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Black soldier fly (*Hermetia illucens*) larvae oil as an alternative to poultry oil in diets for European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*)

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ABSTRACT

Insects have emerged as an alternative protein source for aquafeeds, and more recently, attention has turned into their lipidic fraction. This study evaluated the potential of black soldier fly larvae oil (BSFLO) to replace poultry oil (PO) in diets for two key Mediterranean aquaculture species: European seabass and gilthead seabream juveniles. A control diet containing 5.2% PO was formulated, along with three experimental diets in which PO was replaced by BSFLO at 33%, 66%, and 100% (1.7%, 3.4%, and 5.2% BSFLO dietary inclusion). Fish were fed to satiation for 8 weeks (gilthead seabream) and 12 weeks (European seabass) at 23.0 ± 0.7 °C and 22.8 ± 0.6 °C, respectively. All diets were well accepted by both species, with no adverse effects on feed intake. Growth performance and feed conversion ratio improved with the full replacement of PO by BSFLO, particularly in seabass. Increasing dietary levels of BSFLO led to greater lauric acid deposition in muscle, enhanced antioxidant capacity, and reduced lipid peroxidation in both species. The complete replacement of PO by BSFLO increased the muscle n-3/n-6 PUFA ratio in both seabass and seabream. Overall, BSFLO proved to be a promising lipid source in aquafeeds for seabass and seabream, with the total PO replacement by BSFLO yielding clear performance gains in seabass and contributing to a more favourable and stable muscle lipid profile (characterized by higher n-3/n-6 ratios, improved antioxidant status, and lower lipid peroxidation) which may support product quality in both species.

1. Introduction

The aquaculture sector faces the challenge of meeting the growing demand for high-quality seafood products. This demand is driven not only by population growth but also by changing dietary preferences, as consumers increasingly recognize the health benefits of consuming fish as an exceptional source of essential n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) (FAO, 2024).

Fish oil (FO) has long been considered as the ideal lipid source for marine fish species due to its levels of essential n-3 LC-PUFA, namely eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively), which marine fish species cannot synthesize in sufficient amounts from other fatty acids (FA) (Monroig et al., 2018; Monroig et al., 2022). However, due to limited availability and price fluctuations, FO is strategically included in aquafeeds aiming at meeting fish dietary requirements for essential fatty acids such as DHA and EPA, while other lipid sources, such as plant-based oils (mainly rapeseed oil and soybean oil) or terrestrial animal fats (e.g., poultry oil), have been included in aquafeeds mainly as energy providers (Naylor et al., 2021; Radhakrishnan et al., 2024; Turchini et al., 2009). Despite being

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available in sufficient quantities to support aquafeed production, plant-based and terrestrial animal lipid sources are particularly rich in n-6 LC-PUFA (15–60%) such as linoleic acid (LA; C18:2n-6). In marine fish, the limited activity of Δ5-desaturase restricts the bioconversion of LA to arachidonic acid (ARA; C20:4n-6) (Monroig et al., 2022). Therefore, excessive dietary LA is mainly accumulated into tissues rather than further elongated and desaturated (Benedito-Palos et al., 2008). This accumulation, together with competition with α-linolenic acid (ALA; C18:3n-3) for the already limited enzymatic machinery, further disturbs the n-3/n-6 balance and reduces EPA and DHA deposition in fish flesh (Monroig et al., 2022). Consequently, fillet n-3/n-6 LC-PUFA ratio may reach values as high as 1:20–50, far from the recommended range of 1:4–5 for a healthy human diet (Simopoulos, 2008; González-Becerra et al., 2023). Moreover, such shifts in fatty acid composition may compromise fish physiology, as reduced levels of n-3 LC-PUFA in cellular membranes alter fluidity, and intracellular signalling pathways, with potential implications for growth performance, feed efficiency, immune competence and overall health status (Tocher, 2010; Tocher and Glencross, 2015). Hence, it is urgent to identify new lipid sources to include into aquafeeds not only to compensate for the shortage of FO, but also to mitigate excessive LA loads, such as those associated with plant-based and traditional terrestrial animal lipid sources, and their negative implications for physiological status and flesh quality of marine fish species.

Insect-derived oils – by-products of low-fat insect meal production - have gained attention as potential local and circular feed ingredients. Among these, black soldier fly (*Hermetia illucens*) larvae oil (BSFLO) has emerged as a promising option due to the larvae's high efficiency in converting vegetable by-products or wastes into lipid-rich biomass (Franco et al., 2021; Hawkey et al., 2021; Saviane et al., 2021; Seyedalmoosavi et al., 2022; Sindermann et al., 2021). The FA profile of BSFLO is usually characterized by high levels of saturated FA (SFA; 11.7–87.6%), moderate levels of MUFA (15.1–46.9%), and low levels of n-6 LC-PUFA (1.6–37.2%) (Ewald et al., 2020; Georgescu et al., 2022; Kim et al., 2020; Li et al., 2022; Srisuksai et al., 2024). Among the SFA, lauric acid (LAA; C12:0; 30–70%) is particularly abundant. As a medium-chain FA (MCFA), LAA undergoes rapid catabolism through β-oxidation, a feature that has been proposed to contribute to sparing n-3 LC-PUFA and thereby improving their retention in fish fillet (Schönfeld and Wojtczak, 2016). In addition, BSFLO may also exhibit antioxidant potential, as it can retain bioactive compounds (such as tocopherols and phenolic compounds) originating from the agri-food substrates used during insect larval rearing (Nunes et al., 2018; Zhan et al., 2024). This feature has been associated with improved oxidative stability of the oil itself and may positively influence the preservation and nutritional quality of fish fillets when included in aquafeeds (Hawkey et al., 2021; Jucker et al., 2017; Muangrat and Pannasai, 2024).

Some studies have investigated the effects of dietary inclusion of BSFLO as an alternative to plant-based oils or FO on growth performance, nutrient digestibility, and muscle fatty acid composition across different fish species, including salmonids (Belghit et al., 2018; Dumas et al., 2018; Fawole et al., 2021) and freshwater species (Bakar et al., 2021; Li et al., 2016; Xu et al., 2020a). Despite the importance of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) for European Aquaculture, very few studies have been published on the use of BSFLO in these species grow-out. In gilthead seabream, Moutinho et al. (2023) and Moutinho et al. (2024) evaluated the inclusion of BSFLO, as a replacement for a vegetable oil blend (linseed, palm and rapeseed oils), testing dietary levels of 4, 7.9 and 9.5% BSFLO, which corresponded to the substitution of 42, 84 and 100% of the blend, respectively. These studies reported similar growth performance in fish fed diets with and without BSFLO, together with clear modifications in muscle fatty acid composition. Specifically, muscle LAA deposition increased. Moreover, 100% vegetable blend replacement by BSFLO (9.5% inclusion) enhanced lipid oxidative stability, as shown by reduced muscle lipid peroxidation (LPO). Despite these advances in gilthead seabream, no studies have yet assessed the use of BSFLO in diets for European seabass, leaving an important knowledge gap regarding species-specific responses. In this context, the aim of this study was therefore to assess the impact of replacing PO with BSFLO on growth performance, feed efficiency, nutrient utilisation, muscle fatty acid composition, and antioxidant capacity in both seabass and seabream juveniles.

2. Materials and methods

2.1. Ethics

This study was approved by the Ethics Committee of CIIMAR (ORBEA-CIIMAR 37–2023) and conducted by certified researchers authorized to perform procedures on living animals, in accordance with the guidelines of the Portuguese Directorate-General for Food and Veterinary. The study also followed the Federation of European Laboratory Animal (FELASA) category C recommendations and the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes.

2.2. Ingredients and experimental diets

Based on the nutritional requirements of seabream and seabass juveniles (National Research Council, 2011), four isoenergetic (23 kJ/g dry matter, DM) diets were formulated and extruded by SPAROS Lda (Olhão, Portugal). The control diet (CTRL) was a commercial-like formulation containing 18% of fish protein, 30% of poultry by-products, 2% of single-cells proteins, 36% of vegetable meals, 6.3% FO and 5.2% poultry oil (PO). Three other experimental diets were formulated to include 1.7%, 3.4%, and 5.2% of BSFLO (BSFLO1.7, BSFLO3.4, and BSFLO5.2, respectively), replacing 33%, 66%, and 100% of PO (Table 1). BSFLO was supplied by Ingredient Odyssey S.A. - EntoGreen®, having been extracted from black soldier fly larvae reared on a blend of wheat bran, olive-oil pomace, and other vegetable by-products. All ingredients used in the experimental diets for seabass and seabream, as well as their proximate composition, are presented in Table 1. The absolute FA composition of BSFLO, PO and the experimental diets is presented in Table 2, while the corresponding profiles expressed as percentage of total FAME are shown in Supplementary Table 1.

2.3. In vivo growth trials and sampling

Juveniles of European seabass were obtained from Acuinuga – Acuicultura y Nutrición de Galicia S.L. (A Coruña, Spain) and transported to CIIMAR (Matosinhos, Portugal), while gilthead seabream juveniles were acquired from Marisland – Madeira Mariculture Lda (Madeira, Portugal) and sent to the Calheta Mariculture Centre (Madeira, Portugal). Before growth trials, fish were quarantined for a two-week period and hand-fed a commercial diet (NEOGOLD, AQUASOJA – Soja de Portugal (S. João de Ovar, Portugal) - 55% protein and 15% lipids).

Two independent trials were conducted with juvenile seabream and seabass, under experimental conditions that were kept as similar as possible. In the gilthead seabream trial, 12 homogeneous groups were distributed into 200-L fibreglass tanks (30 fish per tank) within a saltwater flow-through system, at an initial stocking density of 2.7 kg/m^3 (initial body weight (IBW) = 18.3 ± 0.28 g; initial body length (IBL) = 10.4 ± 0.07 cm). In this system, water temperature followed the natural seasonal variation of the incoming seawater, averaging 22.8 ± 0.6 °C; the salinity averaged 39 ± 1 ‰. In a different system, 12 homogeneous groups of 50 European seabass were allocated to twelve 160-L fibreglass tanks connected within a saltwater recirculating aquaculture system (RAS), at an initial stocking density of 3.5 kg/m^3 (initial body weight

Table 1
Ingredients and proximate composition of experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2
Ingredients (%)				
Fishmeal Super Prime [a]	5	5	5	5
Fishmeal 60 [b]	10	10	10	10
Fish protein hydrolysate [c]	3	3	3	3
Poultry meal [d]	20	20	20	20
Poultry blood meal [e]	5	5	5	5
Feather meal hydrolysate [f]	5	5	5	5
Single cell protein concentrate [g]	2	2	2	2
Corn gluten meal [h]	7.2	7.2	7.2	7.2
Guar korma meal	5	5	5	5
Sunflower meal 40 [i]	4	4	4	4
Wheat meal [j]	15.3	15.3	15.3	15.3
Whole peas	5	5	5	5
Vitamin and mineral premix [k]	1	1	1	1
Choline chloride 50% [l]	0.2	0.2	0.2	0.2
Antioxidant [m]	0.2	0.2	0.2	0.2
Sodium propionate [n]	0.1	0.1	0.1	0.1
Rapeseed lecithin [o]	0.5	0.5	0.5	0.5
Fish oil [p]	6.3	6.3	6.3	6.3
Poultry oil [q]	5.2	3.4	1.7	–
Black soldier fly larvae oil [r]	–	1.7	3.4	5.2
Proximate composition (g/100 g dry matter)				
Dry matter (DM; g/100 g feed)	92.1	93.8	93.9	93.1
Crude protein	49.1	50.2	50.9	52.2
Crude fat	18.7	19.2	19.1	18.4
Energy (MJ/100 g DM)	22.4	22.8	22.4	22.5
Ash	6.6	7.2	7.5	7.4
Phosphorus	1.0	1.1	1.1	1.1
European seabass				
Digestible protein (DP; g/100 g DM)	44.7	45.3	46.0	47.2
Digestible energy (DE; MJ/100 g DM)	18.4	18.7	18.8	18.8
DP:DE	2.4	2.4	2.5	2.5
Gilthead seabream				
Digestible protein (DP; g/100 g DM)	41.0	42.0	42.1	43.8
Digestible energy (DE; MJ/100 g DM)	20.7	20.9	20.7	20.8
DP:DE	2.0	2.0	2.0	2.1

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO. Digestible protein and digestible energy were calculated by multiplying crude protein and gross energy content of diets by their respective apparent digestibility coefficients. [a] Fishmeal Super Prime: 66.3% crude protein (CP), 11.5% crude fat (CF), Pesquera Diamante, Peru; [b] Fishmeal 60: 61.2% CP, 8.4% CF, Conserveros Reunidos S.A., Spain; [c] Fish protein hydrolysate (CPSP90): 82.6% CP, 9.6% CF, Sopropêche, France; [d] Poultry meal: 62.4% CP, 12.5% CF, SAVINOR UTS, Portugal; [e] Poultry blood meal: Sorgal S.A. Portugal; [f] Feather meal hydrolysate: 88.8% CP, 1.6% CF, Empro Europe NV, The Netherlands; [g] Aminopro NT70 - *C. glutamicum*: 74.1% CP, 3.1% CF, MAZZOLENI SPA, Italy; [h] Corn gluten meal: 61.2% CP, 5.2% CF, COPAM, Portugal; [i] Sunflower meal 40: 42.9% CP, 3.8% CF, AGP Slovakia, s.r.o., Slovakia; [j] Wheat meal: 11.7% CP, 1.6% CF, Molisur, Spain; [k] Vitamin and mineral premix: Premix Lda, Portugal; [l] Choline chloride 50%: ORFFA, The Netherlands; [m] Antioxidant (Verdilox): Kemin Europe NV, Belgium; [n] Sodium propionate: Disproquímica, Portugal; [o] Rapeseed lecithin (Liquid): 94% CF, Novastell, France; [p] Fish oil: 98.1% CF, Sopropêche, France; [q] Poultry oil: Sorgal S. A. Portugal; [r] Black soldier fly larvae oil:Ingredient Odissey-EntoGreen®, Portugal.

(IBW) = 11.5 ± 0.2 g; initial body length (IBL) = 10.5 ± 0.4 cm). In the system holding the seabass, water temperature was actively controlled and daily adjusted to follow the temperature recorded in the flow-through system holding the seabream, throughout the experimental period, averaging 23.0 ± 0.7 °C; the salinity averaged 35 ± 2.6 ‰. The water flow rate was set at 10 L/min, and the dissolved oxygen level remained above 80% saturation in both trials. A photoperiod of 12 h of light followed by 12 h of darkness was used in both trials. Total ammonia (NH_4^+) and nitrate (NO_3^-) levels were measured twice a week, while pH was monitored daily to ensure conditions remained with the recommended ranges for marine fish species ($NH_4^+ \leq 0.05$ mg/L; $NO_3^- \leq 50$ mg/L; $7.5 \leq pH \leq 8.5$). Mortality was daily checked, and any dead fish were immediately removed and their weight and length registered. In both trials, each experimental diet was randomly assigned to three replicate tanks, and fish were hand-fed ad libitum three times a day, 7 days a week, until their weight quadrupled (8 weeks for seabream and 12 weeks for seabass). Feed intake was monitored daily. Any uneaten pellets were counted, and weight was subtracted from the total feed offered to obtain accurate feed intake values.

The exact same sampling protocols were used in both trials. In both trials, ten fish from the initial stocks were lightly anaesthetised with 2-phenoxyethanol (200 μL L^{-1}), euthanized by a spinal cord section, measured (total length), weighed and stored at −20 °C for subsequent analysis of initial whole-body (WB) composition. At the end of the trials, following a 24-h fasting period, five fish per tank (15 fish per dietary treatment) were lightly anaesthetised with 2-phenoxyethanol (200 μL L^{-1}), weighed and measured and, then, euthanized by a spinal cord section and stored for subsequent WB composition analysis. The whole-bodies of these five fish per tank were later minced using a meat grinder, pooled, homogenised, freeze-dried, and stored at −20 °C. Other five fish per tank (15 fish per dietary treatment) were euthanized the same way, measured and weighed, their viscera and liver collected and weighed for further determination of viscerosomatic (VSI) and hepatosomatic (HSI) indexes, respectively. From these same 5 fish, three portions (~ 1 g each) of skinless white dorsal muscle were collected, flash-frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis of moisture, macronutrients and energy, FA profile, and antioxidant capacity. All the remaining fish were also individually weighed and measured to accurately estimate total biomass and mean body weight per tank. Growth performance and feed efficiency were subsequently calculated

Table 2
Fatty acid profile of poultry oil (PO), black soldier fly larvae oil (BSFLO), (% oil), and the experimental diets (% DM).

	PO	BSFLO	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2
C12:0	–	28.8	–	0.4	0.8	1.2
C14:0	0.8	5.1	0.5	0.6	0.6	0.7
C16:0	21.5	13.6	3.5	3.3	3.2	3.0
C18:0	5.9	1.7	0.8	0.7	0.7	0.6
Σ SFA[a]	28.7	51.0	5.0	5.2	5.5	5.7
C16:1n-7	5.4	1.9	1.0	0.9	0.9	0.8
C18:1n-9	32.6	25.9	3.8	3.7	3.7	3.3
C20:1n-9	–	–	0.1	0.1	0.1	0.1
Σ MUFA[b]	40.6	28.9	5.6	5.4	5.3	4.8
C18:2n-6	20.1	13.0	2.5	2.4	2.2	2.0
C18:3n-3	1.3	1.3	0.2	0.2	0.2	0.2
C18:4n-3	–	–	0.2	0.2	0.2	0.2
C20:4n-6	–	–	0.1	0.1	0.1	0.1
C20:5n-3 (EPA)	–	–	1.2	1.2	1.2	1.2
C22:5n-3	–	–	0.2	0.1	0.1	0.1
C22:6n-3 (DHA)	–	–	0.6	0.6	0.6	0.6
Σ PUFA[c]	25.0	14.3	5.5	5.2	5.1	4.9
Σ n-3	3.6	1.3	2.5	2.3	2.3	2.3
Σ n-6	20.9	13.1	2.8	2.6	2.4	2.2
n-3/n-6	0.2	0.1	0.9	0.9	1.0	1.1
EPA + DHA	–	–	1.8	1.8	1.8	1.8
DHA/EPA	–	–	0.5	0.5	0.5	0.5

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; [a] Includes: C10:0, C11:0, C13.0, C15.0, C17.0, C20.0, C21.0; [b]Includes: C14:1, C17:1n-7, C18:1n-7, C22:1n-11, C22:1n-9; [c] Includes: C16:2n-4, C16:3n-4, C16:4n-1, C18:3n-6, C20:2n-6, C20:3n-6, C22:3n-3, C22:4n-3 and C22:2n-6.

on a tank basis, with each tank considered as one experimental unit ($n = 3$).

2.4. *In vivo digestibility trial*

Digestibility assays were conducted in parallel with the growth trials, using fish from the same initial stock. Groups of 21 fish were placed in 50-L fibreglass tanks was equipped with a sedimentation column (Guelph system), which was designed by Cho and Slinger (1979) for collecting faeces, three replicate tanks being used per dietary treatment. The environmental conditions during the digestibility trial were similar to those of the growth trial. Prior to faecal collection, the fish underwent a 30-day acclimation period to the experimental diets containing 1% Cr_2O_3 as an inert digestibility marker (replacing of 1% wheat meal), allowing dietary adaptation and stabilization of voluntary feed intake. All fish were hand-fed ad libitum three times a day as in the growth

Table 3
Growth performance and somatic indexes of European seabass and gilthead seabream fed the experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2	*p*-value
European seabass					
IBW (g)	11.5 ± 0.1	11.4 ± 0.1	11.4 ± 0.1	11.5 ± 0.1	0.503
IBL (cm)	10.5 ± 0.1	10.5 ± 0.04	10.5 ± 0.01	10.5 ± 0.1	0.977
FBW (g)	53.0 ± 2.0[b]	53.5 ± 1.2[b]	53.8 ± 1.7[b]	57.2 ± 1.1[a]	0.039
FBL (cm)	16.6 ± 0.2[b]	16.6 ± 0.1[b]	16.7 ± 0.2[b]	17.0 ± 0.1[a]	0.029
Final K	1.2 ± 0.02	1.2 ± 0.03	1.2 ± 0.01	1.2 ± 0.02	0.600
TGC ($g^{1/3}$/day × °C)	0.8 ± 0.04	0.8 ± 0.01	0.8 ± 0.02	0.9 ± 0.01	0.170
SGR (%/day)	1.9 ± 0.1	1.9 ± 0.02	1.9 ± 0.04	2.0 ± 0.02	0.198
VFI (%/day)	1.74 ± 0.1	1.73 ± 0.01	1.69 ± 0.02	1.73 ± 0.02	0.436
FCR	1.09 ± 0.02[a]	1.08 ± 0.01[ab]	1.06 ± 0.01[bc]	1.05 ± 0.02[c]	0.042
HSI	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	0.812
VSI	7.3 ± 0.5	7.7 ± 0.1	7.4 ± 0.2	7.2 ± 0.1	0.110
Gilthead seabream					
IBW (g)	18.4 ± 0.2	18.2 ± 0.3	18.4 ± 0.3	18.3 ± 0.3	0.838
IBL (cm)	10.4 ± 0.05	10.4 ± 0.1	10.4 ± 0.02	10.4 ± 0.04	0.864
FBW (g)	79.8 ± 1.4[a]	76.0 ± 1.3[b]	81.7 ± 0.9[a]	81.3 ± 2.2[a]	0.007
FBL (cm)	16.4 ± 0.1[b]	16.2 ± 0.1[c]	16.6 ± 0.1[a]	16.5 ± 0.1[ab]	0.005
Final K	1.8 ± 0.01	1.8 ± 0.02	1.8 ± 0.03	1.8 ± 0.03	0.641
TGC ($g^{1/3}$/day × °C)	1.32 ± 0.01[a]	1.27 ± 0.01[b]	1.34 ± 0.01[a]	1.34 ± 0.04[a]	0.011
SGR (%/day)	2.67 ± 0.01[ab]	2.60 ± 0.01[b]	2.71 ± 0.01[a]	2.71 ± 0.1[a]	0.023
VFI (%/day)	2.5 ± 0.1	2.3 ± 0.1	2.4 ± 0.2	2.4 ± 0.2	0.436
FCR	1.1 ± 0.04	1.0 ± 0.03	1.0 ± 0.1	1.1 ± 0.1	0.611
HSI (%)	1.8 ± 0.1	1.7 ± 0.2	1.8 ± 0.03	1.6 ± 0.1	0.273
VSI (%)	8.2 ± 0.5[a]	7.7 ± 0.2[b]	7.8 ± 0.2[ab]	7.4 ± 0.2[b]	0.037

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; IBW, initial body weight; IBL, initial body length; FBW, final body weight; FBL, final body length; K, final condition factor; TGC, thermal-unit growth coefficient; SGR, Specific growth rate; VFI, voluntary feed intake; FCR, feed conversion ratio; HSI, hepatosomatic index; VSI, viscerosomatic index. Values are presented as means ± standard deviation ($n = 3$). Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan's post-hoc test).

Table 4
Whole-body composition and nutrients balances of European seabass juveniles fed with the experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2	*p*-value
European seabass					
Whole-body composition (% WW)					
Moisture	64.2 ± 0.6	64.6 ± 0.3	64.9 ± 1.0	64.6 ± 0.2	0.594
Protein	17.1 ± 0.1	17.4 ± 0.3	17.2 ± 0.1	17.3 ± 0.2	0.299
Lipids	15.5 ± 0.4	15.0 ± 0.3	14.6 ± 0.9	15.2 ± 0.2	0.288
Energy (kJ/g WW)	9.4 ± 0.1	9.0 ± 0.1	9.0 ± 0.3	9.3 ± 0.2	0.068
Ash	2.9 ± 0.2	3.0 ± 0.1	3.1 ± 0.1	2.9 ± 0.2	0.146
Phosphorus	0.4 ± 0.03	0.4 ± 0.02	0.5 ± 0.02	0.4 ± 0.03	0.211
Nitrogen (N) balance (g/kg ABW/day)					
Digestible N intake (DN)	1.3 ± 0.04	1.3 ± 0.01	1.3 ± 0.01	1.3 ± 0.02	0.057
N gain	0.4 ± 0.01	0.5 ± 0.01	0.4 ± 0.004	0.5 ± 0.01	0.176
N retention efficiency (% DN)	34.8 ± 0.5	35.2 ± 0.5	35.2 ± 0.1	34.6 ± 0.9	0.543
Faecal N losses	0.126 ± 0.004^{c}	0.138 ± 0.001^{ab}	0.133 ± 0.001^{b}	0.141 ± 0.002^{a}	< 0.001
Metabolic N losses	0.8 ± 0.03	0.8 ± 0.001	0.8 ± 0.01	0.9 ± 0.02	0.082
Total N losses	1.0 ± 0.04	1.0 ± 0.0004	1.0 ± 0.01	1.0 ± 0.03	0.051
Lipid (L) balance (g/kg ABW/day)					
Digestible L intake (DL)	3.0 ± 0.1	3.1 ± 0.03	3.0 ± 0.04	2.9 ± 0.04	0.125
L gain	2.8 ± 0.1	2.7 ± 0.1	2.6 ± 0.2	2.8 ± 0.1	0.215
L retention efficiency (% DL)	90.7 ± 1.3^{ab}	84.9 ± 2.0^{b}	85.0 ± 5.9^{b}	92.9 ± 2.5^{a}	0.046
Faecal L losses	0.26 ± 0.01^{a}	0.24 ± 0.002^{b}	0.23 ± 0.003^{b}	0.24 ± 0.003^{b}	< 0.001
Metabolic L losses	0.3 ± 0.03^{ab}	0.4 ± 0.1^{a}	0.4 ± 0.2^{a}	0.2 ± 0.1^{b}	0.044
Total L losses	0.5 ± 0.02	0.7 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.060
Energy (E) balance (kJ/kg ABW/day)					
Digestible E intake (DE)	325.7 ± 11.4	328.0 ± 2.2	322.1 ± 3.6	329.8 ± 4.4	0.531
E gain	156.4 ± 6.0^{ab}	150.1 ± 0.9^{bc}	148.5 ± 3.7^{c}	158.8 ± 3.1^{a}	0.033
E retention efficiency (% DE)	48.0 ± 0.6^{a}	45.8 ± 0.6^{b}	46.1 ± 1.5^{b}	48.2 ± 0.7^{a}	0.026
Metabolizable E	305.2 ± 10.6	307.4 ± 2.2	301.6 ± 3.3	308.3 ± 3.8	0.565
Faecal E losses	70.8 ± 2.5^{b}	73.5 ± 0.5^{a}	64.4 ± 0.7^{c}	66.3 ± 0.9^{c}	< 0.001
Branchial + urinary E losses	20.5 ± 0.8	20.6 ± 0.03	20.5 ± 0.3	21.6 ± 0.6	0.082
Total E losses	240.1 ± 8.4	251.5 ± 3.6	238.0 ± 7.3	237.3 ± 3.8	0.072
Total heat production	148.8 ± 5.3	157.3 ± 3.1	153.1 ± 6.4	149.3 ± 2.7	0.166

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; WW, wet weight; ABW, average body weight. Values are presented as means ± standard deviation ($n = 3$ pools of 15 fish); Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan post-hoc test).

trials. After the last meal of each day, the tanks were thoroughly cleaned to remove any uneaten feed; faeces were collected the next morning before the first meal. Faecal samples were centrifuged at 4170*g* for 10 min at room temperature to remove excess water and stored at −80 °C (pooled by tank). Faeces were collected for 3–5 weeks to obtain the minimum amount required for chemical analyses (approximately 25 g dry weight per tank).

2.5. Chemical analyses

2.5.1. Proximate composition

All the experimental diets, as well as the WB and muscle samples, were analysed for proximate composition using the same procedures. For WB composition analysis, fish sampled at the start of the trial were pooled into a single sample. The five fish per tank sampled at the end of the trial were pooled into 1 pool per tank, resulting in three pools per treatment. For muscle composition analysis, fillets from the five fish sampled per tank were pooled, yielding three samples per treatment. All samples were ground and homogenised prior to analysis. The dry matter (DM) content was determined by drying samples in an oven at 105 °C for 24 h, in accordance with AOAC (2006) method no. 934.01. The ash content of diets and fish WB was determined by incinerating samples in a muffle furnace at 550 °C for 6 h, according to AOAC (2006) method no. 942.05. Crude protein of all samples was measured by quantifying nitrogen (N) through combustion in an N analyser (LECO FP-528; LECO Corporation, Michigan, USA) and applying a 6.25 conversion factor, according to AOAC (2006) method no.990.03. Gross energy of all samples was determined using an automatic isoperibol calorimeter (Parr 6400; Parr Instrument Company, Illinois, USA) following AOAC (2006). Total phosphorus of diets and fish WB was quantified from ash according to AOAC (2006) method no. 942.05, as adapted by Basto et al. (2023). Crude fat content of diets and fish WB was determined using petroleum ether at 140 °C (Soxtec™ 2500, FOSS, Höganäs, Sweden), according to AOAC (2006) method no. 2003.05. Total lipids in muscle samples were extracted and quantified gravimetrically following the method of Folch et al. (1957), using a dichloromethane:methanol solution (2:1 *v*/v) with 0.01% BHT.

2.5.2. Fatty acids profile

FA methyl esters (FAME) from experimental diets and the muscle samples were obtained through acidic methylation according to Lepage and Roy (1986), with modifications. Briefly, 3 mL of a freshly prepared acetyl chloride:methanol solution (2:1 v/v) with BHT (20 mg/L) (methylation solution) was added to the samples, along with 1 mL of tricosanoic acid (C23:0) as an internal standard solution, at concentrations of 0.5 mg/mL for muscle and 1.0 mg/mL for the diets. The samples were vortexed and heated at 100 °C for 1 h. After cooling to room temperature, 5 mL of 6% a K_2CO_3 solution containing 5 mg/L BHT was added, followed by 2 mL of n-hexane with the same BHT concentration. The samples were then vortexed and centrifuged at 1200*g* for 5 min at room temperature. The upper organic layer, containing FAME, was transferred to another tube containing 1 g of anhydrous sodium sulfate. This process was repeated twice before the organic layer was filtered through a 125 mm Ø filter into a new tube and dried under a stream of

nitrogen gas at 37 °C. Finally, FAME was recovered in 1 mL of n-hexane, then injected, identified and quantified by gas chromatography (GC) using a Shimadzu Nexis GC-2030 equipped with a flame-ionization detector (FID) and a Shimadzu AOC-20i auto-injector (Tokyo, Japan), as described in Marques et al. (2022).

2.5.3. Lipid peroxidation and antioxidant capacity

Lipid peroxidation (LPO) levels in muscle were determined by quantifying thiobarbituric acid-reactive substances (TBARS), primarily composed of malondialdehyde (MDA), as described by Filipa-Silva et al. (2023). The muscle radical scavenging potential against the peroxyl radical generated by 2,2′-azobis-(2-amidinopropane) hydrochloride (AAPH) was determined using the oxygen-radical absorbance-capacity (ORAC) assay, with a Trolox solution as the standard, also as outlined by Filipa-Silva et al. (2023).

2.6. Calculations

Growth performance was assessed based on the following parameters: condition index (K) = 100 × (final body weight (FBW) (g) / final body length^3 (cm)); thermal growth coefficient (TGC, $g^{1/3}$ / day × °C) = 1000 × (($FBW^{1/3}$ (g) - initial body weight $(IBW)^{1/3}$ (g)) / (total n° of days of the experiment × temperature (°C))); specific growth rate (SGR, % / day) = 100 × ((Ln (FBW) – Ln (IBW)) / total n° of days of the experiment). Feed efficiency was evaluated based on: voluntary feed intake (VFI, %/day) = 100 × (total feed consumption (g dry matter (DM) per fish) / average FBW / total n° of feeding days); feed conversion ratio (FCR) = total feed consumption (g DM per fish) / (FBW (g) – IBW (g)). Somatic indexes were calculated as: hepatosomatic index (HSI, %) = 100 × (liver weight (g) / FBW (g)); viscerosomatic index (VSI, %) = 100 × (viscera weight (g) / FBW (g). Nutrient digestibility was evaluated based on: nutrient ADC = 100 × (1 - (% chromium in diet / % chromium in faeces) × (faeces nutrient composition (% DM or kJ/g DM) / feed nutrient composition (% DM or kJ/g DM))); Digestible protein (or energy) (DP or DE, mg/g or kJ/g DM) = Total crude protein (or energy) × protein (or energy) ADC (%); Digestible protein to digestible energy ratio (DP:DE, mg/kJ) = DP / DE. Nutrient and energy balance parameters were estimated using an indirect approach based on apparent digestibility coefficients. Nutrient and energy balance were calculated as follows: digestible nutrient intake (g/kg average body weight (ABW)/ day) = crude nutrient intake (g/kg ABW/day) × nutrient ADC; digestible energy intake (kJ/kg ABW/day) = crude energy intake (kJ/kg ABW/day) – faecal energy losses (kJ/kg ABW/day); nutrient or energy gain (g or kJ/kg ABW/day) = (FBW × final nutrient (or energy) composition of carcass (% WW) - IBW × initial nutrient (or energy) composition of carcass (% WW)) / (ABW / days); nutrient or energy retention efficiency (% digestible intake) = nutrient (or energy) gain / digestible nutrient (or energy) intake ×100; faecal nutrient losses (g/kg ABW/day) = crude nutrient intake (g/kg ABW/day) × (1 - nutrient ADC/100); metabolic nutrient losses (g/kg ABW/day) = digestible nutrient intake - nutrient gain; metabolizable energy (kJ/kg ABW/day) = digestible energy intake - metabolic energy loss; faecal energy losses (kJ/kg ABW/day) = crude energy intake (kJ/kg ABW/day) × (1 - energy ADC/100); branchial and urinary energy losses (kJ/kg ABW/day) =

Table 5
Whole-body composition and nutrients balances of gilthead seabream juveniles fed with the experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2	*p*-value
Gilthead seabream					
Whole-body composition (% WW)					
Moisture	66.0 ± 0.7	65.8 ± 0.2	65.9 ± 0.6	66.3 ± 0.7	0.669
Protein	15.8 ± 0.4	16.2 ± 0.01	16.1 ± 0.8	16.1 ± 0.01	0.734
Lipids	14.5 ± 1.0	14.0 ± 0.5	14.5 ± 0.9	14.0 ± 0.7	0.785
Energy (kJ/g WW)	9.0 ± 0.7	8.5 ± 0.2	8.8 ± 0.3	8.5 ± 0.1	0.293
Ash	3.2 ± 0.3	3.3 ± 0.1	3.1 ± 0.3	3.2 ± 0.1	0.627
Phosphorus	0.4 ± 0.03	0.4 ± 0.03	0.4 ± 0.04	0.4 ± 0.01	0.998
Nitrogen (N) balance (g/kg ABW/day)					
Digestible N intake (DN)	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	0.265
N gain	0.6 ± 0.02	0.7 ± 0.003	0.7 ± 0.04	0.7 ± 0.01	0.690
N retention efficiency, %DN	39.6 ± 0.8	42.3 ± 1.2	41.8 ± 4.7	39.3 ± 3.0	0.511
Faecal N losses	0.3 ± 0.01	0.3 ± 0.01	0.3 ± 0.02	0.3 ± 0.02	0.218
Metabolic N losses	1.0 ± 0.04	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.375
Total N losses	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	0.415
Lipid (L) balance (g/kg ABW/day)					
Digestible L intake (DL)	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.3	4.1 ± 0.3	0.632
L gain	4.0 ± 0.3	3.8 ± 0.1	4.0 ± 0.3	3.8 ± 0.2	0.695
L retention efficiency (% DL)	91.6 ± 8.9	91.1 ± 6.2	94.8 ± 5.8	93.7 ± 7.6	0.910
Faecal L losses	0.29 ± 0.01[a]	0.26 ± 0.01[b]	0.30 ± 0.02[a]	0.30 ± 0.02[a]	0.045
Metabolic L losses	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.4	0.754
Total L losses	0.7 ± 0.4	0.6 ± 0.3	0.5 ± 0.3	0.6 ± 0.4	0.950
Energy (E) balance (kJ/kg ABW/day)					
Digestible E intake (DE)	510.0 ± 17.4	480.6 ± 14.7	488.4 ± 30.4	500.7 ± 34.1	0.538
E gain	242.3 ± 20.7	222.5 ± 6.3	237.5 ± 8.5	228.1 ± 4.6	0.241
E retention efficiency (% DE)	47.6 ± 4.8	46.3 ± 0.4	48.7 ± 2.8	45.7 ± 3.6	0.697
Metabolizable E	485.6 ± 16.4	458.5 ± 13.6	465.3 ± 27.0	475.0 ± 31.2	0.539
Faecal E losses	43.7 ± 1.5	44.3 ± 1.4	40.8 ± 2.5	41.1 ± 2.8	0.186
Branchial + urinary E losses	24.4 ± 1.0	22.1 ± 1.1	23.1 ± 3.3	25.5 ± 3.0	0.375
Total E losses	311.3 ± 31.0	302.4 ± 10.3	291.7 ± 30.8	313.6 ± 39.5	0.802
Total heat production	243.2 ± 29.3	236.0 ± 7.8	227.9 ± 25.1	247.1 ± 33.7	0.811

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; WW, wet weight; ABW, average body weight. Values are presented as means ± standard deviation (n = 3 pools of 15 fish); Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan post-hoc test).

metabolizable nitrogen losses × 24.9 kJ / digestible nitrogen; total nutrient or energy losses (g or kJ/kg ABW/day) = nutrient (or energy) intake - nutrient (or energy) gain; total heat production (kJ/kg ABW/day) = metabolizable energy - energy intake;

2.7. Statistical analysis

Firstly, the data were tested for normality and homogeneity using the Kolmogorov-Smirnov and Levene tests, respectively. Differences among dietary treatments were then evaluated using one-way analysis of variance (ANOVA) with a significance level of 95% ($p < 0.05$) in IBM SPSS Statistics 30.0 (IBM Corporation, 2024, USA). When the one-way ANOVA indicated significance, individual comparisons were made using the post- hoc Duncan test. GraphPad Prism 9 (GraphPad Software LLC, USA) was used for graphics design and linear regression calculations. All data are presented as mean ± standard deviation, and different superscript letters indicate significant differences among the experimental groups.

3. Results

3.1. Growth performance and feed efficiency

In both trials, all fish readily accepted the experimental diets, and no significant differences in VFI were observed between fish fed the CTRL diet and those fed BSFLO diets (Table 3). Survival was 100% in seabass and 96% in seabream.

After 12 weeks of feeding, European seabass fed the BSFLO5.2 diet achieved the highest final weight and length (Table 3). SGR and TGC showed an upward (non-significant) trend in fish fed the BSFLO5.2 diet compared to the other groups. Additionally, the BSFLO5.2 resulted in a significantly lower FCR than the CTRL diet. HSI and VSI did not differ among groups.

After 8 weeks of feeding, gilthead seabream fed the BSFLO3.4 and BSFLO5.2 diets reached the highest final weight and length values, although values were not significantly different from those of CTRL group. Fish fed BSFLO1.7 had a significantly lower final weight than all other fish. Accordingly, significant differences were observed for both SGR and TGC among groups: fish fed the BSFLO3.4 and BSFLO5.2 diets showed significantly higher values than those fed on BSFLO1.7, but comparable to the CTRL group. There were no significant differences in FCR between dietary treatments. HSI values did not differ significantly between dietary groups, whereas VSI values were significantly lower in seabream fed the BSFLO5.2 diet compared to those fed the CTRL diet.

3.2. Whole-body composition and nutrient balances

In European seabass, no significant differences in WB composition were observed among the dietary treatments (Table 4). However, there is a trend ($p < 0.1$) for decreased energy content in the WB of fish fed the BSFLO1.7 and the BSFLO3.4 diets. This trend reflected the differences found in energy gain and energy retention efficiency (Table 4). Fish fed the BSFLO5.2 diet exhibited energy gain values comparable to those of the CTRL group. The BSFLO1.7 diet resulted in reduced energy retention efficiency, primarily due to significantly higher energy losses through faeces. With regard to lipid utilisation, digestible lipid intake and lipid gain did not differ among dietary treatments. Although not statistically significant, fish fed the BSFLO5.2 diet showed the highest lipid retention efficiency, together with the lowest faecal and metabolic losses. Faecal nitrogen losses differed significantly in seabass, being highest in BSFLO5.2.

No significant differences in WB composition were observed among dietary treatments in gilthead seabream, indicating that macronutrient composition and energy gain or retention were unaffected by the diets (Table 5). The only difference detected in seabream concerned lipid balance, as faecal lipid losses were lowest in fish fed the BSFLO1.7 diet, while the remaining lipid balance parameters were comparable among treatments.

3.3. Muscle proximate composition and fatty acids profile

No significant differences were observed in the muscle protein or

Table 6
Protein, total lipids, and fatty acid profile (g/100 g of wet weight) of the muscle of European seabass fed the experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2	*p-value*
Protein	20.6 ± 0.3	20.8 ± 0.5	20.6 ± 1.0	20.3 ± 0.7	0.810
Total lipids	3.0 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	3.3 ± 0.5	0.694
C12:0	0.002 ± 0.001^{d}	0.03 ± 0.01^{c}	0.05 ± 0.004^{b}	0.1 ± 0.02^{a}	< 0.001
C14:0	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.228
C16:0	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.934
C18:0	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.733
Σ SFA[1]	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.1	1.0 ± 0.2	0.917
C16:1n-7	0.2 ± 0.04	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.04	0.800
C18:1n-9	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	0.962
C20:1n-9	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.989
Σ MUFA[2]	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.1	1.2 ± 0.3	0.967
C18:2n-6	0.44 ± 0.1	0.41 ± 0.1	0.37 ± 0.03	0.37 ± 0.1	0.685
C18:3n-3	0.043 ± 0.01	0.041 ± 0.01	0.041 ± 0.004	0.043 ± 0.01	0.984
C18:4n-3	0.026 ± 0.01	0.025 ± 0.01	0.025 ± 0.002	0.027 ± 0.01	0.949
C20:4n-6	0.039 ± 0.01	0.036 ± 0.003	0.033 ± 0.002	0.033 ± 0.01	0.321
C20:5n-3 (EPA)	0.268 ± 0.05	0.266 ± 0.04	0.252 ± 0.02	0.276 ± 0.04	0.903
C22:5n-3	0.034 ± 0.01	0.032 ± 0.004	0.031 ± 0.002	0.033 ± 0.01	0.921
C22:6n-3 (DHA)	0.249 ± 0.04	0.237 ± 0.02	0.234 ± 0.02	0.239 ± 0.03	0.931
EPA + DHA	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.944
DHA/EPA	0.94 ± 0.03	0.89 ± 0.04	0.93 ± 0.01	0.87 ± 0.02	0.061
Σ PUFA[3]	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	0.907
Σ n-3	0.63 ± 0.1	0.62 ± 0.08	0.60 ± 0.04	0.63 ± 0.1	0.952
Σ n-6	0.51 ± 0.1	0.48 ± 0.1	0.43 ± 0.03	0.43 ± 0.1	0.693
n-3/n-6	1.3 ± 0.05^{c}	1.3 ± 0.1^{bc}	1.4 ± 0.01^{ab}	1.5 ± 0.1^{a}	0.010

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; [1] Includes: C15:0, C17:0, C20.0; [2] Includes: C14:1, C17:1n-7, C18:1n-7, C22:1n-9; [3] Includes: C16:2n-4, C16:3n-4, C16:4n-1, C18:3n-6, C20:2n-6, C20:3n-6, C20:3n-3; C20:4n-3; C22:3n-3, and C22:4n-3; The abbreviations stand for: WW, wet weight; FA, fatty acid. Values are presented as means ± standard deviation (n = 3 pools of 15 fish); Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan post-hoc test).

Table 7
Protein, total lipids, and fatty acid profile (g/100 g of wet weight) of the muscle of gilthead seabream fed the experimental diets.

	CTRL	BSFLO1.7	BSFLO3.4	BSFLO5.2	*p-value*
Protein	19.6 ± 1.0	19.2 ± 0.5	20.1 ± 0.4	19.5 ± 0.4	0.452
Total Lipids	3.3 ± 0.4	4.0 ± 0.1	3.5 ± 0.6	4.2 ± 0.7	0.398
C12:0	0.002 ± 0.001^{c}	0.05 ± 0.01^{b}	0.08 ± 0.02^{b}	0.2 ± 0.03^{a}	< 0.001
C14:0	0.09 ± 0.02^{b}	0.13 ± 0.02^{b}	0.13 ± 0.02^{b}	0.19 ± 0.03^{a}	0.008
C16:0	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.284
C18:0	0.2 ± 0.03	0.2 ± 0.02	0.2 ± 0.03	0.2 ± 0.03	0.470
Σ SFA[1]	1.0 ± 0.2	1.2 ± 0.1	1.1 ± 0.2	1.5 ± 0.2	0.083
C16:1n-7	0.2 ± 0.1	0.3 ± 0.03	0.2 ± 0.04	0.3 ± 0.05	0.439
C18:1n-9	1.0 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	1.2 ± 0.2	0.461
C20:1n-9	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.354
Σ MUFA[2]	1.4 ± 0.4	1.7 ± 0.2	1.4 ± 0.2	1.7 ± 0.3	0.433
C18:2n-6	0.51 ± 0.1	0.59 ± 0.1	0.47 ± 0.1	0.54 ± 0.1	0.444
C18:3n-3	0.042 ± 0.01	0.051 ± 0.01	0.042 ± 0.01	0.054 ± 0.01	0.264
C18:4n-3	0.028 ± 0.01	0.035 ± 0.004	0.028 ± 0.004	0.036 ± 0.01	0.265
C20:4n-6	0.032 ± 0.01	0.033 ± 0.002	0.029 ± 0.002	0.030 ± 0.003	0.373
C20:5n-3 (EPA)	0.230 ± 0.04	0.266 ± 0.02	0.225 ± 0.02	0.272 ± 0.04	0.273
C22:5n-3	0.062 ± 0.01	0.074 ± 0.01	0.063 ± 0.01	0.078 ± 0.01	0.277
C22:6n-3 (DHA)	0.194 ± 0.03	0.212 ± 0.01	0.185 ± 0.01	0.214 ± 0.02	0.307
EPA + DHA	0.4 ± 0.1	0.5 ± 0.04	0.4 ± 0.04	0.5 ± 0.1	0.287
DHA/EPA	0.9 ± 0.05	0.8 ± 0.02	0.8 ± 0.03	0.8 ± 0.03	0.150
Σ PUFA[3]	1.2 ± 0.3	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	0.473
Σ n-3	0.57 ± 0.1	0.66 ± 0.1	0.56 ± 0.1	0.68 ± 0.1	0.266
Σ n-6	0.57 ± 0.1	0.63 ± 0.1	0.53 ± 0.1	0.55 ± 0.1	0.446
n-3/n-6	1.01 ± 0.1^{b}	1.01 ± 0.01^{b}	1.07 ± 0.03^{b}	1.13 ± 0.02^{a}	0.010

The abbreviations stand for: CTRL, control diet; BSFLO1.7, diet containing 1.7% black soldier fly larvae oil (BSFLO), replacing 33% of the poultry oil (PO); BSFLO3.4, diet containing 3.4% BSFLO, replacing 66% of the PO; BSFLO5.2, diet containing 5.2% BSFLO, totally replacing the PO; [1] Includes: C15:0, C17:0, C20.0; [2] Includes: C14:1, C17:1n-7, C18:1n-7, C22:1n-9; [3] Includes: C16:2n-4, C16:3n-4, C16:4n-1, C18:3n-6, C20:2n-6, C20:3n-6, C20:3n-3; C20:4n-3; C22:3n-3, and C22:4n-3; The abbreviations stand for: WW, wet weight; FA, fatty acid; Values are presented as means ± standard deviation (n = 3 pools of 15 fish); Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan post-hoc test).

total lipids content of either seabass or seabream among the dietary treatments. In seabass, the average muscle protein and lipid contents were approximately 21% and 3% of wet weight, respectively, while in seabream they averaged around 20% and 4% of wet weight, respectively (Tables 6 and 7). Both species exhibited clear changes in muscle fatty acid composition, in response to the progressive replacement of PO with BSFLO (Tables 6 and 7). In both seabass and seabream, LAA content in muscle increased concomitantly with increasing replacement of PO by BSFLO. Total SFA (g/100 g WW) in seabass muscle remained stable across dietary treatments, whereas in seabream a trend ($p < 0.1$) towards a progressive increase in SFA content was observed with increasing dietary BSFLO inclusion (Tables 6 and 7). However, when expressed as a percentage of total FAME (Supplementary Table 2 and Supplementary Table 3), SFA levels increased significantly with increasing BSFLO inclusion in both species. Total MUFA and PUFA, including EPA and DHA, in the muscle of both seabass and seabream were not affected by the replacement of PO with BSFLO. In both species, dietary substitution of PO with BSFLO resulted in an increase in the n-3/n-6 ratio.

3.4. Muscle lipid peroxidation and antioxidant capacity

In both species, muscle LPO differed between diets (Fig. 1A and C), being lower in BSFLO5.2 vs CTRL. Muscle oxygen radical absorbance capacity (ORAC) was higher in BSFLO5.2 (Fig. 1B and D), when compared to the CTRL diet. These results suggest that the BSFLO5.2 diet led to improved antioxidant status and reduced lipid peroxidation in the muscle of both species.

4. Discussion

In seabass, the total PO replacement with BSFLO (5.2% inclusion) significantly increased FBW and length, while reducing FCR. In seabream, both BSFLO3.4 and BSFLO5.2 tended to enhance growth performance and feed efficiency, but without statistical significance. As voluntary feed intake did not differ among dietary treatments, the improved growth observed in seabass is unlikely to be related to increased feed consumption, but rather to differences in nutrient and energy utilisation. In fact, seabass fed the BSFLO5.2 diet showed the highest lipid retention efficiency together with the lowest faecal and metabolic lipid losses, as well as reduced faecal energy losses, indicating a more efficient use of dietary lipids when provided by BSFLO instead of PO. This effect may be associated with the contrasting fatty acid composition of the two oils. BSFLO is characterized by a high content of LAA (28.8%), a medium-chain fatty acid that is rapidly absorbed and preferentially oxidized for energy (Papamandjaris et al., 1998). Unlike long-chain fatty acids, MCFA can enter mitochondrial β-oxidation independently of the carnitine transport system, enabling rapid ATP production and potentially improving metabolic efficiency. In contrast, PO contains higher levels of LA, a LC-PUFA that is more frequently deposited in tissues and requires additional metabolic processing. The stronger response observed when PO was fully replaced by BSFLO suggests a dose-dependent effect of MCFA availability on energy metabolism. Moreover, the more pronounced growth response in seabass compared with seabream may reflect species-specific differences in lipid metabolism, as seabass generally relies more extensively on lipid oxidation to meet its energetic demands, whereas gilthead seabream tends to display a more conservative lipid metabolism with greater lipid deposition and lipogenic activity (Izquierdo et al., 2003; Tocher, 2010). Nevertheless, these interpretations remain hypothetical and should be validated in future studies through targeted analyses of lipid metabolic pathways, including key markers of β-oxidation (e.g., CPT1, ACOX1) and lipogenesis (e.g., ACC, FASN), in order to better elucidate the mechanisms underlying the species-specific responses observed in the present study.

To the best of our knowledge, the inclusion of BSFLO in diets for European seabass has not been previously evaluated. In gilthead seabream, the results of the present study are consistent with those reported by Moutinho et al. (2023), according to which the full replacement of a VO blend (linseed, palm and rapeseed oil) by BSFLO (9.5% inclusion level) did not affect growth performance of gilthead seabream juveniles in a 70 days trial. Although these authors observed that feed efficiency

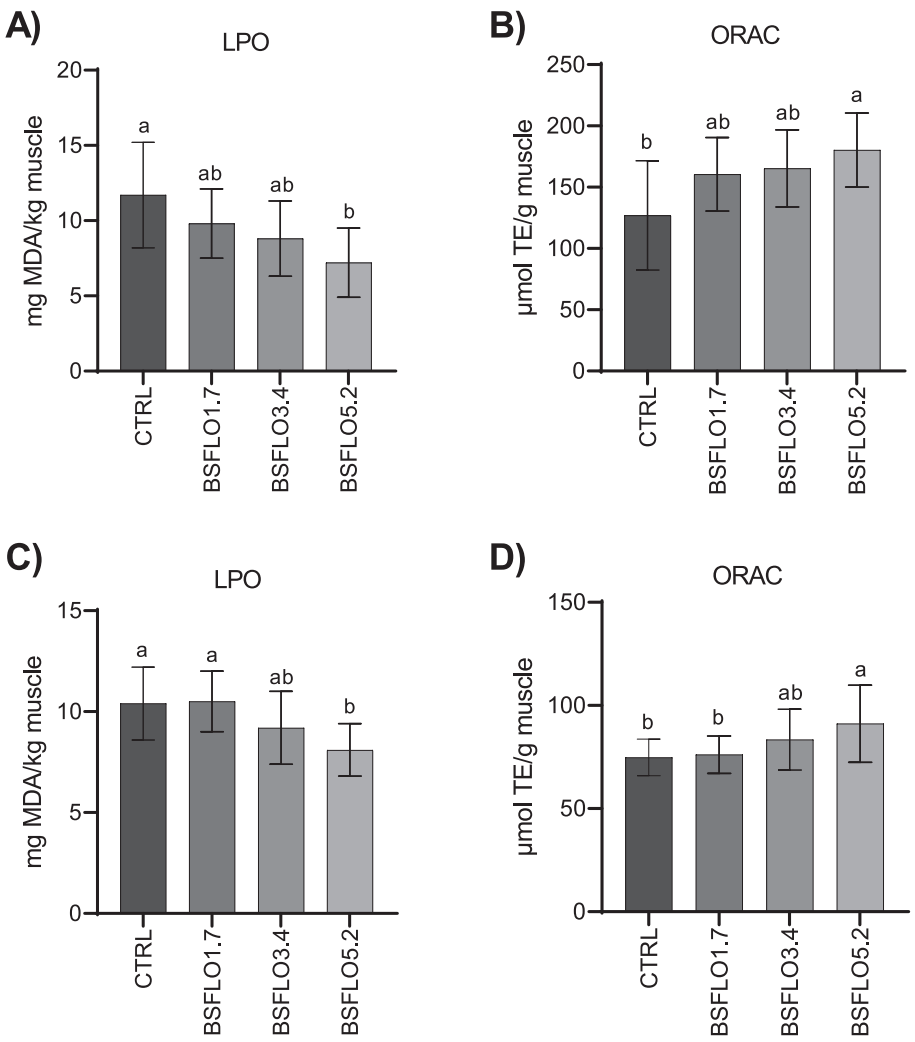

Fig. 1. Lipid peroxidation (LPO) and oxygen radical absorbance capacity (ORAC) of the muscle of European seabass (A-B) and gilthead seabream (C-D). Data expressed as means values ± standard deviation. Different superscript letters represent significant differences among dietary treatments ($p < 0.05$; one-way ANOVA followed by Duncan's post-hoc test). MDA, malondialdehyde; TE, Trolox equivalents.

increased linearly with dietary BSFLO level, the same was not presently observed. In salmonid juveniles, the inclusion of 12–16% BSFLO as a total replacement for VO for 56–70 days has also been reported to be feasible, without negative impacts on fish growth or FCR (Belghit et al., 2018; Fawole et al., 2021). However, when FO is the lipid source being replaced, the results reported in the literature are highly variable. For instance, studies on totoaba (*Totoaba macdonaldi*) have reported a reduced growth when BSFLO replaced more than 60% of FO, with inclusion levels ranging from 4% to 9%, even in diets containing high levels (45%) of fishmeal (Maldonado-Othón et al., 2022). In contrast, in rainbow trout, a 16% inclusion of BSFLO as a complete substitute for FO in diets containing 24% fishmeal (the single source of EPA and DHA) did not compromise growth (Fawole et al., 2021). Similarly, 8% inclusion of BSFLO as a complete replacement for FO in diets containing 35% fishmeal (again the unique source of EPA and DHA) did not negatively affect growth in largemouth bass (*Micropterus salmoides*) (Xia et al., 2024). Totoaba is a marine species with a very limited capability of synthesizing n-3 LC-PUFA from shorter-chain FA precursors, in contrast to rainbow trout and largemouth bass, which are freshwater species capable of such synthesis (Monroig et al., 2018). Nevertheless, the assumption that BSFLO can effectively replace FO in diets for freshwater fish is challenged by previous studies (Bakar et al., 2021), reporting reduced growth in hybrid tilapia (*Oreochromis* sp.) when BSFLO replaced more than 60% of FO, with inclusion levels ranging from 4% to 9%, even in diets containing high levels (45–47%) of fishmeal. Altogether, these findings highlight that growth responses to BSFLO depend on the lipid source being replaced and on whether dietary EPA and DHA requirements are met.

Somatic indices are widely employed as markers of fish nutritional and metabolic status. HSI, reflecting the liver-to-body weight ratio, offers insights into hepatic metabolic activity and adaptive responses to dietary shifts due to its involvement in lipid and energy storage mechanisms (Villasante et al., 2025). VSI provides information on visceral energy reserves and digestive function, encompassing aspects such us enzyme synthesis and nutrient absorption (Ighwela et al., 2014). In the present study, the dietary substitution of PO with BSFLO did not significantly affect HSI in either seabass or seabream, suggesting that it neither promoted nor hindered fat accumulation in the liver. On the other hand, VSI was similar in seabass fed BSFLO diets and those fed the CTRL diet, whereas in seabream this somatic index decreased with the dietary replacement of PO by BSFLO, suggesting species-specific shifts in lipids and energy utilisation. Interestingly, Li et al. (2016) also observed that replacing 75–100% of soybean oil with BSFLO (18.8–25% inclusion level) reduced intraperitoneal fat deposition and VSI in juvenile Jian carp, while HSI remained unaffected.

It is well known that the dietary FA profile plays a crucial role in shaping the FA composition of fish muscle, as dietary lipids are not only key energy sources but also major contributors to tissue lipid deposition

(Qin et al., 2022; Xu et al., 2020b). Accordingly, the progressive increase in dietary SFA associated with the substitution of PO by BSFLO was mirrored by a higher deposition of these FA in the muscle of both seabass and seabream. This pattern has been reported in various fish species, such as gilthead seabream (Moutinho et al., 2023), Jian carp (Li et al., 2016), mirror carp (Xu et al., 2020a), or striped catfish (Sudha et al., 2022), where higher dietary inclusion levels of BSFLO (2.3–25%), at the expense of VO or FO (75–100%), led to increased deposition of SFA in fish muscle. In the present study, the n-3/n-6 ratio increased significantly in the muscle of both seabass and seabream fed the BSFLO5.2 diet, both in absolute terms and when expressed as % of total FAME, mirroring the fatty acid profile of the experimental diets, as commonly observed for C_{18} fatty acids in marine fish, which are readily deposited in muscle due to the limited capacity of these species to endogenously convert these precursors into n-3 LC-PUFA (Tocher, 2010; Du et al., 2025). Although the decrease in LA was not evident in the absolute levels of this fatty acid in muscle, the relative fatty acid profile (% of total FAME) of both species showed a significant reduction in LA, suggesting that the increase in the n-3/n-6 ratio was primarily driven by the decrease in n-6 fatty acids, particularly LA, rather than by an increase in n-3 fatty acids. However, the underlying mechanisms that may explain these changes appear to differ between species. In seabass muscle, fish fed BSFLO5.2 showed significantly higher levels of stearidonic acid (SDA; 18:4n-3) and a slight increase in EPA compared with those fed the CTRL diet. This suggests that the lower availability of dietary n-6 fatty acids may have reduced competition between n-6 and n-3 substrates for shared desaturation and elongation enzymes, particularly Fads2/Δ6-desaturase, potentially favouring the progression of the n-3 biosynthetic pathway from ALA towards longer-chain intermediates (i.e., SDA) and EPA. Similar increases in Δ6-desaturation products have been reported in European seabass juveniles when dietary FO was replaced by alternative lipid sources (Torrecillas et al., 2017). Nevertheless, evidence supporting enhanced endogenous conversion of C_{18} PUFA into longer-chain n-3 fatty acids in marine fish remains inconsistent. For instance, Houston et al. (2024) did not observe a clear stimulation of the n-3 biosynthetic pathway in seabass fed diets with alternative lipid sources. Taken together, these findings suggest a potential shift towards the n-3 metabolic pathway in seabass fed the BSFLO5.2 diet. In contrast, in seabream a reduction in muscle ARA content was observed in fish fed the BSFLO5.2 diet compared to CTRL, suggesting that the lower dietary supply of LA may have more directly constrained the n-6 pathway. Interestingly, Moutinho et al. (2024) reported alterations in the expression of *elovl1b* and *elovl6* in the liver of seabream fed diets containing BSFLO, suggesting that insect-derived lipids may influence fatty acid elongation pathways in this species. Although hepatic gene expression was not assessed in the present study, these findings may indicate that dietary insect oils modulate different steps of LC-PUFA metabolism in seabream and seabass, potentially contributing to the species-specific responses observed here. Moreover, these contrasting responses are consistent with previous studies indicating that seabass and seabream may differ in their metabolic responses to variations in dietary fatty acid composition (Houston et al., 2017; Houston et al., 2022; Monroig et al., 2022; Houston et al., 2024). However, as the present study did not assess the expression of genes or the activity of enzymes involved in fatty acid metabolism, the proposed mechanisms should be regarded as putative and require further validation through studies integrating molecular and enzymatic analyses of LC-PUFA biosynthesis. Overall, despite species-specific responses, full substitution of PO with 5.2% BSFLO appears to be an effective approach to improve the muscle n-3/n-6 PUFA ratio in both seabass and seabream. Similarly, Li et al. (2016) reported that replacing 25–100% of dietary soybean oil with 0.6–2.5% BSFLO in diets completely devoid of FO - where fishmeal (quantity not specified) was the unique source of EPA and DHA - resulted in an increased n-3/n-6 ratio in the muscle of Jian carp, mainly driven by a reduction in LA and an increase in DHA.

In the present study, both seabass and seabream exhibited comparable patterns in muscle oxidative status, with changes in lipid peroxidation and antioxidant capacity suggesting a similar physiological response to this dietary lipid source (Fig. 1). The observed reduction in muscle lipid peroxidation and increase in antioxidant capacity in fish fed BSFLO5.2 may be explained by the possible contribution of bioactive compounds present in BSFLO. The BSFLO used in this study was obtained from larvae reared on a substrate containing 80% olive pomace, a by-product of olive oil production generally rich in tocopherols and polyphenolic compounds with strong antioxidant properties (Nunes et al., 2018; Nunes et al., 2021). Moreover, previous studies have shown that BSF larvae reared on such substrates are able to bioaccumulate these compounds in their lipid fraction (Morand-Laffargue et al., 2023; Rodríguez-González et al., 2025). Therefore, the enhanced antioxidant capacity observed in the muscle of seabass and seabream fed the BSFLO5.2 diet may be attributed, at least partially, to the presence of these bioactive compounds in the dietary oil. Moutinho et al. (2024) has also reported a decrease in muscle lipid peroxidation in seabream juveniles fed diets containing 4–9.5% BSFLO, replacing 42–100% substitution of a VO blend. Moreover, in vitro studies have shown that BSFLO has strong free radical scavenging and lipid peroxidation inhibition properties. Specifically, Phongpradist et al. (2023) reported that BSFLO showed higher DPPH radical scavenging activity (96.61%) than several common oils, including rice bran oil, olive oil, and krill oil. Accordingly, Srisuksai et al. (2024) and Suryati et al. (2023) reported that BSFLO exhibited strong free radical scavenging activities, properties that were attributed to BSFLO high content of tocopherols, and phenolic compounds.

5. Conclusion

In conclusion, provided that dietary macronutrients are properly balanced, totally replacing PO by BSFLO (included at 5.2%) can enhance growth performance and feed efficiency in both European seabass and gilthead seabream juveniles, with more pronounced effects in seabass. The full replacement of PO by BSFLO improved the muscle n-3/n-6 LC-PUFA ratio in juveniles of both species, enhanced antioxidant capacity and reduced lipid peroxidation. These findings open avenues for a potentially improved product stability and shelf-life. However, as these outcomes were not assessed in the present study, such implications remain hypothetical and require dedicated experimental validation in market-sized fish.

CRediT authorship contribution statement

Daniel Acebes: Writing – original draft, Visualization, Validation, Methodology, Formal analysis. **Paula Canada:** Writing – review & editing, Validation, Methodology, Investigation. **Tiago Sá:** Writing – review & editing, Validation, Investigation. **Andreia F. Silva:** Writing – review & editing, Validation, Methodology. **Sara Magalhães:** Writing – review & editing, Resources, Conceptualization. **Daniel Murta:** Writing – review & editing, Resources, Conceptualization. **Ricardo Sousa:** Resources. **Luisa M.P. Valente:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Ana Basto:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2026.744288.

Data availability

Data will be made available on request.

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