish fed KM graded levels along with an increase of FFA and lysophosphatidylcholine (LPC) correlated with the increase of KM in the diets maybe also indicating a higher hydrolyzation of liver PC, in relation to the liver levels found.

We did not observe major differences in fatty acid incorporation in the analyzed tissues of fish, mostly reflecting the dietary pattern. The lack of effect in fatty acid composition among European sea bass fed the different experimental diets seems to contradict the idea that fatty acids via PL intake are more readily incorporated into fish tissues, maybe related to the fact that we only analyzed the fatty acid profile of total lipids. Actually, Atlantic salmon fed KM presented increased levels of EPA and DHA in FFA fraction, however in total lipids their content did not differ among treatments (Mørkøre et al., 2020).

Despite in the present study the dietary levels of EPA + DHA were above the nutritional requirements for this species, *fads2* gene expression was downregulated in a dose-dependent way in fish livers in

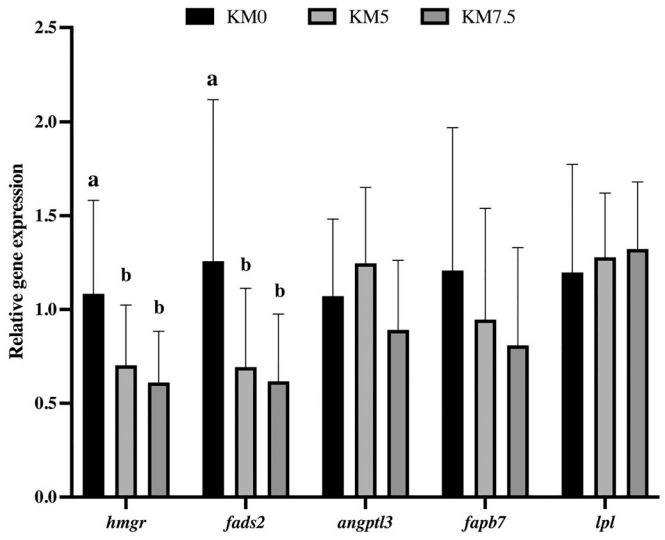

Fig. 3. Relative expression of hepatic genes related with lipid metabolism of European sea bass *Dicentrarchus labrax* juveniles fed the experimental diets for 12 weeks. Values expressed in mean ± SD. Different superscript letters indicate significant differences among dietary treatments ($P < 0.05$) based on Tukey's multiple-range test (one-way ANOVA). *fads2:* fatty acyl desaturase 2; *hmgr*: 3-hydroxy-3-methylglutaryl coenzyme A reductase; *angtpl3*: angiopoietin-like 3; *fabp7*: fatty acid binding protein 7; *lpl*: lipoprotein lipase. KM = control diet; KM5 = krill meal 5 g/kg diet; KM7.5 = krill meal 7.5 g/kg diet.

relation to KM dietary levels, and subsequently to total EPA + DHA dietary levels (+31% and + 27% in KM5 and KM7.5 diets) compared to fish fed control diet. Despite the biosynthesis of EPA, DHA and arachidonic acid (ARA; 20:4n-6) is usually very low and limited in most vertebrates (Tocher et al., 2019), it has been also well described before how dietary LC-PUFA levels regulate *fads2* gene expression (Izquierdo et al., 2008; González-Rovira et al., 2009; Geay et al., 2010; Betancor et al., 2016; Houston et al., 2017; Jin et al., 2017).

Thus, the stimulation of fatty acid β-oxidation and inhibition of fatty acid synthesis by *fads2* associated to KM dietary supplementation may be attributed to a peroxisome proliferator-activated receptor alpha (PPARα) activation and further inhibition of the sterol regulatory element-binding protein 1 (SREBP-1) signaling pathway (Jump, 2008). In this sense, a dietary reduction in LC-PUFA in fish fed KM0 diet may also induce an upregulation of *fabp7* gene expression, which is involved in fatty acid transport and uptake in European sea bass (Geay et al., 2010; Torrecillas et al., 2018).

Certainly, in the present study there is a trend to reduced *fabp7* and *hmgcr* and increased *lpl* gene expression in livers of fish fed KM diets regardless of the KM level fed and in relation only to KM composition. On one hand, an increased HUFA and cholesterol synthesis normally implies a compensation for a reduced dietary intake, through alterations in HUFA and cholesterol biosynthetic pathways via upregulation of SREBPs and *fads2* (Morais et al., 2011; Torrecillas et al., 2018). In mice, it was suggested that n-3 HUFA from krill can increase expression of *insig-1*, which causes the suppression of *hmgcr* and *ldlr* and, consequently, decrease cholesterol synthesis (Burri et al., 2011). Indeed, in the present study cholesterol was strongly correlated with dietary EPA. On the other hand, the high levels of pigmented material in KM, mainly astaxanthin, may be leading to a reduced level of cholesterol in liver as observed in Atlantic salmon (Chimsung et al., 2013; Řehulka, 2000) and yellow croaker *Pseudosciaena crocea* (Li et al., 2014) fed with increasing levels of this carotenoid. Furthermore, the slight increase in *lpl* gene expression in livers of fish fed KM-based diets may be suggesting an increase in lipid transport and metabolism in this tissue, altogether in relation to the healthier liver morphological pattern observed in the present study.

5. Conclusions

The results of the present study showed that not only dietary FM can be partially replaced by KM in European sea bass diets, but also that KM at 5% and 7.5% promotes European sea bass juvenile's growth performance, feed and nutrient utilization. Besides, fish fed KM presented a dose-dependent modulation of liver lipid metabolism and reduced liver hepatocytes vacuolization. Therefore, presenting KM not only as an alternative protein and lipid source in practical diets for European sea bass with low FM/FO content, but also as a potential functional ingredient capable of promoting its liver health status. These results open an interesting alternative not only to replace FM by the partial inclusion of KM, but also other alternative vegetal meals and concentrates, which have a direct implication on growth performance and feed utilization in this fish species.

Authors contribution

ST, DM, MI and TB-S contributed to the conception and design of the study. ST performed formal analysis. ST and MC performed the statistical analyses. ST and MC wrote the first draft of this manuscript. TB-S acquired funding. Resources were provided by TB-S, DM and MI. All authors contributed to manuscript revision, read, and approved the submitted version.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. BioMarine Antartic AS, provided the krill meal and diets, and support in the form of salary for author TB-S, but did not have any additional role in data collection, analysis, and decision to publish. This fact does not alter our adherence to Aquaculture policies on sharing data and materials.

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