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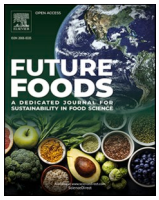

Commercial sea cage farming assessment of sustainable diets on growth performance and fillet quality of gilthead sea bream and European sea bass

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ABSTRACT

The growing need for sustainable aquafeeds has driven the search for alternatives to marine-derived ingredients. This study evaluated the effects of novel diets including poultry by-product meal and *Hermetia illucens* (black soldier fly) larvae meal on growth performance, fillet yield, proximate composition, mineral profile, and fatty acid composition of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) reared under commercial sea-cage farming conditions. In gilthead sea bream, the test diet led to a significantly higher final body weight, specific growth rate, improved feed conversion ratio, and greater fillet yield, along with elevated levels of n-3 polyunsaturated fatty acids. In contrast, European sea bass exhibited similar growth performance across diets, but fish fed the test diet containing farmed salmon oil as the primary lipid source, had lower EPA and DHA content and higher saturated fat levels. Still, fillet yield was higher, and fatty acid profiles across both species remained within the recommended limits for human consumption. These findings demonstrate that alternative feeds based on terrestrial ingredients can sustain or enhance performance and fillet quality in Mediterranean aquaculture species, supporting broader efforts toward environmental sustainability and human nutrition through the maintenance of beneficial n-3 polyunsaturated fatty acids levels.

1. Introduction

In line with the European Green Deal launched by the European Commission (EC, 2019), which presents a comprehensive strategy to achieve environmentally sustainable economic growth in Europe through the implementation of the Circular Economy Action Plan (CEAP), new-generation feeds for mariculture are facing the challenge of transitioning to diets that incorporate substantial levels of new, recycled, or underexploited ingredients. These ingredients must complement conventional protein and lipid sources while minimising or eliminating the use of ocean fishing derivatives such as fishmeal and fish oil (Naylor et al., 2021; Tacon and Metian, 2015). The pressing issue of sustainability is driving research and supply chain companies to explore food matrices derived from the lowest trophic levels and those that align with the principles of the circular bioeconomy (Cashion et al., 2017). In this context, increasing attention has been directed towards processed animal proteins (PAT), including those derived from insects, which fully comply with sustainability and circularity principles (Hancz et al., 2024; Woodgate et al., 2022).

Processed animal proteins, particularly those derived from poultry by-products and insects, have emerged as promising alternatives to traditional fishmeal due to their high protein content, balanced amino acid profile, and digestibility. Poultry by-product meal (PBM) is a well-established ingredient in aquafeeds, supplying essential nutrients such as lysine and methionine, which are vital for fish growth and metabolic processes (Kleijn et al., 2018). Insect meals, especially from *Hermetia*

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illucens, are rich in high-quality proteins, essential amino acids, and bioactive compounds that support gut health and immune function in fish (Gasco et al., 2018; Henry et al., 2015). Furthermore, insect meal production offers significant environmental benefits, as it efficiently utilises organic waste streams while requiring minimal land and water resources, making it a sustainable option for aquaculture (Van Huis, 2020). However, the expansion of the insect farming industry in the EU is slowed by strict regulations, as insects must be approved as novel foods and undergo safety assessments by the European Food Safety Authority (EFSA) before being marketed (Heath et al., 2024). As a result, the adoption of insect meal in European aquaculture remains limited, primarily due to its higher cost and limited availability compared to conventional feed ingredients, such as fishmeal and soybean meal (Biteau et al., 2024).

Previous studies have demonstrated that insect meal combined with PBM serves as a sustainable alternative to fishmeal without adversely affecting the growth performance or intestinal health of Mediterranean aquaculture species, such as gilthead sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) (Anedda et al., 2023; Bušelić et al., 2025; Gasco et al., 2018; Pleić et al., 2022; Randazzo et al., 2023; Rimoldi et al., 2024). Although laboratory-scale studies support their use, evidence from commercial-scale operations remains limited, particularly for Mediterranean fish species farmed in net pens.

Croatia is a significant producer of farmed fish in the Adriatic region, with aquaculture production reaching 27,156 metric tonnes in 2022 and an annual growth rate of 6.4 % (FAO, 2024). The industry is dominated by gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), primarily cultured in floating sea cages. A substantial share of this production is exported to international markets. The continued expansion of Croatian mariculture is driven by the country's highly indented coastline, favourable oceanographic conditions, high-quality seawater, and geographic proximity to major markets. As the sector grows, the demand for sustainable feed alternatives becomes increasingly critical to ensure long-term environmental and economic viability.

The aim of this study was to evaluate the performance of newly formulated diets incorporating poultry by-product meal and *Hermetia illucens* meal, in comparison with standard commercial feeds, for European sea bass and gilthead sea bream. The trials were conducted under practical farming conditions in floating sea cages along the Croatian coastline, where mariculture is a strategically important sector of the national economy. The novel diets were formulated based on findings from previous small-scale experimental studies (Pleić et al., 2022; Randazzo et al. 2021).

This study assessed the growth performance, feed conversion ratio, and fillet quality of fish fed alternative diet formulations. By implementing trials under real-world mariculture conditions, the research bridges the gap between controlled experimental studies and commercial aquaculture practice, providing robust and applicable data on fish performance. The results contribute to the broader effort to reduce dependence on fishmeal and fish oil, support global sustainability objectives, and strengthen the resilience of Mediterranean aquaculture in the face of emerging environmental and economic challenges.

2. Materials and methods

2.1. Diet selection, experimental design and sample collection

For the purpose of diet selection, the study included two commercial and two specially formulated marine aquafeeds. Commercial diets consisted of a gilthead sea bream-specific feed (COM1: 4.5 mm extruded pellet, ForeSea, Naturalleva) and a European sea bass-specific feed (COM2: 4 mm OptiBass, Skretting Ltd). Due to proprietary constraints, only the ingredient lists for the commercial diets were available (Table 1).

The formulated diets, AAN1 for gilthead sea bream and AAN2 for European sea bass, were developed based on findings from previous small-scale experimental studies (Pleić et al., 2022; Randazzo et al. 2021). These diets were produced by the same companies as the commercial feeds, using standard commercial feed ingredients. The diets were formulated to be isoproteic and isolipidic relative to the commercial diets. While protein and lipid levels were closely matched in the gilthead seabream diets, the experimental diet for European sea bass showed a slight reduction in crude protein (~2 % lower) and an increase in crude fat (~2 % higher) compared to the commercial feed (Table 1). Both new formulations featured a high inclusion level of poultry by-product meal (<19 %), a moderate level of insect meal derived from *Hermetia illucens* (<6 %), and a reduced content of marine-derived proteins (5 % in AAN1 and 16 % in AAN2). The difference in the marine-derived protein levels reflected species-specific nutritional requirements, as sea bass generally requires higher fishmeal to meet essential amino acid needs (National Research Council, 2011; Webster and Lim, 2002). Additionally, 30 % of the total lipid content in both diets was derived from plant-based sources (Table 1).

Feeding trials were conducted at two commercial sea cage finfish farms: Farm1, located in the central part of the eastern Adriatic coast (geographical coordinates: 43.509720 N, 15.960778 E), was involved in the gilthead sea bream trial, while Farm2, situated in the northern part of the Adriatic Sea (45.021703 N, 14.350530 E), conducted the trial for European sea bass (Additional file 1: Fig. S1a). Subadult fish were stocked at commercial densities, and each dietary treatment was tested in two replicate sea cages per farm (n = 2), with daily monitoring of water temperature and dissolved oxygen (Additional file 1: Fig. S1b and

Table 1
Ingredients and proximate composition (%) of commercial feeds (COM1 and COM2) and test feeds (AAN1 and AAN2) used in feeding trials with gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). For commercial feeds, only the ingredient list is provided.

	Gilthead seabream feed		European sea bass feed	
Ingredient composition	COM1	AAN1	COM2	AAN2
Poultry by-product meal[1]	+	19.0	+	20.0
Insect meal BSF (Black Soldier Fly)[2]		7.6		6.0
Soybean Meal Non-GMO 48 % CP	+	12.0	+	20.0
Soy protein concentrate		6.0		
Whey protein concentrate	+		+	
Sunflower meal 36 % CP			+	4.2
Rapeseed meal Non-GMO 30 % CP	+	4.0	+	2.0
Maize Gluten Meal Non-GMO 57 % CP		5.0	+	1.8
Whole peas		10.3		
Fish meal GQH max 65 % CP[3]	+	3.0	+	9.0
Fish meal GQL basis 60 % CP			+	6.0
Squid/Krill meal		2.0		1.0
Swine Blood meal	+		+	
Fish oils crude low[4]	+	10.0		3.9
Salmon Oil Farmed Crude Low			+	10.7
Rapeseed oil Hydrogenated HEA Non-GMO	+	3.6	+	0.2
Soybean oil Degummed Non-GMO			+	6.2
Camelina oil		1.0		
Wheat feed grade	+	10.4	+	7.7
Wheat gluten		5.0		
Premixes	+	1.1	+	1.3
Proximate composition				
Dry Matter	92.2	93.7	94.2	94.1
Crude Protein	44.1	44.3	41.1	43.4
Crude fat	21.4	21.5	23.4	21.3
Ash	6.7	7.2	9.6	7.4
Crude Fibre	2.2	2.8	3.5	3.0

[1] Poultry by-product meal from Azienda Agricola Tre Valli; Verona, Italy (CP, 65.6 %; CF, 14.8 % as fed).

[2] ProteinX™, Protix, Dongen, The Netherlands (CP, 55.4 %; CF, 20.8 % as fed).

[3,4] For COM diets, the estimated inclusion levels were ~20–30 % fish meal and ~8–15 % fish oil (Pelusio et al., 2022; Fernandes et al., 2024). CP – crude protein; CF – crude fibre.

Fig. S1c). All experiments involving animals in this study were conducted in accordance with the laboratory animal management principle of Croatia. All experimental protocols were approved by the Ethics Committee of the Institute of Oceanography and Fisheries (approval no 134/2/2018).

Gilthead seabream feeding trial at Farm1. Four square sea-cages, each with a volume of 125 m^3, were stocked with 4000 subadult fish to a 14.5 kg/m^3 final stocking density. The initial mean body weight (± SD) was 122.85 ± 22.3 g, with no significant differences among cages ($F(3, 96) = 1.60$, $P = 0.19$). Each diet (COM1, AAN1) was assigned to two cages. The trial began in May 2020 and lasted 30 weeks with monthly weighing (to the nearest 1.0 g) and measuring (to the nearest 0.1 cm) of a minimum of 30 fish per cage. Fish were manually fed at 1.2 % of biomass daily, six days per week.

European sea bass feeding trial at Farm2. Four circular sea-cages, each with a volume of 4000 m^3, were stocked with 128,000 subadult fish to a final stocking density of 11 kg/m^3. The initial mean body weight (± SD) was 212.32 ± 25.2 g, with no significant differences among cages ($F(3, 116) = 1.53$, $P = 0.2$). Each diet (COM2, AAN2) was assigned to two cages. The trial began in July 2020 and lasted 25 weeks with monthly weighing and measuring of a minimum of 30 fish per cage. Fish were fed 1.2 % of biomass daily, six days per week in July and August, five days per week in September to November, and four days per week in December and January, using automatic feeders.

For monthly biometric monitoring, as well as at the beginning and end of the feeding trials, 30 individuals per species and per cage were euthanised using an overdose of MS-222 (500 mg/L; Sigma-Aldrich, Saint Louis, MO, USA) for weighing and biometric analysis. Additionally, six individuals from each cage were immediately stored at −20 °C for subsequent chemical composition analysis.

2.2. Fish biometric indexes

For each species and dietary treatment, indicators of growth and body performance were calculated as follows: specific growth rate (SGR) = ((ln (final weight) – ln (initial weight)) × 100/number of feeding days); feed conversion ratio (FCR) = (((final weight – initial weight) × 1000)/(initial + final weight)/days)/dry feed intake; condition index (CI) = ((fish weight × 100)/(fish total lengthˆ3)). After dissection, each fish was filleted, and the mass of fillets, gastrointestinal tract, liver, and gonads was recorded. The following biometric indexes were then calculated: dressing index (DI) = (fillet weight/total fish weight) × 100; digestosomatic index (DGI) = (gastrointestinal tract weight / total fish weight) × 100; hepatosomatic index (HSI) = (liver weight / total fish weight) × 100; gonadosomatic index (GI) = (gonads weight / total fish weight) × 100.

2.3. Determination of chemical composition, fatty acid profile and mineral content

The test and commercial diets, and with fillet muscle tissue, were analysed to determine the following chemical parameters: water, total proteins, fat and ash by mean of standard and internal analytical methods. Determination of water and ash content was carried out by gravimetric methods according to ISO 1442:1997 and ISO 936:1998 standards by mean of a drying oven (UF75 plus, Memmert, Germany) and burning furnace (Program Controller LV 9/11/P320, Nabertherm, Germany). The crude fat content was determined by Soxhlet method (HRN ISO 1443:1999) which includes steps of fat hydrolysis followed by fat extraction with petrol ether by mean of an extraction device (Soxtherm 2000, Gerhardt, Germany). Extracted fat was used for fatty acid methyl ester preparation according to ISO 12,966–2: 2015 and ISO 12,966–4:2015 standards, using methanolic potassium hydroxide solution for trans methylation, with minor modifications of the procedure described briefly earlier (Vulić et al., 2021). The protein content was determined according to HRN ISO 937:1999 standard, using the Kjeldahl method, which involves destruction of the sample in a destruction block (Unit 8 Basic, Foss) followed by the distillation and titration in an automatic titration and distillation unit (Vapodest 50 s, Gerhardt, Germany). The in-house Atomic Absorption Spectroscopy (AAS) method was used for mineral content determination. Briefly, the sample was digested in the presence of nitric acid and hydrogen peroxide in a microwave oven (Ethos easy, Milestone, Italy). Samples were then diluted with ultrapure water in a volumetric flask followed by measurement of sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe) by flame AAS (200 Series A4 with SPS 4 Autosampler, Agilent Technologies, USA). For each mineral, an HC coded lamp specific for the given mineral (Agilent Technologies, USA) was used, according to a previously described procedure (Kudumija et al., 2024). The complete list of chemicals, standards, and reagents used in the present study is provided in Additional File 1.

2.4. Determination of lipid quality indexes

The lipid quality indexes, atherogenicity index (AI), thrombogenicity index (TI), and the ratio of hypocholesterolemic and hypercholesterolemic fatty acids (HH) were calculated according to the following equations, given by Ulbritcth and Southgate (1991) and Pleadin et al. (2019):

AI = (C12:0 + 4*(C14:0) + C16:0) / MUFA+PUFA;

TI = C14:0 + C16:0 + C18:0 / (0.5*MUFA + 0.5*n6PUFA + 3*n3PUFA + n3/n6);

HH = C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3 / C14:0 + C16:0;

where MUFA and PUFA stands for monounsaturated and polyunsaturated fatty acids, respectively.

2.5. Statistical analysis

Statistical analyses were performed using the SPSS Statistics Software 22.0 (IBM, New York, NY, USA). Since preliminary growth analysis showed no significant differences between replicate cages within the same diet group, data were pooled to increase sample robustness and statistical power. Differences between dietary treatments were assessed using one-way analysis of variance (ANOVA), with the Kruskal–Wallis H-test applied when normality or homoscedasticity assumptions were not met after transformation. Statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Growth performance

3.1.1. Gilthead sea bream trial

Fish initially accepted the test diet. At the end of the 30-week experiment, the final body weight of fish had nearly tripled compared to the initial weight (Table 2, Additional file 1: Fig. S2a). Both dietary treatments resulted in substantial growth, with fish in the AAN1 group showing a higher final body weight, total length, and specific growth rate compared to those fed the commercial diet (COM1). A significantly lower feed conversion ratio (FCR) was observed in the AAN1 group, indicating improved feed efficiency. Fillet weight and dressing index were also significantly higher in fish fed the test diet, suggesting better fillet yield. Although no significant differences were observed in the hepatosomatic (HSI), digestosomatic (DGI), or gonadosomatic (GSI) indices, a trend toward higher liver and gonad weights was noted in the COM1 group.

3.1.2. European sea bass trial

As shown in Table 3, European sea bass fed the test diet (AAN2)

Table 2
Biometric parameters and indices of gilthead sea bream fed the test diet (AAN1) or commercial-diet (COM1) over 30 weeks. Values are expressed as the pooled mean ± SD of two cages per feed type.

	Initial	COM1 Final	AAN1 Final
BW (g)	134.01 ± 16.70	381.88 ± 46.42	450.21 ± 46.30
TL (cm)	19.03 ± 0.85	26.50 ± 0.85	27.20 ± 0.90
CI	1.94 ± 0.05	2.05 ± 0.19	2.24 ± 0.11
SGR		0.52 ± 0.01	0.57 ± 0.01
FCR		1.89 ± 0.04[a]	1.71 ± 0.03[b]
Fillet weight (g)	63.75 ± 9.14	167.85 ± 29.13[a]	220.96 ± 33.72[b]
GIT weight (g)	5.06 ± 1.62	11.46 ± 1.61	11.08 ± 2.96
Liver weight (g)	2.26 ± 0.97	9.73 ± 2.38	10.17 ± 2.00
Gonad weight (g)	–	10.24 ± 1.31	10.17 ± 1.00
DI	47.48 ± 0.93	43.82 ± 3.42[a]	48.87 ± 3.29[b]
DGI	3.74 ± 0.87	3.02 ± 0.47	2.44 ± 0.20
HSI	1.70 ± 0.79	2.53 ± 0.41	2.25 ± 0.07
GSI	–	2.72 ± 0.39	2.27 ± 0.26

BW, body weight; TL, total length; CI, condition index; SGR, specific growth rate; FCR, feed conversion ratio; GIT, gastrointestinal tract; DI, dressing index; DGI, digestosomatic index; HSI, hepatosomatic index; GSI, gonadosomatic index. Row means of test and commercial diet groups indicated with different superscript letters are significantly different ($P < 0.05$).

Table 3
Biometric parameters and indexes of European sea bass fed the test diet (AAN2) or commercial-diet (COM2) over 25 weeks. Values are expressed as mean ± SD of two cages per feed type.

	Initial	COM2 Final	AAN2 Final
BW (g)	211.61 ± 33.87	357.59 ± 25.71	341.75 ± 37.92
TL (cm)	22.77 ± 1.34	29.65 ± 1.52	29.88 ± 1.08
CI	1.78 ± 0.12	1.39 ± 0.21	1.28 ± 0.05
SGR		0.24 ± 0.01	0.26 ± 0.01
FCR		1.97 ± 0.04	1.85 ± 0.03
Fillet weight (g)	93.83 ± 11.35	176.40 ± 25.14	177.16 ± 18.60
GIT weight (g)	7.52 ± 3.31	8.55 ± 1.64	9.56 ± 2.99
Liver weight (g)	4.59 ± 1.52	9.46 ± 2.39[a]	7.24 ± 2.56[b]
Gonads weight (g)	0.22 ± 0.30	9.25 ± 5.09	10.33 ± 1.76
DI	44.86 ± 3.59	49.23 ± 5.19[a]	51.90 ± 1.93[b]
DGI	3.48 ± 1.20	2.40 ± 0.49	2.80 ± 0.81
HSI	2.21. ± 0.82	2.62 ± 0.51	2.10 ± 0.63
GSI	0.12 ± 0.19	2.54 ± 1.37[a]	3.02 ± 0.32[b]

BW, body weight; TL, total length; CI, condition index; SGR, specific growth rate; FCR, feed conversion ratio; GIT, gastrointestinal tract; DI, dressing index; DGI, digestosomatic index; HSI, hepatosomatic index; GSI, gonadosomatic index. Row means of test and commercial diet groups indicated with different superscript letters are significantly different ($P < 0.05$).

demonstrated growth performance comparable to the COM2 group, with no significant differences in final body weight, total length, SGR, or FCR (Additional file 1: Fig. S2b). The reduction in condition index (CI) observed in both groups may be attributed to a seasonal temperature decline recorded at Farm2 in the northern Adriatic, as final sampling was performed at the end of December (Additional file 1: Fig. S1c). Fillet yield, expressed as the dressing index (DI), and GSI were significantly higher in the AAN2 group, likely reflecting unbalanced sex ratios. Although liver weight was significantly greater in the COM2 group, the hepatosomatic index (HSI) did not differ, indicating that the variation was due to overall body size rather than dietary effect.

3.2. Nutritional characterisation of gilthead sea bream and european sea bass fillets

The nutritional composition and mineral content of gilthead sea bream and European sea bass fillets are presented in Table 4. In gilthead sea bream, fillets from the AAN1 group showed significantly lower iron and zinc concentrations compared to the commercial diet group ($P <$

Table 4
Nutritional composition, mineral content and fat quality indices of gilthead sea bream and European sea bass fillets from the test (AAN) and commercial (COM) diet groups. Results represent the mean ± SD of six individual samples per treatment (total of 12 fillets).

	Gilthead sea bream		European sea bass	
	COM - 1	AAN - 1	COM - 2	AAN - 2
Water (g/100 g)	67.1 ± 0.8	60.6 ± 0.3	69.2 ± 1.1	69.5 ± 0.8
Protein (g/100 g)	20.8 ± 1.3	20.1 ± 0.4	19.8 ± 0.3	19.5 ± 0.6
Ash (g/100 g)	1.4 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Fat (g/100 g)	10.8 ± 1.8	12.6 ± 0.3	10.7 ± 1.1	10.1 ± 1.3
Mineral content				
Sodium (mg/kg)	535.8 ± 70.7	513.7 ± 34.2	544.8 ± 58.2	534.7 ± 61.4
Calcium (mg/kg)	278.3 ± 2.8	242.6 ± 27	538.6 ± 331.1	368.3 ± 164.3
Phosphorus (mg/kg)	2075.9 ± 92.6	2089.6 ± 47.6	2108.2 ± 79.8	2101.3 ± 51.5
Potassium (mg/kg)	4426.5 ± 517.9	4045.2 ± 218.4	4601.8 ± 193.5	4499.2 ± 254.1
Copper (mg/kg)	3 ± 0.3	1.1 ± 0.1	1.1 ± 0.3	1.1 ± 0.2
Iron (mg/kg)	9.7 ± 2.5[b]	6.9 ± 0.6[a]	8.7 ± 1.7	9.2 ± 2.1
Zinc (mg/kg)	11.8 ± 2.1[b]	8.1 ± 0.7[a]	13.45 ± 1.58	12.93 ± 3.2
Magnesium (mg/kg)	252.3 ± 22.5	250.7 ± 7.9	329.8 ± 42.3	317.7 ± 13.2
Fat quality indices				
AI	0.28 ± 0.13	0.36 ± 0.02	0.35 ± 0.01[a]	0.40 ± 0.02[b]
TI	0.32 ± 0.10	0.30 ± 0.02	0.39 ± 0.02[a]	0.48 ± 0.04[b]
HH	4.58 ± 1.44	3.46 ± 0.16	3.16 ± 0.04[b]	2.95 ± 0.12[a]

AI, atherogenic index; TI, thrombogenic index; HH, hypo-/hyper-cholesterolemic fatty acid ratio. For each species, row means of test and commercial diet groups indicated with different superscript letters are significantly different ($P < 0.05$).

0.05). For all other proximate composition parameters, no significant differences were detected between diet groups, although a slight increase in fillet fat content was observed in the AAN1 group. In European sea bass, no significant differences were found between diet groups for proximate composition or mineral content. However, fat quality indexes (AI, TI, and HH) showed significant differences, with AAN2 fillets exhibiting higher atherogenic and thrombogenic indexes and a lower hypo-/hypercholesterolemic fatty acid ratio compared to the commercial group ($P < 0.05$), reflecting subtle dietary influences on lipid health indices.

3.3. Fatty acid profile of diets and gilthead sea bream and european sea bass fillets

The fatty acid composition of gilthead sea bream and European sea bass fillets, and their respective diets, is presented in Table 5 (see Additional file: Table S1-S2, Figure S3). In gilthead sea bream feeds, the AAN 1 diet contained higher proportions of n-3 PUFA, including EPA (C20:5n-3) and DHA (C22:6n-3), compared to the COM 1 diet. This dietary difference was reflected in the fillet composition, with AAN1-fed fish displaying a notably higher total n-3 PUFA content. Despite this, EPA and DHA levels in fillets were lower than in the diets, indicating partial retention efficiency and likely oxidative utilisation of these fatty acids. In European sea bass, this trend was reversed. The COM2 diet had higher EPA and DHA contents than AAN2, resulting in higher n-3 PUFA deposition in fillets from the COM2 group. Fish fed AAN2 exhibited a reduction in EPA and DHA levels and a lower total n-3 PUFA content, consistent with the dietary formulation. Saturated fatty acids (SFA), particularly palmitic acid (C16:0), were higher in fillets from both species fed AAN diets. This likely reflects a higher retention of SFA and limited use of dietary PUFA for structural lipid deposition. Total monounsaturated fatty acids (MUFA), mainly oleic acid (C18:1n-9), remained consistently high across all groups, showing minimal dietary influence. The n-6 PUFA content, predominantly linoleic acid (C18:2n-

Table 5
Fatty acid profiles (% of total fatty acids) of test diets and of fillets from gilthead sea bream and European sea bass fed test (AAN) and commercial (COM) diets. Diets were analysed in replicate; fillet data are presented as mean ± SD of six individual samples per treatment ($n = 12$).

	Gilthead seabream				European seabass			
	COM1 feed	COM1 Fish fillet	AAN 1	AAN1 Fish fillet	COM2 feed	COM2 Fish fillet	AAN2	AAN2 Fish fillet
C12:0	ND	0.1 ± 0.0	1.8 ± 0.0	1.0 ± 0.0	ND	ND	1.5 ± 0.0	0.5 ± 0.1
C14:0	0.8 ± 0.0	2.1 ± 0.2	3.1 ± 0.0	3.0 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.1 ± 0.0	2.6 ± 0.1
C16:0	9.6 ± 0.1	12.3 ± 0.7	11.3 ± 0.0	13.7 ± 0.4	11.3 ± 0.4	16.6 ± 0.7	10.8 ± 0.1	17.9 ± 0.5
C18:0	3.4 ± 0.0	3.1 ± 0.2	2.7 ± 0.0	3.0 ± 0.1	3.2 ± 0.1	4.4 ± 0.2	3.4 ± 0.0	5.0 ± 0.4
Total SFA	15.2 ± 1.0	20.3 ± 6.9	20.9 ± 1.8	23.1 ± 0.7	19.1 ± 0.9	25 ± 0.3[a]	19.7 ± 0.2	26.2 ± 0.9[b]
C18:1n-7	2.5 ± 0.0	2.8 ± 0.1	2.1 ± 0.0	2.5 ± 0.1	2.1 ± 0.1	2.8 ± 0.1	2.0 ± 0.0	2.7 ± 0.1
C18:1n-9	43.1 ± 0.2	44.0 ± 1.0	28.9 ± 0.3	34.9 ± 0.8	30.5 ± 0.5	31.7 ± 1.1	31.2 ± 0.0	34.4 ± 1.1
C20:1n-9	1.4 ± 0.1	2.4 ± 0.2	3.3 ± 0.0	3.1 ± 0.1	1.7 ± 0.2	2.3 ± 0.2	1.9 ± 0.0	2.6 ± 0.1
C22:1	0.8 ± 0.1	0.9 ± 0.1	3.7 ± 0.0	1.5 ± 0.1	1.5 ± 0.1	0.3 ± 0.4	1.7 ± 0.0	0.1 ± 0.1
Total MUFA	50.0 ± 1.0	47.5 ± 15.9	43.4 ± 1.2	45 ± 0.8	40.3 ± 1.1	39.4 ± 0.8[a]	40.8 ± 1.0	41.9 ± 1.8[b]
C18:2n6	23.8 ± 0.3	19.1 ± 1.1	12.6 ± 0.2	16.3 ± 0.5	20.3 ± 1.3	20.0 ± 1.3	22.0 ± 0.1	21.4 ± 0.9
C20:4n6	0.5 ± 0.0	0.2 ± 0.0	1.0 ± 0.0	0.2 ± 0.0	0.7 ± 0.0	0.3 ± 0.1	0.7 ± 0.0	ND
Total PUFAn-6	24.4 ± 1.2	22.4 ± 6.3	13.8 ± 1.2	17.7 ± 0.5	21.3 ± 0.8	24.5 ± 0.3[b]	23.0 ± 0.8	22.4 ± 1.2[a]
C18:3n3	6.8 ± 0.1	5.1 ± 0.7	6.2 ± 0.0	5.2 ± 0.3	5.6 ± 0.2	3.0 ± 0.5	5.9 ± 0.0	3.2 ± 0.3
C18:4n3	0.2 ± 0.1	0.3 ± 0.1	1.3 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.1	0.6 ± 0.0	ND
C20:5n3	1.0 ± 0.0	0.7 ± 0.2[a]	5.3 ± 0.0	1.4 ± 0.1[b]	4.2 ± 0.9	2.4 ± 0.3	3.0 ± 0.0	1.3 ± 0.4
C22:5n3	0.3 ± 0.0	0.2 ± 0.1	0.7 ± 0.0	0.1 ± 0.0	0.8 ± 0.0	0.2 ± 0.1	0.8 ± 0.0	ND
C22:6n3	1.8 ± 0.1	1.4 ± 0.3[a]	7.0 ± 0.1	2.9 ± 0.3[b]	6.6 ± 1.2	3.6 ± 0.9	4.8 ± 0.0	2.2 ± 0.5
Total PUFAn-3	10.2 ± 0.2	7.6 ± 2.1[a]	21.4 ± 0.3	11.1 ± 0.6[b]	18.7 ± 0.6	8.8 ± 0.9[b]	15.9 ± 0.4	6.8 ± 1.6[a]

[a-b], row values under the same fish species with different superscript letters differ significantly ($p < 0.05$)
ND – not detected.

6), was elevated in AAN2 diets and fillets, indicating a shift toward terrestrial lipid sources. Overall, fillet fatty acid profiles were closely aligned with dietary inputs, with modest species-specific differences in the retention and metabolism of individual fatty acids.

4. Discussion

Based on the available literature, the present study is the first to evaluate the performance and fillet quality of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) fed diets containing poultry by-product meal and *Hermetia illucens* insect meal under commercial net-pen farming conditions, thereby bridging the gap between previous experimental studies and practical farming applications. The results provide evidence that terrestrial-based protein ingredients can effectively replace marine-derived sources without compromising production performance or fillet nutritional quality, confirming the practical viability of these sustainable feed formulations.

Due to proprietary restrictions, only the ingredient lists were available for the commercial feeds (COM1 and COM2). Nevertheless, based on typical formulations used in Mediterranean aquaculture, the estimated inclusion levels of fishmeal and fish oil were approximately 20–30 % and 8–15 %, respectively (Fernandes et al., 2024; Pelusio et al., 2022). In contrast, the test diets (AAN1 and AAN2) were formulated with substantially lower levels of marine-derived ingredients: AAN1 contained 5 % total marine protein (3 % fishmeal) and 10 % fish oil, while AAN2 included 16 % marine protein (9 % fishmeal) and 3.9 % fish oil, supplemented with 10.7 % farmed salmon oil as an alternative lipid source. These data highlight a significant reduction in the reliance on wild marine-origin ingredients in the experimental feeds, reflecting the growing shift toward more sustainable and circular feed formulations in aquaculture.

Growth performance was positively influenced by the experimental diets. In gilthead sea bream, a significantly higher final body weight, specific growth rate (SGR), and improved feed conversion ratio (FCR) were observed in the AAN1 group compared to the commercial reference. Enhanced fillet yield, as indicated by a higher dressing index (DI), confirmed that the alternative formulation supported somatic growth. These findings are aligned with prior research demonstrating that poultry by-product and insect meals can effectively support growth performance when properly formulated (Busti et al., 2023, 2024; Donadelli et al., 2024).

In European sea bass, growth parameters did not differ significantly between the AAN2 and COM2 groups, indicating that the alternative diet maintained growth performance at levels comparable to the commercial feed. Similar results under controlled conditions have been reported (Pleić et al., 2022; Serradell et al., 2023; Tulli et al., 2019), demonstrating that European sea bass can efficiently utilise alternative protein sources when diets are formulated to meet essential nutrient requirements. However, a decline in the condition index (CI) was observed during the colder months, aligning with expected seasonal trends associated with metabolic depression at low temperatures (Person-Le Ruyet et al., 2004). Feed intake suppression and reduced metabolism at temperatures below 15 °C are well-documented, and similar seasonal effects were noted in the present study. Farm2 is located in the northern Adriatic, where sea temperatures drop more significantly during the winter months (December and January) compared to the central (Farm1) and southern Adriatic regions. This is due to the shallow depth and exposure to continental air masses, which have a greater impact on water temperature than in the deeper, more thermally stable central and southern Adriatic (Gačić and Civitarese, 2005).

The fatty acid composition of aquafeeds plays a critical role in determining the lipid quality of fish fillets. In this study, both gilthead sea bream and European sea bass exhibited clear diet-dependent variations in fillet fatty acid profiles, with differences primarily driven by the origin and composition of dietary lipids. In gilthead sea bream, fish fed the AAN1 diet, which included a moderate level of fish oil, showed significantly higher fillet levels of long-chain n-3 PUFAs, especially EPA (C20:5n-3) and DHA (C22:6n-3). This indicates successful deposition of health-promoting fatty acids, even with reduced fishmeal inclusion. Similar outcomes were reported in previous studies using poultry and insect meals combined with limited fish oil to sustain n-3 PUFA levels (Anedda et al., 2023; Busti et al., 2024; Donadelli et al., 2024).

In contrast, the AAN2 diet for European sea bass incorporated salmon oil from farmed Atlantic salmon, which has a markedly different fatty acid profile compared to traditional fish oils derived from wild-caught small pelagic species such as anchovy or sardine. Farmed salmon oils typically contain lower levels of EPA and DHA and are enriched in C18:2n-6 (linoleic acid) and C18:3n-3 (alpha-linolenic acid) due to high plant oil content in salmon feeds (Castro et al., 2022; Monteiro et al., 2024). This compositional shift compromises the capacity of salmon oil to support the deposition of long-chain n-3 PUFA in carnivorous fish species like European sea bass, which have limited

ability to elongate and desaturate C18 fatty acids to their longer-chain derivatives (Monroig et al., 2018). As a result, AAN2-fed sea bass showed lower fillet levels of EPA and DHA, and an overall reduction in n-3 PUFA content compared to the COM2 group, indicating that the quality of lipid sources in aquafeeds is critical for nutritional outcomes (Busti et al., 2023; Chen and Liu, 2020).

To address this limitation, future formulations could include targeted amounts of microalgal oil rich in EPA and DHA, either blended into regular feeds or used in finishing diets where economically feasible, to enhance fillet nutritional value while maintaining sustainability goals. Although current costs limit the complete replacement of salmon oil with algal oil, strategic inclusion could restore n-3 LC-PUFA levels to those typically achieved with wild sourced fish oils. In the present study, the combined EPA and DHA content of sea bass fillets (approximately 0.35–0.64 g per 100 g) exceeded the minimum adult daily intake of 250 mg recommended by the World Health Organization and the Food and Agriculture Organization of the United Nations (WHO/FAO, 2010) and remained well within the tolerable upper intake levels set by the European Food Safety Authority (Agostoni et al., 2012), confirming these fillets as a valuable dietary source of essential fatty acids.

Both species displayed a trend toward higher saturated fatty acids (SFA, mainly C16:0) and monounsaturated fatty acids (MUFA, mainly C18:1n-9) in fillets compared to diets. This trend matches patterns described in marine teleosts, as fish often retain SFA and MUFA more effectively than PUFA due to the latter's greater susceptibility to β-oxidation and peroxidation (Chen and Liu, 2020; Khalili Tilami and Sampels, 2017). Importantly, the n-6/n-3 PUFA ratios remained within favourable ranges across all groups, with the most advantageous ratios recorded in gilthead sea bream fillets from the AAN1 group (~0.83). Low n-6/n-3 ratios are associated with improved human health outcomes, particularly reduced risks of cardiovascular and inflammatory diseases (Simopoulos, 2002). Similarly, lipid quality indexes, including the atherogenicity index (AI) and thrombogenicity index (TI), remained within acceptable or favourable ranges for human health across all dietary treatments. Although slight increases in AI and TI were observed in AAN groups, the overall profiles still support cardiovascular health (Chen and Liu, 2020; Pleadin et al., 2017; Ulbritcth and Southgate, 1991).

In terms of mineral content, only gilthead sea bream fillets from the AAN1 group contained significantly lower iron and zinc concentrations than those from the COM1 group. The reduction in iron and zinc levels may be linked with the lower marine-derived ingredient content in the AAN1 diet, as marine fishmeal is a recognised source of bioavailable trace minerals (Latunde-Dada et al., 2016; Öztürk et al. 2019). Nonetheless, the measured concentrations remained within the recommended nutritional ranges for human consumption (Latunde-Dada et al., 2016). The primary dietary sources of iron and zinc in human nutrition are meat, organ meats, shellfish, and dairy products (Gibson, 2006; Hurrell and Egli, 2010), while white fish such as gilthead sea bream contribute minimally to overall intake. Therefore, the observed reductions in fillet iron and zinc are unlikely to impact consumer nutritional status or market acceptance.

This study is characterised by its execution under full-scale commercial farming conditions, a setting not typically represented in prior evaluations of insect and poultry by-product meals, which have often been limited to experimental tanks or semi-controlled environments. Such controlled conditions cannot fully replicate the complexities and operational realities of open sea cage farming, including fluctuating temperatures, hydrodynamics, variable stocking densities, and feeding regimes influenced by weather and logistics (Busti et al., 2024; Donadelli et al., 2024). These environmental and operational variables can markedly influence fish metabolic activity, feeding behaviour, nutrient utilisation, and growth trajectories, yet they are typically controlled or minimised in tank-based experiments.

By conducting trials under operational farm conditions, this research demonstrates that insect and poultry by-product meals are biologically suitable and operationally effective alternative protein sources for marine aquaculture. In gilthead sea bream, the AAN1diet resulted in a significantly higher final body weight, improved feed conversion ratio, and greater fillet yield compared to the commercial control, despite lower inclusion of fishmeal. In European sea bass, growth performance was comparable between diets, with slightly improved fillet yield observed in the AAN2 group, even under lower winter temperatures that affected overall condition. These findings extend the results of previous tank-based studies (Pleić et al., 2022; Randazzo et al. 2021). by demonstrating that sustainable feed formulations can sustain performance under the complex and fluctuating conditions of commercial sea-cage farming, underscoring their applicability in Mediterranean aquaculture systems.

The replacement of marine ingredients with poultry by-product meal and insect meal aligns with the objectives of the European Green Deal and Circular Economy Action Plan (EC, 2019). The use of organic waste streams for insect farming and the valorisation of poultry processing by-products contribute to resource efficiency and reduced environmental impact (Van Huis, 2020). The stable growth performance, fillet yield, and nutritional quality observed in this study support the feasibility of scaling up these alternative feeds in commercial Mediterranean aquaculture. However, continued attention to mineral supplementation and optimisation of fatty acid profiles is needed to align production with consumer health expectations.

This study was limited by the relatively short trial duration and single production cycle, which restricted evaluation of long term impacts on fish performance, health, and product quality. Sensory evaluation and consumer testing were not conducted, and mineral supplementation was not specifically adjusted, which may have contributed to the lower iron and zinc levels observed in gilthead sea bream fillets. Future research should address the long term health effects of alternative diets on fish, while also focusing on optimising lipid sources, including the strategic and cost effective use of microalgal oils to enhance fillet LC n-3 PUFA content, and refining mineral supplementation to ensure consistent micronutrient profiles. These efforts should be supported by longer term trials that incorporate sensory evaluation, storage stability testing, consumer acceptance studies, and full life cycle assessments to provide a comprehensive understanding of the nutritional, market, and environmental implications of alternative diets in commercial farming.

5. Conclusion

The present study demonstrates that alternative aquafeeds incorporating poultry by-product meal and insect meal (*Hermetia illucens*) can maintain or improve growth performance, fillet yield, and nutritional quality of gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) under commercial farming conditions. Species-specific differences were observed, particularly regarding mineral composition and fatty acid profiles, but core nutritional parameters were preserved across both species. Importantly, fillet n-3 PUFA levels remained sufficient to contribute significantly to human dietary intake, even under alternative feeding regimes. The use of terrestrial protein sources aligns with sustainability goals by reducing reliance on marine-derived ingredients and promoting circular resource use. These findings support the broader adoption of insect- and poultry-based feeds in Mediterranean aquaculture, although further optimisation of micronutrient supplementation and detailed consumer acceptance studies remain necessary to ensure full industry and market integration.

CRediT authorship contribution statement

T. Šegvić-Bubić: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **S. Zrnčić:** Project administration, Funding acquisition. **E. Tibaldi:** Writing – review & editing, Supervision, Funding acquisition. **J. Pleadin:** Writing – review &

editing, Supervision. **D. Oraić:** Resources, Methodology, Investigation. **I. Balenović:** Formal analysis. **T. Lešić:** Validation, Software, Formal analysis. **N. Kudumija:** Validation, Formal analysis. **I. Cvitić:** Methodology, Formal analysis. **I.G. Zupičić:** Investigation, Formal analysis. **I. Lepen Pleić:** Investigation, Formal analysis. **G. Cardinaletti:** Writing – review & editing, Resources, Investigation. **A. Vulić:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

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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Ethics

All experimental protocols were approved by the ethics committee on the Institute of Oceanography and Fisheries (No. 134/2/2018).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100747.

Data availability

Data will be made available on request.

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