Fish and Shellfish Immunology 150 (2024) 109607

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Evaluation of quercetin in alleviating the negative effects of high soybean meal diet on spotted sea bass *Lateolabrax maculatus*

Longhui Liu [a,b], Yanbo Zhao [a,b], Zhangfan Huang [a,b], Zhongying Long [a,b], Huihui Qin [a,b], Hao Lin [a,b], Sishun Zhou [a,b], Lumin Kong [a,b], Jianrong Ma [a,b], Yi Lin [a,b], Zhongbao Li [a,b,*]

[a] *Fisheries College, Jimei University, Xiamen, China*
[b] *Fujian Provincial Key Laboratory of Marine Fishery Resources and Eco-environment, Xiamen, China*

ARTICLE INFO

Keywords:
Quercetin
Spotted sea bass
High soybean meal diet
Intestinal microbiota
Metabolomics

ABSTRACT

The aim of this study was to investigate the effects of quercetin (QUE) on alleviating the negative effects of high soybean meal diet for spotted sea bass *Lateolabrax maculatus*. A healthy control group fed a 44% fishmeal diet was used, while the induction control group replaced 50% fishmeal with soybean meal. Subsequently, QUE was added at concentrations of 0.25, 0.50, 0.75, and 1.00 g/kg in the experimental groups. A total of 540 tailed spotted sea bass were randomly divided into 6 groups and fed the corresponding diet for 56 days. The results showed that 40% soybean meal significantly decreased the growth performance and immunity, increased the intestinal mucosal permeability, and caused damage to the intestinal tissue morphology; moreover, there were alterations observed in the composition of the intestinal microbiota, accompanied by detectable levels of saponins in the metabolites. However, the addition of QUE did not yield significant changes in growth performance; instead, it notably reduced the permeability of the intestinal mucosa, improved the body's immunity and the structural integrity of the intestinal tissue, increased the proportion of Proteobacteria, and enhanced the richness and diversity of intestinal microorganisms to a certain extent. In addition, QUE up-regulate the metabolism of amino acids and their derivatives and energy-related metabolites such as uridine and guanosine; furthermore, it appears to regulate transporters through the ABC transporters pathway to promote the absorption and utilization of QUE by enterocytes.

1. Introduction

The growing global demand for seafood depends on the continued growth of aquaculture production [1]. Over the past 30 years, aquaculture has provided more than half of all aquatic products consumed by humans, with fish in particular providing at least 15% of the per capita animal protein intake for more than 4.5 billion people [2]. Because of its high protein content and balanced amino acids, fishmeal is considered the main protein source for many carnivorous fish in aquaculture [3]. However, an aquaculture model that relies on fishmeal as the main protein source is not sustainable due to the dramatic reduction in fishmeal catch production and the continuous increase in price [4]. At present, the plant protein with relatively high effective protein content and balanced amino acids - soybean meal, is considered to be the most promising and potential protein source to replace fishmeal, and has been widely used in production practice [5–7]. Unfortunately, however, soybean meal contains many anti-nutrient factors (ANFs), such as soybean antigen, phytic acid and lectin, which can cause negative effects on many fish, especially carnivorous fish [8,9]. Many studies have reported that high soybean meal diet (HSBMD) causes juvenile obscure puffer (*Takifugu obscurus*) [10], rainbow trout (*Oncorhynchus mykiss*) [11], turbot (*Scophthalmus maximus*) [12], Japanese flounder (*Paralichthys olivaceus*) [13] to produce adverse reactions, including pathological changes of intestinal tissue morphology, increased permeability of intestinal mucosa and disturbance of intestinal microorganisms; the expression of inflammatory factors and tight junction proteins also changed accordingly [14–16].

Quercetin (QUE) is a natural flavonoid widely found in vegetables, fruits, and nuts [17]. QUE has a variety of pharmacological effects, including antioxidant [18], anti-inflammatory [19], immunomodulatory [20], antiviral [21], antibacterial [22] and hypoglycemic [23]. In particular, the effects of QUE were closely related to its bioavailability [24]. In addition, studies have reported that water-soluble dietary fiber (especially soybean fiber) can improve the bioavailability of QUE after

* Corresponding author. Fisheries College, Jimei University, Xiamen, China.
E-mail address: lizhongbao@jmu.edu.cn (Z. Li).

https://doi.org/10.1016/j.fsi.2024.109607
Received 11 January 2024; Received in revised form 22 April 2024; Accepted 2 May 2024
Available online 7 May 2024

long-term feeding, and promote the beneficial effect of quercetin on disease prevention [25]. Jia et al. [26] reported that QUE can interact with two major soybean proteins in a structure-dependent manner and change their secondary structure. Therefore, soybean meal may improve the bioavailability of QUE and thus better exert its beneficial effects, thereby reducing the above series of adverse reactions caused by HSBMD.

Spotted sea bass (*Lateolabrax maculatus*) is a typical carnivorous fish with rich nutrition, delicious meat and strong adaptability which is deeply loved by people. Moreover, its aquaculture output is huge, with the output in China alone reaching 218,100 tons in 2022 [27]. However, HSBMD can also cause intolerance, or even more severe reactions in spotted sea bass, especially in the intestinal mucosal barrier and intestinal microbial disturbances [28]. Therefore, in this study, QUE was added to HSBMD and combined with intestinal microbiome and metabolomics analysis to explore the effect of QUE on alleviating the HSBMD intolerance of spotted sea bass, and to discuss the possibility of combining QUE as a feed additive with soybean meal to reduce the fish meal content in the feed formula.

2. Materials and methods

2.1. Experimental diets

Six groups of feed formulations were designed according to the nutritional requirements of spotted sea bass [29]. 44% fish meal and 0% soybean meal were used as healthy control and labeled as HFM group; in addition, the negative response was induced by 40% soybean meal in spotted sea bass and labeled as HSM group; on the basis of HSM group, 0.25, 0.5, 0.75 and 1 g/kg QUE were gradually added and labeled as QUE1, QUE2, QUE3, and QUE4, respectively. The QUE ($C_{15}H_{10}O_7$, purity: 97%) used in this study was purchased from Wanxiangli Biotechnology Co., Ltd., Xiamen, China. Differences among groups were treated with flour trim (Table 1).

First of all, the raw materials were fully crushed by a pulverizer (SGF130, Tianfan Pharmaceutical Machine Factory, Shanghai, China). After that, each solid component in the formula was fully mixed according to the method of mixing step by step, from small to large. Next, added fish oil, soybean oil, and a certain amount of water (about 30% of the weight of the meal) and stir to combine. The thoroughly mixed feed was put into a granulating machine (CD4 × 1 TS, South China University of Technology, Guangzhou, China) to produce a pellet feed with a particle size of 2.5 mm. The prepared feed was dried in a constant temperature drying oven at 55 °C. When the water content was reduced to 10%, it was taken out and cooled at room temperature, and stored at −20 °C for use.

2.2. Experimental fish and feeding management

The culture experiment was carried out in the seawater test ground of Jimei University, and experimental fish were purchased from a commercial fishery in Fujian, China. The test site and fish tank were cleaned, disinfected and aerated before starting the culture experiment. The fish were fed in a temporary aquarium (1200 L) for two weeks to adapt to the test environment. During the temporary feeding period, the fish were fed twice at 8:30 and 17:30 until they were apparently full. After feeding for half an hour every night, about 30% of the water was changed, and the whole water body was oxygenated.

The fish were fasted for 24 h after the temporary cultivation, and anesthetized with 150 mg/L eugenol [30]. A total of 540 fish with similar size (5.65 ± 0.02 g) were randomly assigned to 18 fish tanks with three replicates per group. The experiment period was 56 days. The feeding management during the formal experiment was basically the same as the temporary feeding. Each group was fed different diets with corresponding labels. All experimental fish tanks were connected to ensure that the water environment of the fish was completely consistent; the water temperature controlled by the chiller was 27.0 ± 0.2 °C; the salinity was maintained between 0.5 and 2.0 ppt; the dissolved oxygen was maintained at about 7 mg/L; the pH was maintained at 7.8–8.2; and the ammonia nitrogen concentration was maintained below 0.3 mg/L.

Table 1
Nutritional composition of experimental diet (Dry matter basis).

Ingredients	Group/Contents (g/kg)					
	HFM	HSM	QUE1	QUE2	QUE3	QUE4
Fish meal	440	220	220	220	220	220
Soybean meal	0	400	400	400	400	400
Casein	110	110	110	110	110	110
Flour	345.00	149.00	148.75	148.50	148.25	148.00
Fish oil	35	50	50	50	50	50
Soybean oil	25	25	25	25	25	25
Mineral premix[a]	6	6	6	6	6	6
Antioxidant	3	3	3	3	3	3
$Ca(H_2PO_4)_2$	12	12	12	12	12	12
Vitamin premix[b]	8	8	8	8	8	8
Choline	6	6	6	6	6	6
Methionine	0	1	1	1	1	1
Lecithin	10	10	10	10	10	10
Quercetin	0	0	0.25	0.50	0.75	1.00
Total	1000					
Proximate composition (%)						
Crude protein	46.04	44.87	44.95	45.37	44.75	45.56
Crude Lipid	11.90	11.40	11.30	11.40	11.20	11.30
Crude Ash	10.20	8.70	8.50	8.60	8.50	8.60
Moisture	4.20	5.20	5.60	4.60	6.60	4.70
Gross energy (MJ/kg)[c]	19.71	19.58	19.56	19.69	19.43	19.69

Note: The proportion of nutrients of the main ingredients in the feed: Soybean meal: Crude fat, 1.9%, Crude protein, 44.2%; Flour: Crude fat, 3 %, Crude protein, 13 %; Fish meal: Crude fat, 8.4%, Crude protein, 67%.

[a] Mineral premix: $MnSO_4{\cdot}4H_2O$ 50 mg/kg, $MgSO_4{\cdot}H_2O$ 4000 mg/kg, KI 100 mg/kg, $CoCl_2$(1%) 100 mg/kg, $CuSO_4{\cdot}5H_2O$ 20 mg/kg, $FeSO_4{\cdot}H_2O$ 260 mg/kg, $ZnSO_4{\cdot}H_2O$ 150 mg/kg, Na_2SeO_3(1%) 50 mg/kg.

[b] Vitamin premix: thiamine 25 mg/kg, riboflavin 45 mg/kg, pyridoxine hydrochloride 20 mg/kg, Vitamin B12 0.1 mg/kg, Vitamin K3 10 mg/kg, inositol 800 mg/kg, pantothenic acid 60 mg/kg, nicotinic acid 200 mg/kg, folic acid 20 mg/kg, biotin 1.2 mg/kg, vitamin A acetate 32 mg/kg, Vitamin D3 5 mg/kg, α-tocopherol 120 mg/kg, ethoxyquin 150 mg/kg.

2.3. Sample collection

After the culture experiment was completed, the spotted sea bass was also fasted for 24 h and anesthetized with 150 mg/L eugenol. Eleven fish were randomly selected from each tank, blood samples were collected by the intravenous blood collection method, and the serum was isolated at 4 °C for 16 h, centrifuged (836×*g*, 4 °C) for 10 min, and stored at −80 °C. The fish were then humanely dissected and the intestines were removed. Nine intestinal samples were briefly stored in liquid nitrogen and transferred to −80 °C for storage. Five intestinal samples were used for the detection of related enzyme activity, and four intestinal samples were used for metabolomics analysis. The remaining two intestinal samples were immobilized with 4% paraformaldehyde for histological observation.

2.4. Analysis of basic dietary components

The contents of crude fat, crude protein, ash and water in dietary samples were determined by standard method [31]. Crude lipid was determined by Soxhlet extraction in ether; protein was determined by Kjeldahl nitrogen determination method (N × 6.25); the samples were burned in a Muffle furnace at 550 °C for 8 h to determine the ash content; and the samples were dried in a 105 °C oven to constant weight for moisture determination.

2.5. Growth performance parameters analysis

The growth indexes in this study included survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and feed intake (FI). The calculation formulas are as follows:

$SR\ (\%) = 100 \times (N_t / N_0)$

$WG = (W_1 - W_0) / W_0$;

$SGR\ (\%/d) = (\ln W_1 - \ln W_0) / d \times 100$;

$FCR = f / (W_1 - W_0)$;

$FI\ (g/d) = f / 56$;

In the formula: N_t is the number of survivors; N_0 is the initial quantity; W_1 is the average final weight (g); W_0 is the average initial weight (g); d is the test days; f is the total food intake (g).

2.6. Analysis of immune parameters

In this study, immune parameters included intestinal and hepatic complement 3 (C3), complement 4 (C4), immunoglobulin M (IgM), alkaline phosphatase (AKP), acid phosphatase (ACP). The principle of C3, C4 and IgM activities detection are that competition method. In short, samples and biotin-labeled recognition antigen were added to the enzyme-labeled pore pre-coated with antibodies, after that, the sample and antigen compete with solid antibodies to form immune complexes. After PBST washed away the unbound biotin antigen, avidin-HRP was added to bind to the antigen and produce a color reaction under specific conditions. There was an absorption peak at 450 nm, and the absorption value was negatively correlated with the antigen concentration [32]. The AKP and ACP activities were determined by colorimetry. Disodium phosphate is decomposed by AKP and ACP to form free phenol and phosphoric acid, and phenol reacts with 4-amino-antipyrine in alkaline solution to oxidize red quinone derivatives [33]. All kits were provided by Nanjing Jiancheng Biotechnology Co., Ltd.

2.7. Analysis of permeability parameters of intestinal mucosal barrier

In this study, intestinal mucosal permeability related indexes included diamine oxidase (DAO), D-lactic acid (D-LA) and albumin (ALB). Commercial kits were used to determine each index. In short, 1, 4-butylenediamine was catalyzed by DAO to produce CH_3, which in turn reacts with α-ketoglutaric acid and NADH to produce glutamate. The rate of NADH decline per minute at 340 nm was measured by the spectrophotometer (UV-1200, MAPADA, China) to calculate DAO activity [34]. The activity of D-LA was determined by enzyme-linked immunosorbent assay (ELISA). The D-LA antibody was pre-coated in micropores, and the substrate changed color under the action of peroxidase and acid. In particular, the concentration of D-LA positively correlated with the color depth. Absorbance was measured at 450 nm using the microplate reader (EPHCH2T, BioTek, USA) [35]. When PH = 4, the color of bromocresol green changes with ALB, and the color depth is positively correlated with ALB. ALB activity was calculated by measuring absorbance at 628 nm wavelength [36]. D-LA kits were purchased from Shanghai Youxuan Biotechnology Co., Ltd., and DAO and ALB kits were purchased from Nanjing Jiancheng Biotechnology Co., Ltd.

2.7.1. Intestinal morphological analysis

Referring to previous laboratory studies, hematoxylin-eosin staining was used to observe intestinal tissue morphology [37]. In short, after the samples were fixed in 4% paraformaldehyde solution for 24 h, they were soaked and dehydrated in ethanol solution of different concentrations. The dehydrated samples were soaked in a mixture of 1:1 ethanol and xylene for 20 min, and soaked in a mixture of xylene and paraffin for 30 min, then removed and quickly embedded with paraffin at 60 °C. After the paraffin was cured, slice it with a microtome, spread the slices in warm water, and collect the slides. After drying in the oven, they were soaked in xylene and ethanol solution for dewaxing, and hematoxylin and eosin were dyed successively. Three sections were selected in each group. The villus height, villus thickness and muscular thickness of each section were measured with Fiji (Image J-win64), and the histological changes of intestinal epithelial cells were evaluated according to the degree of villi changes. The light microscope (Eclipse Ci-L, Nikon, Japan) was used for filming and analysis. CaseViewer Ver 2.2 (The Digital Pathology Corp., Hungary) was used to observe the samples in different multiples.

2.8. High-throughput 16s rRNA gene amplification and sequencing

2.8.1. Total RNA extraction

Stool DNA kit (Tiangen Biotech (Beijing) Co., Ltd.) was used to extract the total DNA of the samples. Meanwhile, the DNA was quantified by Nanodrop, and the quality of the DNA extraction was detected by 1.2% agarose gel electrophoresis.

2.8.2. PCR amplification of the target fragment

The target sequences that can reflect the composition and diversity of bacterial flora, such as microbial ribosomal RNA or specific gene fragments, were taken as targets, and corresponding primers were designed according to the conserved regions in the sequences, and specific barcode sequences were added to the samples, and then PCR amplification was performed on the variable regions of rRNA genes or specific gene fragments. The amplified products were purified and recovered. In short, 25 μl of PCR products were added with Vazyme VAHTSTM DNA Clean beads, which were 0.8 times the volume. After full shock, the beads were absorbed on the magnetic rack for 5 min, and the supernatant was carefully sucked out. After that, 20 μl of 0.8x magnetic bead washing solution was added, and the same full oscillating adsorption was carried out for 5 min, and the supernatant was carefully sucked out. Then, 200 μl of 80% ethanol was added and placed on the magnetic frame in the reverse direction. The magnetic beads were adsorbed on the other side of the PCR tube, and the supernatant was aspirated after full adsorption. The obtained supernatant was left standing at room temperature for 5 min until the alcohol volatilized completely and cracks appeared in the magnetic beads. Finally, 25 μl elution buffer was added for elution, the PCR tube was placed on the adsorption rack for 5 min, the supernatant was fully absorbed, and the supernatant was removed and stored in the centrifuge tube for future use.

The assay Kit was used as the fluorescence reagent and the microplate reader (BioTek, FLx800) was used to quantify the recovered products. According to the fluorescence quantitative results, each sample was mixed according to the corresponding proportion according to the sequencing quantity requirement of each sample.

2.8.3. Sequencing library preparation

Sequencing libraries were prepared using the TruSeq Nano DNA LT library prep kit (Illumina). In short, the end repair of the amplified product was performed first, and the prominent base at the 5′ end of the DNA sequence was removed by End Repair Mix 2 in the kit, and a phosphate group was added to supplement the missing base at the 3′ end. Then, a base was added to the 3′ end of the DNA sequence to prevent the DNA fragment from self-linking and ensure that the target sequence could be in phase with the sequencing splice. A sequencing adapter containing a library specific tag (index sequence) was added at the 5′ end of the sequence to enable DNA molecules to be immobilized on the flow cell. BECKMAN AMPure XP beads were used to remove the joint self-connecting segments by magnetic bead screening, and the library system was purified after adding the joint. The DNA fragments connected to the splices were amplified by PCR and purified by BECKMAN AMPure XP beads. Finally, the library was selected and purified by 2%

agarose gel electrophoresis.

2.8.4. High-throughput sequencing

The Agilent high sensitivity DNA kit and the Quant-iT PicoGreen dsDNA assay kit were used to conduct quality inspection on the Agilent Bioanalyzer and Promega QuantiFluor fluorescence quantification systems, respectively. The qualified libraries were diluted in gradient, mixed in proportion according to the required sequencing amount, and denatured into single strand by NaOH for on-machine sequencing.

2.9. Intestinal metabolomic analysis

2.9.1. Metabolites extraction

After freeze-drying, the weighed samples were placed in 2 mL Eppendorf tube containing a 5 mm tungsten bead for 1 min at 65 Hz in a grinding mill. The metabolites were extracted with a pre-cooled mixture of methanol, acetonitrile and water (v/v/v = 2/2/1), ultrasonic oscillated in an ice water bath for 1 h, placed at −20 °C for 1 h, and centrifugated (14,000×*g*, 4 °C, 20 min). Recovery of supernatant and concentrated to dryness in vacuum.

2.9.2. UHPLC-MS/MS analysis

The UHPLC-ESI-Q-OrbitRAP-MS system (UHPLC, Shimadzu Nexera X2 LC-30AD, Shimadzu, Japan) and Q-Exactive Plus (Thermo Scientific, San Jose, USA) analyzed the extracted metabolites. Specifically, the separation was performed by liquid chromatography (LC) using ACQUITY UPLC®HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters, Milford, MA, USA). The electrospray ionization (ESI) was used for mass spectrometry data acquisition in positive mode and negative mode, respectively. The HESI source conditions were set as follows: spray voltage: 3.8 kv (positive) and 3.2 kv (negative); sheath gas (nitrogen) flow: 30 arb (arbitrary units); capillary temperature: 320 °C; probe heater temperature: 350 °C; aux gas flow: 5 arb; s-lens rf level: 50. The instrument was set to acquire a full mass spectrum of 70–1050 Da in the *m/z* range. The full MS scans were acquired at a resolution of 70,000 at m/z 200, and 17,500 at m/z 200 for MS/MS scan.

2.9.3. Data preprocessing and filtering

MS-dial was used for retention time correction, peak alignment, and peak area extraction of raw mass spectrometry data. The metabolites were identified by MS/MS data (mass tolerance <0.02 Da) and accuracy mass (mass tolerance <10 ppm). After that, it was compared with public databases such as HMDB and Massbank. In the extracted-ion features, only the variables having more than 50% of the nonzero measurement values in at least one group were kept.

2.9.4. Multivariate statistical analysis

All multivariate data analysis and modeling were performed using R (version: 4.0.3) and the R package. Principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and partial least squares discriminant analysis (PLS-DA) were used to establish the model. The descriptive performance of the model was determined by R^2X (cumulative) (perfect model: R^2X (cum) = 1) and R^2Y (cumulative) (perfect model: The R^2Y (cum) = 1) value is determined, while the predictive performance was measured by Q2 (cumulative) (perfect model: Q2 (cum) = 1) and the permutation test (n = 200). Differential metabolites were screened by the combination of difference multiple, P-value and VIP value of OPLS-DA model. The screening criteria were $FC > 1$, $P < 0.05$, $VIP > 1$.

2.9.5. KEGG enrichment analysis

KEGG pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp) for differential metabolite data. KEGG enrichment analyses were carried out with the Fisher's exact test, and FDR correction for multiple testing was performed. It was concluded that the enrichment of KEGG pathway was statistically significant at $P < 0.05$ level.

2.10. Data statistics and analysis

The average value of the experimental data in this study was calculated and expressed as mean ± SD. Independent sample T test was used to analyze the differences between HFM and HSM. One-way analysis of variance was used to compare QUE addition groups with HFM and HSM, respectively. All statistical analyses were performed using SPSS Ver 26 software. When $P < 0.05$, significant differences were considered and Duncan's multiple range test was performed. Fig. 1, Fig. 2, Fig. 3 and Fig. 4 were made by GraphPad Prism 8.

3. Results

3.1. Growth performance parameters analysis

The results of growth performance related indicators are shown in Fig. 1. The SR was 100% in each tank of spotted sea bass. The results showed that compared with the HFM group, WG, SGR and FI in the HSM group and quercetin supplementation group were significantly decreased ($P < 0.05$), and FCR was significantly increased ($P < 0.05$). In addition, HI in HSM and QUE1 groups was significantly higher than that in other QUE groups ($P < 0.05$).

3.2. Analysis of immune parameters

The results of immune parameters related indicators are shown in Fig. 2. The results showed that HSBMD significantly decreased the activities of intestinal C3, IgM, AKP, ACP and hepatic AKP, ACP ($P < 0.05$). The addition of QUE significantly improved the immunity of spotted sea bass. Among them, the intestinal and hepatic ACP activity was significantly increased in all QUE supplementation groups ($P < 0.05$); supplementation levels of 0.5 and 0.75 g/kg significantly increased hepatic and intestinal AKP activity, respectively ($P < 0.05$). In addition, the activities of intestinal C3 and hepatic C3 and C4 were firstly increased and then decreased with the increase of QUE supplemental level; when the supplementation level was 0.25 g/kg, the activities of intestinal C3, hepatic C3 and C4 were significantly higher than HSBMD group and 0.5 g/kg addition group ($P < 0.05$).

3.3. Analysis of permeability parameters of intestinal mucosal barrier

The results of related indexes of intestinal mucosal permeability are shown in Fig. 3. The results showed that HSBMD significantly increased the activities of DAO and D-LA in serum and ALB in intestinal tract ($P < 0.05$). After adding QUE, the activities of DAO and D-LA in serum were significantly decreased ($P < 0.05$). Among them, the serum D-LA activity was significantly decreased in all QUE supplementation groups ($P < 0.05$), and the serum DAO activity was also significantly decreased at 0.25, 0.5 and 0.75 g/kg supplementation levels ($P < 0.05$). There was no significant difference in serum ALB among all groups ($P > 0.05$).

3.3.1. Intestinal morphological analysis

The observation results of intestinal tissue morphology are shown in Figs. 4 and 5. The results showed that the intestinal morphology of spotted sea bass fed whole fish meal was normal, the epithelial cells were intact and orderly, the lamina propria was intact, and no obvious lesions were found. However, the intestinal epithelial cells and lamina propria of spotted sea bass with HSBMD were severely shed, and the villus height and muscular thickness were significantly reduced ($P < 0.05$). After adding QUE, the intestinal tissue morphology was improved and villus height, villus thickness and muscular thickness were significantly enhanced ($P < 0.05$); interestingly, villus height and muscular thickness were significantly increased($P < 0.05$), but villus thickness was not significantly increased when the addition amount was 0.25 g/kg

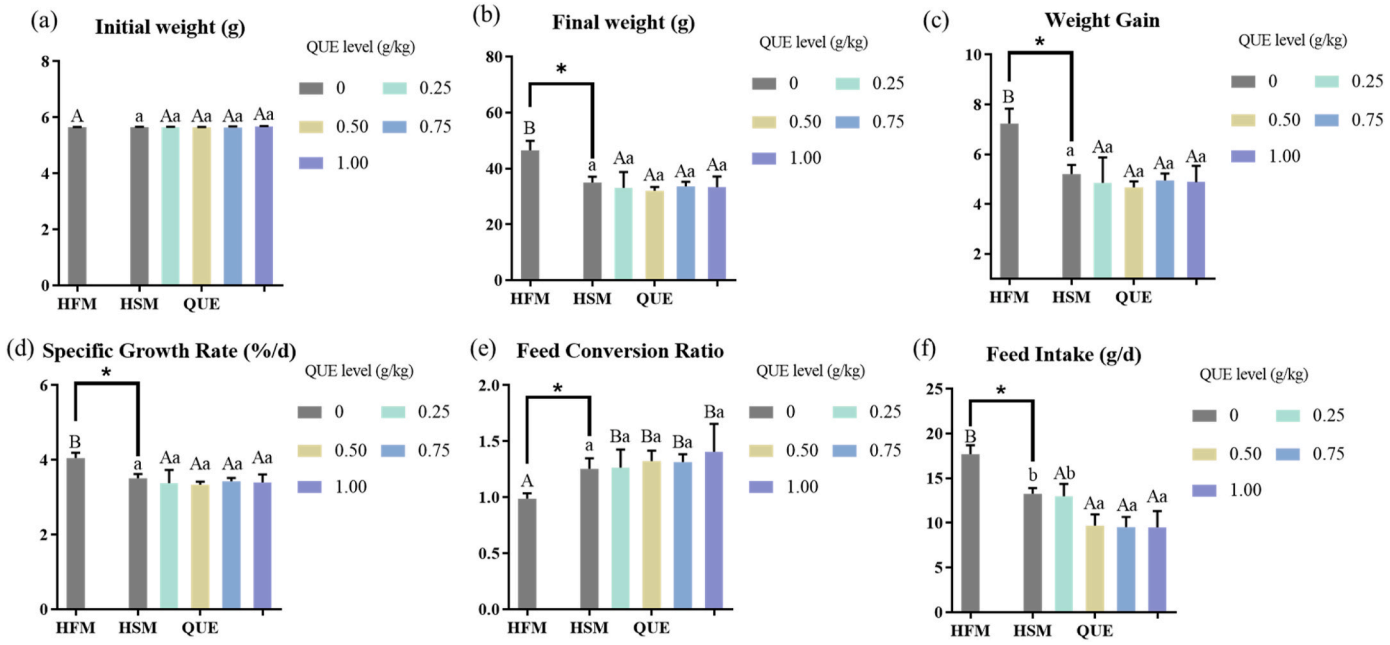

Fig. 1. Effects of QUE in high soybean meal diet on growth parameters of spotted sea bass. Note: Letters in corner marks a to f represent activity of represent initial weight (a), final weight (b), weight gain (WG, c), specific growth rate (SGR, d), feed conversion ratio (FCR, e), feed intake (FI, f), respectively. The discrepancy between the HFM group and HSM group is indicated by * ($P < 0.05$). Capital letters represent the discrepancy between QUE intake groups and HFM group, while different letters indicate significant variation ($P < 0.05$). Lowercase letters represent the discrepancy between QUE intake groups and HSM group, while different letters indicate significant variation, too ($P < 0.05$). Same to the following figures.

($P > 0.05$); however, when the addition was 0.5 g/kg, the villus thickness and muscular thickness increased significantly($P < 0.05$), but the villus height did not increase significantly($P > 0.05$). In addition, a small number of epithelial cells were shed in the QUE supplementation groups, but the degree of lesions was significantly lower than that in spotted sea bass with HSBMD.

3.4. Intestinal microbiota analysis

3.4.1. Microbial species analysis

At the phylum level, the dominant bacteria in HFM group include Proteobacteria, Tenericutes, Fusobacteria, and Firmicutes (Fig. 6a). The dominant microbiota composition of the other groups was similar to that of the HFM group. In addition, HSBMD reduced the proportion of Firmicutes in the gut of spotted sea bass. Among them, the proportion of Firmicutes decreased in all HSBMD groups, and the proportion was similar among HSBMD groups. In particular, it has been observed that the proportion of Proteobacteria in QUE addition group has increased. The specific proportion of dominant microbiota are shown in Table 2.

On the genus level, the dominant bacteria in all groups are *Cetobacterium*. However, after the addition of QUE, the proportion of *Plesiomonas* increased, especially in QUE3 group, reaching the highest level (Fig. 6b).

3.4.2. Alpha diversity analysis

In this study, the richness of Alpha diversity was represented by the index of Chao1 and Observed species [38]; Shanno and Simpson indices represent diversity [39,40]; Faith's PD index represents evolutionarily based diversity [41]; Pielou's evennes index represents evenness [42]; and Good's coverage index represents the extent of coverage.

The results showed that compared with the HFM group, Chao1, Shannon and Observed species in the HSM group were significantly higher, but Good's coverage was significantly lower. When the QUE supplemental level was 0.25 g/kg, compared with the HFM group, Chao1, Shannon and Observed species were also significantly increased, and Good's coverage was significantly decreased. Compared with the HSM group, there was no significant difference in the above indexes. In addition, there was no significant difference in Alpha diversity related indicators between the remaining QUE addition group and the HFM group (Fig. 7).

3.5. Intestinal metabolomic analysis

According to the results of enzyme activities, groups HFM, HSM and QUE2 were selected for metabolomic analysis (QUE group was substituted for QUE2 group in the subsequent results). Metabolomics analysis detected 89, 98 and 90 differential metabolites between the three comparison groups (HFM vs HSM, HSM vs QUE and HSM vs QUE), respectively (Supplementary Table 1, 2, and 3). Significantly upregulated Daidzein and its derivatives, including soyasaponin Ba, malonyldaidzin and daidzein 7-O-glucuronide, were observed in the HSM group compared to the HFM group. In addition, the upregulation of metabolites related to sugar and energy metabolism were also detected, including tetrasaccharides and stachyose. On the other hand, a large amount of soybean meal may lead to the down-regulation of amino acids, their derivatives and metabolites; including tyrosine, prolylleucine, quinolinic acid, 2-hydroxyisovaleric acid, and fructosyl-lysine, which were significantly down-regulated (Fig. 8a). When QUE was added to HSBMD, four lysophosphatidylcholines (LPC (17:1), LPC (18:3), LPC (18:4), and LPC (20: 5)) and two lysophosphatidyl ethanolamine (LPE (14:0) and LPE (22:6)) showed down-regulation. Interestingly, 2-hydroxyisovaleric acid and leucyl-Isoleucine were upregulated; in addition, citric acid, γ-delta-dioxyvalerate, uridine and guanosine, 2-hydroxypurine were also upregulated; however, hypoxanthine was observed to be down-regulated (Fig. 8b). Compared with HFM group, the downregulation of soybean meal metabolites including soyasaponin Ba and daidzein 7-O-glucuronide were also observed in QUE group. Tetrasaccharides, stachyose also showed a downward adjustment; a small number of amino acids and their derivatives were also down-regulated, including prolylleucine, gamma-glutamylcysteine and timonacic. In addition, only one lysophosphatidylcholines (LPC (22:6)) was found to be up-regulated. uridine, guanosine, taurine and

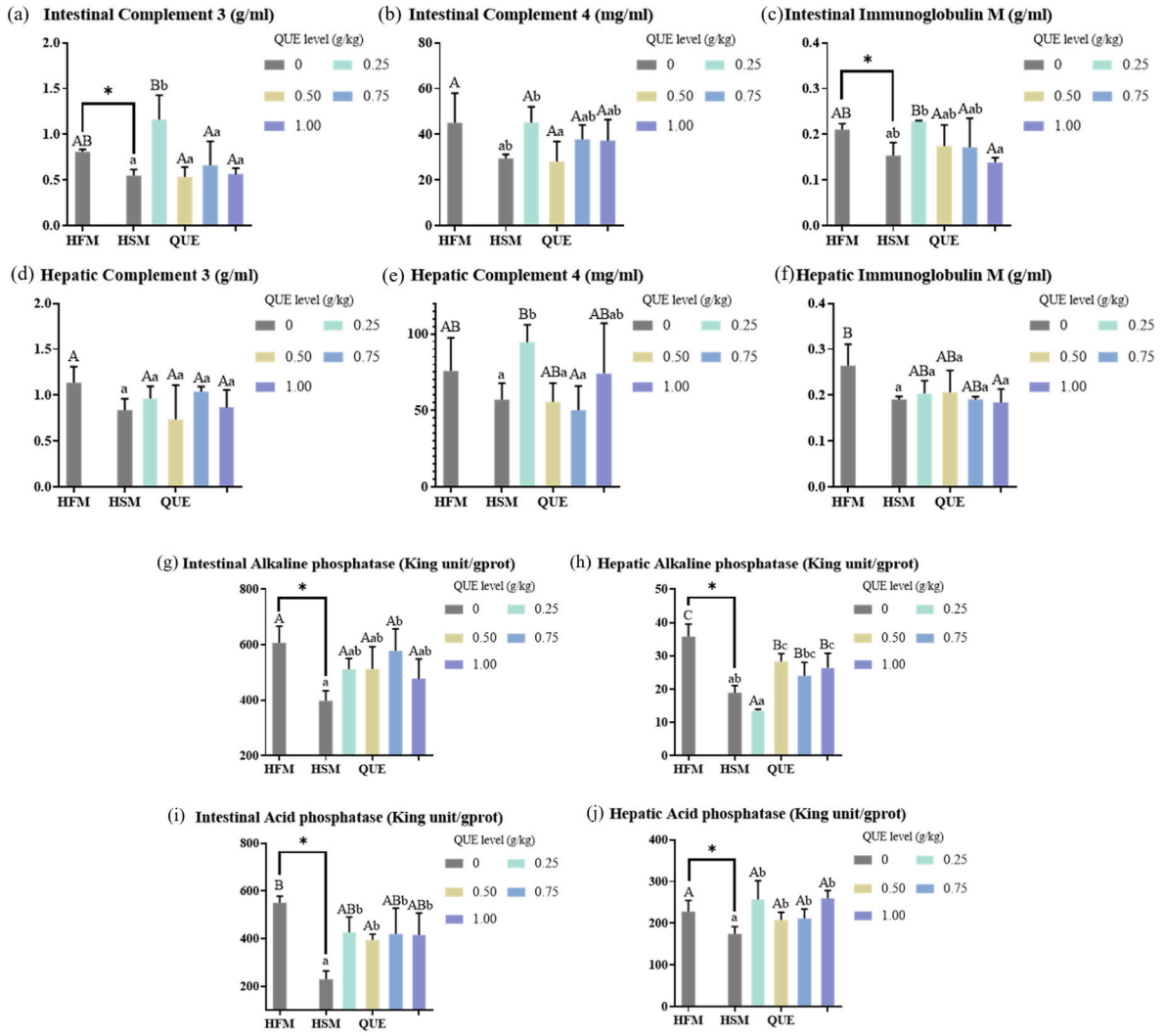

Fig. 2. Effects of QUE in high soybean meal diet on immune parameters of spotted sea bass. Note: Letters in corner marks a to j represent activity of intestinal complement 3 (intestinal C3, a), intestinal complement 4 (intestinal C4, b), intestinal immunoglobulin M (intestinal IgM, c), hepatic complement 3 (hepatic C3, d), hepatic complement 4 (hepatic C4, e), hepatic immunoglobulin M (hepatic IgM, f), intestinal alkaline phosphatase (intestinal AKP, g), hepatic alkaline phosphatase (hepatic AKP, h), intestinal acid phosphatase (intestinal ACP, i), hepatic acid phosphatase (hepatic ACP, j), respectively.

flavin mononucleotide (FMN) were also observed to be upregulated, and xanthine was observed to be upregulated (Fig. 8c).

3.5.1. *Class level classification of HMDB*

Based on the structure and function of metabolites, the HMDB class level classification of metabolites in each comparison group was performed.

The results showed that the differential metabolites in the HFM vs HSM comparison group were mainly concentrated in fatty acyls, prenol lipids and carboxylic acids and derivatives (Fig. 9a). The differential metabolites of HSM vs QUE group were mainly concentrated in fatty acyls, glycerophospholipids and carboxylic acids and derivatives (Fig. 9b). The HFM vs QUE focuses on fatty acyls, Carboxylic acids and derivatives and organooxygen compounds (Fig. 9c).

3.5.2. *KEGG enrichment analysis*

In this study, 10 KEGG pathways with differentially enriched metabolites were screened to analyze the up-down-regulated metabolites and the trend analysis of KEGG pathway levels.

The results showed that the main up-regulated differential metabolite in the HFM vs HSM comparison group was adenosine monophosphate (AMP), and it mainly flows to foxO signaling pathway, mTOR signaling pathway and PI3K-Akt signaling pathway. The main down-regulated metabolite was tyrosine, which plays a role in four pathways (Fig. 10a). The upregulated metabolites in HSM vs QUE group were mainly Prostacyclin, uridine and guanosine; and the main down-regulated metabolite was cytidine; the primary pathway was ABC transporters (Fig. 10b). In the HFM vs QUE comparison group, the up-regulated metabolites were ascorbic acid, which mainly founded metabolic pathways (Fig. 10c).

4. Discussion

As mentioned in the introduction, fish meal contains more protein, more balanced amino acids and better palatability than soybean meal. As a matter of course, fish meal fed spotted sea bass had the higher WG, SGR, FI and lower FCR than HSBMD; and the study's results fully support this point. Zhang et al. [28] reported that replacing 50% fishmeal with soybean meal did not significantly reduce growth performance. The research results of Zhang et al. [43] showed that soybean meal could replace 60% of fishmeal in Japanese sea bass (*Lateolabrax japonicus*) feed. These reports differ from the results of this study. The author thinks that it may be due to the difference in tolerance to soybean meal caused by the initial weight of the test fish. In this experiment, the initial body

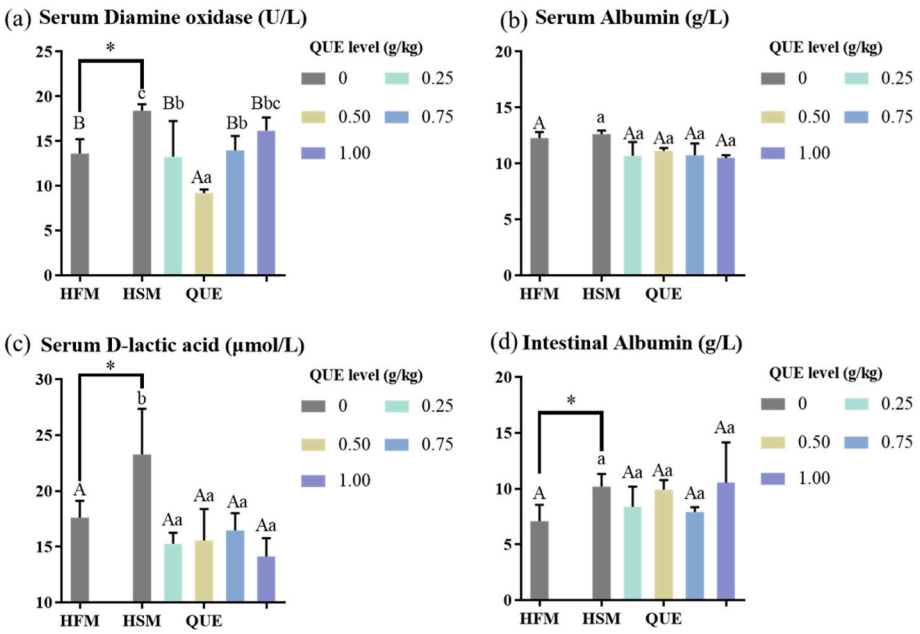

Fig. 3. Effect of QUE on intestinal mucosal permeability of spotted. Note: Letters in corner marks a to d represent activity of serum diamine oxidase (serum DAO, a), serum albumin (serum ALB, b), serum D-lactic acid (serum D-LA, c), intestinal albumin (intestinal ALB, d), respectively.

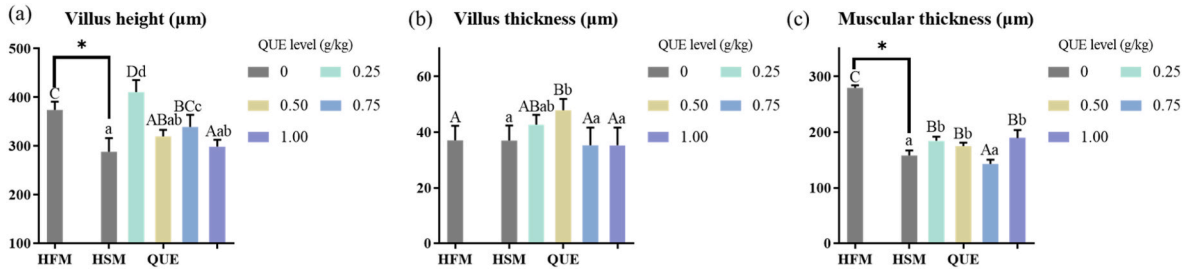

Fig. 4. Intestinal structure of spotted sea bass. Note: Letters in corner marks a to c represent villus height (a), villus thickness (b), muscular thickness (c), respectively.

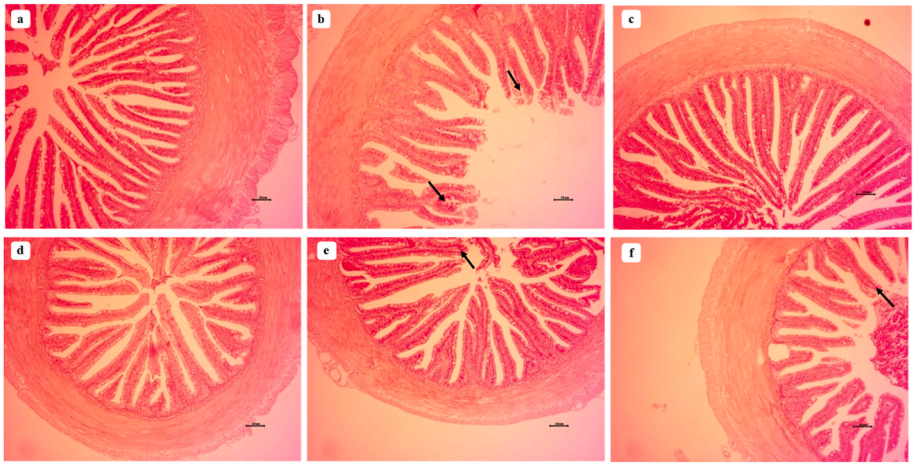

Fig. 5. Intestinal morphological of spotted sea bass (400 ×). Note: Letters in corner marks a to f represent HFM (a), HSM (b), QUE1 (c), QUE2 (d), QUE3 (e), and QUE4 (f) respectively. The black arrows indicate epithelial cells and membranes propria shedding.

weight was small, and the tolerance to soybean meal was low, which affected its growth performance. In addition, Chou et al. reported that replacing half of fishmeal with soybean meal would adversely affect the growth performance of juvenile cobia (*Rachycentron canadum*) [44]. Gu et al. [12] reported that there was a significant negative correlation between the growth performance of turbot (*Scophthalmus maximus*) and the supplemental level of soybean meal. Ma et al. [45] also reported that soybean meal had significant effects on the growth performance of Asian sea bass (*Lates calcarifer*). There are differences in the effects of soybean meal on fish growth performance in the above reports, which may be

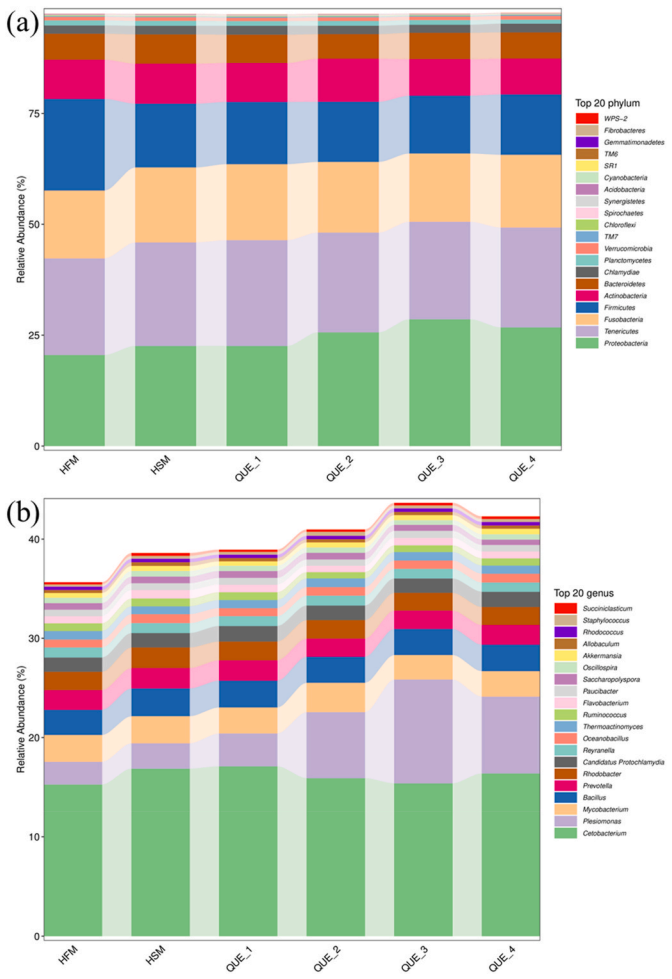

Fig. 6. Phylum and genus horizontal species distribution map (Top 20). Note: Phylum level (a), Genus level (b). The abscissa is the sample name and the ordinate is the relative abundance percentage. Different colors represent different species.

Table 2
Proportion of intestinal microbes at phylum level (%).

Group	Name			
	Proteobacteria	Tenericutes	Fusobacteria	Firmicutes
HFM	20.53 ± 0.02	21.79 ± 0.04	15.29 ± 0.02	20.66 ± 0.11
HSM	22.57 ± 0.01	23.35 ± 0.01	16.91 ± 0.01	14.37 ± 0.01
QUE1	22.56 ± 0.01	23.87 ± 0.01	17.13 ± 0.01	14.03 ± 0.01
QUE2	25.64 ± 0.05	22.50 ± 0.02	15.93 ± 0.01	13.55 ± 0.01
QUE3	28.57 ± 0.07	22.52 ± 0.03	15.39 ± 0.02	13.03 ± 0.02
QUE4	26.75 ± 0.07	22.67 ± 0.02	16.17 ± 0.01	14.88 ± 0.05

related to fish species, age, feed composition, feeding management and ANFs composition in soybean meal in different studies [46]. In addition, several studies have reported the improvement effect of QUE on the growth performance of tilapia (*Oreochromis niloticus*) [47], grass carp (*Ctenopharyngodon idella*) [48], common carp (*Cyprinus carpio*) [49] and snakehead fish (*Channa argus*) [50]. These results indicated that quercetin can promote the growth performance of aquatic animals. However, this study found that growth performance did not change significantly after the addition of QUE to the high-soybean meal feed, which may be due to the small initial body weight of the test bass, resulting in more serious intolerance to soybean meal. The effect of QUE addition could not offset the negative impact of soybean meal on growth performance of spotted sea bass.

The complement system is an important link between adaptive and innate immunity in vertebrates, which plays an important role in pathogen defense, immune enhancement, and cell lysis [51]. C3 and C4 are considered to be the most commonly measured components for monitoring immune diseases; C3 is the core component of the complement system and the central molecule of all known proteins in the complement system; and C4 also plays an important role in activating the complement system [52,53]. Immunoglobulins (Ig) are a class of glycoproteins that play an important role in adaptive immunity by recognizing antigens, including IgM, IgD, and IgT/IgZ; IgM is the main body antibody, involved in mucosal immunity and systemic immunity [54,55]. AKP and ACP can change the surface structure of pathogens, enhance the body's recognition and phagocytosis ability, and thus improve immunity [56]. In this study, HSBMD obviously had a negative effect on the immunity of spotted sea bass to some extent, which was manifested by the decrease in the activities of intestinal C3, IgM, AKP, ACP and hepatic AKP and ACP. Excessive supplementation of soybean meal has a negative effect on the immunity of farmed animals, which has been reported several times in previous studies; Liu et al. reported that replacing fishmeal with high soybean meal could decrease digestive enzyme activity and immunity in silver crucian carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*) [57]; Zhou et al. reported that excessive addition of soybean meal in feed could have a negative impact on the innate immune system of fish [58]. In addition, in this study, the addition of QUE significantly enhanced the immunity of spotted sea bass; when the addition amount was 0.5 and 0.75 g/kg, AKP and ACP activities were significantly increased and the activities of intestinal C3 and hepatic C3 and C4 were firstly increased and then decreased with the increase in QUE supplemental level. Similarly, Armobin et al. reported that supplementation with 400–600 mg/kg QUE could improve the immunity of common carp [59]; Kong et al. reported that QUE could improve the immune-related enzyme activity and immune gene expression of snakehead fish (*Channa argus*), and the best effect was achieved when the supplemental level was 300 mg/kg [60]. These reports confirm that QUE has a strong immunomodulatory effect on fish. However, in this study, the immunomodulatory effect of QUE on the spotted sea bass increased first and then decreased with the increase in supplemental levels. That suggests that the excessive addition of quercetin may have a negative effect on the body's immunity or even immunosuppression. Wang et al. reported that adding 1 μg/L quercetin could significantly enhance the activities of ACP, C3 and C4 of zebrafish (*Danio rerio*), while the activities decreased when the supplemental level was increased to 1000 μg/L [61]; Khan et al. also reported that with the increase of QUE supplemental level, the immunity of the body first increased and then decreased, and reached the highest level when the supplemental level was 4 g/kg [62]; in addition, Xu et al. observed that immune-related enzyme activity increased after 0.4–0.6 g/kg QUE was added to grass carp (*Ctenopharyngodon idella*) feed, and then decreased with the increase of supplemental level [63]. The above studies have different reports on the optimal dosage, which may be related to the different types of fish, diet and purity of QUE; however, there is no doubt that QUE has the effect of improving the body's immunity, and excessive addition may have negative effects on it.

The intestinal mucosal epithelium acts as a physical barrier, allowing the normal absorption of nutrients while preventing the invasion of pathogens [64]; therefore, the intact intestinal mucosal barrier plays an important role in the normal function of the intestine. Serum D-LA and DAO activities are considered to be two important indicators for evaluating intestinal mucosal permeability. D-LA is a metabolite produced by the fermentation of animal intestinal bacteria. DAO is a highly active intracellular enzyme secreted by villus cells in the upper layer of the intestinal mucosa. Normally, they are rarely found in blood serum. However, when the intestinal mucosal barrier is damaged, their activity in serum increases rapidly [65,66]. In this study, the serum D-LA and DAO activities of spotted sea bass with HSBMD were significantly higher than those of the whole fish meal group. The results indicated that the large amount of soybean meal increased the intestinal mucosal

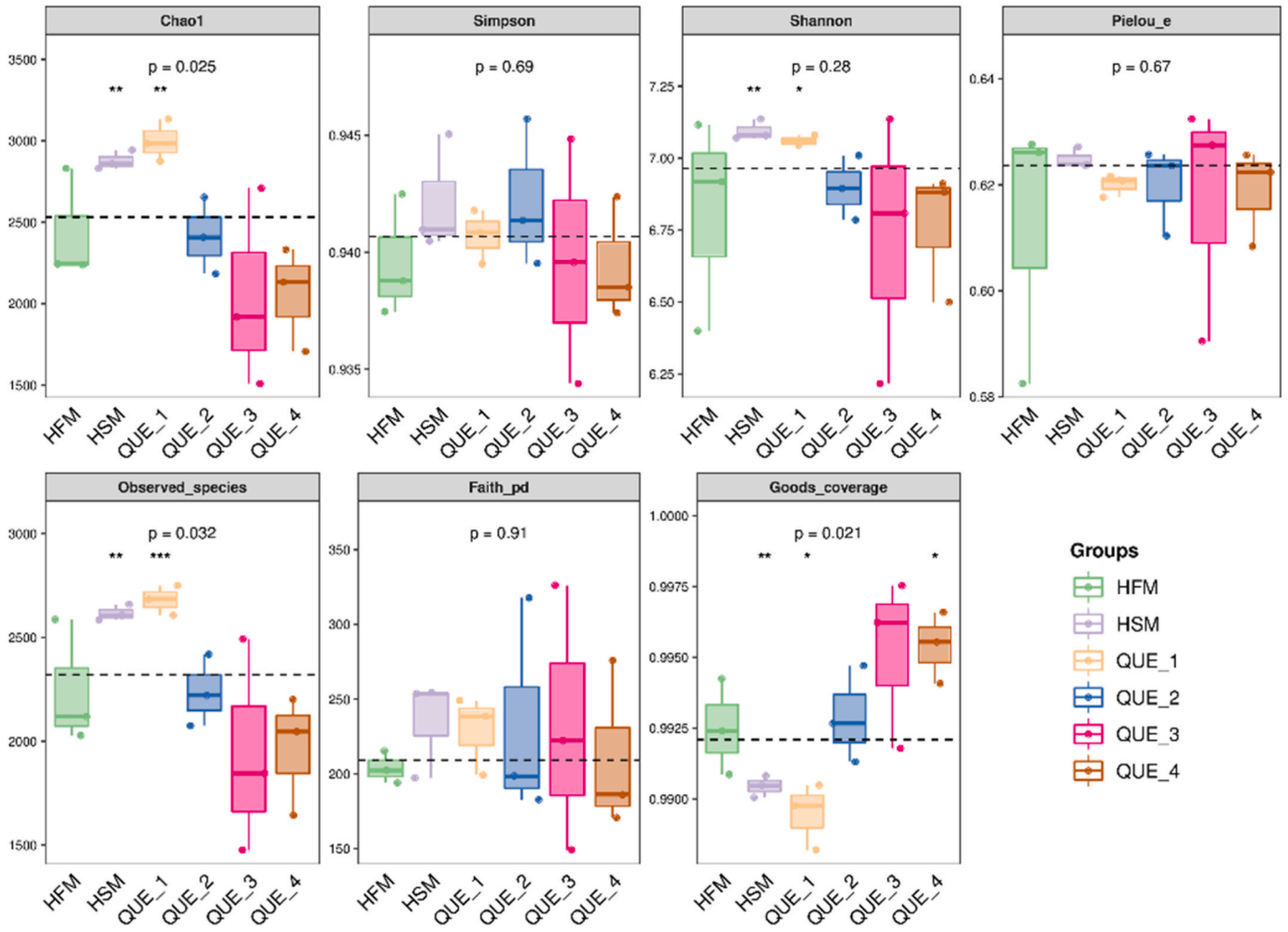

Fig. 7. Boxplot of differences between groups of the Alpha diversity index

Note: Each panel corresponds to an alpha diversity index. In each panel, the horizontal coordinate is the group label, and the vertical coordinate is the value of the corresponding alpha diversity index. In the box diagram, the meanings of symbols are as follows: upper and lower end lines of the box, upper and lower quartile range (IQR); Median line, median; Upper and lower edges, maximum and minimum inner circumference (1.5 times IQR); The points outside the upper and lower edges represent outliers. The numbers under the diversity index label are the P-values of the Kruskal-Wallis test, with * representing significant differences from the other groups.

permeability and damaged the intestinal mucosal barrier. This result has been reported in previous studies [12,28]. In addition, the differential metabolism of saponin metabolites such as soyasaponin Ba in this study proved that the intestinal microbiota or its attached intestinal microbiota could not effectively decompose soybean saponins contained in soybean meal. Therefore, the damage of intestinal structural integrity and the reduction of intestinal mucosal permeability in the spotted sea bass may be the result of ANFs or saponin metabolites [67]. Similarly, Knudsen et al. reported that soy saponins did not degrade or even induce enteritis in the intestines of Atlantic salmon (Salmo salar L.) [68,69]. After adding QUE to high soybean meal, serum D-LA and DAO activities decreased significantly, indicating that QUE reduced intestinal mucosal permeability and had a certain repairing effect on the intestinal mucosal barrier. In addition, the observation results of intestinal tissue morphology also confirmed this. Studies have reported that QUE, on the one hand, can improve the expression of tight junction protein gene and promote the proliferation of intestinal cells [70,71]; on the other hand, it can reduce the infiltration of neutrophils and macrophages in the colon tissue of colitis mice, and improve the secretion capacity of intestinal cells and mucin levels, thereby strengthening the intestinal mucosal barrier [72,73]. Therefore, QUE can reduce the negative effects of soybean meal on the intestinal tract of spotted sea bass by enhancing the intestinal mucosal barrier.

It is well known that the intestinal microbiota plays an important role in the growth and development of the host [74]. As lower vertebrates living completely in water, fish have a large number of microorganisms colonized in their intestines. However, unlike terrestrial animals, the composition of intestinal microbes in fish is influenced by factors such as complex water environment [75], feeding and diet [76], nutrition [77], and environmental stress [78]. At the beginning of feeding, the feed that fish consume can greatly change their gut microbiota, and even determine the composition and structure of the intestinal microbiota [79]. The results of this study showed that the dominant intestinal flora of several different diets were Proteobacteria, Tenericutes, Fusobacteria and Firmicutes. Previous studies have shown that several microorganisms have been identified as dominant microorganisms in fish gut, including Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria [80–82]. In addition, many previous studies have reported that a diet rich in soy meal can cause disruptions in fish gut microbes [83,84]. Serra et al. [85] reported a preference for Firmicutes in the gut microbiome composition of European seabass (*Dicentrarchus labrax*) on a high-soya meal diet; Kononova et al. [86] reported soybean meal diet of rainbow trout in the gut of Firmicutes/Proteobacteria ratio increased; however, this study found

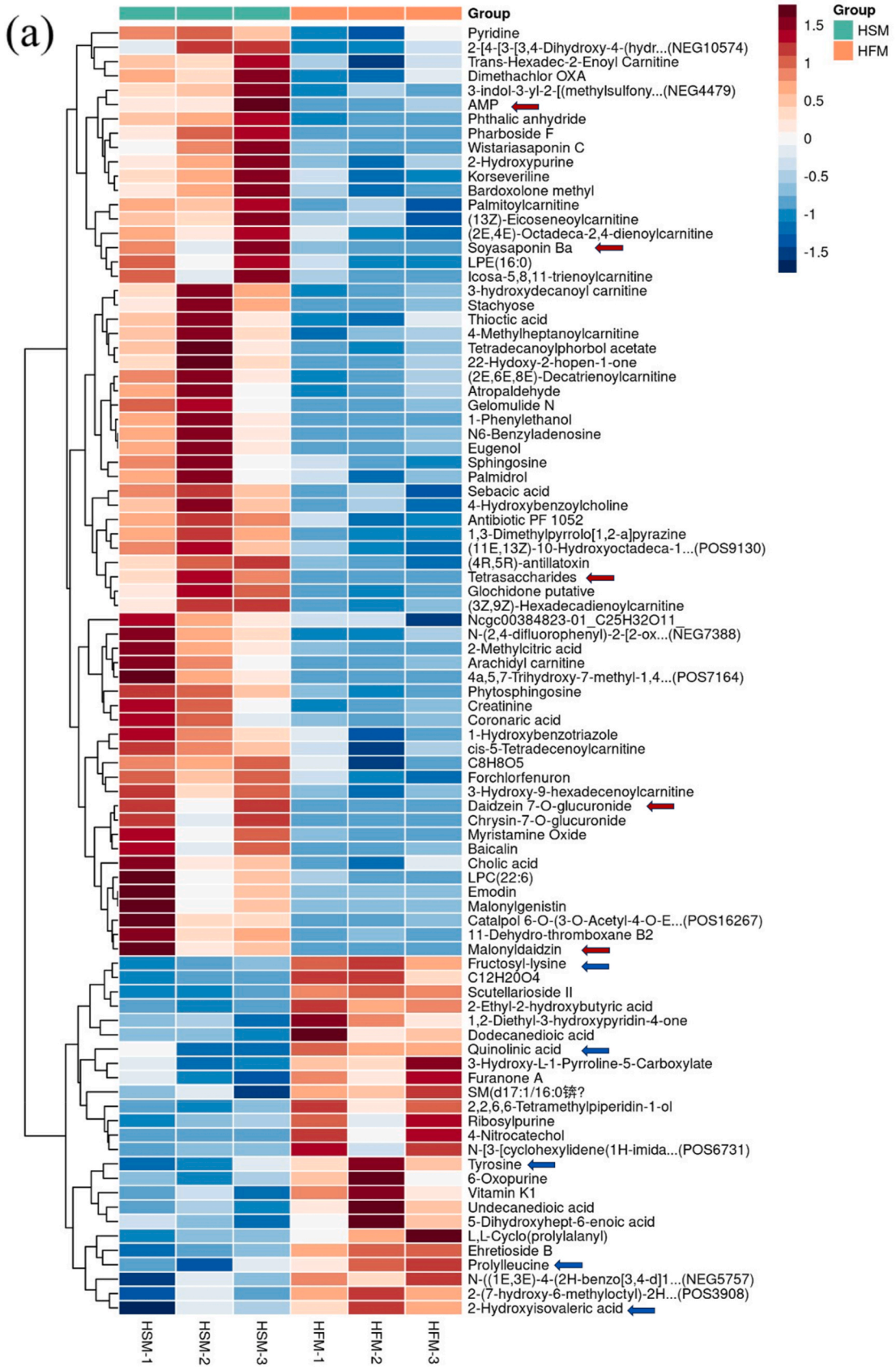

Fig. 8. Differential metabolite hierarchical clustering heat map

Note: Letters in corner marks a to c represent HSM vs HFM (a); QUE vs HSM (b); QUE vs HFM (c). The more red the color, the higher the relative expression, and the more blue the relative expression, the lower the relative expression, and red arrows indicate that key metabolites are up-regulated and blue arrows indicate down-regulated.

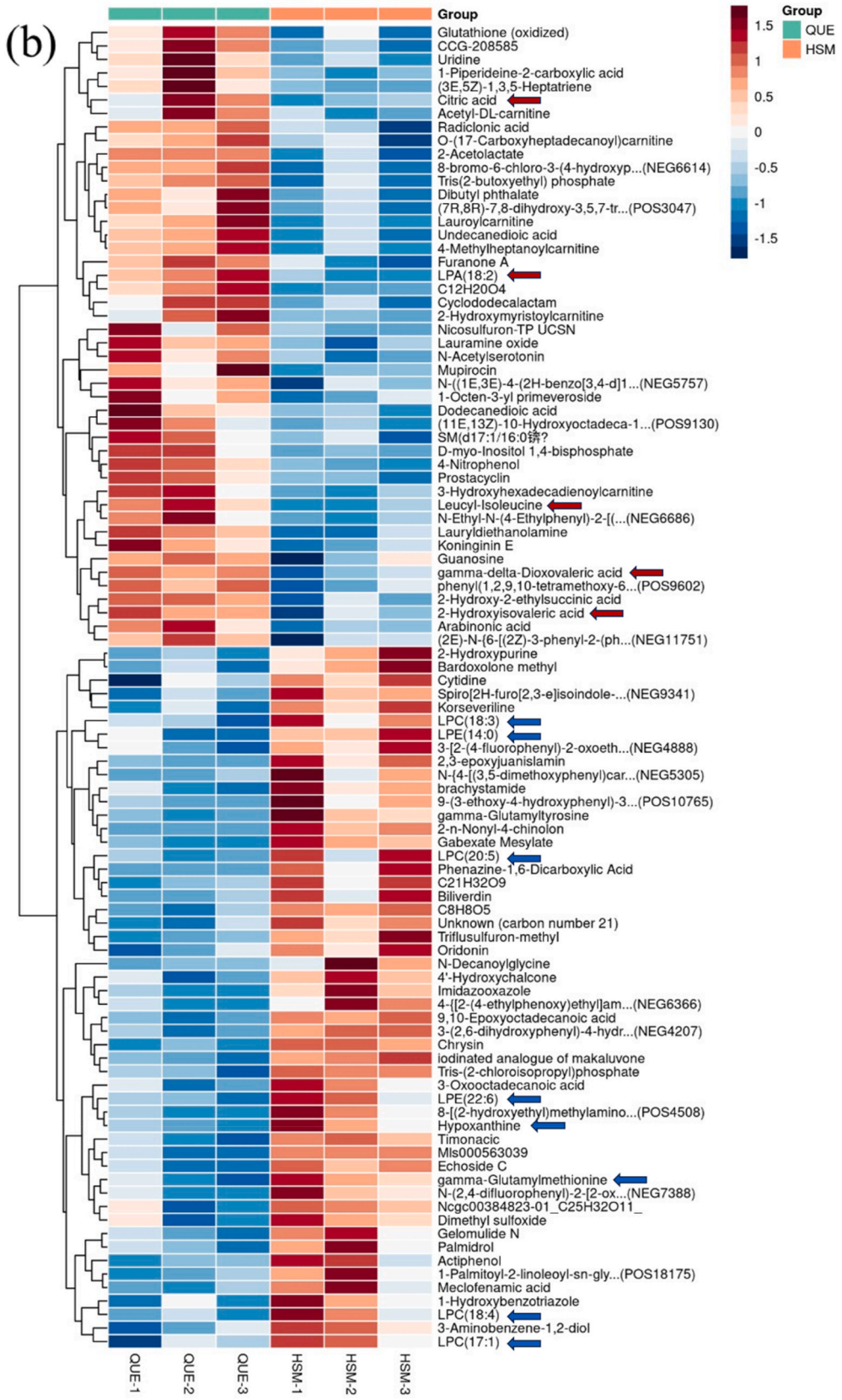

Fig. 8. (*continued*).

that HSBMD reduced the proportion of Firmicutes. Similarly to this study, Liu et al. [83] reported that with the increase in dietary soybean meal, the proportion of Fusobacteria, Proteobacteria and actinobacteria in intestinal microbial composition showed an increasing trend, while the proportion of Firmicutes and Bacteroidetes showed a decreasing trend; Wang et al. [87] reported that replacing fish meal with soy

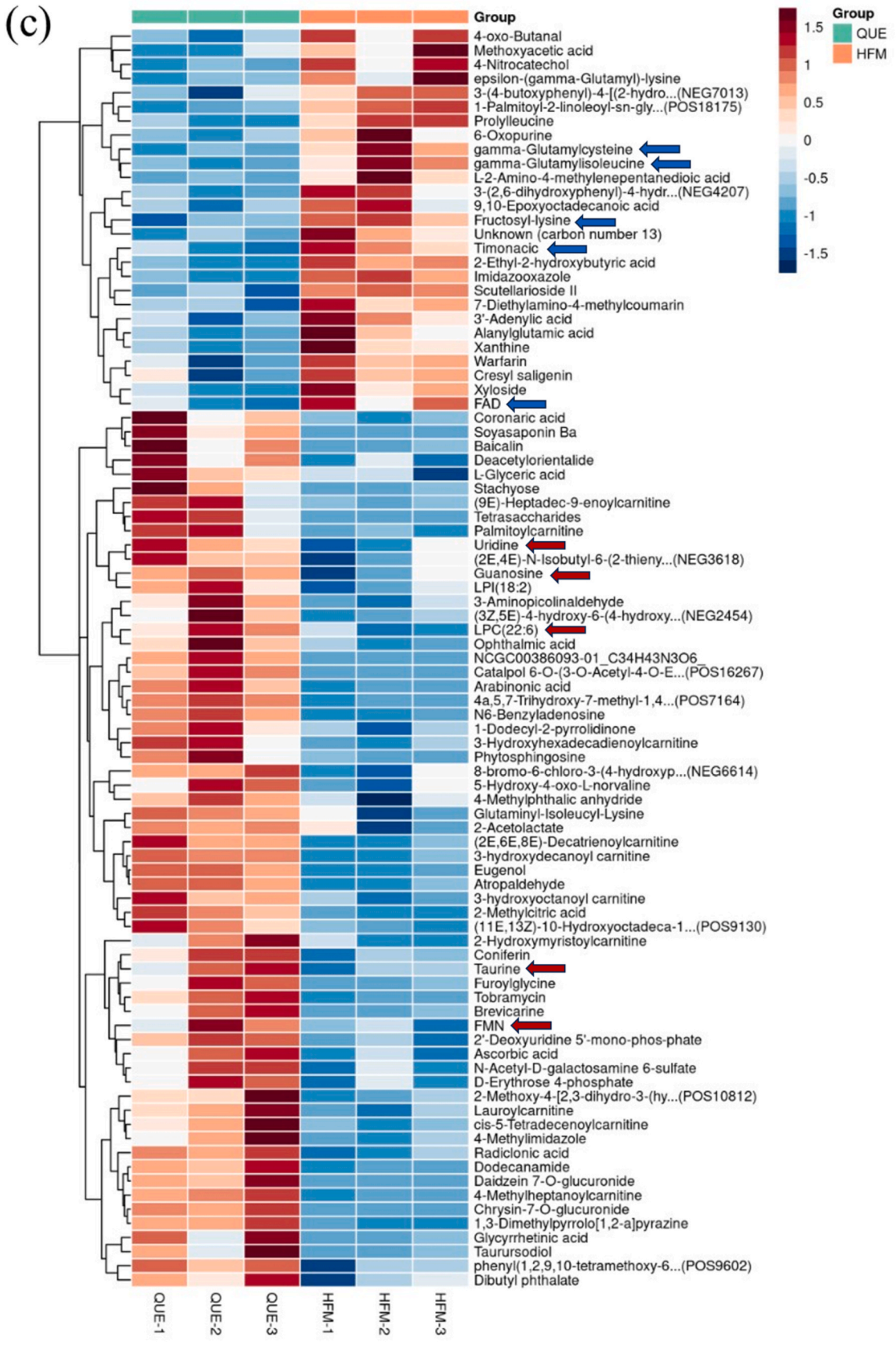

Fig. 8. (*continued*).

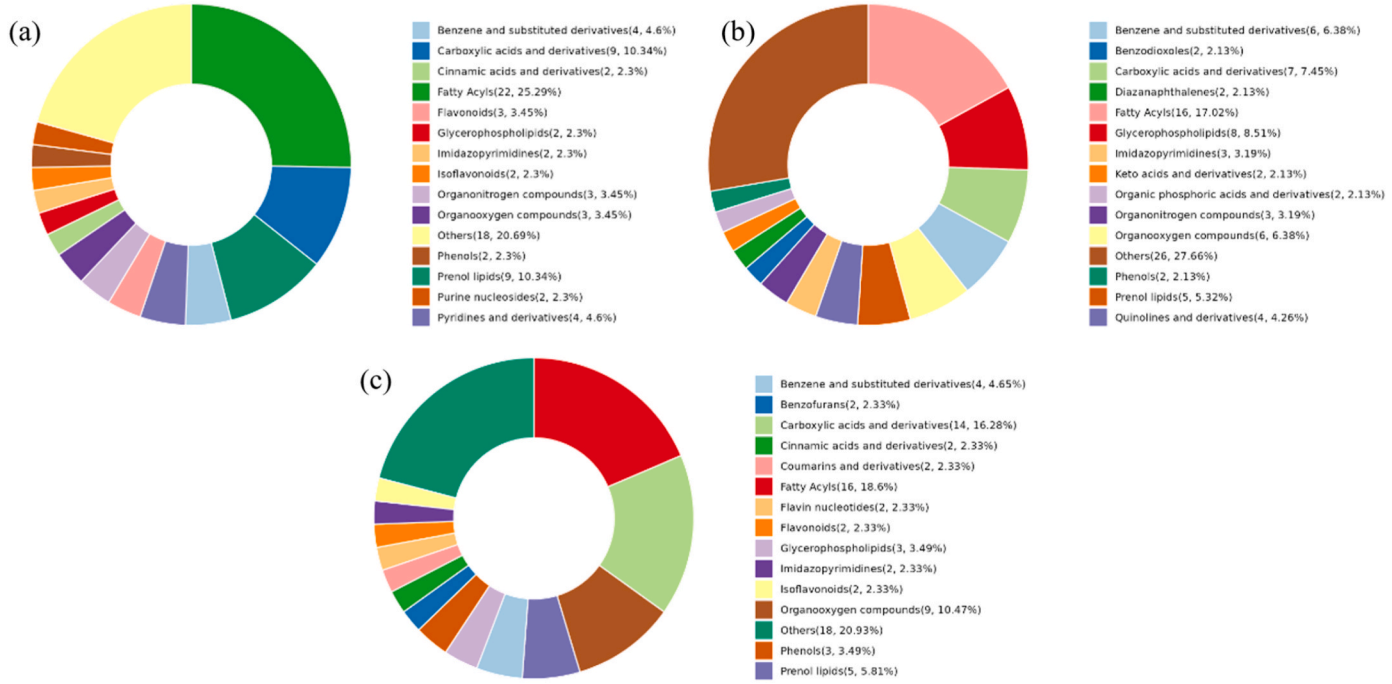

Fig. 9. Differential Metabolite Classification Loop (HMDB Class). Note: Letters in corner marks a to c represent HSM vs HFM (a); QUE vs HSM (b); QUE vs HFM (c). The larger the area, the greater the number of differential metabolites in the classification. The legend indicates the classification name, the number of differential metabolites, and the percentage in the pie chart.

protein isolate could significantly reduce the ratio of Firmicutes/Bacteroidetes. As previously stated, the composition of fish intestinal microbes is influenced by complex factors, so the differences in the above studies may be related to the species of aquatic animals, water environment, feed composition, environmental temperature and other factors. Proteobacteria, as the most abundant phylum in fish microbiota characterization studies, usually dominates the intestinal of marine carnivorous fish [88,89]. In addition, the intestinal microbiota of fish fed on fish meal is thought to be the closest to its native form and is more biased towards Proteobacteria. However, when the content of soybean meal is too high, the original intestinal microbes will decrease or disappear [86]. Therefore, the addition of large amounts of soybean meal to the diet can cause the intestinal microbes of farmed animals to deviate from their original form and produce corresponding changes. However, after QUE was added to this study, it was found that the proportion of Proteobacteria and Chao1, Shannon and Observed increased, but Good's coverage decreased. In this study, the decrease in the proportion of Firmicutes may be caused by the massive addition of soybean meal, while the addition of QUE can increase the proportion of Proteobacteria, making the microflora more similar to that of fish meal. Moreover, the addition of QUE can improve the richness and diversity of intestinal microbes to a certain extent, but its coverage may be reduced. Similarly, Lin et al. [90] reported that QUE can enhance intestinal microbial diversity; Hong et al. [91] also reported that colitis mice on the QUE diet showed higher Alpha diversity. QUE has a certain effect on the composition of intestinal microbes. On the contrary, bacteria also participate in the production and degradation of QUE; for example, the expression of β-glucosidase in bacteria can deglycosylate flavonoids to produce quercetin aglycones [92]; in addition, certain strains (*Clostridium* and *Bacteroides*) are able to split quercetin's C ring and release 3-(3-hydroxyphenyl) propionic acid and 3,4-dihydroxy phenyl acetic acid [93]. There is no doubt that QUE and other natural flavonoids have great potential to study the mechanism of interaction with the intestinal microbes.

Before being absorbed by the intestine, QUE needs to be processed into quercetin glycoside by the digestive fluid or microorganism attached to the intestine. It is then absorbed by intestinal cells in two ways: 1) it is absorbed by transporters and then deglycosylated in intestinal cells by glycosidase; 2) deglycosylation occurs through luminal hydrolases, and then glycoside ligands are transported to intestinal cells by passive diffusion or transporters [94–96]. In addition, further biotransformation of quercetin aglycones involves glucosylation, sulfation and methylation of hydroxyl groups [97]. In this study, many hydroxylated and methylated metabolites were found in the QUE addition group, such as: 2-Hydroxyisovaleric acid, 2-hydroxy-2-ethylsuccinic acid, 2-methylcitric acid and 4-methylphthalic anhydride; these metabolites may be the products of quercetin metabolized by gut microbes; however, no large amount of glucosylated and methylated quercetin was found in quercetin supplementation group. Therefore, the author speculated that on the one hand, quercetin may be used as a feed additive, and the main way that the small amount of quercetin plays its role is to regulate the composition of intestinal microbes and be absorbed by intestinal cells to further enter the blood and transport to the whole body; on the other hand, it may also be due to fasting for 24 h before sample collection, resulting in the complete metabolism of the remaining quercetin glycoside metabolites in the gut of spotted sea bass and their absorption and utilization by intestinal cells. The results of this study showed that a large amount of soybean meal led to the down-regulation of amino acids, their derivatives and metabolites; this may be due to the fact that the amino acid composition of soybean meal is not as balanced as that of fish meal, and the absorption and utilization efficiency of fish are not high. The addition of QUE upregulates these metabolites, but it is still downregulated compared to the whole fish meal diet. These results indicated that QUE could promote the absorption and utilization of amino acids in soybean meal. In addition, four lysophosphatidylcholines (LPC) and two lysophosphatidyl ethanolamines (LPE) showed down-regulation of metabolism after adding QUE. This suggests that QUE may also have the potential to regulate lipid metabolism. On the other hand, the main up-regulated differential metabolite in the HFM vs HSM comparison group was AMP, and the main down-regulated metabolite was tyrosine. The foxO signaling pathway, mTOR signaling pathway and PI3K-Akt signaling pathway were the main metabolic

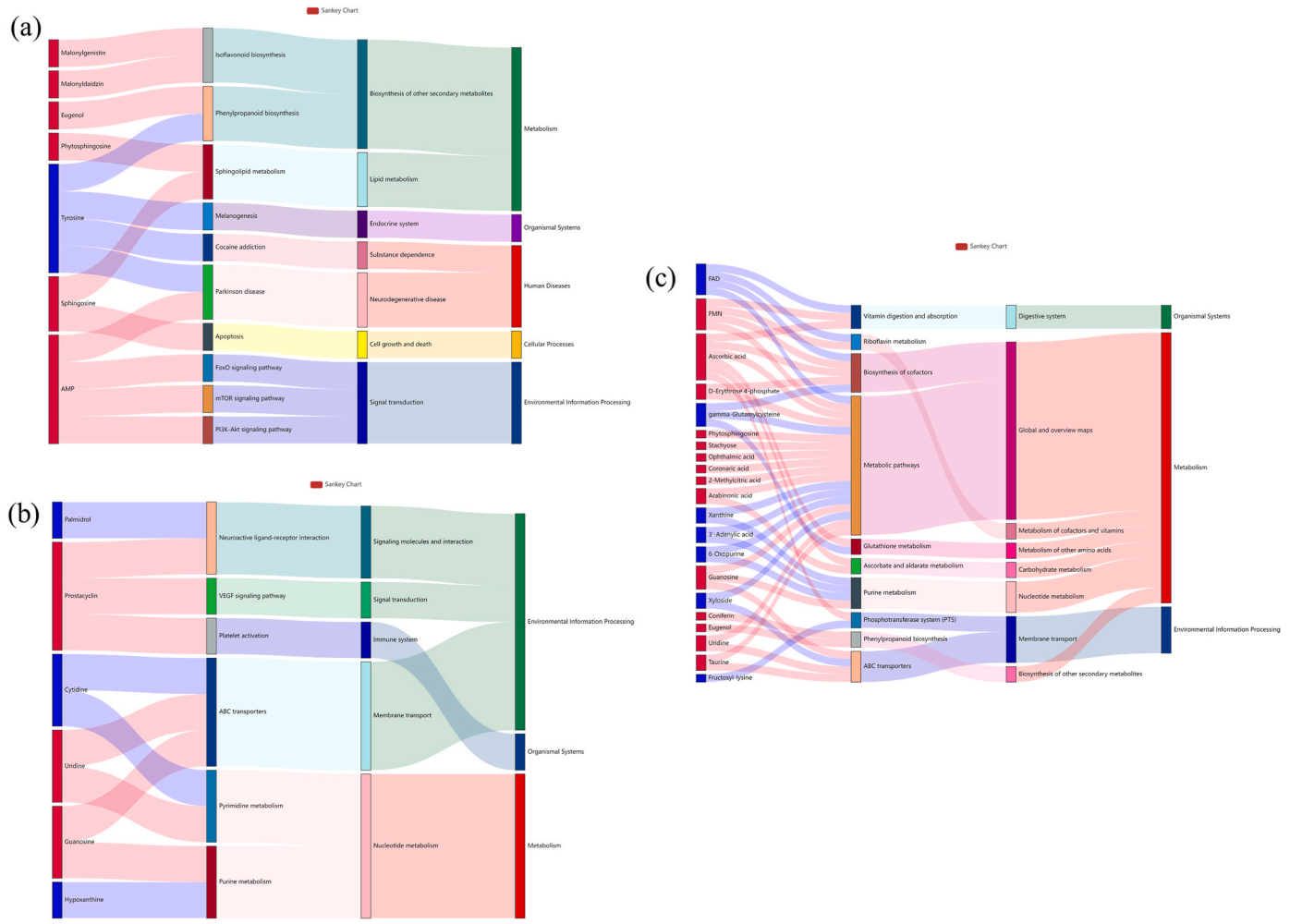

Fig. 10. The top10 pathways of enrichment significance and the Sankey map of differential metabolites in each comparison group. Note: Letters in corner marks a to c represent HSM vs HFM (a); QUE vs HSM (b); QUE vs HFM (c). The first column on the left represents the differential metabolites of up & down, and the level of the box indicates the data flow, that is, the number of metabolites annotated to the pathway. The more corresponding pathways, the higher the box; The red flow line indicates the up-regulated (up) metabolite flow, and the blue flow line indicates the down-regulated (down) metabolite flow. The second, third, and fourth columns represent significantly enriched ($p < 0.05$) metabolic pathways, the second layer KEGG pathway category, and the top KEGG pathway category, respectively.

pathways. AMP can activate AMP-activated protein kinase (AMPK) in the above pathways, and play an important role in a series of reactions [98]. Once the content of AMP is reduced, the activated AMKP is also reduced, and the expression of the above pathway is also blocked. Thus, HSBMD may adversely affect farmed animals by affecting the body's energy metabolism and inhibiting several important expressions. The upregulated metabolites in HSM vs QUE group were mainly prostacyclin, uridine and guanosine. The main down-regulated metabolite was cytidine; the primary transporters pathway is ABC transporters. This suggests that QUE may interfere with the body's energy metabolism and promote the utilization of QUE by intestinal cells by promoting ABC Transporters regulation, thus alleviating the injury of soybean meal to the body. Interestingly, this study also found that QUE also seems to promote the metabolism of intestinal cells or their attached microorganisms to produce ascorbic acid and taurine. Studies have shown that the above two substances are beneficial to the body of farmed fish [99, 100].

In this study, QUE has the ability to alleviate the negative effects of soybean meal on spotted sea bass. Therefore, it is suggested to promote its application in the aquaculture industry, providing more options for the safe application of aquaculture, and looking forward to providing a theoretical basis for the sustainable feed program of replacing fish meal with renewable plant protein. In addition, it is not clear whether QUE can combine with soybean meal fiber to promote the availability of QUE in the body, which is obviously a topic worthy of further study. At the same time, further studies on the short-chain fatty acid metabolism of intestinal microbes, targeted metabolism of intestinal microbes and quantitative analysis of intestinal microbes can be conducted to further study the interaction relationship and mechanism of QUE, intestinal microbes and intestines. In addition, the structural modification of QUE can greatly improve its bioavailability. Therefore, the exploration of the synthesis of QUE derivatives and their structure-activity relationship can accelerate the development and use of QUE. On the other hand, the negative effects of QUE have rarely been reported, especially in the field of practical farming applications. These potential negative effects not only affect the optimal use of QUE, but also limit the practical application of QUE.

5. Conclusion

In conclusion, quercetin can improve the body's immunity, reduce intestinal mucosal permeability, increase the proportion of Proteobacteria, and increase the species richness and diversity of intestinal microorganisms to a certain extent, thus alleviating the intestinal damage caused by the high content of soybean meal, but the growth performance has not changed significantly. On the other hand, QUE may promote the

absorption and utilization of QUE by intestinal cells by up-regulating the metabolism of amino acids and their derivatives and energy-related metabolites such as uridine and guanosine, and by regulating transporters via ABC transporters. These results revealed the potential of QUE in alleviating intestinal damage caused by high soybean meal, and provided a theoretical basis for alleviating fishmeal shortage and realizing sustainable green and healthy culture.

Ethics approval and consent to participate

This experiment was approved by the Animal Ethics Committee of Jimei University (Grant No. JMU202103009).

Availability of data and materials

The analyzed data from this study are available from the corresponding author on request.

Consent for publication

Not applicable.

Funding

This experiment was funded by Science and Technology Planning Project in Fujian, China (Grant No. 2015N0010) and Science and Technology Planning Project in Xiamen, China (Grant No. 3502Z20143017).

CRediT authorship contribution statement

Longhui Liu: conceived and designed the experiments, performed the experiments, participated in the sample collection, Formal analysis, analyzed the data, wrote the paper, and prepared figures and tables. **Yanbo Zhao:** participated in the sample collection. **Zhangfan Huang:** performed the experiments, participated in the sample collection. **Zhongying Long:** participated in the sample collection. **Huihui Qin:** participated in the sample collection. **Hao Lin:** participated in the sample collection. **Sishun Zhou:** participated in the sample collection. **Lumin Kong:** participated in the sample collection. **Jianrong Ma:** participated in the sample collection. **Yi Lin:** Methodology. **Zhongbao Li:** conceived and designed the experiments, reviewed drafts of the paper, All authors discussed the result together, All authors have read and approved this version of the article, and due care has been taken to ensure the integrity of the work.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgements

We greatly appreciate Shanghai bioprofile Technology Co.,Ltd. (Shanghai, China) for their help with data analysis. Thanks to Weihao Wang, Min Liu, Xiuting Wu, Jinsen Kang, Zhenghao Huang and Ying Pan for their help with this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2024.109607.

References

[1] A. Stankus, State of world aquaculture 2020 and regional reviews: fao webinar series, FAO Aquaculture Newsletter 63 (2021) 17–18.
[2] C. Bene, M. Barange, R. Subasinghe, P. Pinstrup-Andersen, G. Merino, G.I. Hemre, et al., Feeding 9 billion by 2050-putting fish back on the menu, Food Secur. 7 (2) (2015) 261–274, https://doi.org/10.1007/s12571-015-0427-z.
[3] J.W. Hertrampf, F. Piedad-Pascual, Handbook on Ingredients for Aquaculture Feeds, Springer Science & Business Media, 2012.
[4] R.L. Naylor, R.W. Hardy, A.H. Buschmann, S.R. Bush, L. Cao, D.H. Klinger, et al., A 20-year retrospective review of global aquaculture, NATURE 591 (7851) (2021) 551, https://doi.org/10.1038/s41586-021-03308-6.
[5] K. Fehrmann-Cartes, M. Coronado, A.J. Hernández, M.L. Allende, C.G. Feijoo, Anti-inflammatory effects of aloe vera on soy meal-induced intestinal inflammation in zebrafish, Fish Shellfish Immunol. 95 (2019) 564–573, https://doi.org/10.1016/j.fsi.2019.10.075.
[6] M.D. Hernández, F.J. Martínez, M. Jover, B. García García, Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (diplodus puntazzo) diet, Aquaculture 263 (1) (2007) 159–167, https://doi.org/10.1016/j.aquaculture.2006.07.040.
[7] M. Peng, W. Xu, Q. Ai, K. Mai, Z. Liufu, K. Zhang, Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (scophthalmus maximus l.), Aquaculture 392–395 (2013) 51–58, https://doi.org/10.1016/j.aquaculture.2013.02.002.
[8] D.M. Gatlin, F.T. Barrows, P. Brown, K. Dabrowski, T.G. Gaylord, R.W. Hardy, et al., Expanding the utilization of sustainable plant products in aquafeeds:: a review, Aquacult. Res. 38 (6) (2007) 551–579, https://doi.org/10.1111/j.1365-2109.2007.01704.x.
[9] I.E. Liener, Implications of antinutritional components in soybean foods, Crit. Rev. Food Sci. Nutr. 34 (1) (1994) 31–67, https://doi.org/10.1080/10408399409527649.
[10] H. Ye, M. Xu, Q. Liu, Z. Sun, C. Zou, L. Chen, et al., Effects of replacing fish meal with soybean meal on growth performance, feed utilization and physiological status of juvenile obscure puffer, takifugu obscurus, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 216 (2019) 75–81, https://doi.org/10.1016/j.cbpc.2018.11.006.
[11] O.H. Romarheim, A. Skrede, M. Penn, L.T. Mydland, Å. Krogdahl, T. Storebakken, Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (oncorhynchus mykiss) fed diets containing defatted soybean meal, Aquaculture 274 (2) (2008) 329–338, https://doi.org/10.1016/j.aquaculture.2007.11.035.
[12] M. Gu, N. Bai, Y. Zhang, Å. Krogdahl, Soybean meal induces enteritis in turbot scophthalmus maximus at high supplementation levels, Aquaculture 464 (2016) 286–295, https://doi.org/10.1016/j.aquaculture.2016.06.035.
[13] W. Chen, Q. Ai, K. Mai, W. Xu, Z. Liufu, W. Zhang, et al., Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (paralichthys olivaceus), Aquaculture 318 (1) (2011) 95–100, https://doi.org/10.1016/j.aquaculture.2011.04.050.
[14] L.E. Ke, Y.M. Qin, T. Song, K. Wang, J.D. Ye, Dietary sodium butyrate administration alleviates high soybean meal-induced growth retardation and enteritis of orange-spotted groupers (*epinephelus coioides*), Front. Mar. Sci. 9 (2022), https://doi.org/10.3389/fmars.2022.1029397.
[15] Y. Wang, X.Y. Jia, Z.X. Guo, L. Li, T.Y. Liu, P.Y. Zhang, et al., Effect of dietary soybean saponin bb on the growth performance, intestinal nutrient absorption, morphology, microbiota, and immune response in juvenile Chinese soft-shelled turtle (*pelodiscus sinensis*), Front. Immunol. 13 (2022), https://doi.org/10.3389/fimmu.2022.1093567.
[16] W. Zhang, B.P. Tan, J.M. Deng, Z. Haitao, Multiomics analysis of soybean meal induced marine fish enteritis in juvenile pearl gentian grouper, *epinephelus fuscoguttatus* ♀ x *epinephelus lanceolatus*, Sci. Rep. 11 (1) (2021), https://doi.org/10.1038/s41598-021-02278-z.
[17] Y. Guo, R.S. Bruno, Endogenous and exogenous mediators of quercetin bioavailability, J. Nutr. Biochem. 26 (3) (2015) 201–210, https://doi.org/10.1016/j.jnutbio.2014.10.008.
[18] M.E. Rubio-Ruiz, V. Guarner-Lans, A. Cano-Martínez, E. Díaz-Díaz, L. Manzano-Pech, A. Gamas-Magaña, et al., Resveratrol and quercetin administration improves antioxidant defenses and reduces fatty liver in metabolic syndrome rats, Molecules No 24 (2019).
[19] E. Middleton, C. Kandaswami, T.C. Theoharides, The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer, Pharmacol. Rev. 52 (4) (2000) 673–751.
[20] P. Shen, W.J. Lin, X. Deng, X. Ba, L. Han, Z. Chen, et al., Potential implications of quercetin in autoimmune diseases, Front. Immunol. 12 (2021), https://doi.org/10.3389/fimmu.2021.689044.
[21] M.Z. Liu, Q. Yu, H.H. Xiao, M.M. Li, Y.M. Huang, Q. Zhang, et al., The inhibitory activities and antiviral mechanism of medicinal plant ingredient quercetin against grouper iridovirus infection, Front. Microbiol. 11 (2020), https://doi.org/10.3389/fmicb.2020.586331.
[22] T. Nguyen, D. Bhattacharya, Antimicrobial activity of quercetin: an approach to its mechanistic principle, Molecules 27 (8) (2022), https://doi.org/10.3390/molecules27082494.

[23] L. Yan, M. Vaghari-Tabari, F. Malakoti, S. Moein, D. Qujeq, B. Yousefi, et al., Quercetin: an effective polyphenol in alleviating diabetes and diabetic complications, Crit. Rev. Food Sci. Nutr. (2022), https://doi.org/10.1080/10408398.2022.2067825.

[24] Y. Guo, R.S. Bruno, Endogenous and exogenous mediators of quercetin bioavailability, J. Nutr. Biochem. 26 (3) (2015) 201–210, https://doi.org/10.1016/j.jnutbio.2014.10.008.

[25] A. Trakooncharoenvit, S. Tanaka, E. Mizuta, T. Hira, H. Hara, Water-soluble dietary fibers enhance bioavailability of quercetin and a fiber derived from soybean is most effective after long-term feeding in rats, Eur. J. Nutr. 59 (4) (2020) 1389–1398, https://doi.org/10.1007/s00394-019-01992-9.

[26] Y. Jia, X. Yan, Y. Huang, H. Zhu, B. Qi, Y. Li, Different interactions driving the binding of soy proteins (7s/11s) and flavonoids (quercetin/rutin): alterations in the conformational and functional properties of soy proteins, Food Chem. 396 (2022) 133685, https://doi.org/10.1016/j.foodchem.2022.133685.

[27] Chinese Fisheries Bureau of Ministry of Agriculture, China Fishery Statistical Yearbook, Beijing, 2022 (in Chinese).

[28] C.X. Zhang, S. Rahimnejad, Y.R. Wang, K.L. Lu, K. Song, L. Wang, et al., Substituting fish meal with soybean meal in diets for Japanese seabass (*lateolabrax japonicus*): effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes, Aquaculture 483 (2018) 173–182, https://doi.org/10.1016/j.aquaculture.2017.10.029.

[29] Q.H. Ai, K.S. Mai, H.T. Li, C.X. Zhang, L. Zhang, Q.Y. Duan, et al., Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, *lateolabrax japonicus*, Aquaculture 230 (1–4) (2004) 507–516, https://doi.org/10.1016/j.aquaculture.2003.09.040.

[30] A.C. Holloway, J.L. Keene, D.G. Noakes, R.D. Moccia, Effects of clove oil and ms-222 on blood hormone profiles in rainbow trout *oncorhynchus mykiss*, walbaum, Aquacult. Res. 35 (11) (2004) 1025–1030, https://doi.org/10.1111/j.1365-2109.2004.01108.x.

[31] AOAC (Asociation of Official Analytical Chemists), Official Methods of Analysis of Official Analytical Chemists International, seventeenth ed., Association of Official Analytical Chemists, Arlington, VA, USA, 2002.

[32] L.H. Liu, Y.B. Zhao, Z.F. Huang, Z.Y. Long, H.H. Qin, H. Lin, et al., Effects of *Lycium barbarum* polysaccharides supplemented to high soybean meal diet on immunity and hepatic health of spotted sea bass *Lateolabrax maculatus*, Front. Immunol. 15 (2024).

[33] M. Powell, M. Smith, The determination of serum acid and alkaline phosphatase activity with 4-AMINOANTIPYRINE (AAP), J. Clin. Pathol. 7 (1954) 245–248.

[34] S. Dupre, S.P. Solinas, P. Guerrieri, G. Federici, D. Cavallini, Nadh-coupled spectrophotometric assay of diamine oxidase, Anal. Biochem. 77 (1) (1977) 68–72, https://doi.org/10.1016/0003-2697(77)90290-1.

[35] L.Q. Zhu, L. Zhang, J. Zhang, G.L. Chang, G. Liu, D.D. Yu, et al., Evodiamine inhibits high-fat diet-induced colitis-associated cancer in mice through regulating the gut microbiota, JOURNAL OF INTEGRATIVE MEDICINE-JIM 19 (1) (2021) 56–65, https://doi.org/10.1016/j.joim.2020.11.001.

[36] B.T. Doumas, W.A. Watson, H.G. Biggs, Albumin standards and measurement of serum albumin with bromocresol green, Clin. Chim. Acta 31 (1) (1971) 87, https://doi.org/10.1016/0009-8981(71)90365-2.

[37] S.S. Zhou, Z.F. Huang, H. Lin, L.M. Kong, J.R. Ma, Z.Y. Long, et al., Effects of mulberry leaf extract on the intestinal health of spotted sea bass (*lateolabrax maculatus*), Front. Mar. Sci. 10 (2023), https://doi.org/10.3389/fmars.2023.1185795.

[38] A. Chao, Nonparametric-estimation of the number of classes in a population, Scand. J. Stat. 11 (4) (1984) 265–270.

[39] C.E. Shannon, A mathematical theory of communication, BELL SYSTEM TECHNICAL JOURNAL 27 (3) (1948) 379–423, https://doi.org/10.1002/j.1538-7305.1948.tb01338.x.

[40] C.E. Shannon, A mathematical theory of communication, BELL SYSTEM TECHNICAL JOURNAL 27 (4) (1948) 623–656, https://doi.org/10.1002/j.1538-7305.1948.tb00917.x.

[41] D.P. Faith, Conservation evaluation and phylogenetic diversity, Biol. Conserv. 61 (1) (1992) 1–10, https://doi.org/10.1016/0006-3207(92)91201-3.

[42] E.C. Pielou, Measurement of diversity in different types of biological collections, J. Theor. Biol. 13 (DEC) (1966) 131, https://doi.org/10.1016/0022-5193(66)90013-0.

[43] Y.Q. Zhang, W.X. Ji, Y.B. Wu, H. Han, J.G. Qin, Y. Wang, Replacement of dietary fish meal by soybean meal supplemented with crystalline methionine for Japanese seabass (*lateolabrax japonicus*), Aquacult. Res. 47 (1) (2016) 243–252, https://doi.org/10.1111/are.12486.

[44] R.L. Chou, B.Y. Her, M.S. Su, G. Hwang, Y.H. Wu, H.Y. Chen, Substituting fish meal with soybean meal in diets of juvenile cobia *rachycentron canadum*, Aquaculture 229 (1–4) (2004) 325–333, https://doi.org/10.1016/S0044-8486(03)00395-8.

[45] Z. Ma, M.M. Hassan, L. Allais, T. He, S. Leterme, A. Ellis, et al., Comparison of partial replacement of fishmeal with soybean meal and enzomeal on growth performance of asian seabass lates calcarifer, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 216 (2019) 29–37, https://doi.org/10.1016/j.cbpc.2018.10.006.

[46] S.M. Lin, L. Luo, Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *oreochromis niloticus* x *o. aureus*, Anim. Feed Sci. Technol. 168 (1–2) (2011) 80–87, https://doi.org/10.1016/j.anifeedsci.2011.03.012.

[47] S.W. Zhai, S.L. Liu, Effects of dietary quercetin on the growth performance, digestive enzymes and antioxidant potential in the hepatopancreas of tilapia (*oreochromis niloticus*), Isr. J. Aquac. Bamidgeh 66 (2014).

[48] Z. Xu, X.Q. Li, H. Yang, G.Y. Liang, B.W. Gao, X.J. Leng, Dietary quercetin improved the growth, antioxidation, and flesh quality of grass carp (*ctenopharyngodon idella*), J. World Aquacult. Soc. 50 (6) (2019) 1182–1195, https://doi.org/10.1111/jwas.12663.

[49] K. Armobin, E. Ahmadifar, H. Adineh, M.N. Samani, N. Kalhor, S. Yilmaz, et al., Quercetin application for common carp (*cyprinus carpio*): i. Effects on growth performance, humoral immunity, antioxidant status, immune-related genes, and resistance against heat stress, Aquacult. Nutr. 2023 (2023), https://doi.org/10.1155/2023/1168262.

[50] Y.X. Kong, J.X. Tian, X.T. Niu, M. Li, Y.D. Kong, R.M. Li, et al., Effects of dietary quercetin on growth, antioxidant capacity, immune response and immune-related gene expression in snakehead fish, *channa argus*, AQUACULTURE REPORTS 26 (2022), https://doi.org/10.1016/j.aqrep.2022.101314.

[51] H. Boshra, J. Li, J.O. Sunyer, Recent advances on the complement system of teleost fish, FISH SHELLFISH IMMUN 20 (2006) 239–262.

[52] D.F. Sesay, H. Habte-Tsion, Q. Zhou, M. Ren, J. Xie, B. Liu, et al., The effect of dietary folic acid on biochemical parameters and gene expression of three heat shock proteins (HSPs) of blunt snout bream (Megalobrama amblycephala) fingerling under acute high temperature stress, Fish Physiol. Biochem. 43 (2017) 923–940.

[53] Y. Xu, Y. Yu, X. Zhang, Z. Huang, H. Li, S. Dong, et al., Molecular characterization and expression analysis of complement component 3 in dojo loach (Misgurnus anguillicaudatus), FISH SHELLFISH IMMUN 72 (2018) 484–493.

[54] C. Uribe, H. Folch, R. Enriquez, G. Moran, Innate and adaptive immunity in teleost fish: a review, VET MED-CZECH. 56 (2011) 486–503.

[55] Q.J. Mu, Z.R. Dong, W.G. Kong, X.Y. Wang, J.Q. Yu, W. Ji, et al., Response of immunoglobulin M in gut mucosal immunity of common carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*, Front. Immunol. 13 (2022).

[56] X. Zhu, R. Hao, J. Zhang, C. Tian, Y. Hong, C. Zhu, et al., Dietary astaxanthin improves the antioxidant capacity, immunity and disease resistance of coral trout (Plectropomus leopardus), FISH SHELLFISH IMMUN 122 (2022) 38–47.

[57] X.R. Liu, B. Han, J. Xu, et al., Replacement of fishmeal with soybean meal affects the growth performance, digestive enzymes, intestinal microbiota and immunity of Carassius auratus gibelio female x Cyprinus carpio male, Aquaculture Reports 18 (2020).

[58] Z. Zhou, E. Ringo, R.E. Olsen, et al., Dietary effects of soybean products on gut microbiota and immunity of aquatic animals: a review, Aquacult. Nutr. 24 (1) (2018) 644–665.

[59] K. Armobin, E. Ahmadifar, H. Adineh, M.N. Samani, N. Kalhor, S. Yilmaz, et al., Quercetin application for common carp (*Cyprinus carpio*): I. Effects on growth performance, humoral immunity. Antioxidant Status, Immune-Related Genes, and Resistance against Heat Stress, AQUACULT NUTR. 2023, 2023 1168262.

[60] Y. Kong, J. Tian, X. Niu, M. Li, Y. Kong, R. Li, et al., Effects of dietary quercetin on growth, antioxidant capacity, immune response and immune-related gene expression in snakehead fish, Channa argus, AQUACULT REP 26 (2022) 101314.

[61] J. Wang, C. Zhang, J. Zhang, J. Xie, L. Yang, Y. Xing, et al., The effects of quercetin on immunity, antioxidant indices, and disease resistance in zebrafish (Danio rerio), Fish Physiol. Biochem. 46 (2020) 759–770.

[62] T. Khan, M. Fatima, S.Z.H. Shah, N. Khan, Ali W. Maryam, et al., Quercetin supplementation in the diet of Labeo rohita: effects on growth, proximate composition, antioxidative indices and immunity, ANIM FEED SCI TECH 303 (2023) 115699.

[63] Z. Xu, X. Li, H. Yang, G. Liang, B. Gao, X. Leng, Dietary quercetin improved the growth, antioxidation, and flesh quality of grass carp (Ctenopharyngodon idella), J. World Aquacult. Soc. 50 (2019) 1182–1195.

[64] Z.W. Sun, X.D. Wang, R. Andersson, Role of intestinal permeability in monitoring mucosal barrier function - history, methodology, and significance of pathophysiology, Dig. Surg. 15 (5) (1998) 386–397, https://doi.org/10.1159/000018651.

[65] G.D. Luk, T.M. Bayless, S.B. Baylin, Diamine oxidase (histaminase) - a circulating marker for rat intestinal mucosal maturation and integrity, J. Clin. Invest. 66 (1) (1980) 66–70, https://doi.org/10.1172/JCI109836.

[66] A. Vella, G. Farrugia, D-lactic acidosis: pathologic consequence of saprophytism, Mayo Clin. Proc. 73 (5) (1998) 451–456, https://doi.org/10.1016/S0025-6196(11)63729-4.

[67] Å. Krogdahl, M. Penn, J. Thorsen, S. Refstie, A.M. Bakke, Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids, Aquacult. Res. 41 (3) (2010) 333–344, https://doi.org/10.1111/j.1365-2109.2009.02426.x.

[68] D. Knudsen, O. Ron, G. Baardsen, J. Smedsgaard, W. Koppe, H. Froklær, Soyasaponins resist extrusion cooking and are not degraded during gut passage in atlantic salmon (*salmo salar* l.), J. Agric. Food Chem. 54 (17) (2006) 6428–6435, https://doi.org/10.1021/jf0604992.

[69] D. Knudsen, P. Urán, A. Arnous, W. Koppe, H. Frokiær, Saponin-containing subfractions of soybean molasses induce enteritis in the distal intestine of atlantic salmon, J. Agric. Food Chem. 55 (6) (2007) 2261–2267, https://doi.org/10.1021/jf0626967.

[70] Y. Dong, Q. Hou, J. Lei, P.G. Wolf, H. Ayansola, B. Zhang, Quercetin alleviates intestinal oxidative damage induced by h2o2 via modulation of gsh: in vitro screening and in vivo evaluation in a colitis model of mice, ACS Omega 5 (14) (2020) 8334–8346, https://doi.org/10.1021/acsomega.0c00804.

[71] T. Suzuki, H. Hara, Role of flavonoids in intestinal tight junction regulation, J. Nutr. Biochem. 22 (5) (2011) 401–408, https://doi.org/10.1016/j.jnutbio.2010.08.001.

[72] S. Damiano, A. Sasso, B. De Felice, I. Di Gregorio, G. La Rosa, G.A. Lupoli, et al., Quercetin increases muc2 and muc5ac gene expression and secretion in intestinal

goblet cell-like ls174t via plc/pkcα/erk1-2 pathway, Front. Physiol. 9 (2018), https://doi.org/10.3389/fphys.2018.00357.
[73] S. Riemschneider, M. Hoffmann, U. Slanina, K. Weber, S. Hauschildt, J. Lehmann, Indol-3-carbinol and quercetin ameliorate chronic dss-induced colitis in c57bl/6 mice by ahr-mediated anti-inflammatory mechanisms, Int. J. Environ. Res. Publ. Health 18 (5) (2021), https://doi.org/10.3390/ijerph18052262.
[74] G.D. Gómez, J.L. Bálcazar, A review on the interactions between gut microbiota and innate immunity of fish, FEMS Immunol. Med. Microbiol. 52 (2) (2008) 145–154, https://doi.org/10.1111/j.1574-695X.2007.00343.x.
[75] K.E. Sullam, S.D. Essinger, C.A. Lozupone, M.P. O'Connor, G.L. Rosen, R. Knight, et al., Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis, Mol. Ecol. 21 (13) (2012) 3363–3378, https://doi.org/10.1111/j.1365-294X.2012.05552.x.
[76] L. Bruni, R. Pastorelli, C. Viti, L. Gasco, G. Parisi, 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 (2018) 56–63, https://doi.org/10.1016/j.aquaculture.2018.01.006.
[77] S. Refstie, O.J. Korsoen, T. Storebakken, G. Baeverfjord, I. Lein, A.J. Roem, Differing nutritional responses to dietary soybean meal in rainbow trout (*oncorhynchus mykiss*) and atlantic salmon (*salmo salar*), Aquaculture 190 (1–2) (2000) 49–63, https://doi.org/10.1016/S0044-8486(00)00382-3.
[78] C. Neuman, E. Hatje, K.Z. Zarkasi, R. Smullen, J.P. Bowman, M. Katouli, The effect of diet and environmental temperature on the faecal microbiota of farmed tasmanian atlantic salmon (*salmo salar* l.), Aquacult. Res. 47 (2) (2016) 660–672, https://doi.org/10.1111/are.12522.
[79] L. Grisez, J. Reyniers, L. Verdonck, J. Swings, F. Ollevier, Dominant intestinal microflora of sea bream and sea bass larvae, from two hatcheries, during larval development, Aquaculture 155 (1–4) (1997) 387–399, https://doi.org/10.1016/S0044-8486(97)00113-0.
[80] J.J. Eichmiller, M.J. Hamilton, C. Staley, M.J. Sadowsky, P.W. Sorensen, Environment shapes the fecal microbiome of invasive carp species, Microbiome 4 (1) (2016) 44, https://doi.org/10.1186/s40168-016-0190-1.
[81] M. Ghanbari, W. Kneifel, K.J. Domig, A new view of the fish gut microbiome: advances from next-generation sequencing, Aquaculture 448 (2015) 464–475, https://doi.org/10.1016/j.aquaculture.2015.06.033.
[82] K.E. Sullam, S.D. Essinger, C.A. Lozupone, M.P. O'Connor, G.L. Rosen, R. Knight, et al., Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis, Mol. Ecol. 21 (13) (2012) 3363–3378, https://doi.org/10.1111/j.1365-294X.2012.05552.x.
[83] X.R. Liu, B. Han, J. Xu, J.Y. Zhu, J.T. Hu, W.L. Wan, et al., Replacement of fishmeal with soybean meal affects the growth performance, digestive enzymes, intestinal microbiota and immunity of carassius auratus gibelio female x cyprinus carpio male, AQUACULTURE REPORTS 18 (2020), https://doi.org/10.1016/j.aqrep.2020.100472.
[84] E. Ringo, Z. Zhou, J. Vecino, S. Wadsworth, J. Romero, Å. Krogdahl, et al., Effect of dietary components on the gut microbiota ofaquatic animals. A never-ending story? Aquacult. Nutr. 22 (2) (2016) 219–282, https://doi.org/10.1111/anu.12346.
[85] C.R. Serra, A. Oliva-Teles, P. Enes, F. Tavares, Gut microbiota dynamics in carnivorous european seabass (dicentrarchus labrax) fed plant-based diets, Sci. Rep. 11 (1) (2021) 447, https://doi.org/10.1038/s41598-020-80138-y.
[86] S.V. Kononova, D.V. Zinchenko, T.A. Muranova, N.A. Belova, A.I. Miroshnikov, Intestinal microbiota of salmonids and its changes upon introduction of soy proteins to fish feed, Aquacult. Int. 27 (2) (2019) 475–496, https://doi.org/10.1007/s10499-019-00341-1.
[87] Z. Wang, L.K. Yang, C.X. Zhang, X.S. Li, L. Wang, K.L. Lu, et al., Substituting dietary fishmeal with soybean meal isolate influences hepatic lipid metabolism and gut microbiota in spotted seabass (*lateolabrax maculatus*), Isr. J. Aquac. Bamidgeh 73 (2021), https://doi.org/10.46989/001c.29803.
[88] S. Egerton, S. Culloty, J. Whooley, C. Stanton, R.P. Ross, The gut microbiota of marine fish, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.00873.
[89] M.C. Piazzon, F. Naya-Catala, P. Simo-Mirabet, A. Picard-Sanchez, F.J. Roig, J. A. Calduch-Giner, et al., Sex, age, and bacteria: how the intestinal microbiota is modulated in a protandrous hermaphrodite fish, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.02512.
[90] R. Lin, M.Y. Piao, Y. Song, Dietary quercetin increases colonic microbial diversity and attenuates colitis severity in *citrobacter rodentium*-infected mice, Front. Microbiol. 10 (2019), https://doi.org/10.3389/fmicb.2019.01092.
[91] Z. Hong, M. Piao, Effect of quercetin monoglycosides on oxidative stress and gut microbiota diversity in mice with dextran sodium sulphate-induced colitis, BioMed Res. Int. 2018 (2018) 8343052, https://doi.org/10.1155/2018/8343052.
[92] P. Gaya, A. Peiroten, J.M. Landete, Expression of a β-glucosidase in bacteria with biotechnological interest confers them the ability to deglycosylate lignans and flavonoids in vegetal foods, Appl. Microbiol. Biotechnol. 104 (11) (2020) 4903–4913, https://doi.org/10.1007/s00253-020-10588-x.
[93] Y.L. Lyu, H.F. Zhou, J. Yang, F.X. Wang, F. Sun, J.Y. Li, Biological activities underlying the therapeutic effect of quercetin on inflammatory bowel disease, Mediat. Inflamm. 2022 (2022), https://doi.org/10.1155/2022/5665778.
[94] A.J. Day, J.M. Gee, M.S. DuPont, I.T. Johnson, G. Williamson, Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter, Biochem. Pharmacol. 65 (7) (2003) 1199–1206, https://doi.org/10.1016/S0006-2952(03)00039-X.
[95] S. Wolffram, M. Blöck, P. Ader, Quercetin-3-glucoside is transported by the glucose carrier sglt1 across the brush border membrane of rat small intestine, J. Nutr. 132 (4) (2002) 630–635, https://doi.org/10.1093/jn/132.4.630.
[96] L. Ziberna, S. Fornasaro, J. Čvorović, F. Tramer, S. Passamonti, Chapter 37 - bioavailability of flavonoids: the role of cell membrane transporters, in: R. R. Watson, V.R. Preedy, S. Zibadi (Eds.), Polyphenols in Human Health and Disease, 2014, pp. 489–511.
[97] M. Lesjak, I. Beara, N. Simin, D. Pintać, T. Majkić, K. Bekvalac, et al., Antioxidant and anti-inflammatory activities of quercetin and its derivatives, J. Funct.Foods 40 (2018) 68–75, https://doi.org/10.1016/j.jff.2017.10.047.
[98] J. Weekes, The Amp-Activated Protein Kinase - Substrate Specificity and Regulation of Reversible Protein Phosphorylation, 1994.
[99] J.H. Blom, K. Dabrowski, Ascorbic acid metabolism in fish: is there a maternal effect on the progeny? Aquaculture 147 (3–4) (1996) 215–224, https://doi.org/10.1016/S0044-8486(96)01409-3.
[100] R.B. Johnson, S.K. Kim, A.M. Watson, F.T. Barrows, E.L. Kroeger, P.M. Nicklason, et al., Effects of dietary taurine supplementation on growth, feed efficiency, and nutrient composition of juvenile sablefish (*anoplopoma fimbria*) fed plant based feeds, Aquaculture 445 (2015) 79–85, https://doi.org/10.1016/j.aquaculture.2015.03.030.