



Modulation of Gilthead Sea Bream Gut Microbiota by a Bioactive Egg White Hydrolysate: Interactions Between Bacteria and Host Lipid Metabolism

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This study aimed to highlight the relationship between diet, animal performance and mucosal adherent gut microbiota (anterior intestine) in fish fed plant-based diets supplemented with an egg white hydrolysate (EWH) with antioxidant and anti-obesogenic activity in obese rats. The feeding trial with juveniles of gilthead sea bream (*Sparus aurata*) lasted 8 weeks. Fish were fed near to visual satiety with a fish meal (FM)/fish oil (FO) based diet (CTRL) or a plant-based diet with/without EWH supplementation. Specific growth rate decreased gradually from 2.16% in CTRL fish to 1.88% in EWH fish due to a reduced feed intake, and a slight impairment of feed conversion ratio. Plant-based diets feeding triggered a hyperplastic inflammation of the anterior intestine regardless of EWH supplementation. However, EWH ameliorated the goblet cell depletion, and the hepatic and intestinal lipid accumulation induced by FM/FO replacement. Illumina sequencing of gut mucosal microbiota yielded a mean of 136,252 reads per sample assigned to 2,117 OTUs at 97% identity threshold. The bacterial diversity was similar in all groups, but a significantly lower richness was found in EWH fish. At the phylum level, Proteobacteria reached the highest proportion in CTRL and EWH fish, whereas Firmicutes were decreased and Actinobacteria increased with the FM/FO replacement. The proportion of Actinobacteria was restored by dietary EWH supplementation, which also triggered a highest amount of Bacteroidetes and Spirochaetes. At a closer look, a widespread presence of Lactobacillales among groups was found. Otherwise, polysaccharide hydrolases secretors represented by *Corynebacterium* and *Nocardioides* were increased by the FM/FO replacement, whereas the mucin-degrading *Streptococcus* was only raised in

fish fed the plant-based diet without EWH. In addition, in EWH fish, a higher abundance of *Propionibacterium* was related to an increased concentration of intestinal propionate. The antagonism of gut health-promoting propionate with cholesterol could explain the inferred underrepresentation of primary bile acid biosynthesis and steroid degradation pathways in the EWH fish microbiota. Altogether, these results reinforce the central role of gut microbiota in the regulation of host metabolism and lipid metabolism in particular, suggesting a role of the bioactive EWH peptides as an anti-obesity and/or satiety factor in fish.

Keywords: bioactive peptide, egg white hydrolysate, gut microbiota, bile salts, lipid metabolism, *Sparus aurata*

INTRODUCTION

European aquaculture is still dependent on marine ingredients for feeds, though a high grade of replacement of marine feedstuffs (more than 70%) has been currently accomplished in Norwegian salmon feeds (Ytrestøyl et al., 2015). A high level of fish meal (FM) and fish oil (FO) replacement has also been achieved in a typically carnivorous marine fish, such as European sea bass (*Dicentrarchus labrax*) (Kousoulaki et al., 2015; Torrecillas et al., 2017). Likewise, plant-based diets with less than 10% of marine ingredients have been proven to support maximum growth from early life stages to completion of sexual maturity in gilthead sea bream (*Sparus aurata*) (Benedito-Palos et al., 2016; Simó-Mirabet et al., 2018). However, wide-serum metabolomics profiling revealed nutritionally mediated effects in processes of mucosal tissue repair and DNA stability (Gil-Solsona et al., 2019). Furthermore, feeding gilthead sea bream with plant-based diets induced negative effects at gut level, including changes in the expression of mucins, mucosal immunoglobulins (IgT) and other immune-relevant genes (Pérez-Sánchez et al., 2013; Piazzon et al., 2016). This biomarker profile leads to a pro-inflammatory condition, with drawback effects in gut integrity and epithelial barrier functions (Estensoro et al., 2016; Piazzon et al., 2017). However, most of these effects, including changes in sex reversal from male to female, or in mucosal adherent intestinal microbiota composition, were restored by dietary sodium butyrate supplementation, resulting in an improved disease outcome in fish exposed to bacteria or myxozoan parasites (Piazzon et al., 2017; Simó-Mirabet et al., 2018).

Gut microbiota has the capacity to modify and possibly activate food constituents, providing benefits for health (Brown et al., 2015; Davis, 2016; Aoun et al., 2020). Certainly, the persistent imbalance of the gut's microbial community resulting from exposure to diverse environmental factors, including unhealthy diets, drugs, toxins, and pathogens, has a major impact on health (Chávez-Talavera et al., 2017; Hasan and Yang, 2019; Lee et al., 2020). Among them, diet micro- and macro-nutrients are considered one of the main factors that modulate gut microbiota (Zhang et al., 2018; Leeming et al., 2019). Therefore, understanding the modulation of the gut microbiome by dietary nutrients and vice-versa becomes essential for the development of novel strategies to improve animal and human health. In fact, the targeting of gut microbiota is a promising tool to improve the health and welfare of farmed fish, and gilthead

sea bream in particular. Certainly, the intestinal gut microbiota is highly modulated by age and sex reversal in a protandrous hermaphrodite fish such as gilthead sea bream (Piazzon et al., 2019). In addition, the gut microbiota of gilthead sea bream families selected for fast-growth showed a high level of plasticity, which makes them more flexible upon dietary changes, showing at the same time, a better ability to deal with intestinal parasites (Piazzon et al., 2020).

In other studies of this special issue of nutrition and gut microbiota in aquaculture, we also analyzed the effect of probiotics and alternative FM replacers on gilthead sea bream gut microbiota (Moroni et al., 2021; Solé-Jiménez et al., 2021). Meanwhile, bioactive peptides derived from food proteins are considered important modulators of various biological processes, which occur both systemically and locally within the gastrointestinal tract (Moughan et al., 2014). Historically, milk proteins have been considered a rich source of bioactive peptides, but there is now evidence of a wide range of animal and plant protein sources for production of biologically active peptides, comprising meat, bone, eggs, cereals, legumes, yeast, seaweed, and fungi (Brown et al., 2015). In particular, a bioactive egg white hydrolysate (EWH) treated with pepsin showed potent *in vitro* and *in vivo* antioxidant and anti-inflammatory properties (Dávalos et al., 2004; Miguel et al., 2004; Garcés-Rimón et al., 2016a), improving oxidative stress and inflammation biomarkers on genetically and diet-induced obese rats (Garcés-Rimón et al., 2016b; Moreno-Fernández et al., 2018a,b). The antioxidant properties of EWH have also been associated with the prevention of metabolic complications arising from the exposure to heavy metals (Rizzetti et al., 2017; Martínez et al., 2019; Gomes Pinheiro et al., 2020). Although earlier studies targeting intestinal microbiota support that EWH has the potential to revert microbial dysbiosis in a rodent model of genetic obesity (Requena et al., 2017), the modulating effects of food-bioactive peptides upon gut microbiota have been much less studied (Wu et al., 2021). Thus, in an attempt to focus on the link between mucosal adherent microbiota and the bioactive properties of EWH in other animal model, we analyzed the potential benefits of dietary EWH supplementation in farmed gilthead sea bream juveniles fed plant-based diets. Special attention was also paid to the evaluation of growth performance, allocation of body fat depots, hepatic and intestinal histopathological scoring, antioxidant status, and intestinal concentration of lactic acid and short chain fatty acids (SCFAs).

MATERIALS AND METHODS

Ethics Statement

Fish manipulation and tissue collection were carried out according to the Spanish (Royal Decree RD53/2013) and the current EU (2010/63/EU) legislations on the handling of experimental fish. All procedures were approved by the Ethics and Animal Welfare Committee of the Institute of Aquaculture Torre de la Sal (IATS-CSIC, Castellón, Spain), CSIC (permit number 869/2019) and “Generalitat Valenciana” (permit number 2020/VSC/PEA/0010).

Animals

Juveniles of gilthead sea bream (March 2020) were purchased from a Mediterranean hatchery (Piscimar, Burriana, Spain) and adapted for 2 months to the indoor experimental facilities of IATS-CSIC under natural photoperiod and temperature conditions (40°5'N; 0°10'E). Seawater was pumped ashore (open system), oxygen content of water effluents was always above 85% saturation, and unionized ammonia remained below 0.02 mg/L. During the acclimation and experimental period (May–July 2020), water temperature increased from 18°C in May to 25°C in July.

Diets

Extruded isoproteic and isolipidic diets (2, 3 mm pellet size) were formulated by Sparos Lda. (Olhão, Portugal), following current industry practices with plant protein and oil sources as main replacers of fish meal (FM) and fish oil (FO) (Table 1). The inclusion level of FM (including fish protein hydrolysates) and FO in the control diet (CTRL) was 35% and 5%, respectively. In the diet named L-FM/FO, the FM and FO inclusion levels were reduced to 12.5% for FM and fish protein hydrolysates, and to 3% for FO, being this diet conveniently supplemented with monocalcium phosphate, L-tryptophan and DL-methionine. The EWH diet was formulated to be a L-FM/FO diet with EWH added at 7.5% instead of plant proteins and fish protein hydrolysates. The EWH was prepared by pepsin hydrolysis of crude egg white as previously described by Garcés-Rimón et al. (2016a). Briefly, commercial pasteurized egg white was hydrolyzed for 8 h with BC Pepsin 1:3000 (E.C. 3.4.23.1; from pork stomach, E:S:2:100 w:w, pH 2.0, 37°C), purchased from Biocatalysts (Cardiff, United Kingdom). Enzyme inactivation was achieved by increasing the pH to 7.0 with 10 N NaOH. The hydrolysate was centrifuged at 2,500 × g for 15 min and the supernatants were frozen and lyophilized until use as fish feed ingredient.

Feeding Trial

In May 2020, fish of 20–24 g body weight were randomly distributed in nine 90 L tanks to establish triplicate groups of 20 fish each (initial rearing density, 4.8–4.9 kg/m³). All fish were tagged with passive integrated transponders (PIT) (ID-100A 1.25 Nano Transponder, Trovan, Madrid, Spain) into the dorsal skeletal muscle, and were individually weighed and measured at initial, intermediate and final sampling points (every 4 weeks),

TABLE 1 | Ingredients and chemical composition of control and experimental diets.

Ingredients	CTRL (%)	L-FM/FO (%)	EWH (%)
Fishmeal super prime	30	10	10
Fish protein hydrolysate	5	2.5	
Soy protein concentrate	12.5	12.5	10
Pea protein concentrate		4.5	2.5
Wheat gluten	5	12.5	10
Corn gluten meal	5	10	10
Soybean meal 48	5	10	10
Rapeseed meal	5	5	5
Sunflower meal 40	5	10	10
Wheat meal	9.07	3.99	5.49
Whole peas	4	2	2
Vitamin and mineral premix*	1	1	1
Vitamin C35	0.03	0.03	0.03
Betaine HCl	0.2	0.2	0.2
Antioxidant powder	0.2	0.2	0.2
Sodium propionate	0.1	0.1	0.1
Monocalcium phosphate	0.6	2.5	2.5
L-Tryptophan		0.03	0.03
DL-Methionine		0.25	0.25
Fish oil	5	3	3
Soybean oil	5.3	8	8.6
Linseed oil	2	1.7	1.6
EGG hydrolysate			7.5
<i>Chemical composition (proximate analyses)</i>			
Dry matter, % feed	92.9	93.2	92.8
Crude protein, % feed	47.9	47.9	47.9
Crude fat, % feed	16.1	16.1	16.1
EPA + DHA, % feed	2.4	1.2	1.2
Ash, % feed	8.7	8.0	7.6

*Vitamin and mineral premix: INVIVONSA Portugal SA, Portugal: Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulfate, 25 mg; retinyl acetate, 20,000 IU; DL-cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 500 mg; inositol, 500 mg; biotin, 3 mg; calcium pantothenate, 100 mg; choline chloride, 1000 mg; betaine, 500 mg. Minerals (g or mg/kg diet): copper sulfate, 9 mg; ferric sulfate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulfate, 7.5 mg; sodium chloride, 400 mg; excipient wheat gluten.

using a FR-200 Fish Reader W (Trovan) for data capture and pre-processing. The trial lasted 8 weeks, and fish were fed by hand once daily (12 a.m.), 6 days per week, near to visual satiety with CTRL or experimental diets for the entire duration of the trial. Feed intake was registered daily, and normal fish behavior was assessed routinely by camera monitoring. No mortalities were registered through the entire experimental period.

Sample Collection

At the end of the trial and following two fasting days, nine fish per diet (three fish/tank) were anaesthetized with 0.1 g/L of tricaine-methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO, United States). Blood was taken from the caudal vessels

with heparinized syringes, centrifuged at $3,000 \times g$ for 20 min at 4°C , and plasma samples were stored at -80°C until analyzed. Before tissue collection, fish were sacrificed by cervical section. Liver, intestine (excluding the pyloric caeca) and mesenteric fat were weighed and measured (intestine length) to calculate the hepatosomatic index (HSI), mesenteric fat index (MSI), and intestine weight (IWI) and length (ILI) indexes. Tissue portions (~ 0.4 cm) of liver, anterior intestine (AI; immediately after the pyloric caeca) and posterior intestine (PI; immediately before the anal ampoule) were fixed in 10% neutral buffered formalin for subsequent histological analyses. The remaining AI was opened and gently washed with sterile Hanks's balanced salt solution to remove non-adherent bacteria. Intestinal mucus was scrapped off using the blunt edge of a sterile scalpel and collected into sterile 1.5 mL tubes. The anterior intestine portion was selected due to its importance in fish nutrient absorption and metabolism (Sundell and Rønnestad, 2011). The autochthonous bacteria were selected, because these populations are capable of colonizing the mucosal surface, directly impacting the fish physiology (Hao and Lee, 2004). Mucus samples were kept on ice and DNA extraction was performed immediately after the sampling. Additional fish (10 fish per diet) were sampled 8 h after feeding for the analysis of intestinal SCFA. Briefly, animals were anesthetized and sacrificed by cervical section, intestine was cut out, and the intestinal content was collected by stripping. During the two sampling days corresponding to fasting and postprandial sample collection, all samples were obtained in a short-period lasting 2–3 h, alternating among replicates of each dietary group to avoid biases due to sampling time.

Histological Analysis

Formalin fixed pieces of liver, AI and PI were processed for paraffin embedment, $4 \mu\text{m}$ -sectioned and stained with Giemsa and periodic acid-Schiff (PAS) following standard procedures. Sections were examined with a Leitz Dialux 22 light microscope connected to an Olympus DP70 camera, and representative microphotographs were taken. The histological alterations observed were scored according to semiquantitative scales. In intestinal sections, cell abundance of differentially stained goblet cells (light- or dark-stained with Giemsa), intraepithelial lymphocytes (IELs) and eosinophilic granular cells (EGCs) were scored ranging from 0 (absence) to 3 (very abundant, meaning 25–30 cells/microscope field at $500\times$ magnification). The degree of lipid vacuolization in enterocytes and the degree of hyperplasia in the lamina propria-submucosa were scored from 0 (absence) to 3 (severe). In liver sections, the degree of lipid and glycogen storage in hepatocytes was scored from 0 (absence) to 3 (pervasive) by Giemsa or PAS staining, respectively. In addition, melanomacrophage centers were quantified in the liver.

Antioxidant Capacity

The oxygen radical absorbance capacity (ORAC) assay was used to measure the total plasma antioxidant capacity as previously described (Garcés-Rimón et al., 2016b). ORAC values were quantified by a fluorimeter Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Germany) with wavelength excitation at 485 nm and wavelength emission measured at 520 nm.

Results were expressed as μmol of Trolox (Sigma, United States) equivalent (eq)/mL of plasma.

Lactic Acid and Short Chain Fatty Acid Determinations

Intestine content (200 mg) was homogenized with 0.1% peptone solution with 0.85% NaCl (500 μL) and centrifuged at $10,000 \times g$ for 5 min at 4°C . The supernatant was filtered and 0.2 μL were injected on a HPLC system (Jasco, Tokyo, Japan) equipped with a UV-975 detector. Lactic acid and SCFA were separated using a Rezex ROA Organic Acids column (Phenomenex, Macclesfield, United Kingdom) following the method described by Sanz et al. (2005). The mobile phase was a linear gradient of 0.005 M sulfuric acid in HPLC grade water, and flow rate was 0.6 mL/min. The elution profile was monitored at 210 nm, and peak identification was carried out by comparing the retention times of target peaks with those of standards. Calibration curves of formic acid, acetic acid, propionic acid, butyric acid and lactic acid were prepared in the concentration range of 1 to 100 mM.

DNA Extraction From Mucus Samples

Intestinal mucus samples (200 μL) were treated with 250 $\mu\text{g}/\text{mL}$ of lysozyme (Sigma) for 15 min at 37°C . Then, DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) following the manufacturer's instructions. DNA concentration, quality and purity were measured using a Nanodrop 2000c (Thermo Scientific), and agarose gel electrophoresis (1% w/v in Tris-EDTA buffer). DNA was stored at -20°C until sequencing.

Illumina MiSeq Sequencing and Bioinformatic Analysis

The V3-V4 region of the 16S rRNA gene (reference nucleotide interval 341–805 nt) was sequenced using the Illumina MiSeq system (2×300 paired-end run) at the Genomics Unit from the Madrid Science Park Foundation (FPCM). The details on the PCR and sequencing of amplicons were described elsewhere (Piazzon et al., 2019). Raw sequence data were uploaded to the Sequence Read Archive (SRA) under Bioproject accession number PRJNA705868 (BioSample accession numbers: SAMN18105342–68). Raw forward and reverse reads were quality filtered using FastQC¹ and pre-processed using Prinseq (Schmieder and Edwards, 2011). Terminal N bases were trimmed in both ends and sequences with $>5\%$ of total N bases were discarded. Reads that were <150 bp long, with Phred quality score <28 in both of the sequence ends and with a Phred average quality score <26 were excluded. Then, forward and reverse reads were merged using fastq-join (Aronesty, 2013).

Bacteria taxonomy assignment was performed using the Ribosomal Database Project (RDP) release 18 as a reference database (Cole et al., 2014). Reads were aligned with a custom-made pipeline using VSEARCH and BLAST (Altschul et al., 1990; Rognes et al., 2016). Alignment was performed establishing high stringency filters ($\geq 90\%$ sequence identity, $\geq 90\%$ query coverage).

¹<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>

Taxonomic assignment results were filtered and data were summarized in an operational taxonomic units (OTUs) table. From the annotation obtained, the discussion and interpretation of the results was based at the level of genus, as taxonomic affiliations with 16S rRNA amplicon sequencing might not be accurate enough at the species level (Winand et al., 2020). Sample depths were normalized by total sum scaling and then made proportional to the total sequencing depth, following the recommendations previously described (McKnight et al., 2019).

Inferred Metagenome and Pathway Analysis

Piphillin was used to normalize the amplicon data by 16S rRNA gene copy number and to infer metagenomic contents (Iwai et al., 2016). This analysis was performed with the OTUs significantly driving the separation by diets in the PLS-DA analysis (described in the “Statistics” section). For the analysis, a sequence identity cut-off of 97% was implemented, and the inferred metagenomic functions were assigned using the Kyoto Encyclopedia of Genes and Genomes database (KEGG, October 2018 Release). Raw KEGG pathway output from Piphillin was analyzed with the R Bioconductor package DESeq2 using default parameters, after flooring fractional counts to the nearest integer (Love et al., 2014; Bledsoe et al., 2016; Piazzon et al., 2020).

Statistics

Data on growth were analyzed by one-way ANOVA using SigmaPlot v14 (Systat Software Inc., San Jose, CA, United States). Normality of the data was verified by Shapiro–Wilk test, and Dunn’s post-test was used for multiple comparisons among groups. Analysis of semiquantitative and quantitative histological data was carried out with the non-parametric Kruskal–Wallis test, followed by Dunn’s post-test for the multiple comparisons. SCFA results were analyzed by one-way ANOVA followed by Holm–Sidak post-test. Rarefaction curves (plotting the number of observed taxonomic assignments against the number of sequences), species richness estimates, and alpha diversity indexes were obtained using the R package phyloseq (McMurdie and Holmes, 2013). Differences in species richness, diversity indexes and phylum abundance were determined by Kruskal–Wallis test using the Dunn’s post-test, with a significance threshold of $P < 0.05$. Beta diversity across groups was tested with permutational multivariate analysis of variance (PERMANOVA), using the non-parametric method *adonis* from the R package Vegan with 10,000 random permutations. To study the separation among groups, supervised partial least-squares discriminant analysis (PLS-DA) and hierarchical clustering of samples were sequentially applied, using EZinfo v3.0 (Umetrics, Umeå, Sweden) and the R package ggplot2, respectively. Values of normalized counts of OTUs present in five or more samples were included in the analyses. The contribution of the different genes to the group separation was determined by the minimum Variable Importance in the Projection (VIP) values achieving the complete clustering of the conditions with a VIP value ≥ 1.2 . Hotelling’s T^2 statistic was calculated by the multivariate software package EZinfo v3.0. All points in the current study were within the 95% confidence limit for T^2 , thus no outliers were

detected and discarded. The quality of the PLS-DA model was evaluated by the parameters R2Y (cum) and Q2 (cum), which indicate the fit and prediction ability, respectively. To assess whether the supervised model was being over-fitted, a validation test consisting on 500 random permutations was performed using SIMCA-P+ v11.0 (Umetrics). The inferred metagenomic pathways were considered differentially represented using a FDR-corrected significance threshold of 0.05.

RESULTS

Growth Performance and Antioxidant Capacity

Data on growth performance are reported in **Table 2**. Final body weight, feed intake and condition factor were significantly lower ($P \leq 0.004$) in EWH fish than in CTRL fish with intermediate values in fish fed the L-FM/FO diet. Specific growth rates (SGR) also varied significantly from 2.16 in CTRL fish to 1.88 in EWH fish, again with intermediate values (2.03) in fish fed the L-FM/FO diet. The opposite trend (not statistically significant, $P = 0.06$) was found for the feed conversion ratio (FCR) that varied from 1.03 in CTRL fish to 1.10 in EWH fish. HSI, MFI, and IWI were not significantly altered by dietary treatment. However, the intestine length of L-FM/FO fish was larger, and the resulting

TABLE 2 | Effects of dietary treatment on growth performance and antioxidant capacity of gilthead sea bream juveniles fed to visual satiety from May to July (8 weeks) with control (CTRL), low fish meal/fish oil (L-FM/FO) diet, and EWH diets.

	CTRL	L-FM/FO	EWH	P^1
Initial body weight (g)	21.93 ± 0.38	21.98 ± 0.43	21.95 ± 0.40	0.996
Final body weight (g)	71.75 ± 1.08 ^a	66.89 ± 1.01 ^b	61.41 ± 0.96 ^c	<0.001
Final condition factor ²	2.67 ± 0.02 ^a	2.68 ± 0.03 ^a	2.58 ± 0.02 ^b	0.004
Feed intake (g DM/fish)	51.09 ± 0.02 ^a	45.84 ± 0.01 ^{ab}	43.46 ± 0.16 ^b	0.004
FCR ³	1.03 ± 0.02	1.02 ± 0.02	1.10 ± 0.02	0.062
SGR (%) ⁴	2.16 ± 0.02 ^a	2.03 ± 0.02 ^b	1.88 ± 0.02 ^c	<0.001
Liver weight (g)	0.73 ± 0.03	0.73 ± 0.03	0.67 ± 0.03	0.232
Mesenteric fat (g)	0.85 ± 0.13	0.77 ± 0.10	0.85 ± 0.11	0.970
Intestine weight (g)	2.60 ± 0.07 ^{ab}	2.79 ± 0.14 ^a	2.32 ± 0.08 ^b	0.008
Intestine length (cm)	10.12 ± 0.53	11.56 ± 0.38	10.31 ± 0.35	0.042
HSI (%) ⁵	1.06 ± 0.04	1.05 ± 0.03	1.13 ± 0.03	0.292
MFI (%) ⁶	1.18 ± 0.14	1.11 ± 0.12	1.37 ± 0.15	0.380
IWI (%) ⁷	3.75 ± 0.10	3.99 ± 0.14	3.91 ± 0.08	0.284
ILI (%) ⁸	73.21 ± 3.86 ^b	84.29 ± 2.34 ^a	75.4 ± 2.68 ^b	0.041
ORAC ⁹	6.42 ± 0.6	6.02 ± 0.67	6.41 ± 0.67	0.881

Data on body weight, feed intake and growth indices are the mean ± SEM of triplicate tanks. Data on organosomatic indices are the mean ± SEM of 18 fish. Different superscript letters in each row indicate significant differences among dietary treatments (Holm–Sidak post-test, $P < 0.05$, stated in bold).

¹ Result values from one-way analysis of variance.

² CF = $100 \times (\text{body weight}/\text{standard length}^3)$.

³ Feed conversion ratio = dry feed intake/wet weight gain.

⁴ Specific growth rate = $100 \times (\ln \text{ final body weight} - \ln \text{ initial body weight})/\text{days}$.

⁵ Hepatosomatic index = $100 \times (\text{liver weight}/\text{fish weight})$.

⁶ Mesenteric fat index = $100 \times (\text{mesenteric fat weight}/\text{fish weight})$.

⁷ Intestinal weight index = $100 \times (\text{intestine weight}/\text{fish weight})$.

⁸ Intestinal length index = $100 \times (\text{intestine length}/\text{standard length})$.

⁹ Oxygen radical absorbance capacity = $\mu\text{mol eq Trolox}/\text{mL plasma}$.

ILI was significantly higher in this group of fish in comparison to CTRL and EWH groups. Intestine weight of L-FM/FO fish was also larger, although this was not reflected in significant differences in IWI among groups. No statistical differences in plasma antioxidant capacity were observed between different groups, with ORAC values around $6 \mu\text{mol eq Trolox/mL}$ plasma.

Histological Scoring

The dietary replacement of FM/FO provoked a hyperplastic inflammation in the intestines of both, L-FM/FO and EWH fish, compared to the CTRL fish (Figures 1, 2). Inflammatory cell infiltrates in the epithelium and lamina propria-submucosa consisted mainly of lymphocytes and eosinophilic granular cells. In the AI, a significant submucosal hyperplasia was found in L-FM/FO and EWH fish, though the increase of EGCs and IELs was not significant. In this segment, the abundance of light-stained goblet cells was significantly reduced by the L-FM/FO diet and this effect reverted by the EWH diet. This goblet cell type presented a PAS+ staining pattern indicative of neutral mucins. By contrast, the hyperplastic effect was less severe (not significant) at the PI, where the increase of intraepithelial lymphocytes was the only significant inflammatory

sign observed in L-FM/FO and EWH fish. The enterocytes of the CTRL and L-FM/FO fish presented a medium degree of lipid vacuolization, which was significantly reduced by the EWH diet. Remarkably, this lipid depletion in EWH PIs co-occurred with a decrease of lipid depots in the hepatocytes of EWH fish, which were increased with L-FM/FO diets. No differences in glycogen storage were found in the liver of fish fed the different diets, though a significant increase of liver melanomacrophage centers was observed in EWH fish, compared to the other two diets.

Intestinal Content in Lactic Acid and Short Chain Fatty Acid

Butyric acid could not be detected in any of the analyzed samples. No statistically significant differences were found among groups for the intestinal concentration of lactic acid, formic acid, acetic acid, or total SCFA (Figure 3 and Supplementary Table 1). The only difference was found in propionic acid, present in significantly higher concentrations in the intestinal content of fish fed EWH ($7.40 \mu\text{mol/g}$) when compared to the CTRL group ($4.14 \mu\text{mol/g}$).

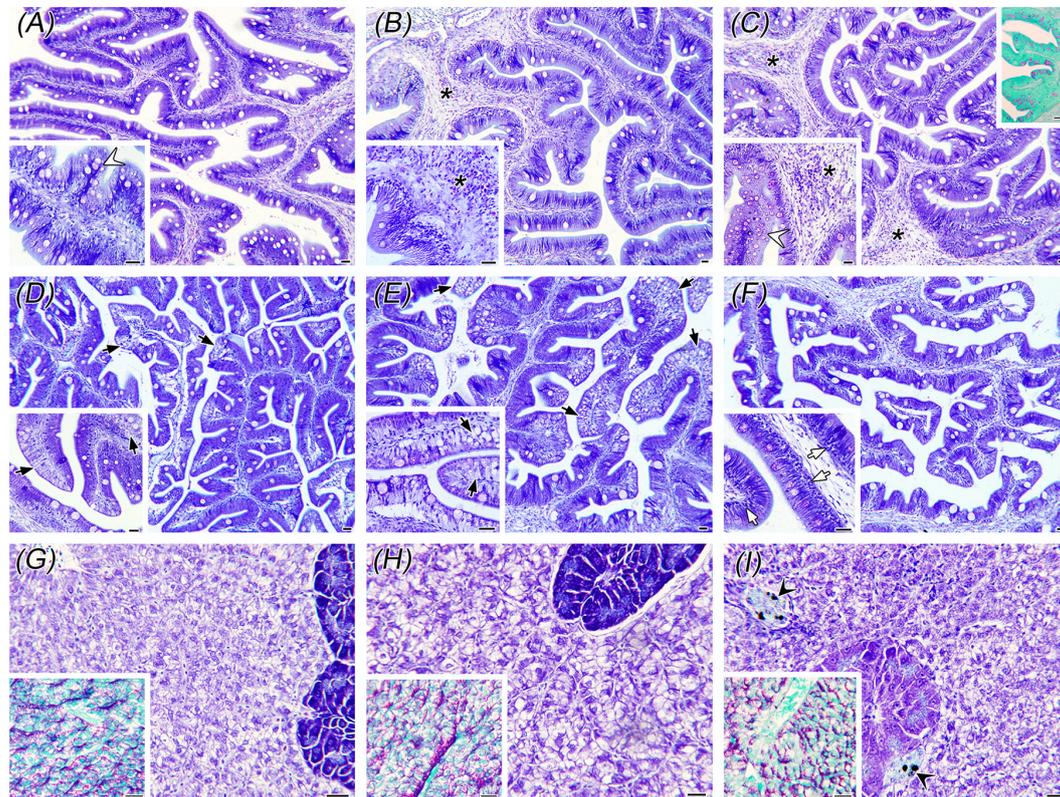
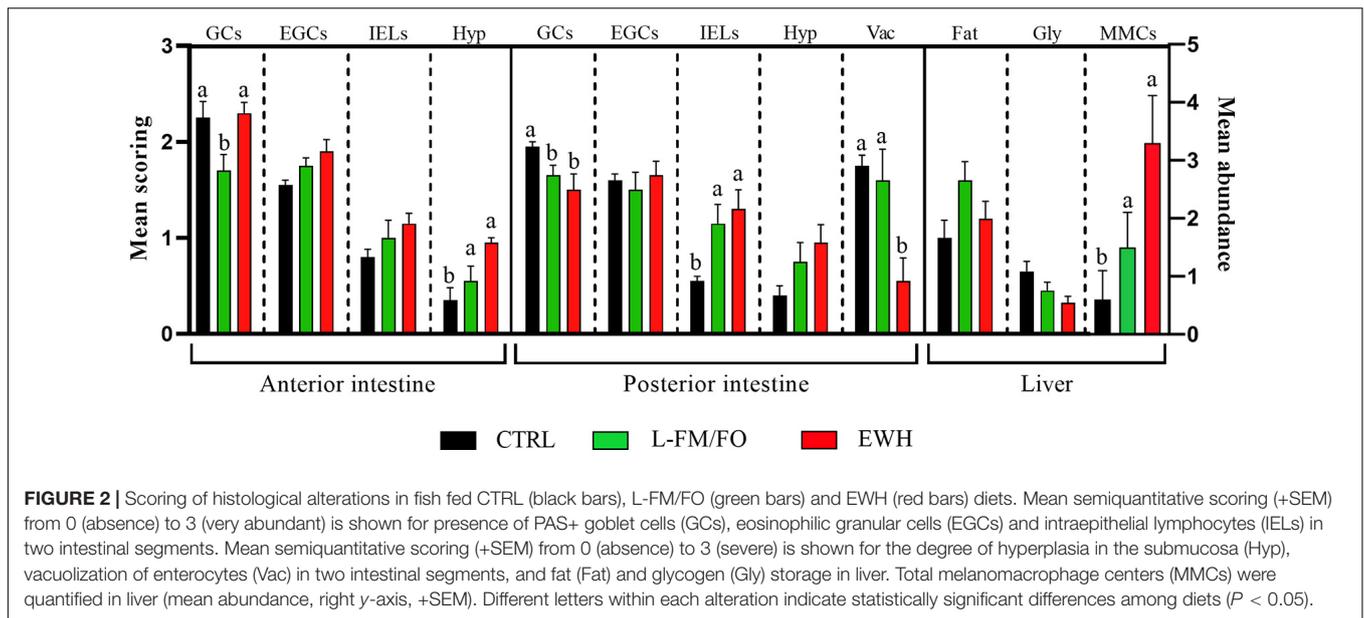


FIGURE 1 | Histological alterations in the AI (A–C), PI (D–F) and liver (G–I) of gilthead sea bream. Panels (A,D,G) correspond to CTRL fish; (B,E,H) to fish fed the L-FM/FO diet; and (C,F,I) to fish fed the EWH diet. In AI, note the high abundance of light-stained goblet cells (white arrowheads) in CTRL (A) and EWH (C) fish, as well as the submucosal hyperplasia (asterisks) in L-FM/FO (B) and EWH (C) fish. The upper insert in panel (C) shows the PAS-stained goblet cells. In the PI, note the presence of lipid vacuolization in enterocytes (black arrows) in CTRL fish (D), which is intensified in L-FM/FO fish (E) and decreased in EWH fish (F). PI of EWH fish presented high abundance of intraepithelial lymphocytes (white arrows). In livers, note the higher lipid storage in L-FM/FO fish (H) and the presence of early melanomacrophage centers (black arrowheads) in EWH fish (I). Glycogen storage did not change among groups (PAS-stained inserts). Scale bars = $20 \mu\text{m}$.

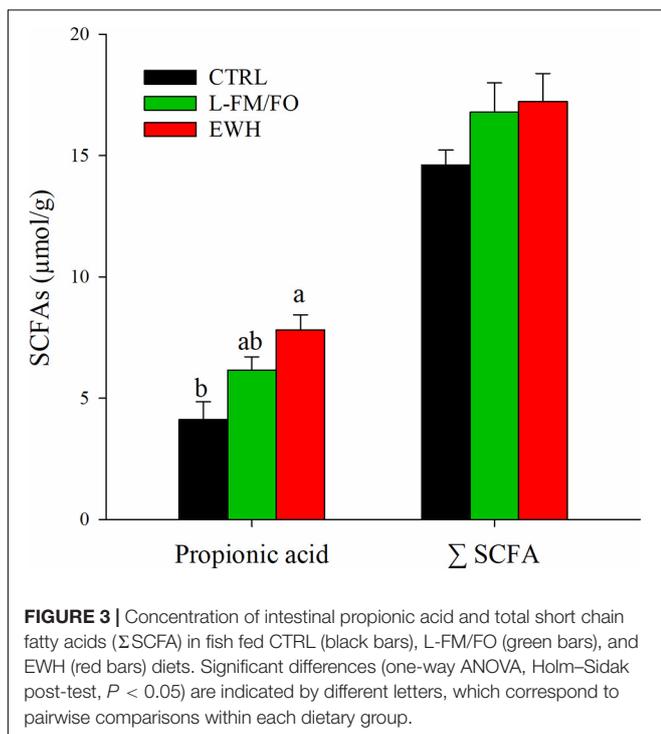


Alpha Diversity and Microbial Composition

Illumina sequencing of the 27 analyzed samples yielded 3,678,804 high quality reads, with a mean of 136,252 reads per sample (Supplementary Table 2). The reads were assigned to 2,117 OTUs at a 97% identity threshold. Rarefaction analysis showed curves that approximated saturation (horizontal asymptote), thus a good coverage of the bacterial community was achieved and

the number of sequences for analysis was considered appropriate (Supplementary Figure 1).

In a first attempt to unravel the effects of dietary intervention on gut mucosal microbiota, we analyzed the bacterial diversity of all dietary groups, and no significant differences were found in Shannon and Simpson diversity indexes, but a significantly lower richness (ACE value, $P < 0.05$) was found in EWH fish (Table 3). At the phylum level (Figure 4), Proteobacteria were the most abundant bacteria, significantly varying from more than 55% in fish fed the L-FM/FO diet to 67.8% in EWH fish. In parallel, a significant decrease in the phylum Firmicutes was found both in L-FM/FO and EWH (16.6–16.8%) groups in comparison to CTRL fish (26.5%). Conversely, the phylum Actinobacteria raised up from ~6% in CTRL and EWH fish to 18.2% in fish fed the L-FM/FO diet. Finally, in EWH fish, the less abundant Bacteroidetes and Spirochaetes phyla were significantly increased, with values of 2.8% and 2.2%, respectively.



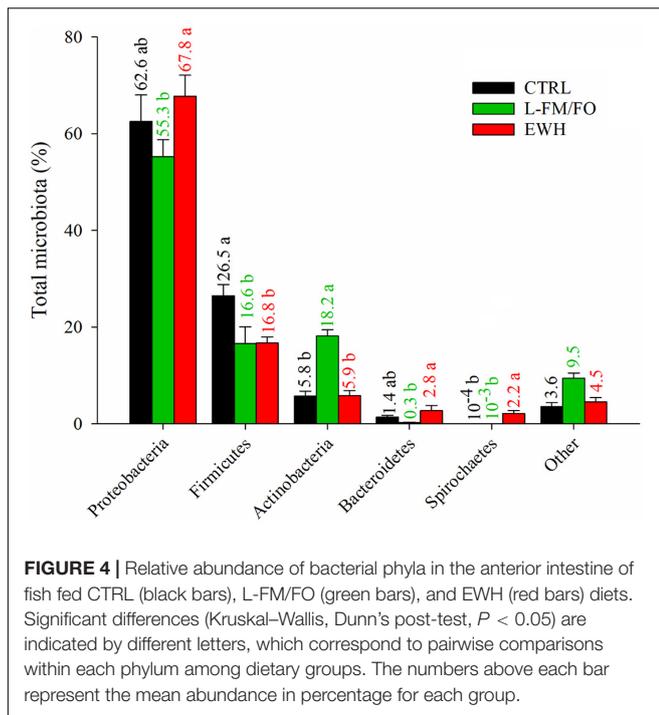
Microbiota Discriminant Analysis

Permutational multivariate analysis of variance test highlighted statistically significant differences in bacterial composition when comparing animals fed different diets ($P = 0.048$, $F = 1.115$,

TABLE 3 | Species richness estimators (observed and ACE) and diversity indexes (Shannon and Simpson) of fish fed CTRL, L-FM/FO, and EWH diets.

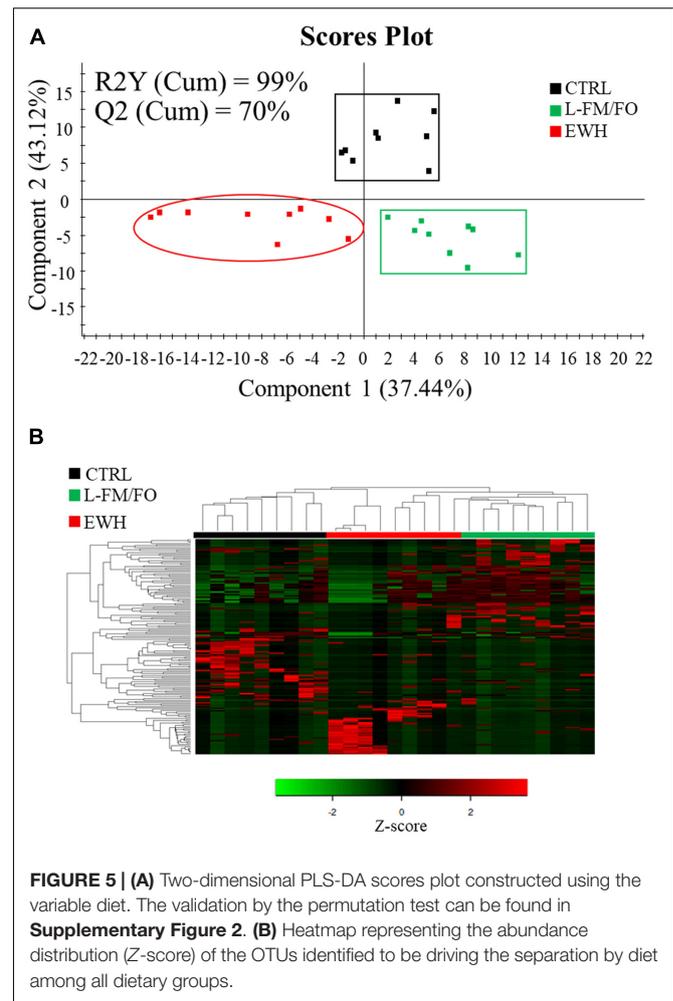
	CTRL	L-FM/FO	EWH	P-value
Observed	278.78 ± 75.29	243.78 ± 55.77	193.56 ± 88.21	0.06
ACE	394.13 ± 81.16 ^a	377.09 ± 70.49 ^a	266.12 ± 115.75 ^b	0.046*
Shannon	2.78 ± 0.26	2.50 ± 0.32	2.60 ± 0.51	0.23
Simpson	0.89 ± 0.04	0.87 ± 0.09	0.86 ± 0.10	0.67

Values are mean ± SEM of 9 fish. Asterisk (*) and bold font indicates significant differences among groups (Holm–Sidak, $P < 0.05$) denoted by different superscript letters.



$R^2 = 0.085$). Although R^2 values detected were quite low, they were in line with what was reported in other microbiota studies (He et al., 2018) due to the complexity and variability of microbiota samples. To validate and study these differences in more detail, a PLS-DA model ($R^2Y = 99\%$, $Q^2 = 70\%$) with three score components was constructed and statistically validated (Figure 5A and Supplementary Figure 2). The first two components explained more than 80% of total variance, clearly separating CTRL fish from fish fed L-FM/FO diets along x -axis (component 1, 37.4%), whereas component 2 (43.2%) separated the L-FM/FO diets with/without EWH along y -axis. To determine which groups of bacteria were driving these separations at a high level of confidence, the minimum VIP value driving the correct separation of groups in the model was determined throughout a heatmap representation (Figure 5B). Such approach disclosed 165 OTUs ($VIP \geq 1.2$), which can be accessed in Supplementary Table 3.

Figure 6 shows the list of most abundant bacteria (at least 1% in one of the groups; 46 OTUs out of the 165 with $VIP \geq 1.2$) that exclusively drove the separation by dietary groups. For these abundant bacteria, a first type of response was mediated by 16 OTUs that were increasing with the FM/FO replacement and decreasing again in EWH fish. In this group, the presence of Neisseriaceae family and species of *Ralstonia*, *Lactobacillus*, *Streptococcus*, *Corynebacterium*, and *Nocardioideis* genera was remarkable. A second type of response grouped 15 OTUs present in a significant proportion in the CTRL group, but decreasing in fish fed the two L-FM/FO diets. In this case, dietary plant ingredients produced the decrease of the Comamonadaceae family and *Novosphingobium*, *Mesorhizobium*, *Klebsiella*, *Acinetobacter*, *Brochotrix*, *Bacillus*, *Clostridium sensu stricto*, and *Exiguobacterium* genera. The remaining 15 OTUs



increased their proportion in fish fed the EWH diet, being in a very low proportion in the other two dietary groups. This response triggered the presence of Bacteroidetes and Spirochaetes phyla, and more specifically of the Flavobacteriaceae family and *Cloacibacterium* genus. The Rhodospirillales order also increased with the addition of EWH, as well as *Granulicatella*, *Serratia*, *Bradyrhizobium*, *Propionibacterium*, and *Photobacterium* genera.

Inferred Metagenome and Pathway Analysis

With the aim of assessing the biological significance of the diet-induced differences in the microbiota of the different groups, a pathway analysis was conducted with the inferred metagenomes of the 165 OTUs that drove the separation by diet (Table 4). The results showed that 15 pathways could be significantly changing in the comparison between fish fed CTRL and the L-FM/FO diet without EWH, whereas the comparison between EWH and CTRL groups rendered 28 pathways. In both comparisons, pathways related to signaling pathways of rat sarcoma (RAS), sphingolipids, GnRH, cAMP, and Fc gamma R-mediated phagocytosis were strongly overrepresented in the two groups of fish fed L-FM/FO diets, whereas Staurosporine biosynthesis, neuroactive ligand–receptor interaction and cholesterol metabolism were

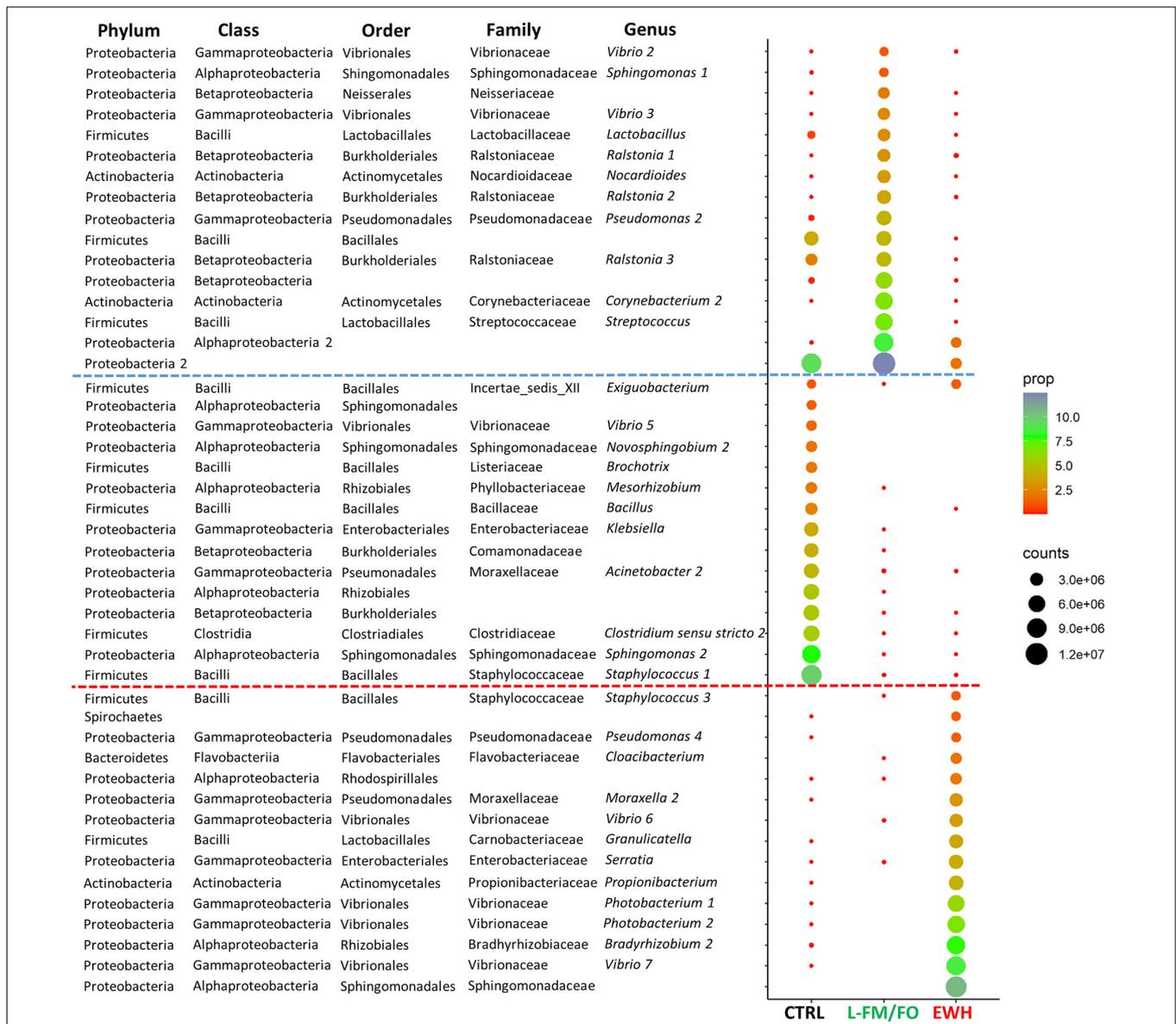


FIGURE 6 | Dotplot map depicting the most abundant genera (more than 1% of the total microbiota in at least one dietary group) from the 165 significant OTUs identified in **Figure 5B**. The size of the dots represents the normalized counts in each dietary group (CTRL, L-FM/FO, and EWH). The color scale represents the mean abundance, in percentage, of each genus within each group. OTUs above the blue dotted line showed an increased abundance in L-FM/FO groups; OTUs between the dotted lines showed an increased abundance in the CTRL group; OTUs below the red dotted line showed an increased abundance in the EWH group. The numbers after the genus names correspond to different OTUs assigned to the same genus that probably belong to different species.

underrepresented. By contrast, only two pathways corresponding to primary bile acid biosynthesis and steroid degradation were consistently underrepresented in the microbiota of EWH fish when comparisons are made with the other two groups. This was coupled to an overrepresentation of the longevity regulating pathway.

DISCUSSION

Enzymatic hydrolysis of animal and plant proteins has been used as a basic method for the conversion of underused

protein products into highly digestible peptides (Benjakul et al., 2014; Egerton et al., 2018a). Additionally, protein hydrolysates containing antioxidant peptides possess a high therapeutic potential for the management of chronic diseases, but also as safe additives to halt lipid peroxidation, improving the quality and consumer satisfaction of several food products (Auwal et al., 2017; Cicero et al., 2017). Thus, the antioxidants and anti-inflammatory properties of EWH have shown beneficial effects in different experimental rat models (Requena et al., 2017), and we discussed herein the potential benefits of dietary EWH supplementation in fish fed experimental diets with a high replacement of marine feedstuffs by alternative plant ingredients.

TABLE 4 | Pathway analysis from predicted metagenome.

Generic	Process	1	2	3
Cellular process	Endocytosis		4.63	
	Flagellar assembly		-1.7	
Environmental information processing	Ras signalling pathway	5.47	7.1	
	Sphingolipid signaling pathway	6.97	4.73	
	cAMP signaling pathway	6.72	4.51	
	Neuroactive ligand-receptor interaction	-3.41	-2.69	
Genetic information processing	Proteasome	4.73	4.28	
	Basal transcription factors	4.19	3.13	
	Ribosome biogenesis in eukaryotes		0.8	
Metabolism	Sulfur relay system		0.75	
	Flavonoid biosynthesis		5.24	
	Stilbenoid, diarylheptanoid and gingerol biosynthesis		5.24	
	Secondary bile acid biosynthesis		4.19	
	Vitamin B6 metabolism		0.63	
	Nitrogen metabolism		0.52	
	Aminobenzoate degradation		-1.01	
	Dioxin degradation		-1.56	
	Bisphenol degradation		-2.81	
	Primary bile acid biosynthesis		-3.22	-3.02
	Steroid degradation		-4.16	-3.41
	Steroid biosynthesis		-6.17	
	Staurosporine biosynthesis	-5.35	-6.72	
	Photosynthesis – antenna proteins	8.23		
Arginine and proline metabolism	-0.61			
Organismal systems	Fc gamma R-mediated phagocytosis	6.16	5.58	
	GnRH signaling pathway	6.14	5.58	
	Cholesterol metabolism	-3.42	-2.7	
	Renin secretion		-4.83	
	Regulation of lipolysis in adipocytes		-5.48	
	Retrograde endocannabinoid signaling	-3.55	-5.85	
	Longevity regulating pathway – multiple species			0.62
	Parathyroid hormone synthesis, secretion and action	4.14		
Bile secretion	4.24			

Values represent the \log_2FC of the comparisons: 1 = L-FM/FO vs. CTRL; 2 = EWH vs. CTRL; 3 = EWH vs. L-FM/FO (FDR < 0.05).

From our results, it is conclusive that dietary EWH supplementation triggered a reduced feed intake and a slight impairment of feed conversion ratio in gilthead sea bream. In rodents, EWH administration reduced body weight gain in obese animals, and this decrease was related to a reduced deposit of fat in different tissues, especially white adipose tissue, but no effects on growth or food intake were observed in obese or control rats fed EWH (Miguel et al., 2006; Moreno-Fernández et al., 2018a). It should be also noted that proteins in their natural state do not contribute to the flavor of food, but hydrolyzed derived peptides can modify the sensory quality of proteins causing food rejection (Iwaniak et al., 2019). Therefore, we cannot exclude a taste effect on the apparent satiety effect of the EWH in our

experimental gilthead sea bream model. Moreover, fish protein hydrolysates, absent in EWH diet, are known feed attractants in aquaculture, which could enhance the feed palatability of CTRL and L-FM/FO diets (Kasumyan and Døving, 2003; Aguila et al., 2007). Alternatively, the modulation of the intestinal microbiota, particularly with respect to production of SCFA, might also contribute to explain the observed effects on growth and feed intake. SCFA, such as butyrate, propionate and acetate, are end products of microbial fermentation implicated in a multitude of physiological functions (Morrison and Preston, 2016), but similar to fiber, protein fermentation also produces SCFA (Macfarlane, 1992). However, while propionate production remains relatively stable, the rate of acetate and butyrate production is lowered when they are generated from protein fermentation (Aguirre et al., 2016). In agreement with this, the intestinal concentration of propionate was much higher than for other SCFA in our model of a carnivorous fish fed hyperproteic diets. It is difficult to categorize individual SCFA into purely obesogenic or anti-obesogenic, though acetate seems to be predominantly obesogenic, whereas butyrate and propionate are broadly anti-obesogenic (Chakraborti, 2015). The beneficial effects of butyrate have been reported in a large extent in gilthead sea bream as a highly promising additive to counteract undesired effects of plant-based diets at the local and systemic level (Robles et al., 2013; Benedito-Palos et al., 2016; Piazzon et al., 2017; Simó-Mirabet et al., 2018). However, we found that intestinal butyrate was below the detection limit in all the studied groups, whereas the highest concentration of propionate was achieved in EWH fish, and its relevance is further discussed later on.

Gut microbiota studies are emerging as effective approaches for promoting farmed fish health, contributing to improve the productivity of the aquaculture sector (Brugman et al., 2018; Egerton et al., 2018b; Egan et al., 2020). In this regard, it is noteworthy that measurements of gut microbiota diversity are considered a good indicator of animal health, becoming dietary factors one of the main regulators of intestinal microbial diversity (Moschen et al., 2012). Thus, the ability of replacement diets and/or feed additives to retain a gut microbiome composition close to that of fish fed diets with high contents of FM and FO is envisaged in gilthead sea bream (Fontinha et al., 2021), as well as in other fish species of interest in aquaculture (Egerton et al., 2020; Niu et al., 2020). Though it exists a high variability of response to protein supplements across different animal models (Clarke et al., 2014; Liu et al., 2014; Butteiger et al., 2016; Beaumont et al., 2017), we did not detect changes in gut microbiota diversity of gilthead sea bream fed EWH diet. Regarding gut microbiota richness, a negative correlation with obesity has been largely reported in humans (Turnbaugh et al., 2008; Le Chatelier et al., 2013; Sze and Schloss, 2016; Peters et al., 2018). However, recent evidence suggests that this association cannot be considered as widespread among the population (Stanislawski et al., 2019). Similarly, the lower microbiota richness of our EWH fish with a reduced feed intake was mainly driven by three animals with extreme low richness values (**Supplementary Figure 1**).

Despite all the above findings, changes in the composition of mucosal adherent bacterial communities are already found at the phylum level (**Figure 4**). Proteobacteria, Firmicutes,

Actinobacteria, and Bacteroidetes Phyla dominated the autochthonous microbiota of the intestine of fish, as it has been also observed in previous studies in this species (Kormas et al., 2014; Estruch et al., 2015; Piazzon et al., 2019), with a fifth phylum, Spirochaetes, increasing in EWH fish. Furthermore, discriminant analysis (Figure 5) helped to disclose 46 dominant bacteria ($VIP \geq 1.2$, $>1\%$ in abundance) (Figure 6) with a significantly higher presence associated to a particular diet, and a decrease in the other groups. This fact revealed a specific organization of the gut microbiota in response to each dietary treatment, which allowed to discover differences at all taxonomic levels. Firstly, Proteobacteria (facultative anaerobic organisms) were presented in all analyzed fish, as it is considered one of the most abundant symbionts in marine fish because of their highly flexible metabolic properties (Tarnecki et al., 2017; Ikeda-Ohtsubo et al., 2018). Within this phylum, high abundance values were found for the Vibrionaceae family in all dietary groups. Species of this family help to digest organic substances due to the production of lipases, amylases and proteases, but some of those species can also produce harmful enzymes like neuraminidases and act as causative agents of fish pathologies (Egerton et al., 2018b).

Firmicutes are also some common intestinal symbionts in fish and mammals (Lozupone et al., 2012; Ghanbari et al., 2015). In our farmed fish, Firmicutes ranged from 26.5% in the CTRL group to $\sim 17\%$ in the L-FM/FO and EWH groups. Both plant-based diets shared important proportions of Lactobacillales, known to inhibit fish pathogens due to the natural production of bacteriocins (Balcázar et al., 2007; Sugita et al., 2007; Shahid et al., 2017; Ringø et al., 2018). This abundance of Lactobacillales in plant-based diets is caused by their ability to use indigestible fiber and fermentable polysaccharides for their metabolism and growth (Gajardo et al., 2017; Theilmann et al., 2017). In the last decade, Lactobacillales have been extensively studied due to their potential use as probiotics (Gillor et al., 2008; Heo et al., 2012), with particular importance to aquaculture, where the avoidance of the use of antibacterial drugs for facing fish pathogens is one of the main challenges (Sahoo et al., 2016). Bacteria of the family Carnobacteriaceae and of the genera *Lactobacillus* and *Streptococcus*, all of them present in our plant-based dietary groups, are prone to produce these antimicrobial agents (Elayaraja et al., 2014). However, the remarkable proportion of *Streptococcus* in L-FM/FO can also display negative effects as this genus has been described among the reduced group of bacteria capable of producing all the enzymes needed for complete mucin degradation (Derrien et al., 2010). Hence, the goblet cell depletion observed on the L-FM/FO fish could be partly explained by the higher abundance of this genus, which is practically not present in the CTRL and EWH groups. Protective mucus at the intestinal mucosa consists of a gel overlying the epithelium based on the production and secretion of mucins, mostly by goblet cells but also by enterocytes (Pelaseyed et al., 2014). The amount of goblet cells of the AI was altered by FM/FO replacement, but interestingly the profile of CTRL fish was restored by EWH supplementation, suggesting that EWH could be re-stimulating mucus secretion in the AI. In a similar manner, milk-derived peptides have already demonstrated to stimulate rat intestinal mucus secretion and improve intestinal

barrier (Giromini et al., 2019). Here we hypothesize that these effects could be, at least in part, induced by the modulation of mucin degrading bacterial populations.

The phylum Bacteroidetes increased in EWH fish, with a predominance of bacteria assigned as *Cloacibacterium*. In gilthead sea bream, fermentation produced by species of this genus is a major process for the metabolism of glucose in SCFA that might be used later in other chemoautotrophic processes (Kormas et al., 2014). The Firmicutes/Bacteroidetes (F/B) ratio is a widely documented factor correlated with obesity in mammals. Changes in these phyla proportions are regarded as dysbiosis (Stojanov et al., 2020), and an increased F/B ratio has been related with obesity in humans (Ley et al., 2006) and rats (Requena et al., 2017). However, the correlation between obesity and F/B ratio in mammals can be controversial (Magne et al., 2020) and has not been demonstrated in fish. In any case, in our fish model, Firmicutes suffered a decrease from 26.5% in CTRL group to $\sim 17\%$ in the plant-based diets, whereas Bacteroidetes were only increased in the EWH group. Thus EWH fish showed the lowest F/B ratio (6 in EWH fish vs. 18.9 and 55.3 in CTRL and L-FM/FO fish, respectively). Firmicutes and Bacteroidetes represent more than 90% of the total bacterial communities in mammals (Magne et al., 2020), whereas, in fish, Proteobacteria are among the most abundant. Thus, although our results are in agreement with previous studies in mammals, further works are needed to determine the validity of this ratio in fish, and the possible implication of Proteobacteria in these correlations.

A wide range of Spirochaetes is found in aquatic habitats, but this phylum usually comprises a low proportion ($<1\%$) of fish intestinal microbiota (Givens et al., 2015; Le and Wang, 2020). In mammals, the presence or increase of Spirochaetes has been associated to lean individuals in fecal microbiomes of captive cynomolgus monkeys (Koo et al., 2019) and in oral microbiomes of diabetes mellitus type 2 patients (Tam et al., 2018). Concordantly, in this study, the addition of EWH in the diet significantly increased the abundance of this phylum up to a 2.2%, pointing to a potential role of these bacteria in the reduced feed intake and decreased weight gain of this group of fish.

The phylum Actinobacteria significantly increased in the L-FM/FO group, mainly due to the increase in *Corynebacterium* and *Nocardioides*, which represented $\sim 10\%$ of the overall bacterial population in this dietary group. These bacteria have been described to produce polysaccharide hydrolases (Anandan et al., 2016), which is compatible with the higher fiber content of our plant-based diets. Lastly, 73% of the total Actinobacteria found in the EWH group belongs to the genus *Propionibacterium*, present in significantly lower proportions in the other two groups. Since *Propionibacterium* is the best natural producer of propionate (Zárate, 2012; González-García et al., 2017), this observation supported the higher concentrations of intestinal propionic acid in fish fed the EWH diet. Microbial production of propionate has been related to a healthier gut state (Hosseini et al., 2011; Louis and Flint, 2017), lowering lipogenesis (Weitkunat et al., 2016) and triggering the secretion of satiety peptides, such as glucagon-like peptide-1 and peptide YY (Chambers et al., 2015). Moreover, propionate acts as an inhibitory factor of food intake *via* its antagonism with the cholesterol synthesis (Harris et al., 2012; Chakraborti, 2015).

In agreement with this, we found herein that primary bile acid biosynthesis and steroid degradation were consistently underrepresented in the inferred metagenome of EWH fish in comparison to the other two groups (Table 4). To clarify, these results do not imply that bacteria are expressing primary bile acid biosynthesis genes, but that some bacteria within the detected populations might be expressing molecules that could affect such pathway. Indeed, bile acids represent a significant host factor that modulates the microbiome of obese mice and the digestion and absorption of dietary lipids (Zheng et al., 2017). Likewise, in the present study, the down-regulation of bile acids biosynthesis, together with the decreased F/B ratio and the increased Spirochaetes phylum in the EWH group could be describing the link between the bioactive egg white hydrolysate and an anti-obesogenic response. This assumption is supported by the reduced lipid vacuolization in intestines, and by the restoration of normal liver fat deposition in association with an increase in the number of hepatic melanomacrophage centers, as already found during feed restriction in lesser guitarfish (*Zapteryx brevirostris*) (Neyrão et al., 2019). Studies addressing the gut metatranscriptome in close association to host changes of metabolism and intestinal transcriptome should be conducted to validate this hypothesis and unravel the molecular interactions behind the effects.

In summary, altogether, these results reinforce the central role of gut microbiota in the regulation of host metabolism and lipid metabolism in particular, which might suggest a role of the EWH derived bioactive peptides as an anti-obesity and/or satiety factor in fish, although the ultimate mechanisms of action still remains to be established. From a practical point of view, the potential use of this functional food ingredient in finishing diets, and the role of gut microbiota in tuning filet fatty acid composition of marketable fish merits further research.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/>, SAMN18105342-68 and PRJNA705868.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics and Animal Welfare Committee of the Institute of Aquaculture

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AUTHOR CONTRIBUTIONS

FN-C, MCP, IE, MM, and JP-S contributed to the formal analysis. FN-C, MM, JP-S, and MCP contributed to the writing-original manuscript. IE, AS-B, GW, ML-M, JC-G, MCP, and TR contributed to the writing-review and editing. GW, MM, AS-B, and JP-S contributed to the conceptualization. All authors read and approved the final manuscript and contributed to the experimental investigation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.698484/full#supplementary-material>

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