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Partial Fishmeal Replacement with Defatted *Hermetia illucens* Meal in Offshore-Farmed Gilthead Seabream (*Sparus aurata*): Effects on Fillet Quality and Microbiological Stability

Marianna Oteri [1,*], Ambra Rita Di Rosa [1], Vittorio Lo Presti [1], Giovanni Toscano [2], Filippo Giarratana [1] and Biagina Chiofalo [1]

[1] Department of Veterinary Sciences, University of Messina, 98168 Messina, Italy; dirosaa@unime.it (A.R.D.R.); vittorio.lopresti@unime.it (V.L.P.); filippo.giarratana@unime.it (F.G.); biagina.chiofalo@unime.it (B.C.)
[2] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98166 Messina, Italy; gtoscano@unime.it
* Correspondence: marianna.oteri@unime.it; Tel.: +39-090-676-6704

Abstract

The search for sustainable alternatives to fishmeal (FM) in aquafeeds represents a major challenge for modern aquaculture. This study evaluated the effects of replacing 35% of FM with defatted *Hermetia illucens* larvae meal (HIM35) in diets of gilthead seabream (*Sparus aurata*) reared under full-scale commercial offshore farming conditions. Fillet nutritional quality, fatty acid and amino acid profiles, mineral composition, and microbiological stability during refrigerated storage were assessed. Dietary HIM35 significantly modified the fatty acid profile, increasing saturated fatty acids, particularly lauric and myristic acids, and n-6 polyunsaturated fatty acids. Despite reductions in eicosapentaenoic and docosahexaenoic acids (EPA and DHA), total PUFA and lipid health indices remained within recommended ranges and EPA + DHA levels were above 8%, supporting both fillet nutritional value and fish physiological requirements. Enzymatic indices based on product-to-precursor fatty acid ratios suggested reduced Δ5 + Δ6-desaturase activity. The amino acid profile showed increases in selected essential and non-essential amino acids, while overall protein quality was preserved. HIM35 fillets showed lower sodium and higher zinc contents, whereas increased aluminum levels warrant further investigation. Microbiological analyses confirmed the absence of foodborne pathogens and no effects on spoilage dynamics. Overall, HIM35 represents a safe and effective partial replacement for FM supporting sustainable aquafeed strategies.

Keywords: *Hermetia illucens*; insect meal; *Sparus aurata*; fishmeal replacement; fatty acids; amino acids; minerals; microbiological stability; offshore aquaculture

Key Contribution: This study provides one of the first integrated full-scale commercial offshore evaluations of a *Hermetia illucens*-based diet in gilthead seabream, demonstrating that a 35% fishmeal replacement maintains fillet nutritional quality, including preservation of nutritionally relevant EPA + DHA levels and microbiological safety under realistic production conditions. These findings support the large-scale adoption of insect-based aquafeeds as a sustainable and circular solution for resilient aquaculture.

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1. Introduction

Aquaculture represents the fastest-growing sector of global food production and currently supplies more than half of the aquatic animal protein consumed worldwide. In 2022, global aquaculture production reached 94.4 million tons, surpassing capture fisheries and contributing to a total aquatic animal output of 185.4 million tons [1]. This sustained expansion is driven by population growth, urbanization, and shifting dietary preferences toward nutrient-dense and health-promoting lipid and protein sources. Accordingly, global per capita fish consumption has more than doubled since 1961, increasing from 9.1 kg to 20.7 kg in 2022, and is projected to reach 21.4 kg by 2030 [1].

Despite its rapid growth, the long-term sustainability of aquaculture remains strongly dependent on the availability of fishmeal (FM) and fish oil (FO), which are primarily derived from wild forage fisheries. These ingredients provide high-quality protein and essential long-chain n-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic (EPAs) and docosahexaenoic acids (DHAs) [2]. However, their continued use exerts pressure on marine ecosystems and contributes to overfishing [3,4]. In 2020, aquaculture accounted for approximately 86% of global FM and 73% of FO consumption and only 64.6% of assessed fish stocks were classified as biologically sustainable [1], highlighting the urgent need for alternative feed resources. In addition, the increasing global demand for EPA and DHA is expected to exceed the supply from marine resources, further emphasizing the need for sustainable and scalable alternative sources of long-chain n-3 PUFA for aquafeeds [5,6].

Plant-based protein sources have been extensively investigated as substitutes for FM; however, their application in carnivorous species such as gilthead seabream (*Sparus aurata*) is limited by the presence of anti-nutritional factors, imbalanced amino acid profiles, and reduced digestibility [7]. Moreover, the large-scale use of crop-derived ingredients raises environmental concerns related to land and freshwater use, as well as competition with human food systems [8].

Among emerging alternatives, insect meals, particularly those derived from the black soldier fly (*Hermetia illucens*, HI), have attracted increasing attention as sustainable aquafeed ingredients. *Hermetia illucens* larvae can be efficiently reared on organic side streams, fitting well within circular economy frameworks and reducing the environmental footprint of feed production [9]. From a nutritional standpoint, *Hermetia illucens* meal (HIM) is rich in protein, lipids, essential minerals, and bioactive compounds such as lauric acid and chitin. While these compounds may exert antimicrobial and immunomodulatory effects, chitin may also interfere with mineral absorption at high dietary inclusion levels [10].

A recognized limitation of HIM is its lipid profile, which is characterized by a high proportion of saturated fatty acids and relatively low levels of n-3 PUFA [11]. The nutritional value of alternative n-3 PUFA sources also depends on lipid class and bioavailability, which may influence their deposition in fish tissues and the final quality of seafood products [12]. To address this issue, HIM has been increasingly incorporated into aquafeed formulations. Moderate FM replacement levels (25–40%) using defatted HIM have generally supported satisfactory growth performance, feed efficiency, and fillet quality in several fish species, including gilthead seabream [13–15]. Conversely, higher inclusion levels have been associated with reduced EPA and DHA deposition and, in some cases, impaired lipid digestibility [11].

In gilthead seabream (*Sparus aurata*), replacement of fishmeal with defatted HIM at levels up to 40% has been shown to preserve fillet nutritional quality and support physiological responses related to gut health and immunity [11,13,15,16]. In contrast, higher inclusion levels (50%) have been associated with modifications in fillet lipid and amino acid composition, although without evidence of adverse effects on microbiological safety [13]. However, most available studies have been conducted under laboratory or pilot-scale

conditions, which may not fully capture the complexity of commercial aquaculture systems. Evidence derived from full-scale commercial offshore farming systems remains limited, particularly with respect to fillet nutritional composition, microbiological stability during refrigerated storage, and lipid nutritional indices relevant to human health [11,13,14]. Therefore, validation of insect-based dietary strategies under full-scale commercial conditions is essential to assess their robustness, reproducibility, and practical applicability. In particular, offshore farming conditions are characterized by environmental variability, commercial stocking densities, and operational-scale feeding management that may influence fish metabolism, nutrient deposition, and post-harvest product quality differently from controlled experimental settings. Therefore, studies conducted under full-scale offshore conditions are essential to verify whether nutritional responses observed in pilot-scale trials remain consistent under realistic production scenarios and to support the practical implementation of insect-based aquafeeds in commercial aquaculture systems.

Regulation (EU) 2017/893 [17] authorised the inclusion of insect-derived Processed Animal Proteins (PAPs) in aquaculture feed. This authorisation was subsequently broadened by Regulation (EU) 2021/1372 [18], adopted in September 2021, which permitted the use of these ingredients in feed for pigs and poultry as well. Later, Regulation (EU) 2021/1925 [19], issued in November 2021, expanded the list of approved insect species for the production of PAPs intended for farmed animals by adding the silkworm (*Bombyx mori*). In 2024, the European Commission's Standing Committee on Plants, Animals, Food and Feed (PAFF Committee) further clarified the legal framework by formally recognising the use of live insects as feed for the aforementioned species within the European Union.

Insect-based diets may influence the n-3/n-6 PUFA ratio of fish fillets, potentially affecting their cardioprotective value. Maintaining adequate dietary levels of EPA and DHA is essential not only for fish physiology but also for ensuring the nutritional benefits of seafood for human health [5,20]. Conversely, HIM inclusion has been associated with increased calcium and iron contents, which may enhance the nutritional value of fish for human consumption, provided that mineral bioavailability is maintained [13,15].

Moderate inclusion levels of defatted *Hermetia illucens* meal (25–40%) have consistently been identified as an optimal range to maintain growth performance and fillet quality while minimizing potential negative effects on lipid composition. Based on this evidence, the 35% fishmeal replacement level adopted in the present study was selected as a biologically relevant intermediate value within this range and evaluated under commercial offshore farming conditions. We hypothesized that such a replacement level would not compromise fillet nutritional quality, while maintaining microbiological stability and lipid-related nutritional indices relevant to human health.

Within this framework, the present study evaluated the partial replacement of fishmeal (35%) with defatted *Hermetia illucens* meal (HIM35) in diets for gilthead seabream reared under commercial offshore farming conditions. The study investigated fillet nutritional quality in terms of fatty acid, amino acid and mineral composition, microbiological stability during refrigerated storage, and lipid-related nutritional indices, including the atherogenic and thrombogenic indices, the hypocholesterolemic/hypercholesterolemic (H/H) ratio, and the peroxidation index. In addition, enzymatic activity related to fatty acid metabolism was estimated using product-to-precursor ratios.

Unlike previous studies conducted under controlled laboratory or pilot-scale conditions, this full-scale offshore trial provides an integrated evaluation of nutritional, biochemical, and microbiological responses under realistic production scenarios, contributing new evidence on the robustness and applicability of insect-based dietary strategies for commercial aquaculture systems.

2. Materials and Methods

2.1. Ethical Statement

All experimental procedures involving animals were approved by the Ethic Committee of the Department of Veterinary Sciences, University of Messina (Italy), granted approval for the experiment procedure (Authorization No. 082/2022).

2.2. Experimental Diets

Two experimental diets were formulated to be isoproteic (42 g/100 g) and isolipidic (~18 g/100 g) and were produced by Veronesi S.p.A. (Verona, Italy) to meet the nutritional requirements of gilthead seabream (*Sparus aurata*). Diets were manufactured as extruded pellets with a diameter of 4 mm.

The insect meal used in the present study was a commercial product (ProteinX®, Protix Ingredients, Bergen op Zoom, The Netherlands), consisting of a milled, partially physically defatted black soldier fly (*Hermetia illucens*) larvae meal obtained through mechanical oil extraction.

The control diet (FM) contained fishmeal as the sole animal-derived protein source at an inclusion level of 250 g/kg. The experimental diet (HIM35) included 11% defatted *Hermetia illucens* meal (HIM), partially replacing fishmeal and accounting for 35% of the total animal protein fraction (Table 1). The dietary formulation corresponds to that previously reported for the same feeding trial [16] and is summarized here for completeness, based on the dietary formulation previously described for the same feeding trial.

Table 1. Ingredients and proximate chemical composition of insect meal (HI) and of the experimental diets (FM and HIM35).

	HI	FM	HIM35
Ingredients (% as feed)			
Fish meal		25.0	16.5
Wheat meal		18.0	14.5
Soybean meal		15.0	15.0
Hermetia illucens meal		-	11.0
Rapeseed oil		10.0	10.0
Sunflower meal		5.0	5.0
Fish oil		5.0	5.0
Wheat gluten		5.0	5.0
Corn gluten		5.0	5.0
SPC (Soy protein concentrate)		5.0	5.0
Pea protein		4.0	4.0
Amino acids [&], vitamin [#], and mineral [&] fraction		3.00	4.00
Proximate analysis, (g/100 g as fed)			
Dry matter	94.45	94.05	94.71
Crude protein	53.77	41.90	41.41
Crude fat [&]	14.20	19.16	18.98
Crude fiber	9.70	1.96	3.14
Ash	6.82	5.95	5.29
Gross Energy (MJ/Kg feed)	n.d.	19.86	19.80

[&] Amino acid, mineral and fatty acid composition has been reported by Rimoldi et al. [16]. [#] Vitamin mixture (IU or mg per Kg): vitamin A 12,000 IU; vitamin D3 2000 IU; vitamin E 160 mg; vitamin C (L-ascorbic acid) 160 mg.

2.3. Chemical Analysis of Insect Meal and Experimental Diets

The proximate composition of HI and the FM and HIM35 diets is summarized in Table 1. In this study, HI refers to the insect meal ingredient itself, whereas HIM35 denotes the experimental diet in which defatted *Hermetia illucens* meal is included as a partial replacement of fishmeal. Detailed compositional data have been previously reported for the same feeding trial [16] and are not reproduced here.

Analytical procedures for determining fatty acid, amino acid, and mineral composition of HI and the experimental diets were performed as previously described by Oteri et al. [21]. The compositional data of the insect meal and experimental diets have already been reported for the same feeding trial [16] and are therefore not repeated here.

2.4. Fish Rearing Conditions and Experimental Design

The feeding trial was conducted over a 25-week period, from 2 November 2021, at the Maricolture Sarde Srl commercial fish farm (Sant'Antioco, Sardinia, Italy). Approximately 60,000 gilthead seabreams with an average initial body weight of 131 ± 1.4 g were randomly distributed into four square offshore sea cages (12 × 12 × 1.5 m), with an initial biomass of approximately 2000 kg per cage. Fish were hand-fed once daily between 08:00 and 10:00 h, six days per week, with either the FM or HIM35 diet (two replicate cages per dietary treatment). Feeding rates ranged from 0.6% to 1.3% of total biomass and were adjusted according to water temperature. During the 25-week feeding trial conducted under commercial offshore farming conditions, water temperature was recorded daily and followed the expected seasonal trend typical of Mediterranean environments, ranging from 10.0 °C in winter to 20.0 °C in late spring. Monthly average temperatures were 17 °C in November, 11 °C in December and January, 12 °C in February, 14 °C in March, 16 °C in April, and 20 °C in early May. Most of the experimental period was characterized by temperatures between 11 and 18 °C, reflecting standard thermal conditions for gilthead seabream (*Sparus aurata*) production cycles under Mediterranean offshore farming conditions. Biomass was monitored monthly by bulk weighing a subsample of 100 fish per cage, while mortality was recorded every three weeks. An overall mortality rate of approximately 10% was observed during the experimental period.

2.5. Sampling Procedures

At the end of the feeding trial, 60 fish (15 fish per cage; two replicate cages per dietary treatment) were randomly sampled and sacrificed for fillet quality evaluation. Of these, a subsample of 40 fish (10 fish per cage) was allocated to chemical composition analyses. Fish were euthanized by immersion in ice water and transported on dry ice to the laboratory. Upon arrival, specimens were gutted, filleted, and deskinned. Fillets were divided into aliquots, vacuum-packed, and freeze-dried prior to analysis. Before analysis, samples were thawed and homogenized using a laboratory knife mill (Grindomix GM 200; Retsch GmbH, Haan, Germany).

The remaining 20 fish (5 fish per cage; two replicate cages per dietary treatment) were allocated to microbiological analyses. Fish were euthanized following the same procedure and transported in sterile plastic bags on dry ice to the laboratory for microbiological evaluation (Section 2.9).

2.6. Fatty Acid Profile, Nutritional Indices, and Enzymatic Activity in Fish Muscle

For determination of total lipids, the aliquots (approximately 2 g) of wet filet muscles were ground, and the oil was extracted using a chloroform/methanol (2:1, *v*/*v*) solution [22]. Each analysis was performed in triplicate. Then, total lipids were used to prepare fatty acid methyl esters (FAMEs) for the analysis of fatty acid (FA) profile, according to the method of Christie [23]. The FAMEs were analyzed with a Trace 1310 gas-chromatograph (Thermo Fisher Scientific, Milan, Italy) provided with a flame ionization detector (FID) according to Oteri et al. [13]. Results are expressed as g/100 g of total identified FAMEs, where 100 g was the total of all areas of the identified FAMEs. All analyses were performed in triplicate.

Lipid nutritional indices relevant to human cardiovascular health were calculated, including the atherogenic index (AI) and thrombogenic index (TI), the hypocholes-

terolemic/hypercholesterolemic (H/H) ratio, and the peroxidation index (PI), which reflects susceptibility to lipid oxidation, as reported by Oteri et al. [13].

The relative activity of enzymes involved in fatty acid elongation and desaturation was estimated using product-to-precursor ratios following [24], as detailed below:

$$\text{Thioesterase activity: C16:0/C14:0} \quad (1)$$

$$\text{Elongase activity: C18:0/C16:0} \quad (2)$$

$$\Delta 9\text{-desaturase (C16): [C16:1/(C16:1 + C16:0)]} \times 100 \quad (3)$$

$$\Delta 9\text{-desaturase (C18): [C18:1/(C18:1 + C18:0)]} \times 100 \quad (4)$$

$$\text{Global } \Delta 9\text{-desaturase: [(C16:1 + C18:1)/(C16:1 + C16:0 + C18:1 + C18:0)]} \times 100 \quad (5)$$

$$\Delta 5 + \Delta 6\text{-desaturase (n-6): [(C20:2n-6 + C20:4n-6)/(C18:2n-6 + C20:2n-6 + C20:4n-6)]} \times 100 \quad (6)$$

$$\Delta 5 + \Delta 6\text{-desaturase (n-3): [(C20:5n-3 + C22:5n-3 + C22:6n-3)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3)]} \times 100 \quad (7)$$

These indices represent indirect estimates of enzymatic activity based on fatty acid composition and have been previously applied in fish studies [25]. Although such indices do not provide direct measurements of enzyme activity, they allow a comparative evaluation of diet-induced metabolic responses. In the present study, their use is further supported by the experimental design, which compares two diets differing in a single key ingredient, thereby enabling the assessment of relative changes in fatty acid metabolism associated with dietary treatment.

2.7. Amino Acid Analysis of Fish Muscle

Amino acid composition was determined using approximately 0.25 g of homogenized wet fillet. Samples were hydrolyzed in 6 M HCl at 110 °C for 24 h. During acid hydrolysis, asparagine and glutamine were converted to aspartic and glutamic acids [26]; therefore, they were calculated as the sum of aspartic acid plus asparagine and of the glutamic acid plus glutamine. For cysteine analysis [26], an oxidation reaction using performic acid was performed for deamination prior to acid hydrolysis with an HCl solution (6 M). For tryptophan analysis, acid hydrolysis was performed using 10 mL of a NaOH solution (4 M) at 112 °C for 16 h; then, cooling and neutralization of each sample were performed with acetic acid [27]. Amino acids were analyzed with a Trace 1310 gas-chromatograph (Thermo Fisher Scientific, Milan, Italy) provided with a flame ionization detector (FID) as reported by Oteri et al. [13].

Results are expressed as g/100 g of wet muscle tissue. All determinations were performed in triplicate.

2.8. Mineral Composition of Fish Muscle

Mineral analysis was performed on fillets after removal of bones and scales. Approximately 0.5 g of homogenized sample was subjected to microwave-assisted acid digestion using ultrapure nitric acid and hydrogen peroxide in PTFE vessels (Ethos 1 microwave system). Digested samples were diluted to 25 mL with Milli-Q ultrapure water.

Certified reference materials (ERM-CE278k and ERM-BB184) were used to verify analytical accuracy. Mineral concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; Avio 200, Perkin Elmer, Waltham, MA, USA) equipped with a DualView optical system and an S10 autosampler, using an external calibration method as described by Oteri et al. [13]. Results are expressed as mg/100 g of wet muscle tissue. Recovery rates exceeded 85% for most elements and reached up to 95% for zinc and copper.

2.9. Microbiological Analysis of Fish Muscle

Microbiological analyses were performed to evaluate the impact of dietary treatments on fillet microbial quality during refrigerated storage. Sampling was carried out at three time points representative of commercial refrigerated storage conditions: 8 days post-harvest (arrival at the laboratory), 12 days (intermediate storage stage), and 21 days (end of shelf-life).

Prior to sampling, the external surface of the fillet was decontaminated with absolute ethanol to minimize surface contamination. The skin was then aseptically removed using sterile scissors and forceps, and the underlying muscle tissue was collected. Each muscle sample was divided into two portions for subsequent microbiological analyses.

One portion was homogenized in buffered peptone water (Biolife, Milan, Italy) at a 1:9 (*w*/*v*) ratio using a stomacher (400 Circulator; International PBI S.p.A., Milan, Italy) for 60 s at 230 rpm. The homogenates were used for enumeration of Enterobacteriaceae [28] on Violet Red Bile Glucose Agar (Biolife), incubated at 37 °C overnight; sulfite-reducing anaerobes [29] on Tryptose Sulfite Cycloserine Agar (Biolife), incubated at 37 °C overnight under anaerobic conditions; detection of *Salmonella* spp. [30] on Chromogenic Salmonella Agar and Xylose Lysine Deoxycholate Agar (Biolife), incubated at 37 °C overnight; enumeration of *Pseudomonas* spp. on Pseudomonas Agar Base (HiMedia Laboratories, Mumbai, India), incubated at 25 °C for 48 h; enumeration of *Aeromonas* spp. on GSP Agar (Merck, Darmstadt, Germany) according to Kielwein, incubated at 30 °C for 48 h; detection and enumeration of *Vibrio* spp. [31] on TCBS agar (bioMerieux, Marcy l'Etoile, France), incubated at 37 °C overnight; and enumeration of specific spoilage organisms (SSOs) [32] on Iron Agar (Lyngby) (Oxoid Ltd., Basingstoke, Hampshire, UK), incubated at 20 °C for 72 h.

The limits of detection corresponded to 10 CFU/g (1.0 log CFU/g) for Enterobacteriaceae, *Aeromonas* spp., sulfite-reducing anaerobes and SSOs.

The second portion of each sample was used for detection of *Listeria monocytogenes* [33]. Samples were first enriched in half Fraser broth (Biolife, Milan, Italy) at 30 °C overnight, followed by secondary enrichment in Fraser broth at 37 °C overnight. Cultures were then streaked onto Agar Listeria according to Ottaviani and Agosti and Listeria Palcam Agar (Biolife), with incubation at 37 °C for 24–48 h.

The sampling design included $n = 4$ fish per dietary treatment at days 8 and 21 (two fish per cage; two replicate cages per treatment) and $n = 2$ fish per dietary treatment at day 12 (one fish per cage; two replicate cages per treatment).

2.10. Statistical Analysis

For the chemical composition of the fillets, all data were analyzed using one-way analysis of variance (ANOVA) with the XLSTAT statistical software version 2021.2.2 (Addinsoft, New York, NY, USA) [34]. Differences between the treatment means were identified using Tukey's test at $p < 0.05$. Results are expressed as mean ± standard deviation (SD).

For microbiological parameters, statistical analysis was performed using a two-tailed paired *t*-test. Data obtained at 8, 12, and 21 days of refrigerated storage were pooled for each dietary group (FM and HIM35) and compared between treatments. Statistical significance was set at $p < 0.05$. All microbiological analyses were conducted using GraphPad Prism software version 9.1.1 (GraphPad Software, San Diego, CA, USA) [35]. Due to the limited number of samples available at each storage time point, microbiological values are reported as individual observations and variance estimates (standard deviation) were not calculated.

3. Results

3.1. Fatty Acid Profiles, Lipid Nutritional Indices, and Enzymatic Activity Indicators of Fillets

3.1.1. Fatty Acid Composition

The fatty acid composition of fillets is presented in Table 2. Dietary inclusion of HIM35 induced a clear shift in lipid composition, characterized by an increase in medium-chain saturated fatty acids and n-6 polyunsaturated fatty acids. In particular, lauric and myristic acids showed the most pronounced increases in the HIM35 group ($p < 0.05$), reflecting the characteristic lipid profile of *H. illucens*.

Table 2. Fatty acid composition (g/100 g, as fed) [#] of *Sparus aurata* fillet muscle fed two experimental diets.

Item	FM	HIM35	*p*-Value
C12:0	0.07 ± 0.01 [b]	0.95 ± 0.08 [a]	<0.0001
C 14:0	2.57 ± 0.06 [b]	2.90 ± 0.10 [a]	<0.0001
C14:1	0.06 ± 0.01	0.06 ± 0.01	0.512
C15:0	0.25 ± 0.01	0.24 ± 0.01	0.260
C16:0	14.32 ± 0.18	14.08 ± 0.54	0.175
C16:1	4.01 ± 0.08	3.95 ± 0.30	0.505
C17:0	0.21 ± 0.01	0.21 ± 0.01	0.860
C17:1	0.05 ± 0.01	0.05 ± 0.001	0.138
C18:0	3.56 ± 0.25	3.38 ± 0.26	0.127
C18:1n9	39.20 ± 0.59	38.78 ± 0.59	0.108
C18:1n7	3.15 ± 0.12 [a]	2.96 ± 0.15 [b]	0.003
C18:2n6	13.68 ± 0.39 [b]	14.15 ± 0.53 [a]	0.027
C18:3n6	0.05 ± 0.001	0.05 ± 0.01	0.059
C18:3n3	3.42 ± 0.09	3.43 ± 0.08	0.908
C20:0	0.26 ± 0.02	0.27 ± 0.05	0.371
C20:1n9	2.40 ± 0.05	2.40 ± 0.27	0.992
C20:2n6	0.40 ± 0.02 [b]	0.45 ± 0.03 [a]	0.001
C20:3n6	0.13 ± 0.01	0.14 ± 0.02	0.538
C20:4n3	0.54 ± 0.04 [a]	0.51 ± 0.04 [b]	0.033
C20:4n6	0.20 ± 0.02	0.21 ± 0.01	0.675
C20:5n3	2.91 ± 0.13 [a]	2.73 ± 0.14 [b]	0.003
C22:0	0.15 ± 0.01	0.17 ± 0.05	0.300
C22:1n9	0.47 ± 0.01	0.48 ± 0.01	0.332
C22:5n3	1.66 ± 0.08	1.69 ± 0.06	0.348
C22:6n3	6.27 ± 0.41 [a]	5.79 ± 0.35 [b]	0.008

FM: Fish meal group; HIM35: *Hermetia illucens* meal at 35% substitution rate of fish meal group. Fish: $n = 20$ fish (10 fish per cage, two replicate cages per dietary treatment). [#] The concentration of fatty acid is expressed as g/100 g, considering 100 g the sum of the areas of all FAME identified. C12:0 = Lauric acid; C14:0 = Myristic acid; C14:1 = Myristoleic acid; C15:0 = Pentadecanoic acid; C16:0 = Palmitic acid; C16:1 = Palmitoleic acid; C17:0 = Heptadecanoic acid; C17:1 = Heptadecenoic acid; C18:0 = Stearic acid; C18:1n9 = Oleic acid; C18:1n7 = Vaccenic acid; C18:2n6 = Linoleic acid; C18:3n6 = γ-Linolenic acid; C18:3n3 = α-linolenic acid; C20:0 = Arachidic acid; C20:1n9 = Gadoleic acid; C20:2n6 = Eicosadienoic acid; C20:3n6 = Eicosatrienoic; C20:4n3 = Eicosatetraenoic acid; C20:4n6 = Arachidonic acid; C20:5n3 = Eicosapentaenoic acid; C22:0 = Behenic acid; C22:1n9 = Erucic acid; C22:5n3 = Docosapentaenoic acid; C22:6n3 = Docosahexaenoic acid. Data are expressed as mean ± SD (standard deviation). Mean values with different superscript letters within rows differ significantly ($p < 0.05$).

Conversely, fillets from FM-fed fish were characterized by higher levels of long-chain n-3 PUFA, including EPA and DHA ($p < 0.05$). Overall, these results indicate a diet-driven redistribution of fatty acids, with HIM35 promoting SFA and n-6 PUFA deposition, while FM supported higher retention of n-3 PUFA.

3.1.2. Fatty Acid Classes and Lipid Nutritional Indices

As summarized in Table 3, HIM35 inclusion resulted in a significant increase in SFA and n-6 PUFA, accompanied by a reduction in MUFA and n-3 PUFA fractions ($p < 0.05$).

Table 3. Fatty acid classes (g/100 g, as fed) [#], and Nutritional Indices in *Sparus aurata* fillet muscle fed two experimental diets.

Item	FM	HIM35	*p*-Value
SFA	21.38 ± 0.35 [b]	22.20 ± 0.38 [a]	<0.0001
MUFA	49.34 ± 0.68 [a]	48.67 ± 0.75 [b]	0.037
PUFA	29.27 ± 0.96	29.13 ± 0.99	0.749
n3-PUFA	14.80 ± 0.64 [a]	14.13 ± 0.50 [b]	0.013
n6-PUFA	14.47 ± 0.39 [b]	15.00 ± 0.55 [a]	0.016
n3/n6 PUFA ratio	1.02 ± 0.03 [a]	0.94 ± 0.02 [b]	<0.0001
EPA + DHA	9.18 ± 0.53 [a]	8.52 ± 0.48 [b]	0.006
AI	0.31 ± 0.001 [b]	0.34 ± 0.01 [a]	<0.0001
TI	0.26 ± 0.01	0.27 ± 0.01	0.164
H/H	3.99 ± 0.06	3.94 ± 0.19	0.433
PI	64.39 ± 2.80	62.39 ± 2.45	0.087

FM: Fish meal group; HIM35: *Hermetia illucens* meal at 35% substitution rate of fish meal group. Fish: n = 20 fish (10 fish per cage, two replicate cages per dietary treatment). SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; n3-PUFA: sum of polyunsaturated fatty acids of the omega-3 series; n6-PUFA: sum of polyunsaturated fatty acids of the omega-6 series; EPA + DHA: sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); AI: Atherogenic Index; TI: Thrombogenic Index; PI: peroxidation index; H/H: hypocholesterolaemic/hypercholesterolaemic ratio. [#] The concentration of fatty acid is expressed as g/100 g, considering 100 g the sum of the areas of all FAME identified. Data are expressed as mean ± SD (standard deviation). Mean values with different superscript letters within rows differ significantly ($p < 0.05$).

This shift led to a lower n-3/n-6 PUFA ratio and reduced EPA + DHA content in the HIM35 group ($p < 0.05$), while total PUFA levels remained unchanged.

Despite these compositional differences, most lipid nutritional indices (TI, H/H, and PI) were not affected by dietary treatment ($p > 0.05$). Only the atherogenic index showed a moderate but significant increase in the HIM35 group.

3.1.3. Enzymatic Activity Indicators

Enzymatic activity indicators (Table 4) showed limited variation between dietary treatments. Most indices related to elongation and desaturation pathways were unaffected ($p > 0.05$).

Table 4. Enzymatic activity indicators in *Sparus aurata* fillet muscle fed two experimental diets.

Item	FM	HIM35	*p*-Value
Thioesters	5.58 ± 0.18 [a]	4.86 ± 0.09 [b]	<0.0001
Elongase	0.25 ± 0.02	0.24 ± 0.03	0.387
Δ9-desaturase (C16)	21.88 ± 0.42	21.87 ± 0.71	0.952
Δ9-desaturase (C18)	92.26 ± 0.45	92.50 ± 0.56	0.281
Global Δ9-desaturase	72.17 ± 0.26	72.35 ± 0.36	0.199
Δ5 + Δ6-desaturase (n6-PUFA pathway)	4.25 ± 0.23	4.42 ± 0.17	0.058
Δ5 + Δ6-desaturase (n3-PUFA pathway)	75.96 ± 1.0 [a]	74.82 ± 1.08 [b]	0.023

FM: Fish meal group; HIM35: *Hermetia illucens* meal at 35% substitution rate of fish meal group. Fish: n = 20 fish (10 fish per cage, two replicate cages per dietary treatment). Data are expressed as mean ± SD (standard deviation). Mean values with different superscript letters within rows differ significantly ($p < 0.05$).

However, HIM35 inclusion was associated with a significant reduction in thioesterase activity and in Δ5 + Δ6-desaturase activity along the n-3 pathway ($p < 0.05$), suggesting a diet-related modulation of fatty acid metabolism.

3.2. Amino Acid Profile of Fillets

The amino acid composition of fillets is reported in Table 5. Overall, HIM35 inclusion resulted in moderate but consistent changes in amino acid profiles. Essential amino acids

such as leucine, methionine, and threonine increased significantly ($p < 0.05$), while arginine and histidine decreased.

Table 5. Amino acid concentration (%, as fed) of fillets of *Sparus aurata* fillet muscle fed two experimental diets.

Item	FM	HIM35	*p*-Value
Essential amino acids			
Arginine (ARG)	1.57 ± 0.10 [a]	1.42 ± 0.06 [b]	<0.0001
Histidine (HIS)	0.80 ± 0.06 [a]	0.76 ± 0.04 [b]	0.032
Isoleucine (ILE)	1.15 ± 0.04	1.15 ± 0.05	0.948
Leucine (LEU)	1.91 ± 0.15 [b]	2.03 ± 0.06 [a]	0.003
Lysine (LYS)	3.46 ± 0.12	3.52 ± 0.08	0.116
Methionine (MET)	0.62 ± 0.04 [b]	0.66 ± 0.03 [a]	0.005
Phenylalanine (PHE)	1.29 ± 0.14	1.30 ± 0.10	0.788
Threonine (THR)	1.04 ± 0.04 [b]	1.16 ± 0.05 [a]	<0.0001
Valine (VAL)	1.13 ± 0.06	1.14 ± 0.08	0.499
Tryptophan (TRP)	0.04 ± 0.001	0.04 ± 0.001	0.631
Non-essential amino acids			
Hydroxylysine (HLY)	0.13 ± 0.06	0.12 ± 0.01	0.199
Alanine (ALA)	0.98 ± 0.02 [b]	1.01 ± 0.03 [a]	0.000
Aspartic acid + Asparagine (ASP)	1.28 ± 0.09 [b]	1.45 ± 0.06 [a]	<0.0001
Cysteine (C-C)	0.05 ± 0.001	0.05 ± 0.001	0.498
Glycine (GLY)	0.85 ± 0.04 [b]	0.92 ± 0.04 [a]	<0.0001
Glutamic acid + Glutamine (GLU)	0.73 ± 0.06	0.75 ± 0.04	0.198
Proline (PRO)	0.68 ± 0.06 [b]	0.75 ± 0.04 [a]	0.000
Hydroxyproline (HYP)	0.35 ± 0.03	0.36 ± 0.03	0.226
Tyrosine (TYR)	1.02 ± 0.06 [b]	1.07 ± 0.03 [a]	0.001
Serine (SER)	1.02 ± 0.05 [b]	1.07 ± 0.06 [a]	0.008
EAA/NEAA	1.84 ± 0.07 [a]	1.75 ± 0.02 [b]	<0.0001

FM: Fish meal group; HIM35: *Hermetia illucens* meal at 35% substitution rate of fish meal group. Fish: $n = 20$ fish (10 fish per cage, two replicate cages per dietary treatment). EAA/NEAA: essential-to-non-essential amino acid ratio. Data are expressed as mean ± SD (standard deviation). Mean values with different superscript letters within rows differ significantly ($p < 0.05$).

A similar trend was observed for non-essential amino acids, with higher levels of alanine, glycine, proline, and aspartic acid in the HIM35 group ($p < 0.05$). Despite these shifts, the overall protein quality remained stable, although the EAA/NEAA ratio was slightly reduced in HIM35-fed fish.

3.3. Mineral and Metal Composition of Fillets

The mineral and trace element composition is shown in Table 6. Most macro- and microelements were not affected by dietary treatment ($p > 0.05$), indicating overall mineral stability.

However, HIM35-fed fish exhibited lower sodium and higher zinc concentrations ($p < 0.05$), along with marked increases in aluminum levels. Additional differences included reduced strontium and increased chromium concentrations in the HIM35 group.

Table 6. Mineral and metal concentrations (mg/kg, fresh weight) of *Sparus aurata* fillet muscle fed two experimental diets.

Item	FM	HIM35	*p*-Value
P—Phosphorus	2193 ± 131	2172 ± 117	0.459
Na—Sodium	870 ± 176 [a]	785 ± 172 [b]	0.033
K—Potassium	4413 ± 373	4358 ± 298	0.473
Mg—Magnesium	345 ± 27	348 ± 29	0.618
Ca—Calcium	154 ± 56	163 ± 64	0.480
Zn—Zinc	29.12 ± 8.3 [b]	34.10 ± 10 [a]	0.020
Fe—Iron	2.76 ± 0.88	2.95 ± 0.90	0.593
Cu—Copper	0.60 ± 0.22	0.47 ± 0.18	0.134
Al—Aluminium	0.82 ± 0.57 [b]	7.64 ± 4.2 [a]	<0.0001
B—Borum	0.23 ± 0.06	0.23 ± 0.04	0.999
Mn—Manganese	0.10 ± 0.03	0.09 ± 0.03	0.122
Sr—Strontium	0.30 ± 0.09 [a]	0.24 ± 0.07 [b]	0.005
Cr—Chromium	0.14 ± 0.04 [b]	0.32 ± 0.09 [a]	<0.0001
Ni—Nickel	0.18 ± 0.05	0.15 ± 0.04	0.552
Ca/P ratio	0.07 ± 0.02	0.08 ± 0.03	0.295

FM: Fish meal group; HIM35: *Hermetia illucens* meal at 35% substitution rate of fish meal group. Fish: $n = 20$ fish (10 fish per cage, two replicate cages per dietary treatment). Ca/P: calcium-to-phosphorus ratio. Data are expressed as mean ± SD (standard deviation). Mean values with different superscript letters within rows differ significantly ($p < 0.05$).

3.4. Microbiological Profile of Fillets

Microbiological analyses were conducted on fillet samples from fish fed either the FM or HIM35 diet to evaluate microbial quality during refrigerated storage (8, 12, and 21 days post-harvest).

Across all samples, microbial loads increased progressively over storage time, with the highest values recorded at day 21. *L. monocytogenes*, *Salmonella* spp., and pathogenic *Vibrio* spp. were absent in any of the analyzed samples. *Aeromonas* spp. and sulfite-reducing anaerobes values were below the limit of detection (LOD = 1.0 log CFU/g). No significant differences ($p < 0.05$) were detected between the FM and HIM35 groups in the counts of SSOs, Enterobacteriaceae and *Pseudomonas* spp. (Table 7).

Table 7. Microbial profile of *Sparus aurata* fillet muscle fed with two experimental diets at 8, 12, and 21 days of refrigerated storage.

Diets	FM			HIM35		
Day	**8**	**12**	**21**	**8**	**12**	**21**
SSO total	3.63	3.54	4.65	2.40	2.84	4.94
Enterobacteriaceae	0.88	1.60	2.20	0.72	<1.00	2.86
Pseudomonas spp.	3.40	3.31	4.56	2.30	2.72	4.89
Sulfite-reducing anaerobes	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Listeria monocytogenes	Absent	Absent	Absent	Absent	Absent	Absent
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent
Aeromonas spp.	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
Pathogenic Vibrio spp.	Absent	Absent	Absent	Absent	Absent	Absent

Values are expressed as $\log_{10}$ CFU/g. Diets: FM = fishmeal-based diet; HIM35 = *Hermetia illucens* meal at 35% substitution level of fishmeal. Sampling time: Day 8 (post-harvest arrival at the laboratory); Day 12 (intermediate storage stage); Day 21 (end of refrigerated storage period). $n = 4$ fish per dietary treatment at days 8 and 21 (2 fish per cage); $n = 2$ fish per dietary treatment at day 12 (1 fish per cage). Values reported as <1.00 correspond to counts below the analytical limit of detection (LOD = 1.0 log CFU/g). Variance estimates (SDs) were not calculated due to the limited number of samples available at each storage time.

4. Discussion

By integrating nutritional, biochemical, and microbiological assessment under real farming conditions, the present study addresses an important knowledge gap in the insect-meal literature, which remains largely dominated by laboratory or pilot-scale investigations [13].

In offshore aquaculture systems, environmental variability and operational conditions such as hydrodynamics, stocking density, and feeding logistics may influence nutrient utilization efficiency and tissue deposition patterns compared with controlled experimental settings. Therefore, datasets generated under commercial offshore conditions are essential to evaluate the robustness and transferability of alternative feeding strategies from experimental environments to large-scale production systems [36,37]. Moreover, the simultaneous evaluation of lipid nutritional indices relevant to human health together with microbiological stability during refrigerated storage under commercial farming conditions represents an aspect that has rarely been investigated in previous studies on insect-based aquafeeds for gilthead seabream.

The fatty acid profile of fillets from HIM35-fed fish reflected the characteristic lipid composition of *H. illucens*, with increased proportions of medium-chain saturated fatty acids, particularly lauric and myristic acids, and higher levels of linoleic acid (n-6 PUFA) [11]. Comparable diet-driven shifts in tissue lipid deposition have been widely reported in fish fed insect-based ingredients [12]. In contrast, fillets from FM-fed fish retained higher concentrations of long-chain n-3 PUFA, especially eicosapentaenoic (EPAs) and docosahexaenoic acids (DHAs), which are central to the recognized health value of seafood [11,14].

A recurring concern in the evaluation of alternative aquafeed ingredients is the trade-off between sustainability and the preservation of fillet n-3 long-chain PUFA. In the present study, EPA and DHA levels declined as expected following partial replacement of marine-derived ingredients; however, total PUFA content remained unchanged, and EPA + DHA concentrations were maintained within ranges considered nutritionally relevant for human consumption [13,38]. Moreover, lipid quality indices closely associated with cardiovascular health, namely the thrombogenic index (TI), hypocholesterolemic/hypercholesterolemic (H/H) ratio, and peroxidation index (PI), were not affected by dietary treatment [13]. These results indicate that, despite compositional shifts, the overall lipid quality of fillets was preserved. From a human nutrition perspective, the maintenance of EPA + DHA levels above commonly recommended thresholds associated with cardiovascular health supports the continued nutritional value of seabream fillets produced with partial fishmeal replacement [38,39]. Although the reduction in the n-3/n-6 PUFA ratio reflects the expected effect of replacing marine-derived ingredients with alternative protein sources, the overall balance between lipid classes remained within ranges considered compatible with the recognized health benefits of fish consumption.

From a physiological standpoint, adequate dietary supply of long-chain n-3 PUFA is essential for marine fish species such as *Sparus aurata*, which have limited capacity for the endogenous synthesis of EPA and DHA from shorter-chain precursors [5,40]. The maintenance of growth performance previously observed in the same feeding trial [16], together with preserved oxidative stability and muscle lipid nutritional indices in the present study, suggests that the partial replacement level adopted here did not compromise essential fatty acid requirements of gilthead seabream under commercial farming conditions.

Although the atherogenic index (AI) increased in fillets from HIM35-fed fish [13,41], this variation should be interpreted in the context of the unchanged TI and PI values, which suggest no generalized deterioration of lipid nutritional quality. Similar patterns have been observed in other studies investigating insect-based diets in aquaculture species [14]. From an applied standpoint, targeted finishing strategies or supplementation with marine oils

have been shown to effectively restore EPA and DHA levels without compromising the sustainability advantages of insect-based feeds representing a feasible option for further optimization of HIM-based formulations [2,5]. In this context, recent global assessments have highlighted that the increasing demand for EPAs and DHAs in aquaculture is expected to exceed the supply from marine resources, reinforcing the need for sustainable and scalable alternative lipid sources [5,6].

The enzymatic indices calculated in the present study provide indirect estimates of endogenous fatty acid metabolism based on muscle fatty acid composition, as they are derived from product-to-precursor ratios rather than direct enzymatic measurements. Within this framework, diet-induced changes in fatty acid profiles were associated with selective modulation of estimated enzymatic activities. Elongase and Δ9-desaturase activities remained unchanged, indicating that partial replacement of fishmeal with HIM35 did not broadly disrupt lipid elongation and desaturation pathways in muscle tissue. In contrast, thioesterase activity was significantly reduced in fillets from HIM35-fed fish, likely reflecting the increased dietary supply of medium-chain fatty acids, which may reduce the requirement for de novo fatty acid synthesis termination [42,43]. Moreover, the observed reduction in Δ5 + Δ6-desaturase activity along the n-3 PUFA pathway likely contributed to the lower deposition of EPAs and DHAs in HIM35-fed fish, consistent with previous evidence linking dietary lipid composition to the regulation of desaturation processes in marine species [44]. Because the experimental diets contained preformed long-chain PUFA, which may be directly deposited in muscle tissue, these indices should be interpreted as relative indicators of metabolic modulation rather than absolute measures of enzymatic activity. Despite these compositional and metabolic adaptations, the absence of differences in the peroxidation index indicates preserved oxidative stability of fillet lipids. This suggests that HIM35-based diets do not increase susceptibility to lipid oxidation under commercial production and refrigerated storage conditions, in line with previous observations on the oxidative resilience of insect-based aquafeeds [43].

The amino acid profile of fillets from HIM35-fed fish exhibited moderate but nutritionally relevant changes. Increased levels of leucine, methionine, and threonine, amino acids essential for muscle protein synthesis, were accompanied by higher concentrations of alanine, glycine, and proline, which are involved in collagen metabolism and osmoregulatory processes. Despite slight reductions in arginine and histidine, the essential-to-non-essential amino acid ratio remained within optimal ranges for *S. aurata*, indicating that protein deposition efficiency and biological value were not compromised [45,46]. From a human nutrition perspective, maintaining balanced amino acid and fatty acid profiles in farmed fish is essential to preserve the recognized health benefits of seafood consumption, particularly in the context of global dietary transitions toward sustainable protein sources [20,47].

Mineral analysis revealed overall stability across most macro- and microelements, supporting the nutritional adequacy of HIM35 diets. The lower sodium content observed in HIM35 fillets may represent a favorable trait from a human health perspective, given the relationship between sodium intake and cardiovascular risk [48], while increased zinc concentrations could contribute to immune-related benefits [49]. In contrast, the higher aluminum concentration detected in HIM35 fillets warrants careful consideration. The relatively high variability observed for aluminum content in *Sparus aurata* fillets is consistent with previous reports indicating that aluminum levels in foods are not physiologically regulated and may vary considerably depending on environmental exposure, feed composition, and technological factors [50,51]. Aluminum accumulation in aquaculture products may originate from multiple factors along the feed–fish continuum, including background levels of trace elements in insect-derived ingredients depending on larval rearing substrates [52], contributions from feed processing equipment, environmental exposure under offshore

farming conditions, or analytical variability [53–55]. In this regard, the higher aluminum concentration observed in fillets from HIM35-fed fish may be partially explained by the higher aluminum content previously measured in the corresponding experimental diet (HIM35) compared with the control diet (FM) in the same feeding trial (251 vs. 194 ppm, respectively; Rimoldi et al. [16]). In addition, structural components of insect meals such as chitin may interact with mineral absorption dynamics in the gastrointestinal tract, potentially influencing trace element availability and tissue deposition. Although the present study was not designed to identify the specific origin or mechanisms underlying aluminum accumulation, these findings highlight the importance of further targeted investigations to clarify trace element transfer pathways and long-term exposure associated with insect-based aquafeeds.

The absence of adverse effects on microbial stability indicates that partial replacement of fishmeal with HIM35 is microbiologically safe and does not compromise post-harvest quality. Although antimicrobial effects of lauric acid and chitin/chitosan derivatives present in *H. illucens* meal have been reported in previous studies [52,56], such effects were not evident under the conditions of the present trial. Nevertheless, the maintenance of comparable microbial profiles between dietary treatments supports the suitability of HIM35 as a sustainable feed ingredient, in line with previous findings in other species showing no adverse effects on fillet quality and shelf-life [14,16]. These results suggest that insect-derived bioactive compounds may contribute to maintaining microbial stability rather than exerting strong antimicrobial effects under commercial conditions, highlighting the importance of evaluating these mechanisms in real farming environments.

Notably, the microbial counts observed in the present study were generally lower than those reported in the literature for gilthead seabream and other fish species during refrigerated storage, where higher levels of SSOs, *Enterobacteriaceae*, and *Pseudomonas* spp. are typically detected after similar or even shorter storage periods [57,58]. Differences in microbial dynamics during storage are known to depend strongly on initial contamination levels at harvest, farming environment, slaughtering procedures, hygienic handling during processing, and storage conditions. In the present study, fish were reared under offshore farming conditions and processed under controlled hygienic procedures, factors that may contribute to reduced initial microbial loads and slower microbial proliferation during refrigerated storage [59]. In addition, some microbial counts fell below the analytical limit of detection at intermediate storage time points, further supporting the hypothesis of low initial contamination rather than analytical inconsistency.

Therefore, while the present results indicate good microbiological stability of fillets from both dietary treatments, they should be interpreted within the context of the specific farming and processing conditions of this commercial offshore trial. Further investigations under different production environments and post-harvest handling conditions are required to confirm the reproducibility of these findings and to better characterize microbial dynamics in seabream fillets produced using insect-based aquafeeds.

The inclusion of *H. illucens* meal in aquafeeds is consistent with circular economy principles, as insect larvae can be produced through the bioconversion of organic side streams, thereby reducing dependence on finite marine resources and land-intensive crops [9]. In the present study, these environmental advantages were associated with maintained growth performance, as previously reported for the same feeding trial [16], preserved fillet nutritional quality, and stable microbiological characteristics under commercial offshore conditions. These findings support the feasibility of insect-based diets in large-scale aquaculture systems and highlight their potential contribution to more resilient and resource-efficient production strategies. However, further research is needed to optimize inclusion levels, improve long-chain n-3 PUFA retention, and better characterize the long-term effects of

insect-based feeds on product quality, safety, and consumer acceptance across different farming environments.

5. Study Limitations

This study presents some limitations that should be acknowledged. First, the enzymatic activity indicators reported herein are indirect estimates derived from product-to-precursor fatty acid ratios and therefore do not represent direct measurements of enzyme activity. In addition, as the experimental diets contained preformed long-chain polyunsaturated fatty acids, the calculated indices may also reflect direct dietary deposition in fish tissues. Consequently, these indices should be interpreted cautiously as indicators of metabolic trends rather than definitive evidence of enzymatic regulation.

Furthermore, although a significant increase in aluminum levels was observed in fillets from HIM35-fed fish, the present study was not designed to identify the specific source of this accumulation. Potential contributing factors may include background mineral levels in insect-derived ingredients depending on larval rearing substrates, environmental exposure during offshore farming, or processing-related contributions.

Further targeted investigations are therefore required to clarify the origin, bioavailability, and potential implications of trace element accumulation in insect-based aquafeeds.

In addition, the relatively low microbial counts observed during refrigerated storage compared with values commonly reported in the literature may reflect the specific farming environment and hygienic handling conditions of the present commercial offshore trial. As microbial dynamics in fish fillets are strongly influenced by initial contamination levels and post-harvest processing procedures, further studies conducted under different production and storage conditions are needed to confirm the reproducibility and broader applicability of these findings.

6. Conclusions

This study demonstrates that defatted *Hermetia illucens* meal can be used as a sustainable and nutritionally viable partial replacement for fishmeal in gilthead seabream (*Sparus aurata*) diets under commercial offshore farming conditions. The inclusion level corresponding to 35% of the animal protein fraction preserved key aspects of fillet nutritional quality, while maintaining microbiological safety during refrigerated storage. Although expected changes in fatty acid composition were observed, the overall lipid, amino acid, and mineral profiles remained within nutritionally relevant ranges and EPA + DHA levels were maintained at concentrations compatible with both human nutritional recommendations and the physiological requirements of gilthead seabream.

Nevertheless, the implementation of targeted finishing strategies and/or short-term marine oil supplementation before harvest may further enhance EPA and DHA levels, thereby maximizing the final nutritional value of the product for consumers under commercial production conditions.

In addition, the maintenance of microbial stability during storage confirms that HIM-based diets do not compromise post-harvest product quality. From an environmental perspective, HIM represents a low-impact ingredient produced through the bioconversion of organic side streams, contributing to reduced reliance on marine resources and supporting circular and climate-resilient aquafeed strategies. However, the significantly higher aluminum levels observed in fillets from HIM35-fed fish warrant further attention. Although no immediate implications for food safety can be drawn from the present data, this finding highlights the importance of monitoring trace element accumulation and identifying potential sources along the feed–fish production continuum. It should also be noted that the enzymatic activity indices reported in this study are based on product-to-precursor

ratios and, therefore, represent indirect indicators of fatty acid metabolism that may be influenced by both endogenous processes and direct dietary fatty acid deposition.

Further research should therefore focus on optimizing formulation strategies to enhance long-chain n-3 PUFA retention, evaluating long-term safety aspects, including trace element bioaccumulation, and validating these findings across diverse aquaculture production systems. These findings provide further evidence supporting the feasibility of insect-based ingredients as functional components of sustainable aquafeed strategies under realistic commercial offshore production conditions. Overall, under commercial offshore conditions, *H. illucens* meal emerges as a promising ingredient for next-generation aquafeeds, combining sustainability, functional value, and product quality, while highlighting the need for continued assessment of trace element dynamics to ensure long-term food safety.

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Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to ongoing research activities and institutional restrictions.

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