



The impact of diets containing *Hermetia illucens* meal on the growth, intestinal health, and microbiota of gilthead seabream (*Sparus aurata*)

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Abstract The present study investigated the effect of replacing fishmeal (FM) with insect meal of *Hermetia illucens* (HI) in the diet of *Sparus aurata* farmed inshore on growth, gut health, and microbiota composition. Two isolipidic (18% as fed) and isoproteic (42% as fed) diets were tested at the farm scale: a control diet without HI meal and an experimental diet with 11% HI meal replacing FM. At the end of the 25-week feeding trial, final body weight, specific growth rate, feed conversion rate, and hepatosomatic index were not affected by the diet. Gross morphology of the gastrointestinal tract and the liver was unchanged and showed no obvious signs of inflammation. High-throughput sequencing of 16S rRNA gene amplicons (MiSeq platform, Illumina) used to characterize the gut microbial community profile showed

that Proteobacteria, Fusobacteria, and Firmicutes were the dominant phyla of the gut microbiota of gilthead seabream, regardless of diet. Dietary inclusion of HI meal altered the gut microbiota by significantly decreasing the abundance of *Cetobacterium* and increasing the relative abundance of the *Oceanobacillus* and *Paenibacillus* genera. Our results clearly indicate that the inclusion of HI meal as an alternative animal protein source positively affects the gut microbiota of seabream by increasing the abundance of beneficial genera, thereby improving gut health and maintaining growth performance of *S. aurata* from coastal farms.

Keywords Aquaculture; Gilthead seabream · Gut microbiota · Intestinal health; *Hermetia illucens*

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Introduction

Aquaculture is one of the most efficient and sustainable methods of producing animal protein for human consumption. According to the Food and Agriculture Organization (FAO), aquaculture is expected to provide about 60% of seafood by 2030 (FAO 2020). However, to ensure sustainable and continued growth of this sector, the limit of feed scarcity must be surpassed. Marine ingredients traditionally used in aqua feeds, *i.e.*, fishmeal (FM) and fish oil (FO), are limited resources with relatively stable production over the last decade. In addition, quotas and demand for human consumption constrain fisheries designated for the production of FM and FO in the European Union (EU).

Therefore, one of the major challenges of modern aquaculture is to find high-quality ingredients based on environmentally friendly resources as an alternative to FM and FO that can be used for feed formulations. The most promising new resources include low-trophic marine species (mesopelagic fish, zooplankton, polychaetes, macroalgae, and crustaceans), microbial ingredients (bacteria, yeast, and microalgae), insects (black soldier fly, yellow mealworm, and crickets), and animal by-products (poultry meal, blood meal, and hydrolyzed feather meal) (Albrektsen et al. 2022). Recently, insects have gained attention as an important sustainable nutrient source for animal feed, especially for fish (Barroso et al. 2014; Henry et al. 2015; Sogari et al. 2019; Alfiko et al. 2022). This is because insects are a natural food source for fish, especially carnivorous and omnivorous species, which are evolutionarily adapted to eat them as part of their regular diet (van Huis 2020).

To date, eight species have been approved by EU legislation (Regulation 2021/1925) for the production of feed in aquaculture, including silkworms (*Bombyx mori*), black soldier fly (*Hermetia illucens*), house fly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*), and Jamaican field cricket (*Gryllus assimilis*). Among these, *H. illucens* (HI) has attracted the most interest from the aqua feed industry as an alternative protein source. HI larvae can efficiently grow on a variety of decomposing organic substrates and convert them into valuable nutrients (Meneguz et al. 2018; Bonelli et al. 2020; Hosseindoust et al. 2023). The HI larval meal

produced from dried insect larvae contains high levels of protein (36–48% DM) and fat (31–33% DM), as well as essential amino acids (EAA), vitamins, and minerals (Oteri et al. 2021). Despite their undeniable nutritional qualities, the use of HIM in animal feed is still limited due to their imbalanced fatty acid profile (Barragan-Fonseca et al. 2017; Hoc et al. 2020). Indeed, larvae of HI are unable to synthesize polyunsaturated fatty acids (PUFAs) and are instead rich in saturated fatty acids (SFAs; mainly lauric, myristic, and palmitic) (Dalle Zotte et al. 2019; Hoc et al. 2020). Therefore, the main drawback is a lower content of omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in final products derived from animals fed with larvae of these diptera. This is undeniably a problem for producers and consumers, especially in the seafood supply chain. Previous research has shown that it is possible to improve the fatty acid profile of HI larvae by enriching their rearing substrate with a source of n-3 PUFA, such as fish waste (Cullere et al. 2019). However, the modulation of the HI fatty acid profile by PUFA-enriched substrates has not always been successful, as recently reported by Ewald et al. (2020) and Ceccotti et al. (2022). Ewald et al. (2020) found that the fatty acid profile of larvae consisted mainly of lauric acid C12:0 and other SFAs endogenously synthesized by larvae, regardless of diet. In addition, the percentage of EPA and DHA tended to decrease with increasing weight, *i.e.*, larvae with a higher weight contained a higher percentage of SFAs and a lower percentage of EPA and DHA. Furthermore, *H. illucens* larvae reared on substrates enriched in monounsaturated fatty acids (MUFAs) and PUFAs tended to bioaccumulate some of these fatty acids to promote the biosynthesis of lauric acid Hoc et al. (2020).

Currently, the potential of HI meal (HIM) as a valuable feed ingredient has been determined for several commercially important farmed fish species, including Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata*), and European seabass (*Dicentrarchus labrax*) (Rimoldi et al. 2019, 2021; Terova et al. 2019; Bruni et al. 2020; Li et al. 2020; Biasato et al. 2022; Oteri et al. 2022; Anedda et al. 2023; Di Rosa et al. 2023; Zaranoniello et al. 2023). The overall results showed that the health status and production performance

of the fish, as well as the quality of the products obtained from them, were satisfactory (Alfiko et al. 2022). Numerous studies on different aquaculture species have also demonstrated that replacing FM with HIM has a positive modulatory effect on gut bacterial communities and increases bacterial richness and abundance of beneficial lactic acid bacteria (Huyben et al. 2019; Rimoldi et al. 2019, 2021; Terova et al. 2019; Weththasinghe et al. 2022; Biasato et al. 2022; Hasan et al. 2023).

The HI contains bioactive compounds such as lauric acid and chitin that can affect the gut microbiota of fish. Lauric acid exerts potent antimicrobial activity against both Gram-positive and Gram-negative pathogens (Skřivanová et al. 2006; Kumar et al. 2020; Borrelli et al. 2021). In contrast, chitin, a biopolymer of glucosamine found in the exoskeletons of arthropods, has shown antimicrobial and bacteriostatic activity against several Gram-negative pathogens in addition to its prebiotic properties (Rangel et al. 2022a; Weththasinghe et al. 2022; Rimoldi et al. 2023). Chitin, which resembles cellulose, acts as an indigestible dietary fiber that promotes the growth of beneficial and chitin-degrading bacteria. Thus, we recently obtained promising results with the inclusion of insect exuviae, the main chitin-rich by-products of insect farming, in the diet of rainbow trout (Rimoldi et al. 2023). In particular, pupal exuviae improved the proportion of Firmicutes and Actinobacteria phyla in the gut of trout.

Although numerous publications have already described the use of insects as sustainable protein sources in fish feeds, most of these studies have been conducted on a small scale in a controlled environment, such as experimental facilities, to minimize variables. To date, there are few findings from large-scale trials. Accordingly, the present study investigated the effect of replacing FM with HIM in the diets of gilthead seabream (*S. aurata*) reared off shore. Growth performance, gut health, and gut microbiota profile were considered to monitor fish health and response to the diet. Two isolipidic and isoproteic diets were tested on farms: a control diet without insect meal and an experimental diet with 11% HIM as a replacement for FM. High-throughput 16S rRNA gene amplicon sequencing (MiSeq platform, Illumina) was used to characterize gut microbial communities.

Materials and methods

Ethics

The experiment was carried out at the “Maricoltura Sarde Srl” farm located in Sant’Antioco, a region in South Sardinia, Italy. The Ethical Committee of the University of Messina, Department of Veterinary Sciences, granted approval for the experimental procedure (Authorization No. 082/2022).

Diets

Two isolipid (approximately 18 g/100 g) and isoprotein (42 g/100 g) diets were manufactured by Veronesi S.p.A. (Verona, Italy). The diets meet the nutritional requirements of gilthead seabream. The extruded feeds were produced in a 4-mm-pellet size. The control basal diet (FM) contained only FM as a protein source of animal origin (250 g/kg), whereas the experimental diet (HIM) contained 11% defatted HIM (used as a basal diet) as a partial substitute for FM, which accounted for 35% of the substitute (Table 1).

Chemical analysis of feeds

All the chemical analyses of the feeds were performed in triplicate.

Dry matter (DM, AOAC #930.15), crude protein (CP, AOAC #2001.11), crude fiber (AOAC #978.10), ash (AOAC #942.05) contents, and ether extract (EE, AOAC #920.39) were determined according to the standard procedures of Association of Official Analytical Chemists (AOAC 2019). Gross energy (GE) was evaluated using an adiabatic calorimetric bomb. The proximate composition of the diets is reported in Table 1. As regards the composition of fatty acids, amino acids, and minerals of the insect meal and the two diets, the analytical methods were described in detail by Oteri et al. (2021), and the results are reported in Tables 2, 3 and 4

Fish rearing conditions and sampling

The feeding experiment started on November 2, 2021, and the 25-week feeding experiment was carried out at the Maricoltura Sarde Srl fish farm (Sant’Antioco, Sardinia, Italy). Approximately 60,000 gilthead sea bream (average initial body weight 131 ± 1.4 g) were randomly distributed to four

Table 1 Raw materials and proximate composition of the diets

	FM	HIM
Ingredients (% as feed):		
Fishmeal	25.0	16.5
Wheat meal	18.0	14.5
Soybean meal	15.0	15.0
<i>Hermetia illucens</i> 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 ^a , vitamin ^b , and mineral ^c fraction	3.0	4.0
Proximate composition (% as feed):		
DM	95.05	94.71
CP	41.9	41.40
EE	19.16	18.98
Fiber	1.96	2.14
Ash	5.95	5.29
Gross energy (MJ/kg feed)	19.86	19.80

^aSee Tables 2 and 3

^bVitamin 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

^cSee Tables 2 and 3

square coastal cages (12×12×1.5 m) with an initial biomass of approximately 2000 kg per cage. During the experimental period, fish were hand-fed either FM or HIM at a daily meal (between 08:00 and 10:00), 6 days a week (two cages/diet). Feeding rates ranged from 0.6 to 1.3% of biomass, depending on water temperature. Biomass in each cage was determined by weighing 100 fish in bulk each month. Fish mortality in the cages was monitored at 3-week intervals. After 25 weeks experimentation, 88 fish per diet were sacrificed for measurement of growth parameters. Specifically, individual final body weight was determined for all fish ($n=88$ per diet) to calculate the following indices for each diet group:

WG (Weight gain, g) = Final body weight(g) – Initial body weight(g)

FCR (Feed conversion rate) = Total feed consumed per cage (g DM)/ Weight gain (g)

SGR(Specific growth rate, %/day) = (ln(final body weight, g) – ln(initial body weight, g))/Number of feeding days) × 100.

Table 2 Fatty acid composition (g/100 g dry matter) of two experimental diets

Item	FM	HIM
C10:0	0.01	0.08
C12:0	0.06	2.81
C13:0	0.02	0.02
C14:0	2.08	2.53
C15:0	0.29	0.26
C16:0	11.53	11.73
C16:1	3.08	3.03
C17:0	0.33	0.26
C18:0	3.42	3.47
C18:1n9	47.08	45.01
C18:1n7	0.14	0.17
C18:2n6	14.81	14.65
C18:3n6	0.06	0.06
C18:3n3	5.29	5.03
C20:0	0.49	0.48
C20:1n9	1.12	1.05
C20:2n6	0.13	0.12
C20:3n3	0.47	0.44
C20:4n6	0.05	0.04
C20:5n3	4.14	3.79
C22:0	0.30	0.28
C22:1n9	0.23	0.22
C22:2n6	0.03	0.02
C23:0	0.03	0.03
C24:0	0.55	0.54
C22:6n3	4.26	3.91
SFA	19.11	22.48
MUFA	51.66	49.48
PUFA	29.23	28.04
SFA/UFA	0.24	0.29
n3-PUFA	14.16	13.16
n6-PUFA	15.08	14.88
EPA/DHA	8.39	7.69

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids, SFA/UFA saturated fatty acids/unsaturated fatty acids ratio, EPA/DHA eicosapentaenoic acid/docosahexaenoic acid ratio

From a subsample of 24 specimens (12 fish per diet, 6 fish per cage), the liver was separated and its weight measured to calculate the hepatosomatic index (HSI) for each fish as follows:

$$\text{HSI (\%)} = (\text{liver weight(g)}/\text{body weight (g)}) \times 100$$

Samples of the liver and the intestine (divided into proximal and distal parts) were collected from ten fish per diet and fixed in a solution of neutral buffered formalin (NBF, 10%) to perform histological analysis.

Similarly, for gut microbiota analysis, 10 fish per diet were sacrificed and the whole intestine (without pyloric ceca) was collected from each fish aseptically. The digesta- and mucosal microbiota were collected and mixed together as described in detail by Rimoldi et al. (2020).

Finally, nine fish were sampled per tank and three fecal samples were collected from each tank for volatile fatty acid analysis. All the fish were killed by putting them in ice water.

Table 3 Amino acid composition (g/100 g dry matter) of the experimental diets

	FM	HIM
Essential amino acids (EAA)		
Arginine	2.29	2.81
Histidine	1.37	1.29
Isoleucine	2.32	2.13
Leucine	4.51	4.40
Lysine	4.34	4.65
Hydroxylysine	0.79	0.57
Methionine	0.63	0.63
Phenylalanine	2.87	3.29
Threonine	1.54	1.49
Valine	2.33	2.05
Tryptophan	0.17	0.15
Non-essential amino acids (NEAA)		
Alanine	2.30	2.14
Aspartic acid	3.94	3.69
Cysteine	0.15	0.20
Glycine	1.71	1.64
Glutamic acid	2.90	3.15
Proline	2.41	2.18
Hydroxyproline	0.90	0.89
Tyrosine	1.77	1.56
Serine	1.88	2.07
EAA/NEAA	1.29	1.34

Histology

NBF-fixed liver and intestinal specimens were embedded in paraffin and cut into 5- μ m slices with a microtome (Leica RM2245). The acquired slides were stained with hematoxylin and eosin (H&E), and the tissue morphology was studied using a CMOS Discovery C30 digital camera under a light microscope (Zeiss Axiophot microscope). Fiji software (an open-source Java-based imaging package) was used to process the captured images. The following intestinal morphological characteristics were examined, as specified by Escaffre et al (2007): villus height (ViH), villus width (ViW), and submucosal layer thickness (SMT). A semi-quantitative grading approach based on a grading score (1=not observed/low, 2=moderate, 3=severe) was employed for the liver (Caballero et al. 2004; Rodrigues et al. 2017; Camargo and Martinez 2007). In particular, vacuolization of hepatocytes (HV), nuclear displacement (ND), cellular hypertrophy (CH), and irregular nuclear shapes (NS) were taken into consideration as histological characteristics in the liver.

Taxonomic and functional characterization of gut microbiota

Bacterial genomic DNA isolation, amplicon library construction, and sequencing.

Table 4 Mineral element profile (mg/kg dry matter) of the experimental diets

Element	FM	HIM
Zn	126	130
Fe	128	148
Ba	0.5	2.1
Ti	<1	<1
Ag	<1	<1
Hg	<1	<1
Mn	64	85
Al	194	251
Cu	16	16
Pb	<1	<1
As	<1	<1
Cd	<1	<1
Cr	3.9	6.5
Ni	<1	<1
Se	<1	<1

Bacterial DNA was extracted from each sample (feces + mucosa) and from three aliquots of 200 mg per feed. The DNeasy® PowerSoil® Pro kit (Qiagen, Italy) was used for extraction according to the manufacturer's instructions, with an additional mechanical lysis step as previously described (Rimoldi et al. 2019). A negative control, with lysis buffer only, was processed in parallel with the biological samples to exclude any external contamination. The extracted DNA was quantified spectrophotometrically and then stored at $-20\text{ }^{\circ}\text{C}$ until use. The 16S amplicon sequencing library was prepared by the microbiome sequencing service GALSEQ srl (Milan, Italy) and sequenced on an Illumina MiSeq sequencing platform. All steps of preparation and sequencing of the 16S rRNA gene library have been described previously (Terova et al. 2019). Briefly, the V4 hypervariable region of the 16S rRNA gene was amplified with oligonucleotides 515F: 5'-GTGYCAGCMGCCGCG GTAA-3' and 806R: 5'-GGACTACNVGGTWTCTAAT-3'. Each library was diluted to 5 ng/ μL , mixed at equimolar concentration (4 nM), and then subjected to multiplex sequencing on an Illumina MiSeq instrument using a $2 \times 150\text{-bp}$ PE strategy.

DNA sequencing data analysis

Raw next generation sequencing data were analyzed using the QIIME2™ pipeline (v. 2020.2) (Bolyen et al. 2019). The entire applied QIIME bioinformatics workflow was previously described by Terova et al. (2021). The final result was an amplicon sequence variant (ASV) table with high-quality filtered reads. Taxonomy was assigned to ASVs using the reference database SILVA (<https://www.arb-silva.de/>) (Quast et al. 2013). ASVs assigned to chloroplasts and mitochondria were removed from the analysis. The qiime 2 core-metrics-phylogenetic pipeline was applied to calculate alpha and beta diversity. Alpha diversity was assessed at a depth of 31,081 reads, and both species richness (Chao 1, observed ASV) and biodiversity (Faith PD, Shannon and Simpson) were calculated. The weighted and unweighted UniFrac distance matrices (Lozupone and Knight 2005; Lozupone et al. 2007) were used to estimate beta diversity.

Only ASVs present in at least 60% of the samples in each group were considered in the analysis of core microbiota.

The free tool Venny 2.1 (<https://bioinfo.gp.cnb.csic.es/tools/venny/index.html>) was used to create a Venn diagram depicting unique and common ASVs between different feeding groups.

Bacterial metabolic pathway prediction

The functional profile of gut microbial communities was predicted using the PICRUST (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) tool (Langille et al. 2013) based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Differences between two experimental groups (FM and HIM) were validated with a two-sided Welch *t*-test using the Statistical Analysis of Metagenomics Profiles software package (STAMP, v.2.1.3) (<http://kiwi.cs.dal.ca/Software/> STAMP) (Parks et al. 2014).

Analysis of fecal short-chain fatty acids (SCFAs)

Qualitative and quantitative determinations of SCFAs (acetate, propionate, iso-butyrate, and butyrate) were performed according to the modified extraction method of Chlebicz-Wójcik and Śliżewska (2020). For this purpose, 0.5 g of fecal matter was placed in a falcon, dissolved with 5 mL of water for HPLC PLUS, and vortexed for 3 min. Immediately thereafter, the samples were centrifuged at 4000 rpm for 30 min. The supernatant was filtered through a 0.22- μm PTFE syringe filter. The extracts obtained were analyzed using a HPLC–UV–VIS system (Shimadzu, Milan, Italy) equipped with two LC -20AD pumps, a CBM-Alite controller, a DGU-20A5 degasser, and an automatic injector.

Chromatographic separation was performed using a 150-mm \times 4.6-mm I.D. and 2.7- μm particle Ascentis Express 90A C18 column (Merck KGaA, Darmstadt, Germany) in isocratic mode. The mobile phase consisted of 0.005 M H_2SO_4 with a flow rate of 0.6 mL/min and a column temperature of 60 $^{\circ}\text{C}$. The injection volume was 10 μL . The wavelength for UV–VIS analysis was set to 210 nm. The software used for data processing is LC solution (Shimadzu, Milan, Italy). Qualitative analyses of SCFAs were performed by comparing the retention times of each certified standard, whereas quantitative evaluations were performed using the calibration curve

method in a range of different concentrations. The results were expressed in millimoles per liter.

Statistics

The Shapiro–Wilk test was employed to assess normality, while homoscedasticity was evaluated using Levene’s test. The application of angular transformation was employed in order to linearize sigmoid distributions and account for variations in the relative frequencies of bacterial species. The statistical tests employed to compare growth performances, alpha diversity indices, and bacterial abundances of the two experimental groups were the Student’s *t*-test or the Mann–Whitney *U*-test, depending on whether the data exhibited a normal distribution. The statistical technique employed in this study to assess variations in beta diversity among different groups was permutational multivariate analysis of variance (PERMANOVA). The statistical analyses were conducted using Past4 v. 4.02 software (Hammer et al. 2001), with a significance level of $p < 0.05$.

Table 5 Growth performance and hepatic somatic index (HSI) of *S. aurata* fed two experimental diets. SGR, specific growth rate; BW_f , final body weight; FCR, feed conversion ratio

	BW_f	SGR	FCR	HSI
FM	246 ± 40	0.22 ± 0.09	1.44 ± 0.43	1.15 ± 0.21
HIM	244 ± 36	0.21 ± 0.09	1.32 ± 0.54	1.20 ± 0.17
<i>p</i> -value	0.707	0.720	0.138	0.512

Results

Growth performance

During the experiment, both diets were well accepted by the fish. Regardless of the feed, a mortality rate of 10% was observed. The results on growth performance of gilthead seabream fed the experimental diets are shown in Table 5. Feeding HIM had no significant effect on growth or somatic index. At the end of the 25-week feeding trial, final body weight (HIM 244 g vs. FM 246, $p=0.707$), specific growth rate (HIM 0.21% vs. FM 0.22%, $p=0.720$), feed conversion ratio (HIM 1.32 vs. FM 1.44, $p=0.138$), and hepato-somatic index (HIM 1.20% vs. FM 1.15%, $p=0.512$) did not differ between the two feeding groups (Table 5).

Fig. 1 Standard hematoxylin and eosin (H&E) staining of proximal and distal intestinal sections of FM (panels A, B) and HIM (panels C, D). M, mucosa; SM, submucosa; CM, circular muscle layer; LM, longitudinal muscle layer. Scale bar, 500 μ m

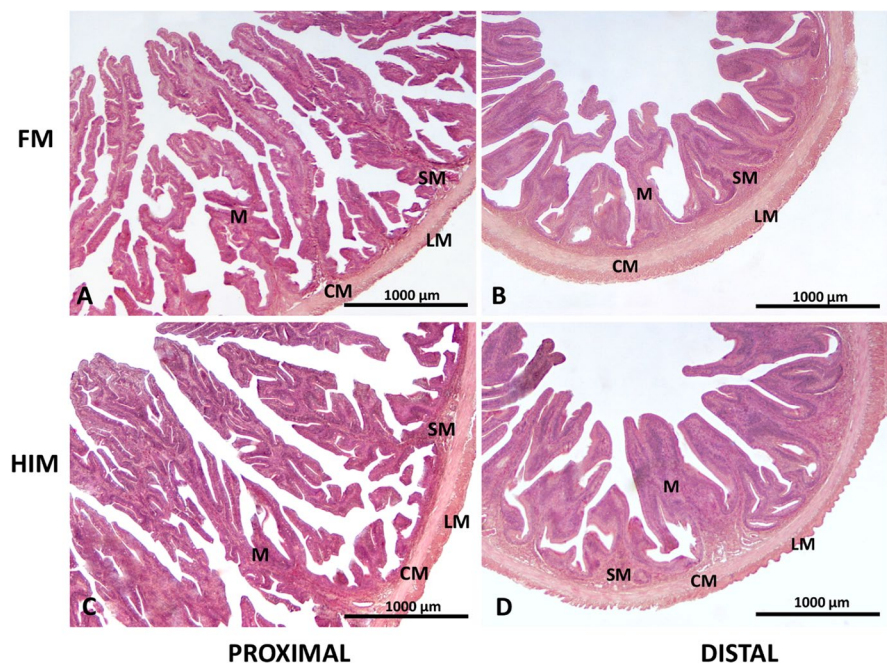
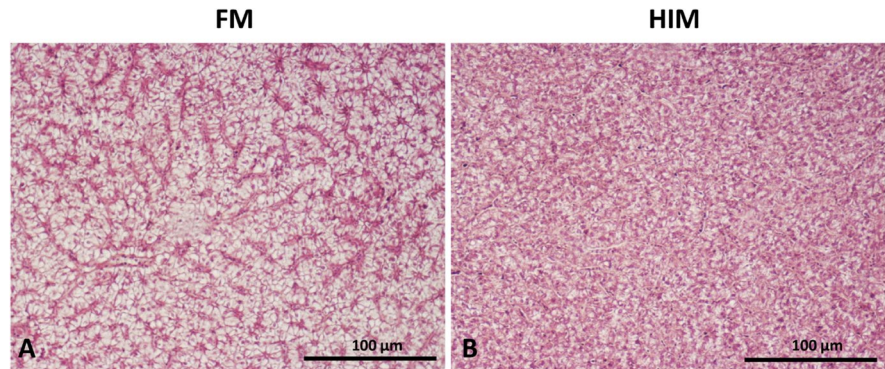


Fig. 2 Standard hematoxylin and eosin (H&E) staining of liver from FM (A) and HIM fish (B). Scale bar, 500 μ m



Gut and liver morphology

The gross morphology of the gastrointestinal system and liver exhibited preservation, without any apparent indications of inflammation (Figs. 1 and 2). The tissue structure of both the proximal and distal regions of the intestine exhibited a well-organized and well-preserved state, devoid of any apparent indications of injury or inflammation, irrespective of the dietary conditions (see Fig. 1). The mucosal folds in the proximal intestine exhibited a higher degree of organization and a greater abundance compared to those in the distal intestine (see Fig. 1A, C). Although the observed trend in muscle layer thickness and villus height in both the proximal and distal intestines of fish fed the HIM diet was not found to be statistically significant, it is worth noting (Fig. 1; Table 6).

Table 6 Morphometry of proximal and distal intestinal segments of *S. aurata* fed two experimental diets ($n = 10$)

	FM	HIM	<i>p</i> -value
Proximal intestine			
Villus height (μ m)	866.40 \pm 63.39	1033.07 \pm 63.59	0.08
Muscle thickness (μ m)	45.12 \pm 1.26	51.27 \pm 4.29	0.19
Villus width (μ m)	136.13 \pm 4.06	139 \pm 5.20	0.67
Distal intestine			
Villus height (μ m)	645.60 \pm 31.81	722.64 \pm 24.85	0.06
Muscle thickness (μ m)	82.75 \pm 1.74	86.43 \pm 1.65	0.13
Villus width (μ m)	109.62 \pm 4.85	113.39 \pm 3.38	0.52

The preservation of the liver parenchyma was observed to be satisfactory in both groups of fish, similar to the condition observed in the gut. The liver in all dietary groups displayed a typical parenchymatic architecture, which was characterized by a consistent arrangement of hepatic cords separated by sinusoids and bile ducts (Fig. 2). The lipid infiltration observed in the fish from FM was of moderate intensity, leading to the relocation of the nuclei towards the outer region of the hepatocytes, as depicted in Fig. 2A. The histological assessment, as presented in Table 7, indicated the absence of apparent indications of structural impairment or inflammation resulting from the consumption of the insect-based diet.

Illumina sequencing efficiency

The sequencing process was conducted with remarkable efficiency, resulting in a total of 1,082,214 high-quality sequences. Among these sequences, 910,820 were from intestinal samples, while the remaining 171,394 were derived from feed pellets. A mean Good's coverage value over 99% was achieved, suggesting that the dataset adequately represents the bacterial communities. The sequencing depth for computing alpha diversity indices was determined to be 31,081 reads based on the analysis of rarefaction curves. The raw sequencing data were deposited in the public database of the European Nucleotide Archive (EBI ENA) with the accession code PRJEB64777.

Profiles of feed-associated microbial communities

The utilization of 16S rDNA gene amplicon sequencing metabarcoding analysis was employed to examine the feed-associated microbiota, leading to the

Table 7 Results of histological scoring of livers of gilthead seabream fed two different experimental diets ($n = 10$)

	FM	HIM	<i>p</i> -value
Nuclear displacement (ND)	1.27	1.14	0.35
Cellular hypertrophy (CH)	1.27	1.16	0.52
Irregular nuclei shapes (NS)	1.18	1.04	0.24
Hepatocytes vacuolization (HV)	1.27	1.22	0.76

Grading scale: 1=not observed/minimal, 2=moderate, 3=severe

identification of noteworthy distinctions between two distinct feeds. The microbial compositions of the feeds were analyzed, focusing on the most prevalent taxa ($\geq 1\%$ at the order level and $\geq 0.5\%$ at the family and genus levels). The analysis revealed the presence of three phyla, three classes, six orders, nine families, and nine genera (Fig. 3A–C). The comprehensive compilation of bacterial taxa categorized by genera is included in Supplementary Data File S1. The results of the alpha diversity analysis revealed that the microbial community linked to the HIM feed had

the greatest levels of species richness, as shown by the Chao1 and observed ASV metrics, as well as bio-diversity, as measured by the Shannon, Simpson, and Faith-PD indices (Table 8).

Consistent with the alpha diversity analysis, a significant decrease in taxa was observed in the FM feed. At the phylum level, the microbiota in the FM feed consisted mainly of Firmicutes (97.6%), with the remainder consisting of Proteobacteria (2.4%) (Fig. 3A). In contrast, diets containing insect meal had a higher proportion of Proteobacteria (32%) and a lower abundance of Firmicutes (60%) compared to FM. In addition, the Actinobacteriota phylum (8%) was found only in association with HIM feeds (Fig. 3A). Bacteria belonging to the families Actinomycetaceae (11.3%), Enterococcaceae (10.8%), Lactobacillaceae (7.8%), Morganellaceae (1.4%), Streptococcaceae (1.2%), and Enterobacteriaceae (1.1%) were detected only in the feed HIM. The family Staphylococcaceae (97.5%) was the major taxa in the control feed FM, followed by Halomonadaceae (2%) (Fig. 3B). In contrast, the Bacillaceae family was the

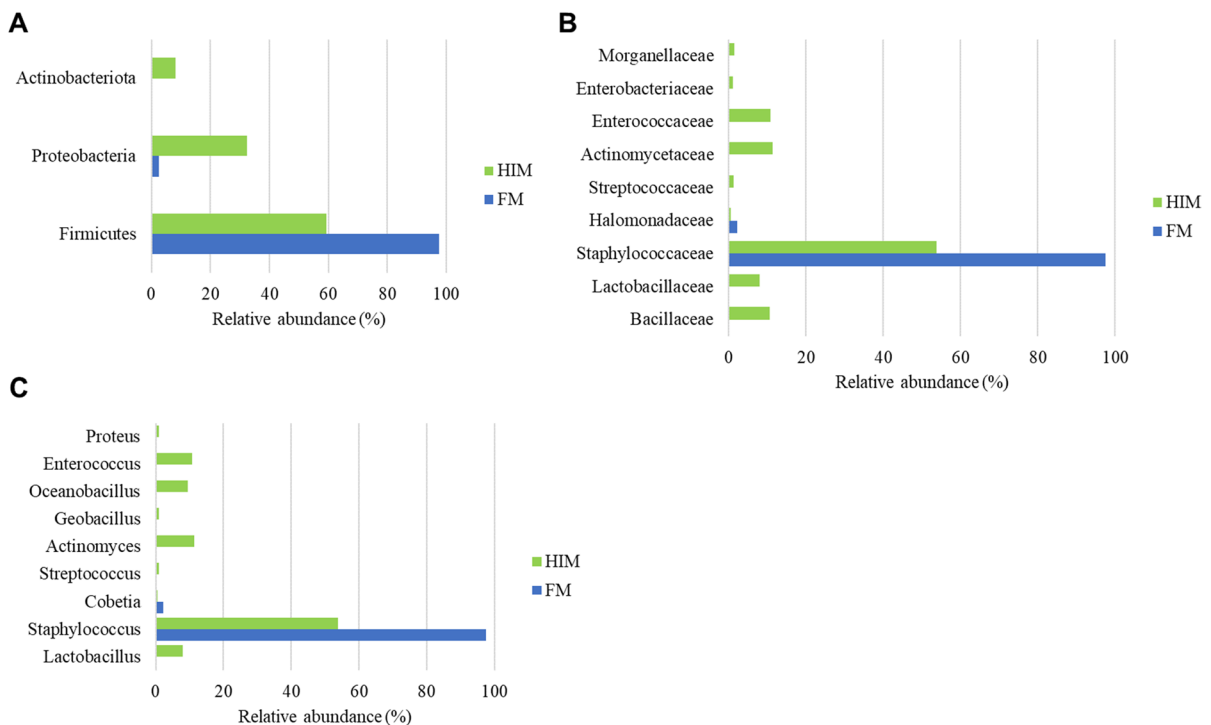


Fig. 3 Profiles of the feed-associated microbiota at the taxonomic level of phylum (A), families (B), and genera (C). Only taxa with a total abundance of $\geq 0.5\%$ are reported. Bacteria with lower abundance are grouped together and referred to as “others”

Table 8 Alpha diversity indexes of feed-associated microbiota ($n=3$) and gut microbial communities ($n=10$). Different letters indicate differences between means ($p < 0.05$)

	Chao1	Obs ASVs	Faith_PD	Shannon	Simpson
Feed samples					
FM	120 ± 19 ^b	109 ± 11 ^b	1.51 ± 0.07 ^b	3.71 ± 0.04 ^b	0.84 ± 0.002 ^b
HIM	390 ± 19 ^a	381 ± 23 ^a	3.58 ± 0.10 ^a	6.10 ± 0.27 ^a	0.95 ± 0.005 ^a
Gut samples					
FM	244 ± 55	227 ± 50	2.44 ± 0.93	4.95 ± 0.46	0.92 ± 0.02
HIM	267 ± 70	249 ± 68	2.90 ± 0.85	4.70 ± 0.61	0.89 ± 0.03

most abundant in the HIM feed (10%). Accordingly, at the genus level, the genera *Actinomyces* (11.3%), *Enterococcus* (10.8%), *Oceanobacillus* (9.4%), *Lactobacillus* (7.8%), *Proteus* (0.97%) *Streptococcus* (0.96%), and *Geobacillus* (0.95%) were only detectable in the HIM feed. In contrast, the control feed FM showed an association with the genera *Staphylococcus* and *Cobetia* (Fig. 1C).

Profiles of gut microbial communities and their dietary modulation

Irrespective of dietary variations, a comprehensive characterization of the microbiota composition in all intestinal samples was achieved, providing detailed information at the genus level. This analysis revealed the presence of 6 phyla, 8 classes, 18 orders, 22 families, and 33 genera, as documented in Supplementary Data File S2. When considering solely the most representative taxa, the assemblage was composed of three phyla, four classes, five orders, nine families, and ten genera. The gut microbiota profiles of two distinct feeding groups are depicted

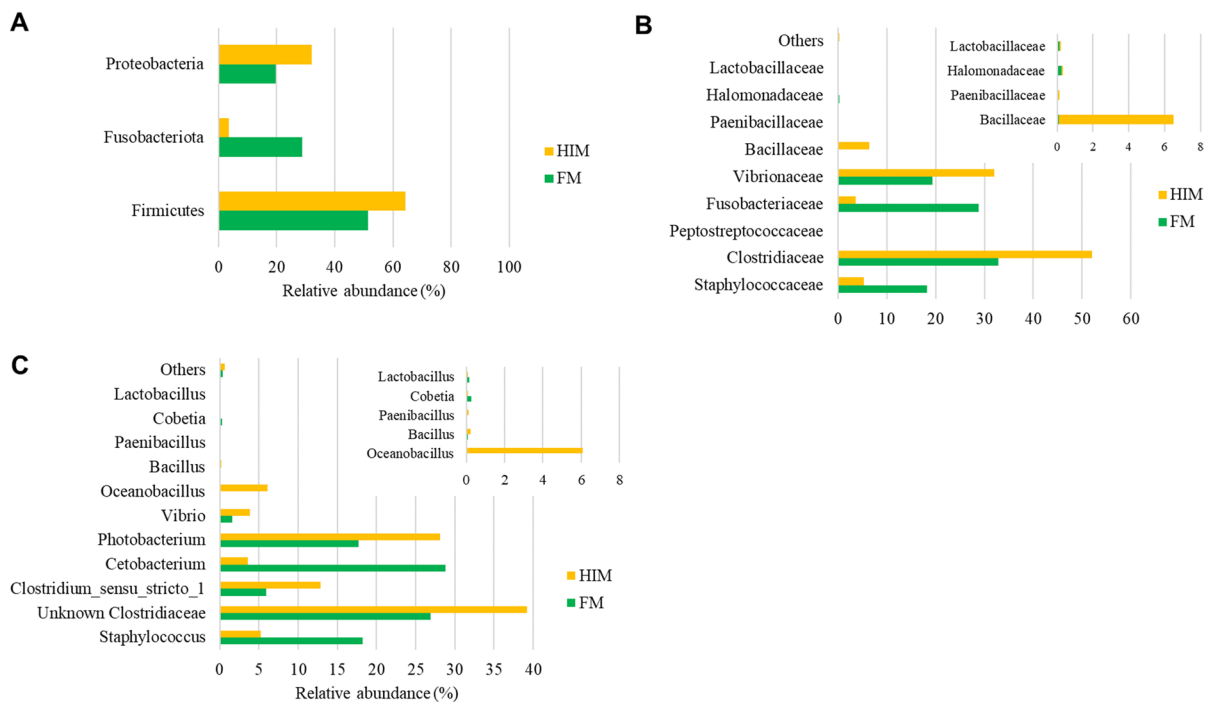


Fig. 4 Profiles of the gut microbiota of gilthead sea bream fed two tested diets. The relative abundance of the overall most abundant bacterial phyla (A), families (B), and genera (C) is

indicated. Bacterial taxa with lower abundance ($< 0.5\%$) are summarized and labelled as “others”

in Fig. 4A–C, showcasing the taxonomic composition at the phylum, family, and genus levels.

The examination of alpha diversity indicated that there were no significant differences observed between the two feeding groups, as shown in Table 5. According to the Venn diagram, it is evident that the common core microbiota consisted of only eight species. Moreover, the HIM fish were found to have six genera solely assigned to them, whereas the FM fish had one genus exclusively assigned to them (refer to Fig. 5). The use of multivariate permutational analysis, utilizing both weighted and unweighted UniFrac metrics, demonstrated statistically significant distinctions ($p < 0.05$) in the gut bacterial communities of the two feeding groups. Additionally, notable dissimilarities were observed between the gut microbiota and the diet microbiota, as indicated in Table 9. Consequently, the use of principal coordinate analysis (PCoA) on the unweighted and weighted UniFrac metrics resulted in distinct clusters for gut and feed samples, with gut samples further grouped based on diet (Fig. 6).

At the taxonomic level of phylum, the three most prevalent phyla identified were Firmicutes, Proteobacteria, and Fusobacteria (Fig. 4A). The dietary composition had an impact on Fusobacteria, as evidenced by the increased proportion of Fusobacteria in the group of fish that were fed the control diet. In accordance with the findings, the bacterial family Fusobacteriaceae exhibited a higher prevalence in the FM group, but Bacillaceae

Fig. 5 Core microbiota. Venn diagram showing unique and common taxa between two experimental groups

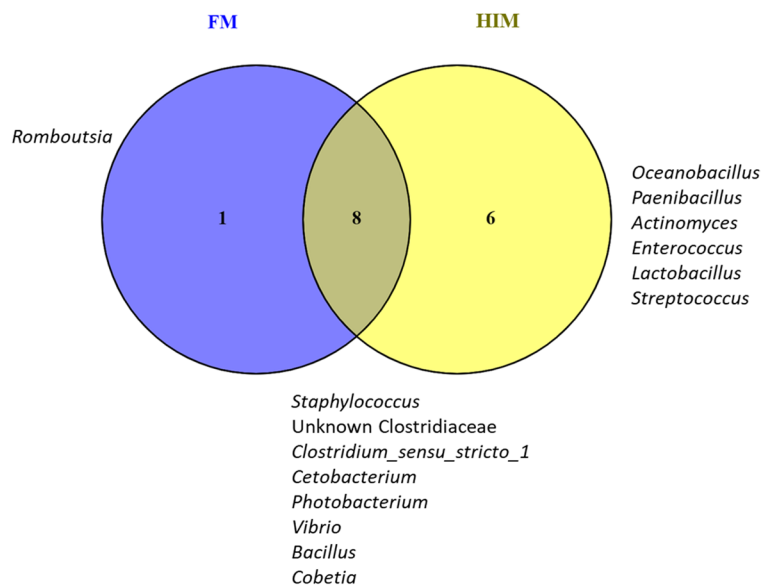


Table 9 Results of PERMANOVA analysis on unweighted and weighted UniFrac metrics data

Group 1	Group 2	Size	Permuta- tions	Pseudo- <i>F</i>	<i>p</i> -value
Unweighted UniFrac					
FM	HIM	20	999	2.224	0.043
FM	Feed FM	13	999	5.616	0.003
HIM	Feed HIM	13	999	3.688	0.015
Feed FM	Feed HIM	6	999	75.116	0.094
Weighted UniFrac					
FM	HIM	20	999	3.653	0.021
FM	Feed FM	13	999	4.546	0.007
HIM	Feed HIM	13	999	24.349	0.004
Feed FM	Feed HIM	6	999	355.807	0.094

and Paenibacillaceae have shown increased abundance in the gastrointestinal tract of fish that were given an insect-based diet (Fig. 4B). At the level of genus, the statistical analysis using Welch's *t*-test indicated that the diet had a significant impact on only three genera ($p < 0.05$) as shown in Fig. 7. The group of fish designated as the control exhibited a greater relative abundance of *Cetobacterium*, but the species *Oceanobacillus* and *Paenibacillus* were mostly discovered exclusively in the fish group HIM (Fig. 7).

Fig. 6 Principal coordinate analysis (PCoA) of unweighted and weighted UniFrac distances of gut microbial communities. The plots show the 2D representation of each fish and feed sample according to their microbial profile at the genus level

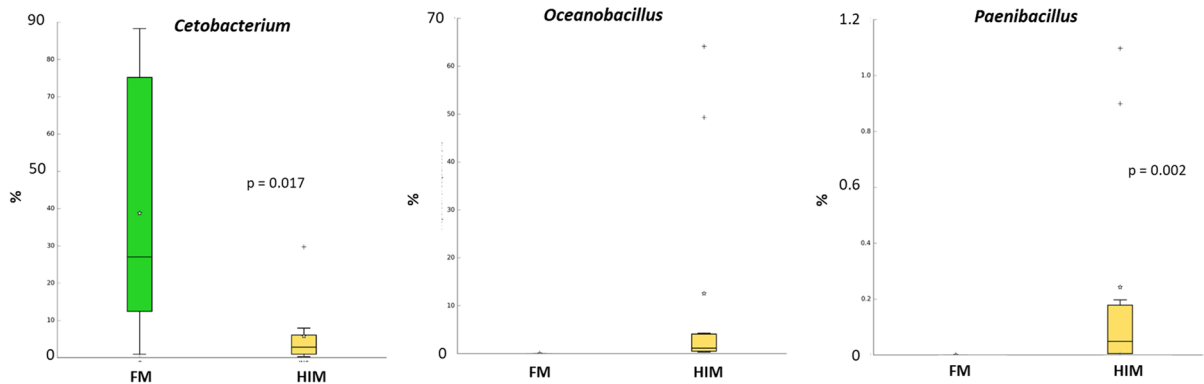
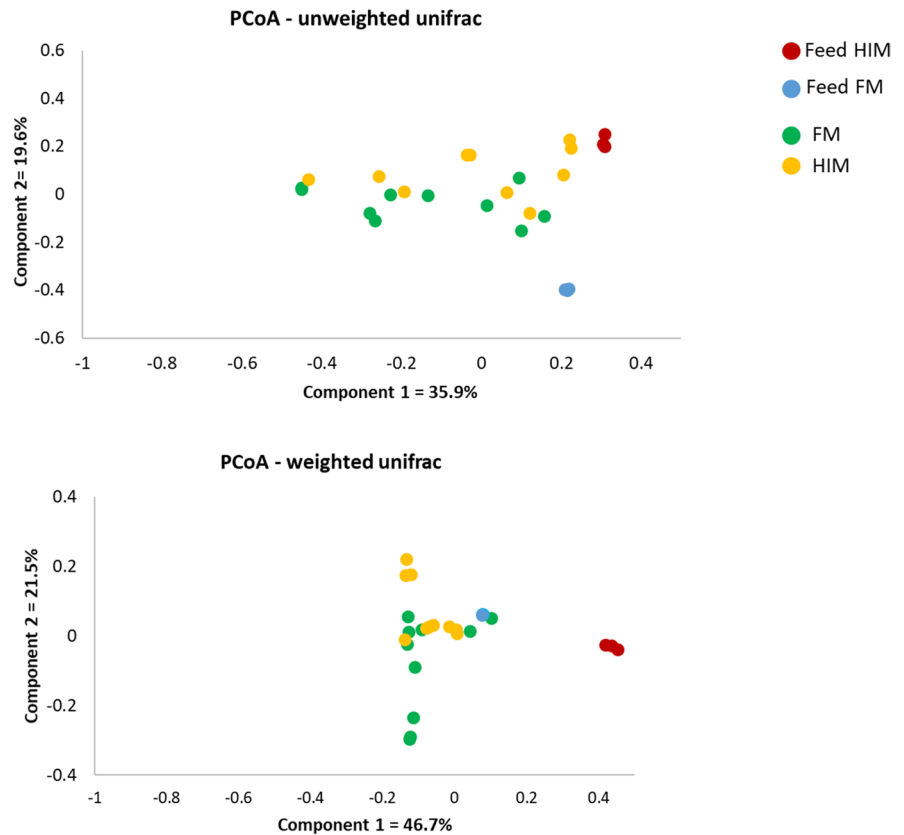


Fig. 7 Bacterial genera with significant influence of diet. Welch's *t*-test ($I < 0.05$)

Microbial metabolic pathway prediction

The metabolic activities of microbial communities were estimated using the PICRUSt software, which utilizes a comparative analysis of discovered 16S rRNA gene sequences with those of known genome-sequenced taxa to infer the potential gene contents of microbial

communities. A statistically significant difference was seen in a total of 18 pathways between the two feeding groups, as depicted in Fig. 8. It is noteworthy that fish that were provided with an insect-based diet had an elevated expression of genes associated with bacterial chemotaxis, motility, and the two-component signal transduction system.



Fig. 8 Results of PICRUSt analysis of predicted functional pathways in gut microbiota of control (FM) and HIM fish groups. Extended error bar graph and statistical analysis were generated using the bioinformatics software STAMP

Volatile SCFAs in fecal samples

The consumption of insects in the HIM group led to a noteworthy rise in short-chain fatty acids (SCFAs), specifically acetic and propionic acids,

in comparison to the FM diet ($p < 0.05$). In contrast, there were no significant differences seen in the amounts of butyric acid and isobutyric acid in fecal samples between the two experimental groups, as indicated in Table 10.

Table 10 Volatile SCFA content in fecal samples of two experimental groups. Results are expressed in millimoles per liter, mean \pm SD ($N=12$). * $p < 0.05$; *** $p < 0.001$; ns, $p > 0.05$

	Acetate (C2:0)	Propionate (C3:0)	Butyrate (C4:0)	Isobutyrate (C4:0)
FM	22.85 \pm 2.00	7.52 \pm 0.81	1.72 \pm 0.16	0.44 \pm 0.24
HIM	24.40 \pm 1.43	9.90 \pm 0.74	1.70 \pm 0.11	0.66 \pm 0.10
Sig	*	***	ns	ns

Discussion

To our knowledge, this is the first farm-scale study to investigate the change in gut microbiota and production of SCFAs following the administration of insect larvae meal in gilthead seabream. Several studies, especially in salmonids, investigated the effects of HI on the gut microbial profile. However, the results were usually obtained in fish reared in indoor or outdoor tanks under controlled environmental conditions, and few of these studies focused on seabream (Fabrikov et al. 2021; Naya-Català et al. 2021; Panteli et al. 2021; Piazzon et al. 2022; Busti et al. 2024).

During the 25-week feeding trial, the experimental diet HIM, which contained 11% insect meal, was well accepted by the fish and did not significantly affect growth performance. Accordingly, it has been recently reported that FM can be replaced (up to 35%) by a moderate admixture of HI meal without negative effects on growth parameters or gut histological characteristics of seabream (Carvalho et al. 2023; Di Rosa et al. 2023). In contrast, gilthead seabream showed lower growth and higher (poorer) feed conversion when fed diets with a low FM content, in which 10% insect meal was equivalent to 66% of the FM replacement (Carvalho et al. 2023). This indicates that the gilthead sea bream is very sensitive to the chitin content in a modern diet with a low FM content (5%) (Carvalho et al. 2023).

Chitin is present in insect meals as it is the main component of the insect exoskeleton. As it is not clear which fish species can digest chitin, it is generally assumed that it could negatively affect the nutrient digestibility of carnivorous fish (Gasco et al. 2019). In fact, it appears that fish tolerance to chitin initially depends on the fish species. For example, studies on Atlantic salmon and rainbow trout showed that a complete replacement of FM with insect meal in practical feeding is possible without negatively affecting growth, while

simultaneously increasing FCR (Lock et al. 2016; Belghit et al. 2019; Chemello et al. 2020). For gilt-head seabream, Carvalho and colleagues (2023) set a tolerance of 0.7% (0.7 g/kg) chitin. Unfortunately, the chitin content in our HIM feed was not measured to confirm this. Furthermore, the optimal level of insect meal seems to depend on the FM content in the feed. In the current study, the HIM diet had a relatively high FM content (16.5%), which was sufficient to meet the total nutritional requirements of the seabream and prevent not only growth losses but also morphometric and histopathological changes in the intestinal tract.

In agreement with our results, HIM was added up to 15% without negative effects on growth performance and feed conversion in gilthead seabream when FM was kept at a minimum of 10% (Busti et al. 2024). The amount of FM was successfully reduced to as low as 5%, even with a 10% proportion of HIM, when processed animal proteins from poultry were used in combination with FM in the diet of gilthead seabream (Bosi et al. 2021). Higher levels of HIM were achieved in gilthead seabream fed diets containing between 25 and 45% FM, a level that is no longer considered sustainable for aquafeed formulation (Panteli et al. 2021; Mastoraki et al. 2022; Karapanagiotidis et al. 2023).

Histological evaluation of the intestinal morphology did not reveal any changes in the mucosa or signs of inflammation. In agreement with our results, no abnormalities in gross intestinal morphology and no obvious signs of inflammation were observed in gilt-head seabream fed a diet containing 10% HIM (Bosi et al. 2021).

To further confirm this, in another pilot experiment conducted in marine RAS, changes in villus morphology were observed only in the anterior intestine of seabream fed a diet in which 50% of FM was replaced with HI meal, corresponding to a proportion of about 16% of insect meal inclusion, but not in a 35% FM

replacement with an 11% proportion of insect meal inclusion (Di Rosa et al. 2023).

Like gut, liver health assessment is also an important indicator of nutritional status and general well-being of fish (Rašković et al. 2011). The HSI showed similar values in fish fed HIM compared to control fish, confirming that there were no problems associated with insect meal ingestion and indicating an adequate supply of taurine or its precursors cysteine and methionine in the HIM diet, as reported by other studies which tested similar diet formulations (Oteri et al. 2021). Taurine is a nonessential metabolite involved in the regulation of lipid metabolism and facilitates digestion and absorption of dietary lipids in the intestine (Ripps and Shen 2012). For example, in Atlantic salmon, partial substitution of FM with high levels of inclusion of insect meal (60%) resulted in negative changes in viscerosomatic index and HSI, and these changes correlated with low dietary taurine content (Lock et al. 2016; Belghit et al. 2018). In the present study, inclusion of insect meal in the diet did not result in significant changes or signs of inflammation in hepatic tissue. Furthermore, histological analysis of the liver revealed no significant differences in lipid accumulation between the two feeding groups. The histomorphological features observed in the livers of our fish are consistent with results previously reported in gilthead seabream (Randazzo et al. 2021; Anedda et al. 2023; Di Rosa et al. 2023;) and in other fish species, such as rainbow trout (Biasato et al. 2022; Palomba et al. 2022), Atlantic salmon (Belghit et al. 2019), and sea bass (Zarantonello et al. 2023). In contrast, in sea bass, the highest FM replacement (20%) with full-fat spirulina-enriched HIM resulted in increased fat accumulation in the liver compared to control fish fed conventional marine diets (Zarantonello et al. 2023). This could be due to the different ratio of n-6/n-3 PUFAs in the diets, which may lead to a high n-6/n-3 ratio and thus impair lipid metabolism and promote hepatic fat accumulation in fish (Ferramosca and Zara 2014; Dong et al. 2023). In the present study, the n-6/n-3 ratio was similar in the two diets tested and the HIM diet included a defatted HI meal, which likely helped prevent hepatic steatosis or hepatocellular disease.

Nowadays, insect meal products are considered a promising alternative for FM, not only as a valuable source of proteins and lipids, but also as a functional ingredient that strengthens the immune system and

protects against pathogens (Somayeh et al. 2020). These health-promoting effects are largely attributed either to the direct broad-spectrum activity of the antimicrobial peptides produced by most insects (Sahoo et al. 2021) or to the indirect release of chitin or other bioactive compounds, such as medium-chain fatty acids. In particular, HI is characterized by a high content of lauric acid, which has been shown to have an inhibitory effect on several Gram-positive bacteria, such as *Clostridium perfringens*, *Staphylococcus aureus*, and *D-Streptococci* (Skrivanova et al. 2006; Spranghers et al. 2018). Indeed, dietary intake of a specific mixture of 1-monoglycerides of short and medium fatty acids (including lauric acid) positively modulated the gut microbiota of seabream by increasing beneficial lactic acid bacteria and reducing potentially pathogenic gamma proteobacteria (Rimoldi et al. 2018). Similarly, the indigestible polysaccharide chitin acts as a prebiotic that stimulates the growth of beneficial bacteria (Rangel et al. 2022a; Rimoldi et al. 2023) and has a bacteriostatic effect against various harmful Gram-negative bacteria (Nawaz et al. 2018).

In the last decade, several studies have shown that feeds enriched with insect meal are able to alter the microbial communities in the fish gut by increasing the abundance of the families Bacillaceae, Lactobacillaceae, and Actinobacteria (Huyben et al. 2019; Rimoldi et al. 2021, 2019; Terova et al. 2021, 2019; Bruni et al. 2020; Li et al. 2021; Gaudioso et al. 2021; Foyosal and Gupta 2022; Rangel et al. 2022a; Hasan et al. 2023). Accordingly, the gut microbial communities of fish fed HIM differed from those of the control groups at the end of the present feeding experiment. Although no increase in species richness or biodiversity was observed, multivariate analysis based on unweighted and weighted UniFrac dissimilarity data revealed differences in response to feeding. Regardless of the diet, the three most frequently observed phyla were Firmicutes, Proteobacteria, and Fusobacteria. The presence of Fusobacteria instead of Actinobacteria, which are often reported in gilthead seabream (Huyben et al. 2020; Parma et al. 2020; Piazzon et al. 2020; Rimoldi et al. 2020; Panteli et al. 2021; Solé-Jiménez et al. 2021; Busti et al. 2024), is remarkable. In contrast to the previous studies conducted in RAS or flow-through systems (FTS), the current feeding trial took place in sea cages under environmental conditions similar to those of commercial seabream farming. Fish reared in sea cages

are exposed to environmental conditions such as water temperature and salinity that can affect their gut microbiota. For example, the transition from freshwater to seawater, which is a common event in the life cycle of Atlantic salmon, has been shown to have profound effects on the host gut microbiota (Wang et al. 2021). In contrast, the gut microbiota of fish in an experimental-scale facility (RAS or FTS) could be influenced by bacterial communities in the rearing water, in biofilters (in RAS) and in biofilms on the tank surface, as well as by suspended solids (Bruno et al. 2023). Therefore, in addition to diet, different rearing conditions could also influence the gut microbiota of fish differently.

At the family level, gilthead seabream fed with HIM showed lower abundance of Fusobacteriaceae and higher abundance of Bacillaceae and Paenibacillaceae compared to controls. As mentioned above, an increase in the proportion of Bacillaceae has often been associated with the feeding of insect meal. Their increase is generally considered beneficial as this bacterial family contains several species with proven probiotic properties, such as *Bacillus subtilis* (Olmos et al. 2020). In contrast to previous data in rainbow trout, an 11% content of HI meal was not sufficient to increase the abundance of lactic acid bacteria in the gut of gilthead seabream (Terova et al. 2019). Unfortunately, very few studies have addressed the effects of feeding insect meal on the gut microbiota of gilthead seabream, especially in the context of novel formulations with low marine components. Furthermore, to our knowledge, none of these studies has been conducted on a farm scale in marine cages. Nevertheless, our results are in agreement with those of Busti et al. (2024), who found a significant enrichment of Bacillaceae and Paenibacillaceae with a concomitant reduction of LAB in insect-fed gilthead seabream. In contrast, Piazzon and colleagues (2022) found an increase in *Lactobacillus* genus in gilthead seabream fed FM-free diets containing 10% meal from HI, but in this case, 15% microbial biomass produced by fermentation of *Corynebacterium glutamicum* and *Methylococcus capsulatus* was also added to the diet. However, because this study evaluated the entire formulation and not the effects of each ingredient, it is not possible to relate this response specifically to the insect meal. In another study, the number of Fusobacteria in the gut microbiota of gilthead seabream increased, while the number of Firmicutes and Actinobacteria decreased in response to the

replacement of FM with HIM (Panteli et al. 2021). In the same study, an increased proportion of Staphylococcaceae was found in gilthead seabream fed with HI meal.

At the genus level, *Oceanobacillus* and *Paenibacillus* have been detected exclusively in the guts of fish fed HIM. Recently, according to our data, a strong increase in the relative abundance of the genera *Oceanobacillus* and *Paenibacillus* was reported in gilthead seabream in response to the inclusion of 5% to 15% HI meal in the diet (Busti et al. 2024). *Oceanobacillus* is considered a probiotic bacterium, and its association with the ingestion of insect meal or exuviae has already been established in rainbow trout (Rimoldi et al. 2021, 2023; Biasato et al. 2022)..

Oceanobacillus is also known for its ability to produce various enzymes, antibiotics, and exopolysaccharides and is significantly positively correlated with sucrose production in rainbow trout (Zhao et al. 2023).

Although the application of *Paenibacillus* in aquaculture is limited, this genus shares several characteristics with *Bacillus* that make *Paenibacillus* a good probiotic candidate. It can produce antimicrobial compounds and volatile organic compounds, improve growth performance and immune status of fish, and degrade non-starch polysaccharides (Gupta et al. 2016; Chen et al. 2019; Amoah et al. 2020).

However, the most interesting ability of the genus *Paenibacillus* is its chitinolytic activity (Grady et al. 2016; Rangel et al. 2022a, b). The presence of chitinolytic bacteria is particularly important when feeding insect-derived ingredients to fish because they promote chitin digestion. In agreement with our results, the modulation of the microbiota in trout digesta by HI meal was closely associated with the presence of *Paenibacillus* (Rimoldi et al. 2021). In European seabass, however, consumption of diets enriched with HI exuviae, but not HI meal, resulted in an increase in this genus (from not detected to 5.5% in relative abundance) (Rangel et al. 2022a).

In contrast, the gut microbiota of gilthead seabream fed FM was dominated by the genus *Cetobacterium*, which belongs to the phylum Fusobacteria. This phylum is widely distributed in the gut of omnivorous and carnivorous freshwater fish, and within Fusobacteria, *Cetobacterium* is the major commensal genus that is dominant in several fish species (Ofek et al. 2021). A *Cetobacterium* species closely related to *C. somerae* has been isolated and

characterized from the digestive tract of five freshwater fish species, demonstrating its ability to produce vitamin B12 (Tsuchiya et al. 2008). *C. somerae* is also involved in glucose homeostasis and carbohydrate metabolism in fish (Wang et al. 2021). In addition, the numerous genes in the genome of *C. somerae* related to protein digestion may indirectly explain the greater abundance of this genus in fish fed an animal diet compared to a plant diet (Hao et al. 2017). In contrast, proteins from plant ingredients, mainly soybean meal, are associated with a high intake of dietary fiber and indigestible polysaccharides. Consequently, carbohydrate-active bacteria such as the genus *Bacillus*, whose genomes contain members of the cellulase, hemicellulose, and pectinase families, are generally positively associated with plant-based diets (Serra et al. 2019).

However, predictive functional profiling of the microbial communities revealed no difference in carbohydrate or protein metabolism between the two feeding groups. PICRUSt analysis revealed that genes associated with motility, chemotaxis, and the two-component signal transduction system were significantly more abundant. In general, bacteria use chemotaxis to enhance the acquisition of high-quality nutrients in their environment, but it could also improve the efficiency of environmental colonization by motile bacteria, swarming, and biofilm formation (Colin et al. 2021). The two-component signal transduction system is instead involved in mediating the response of bacteria to a wide range of signals and stimuli, including nutrients (Laub and Goulian 2007). This result may be due to the higher proportion of indigestible carbohydrates such as chitin in the diet of HIM, which act as probiotics to stimulate the growth and/or activity of certain microorganisms, such as chemotactic bacteria from the Bacillaceae family, which are abundant in the guts of HIM.

Accordingly, the levels of volatile SCFAs, especially acetic and propionic acids, were higher in fecal samples from fish fed HIM. SCFAs, which include butyrate, are the main metabolites produced in the intestine by bacterial fermentation of dietary fiber, and they act as anti-inflammatory agents that play a key role in regulating the immune system of fish (Canani et al. 2011; Terova et al. 2016). They are the main product of bacterial fermentation of chitin produced by chitinolytic bacteria, such as *Paenibacillus*, which were present in greater

abundance in the insect meal feeding group. Similarly, rainbow trout fed diets containing exuviae or shrimp meal had increased levels of acetic acid and propionic acid in feces compared to FM (Rimoldi et al. 2023). Inclusion of insect exuviae in the diet also increased butyrate content, a desirable effect that we were unfortunately unable to achieve in this experiment.

In conclusion, the present study represents one of the few on-farm experiments with gilthead seabream, one of the most important species for aquaculture in the Mediterranean. The results confirmed that HI is a suitable alternative to FM, even at 11%, which is in line with the aqua feed industry's demand to reduce feed production costs. Overall, the data suggest that HI meal may provide benefits to gut health and microbiota profile by enhancing probiotic and chitinolytic bacterial genera, leading to improved utilization of nutrients with the production of SCFAs that are absorbed by the host, contributing to its energy metabolism.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by S. Rimoldi, AR. Di Rosa, G. Terova, M. Saroglia, and B. Chiofalo. The first draft of the manuscript was written by S. Rimoldi, and G. Terova and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability All raw sequencing data were submitted to the European Nucleotide Archive (EBI ENA) public database under accession code PRJEB64777.

Declarations

Ethical approval The experiment was carried out at the “Maricoltura Sarde Srl” farm located in Sant’Antioco, a region in South Sardinia, Italy. The Ethical Committee of the University of Messina, Department of Veterinary Sciences, granted approval for the experimental procedure (Authorization No. 082/2022).

Competing interests Marco Saroglia is an associate editor of the journal Fish Physiology and Biochemistry. The authors have no other relevant financial or nonfinancial interests to disclose.

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