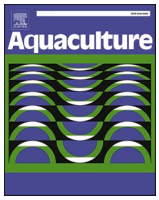
Aquaculture 567 (2023) 739256

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Potential of shrimp waste meal and insect exuviae as sustainable sources of chitin for fish feeds

Simona Rimoldi [a], Chiara Ceccotti [a], Fabio Brambilla [b], Filippo Faccenda [c], Micaela Antonini [a], Genciana Terova [a,*]

[a] Department of Biotechnology and Life Sciences, University of Insubria, Via J.H. Dunant, 3, 21100 Varese, Italy
[b] VRM S.r.l. Naturalleva, 37044 Cologna Veneta, Italy
[c] Aquaculture and Hydrobiology Unit, Edmund Mach Foundation, San Michele all'Adige, Trento, Italy

ARTICLE INFO

Keywords:
Rainbow trout
Insect exuviae
Gut microbiota
Chitin
Prebiotics
SCFA

ABSTRACT

Aquaculture is one of the world's fastest growing food-producing sectors, providing more than half of all fish consumed globally for human nutrition. However, to maintain such growth and meet the increasing demand for aquatic food, sustainable raw materials for fish feeds are needed. In this regard, insects represent one of the most promising alternatives to fish meal (FM) protein source for use in aquafeeds. In addition to protein, insects contain bioactive compounds, such as chitin, which is a natural polysaccharide abundantly present in the pupal exuviae of some insects. Studies have shown that dietary chitin or its derivate chitosan acts as a prebiotic thus modulating the gut microbial communities of fish. Accordingly, the present study aimed to evaluate the effect of two waste products rich in chitin, i.e., shrimp head meal (SHM), and insect (*Hermetia illucens*) pupal exuviae on the gut microbiota of rainbow trout (*Oncorhynchus mykiss*). Three isoproteic, isolipidic, and isoenergetic diets containing either FM, SHD, or a combination of FM and 1.6% of pupal exuviae meal (PEM) were tested through a 91-day feeding trial. At the end of the experiment, no differences in final mean body weight, specific growth rate, and feed conversion ratio values were observed between fish experimental groups. Mortality was <1% and it did not correlate with diet for the entire duration of the trial. However, a modulatory effect of dietary pupal exuviae on fish gut microbiota was detected. Indeed, gut bacterial species richness improved by including insect exuviae. In particular, Firmicutes and Actinobacteria phyla, mainly represented by *Bacillus*, *Facklamia, Brevibacterium*, and *Corynebacterium* genera, were enriched in trout receiving pupal exuviae. These genera are chitinolytic and short-chain fatty acids (SCFAs)-producing bacteria. SCFAs production was confirmed by gas chromatography analysis, which detected the highest amount of butyrate in feces of trout fed with pupal exuviae meal. Functional inference analysis of intestinal microbiota using PICRUST metagenome prediction tool, showed differences in response to diet. In particular, eleven pathways were significantly different between control fish (FM) and fish fed the PEM diet, whereas twenty functional traits were significantly different between the FM and SHM fish groups. Overall, our data confirmed that chitin from insect's pupal exuviae represents a promising functional ingredient, better than SHM, for positively modulating gut microbiota communities of rainbow trout.

1. Introduction

Fish constitute one of the main sources of high-quality proteins for human consumption, supplying 20% of the average per capita intake of animal proteins. In this scenario, aquaculture accounted for 46% of the total fish production and 52% of the global demand for fish for human use (FAO, 2020). To guarantee the rapid growth of this agriculture sector, new strategies and technologies have emerged over the past few decades, which aimed to develop a sustainable aquaculture. In order to minimize the environmental footprint of aquaculture and increase its sustainability, great efforts are being invested to reduce or replace fishmeal (FM) in the diets by developing novel feeds with specialty ingredients that can provide nutritional value and at the same time be cheaper and more sustainable than FM (Oliva-Teles et al., 2015).

Abbreviations: FM, Fishmeal; SCFAs, short chain fatty acids; SHM, Shrimp head meal; PEM, Pupal exuviae meal; FCR, Feed conversion ratio; SGR, Specific growth rate; ASV, amplicon sequence variant; ANOVA, Analysis of variance; PLS-DA, Partial least squares discriminant analysis.

* Corresponding author.
E-mail address: genciana.terova@uninsubria.it (G. Terova).

https://doi.org/10.1016/j.aquaculture.2023.739256
Received 1 November 2022; Received in revised form 29 December 2022; Accepted 12 January 2023
Available online 15 January 2023
0044-8486/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

In this regard, insects have been proven to have high nutritional value, particularly the protein fraction in terms of quantity (range 40–60% of dry weight) and adequate amino acid composition (Makkar et al., 2014). An increasing number of feeding trials have already shown that insect meals can partially or completely replace the FM in feeds for aquaculture (Gasco et al., 2020; Lock et al., 2018; Nogales-Mérida et al., 2019).

Recently, we obtained promising results with the dietary use of black soldier fly (*Hermetia Illucens*) and yellow mealworm (*Tenebrio molitor*) meals in both marine and fresh water carnivorous fish species (Rimoldi et al., 2021, 2019; Terova et al., 2021, 2020, 2019). Insects also contain a significant amount of 'animal' fibers in the form of insoluble chitin. Chitin is Earth's second most abundant polysaccharide after cellulose and is composed of *N*-acetyl-2-amino-2-deoxyglucose (GlcNAc) units linked by β-(1 → 4) bonds (Abdel-Ghany and Salem, 2020; Jiménez-Gómez and Cecilia, 2020). In addition to being present in insect exoskeletons, chitin is the major constituent of crustacean shells and the cell wall of many fungi, too. On an industrial scale, chitin is largely obtained from processing the waste of marine food products, such as shrimp, prawn, crab, lobster, crayfish, squid, cuttlefish, and barnacles, whose chitin content ranges between 15 and 40% (Kurita, 2006). However, interest in edible insects as food and feed has been rising in recent years. In Europe, for the feed sector alone, the annual production of insects amounts to about 6000 tons, with an estimated 24.4% growth per year over the next decade (Gasco et al., 2020). With their content of chitin (up to 35–40%), pupal exuviae are the main chitin-rich by-products of insect farming (Hahn et al., 2020; Kramer et al., 1995), and represent a relevant cost-free and always-available source of chitin with low environmental impact.

Chitin and its deacetylated derivate chitosan have been proven to exhibit positive effects on the performance of cultured fish and are considered to be functional ingredients, or bioactive compounds (Abdel-Ghany and Salem, 2020; Alishahi and Aïder, 2011; Ringø et al., 2012).

Chitin and chitosan supplementation in the diet, indeed, elevates growth rates and feed efficiencies and improves disease resistance in several fish species, including rainbow trout (*Oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), grey mullet (*Liza ramada*), red sea bream (*Pagrus major*), Japanese eel (*Anguilla japonica*), and yellowtail (*Seriola quinqueradiata*) (Ahmed et al., 2021; Dawood et al., 2020; Elserafy et al., 2021; Kono et al., 1987; Qin et al., 2014; Shi et al., 2020). Despite these positive effects, it is generally accepted that chitin is a limiting factor, constraining the use of insect meals in aquafeeds. The presence of chitin in fish diets decrease diet digestibility (Gasco et al., 2019) as most fish species are not able to efficiently digest and absorb chitin. Actually, the ability of fish to digest chitin is a matter of debate as some fish species do show chitinase activity, such as cod (*Gadus morhua*) (Danulat, 1986), juvenile cobia (*Rachycentron canadum*) (Fines and Holt, 2010), channel rockcod (*Sebastolobus alascanus*), splitnose rockfish (*Sebastes diploproa*), and black cod (*Anoplopoma fimbria*) (Gutowska et al., 2004).

Being similar to cellulose, chitin may also act as a prebiotic, increasing bacterial intestinal richness by favoring the growth of beneficial and chitin-degrading bacteria, thus stimulating intestinal fermentation by producing healthy short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, as end products.

In aquatic animals, SCFAs are mainly found in the posterior region of the gut (85% of the total SCFAs) where they are absorbed and act as the main energy source of colonocytes, especially butyrate (Clements et al., 1994). This SCFA exhibits a well-known positive effect on gut, fish health, and the immune system (Gaudioso et al., 2021).

Accordingly, numerous studies have shown that insect-derived ingredients are capable of modulating the microbial communities inhabiting the fish gut. Feeding with insect-supplemented diets usually drives an increasing abundance of the Bacillaceae, Lactobacillaceae, and Actinobacteria families (Bruni et al., 2020; Foysal and Gupta, 2022; Gaudioso et al., 2021; Huyben et al., 2019; Li et al., 2021; Rimoldi et al., 2021, 2019; Terova et al., 2021, 2020, 2019). Similarly, dietary chitosan increases the abundance of beneficial bacteria at the expense of those potentially pathogenic for the host (Ahmed et al., 2019).

The current study aimed to provide knowledge about the use of two waste by-products rich in chitin, i.e., shrimp head meal (SHM) and black soldier pupal exuviae, in aquafeeds and the effect of these ingredients on growth and gut microbiome composition in rainbow trout (*Oncorhynchus mykiss*). For this purpose, a feeding trial was conducted with three isoproteic, isolipidic, and isoenergetic diets containing either FM (control FM-based diet), SHM, or pupal exuviae meal (PEM). Fish growth performances and gut microbiome profiles were evaluated in response to diet. Changes in the amount of intestinal SCFAs were also studied to understand the local effects of gut microbiota modulation.

Our assumption was that these by-product meals, particularly PEM, could positively modulate the intestinal microbiota of rainbow trout by increasing the proliferation of beneficial and chitinolytic bacteria and could be considered valid and more sustainable sources of chitin and chitosan to use as functional ingredients for aquafeeds.

2. Materials and methods

2.1. Ethics approval

This study complies with EU Directive 2010/63/EU and Italian Legislative Decree n°26/2014 for animal used for scientific purposes. All procedures involving fish were performed at Experimental Fish Center of Fondazione Edmund Mach pursuant to the authorization n° 22/2019-UT of the Ministry of Labor, Health, and Social Policies (Art. 20. D.Lgs 26/2014) and approved by the Committee on the Ethics of Animal Experiments of the Edmund Mach Foundation. The procedures described did not cause pain, suffering or any stress in animals as in Art. 2 of Legislative Decree 26/2014 and were therefore not subjected to prior authorization by the Ministry of Health. In order to reduce handling stress and suffering, fish were anesthetized by immersion in tricaine methanesulfonate solution (MS222) before sampling.

2.2. Fish, diets, and feeding trial

The feeding trial and all procedures involving rainbow trout (*O. mykiss*) were conducted at the indoor experimental facility of the Edmund Mach Foundation (FEM) (San Michele all'Adige, Trento, Italy). A total of 360 trout, all females with an initial mean body weight of 290.7 ± 5.4 g were randomly distributed in 9 fiberglass tanks of 800 l (40 fish/tank) connected to a flow-through fish-rearing system.

Experimental tanks were supplied with degassed ground water with an approximately constant temperature of 13.7 ± 0.2 °C and dissolved oxygen concentration at 7.0 ± 0.7 mg/l (DO saturation 68.9 ± 7.2). Fish were acclimatized for 6 days under a natural photoperiod (October 18th 2019 – January 17th 2020) (46°11′30.404" N; 11°8′5.195″ E) and fed to visual satiety with a standard commercial diet (VRM S.r.l, Naturalleva, Italy). After the acclimation period, fish were fed twice daily with a restricted ration (1.5% of biomass, 6 days per week), for 91 days with three different extruded diets in triplicate (3 tanks/diet). For this study, Naturalleva (VRM S.r.l Italy) formulated three isoproteic, isolipidic, and isoenergetic diets containing either fish meal (diet FM), shrimp head meal (diet SHM) (Nutripal Aquativ, Diana Aqua), or FM and 1.6% of pupal exuviae meal (diet PEM) from *Hermetia illucens*. Exuviae were mixed with the oil and added to the feed during the vacuum coater oil addition process. All three diets contained hydrolyzed fish protein and plant proteins to ensure that the protein requirement of trout was met. The formulation and proximate composition of the experimental diets are reported in Table 1. Amino acid, fatty acid, mineral, and vitamin content of the diets are reported in Supplementary Table 1.

To calculate feed rations, the individual weight of 10 randomly chosen fish per tank (30 fish/diet) was assessed weekly, whereas all fish in the tank (120 fish/diet) were weighed at the beginning, after 45 days, and at the end of the experiment (91 days). Feed ingested was recorded

Table 1
Ingredients and proximate composition of experimental diets.

Ingredients	FM	SHM	PEM
Fish meal	25.7	0.0	20.1
Fish protein hydrolysed	4.1	9.0	6.5
Shrimp meal	0.0	20.4	0.0
Haemoglobin porcine	10.4	12.2	12.2
Soybean meal	27.2	26.1	26.1
Wheat meal	10.0	10.0	11.3
Fish oil	10.5	10.3	10.3
Soybean oil	4.8	4.7	4.7
Camelina oil	3.3	3.3	3.2
DL- methionine	0.8	0.8	0.8
Lysine HCl	0.8	0.8	0.8
MAP	1.4	1.4	1.4
Antioxidants premix	0.1	0.1	0.1
Vitamin C	0.1	0.1	0.1
Vitamin & mineral premix	0.8	0.8	0.8
Pupal BSF exuviae	0.0	0.0	1.6
Proximate composition			
DM	93.66	93.66	93.66
CP	43.16	43.13	43.09
EE	21.61	21.58	21.50
Ash	7.27	7.24	7.84
Gross Energy (MJ kg^{-1} DM)	20.04	20.03	20.00

Abbreviations: MAP, monoammonium phosphate; DM, dry matter; CP, crude protein; EE, ether extract.

daily for each tank and uneaten pellets recovered and weighed at the end of each meal. Mortality was also monitored. These data were used as the basis for calculating the feed conversion ratio (FCR = dry feed intake/wet weight gain) and specific growth rate [SGR (%/day) = 100 x [ln (final body weight) ± ln (initial body weight)]/days], for each dietary fish group.

2.3. Sampling

At the end of the feeding trial, two fish from each tank (6 fish/diet) were euthanized with an overdose of tricaine-methasulfonate (MS-222, 400 mg l^{-1}). The external surface of each fish was cleaned with 70% ethanol to prevent external contaminations during dissection. The entire intestine (excluding pyloric ceca) was aseptically removed from each individual fish and the luminal and mucosa-associated microbiota were collected and mixed together in the same sterile tube containing 800 μl of Xpedition™ Lysis/Stabilization Solution (Zymo Research, Irvine, CA, USA). As described in detail by Rimoldi et al. (2020), the fecal matter was recovered by squeezing out, whereas the intestinal mucosa by scrapping with a sterile spatula. An additional six fish per dietary group were sampled and their intestinal content collected in a tube and stored at −80 °C for SCFA quantification.

2.4. Gut microbiota analysis

2.4.1. Bacterial DNA extraction, 16S rRNA library preparation, and sequencing

The bacterial DNA was extracted from each fish intestinal sample (feces + mucosa) and from three aliquots of 200 mg each per feed. DNeasy®PowerSoil® Pro Kit (Qiagen, Italy) was used for extraction following the manufacturer's instructions with only few modifications at the lysis step, as previously described (Rimoldi et al., 2019). A negative control, with only lysis buffer, was carried out in parallel to the biological samples to validate the extraction procedure. The extracted DNA was quantified using a NanoDropTM 2000 Spectrophotometer (Thermo Scientific, Milan, Italy) and then stored at −20 °C until use.

The 16S amplicon sequencing library was prepared by the GALSEQ srl microbiome sequencing service (Milan, Italy) applying the Illumina protocol "16S Metagenomic Sequencing Library Preparation for Illumina MiSeq System" (#15044223 rev. B). All steps of 16S rRNA gene library preparation and sequencing have been previously described (Terova et al., 2019). To characterize microbial community profiles, the hypervariable region V4 of 16S rRNA gene was amplified using the oligonucleotides 515F: 5′-GTGYCAGCMGCCGCGGTAA-3′ and 806R: 5′-GGACTACNVGGTWTCTAAT-3′. Each library was diluted to 5 ng/μL and pooled in one tube at equimolar concentration (4 nM). The mixed libraries were then multiplexed and sequenced on an Illumina MiSeq device, employing a paired-end 2 × 150 bp sequencing strategy.

2.4.2. DNA metabarcoding data analysis

The sequencing raw data analysis was performed using QIIME 2™ (version 2020.2) pipeline (Bolyen et al., 2019) and as previously described by Terova et al. (2021). Briefly, paired-end reads were joined, quality filtered (Q > 30), and denoised using qiime DADA2 denoise-paired command (Callahan et al., 2016).

An amplicon sequence variant (ASV) table with high-quality filtered reads was constructed and the taxonomic information was assigned using the SILVA database (https://www.arb-silva.de/) (Quast et al., 2013). ASVs assigned to chloroplasts and mitochondria were removed from the analysis as they are of eukaryotic origin. To calculate alpha and beta diversity the qiime 2 core-metrics-phylogenetic pipeline was applied.

Alpha diversity was assessed at a depth of 20,000 reads and Chao 1, Faith PD, Shannon, and Simpson indices were calculated. To assess the beta diversity among gut bacterial communities, both weighted and unweighted UniFrac distance matrices (Lozupone and Knight, 2005; Lozupone et al., 2007) were calculated at genus level and results visualized on PCoA plots.

2.4.3. Predictive metagenome functional analysis

The PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) tool (Langille et al., 2013) was used to predict the functional profiles of gut microbial communities based on 16S rRNA amplicon datasets. The inferred metagenomics functions were annotated using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway functions, i.e., EC and KO (KEGG orthology) accessions as previously described (Rimoldi et al., 2021). The differences between the control (FM) and experimental groups (PEM, SHM) were tested by a two-sided Welch *t*-test using the Statistical Analysis of Metagenomics Profiles (STAMP) software package v.2.1.3 (http://kiwi.cs.dal.ca/Software/STAMP) (Parks et al., 2014). Results of the analysis were shown by means of extended error bar plots.

2.5. Quantification of SCFAs in fecal samples

Six rainbow trout per each dietary group were sampled and feces were collected in 5-ml tubes. Then, the samples were transferred and stored at −80 °C until analysis to prevent the volatilization of volatile fatty acids. To obtain a maximum extraction yield, fecal samples from two fish were pooled (3 pools/diet). Each fecal pool was then centrifuged at 3500*g* for 10 min, and 25% meta-phosphoric acid was added at a ratio of 5 parts of feces to 1 part of acid. The tubes were then covered and allowed to stand for 30 min at room temperature. The fecal fluid was centrifuged again at 3500*g* for 10 min and the clear supernatant was collected and frozen. Before the analysis, each frozen supernatant was thawed and centrifuged again as described above.

SCFAs were quantified by using a VARIAN CP-3800 gas chromatograph equipped with a Nukol column (30 m × 0.25 mm × 0.25 μM) (SUPELCO, Sigma-Aldrich Co., Milan, Italy).

The GC program was as follows: initial temperature of 100 °C, hold for 2 min; ramp to 140 °C at a rate of 5 °C/min and hold for 1 min; and then ramp to 200 °C at a rate of 7 °C/min.

The temperatures of detector and injector were kept at 250 °C and at 220 °C, respectively. Helium was used as a carrier gas at a flow rate of 4 ml/min. Samples were analyzed with a run time of 16 min and SCFAs

were quantified in accordance with external calibration standard curves for acetic, propionic and butyric acid.

2.6. Statistics

Normality and homoscedasticity of all data were checked by Shapiro-Wilk and Levene's test, respectively. One-way ANOVA with Tukey's test or nonparametric Kruskal-Wallis followed by Dunn's post hoc test were applied to assess growth performance, feed conversion, and α-diversity and bacteria-relative abundance data using Past4 software version 4.02 (Hammer et al., 2001). To compare gut bacterial abundances of experimental feeding groups (PEM, SHM) with their control (FM), the STAMP v.2.1.3 program was also used (Parks et al., 2014), applying two-sided Welch's *t*-test with Benjamin-Hochberg False Discovery Rate (FDR) correction. The significance of differences in the beta diversity of bacterial communities was assessed using the nonparametric Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations. Statistical significance was set at $p < 0.05$ for all tests. To better evaluate the separation among gut bacterial communities, a partial least-squares discriminant analysis (PLS-DA) was performed using R package mix Omics v.6.22.0. The contribution of the different taxa to the group separation was determined by the minimum variable importance in the projection (VIP) values. A VIP value ≥1 was considered an adequate threshold to define discriminant variables in the PLS-DA model.

3. Results

3.1. Growth and fish efficiency

Mortality remained negligible and did not correlate with diet for the entire duration of the trial (< 1 percentage). At the end of the 91-day trial, no differences in final mean body weight, SGR, and FCR values were observed among feeding groups (Table 2). The results revealed that the weights of all fish groups doubled after 91 days of feeding.

3.2. Illumina MiSeq sequencing data output

All feed and intestinal samples were efficiently sequenced, obtaining 941,495 high-quality sequences corresponding to an average of 34,870 ± 5142 sequences per sample. A Good's coverage value >99% indicated that the bacterial communities were well representative. Based on rarefaction curves, the sequencing depth to calculate alpha diversity indices was set at 20,000 reads. All sequencing raw data were submitted and are freely available on the European Nucleotide Archive (EBI ENA) public database, under the accession code PRJEB55867.

3.3. Characterization of feed-associated bacterial communities

From the feed samples, 336,091 valid and trimmed sequences were obtained and correctly assigned at different taxonomical ranks. The 16S rDNA gene amplicon sequencing of the microbial communities revealed significant differences between feed samples. The microbial profiles of feeds were mainly comprised of 4 phyla (Fig. 1), 8 classes, 15 orders, 31 families, and 37 genera. The complete list of bacteria taxa found in feeds for each taxonomical level is given in Supplementary Data File 1. Alpha diversity analysis showed that the microbial community associated with FM feed had the lowest species richness (Chao1) and biodiversity (Shannon, Simpson, and Faith-PD) values. Highest level of bacterial diversity was found in PEM feed followed by SHM samples (Table 3).

Table 2
Growth parameters of fish fed tested diets. Average initial (W_i) and final (W_f) body weight, SGR, and FCR are reported as mean ± SD ($n = 3$ tanks, 120 fish).

	FM	PEM	SHM
W_i (g)	295.40 ± 2.11	284.71 ± 15.06	292.03 ± 12.38
W_f (g)	639.95 ± 0.54	623.05 ± 27.60	620.01 ± 25.54
SGR	0.85 ± 0.01	0.86 ± 0.02	0.83 ± 0.02
FCR	1.13 ± 0.02	1.09 ± 0.04	1.16 ± 0.05

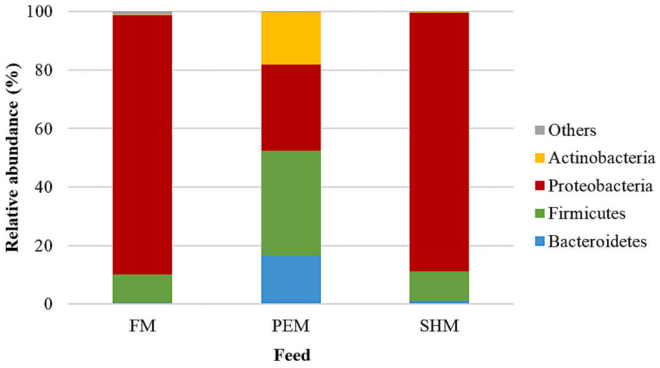


Fig. 1. Taxonomic composition of feed-associate microbiota at phylum level. Only bacteria with an overall abundance ≥1% are reported. Bacteria with lower abundance are pooled and indicated as "Others".

At phylum level, FM and SHM feeds were very similar, with a high proportion of Proteobacteria (89%) and 10% of Firmicutes (Fig. 1) or following the new phylum nomenclature set by the International Committee on Systematics of Prokaryotes (Oren and Garrity, 2021), 89% of Pseudomonadota and 10% of Bacillota. In contrast, bacterial communities associated with PEM feed differed significantly from the other two feeds, composed of a low percentage of Proteobacteria (29%) and higher percentage of Firmicutes (36%). Actinobacteria (Actinomycetota) (18%) and Bacteroidetes (Bacteroidota) (17%) were only well represented in PEM feed samples (Fig. 1). Bacterial communities associated with FM feed were especially enriched in *Vibrio* and *Lactobacillus* genera. *Corynebacterium*, *Facklamia*, *Pseudomonas*, *Brevibacterium*, *Bacillus*, and *Alcaligenes* genera were more abundant in PEM feed. SHM feed showed instead an association with *Aeromonas*, *Acinetobacter*, and *Photobacterium* genera (Supplementary Table 2).

3.4. Characterization of gut bacterial communities and their dietary modulation

The total number of high-quality reads obtained from fecal samples was 605,404. Their microbial profiles were mainly formed by 6 phyla, 9 classes, 10 orders, 18 families, and 20 genera. In Fig. 2 the gut microbial profiles are reported at the phylum and family level for each feeding group. The complete profiles of bacterial communities are detailed in Supplementary Data File 1. The analysis of alpha diversity revealed an increased species richness (Chao1) in fecal samples from the PEM feeding group. In contrast, the gut microbial communities of fish fed the SHM diet had the lowest chao1 index value (Table 3). Analysis of beta diversity only showed significant differences among bacterial

Table 3
Alpha diversity indices of microbial communities of feeds and gut samples. Different letters indicate significant differences between means within the same column ($p < 0.05$). values are reported as mean ± SD ($n = 6$).

	Chao1	Faith-PD	Shannon	Simpson
Feed samples				
FM	194.50 ± 8.02^{c}	7.41 ± 0.25^{c}	2.26 ± 0.08^{c}	0.65 ± 0.01^{c}
PEM	280.48 ± 9.60^{a}	9.37 ± 0.31^{a}	3.86 ± 0.08^{a}	0.79 ± 0.01^{a}
SHM	233.01 ± 16.98^{b}	8.19 ± 0.33^{b}	2.72 ± 0.04^{b}	0.70 ± 0.01^{b}
Gut samples				
FM	115.98 ± 69.08^{ab}	6.34 ± 2.46	2.48 ± 0.67	0.72 ± 0.09
PEM	248.23 ± 116.60^{a}	9.97 ± 3.13	3.53 ± 0.92	0.81 ± 0.07
SHM	114.43 ± 71.56^{b}	6.34 ± 2.46	2.79 ± 0.84	0.77 ± 0.12

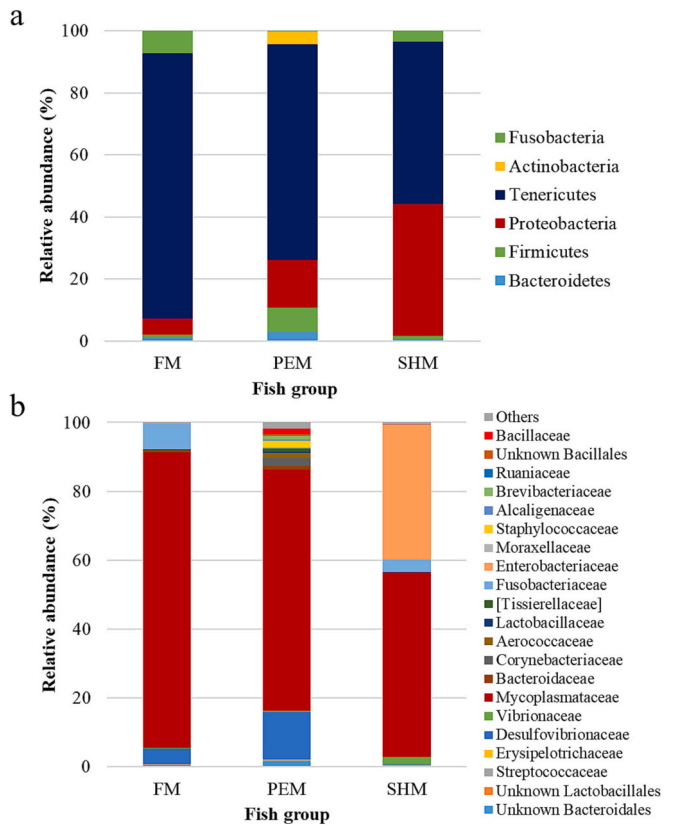


Fig. 2. Taxonomic composition of gut microbiota of trout fed three tested diets. The relative abundance of the overall most prevalent bacterial phyla (a) and families (b) are reported. Bacteria taxa with lower abundance (< 1%) are pooled and indicated as "Others".

communities for the unweighted UniFrac metric. The PERMANOVA non-parametric test indicated that gut bacterial communities of fish fed with the PEM diet differed significantly from those of FM and SHM groups (Table 4). Accordingly, the principal coordinate analysis (PCoA) plot of unweighted UniFrac distances of gut microbial communities revealed a separate cluster of PEM samples, with the exception of two outliers, from other sample groups (Fig. 3), whereas the PCoA plot based on weighted UniFrac distances showed no clustering by diet (data not shown). To further evaluate differences in the bacterial composition of gut samples, PLS-DA was performed based on relative taxa abundance of the most representative taxa (relative abundance >0.1%) at genus level.

The PLS-DA clearly separated the three experimental groups along component 1 (51% explained variance) and component 2 (21% explained variance) (Fig. 4a). Differences among gut bacterial communities were driven by 5 and 15 taxa (VIP > 1) for component 1 and 2, respectively (Fig. 4b). At the phylum level, the six most abundant phyla identified were Tenericutes (Mycoplasmatota), Proteobacteria, Firmicutes, Fusobacteria, Actinobacteria, and Bacteroidetes, in that order (Fig. 2). Among them, Actinobacteria were influenced by diet, resulting in a higher amount in fish fed insect exuviae (Fig. 2; Table 5). At the order level, the PEM fish group showed an enrichment of Actinomycetales and Bacillales, whereas Enterobacteriales were more abundant in intestines of fish fed SHM diet as compared to other groups of fish (Table 5). Representation of the Desulfovibrionales order was significantly lower in gut of SHM fish. Accordingly, the Desulfovibrinaceae family was reduced in this fish group, whereas Corynebacteriaceae, Aerococcaceae, Staphylococcaceae, and Bacillaceae were in higher abundance in PEM samples (Fig. 2; Table 5). At the genus level, *Corynebacterium* and *Facklamia* were enriched in PEM gut samples; moreover, only in these fish were *Bacillus*, *Jeotgalicoccus*, *Brevibacterium*,

Table 4
Results of PERMANOVA analysis performed on Unweighted and Weighted UniFrac distance data. The significant *p*-values ($p < 0.05$) are reported in bold.

PERMANOVA analysis		
Unweighted UniFrac distances		
Permutation N: 999		
	Pseudo-F	*p*-value
PEM vs FM	3.67	**0.018**
PEM vs SHM	4.15	**0.006**
SHM vs FM	2.06	0.064
feed FM vs feed PEM	14.36	0.109
feed FM vs feed SHM	7.34	0.095
feed PEM vs feed SHM	39.06	0.104
FM vs feed FM	9.85	**0.013**
PEM vs feed PEM	5.66	**0.010**
SHM vs feed SHM	3.81	**0.017**
Weighted UniFrac distances		
Permutation N: 999		
	Pseudo-F	*p*-value
PEM vs FM	1.17	0.304
PEM vs SHM	2.55	0.057
SHM vs FM	3.25	0.050
feed FM vs feed PEM	196.84	0.100
feed FM vs feed SHM	56.42	0.120
feed PEM vs feed SHM	264.99	0.086
FM vs feed FM	17.53	**0.018**
PEM vs feed PEM	24.27	**0.010**
SHM vs feed SHM	5.142	**0.013**

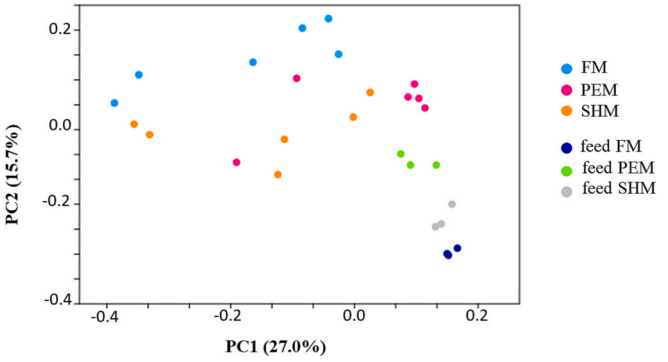


Fig. 3. Principal coordinate analysis (PCoA) of Unweighted Unifrac distances of gut microbial communities associated to different diet. The figures show the 2D plot of individual fish and feed samples according to their microbial profile at genus level.

Oceanobacillus, and *Atopostipes* genera detected. Relative abundance of *Photobacterium* was higher in intestines of trout fed SHM, in which *Serratia* genus was also found. Genus *Cetobacterium* was only detectable in great relative abundance in control fish FM (Table 5). Results of Kruskal-Wallis's analysis were checked/confirmed by pairwise comparison using Welch's *t*-test, which detected that 13 and 6 taxa differed between control and PEM or SHM groups, respectively (Supplementary Tables 3, 4).

3.5. *Predictive functional profiling of gut microbial communities*

PICRUSt analysis results of predicted functional pathways in the gut microbiota showed differences in response to diet. Specifically, eleven pathways significantly differed between the control group FM and fish fed the PEM diet (Fig. 5). Gene-encoded proteins involved in nitrogen, sulfur, glyoxylate and dicarboxylate metabolism, and cell growth were

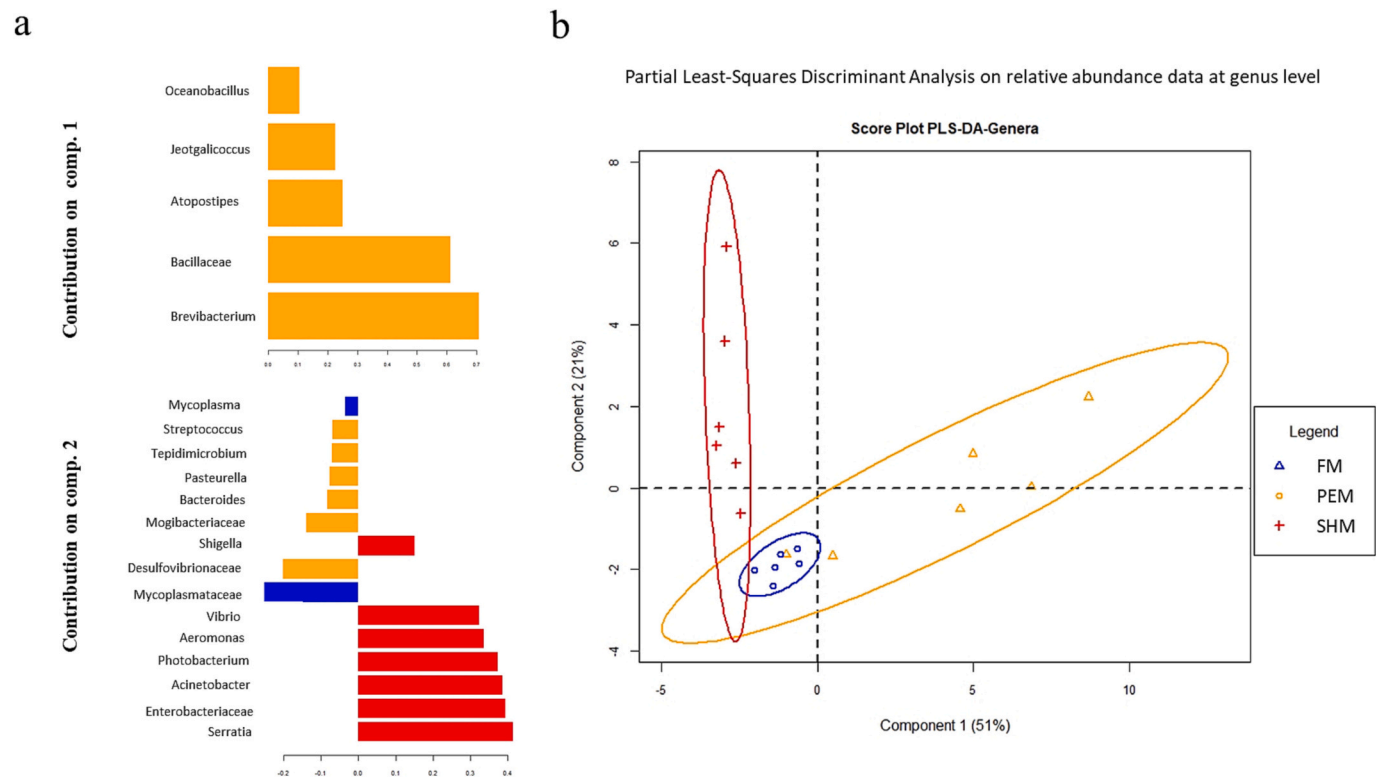


Fig. 4. Score plots of variable importance in projection (VIP) (a) and PLS-DA (b) of gut microbial communities.

in greater abundance in gut microbiota of trout fed PEM than in FM controls. In the latter, in contrast, a higher number of genes were involved in DNA replication, RNA translation, and starch and sucrose metabolism. Twenty functional traits were significantly different between the FM and SHM fish groups; thirteen of them were improved in controls (Fig. 6). The total substitution of FM with SHM led to an increase in genes engaged in sulfur and propanoate metabolism, secretion system, ABC transporters, and folding catalysis. In contrast, in comparison with the FM group, glycolysis, lysine and fatty acid biosynthesis, in addition to DNA replication and RNA translation pathways, were represented to a lesser degree in the intestinal microbial communities.

3.6. Quantification of SCFAs in fecal samples

Considering the well-known beneficial effects of SCFAs at the intestinal level, the effect of dietary chitin derived from PEM and SHM intake was evaluated by quantifying the fecal content of SCFA in rainbow trout. The dietary addition of PEM caused a significant increase in fecal acetic acid, propionic acid, and butyric acid with respect to SHM and FM diets ($p < 0.01$). Butyric acid concentration was the lowest in feces collected from fish fed SHM ($p < 0.01$). Additionally, the FM-based diet significantly reduced the concentrations of both acetic acid and propionic acid (Table 6).

4. Discussion

In this study, we evaluated the effects of including *Hermetia illucens* exuviae on gut-associated microbial communities of rainbow trout and compared these effects with another waste by-product rich in chitin, i.e., SHM. For this purpose, a feeding trial was conducted with three isoproteic, isolipidic, and isoenergetic diets containing either FM (control diet), SHM, or PEM (FM and 1.6% of pupal exuviae meal).

Overall, at the end of the 91-day feeding trial, fish showed good performance in response to the test diets PEM or SHM when compared to the FM control formulation. This is not surprising and confirms what has been found in our previous studies and in others already reported in literature, i.e., partial or total substitution of FM with chitin-containing insect meals does not negatively affect rainbow trout growth performance (Rimoldi et al., 2019; Terova et al., 2021, 2019). However, it is also true that chitin has been hypothesized to have a potentially negative impact on fish performance and nutrient digestibility. For instance, it was recently found in both Nile tilapia (*Oreochromis niloticus*) and rainbow trout that chitin can act as a nutrient source as well as an anti-nutrient because its digestibility is negatively related to dietary inclusion levels (Eggink et al., 2022). The indications that chitin, as a non-digestible fiber, negatively influences the digestibility of lipids and proteins have led to a tendency to remove chitin from food and feed. However, this tendency is changing in light of recent studies showing the potential application of insect meal and chitin and its derivatives as effective prebiotics and antibacterial and immunomodulatory compounds in fish feeds (Ahmed et al., 2021; Dawood et al., 2020; Gaudioso et al., 2021; Rimoldi et al., 2021; Terova et al., 2021, 2019).

As reported in the literature, the chitin content of pupal exuviae from black soldier fly is approximately 25% (Triunfo et al., 2022), whereas the SHM used in this trial contained 35% chitin.

Regardless of the diet, a high abundance of Tenericutes followed by Proteobacteria and Firmicutes phyla was found by characterizing the microbial communities in the gut of rainbow trout, which is in agreement with previous reports on the same species (Gaudioso et al., 2021; Rimoldi et al., 2021, 2019, 2018; Terova et al., 2021, 2019). Tenericutes were mainly represented by the Mycoplasmataceae family in which, not surprisingly, *Mycoplasma* was the dominant genus found in the distal intestine of rainbow trout (Antonopoulou et al., 2019; Lyons et al., 2017; Mente et al., 2018). It is considered a gut symbiont of rainbow trout, which contributes to the host metabolism using dietary substrates to produce lactic and acetic acid as the main fermentation end-products (Agerbo Rasmussen et al., 2021; Bolyen et al., 2019; Lyons et al., 2017). Moreover, *Mycoplasma* seems to have an essential role in maintaining gut homeostasis in rainbow trout and a decrease in its abundance has been related to higher susceptibility to disease and inflammation in

Table 5
Mean relative abundance (%) ± SE (n = 6) of the most prevalent phyla, orders, classes, families, and genera found in the intestine of trout fed with three tested diets. Different letters indicate differences among means in the same row. ANOVA significance: (*) $p < 0.05$, (**) $p < 0.01$.

Taxonomy	FM			PEM			SHM			*Sig.*
Phylum										
Bacteroidetes	1.08	±	0.50	2.95	±	1.16	0.29	±	0.17	
Firmicutes	0.89	±	0.35	7.73	±	2.92	1.27	±	0.73	
Proteobacteria	5.17	±	2.75	15.37	±	5.77	42.47	±	15.00	
Tenericutes	85.66	±	8.22	69.50	±	4.52	52.45	±	15.52	
Actinobacteria	0.03	±	0.01^{b}	4.31	±	1.57^{a}	0.07	±	0.04^{b}	*
Fusobacteria	7.18	±	7.08	0.14	±	0.05	3.45	±	3.30	
Class										
Bacteroidia	1.07	±	0.49	2.86	±	1.14	0.20	±	0.15	
Bacilli	0.30	±	0.13^{b}	6.16	±	2.53^{a}	0.91	±	0.52^{ab}	*
Clostridia	0.51	±	0.20	1.37	±	0.49	0.27	±	0.16	
Alphaproteobacteria	0.27	±	0.16	0.69	±	0.27	1.60	±	0.69	
Deltaproteobacteria	4.56	±	2.80^{a}	13.68	±	6.11^{a}	0.26	±	0.10^{b}	**
Gammaproteobacteria	0.30	±	0.10^{b}	0.78	±	0.28^{b}	40.59	±	14.98^{a}	**
Mollicutes	85.66	±	8.22	69.50	±	4.52	52.45	±	15.52	
Actinobacteria	0.02	±	0.01^{b}	4.29	±	1.56^{a}	0.06	±	0.04^{b}	**
Fusobacteriia	7.18	±	7.08	0.14	±	0.05	3.45	±	3.30	
Order										
Bacteroidales	1.08	±	0.50	2.90	±	1.16	0.21	±	0.15	
Lactobacillales	0.29	±	0.12	2.25	±	0.93	0.76	±	0.42	
Clostridiales	0.52	±	0.20	1.38	±	0.50	0.28	±	0.17	
Desulfovibrionales	4.57	±	2.80^{a}	13.75	±	6.10^{a}	0.26	±	0.10^{b}	**
Vibrionales	0.12	±	0.004	0.29	±	0.17	1.94	±	1.02	
Mycoplasmatales	85.85	±	8.15	70.09	±	4.49	53.28	±	15.57	
Actinomycetales	0.02	±	0.01^{b}	4.27	±	1.55^{a}	0.06	±	0.04^{b}	**
Fusobacteriales	7.27	±	7.17	0.14	±	0.05	3.48	±	3.33	
Enterobacteriales	0.05	±	0.02^{b}	0.21	±	0.13^{b}	38.77	±	15.18^{a}	**
Bacillales	0.00	±	0.00^{b}	3.98	±	1.64^{a}	0.15	±	0.09^{ab}	**
Family										
Streptococcaceae	0.17	±	0.07	0.22	±	0.09	0.10	±	0.05	
Erysipelotrichaceae	0.08	±	0.03	0.21	±	0.09	0.09	±	0.06	
Desulfovibrionaceae	4.57	±	2.80^{a}	13.74	±	6.10^{a}	0.26	±	0.10^{b}	**
Vibrionaceae	0.12	±	0.04	0.29	±	0.17	1.95	±	1.03	
Mycoplasmataceae	85.88	±	8.16	70.06	±	4.49	53.51	±	15.58	
Bacteroidaceae	0.48	±	0.23	1.15	±	0.43	0.11	±	0.07	
Corynebacteriaceae	0.02	±	0.01^{b}	2.32	±	0.84^{a}	0.03	±	0.02^{b}	**
Aerococcaceae	0.00	±	0.00^{b}	1.37	±	0.56^{a}	0.03	±	0.02^{b}	*
Lactobacillaceae	0.05	±	0.04	0.31	±	0.12	0.07	±	0.04	
[Tissierellaceae]	0.27	±	0.11^{ab}	0.95	±	0.37^{a}	0.08	±	0.05^{b}	*
Fusobacteriaceae	7.27	±	7.17	0.14	±	0.05	3.49	±	3.33	
Enterobacteriaceae	0.05	±	0.02^{b}	0.21	±	0.13^{b}	38.94	±	15.58^{a}	**
Moraxellaceae	0.03	±	0.01	0.05	±	0.02	0.30	±	0.07	
Staphylococcaceae	0.00	±	0.00^{b}	1.95	±	0.84^{a}	0.03	±	0.02^{b}	**
Alcaligenaceae	0.02	±	0.01	0.21	±	0.08	0.00	±	0.00	
Brevibacteriaceae	0.00	±	0.00	1.10	±	0.38	0.00	±	0.00	
Ruaniaceae	0.00	±	0.00	0.21	±	0.08	0.00	±	0.00	
Bacillaceae	0.00	±	0.00	1.55	±	0.65	0.02	±	0.02	
Genus										
Streptococcus	0.24	±	0.07	0.33	±	0.11	0.07	±	0.04	
Vibrio	0.14	±	0.02	0.19	±	0.06	2.56	±	1.12	
Mycoplasma	12.54	±	4.50	23.37	±	8.14	32.94	±	2.09	
Bacteroides	0.89	±	0.22	1.67	±	0.55	0.08	±	0.04	
Corynebacterium	0.03	±	0.01^{b}	3.22	±	1.17^{a}	0.05	±	0.03^{b}	**
Facklamia	0.01	±	0.00^{b}	1.07	±	0.45^{a}	0.05	±	0.03^{ab}	*
Lactobacillus	0.09	±	0.05	0.44	±	0.16	0.08	±	0.05	
Tepidimicrobium	0.30	±	0.09	0.82	±	0.41	0.11	±	0.07	
Aeromonas	0.02	±	0.01	0.04	±	0.02	0.27	±	0.14	
Shigella	0.01	±	0.01	0.16	±	0.11	1.23	±	0.84	
Pasteurella	0.10	±	0.03	0.22	±	0.07	0.00	±	0.00	
Acinetobacter	0.04	±	0.01	0.04	±	0.01	0.35	±	0.16	
Jeotgalicoccus	0.00	±	0.00	0.28	±	0.10	0.00	±	0.00	
Cetobacterium	14.53	±	10.27	0.00	±	0.00	0.00	±	0.00	
Bacillus	0.00	±	0.00	0.74	±	0.35	0.07	±	0.00	
Photobacterium	0.02	±	0.01^{b}	0.02	±	0.01^{ab}	0.48	±	0.26^{a}	*
Brevibacterium	0.00	±	0.00	1.46	±	0.54	0.00	±	0.00	
Oceanobacillus	0.00	±	0.00	0.70	±	0.29	0.01	±	0.01	
Atopostipes	0.00	±	0.00	0.90	±	0.36	0.00	±	0.00	
Serratia	0.00	±	0.00	0.00	±	0.00	0.32	±	0.22	

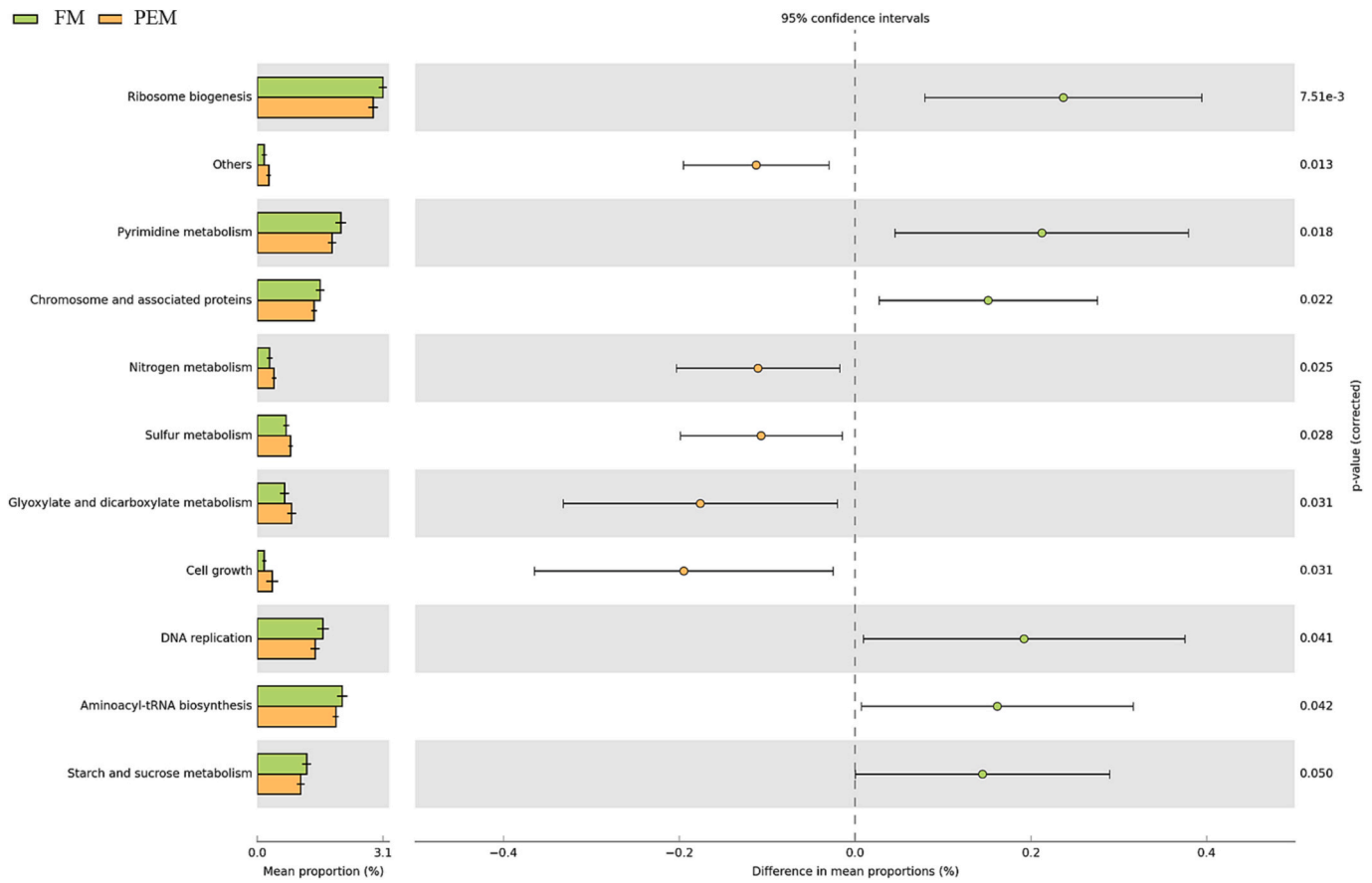


Fig. 5. Inferred metagenome pathway analysis with PICRUSt between gut microbiome profiles of control (FM) and PEM groups of fish. The extended error bar graph and statistical analysis were made using STAMP bioinformatics software.

this species (Brown et al., 2019).

When compared to the control diet, we observed more pronounced changes in the gut microbiota community in response to the PEM diet than to totally replacing FM with SHM. Trout fed the PEM diet showed an increase in bacterial species richness as estimated by the Chao1 index and major shifts in gut microbial community composition. Accordingly, multivariate analysis clustered the PEM dietary group separately from the other two. Specifically, including 1.6% of exuviae meal led to an increase in the relative abundance of Actinobacteria families Corynebacteriaceae and Brevibacteriaceae, and Firmicutes families Staphylococcaceae, Aerococcaceae, and Bacillaceae. Similarly, consumption of a diet containing 25% of *H. illucens* exuviae meal increased the abundance of the Bacillaceae, Staphylococcaceae, Paenibacillaceae families as well as the Actinobacteria family of Brevibacteriaceae in the digesta community of sea bass (Rangel et al., 2022a). An increase in Actinobacteria relative abundance was found in the gut of trout fed *Tenebrio molitor* larvae meal supplemented diet, but not so relevant in sea bass and sea bream (Antonopoulou et al., 2019). Actually, including dietary fiber, such as chitin, generally shifts the intestinal microbiota towards Firmicutes and/or Actinobacteria (Bruni et al., 2018; Gaudioso et al., 2021; Huyben et al., 2019; Rimoldi et al., 2021; Terova et al., 2019). Interestingly, Firmicutes and Actinobacteria phyla include several chitin-degrading bacteria species belonging to the genera *Paenibacillus*, *Actinomyces*, *Bacillus*, and *Brevibacterium,* which usually became enriched in the intestines of those fish fed a chitin-containing diet (Callegari et al., 2020; Gaudioso et al., 2021; Rangel et al., 2022a, 2022b; Rimoldi et al., 2021). Accordingly, in the present study, dietary inclusion of exuviae meal promoted an increase in *Corynebacterium* and *Facklamia* genera compared to other feeding groups, whereas *Bacillus*, *Brevibacterium, Jeotgalicoccus*, *Oceanobacillus*, and *Atopostipes* genera were exclusively related to the PEM diet. From our data, it was evident that SHM did not lead to an increase in the same bacteria taxa with notorious chitinolytic capabilities despite its higher chitin content. Indeed, in trout fed SHM we found that the Gammaproteobacteria class was enriched with an increase in the relative abundance of the *Photobacterium* genus and the exclusive presence of *Serratia* genus in their intestinal microbiota. However, it is interesting to note that, although the Gammaproteobacteria class comprises several potential pathogenic genera, both the *Photobacterium* and *Serratia* genera include bacteria species producing chitinases (Paulsen et al., 2016; Swiontek Brzezinska et al., 2014).

Similarly, Rangel and colleagues (Rangel et al., 2022a) observed distinct changes in the digesta gut microbiota profiles in response to diets tested even when the chitin content of the diets was similar (1.3–1.8%). Unexpectedly, in their study, the inclusion of 5% of commercial chitin did not lead to an increase in either Firmicutes or Actinobacteria phyla, indicating that the composition and predicted metabolic capacity of gut microbiota can be modulated differently by different chitin sources. Actually, the physicochemical properties of chitin and chitosan may vary among samples, being affected by many factors, such as the source of chitin and the insect's life stage. The exoskeleton of arthropods is naturally formed by various chitin-associated proteins and lipids and varying degrees of chitin acetylation (Abidin et al., 2020; Soetemans et al., 2020; Zhang et al., 2000). In addition, it has been reported that morphology in *H. illucens* samples is different than that of shrimp chitin. Chitin of shrimp simply consisted of different nanofibers with variable thickness without any pores, which are instead present in *H. illucens* chitin (Soetemans et al., 2020). The common idea is that differences in chemical composition, structure (surface, porosity), and/or solubility of chitin can affect its bio

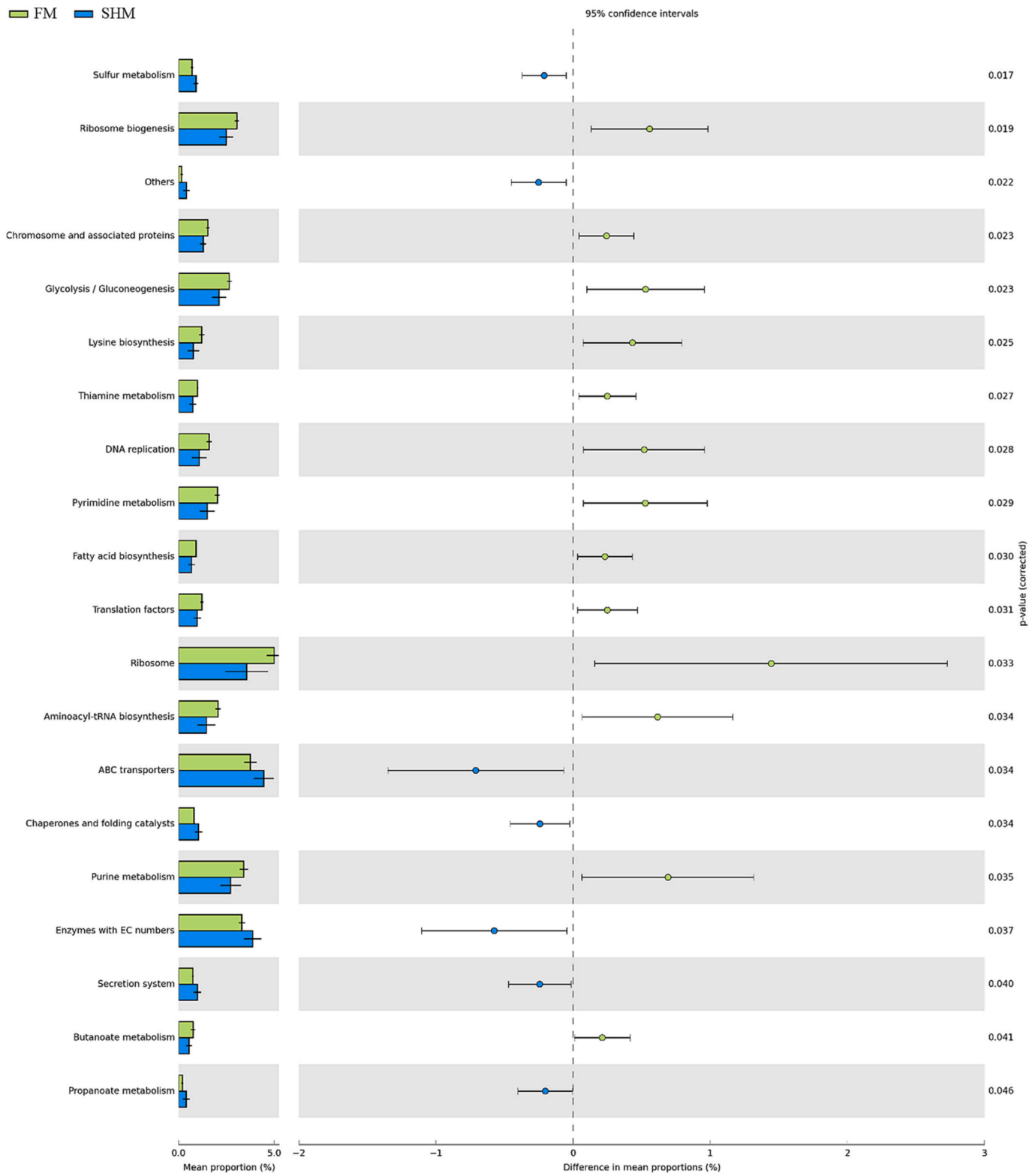


Fig. 6. Inferred metagenome pathway analysis with PICRUSt between gut microbiome profiles of control (FM) and SHM groups of fish. The extended error bar graph and statistical analysis were made using STAMP bioinformatics software.

accessibility.

It is also true that feed pellets containing insect exuviae (PEM) contained a higher amount of both Firmicutes and Actinobacteria phyla, whereas microbiota associated with FM and SHM feeds was mainly composed of Proteobacteria. At the genus level, *Corynebacterium*, *Bacillus*, *Facklamia*, and *Brevibacterium* were found to be associated with PEM feed. SHM-based feed showed instead an association with *Aeromonas*, *Acinetobacter*, and *Photobacterium* genera. These results could indicate that a microbiota transfer effect takes place from feed to the gut allochthonous bacterial community. This feed-borne microbiota effect has been previously reported in rainbow trout and Atlantic salmon (Gaudioso et al., 2021; Li et al., 2021; Mente et al., 2018). Indeed, feed

Table 6
Acetic acid, propionic acid and butyric acid amount in fecal samples of fish fed on FM, PEM, and SHM. Data are shown as mean values ± SD (n = 3). Different letters in the same column indicate significant difference between means ($p < 0.05$).

Feeding group	Acetic acid (mM)	Propionic acid (mM)	Butyric acid (mM)
FM	6.42 ± 0.84^{c}	0.01 ± 0.01^{c}	0.52 ± 0.19^{b}
PEM	16.24 ± 0.71^{a}	1.23 ± 0.30^{a}	2.04 ± 0.21^{a}
SHM	13.76 ± 0.58^{b}	0.73 ± 0.08^{b}	0.32 ± 0.07^{c}

can contain genetic material from animal and plant species that were used in its formulation, as well as the microbes associated to those ingredients (Karlsen et al., 2022). Although microbial contaminants may be killed by the high temperature during feed extrusion process, their DNA fragments may remain. However, this does not mean that the observed differences at the gut level were simply a consequence of microbiota transfer from feed; indeed, the PERMANOVA analysis and the PCoA results confirmed that intestinal communities were significantly different from feed-associated bacterial communities.

In fact, predictive functional analysis revealed unique features for each experimental group. Compared to controls, nitrogen, sulfur, glyoxylate and dicarboxylate metabolism, and cell growth pathways were enriched in fish receiving the PEM diet. Whereas the same analysis revealed that transport system (including ABC transporters), sulfur and propanoate metabolism, secretion, and folding catalysis were enhanced in the intestinal microbiota of trout fed the SHM-based diet. In any case, no predicted functional categories influenced by diet were related to chitin's degradation and/or metabolism.

On the other hand, the differences in abundance of taxa belonging to the Actinobacteria phylum and *Bacillus* genus between dietary groups may be indicative of the microbial responses to prebiotic chitin from insect exuviae meal. The Bacillaceae family includes species, such as *Bacillus subtilis*, that represent ideal multifunctional probiotic bacteria with beneficial effects on nutrient digestion and assimilation and prevention of pathogens development (Olmos et al., 2020; Soltani et al., 2019; Van Doan et al., 2019). Moreover, it is well known that the dietary administration of probiotics can also exert a positive effect on the production of volatile SCFAs, such as acetate, propionate, and butyrate in fish intestine. SCFAs are the principal end products of chitin bacterial fermentation and, in general, of anaerobic bacterial fermentation of dietary fibers in the gastrointestinal tract. It is well established that SCFAs, especially butyrate, have several beneficial roles promoting fish intestinal health, being an energy source for colonocytes, regulating mucosal homeostasis, and exerting anti-inflammatory effects (Canani et al., 2011; Hamer et al., 2008; Koh et al., 2016; Rimoldi et al., 2016).

In our study, total SCFA levels, in particular butyrate, were higher in the feces of trout fed the exuviae-containing diet. In contrast, the total substitution of FM with SHM positively affected only the amount of acetic and propionic acid, but not butyrate, which was lower than in control fish. This increase in SCFA concentrations observed in PEM fish may be the result of modulation in the microbial population induced by the insect exuviae. Specifically, *Corynebacterium*, *Bacillus*, and *Brevibacterium* genera represent the major contributors to changes in gut microbiota composition of the PEM group and may thus connect the chitin degradation with high SCFA production. Actually, Bacillus and bacteria genera affiliated with Actinobacteria actinomycetes are generally identified as chitin degraders (Swiontek Brzezinska et al., 2014; Lacombe-Harvey et al., 2018). The capacity to produce volatile SCFAs, specifically acetate, was recently demonstrated for Bacillus sp. isolated from the gastrointestinal tract of adult Malaysian Mahseer (*Tor tambroides*) (Asaduzzaman et al., 2018). Similarly, an insect-based diet modulated gut microbiota composition and SCFA production by increasing propionate and butyrate in the ceca of laying hens (Borrelli et al., 2017). Additionally, in weaned piglets, chitosan-chelated zinc modulated ileal microbiota and microbial metabolites propionate, butyrate, and lactate (Hou et al., 2021). In contrast, there is still a lack of knowledge surrounding the importance and functionality of the gut microbiota in fish nutrition (Lokesh et al., 2022).

5. Conclusions

In summary, the present study demonstrates that *H. illucens*-derived exuviae exert selective pressure on fish gut microbiota to increasing bacteria belonging to the Firmicutes and Actinobacteria phyla. In particular, insect exuviae are a credible candidate prebiotic greatly affecting the fish gut microbiota by increasing the gut bacterial richness and the number of beneficial chitin-degrading bacteria, such as *Bacillus* genera, thus promoting the microbial synthesis of SCFAs, primarily butyrate.

Our study has discovered encouraging prospects for the use of insect exuviae in fish diets as a functional ingredient and may open new opportunities for microbiota mediated nutritional optimization in aquaculture.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739256.

Funding

The current study was supported by the EU Horizon 2020 AQUA-IMPACT (Genomic and nutritional innovations for genetically superior farmed fish to improve efficiency in European aquaculture), number: 818367.

Author's contributions

Conceptualization: G.T., F.B., and F.F.; methodology: S.R., C.C., F.F., M.A., and F.B.; Data collection, curation, and analysis, S.R., C.C., G.T., M.A.; writing—original draft preparation, S.R.; writing—review and editing, G.T.; funding acquisition, F.B., and G.T. All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Simona Rimoldi: Methodology, Data curation, Writing – original draft. **Chiara Ceccotti:** Methodology, Data curation, Formal analysis. **Fabio Brambilla:** Conceptualization, Methodology, Funding acquisition. **Filippo Faccenda:** Conceptualization, Methodology. **Micaela Antonini:** Methodology, Data curation. **Genciana Terova:** Conceptualization, Data curation, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

F.B. is employed by Naturalleva VRM Srl. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

All sequencing raw data were submitted and are freely available on the European Nucleotide Archive (EBI ENA) public database, under the accession code PRJEB55867.

Acknowledgements

Authors are thankful to Prof. Marco Saroglia for helpful discussions and expert guidance.

References

Abdel-Ghany, H.M., Salem, M.E.S., 2020. Effects of dietary chitosan supplementation on farmed fish; a review. Rev. Aquac. 12, 438–452. https://doi.org/10.1111/RAQ.12326.

Abidin, Nurul Alyani Zainol, Kormin, F., Abidin, Nurul Akhma Zainol, Anuar, N.A.F.M., Bakar, M.F.A., 2020. The potential of insects as alternative sources of chitin: an overview on the chemical method of extraction from various sources. Int. J. Mol. Sci. 21, 1–25. https://doi.org/10.3390/IJMS21144978.

Agerbo Rasmussen, J., Rømer Villumsen, K., Duchêne, D.A., Christine Puetz, L., Delmont, T.O., Sveier, H., von Gersdorff Jørgensen, L., Praebel, K., Martin, M.D., Miki Bojesen, A., Thomas Gilbert, M.P., Kristiansen, K., Morten Limborg, T., 2021. Genome-resolved metagenomics suggests a mutualistic relationship between Mycoplasma and salmonid hosts. Commun. Biol. 4, 579. https://doi.org/10.1038/s42003-021-02105-1.

Ahmed, F., Soliman, F.M., Adly, M.A., Soliman, H.A.M., El-Matbouli, M., Saleh, M., 2019. Recent progress in biomedical applications of chitosan and its nanocomposites in aquaculture: a review. Res. Vet. Sci. 126, 68–82. https://doi.org/10.1016/J.RVSC.2019.08.005.

Ahmed, F., Soliman, F.M., Adly, M.A., Soliman, H.A.M., El-Matbouli, M., Saleh, M., 2021. Dietary chitosan nanoparticles: potential role in modulation of rainbow trout (*Oncorhynchus mykiss*) antibacterial defense and intestinal immunity against enteric redmouth disease. Mar. Drugs 19. https://doi.org/10.3390/MD19020072.

Alishahi, A., Aïder, M., 2011. Applications of chitosan in the seafood industry and aquaculture: a review. Food Bioprocess Technol. 5, 817–830. https://doi.org/10.1007/S11947-011-0664-X.

Antonopoulou, E., Nikouli, E., Piccolo, G., Gasco, L., Gai, F., Chatzifotis, S., Mente, E., Kormas, K.A., 2019. Reshaping gut bacterial communities after dietary Tenebrio molitor larvae meal supplementation in three fish species. Aquaculture 503, 628–635. https://doi.org/10.1016/j.aquaculture.2018.12.013.

Asaduzzaman, M., Iehata, S., Akter, S., Kader, M.A., Ghosh, S.K., Khan, M.N.A., Abol-Munafi, A.B., 2018. Effects of host gut-derived probiotic bacteria on gut morphology, microbiota composition and volatile short chain fatty acids production of Malaysian Mahseer Tor tambroides. Aquac. Rep. 9, 53–61. https://doi.org/10.1016/j.aqrep.2017.12.003.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. https://doi.org/10.1038/s41587-019-0209-9.

Borrelli, L., Coretti, L., Dipineto, L., Bovera, F., Menna, F., Chiariotti, L., Nizza, A., Lembo, F., Fioretti, A., 2017. Insect-based diet, a promising nutritional source, modulates gut microbiota composition and SCFAs production in laying hens. Sci. Rep. 7, 1–11. https://doi.org/10.1038/s41598-017-16560-6.

Brown, R.M., Wiens, G.D., Salinas, I., 2019. Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol. 86, 497–506. https://doi.org/10.1016/J.FSI.2018.11.079.

Bruni, L., Pastorelli, R., Viti, C., Gasco, L., Parisi, G., 2018. Characterisation of the intestinal microbial communities of rainbow trout (Oncorhynchus mykiss) fed with Hermetia illucens (black soldier fly) partially defatted larva meal as partial dietary protein source. Aquaculture 487, 56–63. https://doi.org/10.1016/j.aquaculture.2018.01.006.

Bruni, L., Belghit, I., Lock, E., Secci, G., Taiti, C., Parisi, G., 2020. Total replacement of dietary fish meal with black soldier fly (Hermetia illucens) larvae does not impair physical, chemical or volatile composition of farmed Atlantic salmon (Salmo salar L.). J. Sci. Food Agric. 100, 1038–1047. https://doi.org/10.1002/jsfa.10108.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 137 (13), 581–583. https://doi.org/10.1038/nmeth.3869.

Callegari, M., Jucker, C., Fusi, M., Leonardi, M.G., Daffonchio, D., Borin, S., Savoldelli, S., Crotti, E., 2020. Hydrolytic profile of the culturable gut bacterial community associated with Hermetia illucens. Front. Microbiol. 11 https://doi.org/10.3389/FMICB.2020.01965.

Canani, R.B., Di Costanzo, M., Leone, L., Pedata, M., Meli, R., Calignano, A., 2011. Potential beneficial effects of butyrate in intestinal and extraintestinal diseases. World J. Gastroenterol. 17, 1519–1528. https://doi.org/10.3748/wjg.v17.i12.1519.

Clements, K.D., Gleeson, V.P., Slaytor, M., 1994. Short-chain fatty acid metabolism in temperate marine herbivorous fish. J. Comp. Physiol. B. 1645 (164), 372–377. https://doi.org/10.1007/BF00302552.

Danulat, E., 1986. The effects of various diets on chitinase and ß-glucosidase activities and the condition of cod, Gadus morhua (L.). J. Fish Biol. 28, 191–197. https://doi.org/10.1111/J.1095-8649.1986.TB05157.X.

Dawood, M.A.O., Gewaily, M.S., Soliman, A.A., Shukry, M., Amer, A.A., Younis, E.M., AbdelWarith, A.W.A., Van Doan, H., Saad, A.H., Aboubakr, M., Abdel-Latif, H.M.R., Fadl, S.E., 2020. Marine-derived chitosan nanoparticles improved the intestinal histo-morphometrical features in association with the health and immune response of grey mullet (Liza ramada). Mar. Drugs Vol. 18. https://doi.org/10.3390/MD18120611. Page 611 18, 611.

Eggink, K.M., Pedersen, P.B., Lund, I., Dalsgaard, J., 2022. Chitin digestibility and intestinal exochitinase activity in Nile tilapia and rainbow trout fed different black soldier fly larvae meal size fractions. Aquac. Res. 53, 5536–5546. https://doi.org/10.1111/ARE.16035.

Elserafy, S.S., Abdel-Hameid, N.A.H., Abdel-Salam, H.A., Dakrouni, A.M., 2021. Effect of shrimp waste extracted chitin on growth and some biochemical parameters of the Nile tilapia. Egypt. J. Aquat. Biol. Fish. 25, 313–329. https://doi.org/10.21608/EJABF.2021.143244.

FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fines, B.C., Holt, G.J., 2010. Chitinase and apparent digestibility of chitin in the digestive tract of juvenile cobia, Rachycentron canadum. Aquaculture 303, 34–39. https://doi.org/10.1016/J.AQUACULTURE.2010.03.010.

Foysal, M.J., Gupta, S.K., 2022. A systematic meta-analysis reveals enrichment of Actinobacteria and Firmicutes in the fish gut in response to black soldier fly (Hermetica illucens) meal-based diets. Aquaculture 549, 737760. https://doi.org/10.1016/J.AQUACULTURE.2021.737760.

Gasco, L., Biasato, I., Dabbou, S., Schiavone, A., Gai, F., 2019. Animals fed insect-based diets: state-of-the-art on digestibility, performance and product quality. Anim. Vol. 9 https://doi.org/10.3390/ANI9040170. Page 170 9, 170.

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P.P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., Roncarati, A., 2020. Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. Ital. J. Anim. Sci. 19, 360–372. https://doi.org/10.1080/1828051X.2020.1743209.

Gaudioso, G., Marzorati, G., Faccenda, F., Weil, T., Lunelli, F., Cardinaletti, G., Marino, G., Olivotto, I., Parisi, G., Tibaldi, E., Tuohy, K.M., Fava, F., 2021. Processed animal proteins from insect and poultry by-products in a fish meal-free diet for rainbow trout: impact on intestinal microbiota and inflammatory markers. Int. J. Mol. Sci. 22 https://doi.org/10.3390/IJMS22115454.

Gutowska, M.A., Drazen, J.C., Robison, B.H., 2004. Digestive chitinolytic activity in marine fishes of Monterey Bay, California. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 139, 351–358. https://doi.org/10.1016/J.CBPB.2004.09.020.

Hahn, T., Tafi, E., Paul, A., Salvia, R., Falabella, P., Zibek, S., 2020. Current state of chitin purification and chitosan production from insects. J. Chem. Technol. Biotechnol. 95, 2775–2795. https://doi.org/10.1002/JCTB.6533.

Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F.J., Brummer, R.-J., 2008. Review article: the role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27, 104–119. https://doi.org/10.1111/j.1365-2036.2007.03562.x.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4, 9.

Hou, G., Zhang, M., Wang, J., Zhu, W., 2021. Chitosan-chelated zinc modulates ileal microbiota, ileal microbial metabolites, and intestinal function in weaned piglets challenged with Escherichia coli K88. Appl. Microbiol. Biotechnol. 105, 7529–7544. https://doi.org/10.1007/S00253-021-11496-4.

Huyben, D., Vidaković, A., Werner Hallgren, S., Langeland, M., 2019. High-throughput sequencing of gut microbiota in rainbow trout (Oncorhynchus mykiss) fed larval and pre-pupae stages of black soldier fly (Hermetia illucens). Aquaculture 500, 485–491. https://doi.org/10.1016/j.aquaculture.2018.10.034.

Jiménez-Gómez, C.P., Cecilia, J.A., 2020. Chitosan: a natural biopolymer with a wide and varied range of applications. Molecules 25 (17), 3981. https://doi.org/10.3390/molecules25173981.

Karlsen, C., Tzimorotas, D., Robertsen, E.M., Kirste, K.H., Bogevik, A.S., Rud, I., 2022. Feed microbiome: confounding factor affecting fish gut microbiome studies. ISME Commun. 2, 1–4. https://doi.org/10.1038/s43705-022-00096-6.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., Bäckhed, F., 2016. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165, 1332–1345. https://doi.org/10.1016/j.cell.2016.05.041.

Kono, M., Shimizu, C., Matsui, T., 1987. Effect of chitin, chitosan, and cellulose as diet supplements on the growth of cultured fish. undefined 53, 125–129. https://doi.org/10.2331/SUISAN.53.125.

Kramer, K.J., Hopkins, T.L., Schaefer, J., 1995. Applications of solids NMR to the analysis of insect sclerotized structures. Insect Biochem. Mol. Biol. 25, 1067–1080. https://doi.org/10.1016/0965-1748(95)00053-4.

Kurita, K., 2006. Chitin and chitosan: functional biopolymers from marine crustaceans. Mar. Biotechnol. (NY). 8, 203–226. https://doi.org/10.1007/S10126-005-0097-5.

Lacombe-Harvey, M.È., Brzezinski, R., Beaulieu, C., 2018. Chitinolytic functions in actinobacteria: ecology, enzymes, and evolution. Appl. Microbiol. Biotechnol. 102 (17), 7219–7230. https://doi.org/10.1007/s00253-018-9149-4.

Langille, M.G.I., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Vega Thurber, R.L., Knight, R., Beiko, R.G., Huttenhower, C., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat. Biotechnol. 31, 814–821. https://doi.org/10.1038/nbt.2676.

Li, Y., Bruni, L., Jaramillo-Torres, A., Gajardo, K., Kortner, T.M., Krogdahl, Å., 2021. Differential response of digesta- and mucosa-associated intestinal microbiota to

dietary insect meal during the seawater phase of Atlantic salmon. Anim. Microbiome 3, 8. https://doi.org/10.1186/S42523-020-00071-3.
Lock, E.J., Biancarosa, I., Gasco, L., 2018. Insects as raw materials in compound feed for aquaculture. In: Edible Insects in Sustainable Food Systems. Springer International Publishing, pp. 263–276. https://doi.org/10.1007/978-3-319-74011-9_16.
Lokesh, J., Ghislain, M., Reyrolle, M., Le Bechec, M., Pigot, T., Terrier, F., Roy, J., Panserat, S., Ricaud, K., 2022. Prebiotics modify host metabolism in rainbow trout (Oncorhynchus mykiss) fed with a total plant-based diet: potential implications for microbiome-mediated diet optimization. Aquaculture 561, 738699. https://doi.org/10.1016/J.AQUACULTURE.2022.738699.
Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71, 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005.
Lozupone, C.A., Hamady, M., Kelley, S.T., Knight, R., 2007. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. Appl. Environ. Microbiol. 73, 1576–1585. https://doi.org/10.1128/AEM.01996-06.
Lyons, P.P., Turnbull, J.F., Dawson, K.A., Crumlish, M., 2017. Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout Oncorhynchus mykiss from both farm and aquarium settings. J. Appl. Microbiol. 122, 347–363. https://doi.org/10.1111/jam.13347.
Makkar, H.P.S., Tran, G., Heuzé, V., Ankers, P., 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197, 1–33. https://doi.org/10.1016/J.ANIFEEDSCI.2014.07.008.
Mente, E., Nikouli, E., Antonopoulou, E., Martin, S.A.M., Kormas, K.A., 2018. Core vs. diet-associated and postprandial bacterial communities of the rainbow trout (Oncorhynchus mykiss) midgut and faeces. Biol Open. 13 (7(6)), bio034397 https://doi.org/10.1242/bio.034397.
Nogales-Mérida, S., Gobbi, P., Ozefiak, D.J., Mazurkiewicz, J., Dudek, K., Rawski, M., Kiero Nczyk, B., Ozefiak, A.J., 2019. Insect meals in fish nutrition. Rev. Aquac. 11, 1080–1103. https://doi.org/10.1111/RAQ.12281.
Oliva-Teles, A., Enes, P., Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. In: Feed and Feeding Practices in Aquaculture. Elsevier, pp. 203–233. https://doi.org/10.1016/b978-0-08-100506-4.00008-8.
Olmos, J., Acosta, M., Mendoza, G., Pitones, V., 2020. Bacillus subtilis, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. Arch. Microbiol. 202, 427–435. https://doi.org/10.1007/S00203-019-01757-2.
Oren, A., Garrity, G.M., 2021. Valid publication of the names of forty-two phyla of prokaryotes. Int. J. Syst. Evol. Microbiol. 71 (10) https://doi.org/10.1099/ijsem.0.005056.
Parks, D.H., Tyson, G.W., Hugenholtz, P., Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30, 3123–3124. https://doi.org/10.1093/bioinformatics/btu494.
Paulsen, S.S., Andersen, B., Gram, L., MacHado, H., 2016. Biological potential of chitinolytic marine bacteria. Mar. Drugs 14. https://doi.org/10.3390/MD14120230.
Qin, C., Zhang, Y., Liu, W., Xu, L., Yang, Y., Zhou, Z., 2014. Effects of chito-oligosaccharides supplementation on growth performance, intestinal cytokine expression, autochthonous gut bacteria and disease resistance in hybrid tilapia Oreochromis niloticus ♀ × Oreochromis aureus ♂. Fish Shellfish Immunol. 40, 267–274. https://doi.org/10.1016/J.FSI.2014.07.010.
Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web based tools. Nucleic Acids Res. 41 https://doi.org/10.1093/NAR/GKS1219.
Rangel, F., Enes, P., Gasco, L., Gai, F., Hausmann, B., Berry, D., Oliva-Teles, A., Serra, C. R., Pereira, F.C., 2022a. Differential modulation of the European sea bass gut microbiota by distinct insect meals. Front. Microbiol. 13, 1249. https://doi.org/10.3389/FMICB.2022.831034/BIBTEX.
Rangel, F., Santos, R.A., Monteiro, M., Lavrador, A.S., Gasco, L., Gai, F., Oliva-Teles, A., Enes, P., Serra, C.R., 2022b. Isolation of chitinolytic bacteria from European sea bass gut microbiota fed diets with distinct insect meals. Biology (Basel). 11 https://doi.org/10.3390/BIOLOGY11070964.
Rimoldi, S., Finzi, G., Ceccotti, C., Girardello, R., Grimaldi, A., Ascione, C., Terova, G., 2016. Butyrate and taurine exert a mitigating effect on the inflamed distal intestine of European sea bass fed with a high percentage of soybean meal. Fish. Aquat. Sci. 19, 1–14. https://doi.org/10.1186/s41240-016-0041-9.
Rimoldi, S., Terova, G., Ascione, C., Giannico, R., Brambilla, F., 2018. Next generation sequencing for gut microbiome characterization in rainbow trout (Oncorhynchus mykiss) fed animal byproduct meals as an alternative to fishmeal protein sources. PLoS One 13, 1–29. https://doi.org/10.1371/journal.pone.0193652.
Rimoldi, S., Gini, E., Iannini, F., Gasco, L., Terova, G., 2019. The effects of dietary insect meal from Hermetia illucens prepupae on autochthonous gut microbiota of rainbow trout (Oncorhynchus mykiss). Animals 9. https://doi.org/10.3390/ani9040143.
Rimoldi, S., Gini, E., Koch, J.F.A., Iannini, F., Brambilla, F., Terova, G., 2020. Effects of hydrolyzed fish protein and autolyzed yeast as substitutes of fishmeal in the gilthead sea bream (Sparus aurata) diet, on fish intestinal microbiome. BMC Vet. Res. 16, 118. https://doi.org/10.1186/s12917-020-02335-1.
Rimoldi, S., Antonini, M., Gasco, L., Moroni, F., Terova, G., 2021. Intestinal microbial communities of rainbow trout (Oncorhynchus mykiss) may be improved by feeding a Hermetia illucens meal/low-fishmeal diet. Fish Physiol. Biochem. 47, 365–380. https://doi.org/10.1007/s10695-020-00918-1.
Ringø, E., Zhou, Z., Olsen, R.E., Song, S.K., 2012. Use of chitin and krill in aquaculture – the effect on gut microbiota and the immune system: a review. Aquac. Nutr. 18, 117–131. https://doi.org/10.1111/J.1365-2095.2011.00919.X.
Shi, F., Qiu, X., Nie, L., Hu, L., Babu, V.S., Lin, Q., Zhang, Y., Chen, L., Li, J., Lin, L., Qin, Z., 2020. Effects of oligochitosan on the growth, immune responses and gut microbes of tilapia (Oreochromis niloticus). Fish Shellfish Immunol. 106, 563–573. https://doi.org/10.1016/J.FSI.2020.07.049.
Soetemans, L., Uyttebroek, M., Bastiaens, L., 2020. Characteristics of chitin extracted from black soldier fly in different life stages. Int. J. Biol. Macromol. 165, 3206–3214. https://doi.org/10.1016/J.IJBIOMAC.2020.11.041.
Soltani, M., Ghosh, K., Hoseinifar, S.H., Kumar, V., Lymbery, A.J., Roy, S., Ringø, E., 2019. Genus Bacillus, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. Rev. Fish. Sci. Aquac. https://doi.org/10.1080/23308249.2019.1597010.
Swiontek Brzezinska, M., Jankiewicz, U., Burkowska, A., Walczak, M., 2014. Chitinolytic microorganisms and their possible application in environmental protection. Curr. Microbiol. 68, 71. https://doi.org/10.1007/S00284-013-0440-4.
Terova, G., Rimoldi, S., Ascione, C., Gini, E., Ceccotti, C., Gasco, L., 2019. Rainbow trout (Oncorhynchus mykiss) gut microbiota is modulated by insect meal from Hermetia illucens prepupae in the diet. Rev. Fish Biol. Fish. 29, 465–486. https://doi.org/10.1007/s11160-01909558-y.
Terova, G., Ceccotti, C., Ascione, C., Gasco, L., Rimoldi, S., 2020. Effects of partially defatted Hermetia illucens meal in rainbow trout diet on hepatic methionine metabolism. Animals 10, 1–13. https://doi.org/10.3390/ANI10061059.
Terova, G., Gini, E., Gasco, L., Moroni, F., Antonini, M., Rimoldi, S., 2021. Effects of full replacement of dietary fishmeal with insect meal from Tenebrio molitor on rainbow trout gut and skin microbiota. J. Anim. Sci. Biotechnol. 121 (12), 1–14. https://doi.org/10.1186/S40104-021-00551-9.
Triunfo, M., Tafi, E., Guarnieri, A., Salvia, R., Scieuzo, C., Hahn, T., Zibek, S., Gagliardini, A., Panariello, L., Coltelli, M.B., De Bonis, A., Falabella, P., 2022. Characterization of chitin and chitosan derived from Hermetia illucens, a further step in a circular economy process. Sci. Report. 121 (12), 1–17. https://doi.org/10.1038/s41598-022-10423-5.
Van Doan, H., Hoseinifar, S.H., Ringø, E., Ángeles Esteban, M., Dadar, M., Dawood, M.A. O., Faggio, C., 2019. Host-associated probiotics: a key factor in sustainable aquaculture, 28, pp. 16–42. https://doi.org/10.1080/23308249.2019.1643288.
Zhang, M., Haga, A., Sekiguchi, H., Hirano, S., 2000. Structure of insect chitin isolated from beetle larva cuticle and silkworm (Bombyx mori) pupa exuvia. Int. J. Biol. Macromol. 27, 99–105. https://doi.org/10.1016/S0141-8130(99)00123-3.