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Replacing fishmeal with an insect meal blend: Implications for intestinal microbiota in European seabass

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ABSTRACT

In this study, we investigated the effects of an insect meal (IM) blend containing larvae of *Hermetia illucens* and *Tenebrio molitor* on the gut microbiota of European seabass (*Dicentrarchus labrax*), a key species in Mediterranean marine aquaculture. This research stands out by examining a combination of IMs rather than a single source, thereby addressing an underexplored area in the current literature. In a feeding trial that lasted 75 days, European seabass were fed three different diets: a commercial-like control diet and two experimental diets containing partial replacement of FM with IM (25 % and 50 %). The experimental diets showed no adverse effects on feed intake or overall fish health. Analysis of the microbiota revealed significant changes in gut microbial communities, with a trend towards increased bacterial richness and diversity in fish fed IM. Beta diversity analysis showed that the mucosa-associated microbial communities were stable across diets, while the digesta-associated microbiota showed notable shifts in the IM25 and IM50 groups, suggesting that the transient microbiota is more sensitive to dietary changes. The study also found an enrichment of beneficial bacterial genera, particularly *Bacillus* and *Paenibacillus*, in fish fed IM. These genera, known for their chitinolytic activity, have likely adapted to the increased chitin content in IM diets. *Oceanobacillus* (*Bacillaceae*) and *Brevibacterium* (*Brevibacteriaceae*) were more abundant in the digesta of fish from the IM25 and IM50 groups, but not in the mucosa. Their presence indicates that they react more strongly to changes in diet than to a stable mucosal environment. Overall, the study highlights the potential of an IM-based diet to support fish health and growth while promoting a favorable gut microbiota.

1. Background

The success of the aquafeed industry depends on the availability of high-quality raw materials. Fishmeal (FM) has a high nutritional value with a well-balanced amino acid profile and a variety of micronutrients. However, its limited availability to address a rising demand, associated with sustainability perception and prices volatility, underscores the need for alternative protein sources in fish diets (Glencross et al., 2024; Serra et al., 2024). Since the EU's approval in 2017 of the inclusion of insect meals (IMs) in aquafeeds, insects such as *Hermetia illucens* (black soldier fly larva, BSFL) and *Tenebrio molitor* (yellow mealworm, YM) have emerged as some of the most widely explored protein sources (Gasco et al., 2023). BSFL is composed of 50–60 % crude protein and is a rich source of essential amino acids, including glutamate, aspartate, alanine, lysine, and leucine (Siddiqui et al., 2024). Similarly, YM meal is another promising insect protein, with a high crude protein content (40–63 %) and an amino acid profile comparable to FM (Makkar, 2018). These IMs also provide bioactive compounds that, even at low inclusion levels, may stimulate immune responses, modulate gut microbiota, and offer antioxidant and anti-inflammatory benefits. These may include chitin, antimicrobial peptides, and medium-chain fatty acids like lauric acid (da Silva Lucas et al., 2020; Gasco et al., 2023).

Despite their promising nutritional profiles, both BSFL and YM meals still face challenges that may limit their broader adoption in aquafeeds.

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Both protein and lipid levels in BSFL and YM meals vary significantly depending on rearing conditions and processing technologies (Oonincx and Finke, 2021). Additionally, these IMs are relatively low in lysine and methionine – two essential amino acids in diets for marine fish species like European seabass (*Dicentrarchus labrax*) – requiring supplementation from other sources to meet the dietary needs of these species when used at high inclusion levels (Basto et al., 2021, 2023). Furthermore, the use of YM in aquafeeds presents a potential conflict, as it competes with its rising demand for human consumption (Gasco et al., 2023). Despite the rapid growth of the insect protein sector, current production volumes remain insufficient for widespread use in aquafeeds (Glencross et al., 2024). To overcome these challenges, combining IMs from different species, leveraging their distinct nutritional profiles, could offer feed manufacturers greater flexibility in feed formulation and reduce reliance on a single source (Basto et al., 2021; Oonincx and Finke, 2021).

When testing feed formulations, besides the zootechnical performance and fish physiological status, the characterization of the intestinal microbiota can be useful in evaluating their effect on fish health and welfare. Several features of the gut microbiota have been investigated as markers of host health and disease. These include the *Firmicutes*: *Proteobacteria* and *Firmicutes*: *Bacteroidetes* ratios, changes in alpha and beta diversity, and the abundance of specific taxa. An increase in *Proteobacteria*, together with a decrease in *Firmicutes* and *Bacteroidetes*, was associated with intestinal disease in grass carp (*Ctenopharyngodon idella*) (Tran et al., 2018). In humans, *Firmicutes*: *Bacteroidetes* ratio has been associated with energy harvest and homeostasis (Shortt et al., 2018). Changes in the microbiota of diseased rainbow trout (*Oncorhynchus mykiss*) were found by Parshukov et al. (2019), including a decrease in alpha diversity compared to healthy controls, as well as an increase in *Pseudomonas*, a potential pathogen. Indeed, today, the characterization of intestinal bacterial communities is becoming a common practice in studies that aim to evaluate experimental aquaculture feeds in various fish species, including rainbow trout (Giannenas et al., 2012; Tefal et al., 2024), gilthead seabream (*Sparus aurata*) (Fontinha et al., 2021; Rimoldi et al., 2024a), and European seabass (Torrecillas et al., 2017; Serra et al., 2021; Pleić et al., 2022). The composition, diversity and function of the gut microbiota ultimately impact feed utilization and efficiency. Li et al. (2025) found that bacterial species richness and diversity were higher in the gut of fish with a higher feed efficiency when fed a FM-free diet. In Atlantic salmon (*Salmo salar)*, a significant association was found between taxa present in the gut microbiota and host lipid carbon metabolism, possibly due to a direct contribution of bacteria in the gut to nutrient absorption (Dvergedal et al., 2020).

The status of the intestinal microbiota is of particular interest when it comes to investigating feeds containing insects as a primary source of protein. Chitin, the polysaccharide that makes up the exoskeleton of insects, has an inherently resistant structure that renders its digestion difficult especially for carnivorous fish, and some studies have suggested that its presence in feeds may hinder fish growth and performance (Freccia et al., 2020). On the other hand, many recent studies have concluded that the chitin present in IM feeds can be beneficial in various species, thanks to its prebiotic effect (Freccia et al., 2020; Fantatto et al., 2024). Understandably, results vary depending on feed formulations, inclusion rates and fish species. What is undeniable, however, is the role of the gut microbial community in metabolizing chitin and releasing beneficial short-chain fatty acids (SCFAs) in the process, thus managing to improve feed utilization and energy uptake from IM feed (Biasato et al., 2022; Stull and Weir, 2023; Rimoldi et al., 2024a).

This study aims to explore the potential of using a mixture of BSFL and YM meals to partially replace FM in diets for European seabass, hypothesizing that this mixture will support fish growth and promote a beneficial gut microbiota.

2. Materials and methods

2.1. Ingredients and diets

Three diets were formulated to be isoproteic (45.9 % dry matter, DM), isolipidic (18.7 % DM), and isoenergetic (22.5 kJ/g DM). A practical control diet (CTRL) was formulated to reflect a typical commercial aquafeed and included 15 % fishmeal. Two other experimental diets included a 50:50 mixture of defatted BSFL meal and YM meal to replace 25 %, and 50 % (IM25 and IM50, respectively) of the FM protein of the CTRL. The defatted BSFL and YM meals were supplied by Entogreen®-Ingredient Odyssey S.A. (Santarém, Portugal) and ThunderFoods Lda. (Santarém, Portugal), respectively. All diets met the nutritional requirements of European seabass juveniles and were extruded by SPAROS Lda. (Olhão, Portugal). The chemical composition and amino acid

Table 1
Ingredients (%) and proximate composition [% dry matter (DM)] of the black soldier fly larva (BSFL) and yellow mealworm (YM) meals and diets.

	Insect meals (IMs)		Diets		
	BSFL	YM	CTRL	IM25	IM50
Ingredients					
BSFL meal[a]	100	-	-	2.15	4.20
YM meal[b]	-	100	-	2.15	4.20
Fishmeal Super Prime[c]	-	-	15.00	11.25	7.50
Fish protein hydrolysate[d]	-	-	3	3	3
Poultry meal[e]	-	-	6	6	6
Poultry blood meal[f]	-	-	3	3	3
Soy protein concentrate[g]	-	-	10	10	10
Wheat gluten[h]	-	-	5.7	5.7	5.7
Corn gluten meal[i]	-	-	12	12	12
Soybean meal[j]	-	-	12.5	12.5	12.5
Sunflower meal 40[k]	-	-	4	4	4
Wheat meal[l]	-	-	6.10	5.25	4.45
Whole peas[m]	-	-	5	5	5
Vitamin and mineral premix[n]	-	-	1	1	1
Choline chloride 50	-	-	0.2	0.2	0.2
Antioxidant	-	-	0.2	0.2	0.2
Sodium propionate	-	-	0.1	0.1	0.1
Monocalcium phosphate	-	-	1.00	1.40	1.85
Sardine oil[o]	-	-	4	4	4
Salmon oil[p]	-	-	8.2	8.6	9.3
Rapeseed oil[q]	-	-	3.0	2.5	1.8
Chemical composition					
Dry matter (%)	93.9	90.5	94.7	93.9	93.0
Crude protein (N x 6.25)	55.1	76.1	45.6	45.7	46.3
Crude protein (N x 5.33)	47.0	64.9	-	-	-
Crude fat	8.98	6.17	18.7	18.5	18.7
Ash	10.3	5.93	8.32	8.06	7.52
Phosphorus	0.72	0.98	0.93	0.94	0.94
Chitin[r]	6.28	5.59	0.71	0.82	0.95
Gross energy (kJ/g DM)	19.0	20.0	22.5	22.5	22.7

[a] Entogreen, Portugal, [b] ThunderFoods, Portugal, [c] Diamante, Pesquera Diamante, Peru (Crude protein, CP: 66.3 %; Crude fat, CF: 11.5 %), [d] CPSP90, Sopropêche, France (CP: 82.6 %; CF: 9.6 %), [e] Savinor UTS, Portugal (CP: 62.4 %; CF: 12.5 %), [f] Savinor UTS, Portugal, [g] Soycomil P, ADM, The Netherlands (CP: 62.2 %; CF: 0.7 %), [h] Vital, Roquette, France (CP: 80.4 %; CF: 5.8 %), [i] Copam, Portugal (CP: 61.2 %; CF: 5.2 %), [j] Fiskå Mølle, Norway, [k] AGP Slovakia, s.r.o., Slovakia (CP: 42.9 %; CF: 3.8 %), [l] Molisur, Spain (CP: 11.7 %; CF: 1.6 %), [m] Ribeiro e Sousa Lda, Portugal, [n] Premix Lda, Portugal. Vitamins (IU or mg/kg diet): 100 mg DL-alphatocopherol acetate; 25 mg sodium menadione bisulphate; 20000 IU retinyl acetate; 2000 IU DL-cholecalciferol; 30 mg thiamine; 30 mg riboflavin; 20 mg pyridoxine; 0.1 mg cyanocobalamin; 200 mg nicotidin acid; 15 mg folic acid; 1000 mg ascorbic acid; 500 mg inositol; 3 mg biotin; 100 mg calcium panthotenate; 1000 mg choline chloride; 500 mg betaine. Minerals (g or mg/kg diet): 0.65 mg cobalt carbonate; 9 mg copper sulphate; 6 mg ferric sulphate; 0.5 mg potassium iodide; 9.6 mg manganese oxide; 0.01 mg sodium selenite; 7.5 mg zinc sulphate; 400 mg sodium chloride; 1.86 g calcium carbonate; excipient wheat middling's.[o, p] Sopropêche, France, [q] JC Coimbra, Portugal. [r] Chitin levels of IM25 and IM50 were determined by subtracting the hexosamine content in CTRL from that of the respective diet.

profile of the IMs and diets are listed in Tables 1 and 2, respectively.

2.2. Chemical analysis of the insect meals and diets

The proximate composition of the BSFL and YM meals and diets was assessed in conformity with the AOAC (2006) methodology. DM was determined after drying at 105 ºC (24 h); ash was determined after combustion in a muffle furnace at 550 ºC (6 h); phosphorus was quantified in ash samples, after digestion with 6 M HCl at 150 ºC, and following the spectrophotometric method described by Marques et al. (2023) (820 nm); gross energy was quantified with an adiabatic bomb calorimeter (C2000, IKA-Werke GmbH & Co. KG, Germany); crude protein was determined by nitrogen quantification through the Dumas method (FP-528, Leco Corporation, U.S.A.) and multiplication by a nitrogen-to-protein conversion factor (K_p). A K_p of 5.33 was used for the IMs, as suggested by Boulos et al. (2020), and a K_p of 6.25 was used for the diets. Crude fat was quantified by petroleum ether extraction, at 40–60 ºC (SoxtecTM 2055, Foss, Sweden). However, for the IMs, a hydrolysis was first conducted (2.5 g of sample in 100 mL of 3 M HCl at 100 ºC for 1 h), the hydrolysate was filtered (filter paper with 5–8 μm of pore diameter), and the retentate was dried overnight (50 ºC). Chitin levels of the IMs and diets were estimated as described by Guerreiro et al. (2020).

The amino acid composition of the IMs and diets was assessed by ultra-high-performance liquid chromatography on a Waters Reversed-Phase Amino Acid Analysis System, after hydrolysis with 6 M HCl at 116 ºC (72 h), as described by Teodósio et al. (2021). The chromatographic peaks were analysed using the EMPOWER software (Waters, U.S.A.), and amino acid levels were expressed as % DM. Tryptophan levels are not shown because of partial loss during hydrolysis.

2.3. Feeding trial and sampling

European seabass juveniles (13 ± 1 g), acquired from Culmárex S.A.U. (Águilas, Spain) and transported to the Fish Culture Experimental Unit of CIIMAR (Matosinhos, Portugal), were distributed in 250-L fiberglass tanks (75 fish per tank) within a saltwater recirculation system (22.1 ± 0.3 °C, 35.2 ± 0.6 ppt, and 12-h of light/12-h of dark photoperiod), establishing homogeneous groups. Each experimental diet was randomly assigned to three groups of fish and hand-fed until visual satiety three times daily (8:30, 12:00, and 16:00) for 75 days. At the end of the feeding trial, and 6 h after feeding, all fish were lightly anesthetised (2-phenoxyethanol, 300 μL/L) for individual weight measurement (g). After weighing, five fish per tank were euthanised by a spinal cord section, and intestinal mucosa along with digesta were collected from each fish, as described by Monteiro et al. (2023). Additionally, 10 g of each experimental diet were collected for analysis of the feed microbiota. All samples were immediately frozen in liquid nitrogen and stored at −80 °C until DNA extraction.

Table 2
Amino acid profile [% amino acids] of the black soldier fly larva (BSFL) and yellow mealworm (YM) meals and diets.

	Insect meals		Diets		
	BSFL	YM	CTRL	IM25	IM50
Essential amino acids (EAA)					
Arginine	5.50	5.59	5.86	5.97	5.94
Histidine	3.13	3.35	2.56	2.64	2.73
Lysine	6.60	5.93	5.99	5.98	5.86
Threonine	4.47	4.43	4.13	4.23	4.18
Isoleucine	5.02	4.84	4.91	4.99	4.96
Leucine	7.13	7.41	9.13	8.89	8.86
Valine	6.54	6.88	5.13	5.21	5.29
Methionine	2.37	1.81	2.54	2.56	2.59
Phenylalanine	5.05	4.30	5.22	5.23	5.26
ΣEAA	45.8	44.6	45.5	45.7	45.7
Non-essential amino acids (NEAA)					
Cystine	1.69	1.47	2.21	2.12	2.33
Tyrosine	8.49	8.54	4.38	4.49	4.69
Aspartic acid + Asparagine	10.1	8.60	9.00	9.16	9.12
Glutamic acid + Glutamine	11.9	11.9	17.1	16.7	16.4
Alanine	6.15	7.64	5.88	5.84	5.86
Glycine	5.35	5.56	4.88	4.91	4.85
Proline	5.69	6.38	5.94	5.94	5.94
Serine	4.83	5.39	5.13	5.13	5.12
ΣNEAA	5.50	5.59	5.86	5.97	5.94
Total	100	100	100	100	100

2.4. Feed efficiency and growth performance

Voluntary feed intake (VFI), feed conversion ratio (FCR), daily growth index (DGI), and fish weight gain were calculated as described by Basto et al., (2023). W_i and W_f are the fish's initial and final weights (g), respectively, and ABW the average body weight (g) [$(W_f + W_i) / 2$]: voluntary feed intake (VFI, % day^{-1}) = 100 × feed intake / ABW / days; feed conversion ratio (FCR) = dry feed intake / (W_f - W_i); daily growth index (DGI, g day^{-1}) = 100 × ($W_f^{1/3}$ - $W_i^{1/3}$) / days; weight gain (g) = W_f – W_i.

2.5. DNA extraction, next generation sequencing, and raw data analysis

Bacterial metagenomic DNA was extracted from 300 μL of intestinal mucosa samples, 250 mg of gut content samples, and 150 mg of each feed (in triplicate). The DNeasy PowerSoil Pro kit (Qiagen, Italy) was used following the instructions provided by the manufacturer. An additional step of mechanical lysis with TissueLyserII (Qiagen) was employed. 16S rRNA V4 amplicon libraries were prepared with the metagenomic DNA and sequenced with next generation sequencing (NGS) by GALSEQ Srl (Milan, Italy) on the Illumina NovaSeq 6000 system (San Diego, CA, USA). A paired-end 2 × 150 bp approach was used. All sequences were submitted to European Nucleotide Archive (EBI ENA) under the accession code **PRJEB81551**.

Raw sequencing data was processed with the QIIME 2™ pipeline. Following quality control and denoising, samples were grouped based on sequence identity using the Amplicon Sequence Variant (ASV) approach. The ASVs were compared to known sequences in the SILVA database and taxa were assigned down to the genus level (https://www.arb-silva.de/). Alpha diversity indices, as well as beta diversity based on unweighted and weighted UniFrac distances, were calculated. DNA extraction, library preparation for NGS, and raw data analysis have been described in detail by Rimoldi et al. (2021).

2.6. Predictive functional analysis of intestinal microbiota

PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used to predict the functional potential of gut microbial communities, based on the KEGG database (Douglas et al., 2020). Extended error bar plots were then generated with the Statistical Analysis of Metagenomic Profiles (STAMP) software.

2.7. Statistics

All data were tested for normality with the Shapiro-Wilk test. One-way analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test was used for statistical comparisons, depending on the normality of the data. Welch's two-sided *t*-test was used to make pairwise comparisons with STAMP. Permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM) were used to test the significance of differences in beta diversity. All tests were performed with Past4 v. 4.02 using a $p < 0.05$ as a threshold for significance.

3. Results

3.1. Feed efficiency and growth performance

After 75 days of feeding, fish mortality rate was negligible (< 1 %), and all diets were equally well-accepted by European seabass juveniles, resulting in similar VFI and FCR (Fig. 1A and Fig. 1B, respectively) across all groups. All fish had similar DGI and almost quintuplexed their initial average body weight (average weight gain of 45 g), reaching a final body weight of 58 g (Fig. 1C-E).

3.2. Sequencing efficiency

A total of 63 samples were successfully sequenced and analysed, with 27 being intestinal mucosa samples (9 per group), 27 gut content samples (9 per group), and 9 feed samples (3 per feed). As a result, 5177,184 sequencing reads were obtained. The good's coverage index was above 99 % for all mucosa samples and above 98 % for gut content and feed samples. These values indicate a sufficient sequencing depth for revealing all bacteria present in the analysed samples. To calculate the alpha diversity indices, the sequencing depth was set to 46,104, based on rarefaction curves.

3.3. Microbial profiles of feeds

Considering only the taxa with a mean relative abundance equal to or higher than 1 % (phylum, class, and order), or 0.2 % (family and genus), the microbial community of the feeds comprised 5 phyla, 7 classes, 9 orders, 22 families, and 38 genera (Additional file 1). The most abundant phyla in all three feeds were *Firmicutes* and *Protebacteria* (Fig. 2A). Pairwise comparisons revealed a significant decrease in the proportion of *Firmicutes* in samples from the IM25 group (51.6 %) as compared to those of the CTRL (54.7 %). Such a decrease was not observed in samples from the IM50 feeding group. At the same time, a significant decrease in the proportion of *Proteobacteria* was observed in IM50 samples (37.9 %) as compared to IM25 samples (42 %). Some of the most abundant families were *Lactobacillaceae* (*Lactobacillus*), *Streptococcaceae* (*Lactococcus*, *Streptococcus*), and *Peptostreptococcaceae* (*Peptostreptococcus*), all of which belong to *Firmicutes* (Fig. 2B). At the genus level, the relative abundance of 13 and 12 genera differed significantly between the CTRL feed and the IM25 and IM50 feeds, respectively. Alpha indices of both species richness and biodiversity did not significantly differ between the feeds (Table 3). As for beta diversity, PERMANOVA and ANOSIM indicated no significant differences in the Unweighted and Weighted UniFrac distances (Additional file 2). Furthermore, statistical tests revealed significant differences between the Unweighted and Weighted UniFrac distances between feed samples, and gut mucosa and digesta samples revealed ($p < 0.05$, Additional file 3).

3.4. Mucosa- and gut content- associated microbial communities

Mucosa-associated communities comprised 4 phyla, 6 classes, 15 orders, 41 families, and 41 genera when considering only the taxa with a mean relative abundance above the set thresholds (see 3.3) (Additional file 4). Similar numbers were observed in gut content samples, with 4 phyla, 6 classes, 13 orders, 31 families, and 33 genera (Additional file 5). According to comparison tests of alpha indices, there was no variation in the alpha diversity ($p > 0.05$) of mucosa or gut content samples from the CTRL, IM25, and IM50 groups (Additional file 6).

Unweighted and weighted UniFrac distances between all samples are graphically represented by Principal Coordinates Analysis (PCoA) plots (Fig. 3). The UniFrac distances of gut mucosa and digesta samples appear to be separated from feed samples in the plot, in particular the weighted distance (Fig. 3B). Beta diversity of the mucosa-associated

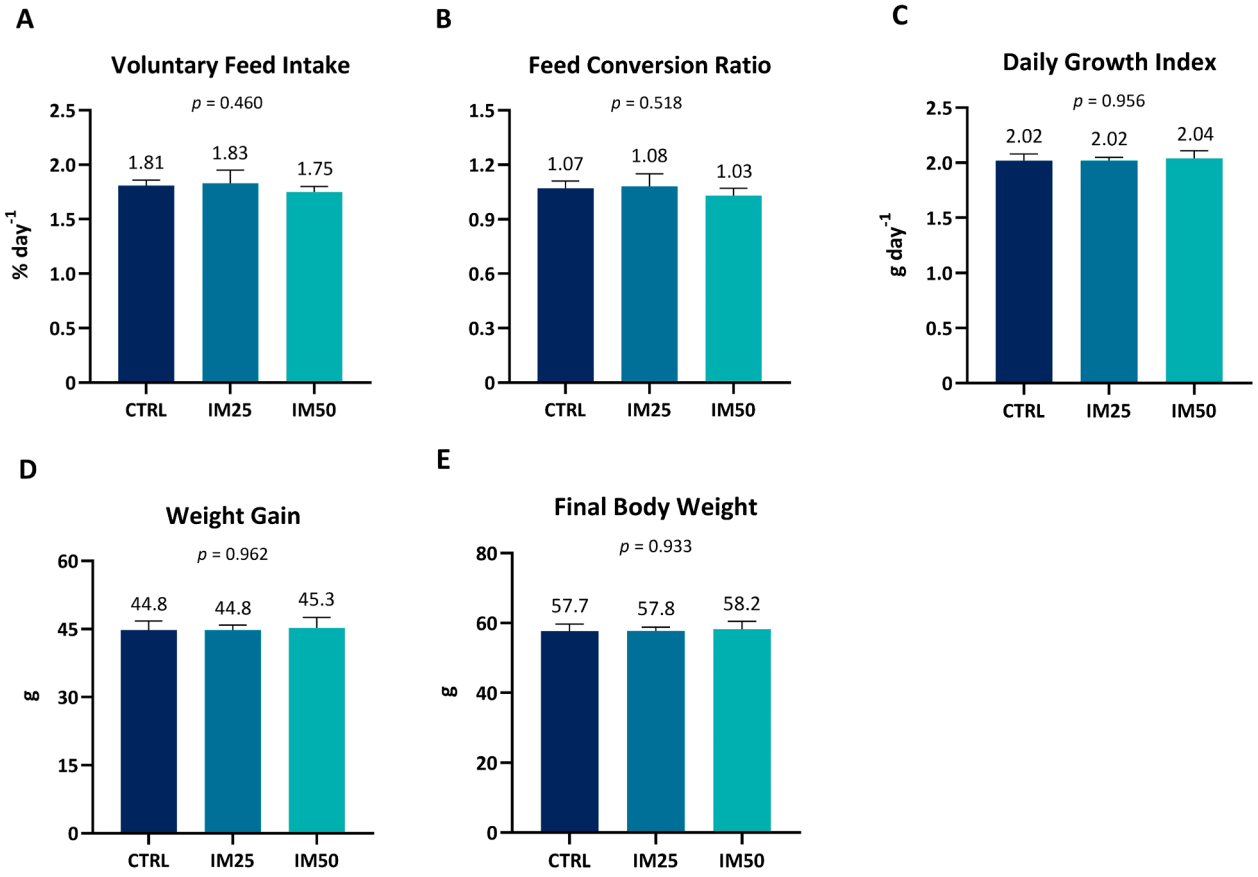

Fig. 1. Growth performance and feed efficiency of European seabass fed the experimental diets. Values are expressed as mean ± standard deviation (n = 3 tanks).

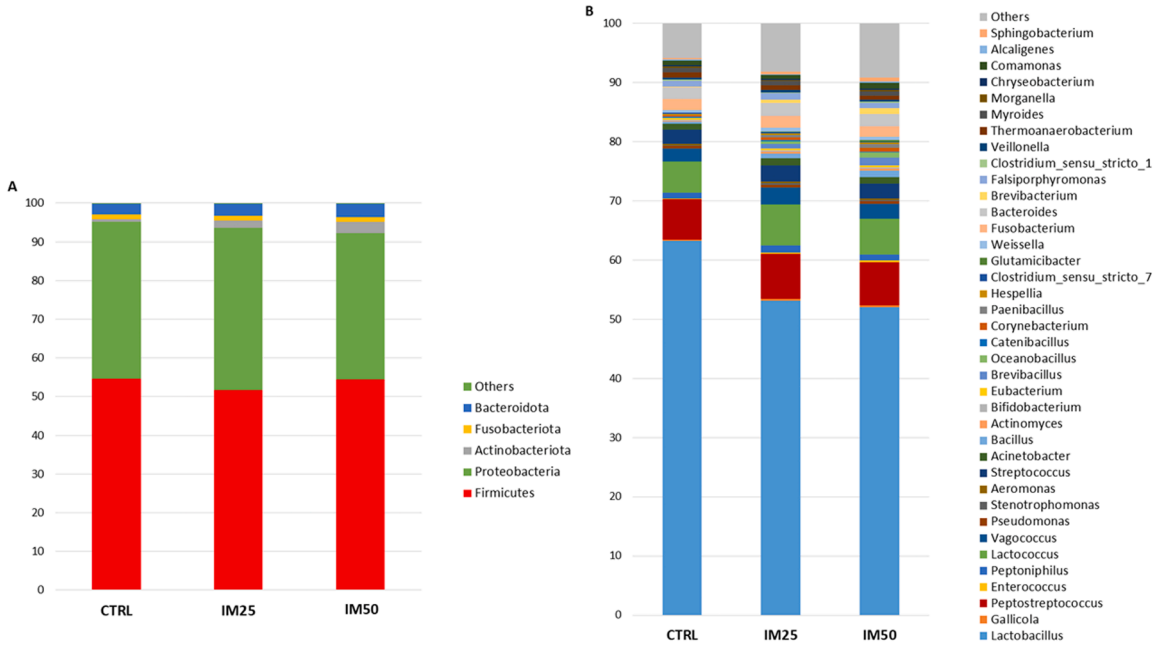

Fig. 2. Stacked bar chart of the mean relative abundance (%) of most abundant bacterial phyla (A) and genera (B) in feed samples.

Table 3
Alpha diversity indices of feed bacterial communities (mean ± standard deviation).

	CTRL	IM25	IM50	*p* value
Observed OTUs	1517 ± 23	1810 ± 40	1913 ± 72	> 0.05
Chao1	2102 ± 23	2562 ± 60	2631 ± 86	> 0.05
Faith PD	26.4 ± 0.4	28.7 ± 0.6	29.1 ± 0.7	> 0.05
Shannon	6.16 ± 0.01	6.25 ± 0.02	6.46 ± 0.03	> 0.05
Simpson	0.95 ± 0.00	0.94 ± 0.00	0.95 ± 0.00	> 0.05

communities did not seem to be impacted by diet, both qualitatively and quantitatively. However, some differences were observed in the case of gut content samples. Unweighted UniFrac distances of samples from the treatment feeding groups were separated from those from CTRL in the PCoA plot (Fig. 3A). While qualitative differences were present, there were no quantitative differences in the beta diversity between the three groups (Fig. 3B). The differences in beta diversity, or lack thereof, were confirmed by ANOSIM and PERMANOVA (Table 4).

3.5. Effect of diet on mucosa-associated and gut content- associated microbiota

A look into the relative abundances of taxa in our samples revealed that the most abundant phyla were *Firmicutes* and *Proteobacteria*, followed by *Actinobacteriota*, in spite of diet or sample origin (Fig. 4A, C). The ratio between the first two showed a tendency to increase in treatment groups compared to CTRL. However, these differences were found to be statistically insignificant. In all cases, *Firmicutes* was represented by *Lactobacillus*, *Peptostreptococcus* and *Lactococcus*, which were the most abundant genera across all samples (Fig. 4B, D).

To assess the possible effects of the treatment diets on the composition of the intestinal microbiota, we performed pairwise comparisons of the relative abundances between the CTRL group, and the IM25 and IM50 groups. The genera for which significant differences were revealed ($p < 0.05$) are listed in Table 5. An increased fraction of *Bacillus* was observed in the mucosa and gut content samples from the IM25 and IM50 groups. Although the relative abundance was generally higher in mucosa samples, the observed increase was higher in gut content samples (8- and 9- fold increase compared to CTRL). At the same time,

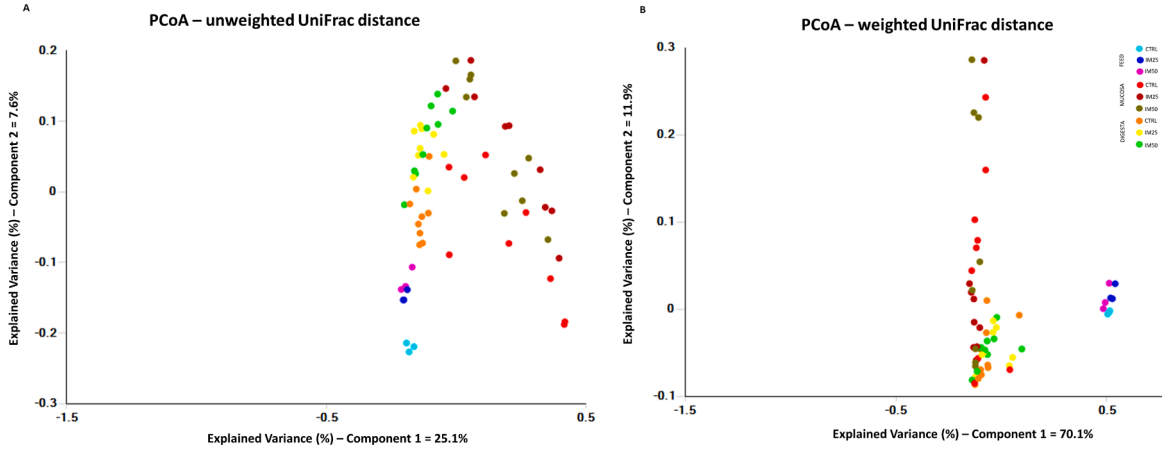

Fig. 3. Principal Coordinate Analysis (PCoA) plot based on unweighted and weighted UniFrac distances of mucosa, digesta, and feed microbial communities at the genus level.

Table 4
Verification of differences in beta diversity by ANOSIM and PERMANOVA on unweighted and weighted UniFrac distances, mucosa and digesta samples.

Unweighted UniFrac distances					
ANOSIM					
Mucosa		Sample size	Permutations	R	*p* value
CTRL	IM25	18	999	0.130	0.089
CTRL	IM50	18	999	0.127	0.053
IM25	IM50	18	999	0.000	0.377
Digesta		Sample size	Permutations	R	*p* value
DCT	DIM2	18	999	0.350	**0.001**
DCT	DIM5	18	999	0.426	**0.001**
DIM2	DIM5	18	999	−0.014	0.541
PERMANOVA					
Mucosa		Sample size	Permutations	pseudo-F	*p* value
CTRL	IM25	18	999	1.391	0.100
CTRL	IM50	18	999	1.525	0.054
IM25	IM50	18	999	0.988	0.384
Digesta		Sample size	Permutations	pseudo-F	*p* value
CTRL	IM25	18	999	2.359	**0.001**
CTRL	IM50	18	999	2.613	**0.001**
IM25	IM50	18	999	0.982	0.499
Weighted UniFrac distances					
ANOSIM					
Mucosa		Sample size	Permutations	R	*p* value
CTRL	IM25	18	999	0.093	0.060
CTRL	IM50	18	999	0.035	0.224
IM25	IM50	18	999	−0.014	0.461
Digesta		Sample size	Permutations	R	*p* value
CTRL	IM25	18	999	0.126	**0.037**
CTRL	IM50	18	999	0.140	**0.015**
IM25	IM50	18	999	−0.047	0.679
PERMANOVA					
Mucosa		Sample size	Permutations	pseudo-F	*p* value
CTRL	IM25	18	999	1.335	0.258
CTRL	IM50	18	999	1.179	0.323
IM25	IM50	18	999	0.776	0.542
Digesta		Sample size	Permutations	pseudo-F	*p* value
CTRL	IM25	18	999	1.185	0.336
CTRL	IM50	18	999	1.531	0.216
IM25	IM50	18	999	0.349	0.754

Bacillus was found to be more abundant in the mucosa of fish from the IM25 group (3.90 %) than of the IM50 (2.44 %). *Paenibacillus*, *Oceanobacillus*, and *Brevibacterium* were all positively associated with IM diets. A decrease was detected in the proportion of *Lactobacillus* in digesta samples from the IM50 group (30.55 %) compared to CTRL (37.84 %), but not in those from IM25 (34.11 %).

3.6. Functional potential of mucosa- and gut content- associated microbiota

We predicted the metabolic pathways present in the gut microbiota of our seabass with PICRUSt2. Pairwise comparison with Welch's two-sided *t*-test revealed differences in the abundance of some pathways between feeding groups ($p < 0.05$). The differences are graphically represented with extended error bar plots that were generated with STAMP (Fig. 5). In mucosa samples, 9 and 5 pathways differed significantly between the CTRL and the IM25 and IM50 groups, respectively. Pathways related to carbohydrate metabolism, transcription factors, transcription machinery, and cell growth were more expressed in the gut of seabass from treatment groups compared to those from CTRL. Cell growth pathways appeared to be especially pronounced in the gut of IM25 compared to IM50 fish. A decrease in glutathione metabolism was observed in IM25 fish in comparison with the CTRL. At the same time, benzoate degradation was enhanced in the mucosa-related microbiota of IM50 seabass, as compared to those of IM25.

More differences were observed in digesta samples, with 7 and 16 pathways differing significantly between CTRL and IM25 and IM50, respectively. Again, pathways related to cell growth and transcription were positively related to the treatment groups. Here, no differences were observed in carbohydrate metabolism. Pathways related to flagellar assembly and the two-component system appeared more pronounced in the treatment feeding groups compared to the CTRL. No significant differences were found between the predicted metabolic pathways of the IM25 and IM50 groups.

4. Discussion

The results of this study present several key findings that shed light on the potential of incorporating insect meal (IM) in the diets of European seabass and its effects on growth performance and gut microbiota. The negligible mortality rates and well-accepted experimental diets suggest that the inclusion of BSFL and YM does not negatively impact feed intake or overall fish health. Additionally, the similarity in VFI, FCR, and DGI across all dietary treatments implies that the partial replacement of FM protein with IM in the IM25 and IM50 diets effectively supports growth performance and feed efficiency. These findings are consistent with earlier studies on European seabass, showing that partial replacement of FM (up to 50 %) by BSFL or YM did not negatively affect fish growth (Magalhães et al., 2017; Mastoraki et al., 2020; Abdel-Latif et al., 2021; Basto et al., 2021, 2023; Randazzo et al., 2023; Zarantoniello et al., 2023).

The feed microbiota analysis revealed a diverse bacterial community across all diets—dominated by Firmicutes and Proteobacteria—with significant differences in the relative abundance of specific phyla and genera between feeds, although overall species richness, biodiversity, and beta diversity did not differ significantly. The presence of diverse microbial communities in the experimental feeds despite extrusion is indeed an intriguing result. It is well known that extrusion involves the application of high temperatures and high pressure, which are generally expected to inactivate or eliminate most viable microorganisms. However, it is important to clarify that the microbiota profiles described in this study are based on the sequencing of the 16S rRNA gene, which detects bacterial DNA independently of cell viability. Therefore, the microbial signatures identified in the feeds may reflect DNA from non-viable, heat-killed bacteria that were present in the raw materials prior to extrusion. This residual DNA may remain stable and amplifiable even after thermal processing. Furthermore, contamination after extrusion, even if minimized by careful handling, cannot be completely excluded, especially during the cooling, drying or storage phases. Therefore, the different microbial profiles observed in the feeds probably represent a combination of DNA from inactivated microbes initially present in the raw materials and, to a lesser extent, potential minor post-extrusion contamination. These considerations help to explain the observed taxonomic diversity and compositional differences between feed types, even in the absence of significant differences in alpha or beta diversity.

Microbiota analysis, particularly the sequencing data, highlighted changes in gut microbial communities, with specific shifts observed in response to the IM inclusion. Although alpha diversity indices did not reveal significant differences between the control and treatment groups, an increasing trend in richness and diversity was noted. Alpha and beta diversity of gut samples are good indicators of dietary impact on the intestinal bacterial community. Higher alpha diversity indices, that is, a richer and more diverse bacterial community, are considered markers of a healthy microbiota and host. Indeed, Parshukov et al. (2019) found a significant decrease in Chao1 and Shannon indices in diseased rainbow trout compared to healthy controls. Here, we found that alpha diversity of digesta- or mucosa- associated communities did not significantly differ between the three feeding groups, although an increasing trend was observed for the Chao1 and Shannon indices. Similarly, a meta-analysis of four studies on rainbow trout fed BSF diets concluded that a 10–30 % inclusion of BSF did not significantly alter alpha diversity indices compared to fish fed a BSF-free diet (Foysal and Gupta, 2022). Other studies, including our previous works, have found an association between the inclusion of BSF in feeds and a higher microbial

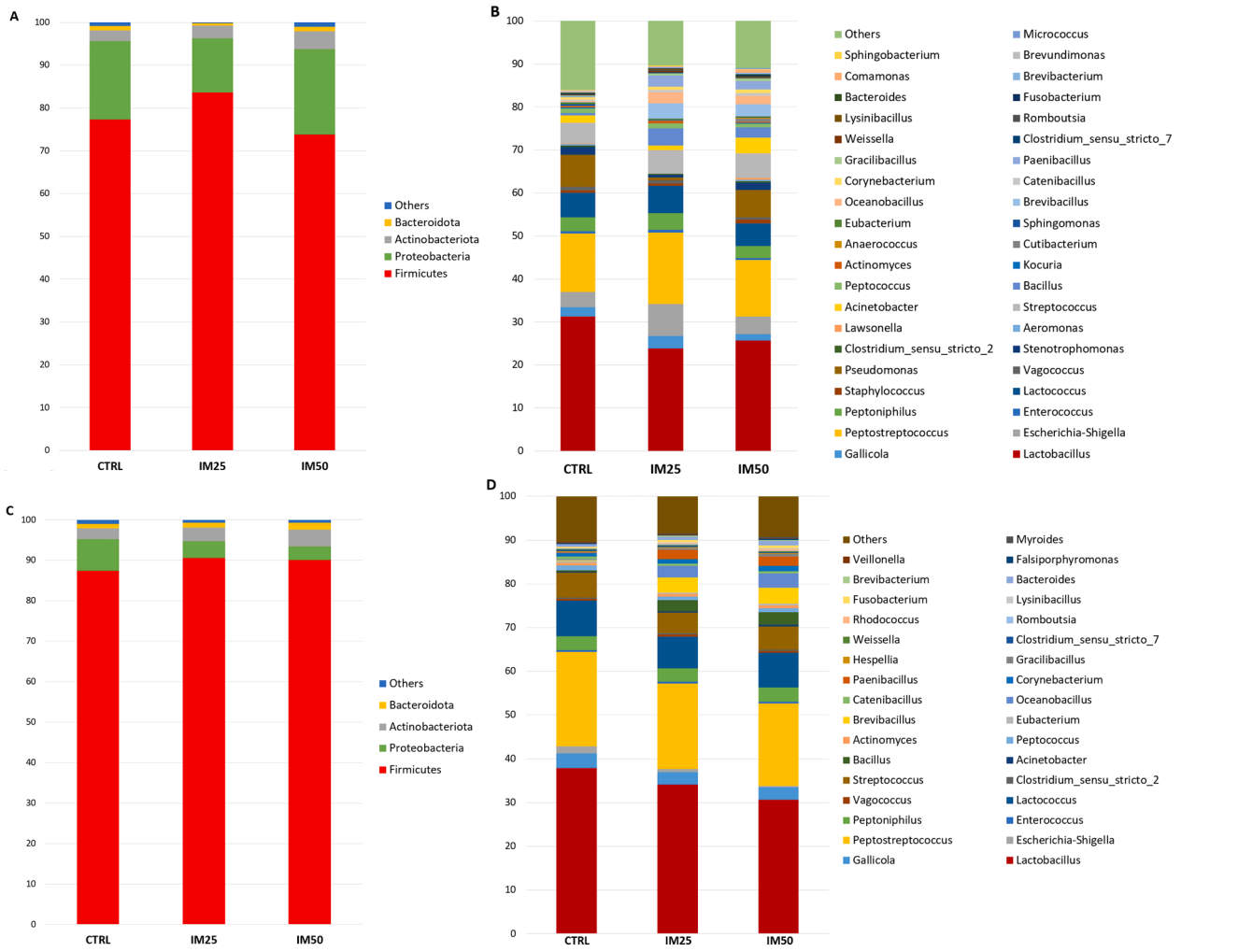

Fig. 4. Stacked bar chart of the mean relative abundance (%) of most abundant bacterial taxa in mucosa (A and B) and gut content samples (C and D).

diversity in the gut of Atlantic salmon (Li et al., 2021) and rainbow trout (Bruni et al., 2018; Terova et al., 2019).

The beta diversity of mucosa-associated microbial communities was similar between the feeding groups, both in terms of taxa present (unweighted UniFrac distance) and abundance (weighted UniFrac distance). Some significant qualitative differences were observed in the case of digesta-associated communities, for which the IM25 and IM50 feeding groups were separated from the CTRL. This was an expected result as the digesta-associated microbiota i.e., the transient microbiota, is less stable than the resident, mucosa-associated microbiota, and more prone to changes with diet (Viver et al., 2023). With that said, the gut content and mucosa samples were significantly distant from feed samples in the UniFrac matrix. This could indicate that changes in the gut microbiota composition are not merely a reflection of the feed microbial community, but rather a result of a complex interaction between host metabolism, gut microbes, and feed.

Despite the lack of differences in diversity indices, we observed differences in the mucosa- and gut- associated bacterial communities of different feeding groups at a taxonomic level. Several genera of the *Bacillaceae* family were significantly more abundant in the gut of fish that were fed insect-containing feeds. Notably, the relative abundance of *Bacillus* was higher in the gut of fish from IM25 and IM50, both in the lumen and mucosa. Comparable results about digesta-associated *Bacillus* have been obtained in several species fed BSFL, including rainbow trout (Rimoldi et al., 2021; Biasato et al., 2022), Atlantic salmon (Li et al., (2022);, seabream (Busti et al., 2024), and European seabass (Rangel et al., 2024). It is known that several species of *Bacillus* are chitinolytic (Cody, 1989). Since our treatment feeds contain 0.11 % (IM25) and 0.25 % (IM50) of chitin, it can be inferred that this enrichment of the gut with *Bacillus* is an adaptation of the microbiota to the increased content of chitin in the diet.

Interestingly, a differential abundance of *Bacillus* in the mucosa was observed between the IM25 and IM50 groups, with the IM25 group exhibiting a greater relative abundance. This suggests that factors beyond chitin content and feed composition may be influencing the proliferation of resident species belonging to *Bacillus*. In fact, it is known that the mucosa-associated microbiota is shaped by external and host-related factors (Luan et al., 2023). In previous studies about IM inclusion in the feeds of rainbow trout, we did not find any association between mucosa-associated *Bacillus* and IM inclusion (Rimoldi et al., 2019; Terova et al., 2021). Similarly, Rangel et al., (2022) did not observe any differential abundance of this genus in the intestinal mucosa of European seabass, despite the chitin content of their IM feeds. It could be hypothesized that the combination of BSFL and YM meal in this study caused a significant enrichment of *Bacillus* not only in the digesta-associated microbiota, but also in the resident, mucosa-associated microbiota.

Overall, the proliferation of the *Bacillus* genus is a highly positive and desirable effect, as several species within this genus are recognised as beneficial probiotics for fish, known for enhancing both gut health and immune response (Van Doan et al., 2020). Inclusion of two chitinolytic *Bacillus* spp. to *H. illucens* feeds of European seabass led to improved chitin and protein digestibility, and increased seabass survivability to *Vibrio anguillarum*, a common fish pathogen (Rangel et al., 2024).

Table 5
Mean relative abundance, standard deviation and taxonomic classification of genera with a differential abundance between feeding groups.

Mucosa-associated genera that differ between the CTRL and IM25 group									
Phylum	**Class**	**Order**	**Family**	**Genus**	**CTRL (%)**	**SD (%)**	**IM25 (%)**	**SD (%)**	***p* value**
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.58	0.41	3.90	1.18	2.17E−05
Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Paenibacillus	0.26	0.27	2.59	1.25	0.0007
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	0.03	0.03	0.23	0.12	0.0013
Firmicutes	Bacilli	Brevibacillales	Brevibacillaceae	Brevibacillus	0.10	0.16	3.55	2.16	0.0020
Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Peptostreptococcaceae	Romboutsia	0.10	0.09	0.34	0.24	0.0263
Mucosa-associated genera that differ between the CTRL and IM50 group									
Phylum	**Class**	**Order**	**Family**	**Genus**	**CTRL (%)**	**SD (%)**	**IM50 (%)**	**SD (%)**	***p* value**
Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Paenibacillus	0.26	0.27	2.11	0.63	1.06E−05
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.58	0.41	2.44	1.01	0.0006
Firmicutes	Bacilli	Brevibacillales	Brevibacillaceae	Brevibacillus	0.10	0.16	3.03	1.58	0.0008
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	0.03	0.03	0.22	0.14	0.0056
Firmicutes	Bacilli	Bacillales	Bacillaceae	Gracilibacillus	0.14	0.38	0.68	0.58	0.0452
Gut content-associated genera that differ between the CTRL and IM25 group									
Phylum	**Class**	**Order**	**Family**	**Genus**	**CTRL (%)**	**SD (%)**	**IM25 (%)**	**SD (%)**	***p* value**
Firmicutes	Bacilli	Bacillales	Bacillaceae	Gracilibacillus	0.01	0.02	0.46	0.21	0.0003
Firmicutes	Bacilli	Brevibacillales	Brevibacillaceae	Brevibacillus	0.14	0.18	3.40	1.54	0.0003
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	0.04	0.03	0.19	0.08	0.0004
Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Paenibacillus	0.12	0.12	2.11	1.00	0.0005
Firmicutes	Bacilli	Bacillales	Bacillaceae	Oceanobacillus	0.25	0.52	2.70	1.33	0.0006
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.31	0.18	2.50	1.19	0.0008
Actinobacteriota	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	0.04	0.03	0.25	0.12	0.0008
Gut content-associated genera that differ between the CTRL and IM50 group									
Phylum	**Class**	**Order**	**Family**	**Genus**	**CTRL (%)**	**SD (%)**	**IM50 (%)**	**SD (%)**	***p* value**
Firmicutes	Bacilli	Paenibacillales	Paenibacillaceae	Paenibacillus	0.12	0.12	2.13	0.39	1.13E−07
Actinobacteriota	Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	0.04	0.03	0.23	0.05	6.16E−07
Firmicutes	Bacilli	Brevibacillales	Brevibacillaceae	Brevibacillus	0.14	0.18	3.68	0.89	2.1E−06
Firmicutes	Bacilli	Bacillales	Bacillaceae	Oceanobacillus	0.25	0.52	3.29	0.97	4.29E−06
Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	0.31	0.18	2.82	0.87	2.84E−05
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	0.04	0.03	0.27	0.09	4.56E−05
Firmicutes	Bacilli	Bacillales	Bacillaceae	Gracilibacillus	0.01	0.02	0.59	0.21	4.93E−05
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	37.84	5.12	30.55	3.44	0.0048
Firmicutes	Clostridia	Lachnospirales	Lachnospiraceae	Catenibacillus	0.59	0.11	0.45	0.10	0.0136

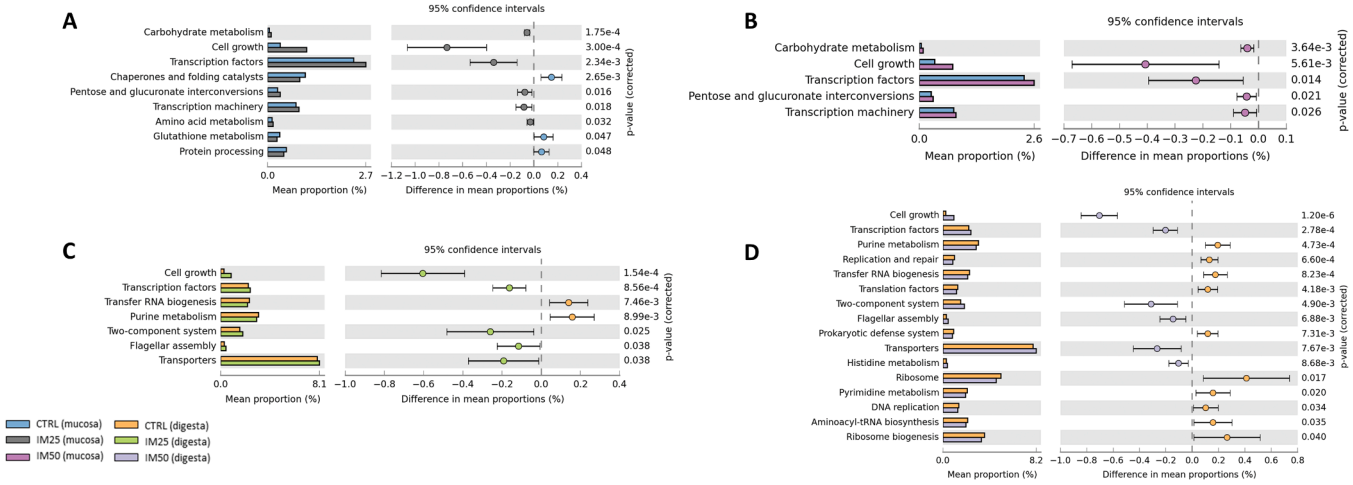

Fig. 5. Extended error bar plots depicting pairwise comparisons of predicted metabolic pathways in mucosa (A and B) and digesta (C and D) samples.

Although our study did not directly assess the immune response of European seabass to insect-based diets, the enrichment of the gut with *Bacillus* spp. and other probiotic bacteria through feed formulations is of key importance in aquaculture. This is because enhanced immunity can prevent disease outbreaks and thus avoid the use of antibiotics, which are not only an economic strain to the producers, but also a major environmental and food security concern (Ghosh, 2025).

Other beneficial bacteria were associated with IM diets. *Paenibacillus* showed a similar trend of differential abundance to *Bacillus*, with an enrichment of the mucosa and the gut content of fish from the two treatment groups. *Paenibacillus* is a genus known for its probiotic potential, particularly in enhancing nutrient digestion and promoting host health through various mechanisms, such as antimicrobial activity and immunomodulation. *Paenibacillus* species are gram-positive, endospore-forming bacteria that were initially classified under the *Bacillus* genus due to their similarity to *Bacillus subtilis* (Lin et al., 2022). In the early

1990s, they were reclassified as a separate genus based on phylogenetic analysis of 16S rRNA sequences. *Paenibacillus* is known for producing bacteriocins, which have potential applications as antimicrobial agents in medicine and agriculture (Olishevska et al., 2019). However, their use in aquaculture for disease control is still limited. In addition to bacteriocins, *Paenibacillus* produces hydrolytic enzymes like amylase, cellulase, protease, and chitinase, which have demonstrated antagonistic activity against fish pathogens (Midhun et al., 2017).

The enrichment of *Paenibacillus* in both the mucosa and gut content of fish from the IM25 and IM50 treatment groups suggests that the IM blend has a positive impact on the gut environment, fostering beneficial microbial populations. As aforementioned, *Paenibacillus* can produce enzymes such as cellulase and chitinase, which contribute to the breakdown of complex carbohydrates and chitin, a key component of insect exoskeletons (Midhun et al., 2019; Lin et al., 2022; Hasan et al., 2023; Busti et al., 2024; Siddiqui et al., 2024). This enzymatic activity may improve nutrient bioavailability and absorption, which can result in higher overall fish growth. However, in the present study this could not be perceived as fish had a similar growth rate. An evaluation of the feed digestibility and/or a longer feeding period is warranted to clarify this trend. Furthermore, the consistent presence of *Paenibacillus* in both the mucosa and gut content underscores its adaptability to different gut environments and its potential to play a crucial role in the host's digestive and immune functions. Although our study did not specifically assess immune responses, the enrichment of *Paenibacillus* and other beneficial bacteria may have indirect positive effects on fish health, warranting further investigation into their role in immunomodulation in aquaculture.

In contrast to *Paenibacillus*, *Oceanobacillus* from the Bacillaceae family, and *Brevibacterium* from the Brevibacteriaceae family appeared to be higher only in the digesta of fish from the IM25 and IM50 groups, but not in the mucosa. *Oceanobacillus* and *Brevibacterium* both belong to the transient or stable gut microbial population, but their higher abundance in the digesta suggests that they are more responsive to changes in diet than to the stable mucosal environment. This distinction is supported by studies showing that the microbiota associated with digesta, which includes transient dietary microbes, often reflects short-term changes in diet composition (Leeming et al., 2019). As in humans, the composition of the gut microbiota in fish is influenced by diet, with transient fluctuations in microbial populations observed in response to changes in diet. However, these shifts are often transient, and it remains unclear whether prolonged dietary changes can lead to permanent changes in the gut microbiota of fish. Habitual diets may have a more lasting impact on microbial composition than short-term interventions (Leeming et al., 2019). Understanding how long-term dietary strategies can shape the gut microbiota of fish, just as in humans, is key to optimizing the health and growth of fish in aquaculture. Further research into the long-term effects of diet on the gut microbiota of fish could shed light on how sustained dietary changes could permanently alter gut health and resilience.

The increase in *Brevibacterium* in the digesta, rather than the gut mucosa, of fish fed IM is another intriguing finding that aligns with recent literature on microbial dynamics (Li et al., 2022; Rimoldi et al., 2024b). This genus has been associated with improved nutrient uptake in fish, particularly in fish fed alternative protein sources such as IM (Li et al., 2022; Rimoldi et al., 2024b). This is consistent with the known enzymatic activity of *Brevibacterium*, particularly its ability to break down proteins and lipids by proteases and lipases, making it beneficial for the digestion of fish when fed alternative protein sources such as IM. In fact, although no significant differences in macronutrient digestibility were found, our previous study, based on the same feeding trial, reported lower fecal lipid and phosphorus losses and the lowest nitrogen losses in the IM50 group. The enrichment of *Brevibacterium* in the gut microbiota of fish could contribute to improved growth performance and feed efficiency, at longer term, which are crucial for sustainable aquaculture practices.

The fact that both *Oceanobacillus* and *Brevibacterium* genera were found in the digesta rather than the mucosa of seabass in the present study suggests that their activity is closely linked to the feed components that pass through the gut and not to the formation of stable, resident populations in the mucosa. This is consistent with the notion that the intestinal mucosa is more selective and harbors bacteria that interact directly with the host's immune system and intestinal mucosa, whereas the digestive tract primarily reflects the immediate effects of food (Viver et al., 2023). These dynamics call for additional investigation to understand the specific role of both genera in improving feed efficiency and gut health in aquaculture species fed insect-based diets.

The variable abundance of *Lactobacillus*, another well-known probiotic in fish, was interesting. It was one of the most abundant genera in the guts of our seabass. The only exception was the digesta of the fish from the IM50 group, in which we observed a significant decrease in *Lactobacillus* abundance compared to the CTRL group. This raises interesting questions about the microbial response to IM-based diets. *Lactobacillus* is a well-known probiotic in fish that is recognised for its role in maintaining gut health by balancing the microbial community, enhancing nutrient absorption and promoting immune response (Rimoldi et al., 2018, 2019; Terova et al., 2019, 2021). A decline in *Lactobacillus* abundance could be due to several factors, although the exact cause remains unclear. One possibility is that the increased presence of insect-derived chitin and other components in the IM50 diet might have created a gut environment that is less favorable for *Lactobacillus* colonization. It is also plausible that IMs—particularly those derived from species such as BSFL—contain bioactive compounds, including antimicrobial peptides and medium-chain fatty acids, which might selectively inhibit certain bacterial populations. Additionally, the dietary shift toward insect proteins could potentially promote the proliferation of other bacteria, such as *Bacillus* or *Paenibacillus*, which may, in turn, competitively exclude *Lactobacillus* under these conditions. However, despite this decline, *Lactobacillus* remained one of the most abundant genera in the gut, suggesting that its fundamental role in gut health was not completely compromised. This observation suggests a certain degree of resilience of the microbial community that can adapt to changes in diet, even when the diet deviates significantly from the traditional FM-based diet. The specific mechanisms underlying this change and whether they have long-term effects on fish health and performance should be further explored in order to optimize the balance of beneficial bacteria in the aquaculture diet.

Based on 16S rRNA gene sequencing information, PICRUSt provides information on how different feeds influence the functional content of the intestinal metagenome in seabass. The data show that the gut metagenome of seabass responds to changes in diet, particularly in the pathways of cell growth, transcription and metabolism. Both the mucosal and digesta-associated microbiota were affected by the treatments, with digesta samples showing a wider range of differences, possibly due to their more direct exposure to the dietary components. The increased occurrence of transcriptional and cell growth pathways in the treatment groups may indicate that the IM diets promote a more active or robust microbiota, which could contribute to improved gut health or nutrient utilization in the fish. The differential effects on glutathione metabolism and benzoate degradation suggest that oxidative stress and xenobiotic processing are also modulated by diet, potentially affecting fish health and resilience. Finally, the lack of differences between the IM25 and IM50 groups in digesta suggests that there may be a threshold effect where higher levels of IM do not lead to additional metabolic changes. This could be important for optimizing raw materials in the diet to achieve the desired results without unnecessary overdosing.

The high abundance of *Firmicutes*, known for their ability to break down complex carbohydrates, can explain the upregulated carbohydrate metabolism in the mucosa-associated microbiota of our seabass (Tao et al., 2022). Meanwhile, the upregulated amino acid metabolism may be due to the high abundance of *Peptostreptococcus* which, in humans, is

known to ferment amino acids in the gut (Smith and Macfarlane, 1998). Both the mucosal and digesta-associated microbiota responded to changes in diet, with digesta samples showing a broader range of differences due to their direct exposure to the diet. The IM diets promoted increased transcriptional activity and cell growth, potentially improving gut health and nutrient utilization.

Overall, these results suggest that IM diets affect the metabolic functions of the gut microbiota in a way that could potentially improve fish health.

5. Conclusions

The results of this study highlight the potential benefits of including IM, particularly a mixture of BSFL and YM meals, in the diet of seabass. Specifically, the addition of IM did not negatively impact feed intake, mortality rates, or overall health of seabass.

Analysis of the gut microbiota revealed significant shifts associated with the dietary changes. The proliferation of beneficial genera such as *Bacillus* and *Paenibacillus* in the guts of fish fed IM suggests their potential role as probiotics that promote nutrient digestion and contribute to an improved immune response.

Interestingly, the presence of *Oceanobacillus* and *Brevibacterium* predominantly in the digesta rather than in the mucosa suggests that these genera respond rapidly to changes in diet and may play a key role in immediate digestive processes. This distinction underscores the complexity of the dynamics of the gut microbiota, in which transient microbes may reflect short-term dietary influences rather than form stable populations. Furthermore, the resilience of *Lactobacillus* highlights the adaptability of the microbial community to changes in diet, although further investigation is required to understand the long-term effects on fish health.

Overall, this study underscores the promise of a mixture of BSFL and YM meals as a sustainable nutritional ingredient in aquaculture feeds, promoting a beneficial gut microbiota that can improve fish health and performance. Further research is essential to unravel the intricate relationships between dietary components and microbial communities, paving the way for optimised aquaculture practices.

CRediT authorship contribution statement

Luisa M.P. Valente: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Simona Rimoldi:** Writing – review & editing, Validation, Methodology. **Rafaela S. Costa:** Writing – review & editing, Formal analysis. **Violeta Kalemi:** Writing – original draft, Validation, Methodology. **Ana Basto:** Writing – review & editing, Writing – original draft, Investigation. **Marta Monteiro:** Writing – review & editing, Writing – original draft, Investigation. **Genciana Terova:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Ethics approval and consent to participate

The fish experiment was approved by the CIIMAR Ethics Committee for Animal Welfare (ORBEA_CIIMAR-37–2023) and conducted by accredited scientists (1005/92, DGAV Portugal) in accordance with European (Directive 2010/63/EU) and Portuguese (Decree-Law No. 113/2013) animal welfare guidelines.

Consent for publication

Not applicable.

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Declaration of Competing Interest

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

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Violeta Kalemi is a doctorate student of the PhD in "Life Sciences and Biotechnology" at the "University of Insubria", Varese, Italy.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102939.

Data availability

I have shared the link to my data/code

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