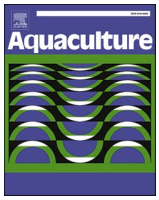
Aquaculture 560 (2022) 738452

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Effect of combined fishmeal and fish oil replacement on growth performance, flesh quality and shelf life of European sea bass (*Dicentrarchus labrax*)

P.L. Castro *, S. Torrecillas, D. Montero, M.S. Izquierdo, R. Ginés

GIA-ECOAQUA Universidad de Las Palmas de Gran Canaria, Muelle de Taliarte s/n, 35214 Telde, Spain

ARTICLE INFO

Keywords:
European sea bass
Alternative meals
Alternative oils
Growth performance
Quality
Histology
Fatty acid profiles

ABSTRACT

The present study was aimed to evaluate the effect on growth performance, muscle integrity and quality traits throughout ice storage of distinct levels of substitution of fish meal (FM) and fish oil (FO) by sustainable plant raw materials (plant meal and oils) on European sea bass (*Dicentrarchus labrax*) in long-term feeding of 18 months. Six experimental diets, isonitrogenous (45% crude protein) and isolipidic (21%), containing gradually reduced levels of FM and FO as follows (%FM/%FO): 20/6, 20/3, 10/6, 10/3, 5/6 and 5/3 were evaluated. The reduction of FM resulted in a significant decrease (17–20%) of fish growth performance. There were no differences among the experimental diets for myofibrillar or endoproteases antibodies (anti-desmin, anti-dystrophin, anti-calpastatin, anti-M-calpain, anti-Micro-calpain) after 8 days of storage period. FO substitution increased *n-6* fatty acids (FA) and reduced saturated FA, n-3 LC-PUFA (Long-chain polyunsaturated fatty acid) and n-3/n-6 ratio. Monounsaturated fatty acids were not affected by the content of the diet. No organoleptic changes were observed following the Quality Index Method (QIM) scheme, but a higher hardness measured by instrumental texture in whole fish and the raw fillet was associated with diets with high inclusion of FM at the beginning of storage, not perceptible in the cooked fillet.

1. Introduction

Aquaculture is the major consumer of fish meal that supplies the largest portion of dietary protein in fish diets. However, fish meal is a limited resource with inherent variability in composition. Most of the future changes in developing novel aquafeeds should be focused on alternative protein and lipid sources (Tacon and Metian, 2015; Álvarez et al., 2020). The fillet fatty acid composition of cultured fish fed a vegetable meal and oil-based diets are normally characterized by significantly decreased levels of n-3 LC-PUFA compared to fish fed a fish meal and oil-based diet.

The European seabass, *Dicentrarchus labrax*, a widely distributed marine teleost, is considered of great commercial importance. The species has high growth rates and feed efficiency, having a high demand in the market with an average commercial size of 400 g. The effects of a combined FM and FO reduction at extremely low inclusion levels have hardly been studied in European seabass (Torrecillas et al., 2017). A complete FM and FO free feed led to reduced growth after nine months of feeding (Geay et al., 2010; Geay et al., 2011). Feeding young European sea bass of 2.5 g initial body mass, either an FM/FO based diet or a totally plant-based diet with no FM or FO, led to a significant reduction in survival and growth of fish fed the zero FM/FO diet (Le Boucher et al., 2013). In bigger fish, weighing about 194 g, the differences in growth rates were reduced with final individual body weights of sea bass fed the FM/FO diet having only slightly higher final body weights than when fed the all plant-based diet (Le Boucher et al., 2011).

FM and FO replacement additionally can alter the resulting quality (Rincón et al., 2016). The effects in the fillet, apart from changes in fatty acid profiles, are related to the sensory properties, namely the appearance, odour, flavour, and texture (Matos et al., 2017) having an impact on fillet quality (Castro et al., 2015) and consumer acceptance (Turchini et al., 2009). Fish muscle rapidly softens during postmortem storage due

Abbreviations: FO, Fish oil; FM, Fish meal; VM, Vegetable meal; VO, Vegetable oil; LC-PUFA, Long-chain polyunsaturated fatty acid; SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; DHA, Docosahexaenoic acid; ARA, Arachidonic acid; EPA, Eicosapentaenoic acid; FA, Fatty acid; dph, days post-harvest; QIM, Quality Index Method..

* Corresponding author.
E-mail address: pedro.castro@ulpgc.es (P.L. Castro).

https://doi.org/10.1016/j.aquaculture.2022.738452
Received 11 February 2022; Received in revised form 20 May 2022; Accepted 31 May 2022
Available online 8 June 2022

to proteolytic degradation (Caballero et al., 2009) with the initial phenomena of texture softening and water loss of muscle, leading to the deterioration of flesh quality (Yu et al., 2022). These changes can be monitored by instrumental texture (Cheng et al., 2014), specific Quality Index Method (QIM) (Knowles et al., 2007) or histological or immunohistochemical methods (Caballero et al., 2009; Ayala et al., 2010) to outline quality losses. The first changes occurring are due to endogenous enzymes promoting proteolysis of muscle proteins and connective tissue as well as fat hydrolysis (Delbarre-Ladrat et al., 2006). Understanding the postmortem process and the relationship between this disorganization, loss of freshness, and textural degradation is of great importance to find new markers and develop methods for the evaluation of fish freshness (Tie et al., 2022).

The effect of dietary changes has been evaluated mainly in juvenile fish, with limited studies examining the effect of diet in the period from juvenile to commercial-size bass. Thus, the present study was designed to determine the optimal levels of FM and FO replacement based on a long-term feeding production cycle for European sea bass. Besides, to monitor the structural and functional changes occurring in muscle during its shelf life, to assess the effects of dietary modifications on flesh texture and overall quality.

2. Materials and methods

2.1. Experimental diets

Six experimental diets were formulated to contain graded levels of FM combined with several levels of FO as follows (%FM/%FO): 20/6, 20/3, 10/6, 10/3, 5/6 and 5/3. The diets were isoenergetic and isonitrogenous, balanced in several macro-and micro-nutrients including amino acids. They were formulated to meet the respective known fish nutritional requirements (Jobling, 2012) and manufactured by BioMar (BioMar Tech-Centre, Brande, Denmark). The diets formula, chemical composition and fatty acid profile are shown in Tables 1 and 2.

Table 1
Ingredients (%) and chemical composition of the experimental diets.

	Diets (%FM/%FO)					
Ingredients	20/6	20/3	10/6	10/3	5/6	5/3
Fish meal[1]	20.00	20.00	10.00	10.00	5.00	5.00
Blood meal spray	5.00	5.00	6.00	6.00	7.00	7.00
Soya concentrate	14.20	14.20	18.30	18.30	20.00	20.00
Corn gluten	14.00	14.00	18.40	18.40	22.00	22.00
Wheat gluten	5.00	5.00	6.00	6.00	5.50	5.50
Rapeseed cake	13.00	13.00	12.70	12.60	11.30	11.30
Wheat	10.00	9.59	7.44	7.35	6.79	6.89
Fish oil[2]	6.00	3.00	6.00	3.00	6.00	3.00
Rapeseed oil	4.00	5.40	4.00	5.30	4.00	5.20
Linseed oil	2.00	2.70	2.00	2.65	2.00	2.60
Palm oil	4.00	5.40	4.00	5.30	4.00	5.20
Microingredients[3]	1.98	1.88	4.33	4.28	5.58	5.49
Premix[4]	0.75	0.75	0.75	0.75	0.75	0.75
Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05
Yttrium	0.03	0.03	0.03	0.03	0.03	0.03
Crude lipids	24.37 ± 0.7	23.24 ± 0.6	24.66 ± 0.2	25.17 ± 0.1	28.21 ± 0.2	28.78 ± 0.3
Crude protein	45.56 ± 0.3	46.06 ± 0.2	45.21 ± 0.2	45.19 ± 0.5	46.33 ± 0.3	46.21 ± 0.2
Ash	6.32 ± 0.07	6.04 ± 0.05	5.71 ± 0.07	5.61 ± 0.04	5.4 ± 0.02	5.33 ± 0.04
Moisture	6.68 ± 0.06	8.76 ± 0.1	8.19 ± 0.05	7.86 ± 0.08	8.80 ± 0.02	8.81 ± 0.2

[1] South-American, Superprime – Feed Service Bremen, Germany.
[2] South American fish oil, LDN Fish Oil, Denmark.
[3] Contains lysine and methionine, monocalcium phosphate, choline, inositol, phospholipids (Emulthin G35). Vilomix (Denmark), Evonik Industries (Germany), Pöhner (Germany).
[4] Supplied the following vitamins (mg/kg): A 3.8, D 0.05, E 102.4, K3 9.8, B1 2.7, B2 8.3, B6 4.8, B12 0.25, B3 24.8, B5 17.2, folic acid 2.8, H 0.14, C 80; minerals (mg/kg): cobalt 0.94, iodine 0.7, selenium 0.2, iron 32.6. manganese 12, copper 3.2, zinc 67; other (g/kg): taurine 2.45, methionine 0.5, histidine 1.36, cholesterol 1.13. DSM, (Netherlands), Evonik Industries (Germany), Deutsche Lanolin Gesellschaft (Germany).

2.2. Animals and housing

Experiments were conducted following FELASA category C recommendations and according to the European Economic Community animal experimentation guidelines directive of 24 November 1986 (86/609/EEC). European sea bass juveniles of 9.8 ± 1.5 g and 9.1 ± 0.5 cm were randomly distributed in 18 indoor cylindroconical 500 L fibreglass tanks at an initial stocking density of 1.8 kg m^{-3} (90 fish per tank). Tanks were supplied in a flow-through system with filtered seawater, at a temperature of 25 °C, and natural photoperiod. Fish were manually fed until apparent satiation with one of the six experimental diets for seventy-eight weeks (3 times a day, 6 days a week). Each diet was fed to triplicate tanks.

2.3. Sampling procedures

At the end of the trial (556 days of feeding), the fish were sacrificed by administering an overdose of anesthetic (clove oil) by immersion and individually weighed (growth parameters were determined every two months). Fish were packed ungutted with flaked ice into polystyrene boxes stored at 4 °C, replacing the ice as needed. During storage, five randomly chosen fish per group were obtained at 0, 2, 5, 8 and 12 dph (days post-harvest) and individually subjected to QIM assessment. The Texture Profile Analysis (TPA), proximate composition and fatty acid profile in raw fillets were also determined at 0, 2, 5, 8 and 12 dph, while cooked fillets were only analyzed for the fatty acid profile on the 0 dph. Immunohistochemical analysis was developed on 0 and 8 dph.

2.4. Muscle immunohistochemical analyses

Muscle samples were fixed in 10% neutral-buffered formalin, dehydrated in ethanol series, and embedded in paraffin wax. Sections of 3 μm were prepared with a Leica microtome (Leica Instruments GmbH, Hubloch, Germany) and mounted on Vectabon-coated slides (Sigma Diagnostics, St. Louis, MO). Immunohistochemical staining was carried out using the horseradish peroxidase (HRP) anti-rabbit EnVision (Dako, Denmark). After antigen retrieval (High pH), endogenous peroxidase activity was blocked. The primary antibody used were, for the anti-desmin polyclonal antibody (Euro-diagnostica, Malmö, Sweden, diluted 1:300), anti-dystrophin (Sigma, Saint Louis, Missouri, USA, clone MANDRA1, diluted 1:350), anti-calpastatin (Affinity BioReagents, Golden, USA, clone 2G11D6, diluted 1:1000), anti-M-calpain (Affinity BioReagents, Golden, USA, clone 107–82, diluted 1:100) or anti-Micro-calpain (Affinity BioReagents, Golden, USA, clone 9A4H8D3, diluted 1:100). Diaminobenzidine and aminoethyl carbazole (Dako, Denmark) was used as chromogen and slides were counterstained with Harris hematoxylin. Negative controls were run by replacing each primary antibody with PBS. In each section (3 sections per staining method and time point, 0 and 8 dph), 10 fields were examined under a light microscope separately by two pathologists. The mean proportions of positive cells (brown staining of the sarcolemma membrane and/or cytoplasm) were scored as follows: +++, > 70%; ++, 30 to 70%; +, < 30%; −, negative.

2.5. Biochemical analyses

The feed and the fish biochemical composition were analyzed following standard procedures (AOAC, 2000). Dry matter content was determined by drying in an oven (110 °C) until constant weight, ash content by combustion in a muffle furnace (600 °C for 12 h), crude protein content (Nx6.25) was determined by Kjeldahl method and crude

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

Diets (%FM/%FO)						
	20/6	20/3	10/6	10/3	5/6	5/3
14:0	7.29	6.85	6.61	6.48	6.47	6.62
14:1n-7	0.02	0.01	0.02	0.01	0.02	0.01
14:1n-5	0.10	0.06	0.10	0.05	0.09	0.05
15:0	0.26	0.17	0.25	0.15	0.23	0.14
15:1n-5	0.01	0.01	0.01	0.01	0.01	0.01
16:0ISO	0.04	0.03	0.04	0.02	0.04	0.02
16:0	14.28	12.42	14.09	12.40	13.94	12.32
16:1n-7	4.14	2.52	3.84	2.23	3.55	2.05
16:1n-5	0.18	0.11	0.16	0.09	0.12	0.09
16:2n-6	0.01	0.01	0.01	0.00	0.01	0.00
16:2n-4	0.50	0.30	0.44	0.25	0.40	0.23
17:0	0.58	0.34	0.50	0.28	0.45	0.26
16:3n-4	0.09	0.08	0.10	0.07	0.09	0.07
16:3n-3	0.08	0.05	0.07	0.04	0.07	0.04
16:3n-1	0.06	0.04	0.04	0.02	0.03	0.02
16:4n-3	0.82	0.50	0.66	0.38	0.60	0.36
16:4n-1	0.00	0.00	0.00	0.00	0.00	0.00
18:0	3.42	3.25	3.43	3.29	3.42	3.24
18:1n-9	26.83	32.50	27.86	32.76	28.26	32.29
18:1n-7	2.80	2.56	2.74	2.45	2.63	2.34
18:1n-5	0.06	0.04	0.06	0.04	0.05	0.04
18:2n-9	0.06	0.03	0.06	0.03	0.05	0.02
18:2n-6	14.34	16.75	16.46	18.88	18.18	20.31
18:2n-4	0.19	0.12	0.16	0.09	0.15	0.08
18:3n-6	0.15	0.09	0.14	0.08	0.12	0.07
18:3n-4	0.08	0.05	0.06	0.05	0.07	0.04
18:3n-3	8.43	11.03	9.31	11.75	9.53	11.81
18:3n-1	0.01	0.01	0.01	0.00	0.01	0.00
18:4n-3	0.84	0.48	0.71	0.39	0.65	0.37
18:4n-1	0.09	0.05	0.07	0.04	0.06	0.04
20:0	0.39	0.41	0.39	0.41	0.40	0.40
20:1n-9	0.05	0.04	0.04	0.03	0.04	0.03
20:1n-7	1.04	1.09	1.05	1.06	1.04	1.01
20:1n-5	0.18	0.12	0.16	0.11	0.15	0.10
20:2n-9	0.06	0.03	0.06	0.03	0.05	0.03
20:2n-6	0.12	0.11	0.12	0.10	0.11	0.10
20:3n-9	0.04	0.02	0.03	0.01	0.03	0.01
20:3n-6	0.07	0.04	0.06	0.03	0.05	0.03
20:4n-6	0.44	0.25	0.35	0.20	0.31	0.18
20:3n-3	0.04	0.04	0.04	0.04	0.04	0.04
20:4n-3	0.27	0.17	0.25	0.14	0.22	0.12
20:5n-3	6.02	3.62	4.86	2.72	4.32	2.51
22:1n-11	0.15	0.09	0.15	0.09	0.14	0.08
22:1n-9	0.21	0.28	0.30	0.32	0.31	0.31
22:4n-6	0.05	0.03	0.05	0.03	0.04	0.04
22:5n-6	0.11	0.07	0.09	0.05	0.08	0.05
22:5n-3	0.77	0.47	0.63	0.35	0.53	0.30
22:6n-3	4.23	2.67	3.35	1.93	2.87	1.73
SFA	26.25	23.47	25.32	23.03	24.95	23.01
MUFA	35.78	39.44	36.49	39.26	36.40	38.40
Σ n-6	15.30	17.35	17.28	19.37	18.90	20.77
Σ n-3	20.66	18.54	19.17	17.34	18.17	16.91
Σ n-3LC-PUFA	11.33	6.97	9.13	5.18	7.98	4.70
Σ n-3/Σ n-6	1.35	1.07	1.11	0.90	0.96	0.81

FM: Fishmeal; FO: Fish oil; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids.

lipid was extracted as described by Folch et al. (1957). Fatty acid methyl esters were obtained by transmethylation with 1% sulphuric acid in methanol as described by Christie (1982) and separated by gas chromatography (GC-14A, Shimadzu, Japan) in a Supercolovax-10-fused silica capillary column (Supelco, Bellefonte, USA) using helium as the carrier gas, following the conditions described by Izquierdo et al. (1992). Fatty acid methyl esters were quantified by a flame ionization detector and identified by comparison with external and well-characterized fish oils standards (EPA 28, Nippai, Ltd. Tokyo, Japan).

2.6. Quality index method

Six laboratory-trained panellists evaluated sensory attributes dependent on fish freshness, applying the QIM performed for this species (Knowles et al., 2007), blind and unaware of dietary treatments. They applied a scale of 22 demerit points, assessing 10 parameters.

2.7. Texture profile analysis (TPA)

Texture analyses were carried out on the whole fish and both raw and cooked fillets. TPA was made with a TA.XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK). Ungutted whole fish texture studies were developed over the lateral line, on the left side, 1 cm from the operculum. Each fish was compressed in two consecutive cycles to a depth of 7 mm with a plunger of 1.2 cm in diameter at a constant speed of 0.8 mm/s and 5 s between cycles. The pattern attempted to mimic the pressure that might be applied by a person with their finger (Ginés et al., 2002). Differently, for fillet texture studies, the skin was removed and three-square pieces (cranial, central, and caudal, 2.5 × 2.5 × 1.5 cm) above the lateral line were collected from the left fillets raw and right fillet for cooked. The force-deformation curve was analyzed to determine four texture parameters (fracturability, hardness, springiness, and adhesiveness). This time, compression plate and speed were 100 mm Ø and 0.8 mm/s applying a deformation of 60% of the original thickness for raw fillet (Ginés et al., 2004). Cooked fillet samples were prepared in a steam oven (Compact; Eurofred, Barcelona, Spain) for 10 min at 115 °C in lidded aluminium boxes. The applied deformation was 80% of the original thickness. Fracturability on cooked fillet was not determined.

2.8. Statistical analyses

Data were analyzed with IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp.) testing for normality and homogeneity of variance. A General Linear Model (GLM) with fish meal and fish oil content in the diet as fixed factors and body weight as a covariate was used to determine effects in growth performance parameters and proximate and fatty acid composition of muscle at 0 dph. Data of evolution over time during storage about fillet composition, texture and spoilage were subjected to one-way ANOVA. Significant differences between the means were evaluated by Duncan's multiple range tests.

3. Results

3.1. Growth parameters

The dietary FM content affected growth parameters and feed efficiency (Table 3). Throughout the experimental period, European sea bass fed with the three dietary levels of FM were progressively differentiated according to weight and SGR, regardless of the FO content. After 153 days of experimentation, significant differences were only observed between the European sea bass fed the diets with 20% FM compared to those that consumed the diets formulated with a 5% FM. These differences became more pronounced after 355 and 556 days of experimentation. At this stage, weight and SGR reached the highest values in the fish fed diets containing 20% FM and the lowest values for fish fed diets containing 5% FM (Table 3). The condition factor (K) was influenced by the FO content of the diets up to 153 days of experimentation, being significantly higher in European sea bass fed a dietary 3% FO. From 153 days onwards, the dietary FO content did not produce any significant effect on the parameters evaluated. Regarding the feed efficiency (FE), FM content produced a significant outcome from the first part of the trial, after 153 days of experimentation, although only comparing fish fed the 20/6 diet with 5/3 diet. After 355 days of feeding, FE was significantly lower for those diets containing a 5% FM. At the end of the experiment, at 556 days, although the fish fed the diets with a 5% FM presented the lowest efficiency, they were only significantly

Table 3
Growth performance and feed utilization of European sea bass fed diets with several FM/FO contents.

		Diets (%FM/%FO)						*P*-value		
		20/6	20/3	10/6	10/3	5/6	5/3	FM	FO	FMxFO
Initial	Weight (g)	9.77 ± 1.62	9.86 ± 1.47	9.86 ± 1.54	9.75 ± 1.59	9.84 ± 1.49	9.78 ± 1.51	NS	NS	NS
	K[1]	1.29 ± 0.11	1.30 ± 0.12	1.28 ± 0.13	1.30 ± 0.11	1.28 ± 0.12	1.30 ± 0.12	NS	NS	NS
153 days	Weight (g)	96.31 ± 24.74^{a}	95.00 ± 21.53^{a}	86.45 ± 18.34^{ab}	88.96 ± 19.26^{ab}	82.61 ± 20.85^{b}	80.67 ± 19.50^{b}	0.000	NS	NS
	K[1]	1.62 ± 0.14^{ab}	1.66 ± 0.16^{a}	1.57 ± 0.14^{ab}	1.61 ± 0.15^{ab}	1.54 ± 0.16^{b}	1.60 ± 0.17^{ab}	0.000	0.000	NS
	SGR[2]	1.50 ± 0.02^{a}	1.48 ± 0.03^{a}	1.42 ± 0.03^{ab}	1.44 ± 0.03^{ab}	1.39 ± 0.02^{b}	1.38 ± 0.04^{b}	0.000	NS	NS
	FE[3]	0.85 ± 0.02^{a}	0.83 ± 0.03^{ab}	0.82 ± 0.02^{ab}	0.83 ± 0.04^{ab}	0.80 ± 0.04^{ab}	0.79 ± 0.03^{b}	0.044	NS	NS
355 days	Weight (g)	258.82 ± 44.36^{a}	258.47 ± 48.76^{a}	236.98 ± 59.28^{b}	240.12 ± 44.01^{b}	216.00 ± 41.89^{c}	207.29 ± 40.52^{c}	0.000	NS	NS
	K[1]	1.65 ± 0.15^{a}	1.65 ± 0.17^{a}	1.61 ± 0.20^{a}	1.63 ± 0.17^{a}	1.49 ± 0.17^{b}	1.52 ± 0.16^{b}	0.000	NS	NS
	SGR[2]	0.92 ± 0.01^{a}	0.92 ± 0.01^{a}	0.90 ± 0.01^{b}	0.90 ± 0.00^{b}	0.87 ± 0.00^{c}	0.86 ± 0.01^{c}	0.000	NS	NS
	FE[3]	0.71 ± 0.03^{a}	0.70 ± 0.02^{ab}	0.64 ± 0.04^{b}	0.65 ± 0.03^{ab}	0.56 ± 0.04^{c}	0.54 ± 0.04^{c}	0.000	NS	NS
556 days	Weight (g)	552.98 ± 97.77^{a}	560.53 ± 88.32^{a}	492.73 ± 50.90^{b}	490.10 ± 65.71^{b}	416.93 ± 74.66^{c}	432.20 ± 78.31^{c}	0.000	NS	NS
	K[1]	1.65 ± 0.14^{a}	1.70 ± 0.14^{a}	1.57 ± 0.16^{b}	1.60 ± 0.14^{ab}	1.53 ± 0.15^{b}	1.59 ± 0.12^{ab}	0.006	NS	NS
	SGR[2]	0.73 ± 0.02^{a}	0.73 ± 0.01^{a}	0.70 ± 0.02^{b}	0.70 ± 0.01^{b}	0.67 ± 0.01^{c}	0.68 ± 0.02^{c}	0.007	NS	NS
	FE[3]	0.55 ± 0.05^{a}	0.56 ± 0.04^{a}	0.52 ± 0.03^{ab}	0.51 ± 0.06^{ab}	0.46 ± 0.03^{b}	0.47 ± 0.04^{b}	0.004	NS	NS

Values expressed in mean ± SD. ($n = 75$ fish per diet in Weight and K; $n = 3$ tanks per diet in SGR and FE). Different letters within a line denote significant differences among dietary treatments ($P < 0.05$). NS: not significant.
[1] K (Condition factor) = [(weight)/(length)3].
[2] SGR (Standard Growth Rate) = [(ln final weight – ln initial weight)/number of days] × 100.
[3] FE (Feed Efficiency) = 1/(ingested feed/gain weight).

different from the European sea bass fed the diets with the highest level of FM (Table 3).

3.2. Muscle immunohistochemical analyses: Fillet degradation

Immunohistochemical studies were undertaken at 0 and 8 dph. At 0 dph all samples studied presented intact muscle fibers with a typical polygonal shape surrounded by a thin connective tissue layer (endomysium). Similarly, well-preserved myocommata of connective tissue were observed at this stage (Figs. 1 and 2). At 8 dph, myotomes appeared detached from the myocommata, and autolytic alterations became more evident, such as the separation of myotomes. Thus, some groups of myofibers showed impaired cellular integrity reflected in a hyperacidophilic cytoplasm, with loss of cell boundaries and cytoplasmic details (Figs. 1 and 2) regardless of dietary treatment. An intense immunoreactivity to anti-desmin was observed in the periphery and cytoplasm of red and white muscle fibers in all the sections observed (Fig. 1a), which persisted although reactivity intensity decreased at 8 dph (Fig. 1b) when the fibre-fibre adhesion was reduced and disintegration of myocommata was apparent (Fig. 1b). Immunoreactivity for anti-dystrophin was intense and located mainly at the periphery of the red and white muscle cells (Fig. 1c). This immunoreactivity was disappearing at the end of the postmortem period concerning the tissue itself degradation (Fig. 1d). About muscle morphology deterioration, no differences were observed comparing the experimental diets throughout the storage period. The immunoreaction against anti-m-calpain, anti-μ-calpain and anti-calpastatin, at 0 dph appeared as a microgranular deposit preferentially localized in the fibre's periphery (Fig. 2a, c, e). No remarkable changes in the patterns of m-calpain and μ-calpain staining were observed throughout the storage period apart from some degradation indicators connected with the cytoplasmatic loss of the membrane components (Fig. 2b, d). Anti-calpastatin immunolabelling progressed as increased the homogenization of the cytoplasm. Thus, at 8 dph the immunoreactivity was clearly detected in the cytoplasm of

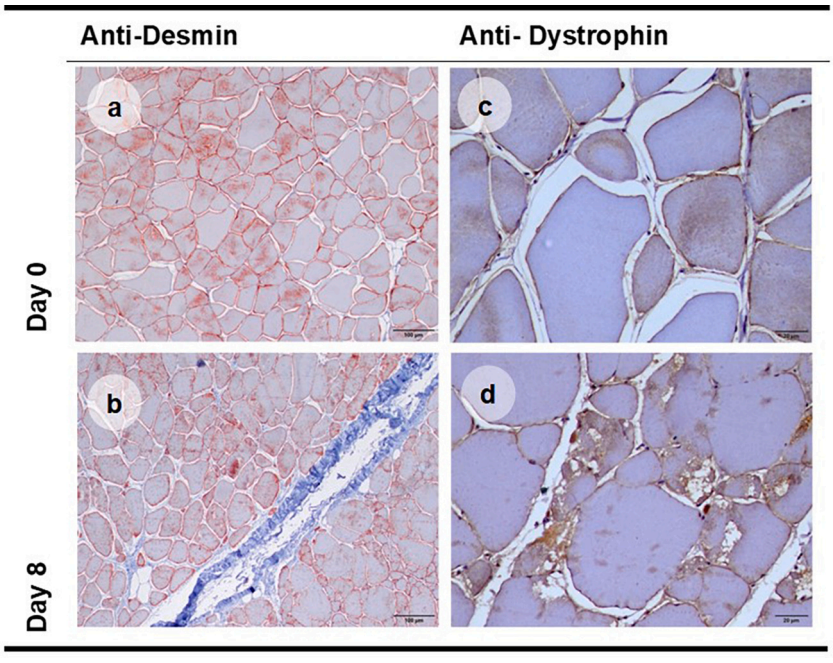


Fig. 1. Immunohistochemistry against myofibrilar antibodies of desmin at 0 (a) and 8 dph (b), and dystrophin at 0 (c) and 8 dph (d) in white muscle.

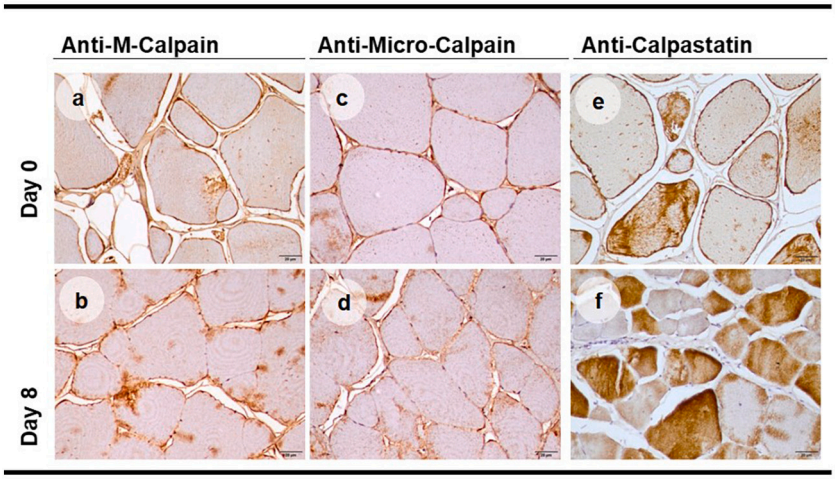


Fig. 2. Immunohistochemistry against endoproteases enzymes antibodies of M-calpain at 0 (a) and 8 dph (b), Micro-calpain at 0 (c) and 8 dph (d), and calpastain at 0 (e) and 8 dph (f) in white muscle.

muscle fibers which showed degradation signs (Fig. 2 f). The endoproteases immunoreactivity pattern along the storage period evaluated was indistinguishable comparing the dietary groups.

3.3. Proximate composition and fatty acid profile

No significant differences were found in muscle total lipid content among fish fed different experimental FM or FO levels (Table 4). However, the reduction of FM promoted a reduction of protein and ash content in muscle in fish fed a 5% FM based diet compared to fish fed 20/3 diet. These differences were maintained for protein and ash content along the period of ice storage (Table S1). Raw fillet fatty acid classes at 0 dph were affected by dietary FM and/or FO replacement (Table 4). Thus, the lowest values of SFA were found in fish fed diets with 10% of FM. Relative to FO level, fish fed diets with a 6% FO showed higher SFA content than those fed with a 3% of FO within the same FM dietary percentage due to the reduction in 15:0, 16:0 and 17:0 (Table S2). During ice storage (Table S3), SFA content did not differ between fish fed a 20 or a 10% of FM, showing the highest SFA values fish fed a 5% FM. With FO replacement, fish fed a 6% FO had a higher content of SFA than fish fed a 3% FO at 2, 5, 8 and 12 dph (Table S3). Dietary replacement of FO only mildly increased monounsaturated and n-9 fatty acids contents when fed 10/3, mainly due to the increase in 18:1n-9 (Table S1). During ice storage, those differences were sustained depending on FO replacement (Table S3). Both FM and FO reduction (especially FO) in the diet significantly increased 18:2n-6 and, subsequently, n-6 fatty acids in the muscle with reduced levels of ARA, EPA, DHA, and n-3 LC-PUFA (Table S2), in agreement with dietary levels of these fatty acids (Table 2). Dietary FM and FO reduction increased 18:2n-9 and 18:3n-6, as well as 20:2n-6 and 20:3n-3, products of Δ6-desaturase and Elovl6, respectively, despite their low levels in the diet (Table S2). The Σn-6 was higher in fish fed 3% FO than fish fed 6% FO at

Table 4
Muscle proximate composition (wet-weight basis) and fatty acid composition (% of total identified fatty acids) in raw and cooked fillets of European sea bass fed diets with several FM/FO contents at 0 dph.

	Diets (%FM/%FO)						*P*-value		
	20/6	20/3	10/6	10/3	5/6	5/3	FM	FO	FMxFO
Proximate composition (wet-weight basis)									
Lipids	7.75 ± 2.40	6.67 ± 2.73	7.36 ± 3.06	8.84 ± 2.65	8.89 ± 2.06	8.23 ± 3.11	NS	NS	NS
Protein	20.67 ± 1.24^{ab}	21.74 ± 0.88^{a}	20.74 ± 0.61^{ab}	20.99 ± 0.70^{ab}	19.96 ± 0.74^{b}	20.23 ± 0.53^{b}	0.017	NS	NS
Ash	1.40 ± 0.03^{a}	1.35 ± 0.04^{ab}	1.29 ± 0.03^{c}	1.28 ± 0.04^{c}	1.27 ± 0.07^{c}	1.29 ± 0.06^{bc}	0.000	NS	NS
Moisture	70.18 ± 1.35	70.23 ± 1.89	70.61 ± 2.56	68.89 ± 1.93	69.88 ± 1.51	70.24 ± 2.66	NS	NS	NS
Fatty acid in raw fillet (% of total identified fatty acids)									
SFA	26.55 ± 0.53^{ab}	25.93 ± 0.40^{b}	25.68 ± 0.85^{bc}	25.04 ± 0.33^{c}	26.94 ± 0.73^{a}	26.03 ± 0.73^{b}	0.001	0.004	NS
MUFA	39.27 ± 1.45^{c}	39.53 ± 1.21^{bc}	40.61 ± 1.86^{bc}	43.93 ± 0.99^{a}	41.05 ± 0.34^{b}	41.03 ± 0.52^{b}	0.000	0.011	0.007
Σ n-6	12.13 ± 0.35^{d}	13.41 ± 0.35^{c}	14.77 ± 0.66^{b}	15.72 ± 0.68^{a}	14.66 ± 0.49^{b}	16.31 ± 0.51^{a}	0.000	0.000	NS
Σ n-3	20.33 ± 0.94^{a}	19.51 ± 1.15^{a}	17.48 ± 1.46^{b}	14.03 ± 0.70^{d}	15.55 ± 0.49^{c}	15.12 ± 0.35^{cd}	0.000	0.000	0.002
Σ n-3LC-PUFA	11.92 ± 1.01^{a}	9.90 ± 1.08^{b}	10.38 ± 1.41^{b}	5.92 ± 0.51^{d}	8.11 ± 0.58^{c}	6.07 ± 0.27^{d}	0.000	0.000	0.007
Σ n-3/Σ n-6	1.68 ± 0.06^{a}	1.45 ± 0.06^{b}	1.18 ± 0.05^{c}	0.89 ± 0.02^{e}	1.06 ± 0.05^{d}	0.93 ± 0.02^{e}	0.000	0.000	0.005
Fatty acid in cooked fillet (% of total identified fatty acids)									
SFA	28.31 ± 0.57^{ab}	28.04 ± 0.94^{b}	29.05 ± 0.45^{a}	27.41 ± 1.02^{bc}	27.56 ± 0.60^{bc}	26.90 ± 0.47^{c}	0.007	0.003	NS
MUFA	37.65 ± 2.52^{d}	38.91 ± 2.03^{cd}	42.39 ± 0.81^{ab}	44.08 ± 1.52^{a}	41.28 ± 0.60^{b}	40.89 ± 0.61^{bc}	0.000	NS	NS
Σ n-6	12.70 ± 0.49^{d}	13.75 ± 0.57^{c}	14.99 ± 0.96^{b}	15.56 ± 0.42^{b}	15.03 ± 0.47^{b}	16.59 ± 0.32^{a}	0.000	0.000	NS
Σ n-3	19.87 ± 2.08^{a}	18.16 ± 1.25^{b}	12.55 ± 0.40^{d}	12.10 ± 0.43^{d}	14.92 ± 0.69^{c}	14.62 ± 0.28^{c}	0.000	0.045	NS
Σ n-3LC-PUFA	12.70 ± 2.36^{a}	9.69 ± 1.64^{b}	6.43 ± 0.10^{cd}	4.95 ± 0.80^{d}	7.50 ± 0.64^{c}	5.92 ± 0.34^{cd}	0.000	0.000	NS
Σ n-3/Σ n-6	1.57 ± 0.09^{a}	1.32 ± 0.06^{b}	0.84 ± 0.04^{d}	0.78 ± 0.03^{d}	0.99 ± 0.02^{c}	0.88 ± 0.02^{cd}	0.000	0.000	NS

Values expressed in mean ± SD. (n = 6 fish per diet). Different letters within a line denote significant differences among dietary treatments ($P < 0.05$). NS: not significant; dph: days post-harvest; FM: Fishmeal; FO: Fish oil; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; LC-PUFA: long chain polyunsaturated fatty acids.

0 dph (Table 4) and during ice storage (Table S3), although from the 8th dph on, the differences were not significant. On the other hand, fish fed 20% FM showed the lowest values at 0 dph (Table 4) and during the ice storage period (Table S3). On the contrary, Σn-3 had the highest contents in 20% FM and 6% FO (Table 4). A comparable situation could be explained both for Σn-3LC-PUFA and Σn-3/Σn-6. Remarkably, the differences between fish fed 20/6 diet with the highest value of the relationship Σn-3/Σn-6 was decreasing from 0 to 12 dph (Fig. 3), although differences were always significant.

The fatty acid profile of the cooked fillet was analyzed on the 0 dph (Table 4). The percentage of SFA showed a slight but significant increase after cooking in the fish fed the diets 20 and 10% FM (Table S4). Diets with 20% FM had the lowest values of MUFA and Σn-6, but the highest of Σn-3 and Σn-3LC-PUFA. The highest relationship Σn-3/Σn-6 was for the diet 20/6. It can also appreciate a better n-3/n-6 relationship for raw fillets due to the slight reduction in the proportion of Σ n-3 after cooking (Table S4). Fish fed diets with 3% FO showed the lowest contents of SFA, Σn-3 and Σn-3LC-PUFA, but the highest of Σn-6 (Table 4).

3.4. Quality index method (QIM)

No significant differences were observed in the evolution of sensory freshness during ice storage of European sea bass fed the six experimental diets tested (Fig. 4). The coefficient of variation between scores at any postmortem day was below 10%, indicating a clear consistency of the valuation promoted by an accurate assessment of the panelist members.

3.5. Texture profile analysis

The values of the hardness registered for whole fish were the highest for diets with 20% FM in the 0 and 2 dph (Fig. 5). Throughout ice storage, those initial differences disappeared. In the case of springiness, no differences in any of the samples were found. In the raw fillet, there were no differences between diets according to FM levels tested in 0 dph but they were significant at 2 dph. Fish fillets fed 20 and 10% FM diets required a higher compressive force to deform than fish fillets fed 5% FM (Fig. 6). These differences melted progressively afterwards. The evolution of the other texture parameters throughout the shelf life in the raw fillet showed the same tendency, although gumminess was maintained from 2 until 5 dph higher with higher values for the 20 and 10% FM diets than for the 5% FM diet (Table S5). No significant differences in texture parameters were found studying the cooked fillets, neither attributable to the diet nor the interval of ice storage.

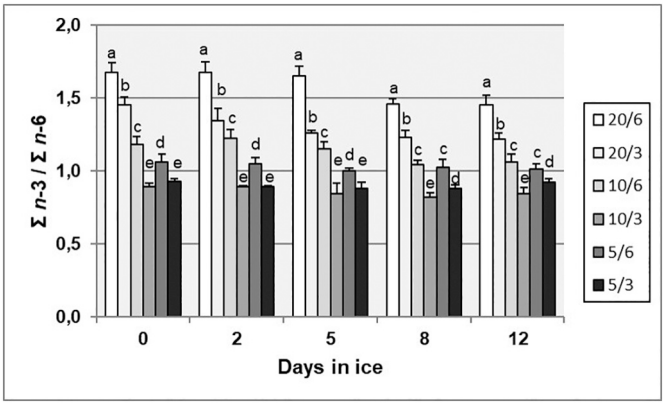


Fig. 3. Evolution of *n*-3/*n*-6 ratio in the muscle of European sea bass fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

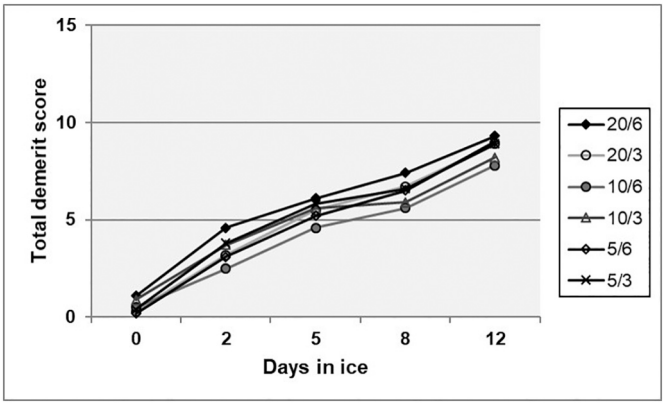


Fig. 4. QIM scores in whole fish of European sea bass fed different diets throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

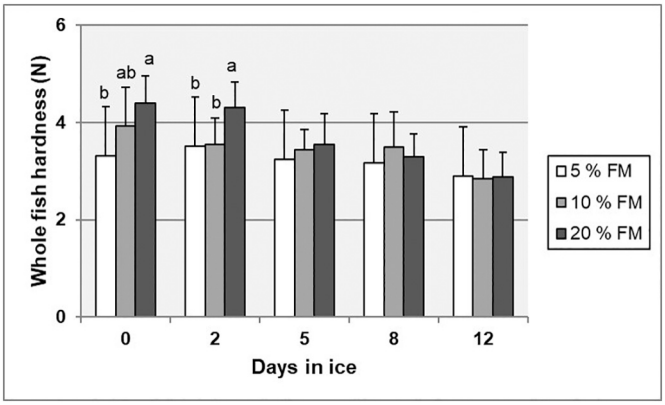


Fig. 5. Evolution of whole fish hardness (N) in the muscle of European sea bass fed diets with several FM/FO contents throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

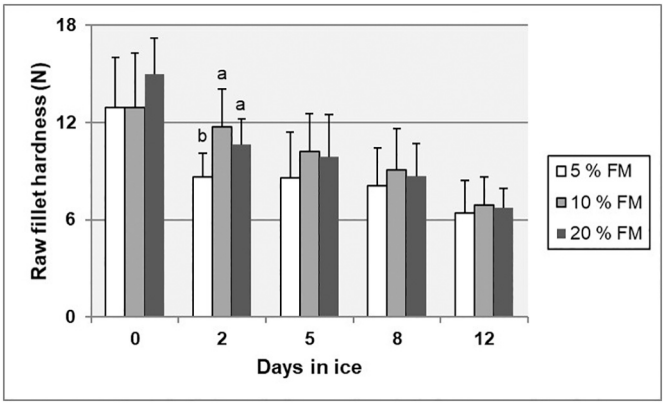


Fig. 6. Evolution of raw fillet hardness (N) in the muscle of European sea bass fed diets with several FM/FO contents throughout ice storage. Different letters in the same day denote statistically significant differences ($P < 0.05$).

4. Discussion

4.1. Growth parameters

The results show that a reduction of raw materials of marine origin in the diets of European seabass affects growth performance, being especially appreciable for extended periods. The data obtained during the

period from juvenile until commercial weight goes beyond the results of an earlier study where similar diets were used to rear sea bass until 50 g (Torrecillas et al., 2017). In that study the growth performance was less influenced than at the present one, underscoring the importance of monitoring the complete growth period. Conversely, and regarding the stage of development, Le Boucher et al. (2013), showed that complete substitution of marine ingredients leads to a reduced weight gain in European seabass, more pronounced between 2.5 and 100 g of weight than when it was carried out from 194 to 647 g (Le Boucher et al., 2011). However, it is opportune to distinguish the effect of FM from the FO effect, even if combined in diets. In the present study, the growth of European sea bass was highly dependent on dietary FM to reach a commercial size >500 g. Thus, those fish fed low FM diets had 20% less growth. A reduction of FM up to 10% implies a 7% less growth at 556 days, reaching up to 17% less with a 5% FM content. Likewise, feed efficiency was conditioned by dietary FM content, although only on fish fed diets formulated with higher substitution levels. The poor growth performances found in previous research in the species after a high-level FM replacement by a single plant-protein source (Dias et al., 2005) were associated with an essential amino acid imbalance and impaired phosphorus availability. Appropriate supplementation of essential amino acids in plant-based diets for European sea bass leads to proper somatic growth or nitrogen utilization (Kaushik et al., 2004). For instance, dietary inclusion of blood meal and the progressive supplementation with lysine and methionine according to FM reduction were efficient to guarantee similar growth rates with 20 and 10% content of FM from 9 to 55 g of body weight (Torrecillas et al., 2017).

On the other hand, the FO replacement by a blend of rapeseed, linseed and palm oils did not affect growth and feed efficiency, even with a reduction to 3%. However, it entails changes in the structure of the intestinal membrane and alterations in the functionality of the local and systemic immune system (Torrecillas et al., 2017b), which could affect the fish performance in stressful situations under farm conditions. The content of n-3 LC-PUFA in the diet 5/3 FM/FO (0.9% d.w.) was effective to cover the requirements for European sea bass, not only on the juvenile as shown by Skalli and Robin (2004) with a 0.7% but also in a long-term feeding experience for commercial-size fish as in the present study. Adverse effect on the fish growth performance was presented after a total marine oils' replacement with VO (Sales and Glencross, 2011). However, if the diet provides a minimum content of essential fatty acids, fish growth and feed utilization are not compromised (Izquierdo et al., 2003; Richard et al., 2006), being able to achieve up to a 75% of dietary FO substitution (Turchini et al., 2009).

4.2. Muscle immunohistochemical analyses: Fillet degradation

Desmin labelling did not decrease during the storage of the fish muscle, in agreement with previous observations indicating that desmin is stable in chill-stored fish muscle (Verrez-Bagnis et al., 1999). On the contrary, the immunodetection of dystrophin, which ensures the link between the actin cytoskeleton and the extracellular matrix, was limited to the first 24 h after slaughtering, that is 0 dph. Dystrophin was no longer detected throughout the storage at 4 °C in agreement with previous studies (Bonnal et al., 2001; Bao et al., 2020). This breakdown allows monitoring early proteolysis during fish storage, which was not apparently modified by the dietary content of FM and FO. Likewise, calpains cleaved the protein structure bonded to sarcolemma with changes microscopically independent of the diet. Kinetic analysis of European sea bass white muscle showed that 60% of dystrophin was cleaved during the first 24 h, and total disappearance was observed after 2 days of storage at 4 °C (Bonnal et al., 2001). The analysis of vacuoles, spaces, interstitial space or myofiber empty spaces (Tinacci et al., 2018) showed no effect of the dietary treatment fed. Post-mortem changes of flesh quality under the microscope are tightly coupled to organizational and structural changes, which have been reported to be highly associated with biological reactions, including endogenous enzymatic reactions (Tie et al., 2022). Structural proteins are increasingly studied as biomarkers to monitor fish muscle quality during post-mortem storage. The fact that there are no visually appreciable differences when comparing dietary treatments should not discourage us from continuing to try to understand the effect on post-mortem interval. Understanding the contribution and interaction between proteases is of particular importance for the exploitation of more refined strategies aimed at protease control for better quality of fishery products (Ge et al., 2018).

4.3. Proximate composition and fatty acid profile

The formulation of the diets was intended to rationalize the cost of ingredients and the present results indicate that a considerable reduction of up to 10% in FM levels returns only a 10% decrese in fish performance at the end of the experimental period. Previous studies have shown that replacing FM alone with plant protein sources did not affect muscle protein content in European sea bass at reduction levels of 5% (Kaushik et al., 2004), 10% (Dias et al., 2005) and 25–50% (Tibaldi et al., 2015) or with related species such as Japanese sea bass (*Lateolabrax japonicas*) (Hu et al., 2013). Likewise, in the present study, fish fed diets with 10 or 20% of FM content did not register changes in the muscle protein content. Protein accretion is determined by the balance between the processes of protein synthesis and degradation, regulated by interactions among hormonal, nutritional and cellular signalling pathways (Liu and Barrett, 2002). When proper consideration is given in meeting the requirements in essential nutrients, there is much potential for developing low FM/FO feeds for most species without adverse effects (Halver and Hardy, 2003; Krogdahl et al., 2010; Torrecillas et al., 2017).

The fact that no change in lipid content was recorded in the muscle denotes that lipid content is stable despite the variation of ingredients when a careful formulation is applied. FO was substituted by a blend of rapeseed, linseed and palm oils that kept saturated and monounsaturated fatty acids profile balanced in fish tissues, and diets were progressively complemented with micronutrients. Thus, the SFA reduction in the muscle with no connection with any experimental diet, confirmed that a mixture of different alternative sources could substitute FM and FO using practical ingredients readily available to the aquafeed industry (Kaushik et al., 2004; Jobling, 2012). The MUFA fatty acid profile, when soybean meal progressively replaces FM, tends to increase significantly, and this effect was mainly due to 18:1n-9, oleic acid. Rapeseed oil, a traditional source of MUFA, (Bell and Sargent, 2003) did not mediate in the 18:1n-9 increase. The mitochondrial β-oxidation of 18:1n-9 as an energy source diminished under 10% of FM concentration being an object of bioaccumulation. When the diets were combined with higher FO presence (6%) into the dietary formulation fortified or maintained this effect. This result entails a change in adult European sea bass metabolism different from that determined in juvenile where changes in the source of FO do not promote modifications of the oleic acid deposited in the muscle (Eroldoğan et al., 2013). In the same vein, dietary FM content lower than 10% did not affect total n-6 fatty acids, the main energy source of European seabass, which efficiently uses n-6 PUFAs as an energy substrate (Eroldoğan et al., 2013) helping to minimize the β-oxidation of n-3 LC-PUFA allowing a deposition in body compartments. This total content of n-6 fatty acids, especially pronounced in the case of linoleic acid 18:2n-6, even though incremented in fish fatty acids profile was only half of those observed in the diet. In addition, marine finfish have a limited ability to biosynthesize LC-PUFA from C18 PUFA precursors due to apparent limited fatty acid elongase and desaturase enzymatic activities (Izquierdo et al., 2005; Richard et al., 2006; Montero et al., 2010). As commented, soybean meal protein percentages resulted in lower n-3 PUFA levels in both diet and fish muscle, however, the decrease was less pronounced than expected due to endogenous synthesis. The present study shows that FO substitution by VO caused a significant increase in the fatty acid products of Δ6-desaturase and Elovl6. Delta-6 FA desaturase catabolizes two different steps of the LC-PUFA biosynthesis acting over C18 PUFA, such as 18:2n-

6 and 18:3n-3 and on C24 PUFA, such as 24:4n-6 or 24:5n-3 (Sargent et al., 2003). Thus, the relative increase in 18:2n-9 and 18:3n-6, intermediate metabolites of Δ6-desaturase, as well as the intermediate 20:2n-6 from Elovl6 suggest the increased activity of these enzymes as described for other species such as Atlantic salmon or Japanese sea bass (Bell et al., 2002; Xu et al., 2014). Thus, FO substitution by VO activates LC-PUFA synthesis also in European sea bass. This effect was also found in the liver and muscle in juvenile sea bass (Torrecillas et al., 2017). The present study verifies the persistence of the effect up to commercial weight but shows that, at this age, the activity of Elovl5, more active in juveniles, was reduced. The levels of n-3 PUFA determined in the muscle even with the more restrictive diets have shown excellent perspectives for these formulations where higher accumulation was determined than that offered by experimental diets, especially under low FM concentrations. Our results show that increasing the dietary proportion of soybean meal (and corn) leads to an n-3/n-6 relationship reduction. This is due to the loss of LC-PUFA n-3 fatty acids that accompanies the reduction of FM.

Proximate composition and fatty acid profile of raw fillet are sensible to heat treatment, varying its nutritive value depending on the different cooking methods (Türkkan et al., 2008). The fatty acid profile could maintain the same proportions after a light cooking process (Şengör et al., 2013), as it has been carried out in this experiment using a steam oven. The SFA showed a higher proportion in cooked fillets than raw ones but only in those fillets from fish fed the diets with higher FM content, accordingly with a higher amount of n-3 LC-PUFA. It was due to SFA being fairly heat stable at habitual cooking temperatures (Larsen et al., 2010), while unsaturated fatty acids usually become more labile when the degree of unsaturation increases (Koubaa et al., 2012). The final consequence is a poor ratio of n-3/n-6 in the cooked fillet (Yu et al., 2017). In any case, the best nutritional balance was shown by cooked fillets from fish fed the diet with 20% FM.

4.4. Quality index method (QIM)

The assessment of spoilage, and therefore the fish acceptability during ice storage showed a linear relationship with a high correlation between the average QIM scores for each storage day and storage time in ice (Sveinsdottir et al., 2003). However, the spoilage into the same species could be different according to the origin, wild or farmed (Alasalvar et al., 2002), season (Tejada and De Las Heras, 2007), sexually mature (Nielsen and Hyldig, 2004), perimortem conditions (Erikson et al., 2018) or processing methods after slaughtering (Erikson et al., 2019). The linear relation between QIM scores and time is strongly affected by the bacterial spoiling activity (Giuffrida et al., 2013), along with the biochemical composition (Castro et al., 2018), which allows decoding the bacterial concentrations into QIM scores (Valenti et al., 2016). In the present study, the experimental diets influenced the fatty acid profile but without changes on the fish freshness assessment, probably attached with the low level of variation promoted between dietary groups. In this sense, a high proportion of LC-PUFA might cause a faster deterioration (Alasalvar et al., 2002) and presents a positive correlation with QIM score (Castro et al., 2018). Fish fed diet 20/6 showed the highest demerit points in all sampled days, but without significant differences throughout shelf life, attributable to individual variation of spoilage.

4.5. Texture profile analysis

The texture is one of the most important quality parameters of fish for producers, processors, and consumers (Hyldig and Nielsen, 2001) hence, texture assessment makes particular sense at the commercial size. The long feeding period displayed in the present study and the amply substitution levels has been an opportunity to review the effect of the dietary variations in flesh features. Thus, the replacement of FM by plant proteins indicated no texture differences compared with a standard commercial diet after adding a finishing diet (Matos et al., 2019) or with an enriched standard commercial diet with fish meal and final replacement (Cai et al., 2018; Johnsen et al., 2011). However, starting with juvenile individuals, fish fed with the highest FM diets showed the greatest flesh hardness (Liang et al., 2017). In our case, starting with juvenile fish, fewer than ten grams of weight and reviewing a feeding trial of eighteen months, the effect of FM content in the diet was manifest. Both whole body and raw fillets fish fed diets with a 20 and a 10% FM required a higher compression force to deformation than fish fed diets with a 5% FM. In any case, those differences were only significant at the start of ice storage subsiding throughout the shelf life. In shorter experimental periods (three months) and FM diet contents ranging from 26 up to 40%, De la Serrana et al. (2013) did not find differences in the raw fillet texture of gilthead sea bream.

The dietary proportion of amino acids may be beneficial to improve fillet texture (Larsson et al., 2014). Fish fed diets exceeding a certain level of plant proteins could have a compromised collagen biosynthesis (Liu et al., 2014), essential to maintain the normal structure and strength of connective tissue (Li and Wu, 2018). Even though proline plus hydroxyproline, the most abundant amino acids in collagen proteins (Wu et al., 2011), normally can be synthesized in adequate amounts by the organism, they must be provided through the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis (Castro et al., 2021). This phenomenon is characteristic of those considered as conditionally essential amino acids in fish (Wu, 2009). For that, the level of inclusion of fish meal in plant protein-based diets plays a key role since it contains connective tissue, an excellent source of proline plus hydroxyproline (Li et al., 2011). This is central as plant protein sources normally used in aquaculture feed, contain by nature low levels or no hydroxyproline (Aksnes et al., 2008). Unlike that of raw fillet results, the cooked fillet texture was not affected by diet. A similar result has been shown in Atlantic salmon (*Salmo salar*) fed a fish meal-free diet and fish meal-based diet (Davidson et al., 2018). After cooking, the collagen shrinks then soften, whereas the actomyosin complex changes from a soft gel to a firmer denatured complex, making it exceedingly difficult to relate the texture attributes of raw flesh to the attributes once the fillet is heated (Castro et al., 2015).

In conclusion, the present study demonstrated that long-term FM feed replacement influenced growth performance, whereas FO replacement affected fatty acid profile by increasing n-6 fatty acids and reducing saturated, n-6 fatty acids. 3 LC-PUFA and n-3/n-6 ratio. No differences were found when reviewing myofibrillar antibodies or endoproteases labelling comparing diets at two different experimental days. No organoleptic changes were observed when comparing experimental diets, except for muscle texture in whole fish and raw fillet, not perceptible in cooked fillet.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research is part of a series of studies resulting from the EU 7FP funded ARRAINA project (N288925: Advanced Research Initiatives for Nutrition & Aquaculture) aiming to determine the effects of high FM and FO replacement on European sea bass growth, metabolism, health welfare and quality along the whole life cycle. The views expressed in this work are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.aquaculture.2022.738452.

References

Aksnes, A., Mundheim, H., Toppe, J., Albrektsen, S., 2008. The effect of dietary hydroxyproline supplementation on salmon (*Salmo salar* L.) fed high plant protein diets. Aquaculture 275, 242–249. https://doi.org/10.1016/j.aquaculture.2007.12.031.

Alasalvar, C., Taylor, K.D.A., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): Total lipid content, fatty acid and trace mineral composition. Food Chem. 79, 145–150. https://doi.org/10.1016/S0308-8146(02)00122-X.

Álvarez, A., Fontanillas, R., Hernández-Contreras, A., Hernández, M.D., 2020. Partial replacement of fish oil with vegetal oils in commercial diets: the effect on the quality of gilthead seabream (*Sparus aurata*). Anim. Feed Sci. Technol. 265, 114504 https://doi.org/10.1016/j.anifeedsci.2020.114504.

AOAC, 2000. Official Methods of Analysis, 17th ed. Association of official Analytical chemists, Washington, D.C.

Ayala, M.D., Abdel, I., Santaella, M., Martínez, C., Periago, M.J., Gil, F., Albors, O.L., 2010. Muscle tissue structural changes and texture development in sea bream, *Sparus aurata* L., during post-mortem storage. LWT Food Sci. Technol. 43, 465–475. https://doi.org/10.1016/j.lwt.2009.08.023.

Bao, Y., Wang, K., Yang, H., Regenstein, J.M., Ertbjerg, P., Zhou, P., 2020. Protein degradation of black carp (*Mylopharyngodon piceus*) muscle during cold storage. Food Chem. 308, 125576 https://doi.org/10.1016/j.foodchem.2019.125576.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture 218, 491–499. https://doi.org/10.1016/S0044-8486(02)00370-8.

Bell, J.G., Henderson, R.J., Tocher, D.R., McGhee, F., Dick, J.R., Porter, A., Sargent, J.R., 2002. Substituting fish oil with crude palm oil in the diet of Atlantic salmon (*Salmo salar*) affects muscle fatty acid composition and hepatic fatty acid metabolism. J. Nutr. 132, 222–230. https://doi.org/10.1093/jn/132.2.222.

Bonnal, C., Raynaud, F., Astier, C., Lebart, M.C., Marcilhac, A., Coves, D., Benyamin, Y., 2001. Postmortem degradation of white fish skeletal muscle (Sea bass, *Dicentrarchus labrax*): fat diet effects on in situ dystrophin proteolysis during the prerigor stage. Mar. Biotechnol. 3, 172–180. https://doi.org/10.1007/s101260000059.

Caballero, M.J., Betancor, M., Escrig, J.C., Montero, D., Espinosa de los Monteros, A., Castro, P., Izquierdo, M., 2009. Post mortem changes produced in the muscle of sea bream (*Sparus aurata*) during ice storage. Aquaculture 291, 210–216. https://doi.org/10.1016/j.aquaculture.2009.03.032.

Cai, W.C., Jiang, G.Z., Li, X.F., Sun, C.X., Mi, H.F., Liu, S.Q., Liu, W.B., 2018. Effects of complete fish meal replacement by rice protein concentrate with or without lysine supplement on growth performance, muscle development and flesh quality of blunt snout bream (*Megalobrama amblycephala*). Aquac. Nutr. 24, 481–491. https://doi.org/10.1111/anu.12581.

Castro, P.L., Caballero, M.J., Ginés, R., Penedo, J.C., Montero, D., Lastilla, M.T., Izquierdo, M., 2015. Linseed oil inclusion in sea bream diets: effect on muscle quality and shelf life. Aquac. Res. 46, 75–85. https://doi.org/10.1111/are.12161.

Castro, P.L., Rincón, L., Álvarez, B., Rey, E., Ginés, R., 2018. Blackspot seabream (*Pagellus bogaraveo*) fed different diets. Histologic study of the lipid muscle fiber distribution and effect on quality during shelf life. Aquaculture 484, 71–81. https://doi.org/10.1016/j.aquaculture.2017.10.042.

Castro, P.L., Plasencia, S., Zamorano, M.J., Guerrero, L., Claret, A., Beltrán, J.A., Ginés, R., 2021. Effect of L-Hyp supplementation on collagen muscle histology, gene expression, growth performance, body composition and fillet texture on big size European sea bass (Dicentrarchux labrax). Aquac. Rep. 21, 100787 https://doi.org/10.1016/j.aqrep.2021.100787.

Cheng, J.-H., Sun, D.-W., Han, Z., Zeng, X.-A., 2014. Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. Compr. Rev. Food Sci. Food Saf. 13, 52–61. https://doi.org/10.1111/1541-4337.12043.

Christie, W.W., 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipid Res. 23, 1072–1075. https://doi.org/10.1016/S0022-2275(20)38081-0.

Davidson, J., Kenney, P.B., Barrows, F.T., Good, C., Summerfelt, S.T., 2018. Fillet quality and processing attributes of Postsmolt Atlantic Salmon, *Salmo salar*, fed a fishmeal-free diet and a fishmeal-based diet in recirculation aquaculture systems. J. World Aquacult. Soc. 49, 183–196. https://doi.org/10.1111/jwas.12452.

De la Serrana, D.G., Fontanillas, R., Koppe, W., Fernández-Borràs, J., Blasco, J., Martín-Pérez, M., Gutiérrez, J., 2013. Effects of variable protein and lipid proportion in gilthead sea bream (*Sparus aurata*) diets on fillet structure and quality. Aquac. Nutr. 19, 368–381. https://doi.org/10.1111/j.1365-2095.2012.00966.x.

Delbarre-Ladrat, C., Chéret, R., Taylor, R., Verrez-Bagnis, V., 2006. Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. Crit. Rev. Food Sci. Nutr. 46, 409–421. https://doi.org/10.1080/10408390591000929.

Dias, J., Alvarez, M.J., Arzel, J., Corraze, G., Diez, A., Bautista, J.M., Kaushik, S.J., 2005. Dietary protein source affects lipid metabolism in the European seabass (*Dicentrarchus labrax*). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 142, 19–31. https://doi.org/10.1016/j.cbpb.2005.07.005.

Erikson, U., Shabani, F., Beli, E., Muji, S., Rexhepi, A., 2018. The impacts of perimortem stress and gutting on quality index and colour of rainbow trout (Oncorhynchus mykiss) during ice storage: a commercial case study. European Food Research and Technology 244 (2), 197–206. https://doi.org/10.1007/s00217-017-2944-9.

Erikson, U., Truong, H.T., Le, D.V., Pham, P.D., Svennevig, N., Phan, V.T., 2019. Harvesting procedures, welfare and shelf life of ungutted and gutted shortfin pompano (Trachinotus falcatus) stored in ice. Aquaculture 498, 236–245. https://doi.org/10.1016/j.aquaculture.2018.06.085.

Eroldoğan, T.O., Yilmaz, A.H., Turchini, G.M., Arslan, M., Sirkecioğlu, N.A., Engin, K., Mumoğullarinda, P., 2013. Fatty acid metabolism in European sea bass (*Dicentrarchus labrax*): effects of n-6 PUFA and MUFA in fish oil replaced diets. Fish Physiol. Biochem. 39, 941–955. https://doi.org/10.1007/s10695-012-9753-7.

Folch, J., Lees, M., Stanley Sloane, G.H., 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497–509. https://doi.org/10.1016/s0021-9258(18)64849-5.

Ge, L., Xu, Y., Xia, W., Jiang, Q., 2018. Synergistic action of cathepsin B, L, D and calpain in disassembly and degradation of myofibrillar protein of grass carp. Food Res. Int. 109, 481–488. https://doi.org/10.1016/j.foodres.2018.04.067.

Geay, F., Santigosa I Culi, E., Corporeau, C., Boudry, P., Dreano, Y., Corcos, L., Cahu, C. L., 2010. Regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 156, 237–243. https://doi.org/10.1016/j.cbpb.2010.03.008.

Geay, F., Ferraresso, S., Zambonino-Infante, J.L., Bargelloni, L., Quentel, C., Vandeputte, M., Mazurais, D., 2011. Effects of the total replacement of fish-based diet with plant-based diet on the hepatic transcriptome of two European sea bass (*Dicentrarchus labrax*) half-sibfamilies showing different growth rates with the plant-based diet. BMC Genomics 12, 1–18. https://doi.org/10.1186/1471-2164-12-522.

Ginés, R., Palicio, M., Zamorano, M.J., Argüello, A., López, J.L., Afonso, J.M., 2002. Starvation before slaughtering as a tool to keep freshness attributes in gilthead sea bream (*Sparus aurata*). Aquac. Int. 10, 379–389. https://doi.org/10.1023/A:1023365025292.

Ginés, R., Valdimarsdottir, T., Sveinsdottir, K., Thorarensen, H., 2004. Effects of rearing temperature and strain on sensory characteristics, texture, colour and fat of Arctic charr (*Salvelinus alpinus*). Food Qual. Prefer. 15, 177–185. https://doi.org/10.1016/S0950-3293(03)00056-9.

Giuffrida, A., Valenti, D., Giarratana, F., Ziino, G., Panebianco, A., 2013. A new approach to modelling the shelf life of gilthead seabream (*Sparus aurata*). Int. J. Food Sci. Technol. 48, 1235–1242. https://doi.org/10.1111/ijfs.12082.

Halver, J.E., Hardy, R.W., 2003. Nutrient flow and retention. In: Fish Nutrition. Elsevier, pp. 755–770. https://doi.org/10.1016/b978-012319652-1/50015-x.

Hu, L., Yun, B., Xue, M., Wang, J., Wu, X., Zheng, Y., Han, F., 2013. Effects of fish meal quality and fish meal substitution by animal protein blend on growth performance, flesh quality and liver histology of Japanese seabass (*Lateolabrax japonicus*). Aquaculture 372–375, 52–61. https://doi.org/10.1016/j.aquaculture.2012.10.025.

Hyldig, G., Nielsen, D., 2001. A review of sensory and instrumental methods used to evaluate the texture of fish muscle. J. Texture Stud. 32, 219–242. https://doi.org/10.1111/j.1745-4603.2001.tb01045.x.

Izquierdo, M.S., Arakawa, T., Takeuchi, T., Haroun, R., Watanabe, T., 1992. Effect of n-3 HUFA levels in Artemia on growth of larval Japanese flounder (*Paralichthys olivaceus*). Aquaculture 105, 73–82. https://doi.org/10.1016/0044-8486(92)90163-F.

Izquierdo, M.S., Obach, A., Arantzamendi, L., Montero, D., Robaina, L., Rosenlund, G., 2003. Dietary lipid sources for seabream and seabass: growth performance, tissue composition and flesh quality. Aquac. Nutr. 9, 397–407. https://doi.org/10.1046/j.1365-2095.2003.00270.x.

Izquierdo, M.S., Montero, D., Robaina, L., Caballero, M.J., Rosenlund, G., Ginés, R., 2005. Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. Aquaculture 250, 431–444. https://doi.org/10.1016/j.aquaculture.2004.12.001.

Jobling, M., 2012. National Research Council (NRC): nutrient requirements of fish and shrimp. Aquac. Int. https://doi.org/10.1007/s10499-011-9480-6.

Johnsen, C.A., Hagen, O., Bendiksen, E.Å., 2011. Long-term effects of high-energy, low-fishmeal feeds on growth and flesh characteristics of Atlantic salmon (*Salmo salar* L.). Aquaculture 312, 109–116. https://doi.org/10.1016/j.aquaculture.2010.12.012.

Kaushik, S.J., Covès, D., Dutto, G., Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391–404. https://doi.org/10.1016/S0044-8486(03)00422-8.

Knowles, T.G., Brown, S.N., Warriss, P.D., Lines, J., Tinarwo, A., Bravo, A., Gonçalves, A., 2007. Effect of electrical stunning at slaughter on the carcass, flesh and eating quality of farmed sea bass (*Dicentrarchus labrax*). Aquac. Res. 38, 1732–1741. https://doi.org/10.1111/j.1365-2109.2007.01846.x.

Koubaa, A., Mihoubi, N.B., Abdelmouleh, A., Bouain, A., 2012. Comparison of the effects of four cooking methods on fatty acid profiles and nutritional composition of red mullet (*Mullus barbatus*) muscle. Food Sci. Biotechnol. 21, 1243–1250. https://doi.org/10.1007/s10068-012-0163-5.

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquac. Res. 41, 333–344. https://doi.org/10.1111/j.1365-2109.2009.02426.x.

Larsen, D., Quek, S.Y., Eyres, L., 2010. Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). Food Chem. 119, 785–790. https://doi.org/10.1016/j.foodchem.2009.07.037.

Larsson, T., Koppang, E.O., Espe, M., Terjesen, B.F., Krasnov, A., Moreno, H.M., Mørkøre, T., 2014. Fillet quality and health of Atlantic salmon (*Salmo salar* L.) fed a diet supplemented with glutamate. Aquaculture 426–427, 288–295. https://doi.org/10.1016/j.aquaculture.2014.01.034.

Le Boucher, R., Vandeputte, M., Dupont-Nivet, M., Quillet, E., Mazurais, D., Robin, J., Chatain, B., 2011. A first insight into genotype × diet interactions in European sea bass (*Dicentrarchus labrax* L1756) in the context of plant-based diet use. Aquac. Res. 42, 583–592. https://doi.org/10.1111/j.1365-2109.2010.02654.x.

Le Boucher, R., Vandeputte, M., Dupont-Nivet, M., Quillet, E., Ruelle, F., Vergnet, A., Chatain, B., 2013. Genotype by diet interactions in European sea bass (*Dicentrarchus labrax* L.): nutritional challenge with totally plant-based diets. J. Anim. Sci. 91, 44–56. https://doi.org/10.2527/jas.2012-5311.

Li, P., Wu, G., 2018. Roles of dietary glycine, proline, and hydroxyproline in collagen synthesis and animal growth. Amino Acids 50, 29–38. https://doi.org/10.1007/s00726-017-2490-6.

Li, X., Rezaei, R., Li, P., Wu, G., 2011. Composition of amino acids in feed ingredients for animal diets. Amino Acids 40, 1159–1168. https://doi.org/10.1007/s00726-010-0740-y.

Liang, X.F., Hu, L., Dong, Y.C., Wu, X.F., Qin, Y.C., Zheng, Y.H., Liang, X.F., 2017. Substitution of fish meal by fermented soybean meal affects the growth performance and flesh quality of Japanese seabass (*Lateolabrax japonicus*). Anim. Feed Sci. Technol. 229, 1–12. https://doi.org/10.1016/j.anifeedsci.2017.03.006.

Liu, Y., He, G., Wang, Q., Mai, K., Xu, W., Zhou, H., 2014. Hydroxyproline supplementation on the performances of high plant protein source based diets in turbot (*Scophthalmus maximus* L.). Aquaculture 433, 476–480. https://doi.org/10.1016/j.aquaculture.2014.07.002.

Liu, Z., Barrett, E.J., 2002. Human protein metabolism: its measurement and regulation. Am. J. Physiol. Endocrinol. Metab. 283, E1105–E1112. https://doi.org/10.1152/ajpendo.00337.2002.

Matos, E., Dias, J., Dinis, M.T., Silva, T.S., 2017. Sustainability vs. Quality in gilthead seabream (*Sparus aurata* L.) farming: are trade-offs inevitable? Rev. Aquac. 9, 388–409. https://doi.org/10.1111/raq.12144.

Matos, E., Gonçalves, A., Bandarra, N., Colen, R., Nunes, M.L., Valente, L.M.P., Dias, J., 2019. Corrigendum: (Plant proteins and vegetable oil do not have detrimental effects on post-mortem muscle instrumental texture, sensory properties and nutritional value of gilthead seabream (2012) 358–359 (205–212), (S0044848612004103), (10.1016/j.aquaculture.2012.07.009)). Aquaculture 512, 205–212. https://doi.org/10.1016/j.aquaculture.2019.734407.

Montero, D., Mathlouthi, F., Tort, L., Afonso, J.M., Torrecillas, S., Fernández-Vaquero, A., Izquierdo, M.S., 2010. Replacement of dietary fish oil by vegetable oils affects humoral immunity and expression of pro-inflammatory cytokines genes in gilthead sea bream *Sparus aurata*. Fish Shellfish Immunol. 29, 1073–1081. https://doi.org/10.1016/j.fsi.2010.08.024.

Nielsen, D., Hyldig, G., 2004. Influence of handling procedures and biological factors on the QIM evaluation of whole herring (*Clupea harengus* L.). Food Res. Int. 37, 975–983. https://doi.org/10.1016/j.foodres.2004.06.006.

Richard, N., Mourente, G., Kaushik, S., Corraze, G., 2006. Replacement of a large portion of fish oil by vegetable oils does not affect lipogenesis, lipid transport and tissue lipid uptake in European seabass (*Dicentrarchus labrax* L.). Aquaculture 261, 1077–1087. https://doi.org/10.1016/j.aquaculture.2006.07.021.

Rincón, L., Castro, P.L., Álvarez, B., Hernández, M.D., Álvarez, A., Claret, A., Ginés, R., 2016. Differences in proximal and fatty acid profiles, sensory characteristics, texture, colour and muscle cellularity between wild and farmed blackspot seabream (*Pagellus bogaraveo*). Aquaculture 451, 195–204. https://doi.org/10.1016/j.aquaculture.2015.09.016.

Sales, J., Glencross, B., 2011. A meta-analysis of the effects of dietary marine oil replacement with vegetable oils on growth, feed conversion and muscle fatty acid composition of fish species. Aquac. Nutr. 17, e271–e287. https://doi.org/10.1111/j.1365-2095.2010.00761.x.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2003. The lipids. Fish Nutr. 181–257 https://doi.org/10.1016/b978-012319652-1/50005-7.

Şengör, G.F.Ü., Alakavuk, D.Ü., Tosun, Ş.Y., 2013. Effect of cooking methods on proximate composition, fatty acid composition, and cholesterol content of Atlantic salmon (*salmo salar*). J. Aquat. Food Prod. Technol. 22, 160–167. https://doi.org/10.1080/10498850.2011.635839.

Skalli, A., Robin, J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquaculture 240, 399–415. https://doi.org/10.1016/j.aquaculture.2004.06.036.

Sveinsdottir, K., Hyldig, G., Martinsdottir, E., Jørgensen, B., Kristbergsson, K., 2003. Quality Index Method (QIM) scheme developed for farmed Atlantic salmon (*Salmo salar*). Food Qual. Prefer. 14, 237–245. https://doi.org/10.1016/S0950-3293(02)00081-2.

Tacon, A.G.J., Metian, M., 2015. Feed matters: satisfying the feed demand of aquaculture. Rev. Fish. Sci. Aquac. 23, 1–10. https://doi.org/10.1080/23308249.2014.987209.

Tejada, M., De Las Heras, C., 2007. Sensory changes in farmed senegalese sole (*Solea senegalensis*) during ice storage. Food Sci. Technol. Int. 13, 117–124. https://doi.org/10.1177/1082013207078594.

Tibaldi, E., Chini Zittelli, G., Parisi, G., Bruno, M., Giorgi, G., Tulli, F., Poli, B.M., 2015. Growth performance and quality traits of European sea bass (*D. labrax*) fed diets including increasing levels of freeze-dried Isochrysis sp. (T-ISO) biomass as a source of protein and n-3 long chain PUFA in partial substitution of fish derivatives. Aquaculture 440, 60–68. https://doi.org/10.1016/j.aquaculture.2015.02.002.

Tie, H., Dong, J., Jiang, Q., Yu, D., Yang, F., Xu, Y., Xia, W., 2022. Profound changes of mitochondria during postmortem condition used as freshness indicator in grass carp (*Ctenopharyngodon idella*) muscle. Food Biosci. 48, 101749 https://doi.org/10.1016/J.FBIO.2022.101749.

Tinacci, L., Armani, A., Guidi, A., Nucera, D., Shvartzman, D., Miragliotta, V., Abramo, F., 2018. Histological discrimination of fresh and frozen/thawed fish meat: European hake (*Merluccius merluccius*) as a possible model for white meat fish species. Food Control 92, 154–161. https://doi.org/10.1016/j.foodcont.2018.04.056.

Torrecillas, S., Robaina, L., Caballero, M.J., Montero, D., Calandra, G., Mompel, D., Izquierdo, M.S., 2017a. Combined replacement of fishmeal and fish oil in European sea bass (*Dicentrarchus labrax*): production performance, tissue composition and liver morphology. Aquaculture 474, 101–112. https://doi.org/10.1016/j.aquaculture.2017.03.031.

Torrecillas, S., Caballero, M.J., Mompel, D., Montero, D., Zamorano, M.J., Robaina, L., Izquierdo, M., 2017b. Disease resistance and response against *Vibrio anguillarum* intestinal infection in European seabass (*Dicentrarchus labrax*) fed low fish meal and fish oil diets. Fish & Shellfish Immunol. 67, 302–311. https://doi.org/10.1016/j.fsi.2017.06.022.

Turchini, G.M., Torstensen, B.E., Ng, W.K., 2009. Fish oil replacement in finfish nutrition. Rev. Aquac. 1, 10–57. https://doi.org/10.1111/j.1753-5131.2008.01001.x.

Türkkan, A.U., Cakli, S., Kilinc, B., 2008. Effects of cooking methods on the proximate composition and fatty acid composition of seabass (*Dicentrarchus labrax*, Linnaeus, 1758). Food Bioprod. Process. 86, 163–166. https://doi.org/10.1016/j.fbp.2007.10.004.

Valenti, D., Denaro, G., Giarratana, F., Giuffrida, A., Mazzola, S., Basilone, G., Spagnolo, B., 2016. Modeling of sensory characteristics based on the growth of food spoilage Bacteria. Math. Model. Nat. Phenom. 11, 119–136. https://doi.org/10.1051/mmnp/201611508.

Verrez-Bagnis, V., Noel, J., Sautereau, C., Fleurence, J., 1999. Desmin degradation in postmortem fish muscle. J. Food Sci. 64, 240–242. https://doi.org/10.1111/j.1365-2621.1999.tb15873.x.

Wu, G., 2009. Amino acids: metabolism, functions, and nutrition. Amino Acids 37, 1–17. https://doi.org/10.1007/s00726-009-0269-0.

Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D.A., Spencer, T. E., 2011. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino Acids 40, 1053–1063. https://doi.org/10.1007/s00726-010-0715-z.

Xu, H., Dong, X., Ai, Q., Mai, K., Xu, W., Zhang, Y., Zuo, R., 2014. Regulation of tissue LC-PUFA contents, Δ6 Fatty Acyl Desaturase (FADS2) gene expression and the methylation of the putative FADS2 gene promoter by different dietary fatty acid profiles in Japanese seabass (*Lateolabrax japonicus*). PLoS One 9, e87726. https://doi.org/10.1371/journal.pone.0087726.

Yu, D., Zhao, W., Dong, J., Zang, J., Regenstein, J.M., Jiang, Q., 2022. Multifunctional bioactive coatings based on water-soluble chitosan with pomegranate peel extract for fish flesh preservation. Food Chem. 374 https://doi.org/10.1016/j.foodchem.2021.131619.

Yu, L.C., Zzaman, W., Akanda, M.J.H., Yang, T.A., Easa, A.M., 2017. Influence of superheated steam cooking on proximate, fatty acid profile, and amino acid composition of catfish (Clarias batrachus) fillets. Turkish Journal of Fisheries and Aquatic Sciences 5, 935–943. https://doi.org/10.4194/1303-2712-v17_5_09.