Aquaculture Reports 39 (2024) 102429

Contents lists available at ScienceDirect

Aquaculture Reports

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

High soybean dietary supplementation with quercetin improves antioxidant capacity of spotted sea bass *Lateolabrax maculatus*

Longhui Liu [a,b,1], 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
Antioxidant
Transcriptomics

ABSTRACT

This study aimed to examine the effect of quercetin (QUE) on the antioxidant capacity of spotted sea bass that were fed a high soybean meal diet. 44 % fish meal was used as a healthy control, and soybean meal instead of 50 % fish meal induced a negative reaction. On this basis, 0.25, 0.50, 0.75, and 1.00 g/kg QUE were added, respectively. A total of 540 spotted sea bass were randomly allocated into 6 groups and provided with diets that corresponded to their respective groups for a duration of 56 days. The results showed a significant decrease in the antioxidant capacity of spotted sea bass fed a high soybean meal diet. The inclusion of QUE considerably improved the antioxidant capacity of spotted sea bass. This was evidenced by the notable rise in glutathione (GSH) levels in the intestine and liver, as well as the elevated levels of catalase (CAT) and total antioxidant capacity (T-AOC) in the liver. Furthermore, transcriptome results showed that the S-transferase (GST) gene in the glutathione metabolism pathway was significantly down-regulated by high soybean meal, while with the addition of QUE, noteworthy alterations in genes associated with the PI3K-Akt signaling pathway, Foxo signaling pathway, and AMPK signaling pathway. These signaling pathways are involved in signal transduction, apoptosis, and oxidative stress. On the other hand, genes related to energy metabolism, such as phosphoenolpyruvate carboxykinase (PEPCK), were observed to be upregulated. These pathways could potentially contribute to the antioxidant properties of QUE.

1. Introduction

Aquaculture has been responsible for producing almost 50 % of all aquatic goods consumed by humans in the last 30 years. Fish, in particular, have contributed to at least 15 % of the per capita animal protein intake for over 4.5 billion people globally (Gephart et al., 2020). Aquaculture has always extensively depended on fish meal as a source of high-quality dietary nutrients. This is because fish meal is rich in protein, has a well-balanced amino acid composition, and contains plentiful minerals and vitamins (Macusi et al., 2023). However, the price of fish meal has skyrocketed due to the suspension of supply and strong demand, and aquaculture with fishmeal as the main protein source is not sustainable (Gao et al., 2024). At present, because of its relatively balanced amino acid composition and low price, plant-based protein-soybean meal has been identified as one of the most promising alternatives to fishmeal and has been widely used in production practice (Fehrmann-Cartes et al., 2019). Nevertheless, the utilization of soybean meal as a protein source in aquaculture animals, particularly carnivorous fish, leads to a cascade of adverse reactions. These include heightened permeability of the intestinal mucosa, disruption of intestinal microbial balance, inflammatory response, and alterations in inflammatory factors (Antonopoulou et al., 2017; Chen et al., 2018; Wang et al., 2021). In addition, reduced antioxidant capacity and oxidative stress have also been identified as negative effects of soybean meal on aquaculture animals (Jiang et al., 2018). Prior research has demonstrated that substituting fish meal with soy meal leads to heightened oxidative stress and consequent oxidative harm in various fish species, such as darkbarbel catfish (*Pelteobagrus vachelli*) (Dong et al., 2013), white shrimp (*Litopenaeus vannamei*) (Lin and Mui, 2017), and turbot (*Scophthalmus maximus* L.) (Yu et al., 2021).

Quercetin (QUE) is a natural flavonoid that is widely found in vegetables and fruits (Guo, Bruno, 2015). QUE, a dietary antioxidant, has

* Correspondence to: Fisheries College, Jimei University, Xiamen, Fujian, China.
E-mail address: lizhongbao@jmu.edu.cn (Z. Li).
[1] ORCID iD : 0009–0004-2440–7857

https://doi.org/10.1016/j.aqrep.2024.102429
Received 25 July 2024; Received in revised form 6 September 2024; Accepted 11 October 2024
Available online 14 October 2024

the ability to eliminate oxygen-free radicals, decrease oxidative stress, and stimulate the synthesis of antioxidant enzymes (Kobori et al., 2015). Nevertheless, QUE exhibits limited bioavailability and mostly operates by undergoing conversion into several metabolites (Lesjak et al., 2018). Previous studies have shown that QUE can interact with two soybean proteins and alter their secondary structures in a structure-dependent manner (Jia et al., 2022). Trakooncharoenvit et al. (2020) also reported that soybean fiber can improve the bioavailability of QUE after long-term feeding and even promote the beneficial effect of quercetin in preventing disease. Therefore, quercetin may have the potential to be combined with soybean meal to improve its bioavailability. On the other hand, the increased bioavailability of QUE has correspondingly enhanced its antioxidant and oxidative stress reduction capabilities, thus alleviating the above negative effects caused by soybean meal on aquaculture animals.

Transcriptome analysis offers distinct and advantageous perspectives in the examination of fish physiology and nutrition (Chen et al., 2024; Huang et al., 2022; Liu et al., 2024; Yan et al., 2023). According to Wang et al. (2023), xylanase was found to enhance the intestinal barrier function of nile tilapia (*Oreochromis niloticus*) that were fed with soybean meal. This conclusion was reached through transcriptome analysis. Jin et al. (2023) used transcriptome research to investigate the impact of various dietary fat sources on spotted sea bass (*Lateolabrax maculatus*). Transcriptomic techniques offer novel insights into the examination of alterations in fish under varying nutritional conditions, such as a diet rich in soybean meal or the incorporation of additives. Our previous results showed that QUE had no significant effect on the growth performance of spotted sea bass on a high soybean meal diet (Liu et al., 2024). However, QUE, as a natural antioxidant, may have a beneficial effect on improving the antioxidant capacity of spotted sea bass (*Lateolabrax maculatus*), but this still needs to be proved by research. Spotted sea bass is a typical carnivorous fish. It is rich in nutrition, delicious meat, and strong adaptability, but also widely cultured as an economic fish (Wang et al., 2023). However, the high content of soybean meal in the diet was still associated with a range of negative effects on the spotted sea bass, including reduced antioxidant capacity and oxidative stress in the gut (Peng et al., 2022; Zhang et al., 2018). Therefore, in this study, the effects of quercetin on the antioxidant capacity of spotted sea bass on a high-soybean meal diet were investigated by adding QUE, and combined with transcriptomic analysis, the possibility of combining quercetin with soybean meal to reduce the negative effects of soybean meal on spotted sea bass was discussed.

2. Materials and methods

2.1. Experimental diets

The dietary formula for this study was designed according to the nutritional requirements of spotted sea bass (Ai et al., 2004). 44 % fish meal and 0 % soybean meal were used as healthy controls and labeled as HFM group. After that, according to the research of Zhang et al. (2018), half of the fish meal was replaced with 40 % soybean meal, which induced the negative reaction of the spotted sea bass and was labeled as HSM group. In addition, on the basis of HSM, 0.25, 0.5, 0.75, and 1 g/kg quercetin were added as experimental groups 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 between groups were balanced using flour (Table 1).

The feed raw materials were fully pulverized by a grinder (SGF130, Tianfan Pharmaceutical Machine Factory, Shanghai, China), and each component was thoroughly mixed according to the principle of mixing step by step from small to large. After that, add the fish oil, soybean oil, and water in turn, and stir well again. A granulator (CD4×1TS, South China University of Technology, Guangzhou, China) was used to prepare 2.5 mm particle size from completely mixed feed. After drying the prepared feed in a constant temperature oven at 55°C, it was removed and stored at −20°C for later use.

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: $MnSO_4{\cdot}4\ H_2O$ 50 mg/kg, $MgSO_4{\cdot}H_2O$ 4000 mg/kg, KI 100 mg/kg, $CoCl_2$(1 %) 100 mg/kg, $CuSO_4{\cdot}5\ H_2O$ 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.

[c] Gross energy (MJ/kg) = protein content × 23.6 + lipid content × 39.5 + carbohydrate content × 17.2.

2.2. Experimental fish and feeding management

The culture experiment was carried out on the seawater test grounds of Jimei University. The experimental spotted sea bass was purchased from a commercial fishery in Zhangpu, Fujian Province, China. The experimental fish were stocked into two 1200 L tanks and fed basal feed twice a day for two weeks to acclimate to the experimental facility and culture environment. After the temporary feeding period, the fish were fasted for 24 h, and they were anesthetized with 150 mg/L eugenol (Holloway et al., 2004). In the circulation system, 540 healthy fish with an average weight of 5.65 ± 0.02 g were placed in 18 tanks (80 cm × 45 cm × 45 cm) at a density of 30 fish per tank. The circulating system consists of 18 fish tanks, two large water storage tanks, a circulating water pump, and a thermal circulating heater. In addition, all the fish tanks were connected to ensure that the experimental fish water environment was consistent. The study was divided into 6 groups with 3 replicates per group, and the trial period was 56 days. The corresponding feed of each group was fed twice a day at regular times (8:30 and 17:30) until they were obviously full. After half an hour of feeding, sewage suction and water change were carried out, and the water

change volume was about 30 % of the water volume in the cylinder. The water salinity was maintained between 0.5 and 2.0 ppt, the temperature was controlled at 27.0 ± 0.2°C, the dissolved oxygen was maintained at about 7 mg/L, the pH was maintained at 7.8 and 8.2, and the ammonia nitrogen concentration was maintained below 0.3 mg/L.

2.3. Sample collection

After the end of the feeding period, the experimental fish were fasted and anesthetized using the same method as in 2.2. A total of 11 fish were grabbed at random from each tank, and blood samples were collected using the venous blood sampling method. After standing for 16 h, the blood samples were centrifuged (836×g, 4°C) for 10 minutes to separate serum and stored at −80°C for later use. After that, the fish was humanely dissected, and the intestines and liver were collected. All samples were stored in liquid nitrogen for a short time after collection and then transferred to −80°C for storage. Among them, 5 intestinal samples were used for related enzyme activity detection, 4 intestinal samples were used for transcriptomic analysis, and all liver samples were tested for enzyme activity.

2.4. Analysis of basic dietary components

The contents of crude protein, crude fat, ash, and water in dietary samples were determined by the standard method (AOAC, 2002). Protein was determined by the Kjeldahl nitrogen determination method (N × 6.25). Crude lipid was determined by Soxhlet extraction in ether. The samples were burned in a Muffle furnace at 550℃ for 8 h to determine the ash content. And the samples were dried in a 105°C oven to a constant weight for moisture determination.

2.5. Analysis of antioxidant parameter

Antioxidant related indexes in this study included serum, hepatic, and intestinal glutathione (GSH), catalase (CAT), malondialdehyde (MDA), and total antioxidant capacity (T-AOC). Commercial kits were used to detect the activity of these enzymes. GSH reacted with dithiodinitrobenzoic acid (DTNB) to produce a yellow compound; the activity of GSH was quantitatively determined by colorimetry at 405 nm (Harrap, 1967). The decomposition reaction of H_2O_2 by CAT was terminated immediately when ammonium molybdate was added, and the remaining H_2O_2 and ammonium molybdate formed a yellow substance, which was also determined by the colorimetric method (Hamza and Hadwan, 2020). The determination principle of MDA was that it condensates with thiobarbituric acid (TBA) to form a red product, and the maximum absorption peak was at 532 nm. Therefore, a spectrophotometer (UV-1200, MAPADA, China) was used to determine the absorbance and calculate the MDA activity (Buege and Aust, 1978). Antioxidants inhibit the oxidation of ABTS to green $ABTS_+$, so the total antioxidant capacity of the sample can be calculated by measuring the absorbance of $ABTS_+$ (Re, Pellegrini et al., 1999). All kits were provided by Nanjing Jiancheng Biotechnology Co., Ltd. (Item No.: GSH: A006–2–1, CAT: A007–1–1, MDA: A003–1, T-AOC: A015–2–1).

2.6. Intestinal transcriptome analysis and RT-qPCR

2.6.1. Total RNA extraction and detection

Total RNA was extracted by the TRIzol kit. Integrity was tested using RNA-specific agarose electrophoresis or Agilent 2100 Bioanalyzer.

2.6.2. Library construction and quality control

The mRNA with polyA structure in total RNA was enriched by Oligo (dT) magnetic beads, and the RNA was broken into fragments of about 300 bp in length by ion interruption. Using RNA as a template, 6-base random primers and reverse transcriptase were used to synthesize the first-strand cDNA, and the second-strand cDNA was synthesized using the first-strand cDNA as a template. After the completion of the library construction, PCR amplification was used to enrich the library fragments, and then the library was selected according to the fragment size, the library size was 450 bp. Then, the library was inspected by an Agilent 2100 Bioanalyzer, and the total concentration and effective concentration of the library were detected. Then, according to the effective concentration of the library and the amount of data required by the library, the libraries containing different index sequences (each sample adds a different index and finally distinguishes the data of each sample according to the index) are mixed proportionally. The hybrid library was uniformly diluted to 2 nm, and the single strand library was formed by alkali denaturation.

2.6.3. Sequencing

These libraries were paired-end (PE) sequenced using next-generation sequencing (NGS) based on the Illumina sequencing platform.

2.6.4. Reference transcriptome alignment

The raw data was filtered, and using TopHat2 upgrade HISAT2 (http://ccb.jhu.edu/software/hisat2/index.shtml), the software will be filtered. Reads the comparison to the reference genome. Reference genome details are from Genome Warehouse (GWH) (https://ngdc.cncb.ac.cn/gwh/Assembly/37551/show). According to the comparison results, the expression amount of each gene was calculated.

2.6.5. Differential expression analysis

DESeq was used for differential analysis of gene expression, and the conditions for screening differentially expressed genes were as follows: expression difference multiple $|\log_2\text{FoldChange}| > 1$, with a significant P-value < 0.05.

2.6.6. Functional enrichment analysis of differentially expressed genes

Gene function annotation was based on the following databases: GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), NR (NCBI non-redundant protein sequences), KOG/COG (Clusters of Orthologous Groups of Proteins), Pfam (Protein family), Swiss-Prot (a manually annotated and reviewed protein sequence database).

2.6.7. RT-qPCR

In this study, an RT-qPCR reaction was performed using the SYBR Green I chimeric fluorescence method. In short, 0.4 μl of forward and reverse primer (10 μm) and 2 μl of template cDNA were added to the reaction system. 10 μl of 2 × ChamQ Universal SYBR qPCR Master Mix, 7.2 μl RNase-free dd H_2O. The reaction was amplified by fluorescent quantitative PCR (LC480, Roche, Switzerland). The reaction conditions were: predenaturation at 95°C for 30 s, 40 cycles were performed at 95°C for 10 s, and 60°C for 30 s. Finally, the dissolution curves were collected under the reaction conditions of 95°C 15 s, 60°C 60 s, and 95°C 15 s. The relative expression was calculated by 2-ΔΔCt. Detailed primer

Table 2
Information of primers.

Gene	Primer sequence (5'-3')	Annealing temperature (℃)
β-actin	F : AACTGGGATGACATGGAGAAG R : TTGGCTTTGGGGTTCAGG	60
evm.TU.scaffold_217.7	F : AGATGTGCTGAATACGGATGAGT R : TGTTCCTCCTGTTCGTGTTGA	60
evm.TU.scaffold_180.27	F : ATGTATAGAGTGATGCTGAGGAGTG R : TTGGCAGGCGATGTCTACTAG	60
evm.TU.scaffold_308.14	F : CACTCCTTCCATCCTGTTCCA R : AGGTGTGGCGTTAGAATCTGG	60
evm.TU.scaffold_29.161	F : CCAGTGTGAAGGAGAGGTGTC R : GGATGAAGGAGCCGATGAAGAT	60

Note: F means forward primer, while R means reverse primer.

information is shown in Table 2.

2.7. Data statistics and analysis

Data are presented as mean ± standard error of the mean (SE). SPSS Ver26 software was used to analyze the significance of enzyme activity data. The independent sample T test was used to analyze the differences between HFM and HSM groups. The difference between the QUE addition group and HFM, HSM group was analyzed by the one-way ANOVA. When $p < 0.05$, a significant difference was considered, and a Duncan multiple range test was performed. The results of enzyme activities were mapped using GraphPad Prism 8.

3. Results

3.1. Analysis of antioxidant parameter

In this study, the results of serum, intestinal, and liver antioxidant-related indexes were shown in Fig. 1, Fig. 2, and Fig. 3, respectively. The results showed that, compared with the whole fish meal diet, the serum, intestinal, and liver GSH levels of the high soybean meal diet were significantly decreased ($p < 0.05$). Serum T-AOC and liver CAT, T-AOC activities were also significantly decreased ($p < 0.05$). However, after adding QUE, the antioxidant capacity of spotted sea bass was greatly improved. Among them, MDA activities in serum and liver were significantly decreased when the supplemental level was 0.25 g/kg ($p < 0.05$), and had a downward trend in other supplemental groups, but were not significant ($p > 0.05$). The activities of intestinal GSH and liver GSH, CAT, and T-AOC in all QUE supplementation groups were significantly increased ($p < 0.05$). In addition, when the QUE supplemental level was 0.5 g/kg, serum CAT activity was significantly decreased ($p < 0.05$). Compared with the HFM group, GSH level in liver supplemented with 1.0 g/kg was significantly decreased ($p < 0.05$), and CAT activity in liver supplemented with 0.75 g/kg was significantly increased ($p < 0.05$). There were no significant differences in other indexes between the HFM group and the QUE group ($p > 0.05$). The statistical values have been added in supplemental Table 1.

3.2. Intestinal transcriptome analysis

According to the above results, this study selected three groups of intestinal samples of HFM, HSM, and QUE2 (QUE was used to replace QUE2 in the subsequent results) for transcriptome sequencing and pound-for-pair comparison. The three comparison groups were HFM vs HSM, HSM vs QUE, and HSM vs QUE.

3.2.1. Differentially Expressed Genes (DEGs)

Compared with the HFM group, there were 127 DEGs in the HSM group, including 56 up-regulated genes and 71 down-regulated genes. Compared with the HSM group, the QUE group had 168 DEGs, 95 up-regulated genes, and 73 down-regulated genes. Compared with the HFM group, the QUE group had 133 DEGs, 73 up-regulated genes, and 60 down-regulated genes (Table 3). In addition, the total DEGs between the pairings of the three comparison groups were 11, 20, and 25, respectively (Fig. 4).

3.2.2. GO classification enrichment analysis

The results of GO enrichment analysis in the classification of cellular component (CC), molecular function (MF), and biological process (BP) are shown in Figs. 5 and 6. The results showed that the up-regulated genes in the HFM vs HSM comparison group were mainly enriched in terms of perinucleolar compartment and prominosome, and down-regulated genes are mainly enriched in toll-like receptor 1- toll-like receptor 2 protein and melanosome in the CC classification. In the MF classification, up-regulated genes are mainly concentrated in oxidoreductase activity, acting on paired donors, catalase activity, and 3-oxoacid COA transferase activity. The down-regulated genes are mainly enriched in interleukin-10 receptor binding, glycogenin glucosyltransferase activity, and galanin receptor binding terms. In the BP classification, up-regulated genes were mainly enriched in keratinocyte migration, phototaxis, and other terms, while down-regulated genes were mainly enriched in interleukin-18 production, regulation of interleukin-1 production, and regulation of interleukin-12 production, and so on. In the HSM vs QUE comparison group, in the CC classification, the up-regulated genes were mainly enriched in extracellular

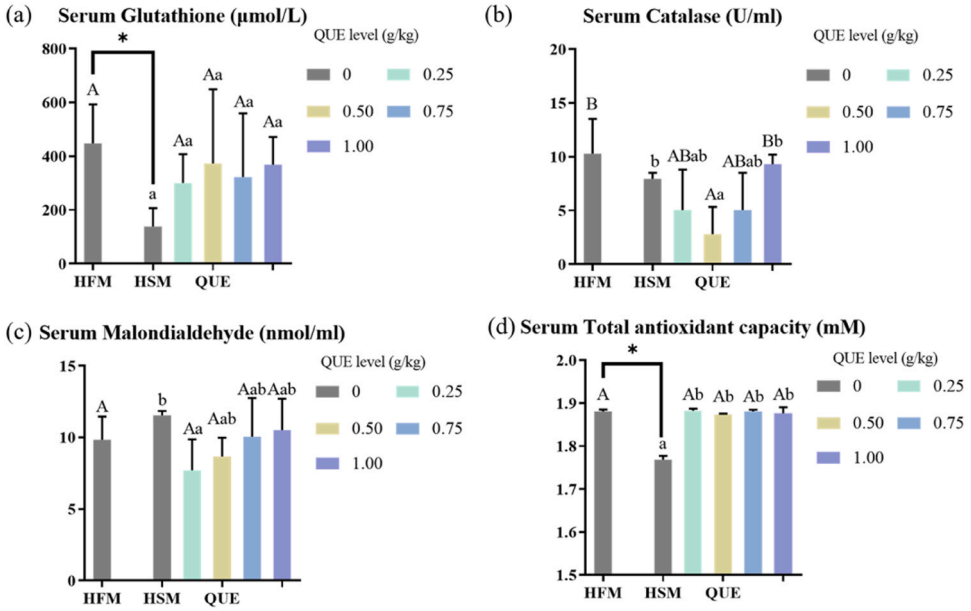

Fig. 1. Effects of QUE supplementation in high soybean meal diet on serum antioxidant of spotted sea bass. Note: Letters in corner marks a to d represent activity of serum glutathione (serum GSH, a), serum catalase (serum CAT, b), serum malondialdehyde (serum MDA, c), serum total antioxidant capacity (serum T-AOC, d), respectively. The discrepancy between the HSM group and HFM group is indicated by * ($P < 0.05$). Capital letters represent the discrepancy between HFM group and QUE intake groups, while different letters indicate significant variation ($P < 0.05$). Lowercase letters represent the discrepancy between HSM group and QUE intake groups, while different letters indicate significant variation, too ($P < 0.05$). Same to the following figures.

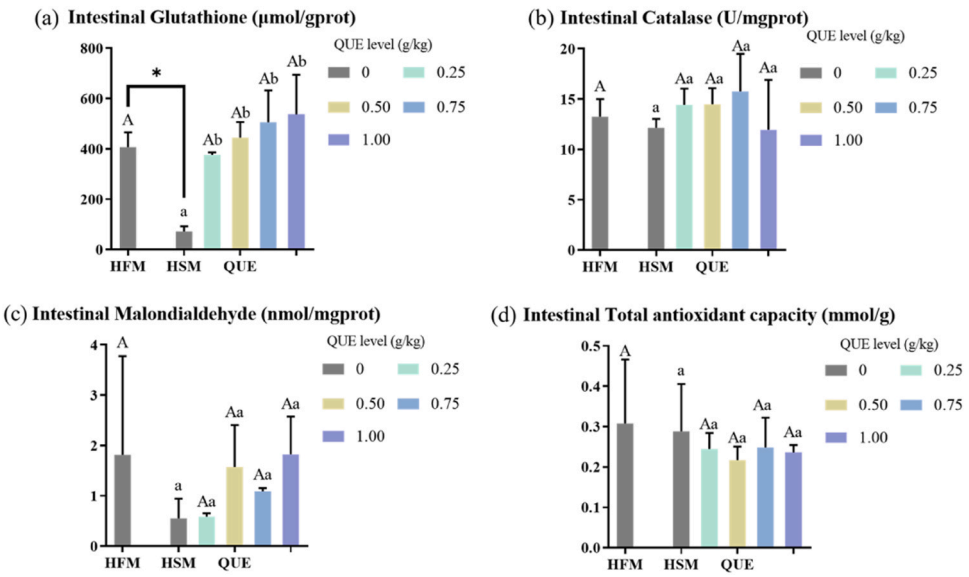

Fig. 2. Effects of QUE supplementation in high soybean meal diet on intestinal antioxidant of spotted sea bass. Note: Letters in corner marks a to d represent activity of intestinal glutathione (intestinal GSH, a), intestinal catalase (intestinal CAT, b), intestinal malondialdehyde (intestinal MDA, c), intestinal total antioxidant capacity (intestinal T-AOC, d), respectively.

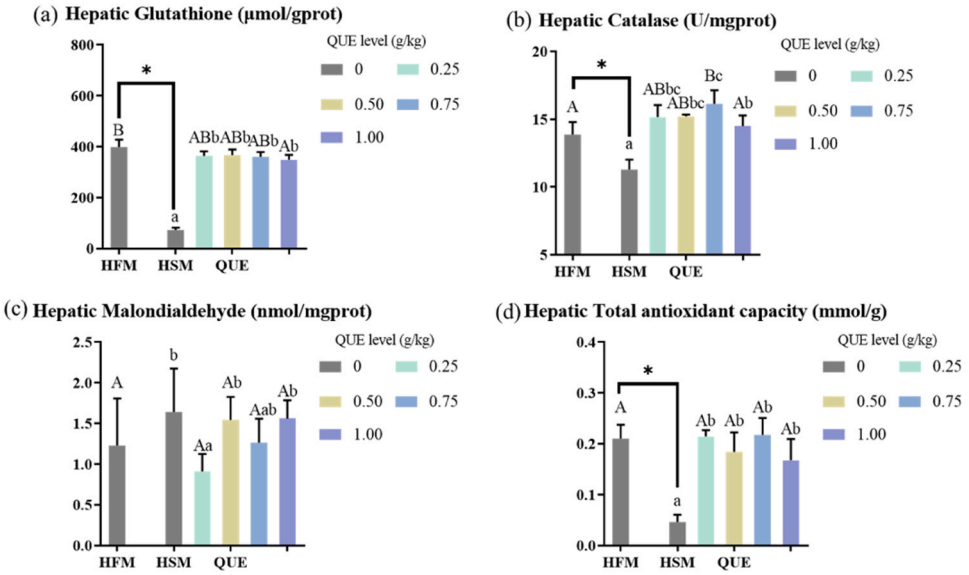

Fig. 3. Effects of QUE supplementation in high soybean meal diet on hepatic antioxidant of spotted sea bass. Note: Letters in corner marks a to d represent activity of hepatic glutathione (hepatic GSH, a), hepatic catalase (hepatic CAT, b), hepatic malondialdehyde (hepatic MDA, c), hepatic total antioxidant capacity (hepatic T-AOC, d), respectively.

Table 3
Statistics of the number of differentially expressed genes.

Control	Treat	Up-regulated	Down- regulated	Total
HFM	HSM	56	71	127
HSM	QUE	95	73	168
HFM	QUE	73	60	133

Note: Up-regulated: the number of genes up-regulated in Treat compared to Control. Down-regulated: the number of genes down-regulated in Treat compared to Control. Total: the number of differentially expressed genes in Treat compared to Control.

region and extracellular space terms, while the down-regulated genes were mainly enriched in rod spherule, connexin complex, cell surface, etc. The main enrichment terms of up-regulated genes in the MF classification include epidermal growth factor receptor activity, phosphoenolpyruvate carboxykinase activity, and signaling receptor bingding. The down-regulated genes are mainly enriched in terms of dihydrofolate reductase activity, glycine N-acyltransferase activity, and NAD+ nucleosidase activity. The up-regulated genes in the BP classification were mainly enriched in terms of protein homotrimerization, positive regulation of blood circulation, positive regulation of vasoconstriction, and down-regulated gene enrichment in terms of histamine biosynthetic process, cyclic-nucleotide-mediated signaling, and thymidine biosynthetic process.

3.2.3. *KEGG annotation analysis*

The KEGG enrichment results of up-regulated and down-regulated genes in this study are shown in Fig. 7, respectively. The results

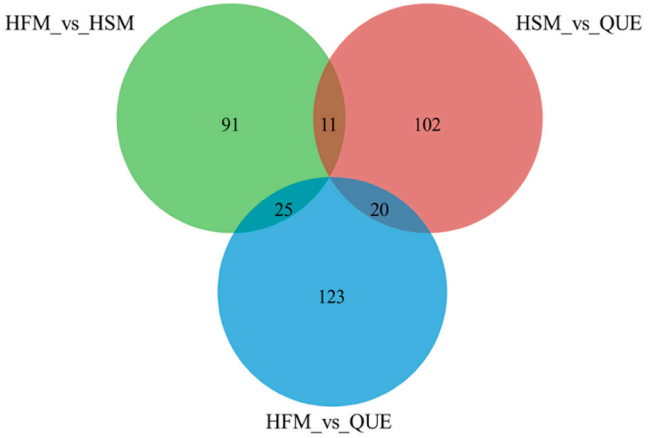

Fig. 4. Veen of common differential genes. Note: The sum of the numbers in each circle represents the total number of differential genes in the comparison group, and the overlapping part of the circle represents the common differential genes between the two comparison groups.

showed that upregulated DEGs were mainly enriched in metabolically-related pathways by the addition of high soybean meal, including protein processing in endoplasmic reticulum, apoptosis, and arginine and proline metabolism. The main enrichment pathways that down-regulate DEGs include the forkhead box O (Foxo) signaling pathway, the phosphatidylinositol-3-kinase-protein kinase B (PI3K-Akt) signaling pathway, the p53 signaling pathway, and neutrophil extracellular trap formation. When QUE was added, PI3K-Akt signaling pathway and Foxo signaling pathway were found in the significantly enriched pathway of up-regulated DEGs. In addition, it also includes cytokine-cytokine receptor interaction, extracellular matrix (ECM)-receptor interaction. The down-regulation of DEGs significantly enriched pathways associated with metabolism, including protein processing in endoplasmic reticulum, and histidine metabolism. In the HFM vs QUE group, up-regulated DEGs were mainly enriched in lysosome and cell adhesion molecules. Down-regulated DEGs were mainly enriched in B cell receptor signaling pathway, bile secretion, and cytokine-cytokine receptor interaction.

In this study, it was found that a high soybean meal diet resulted in a significant down-regulation of anti-inflammatory factor interleukin-10 (IL-10), while there was no significant difference in IL-10 level between the QUE group and the HFM group. On the other hand, the addition of high soybean meal decreased several important pathway-

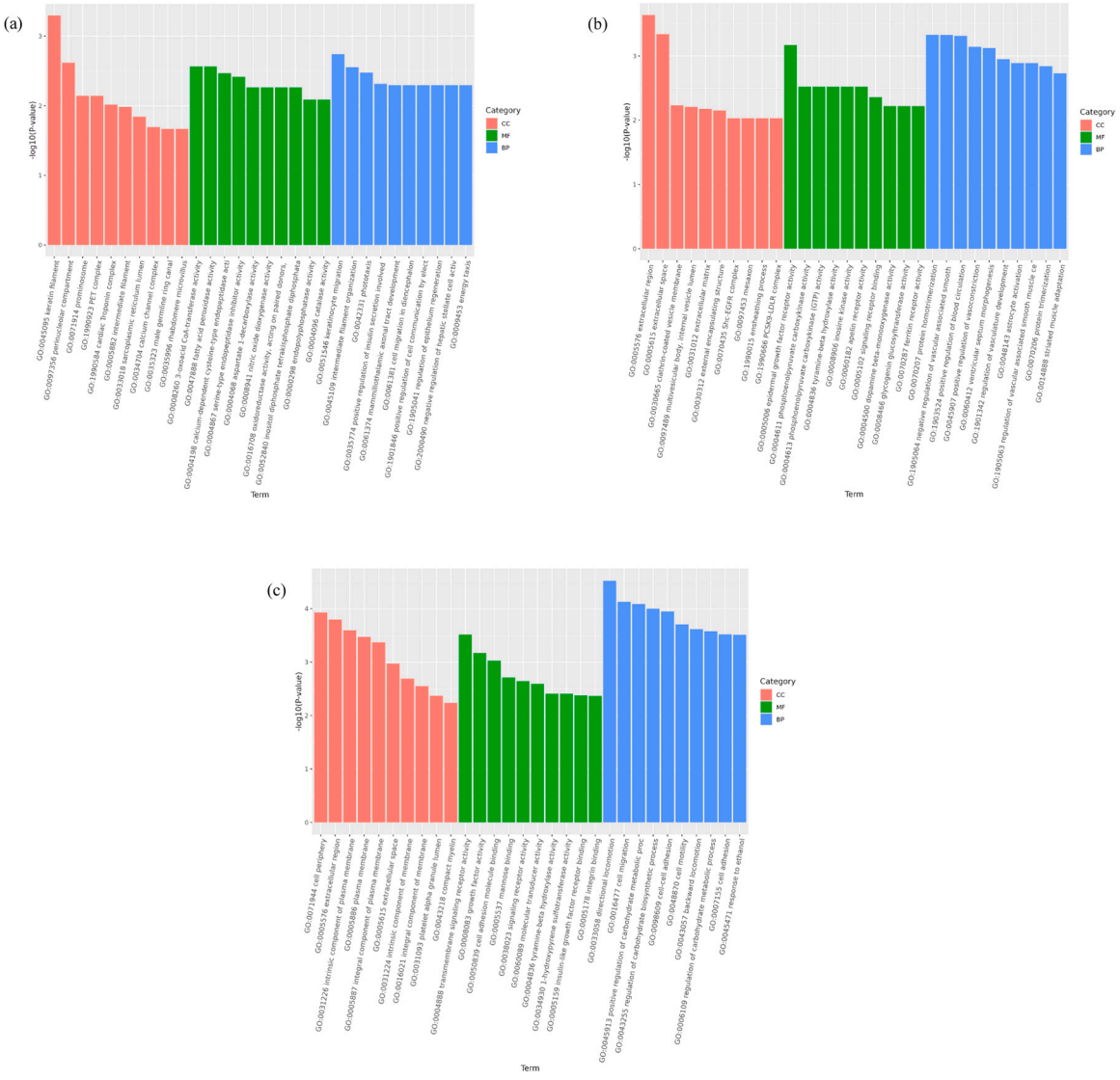

Fig. 5. GO enrichment classification up-regulated terms (Top10). Note: Letters in corner marks a to c represent HFM vs HSM (a), HSM vs QUE (b), HFM vs QUE (c), respectively. Red is cellular component (CC), green is molecular function (MF), and blue is biological process (BP). Same to the Fig. 6.

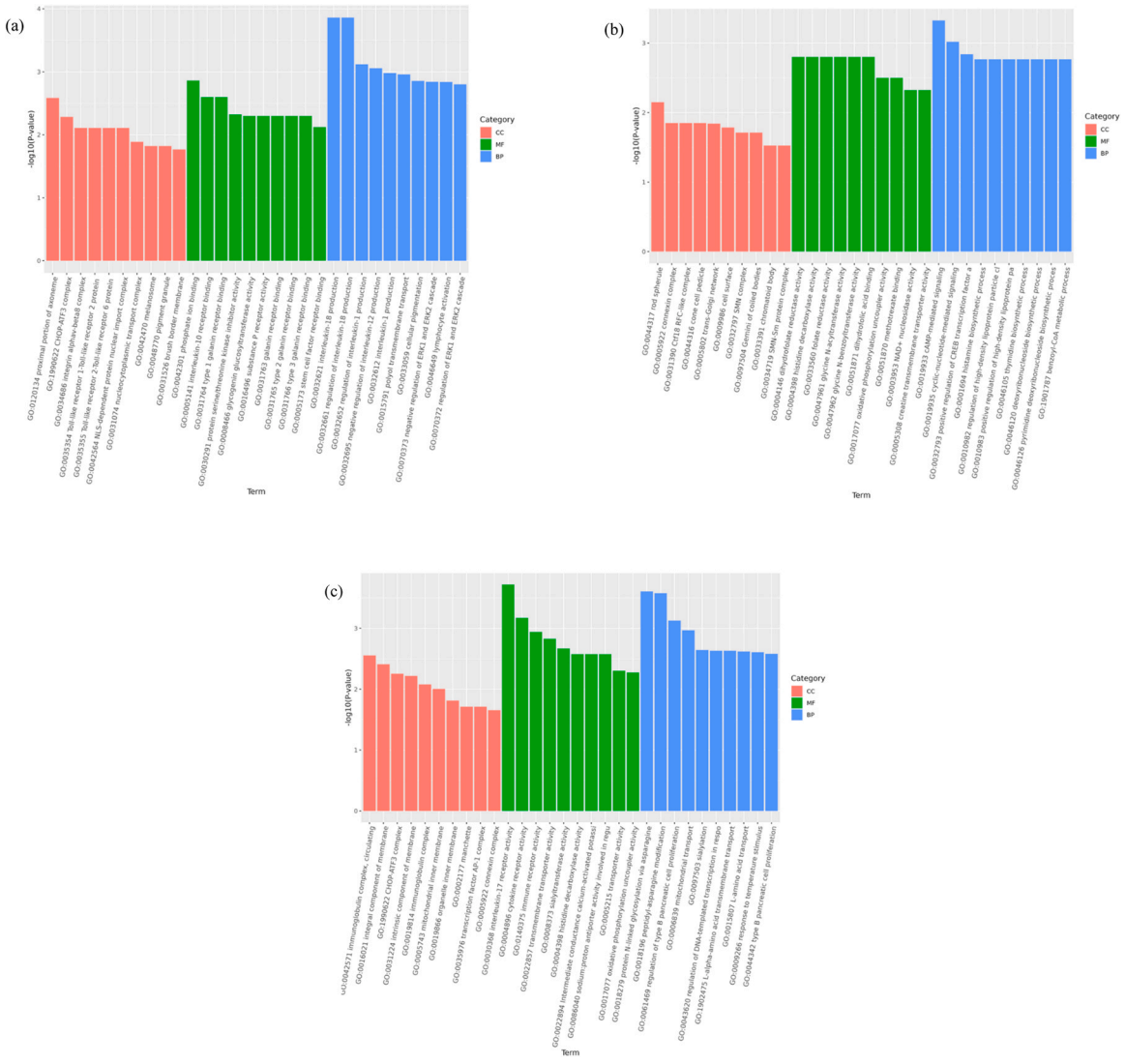

Fig. 6. GO enrichment classification down-regulated terms (Top10).

related DEGs, including serum/glucocorticoid-regulated kinase 1 (SGK1) in the Foxo signaling pathway, the toll-like receptor 2 (TLR2) in toll-like receptor signaling pathway, and cyclin-dependent kinase 2 (CDK2) in the PI3K-Akt signaling pathway. In addition, the DEGs of several pathways related to energy metabolism, including arginine and proline dehydrogenase (PRODH) in proline metabolism, aspartate 1-decarboxylase (PAND), and glutathione S-transferase (GST) in β-alanine metabolism were up-regulated. However, after adding QUE, the expression of nuclear factor of activated T-cells, cytoplasmic 2 (NFATC2) was up-regulated in T and B cell receptor signaling pathway, and AMPK signaling pathway. In addition, phosphoenolpyruvate carboxykinase (PEPCK) was observed to be upregulated in the PI3K-Akt signaling pathway, Foxo signaling pathway, gluconeogenic pathway, and peroxisome proliferators-activated receptors (PPAR) signaling pathway. These pathways play an important role in energy metabolism and oxidative damage/stress.

3.2.4. RT-qPCR verification

In order to verify the authenticity and reliability of RNA-Seq results, RT-qPCR was used in this study to verify the expression of parts of DEGs between the three groups (biological repeat n = 6). The results confirmed that the differences in the expression of some DEGs among the three groups were similar to the results of RNA-Seq. HFM vs HSM, R^2=0.9202, HSM vs QUE, R^2=0.9954, HFM vs QUE, R^2=0.9847 (Fig. 8).

4. Discussion

Reactive oxygen species (ROS) are produced as a result of regular cellular metabolism. ROS in small and moderate quantities have positive effects on several physiological processes, such as tissue regeneration, wound healing, and elimination of harmful microorganisms. Oxidative stress arises when the body generates reactive oxygen species (ROS) at a rate that surpasses the capacity of the antioxidant system to eliminate them (Jiang et al., 2016). The substances ingested by the body may induce oxidative damage and intestinal inflammation of intestinal epithelial cells, immune cells, and inflammatory cells. Therefore, oxidative stress is one of the important causes of intestinal mucosal injury and intestinal inflammation (Bhattacharyya et al., 2014). In vivo, free radicals act on lipids, peroxidation occurs, and the final product is MDA, which leads to cell damage. Therefore, MDA is used as an important index to measure oxidative damage in the body (Wang, Powell, 2010). GSH is an important intracellular antioxidant involved in a variety of cellular pathways (Leggatt and Iwama, 2009). T-AOC is a comprehensive embodiment of various antioxidant substances and systems in the body to complete antioxidant effects (Zhou et al., 2014). CAT can convert superoxide free radicals into H_2O_2, which can effectively avoid or repair oxidative damage caused by the body (Bruno-Bárcena et al., 2004; Duan et al., 2017). In general, the antioxidant capacity of fish depends to some extent on nutrient levels (Sheikhzadeh et al.,

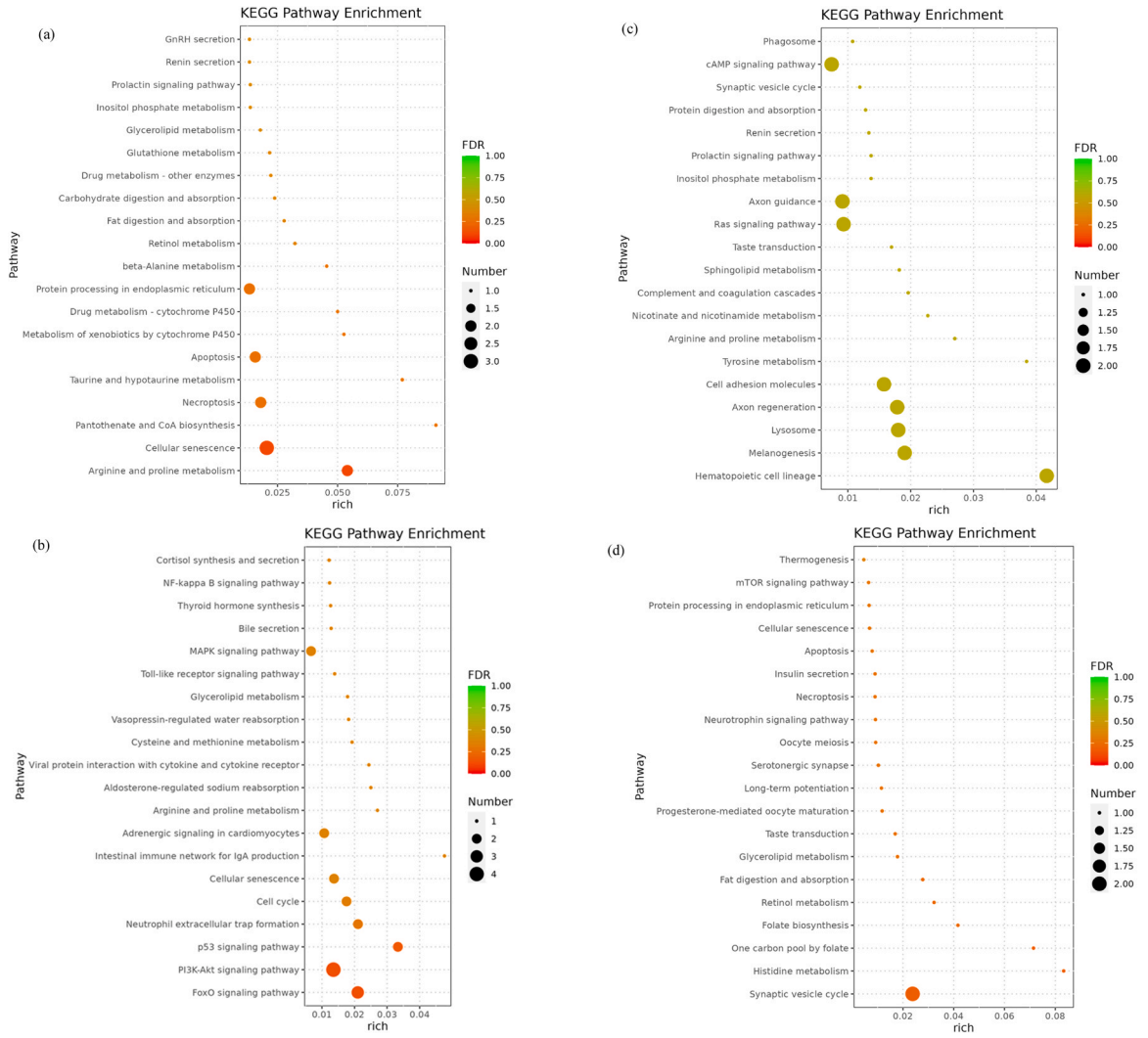

Fig. 7. Enrichment bubble map of up-regulated and down- regulated gene KEGG pathway (Top 20). Note: Letters in corner marks a to f represent HFM vs HSM (up-regulated: a, down-regulated: b), HSM vs QUE (up-regulated: c, down-regulated: d), HFM vs QUE (up-regulated: e, down-regulated: f), respectively. The greater the Rich factor, the greater the degree of enrichment. FDR generally ranges from 0 to 1, and the closer it is to zero, the more significant the enrichment.

2012). In this study, the levels of GSH and T-AOC in serum and GSH, T-AOC, and CAT in liver were also significantly decreased after a large amount of soybean meal was added to the diet of spotted sea bass. The findings demonstrated a significant decrease in the antioxidant capacity of spotted sea bass due to the high quantity of soybean meal. In a similar vein, Hsieh et al. (2017) found that soybean, when used as the primary protein source, caused oxidative stress in juvenile hybrid tilapia (*Orechromis nitoticus* x *O-aureus*). Additionally, Chen et al. (2018) discovered that soybean meal can lead to oxidative damage in juvenile turbot (*Scophthalmus maximus* L) and diminish its antioxidant capacity. Similarly, the research conducted by Jiang et al. (2018) demonstrated that a high concentration of soybean meal can diminish the antioxidant capacity of the intestines and provoke oxidative damage. The results of the above studies are similar to the results of this study, indicating that soybean meal can reduce the antioxidant capacity of farmed animals. Several studies have shown that dietary β-accompanied daidzein and glycine can reduce the expression of genes related to glutathione biosynthesis and antioxidant enzymes, thereby reducing the body's antioxidant capacity and causing oxidative damage in the gut (Jiang et al., 2015; Zhang et al., 2013). Therefore, the rich anti-nutrient factors and unbalanced amino acid composition in soybean meal may reduce the antioxidant capacity of farmed animals and even produce oxidative damage. In the GO enrichment results of this study, up-regulated genes were found to be enriched in oxidoreductase activity, acting on paired donors, catalase activity, and 3-oxoacid COA transferase activity terms. Down-regulated interleukin-10 receptor binding, glycogenin glucosyltransferase activity, and galanin receptor binding terms. These results fully indicate that the addition of high soybean meal in the diet can affect the activity of antioxidant-related enzymes and the overall antioxidant capacity of the body. The results of KEGG enrichment showed that DEGs were mainly concentrated in energy metabolism, inflammation, and apoptosis. The primary protein source in the diet shifted from fish meal to soybean meal, resulting in adaptive modifications in the spotted sea bass. These changes include the regulation of protein and amino acid metabolism, as well as corresponding alterations in antioxidant capacity. Similar results have been reported in several studies. Kiron et al. (2020) reported that soybean derivatives can cause significant changes in the intestinal transport mechanisms and metabolic pathways of Atlantic salmon (*Salmo salar*). Yoshinaga et al. (2023) reported that red seabream (*Pagrus major*) could affect glutathione and glycine metabolism after consuming a large amount of soybean meal. Wang et al. (2023) reported that feeding Sturgeon (*Acipenser schrenckii*) plant protein resulted in upregulation of metabolically related genes, including pyruvate metabolism and amino acid biosynthesis. All the

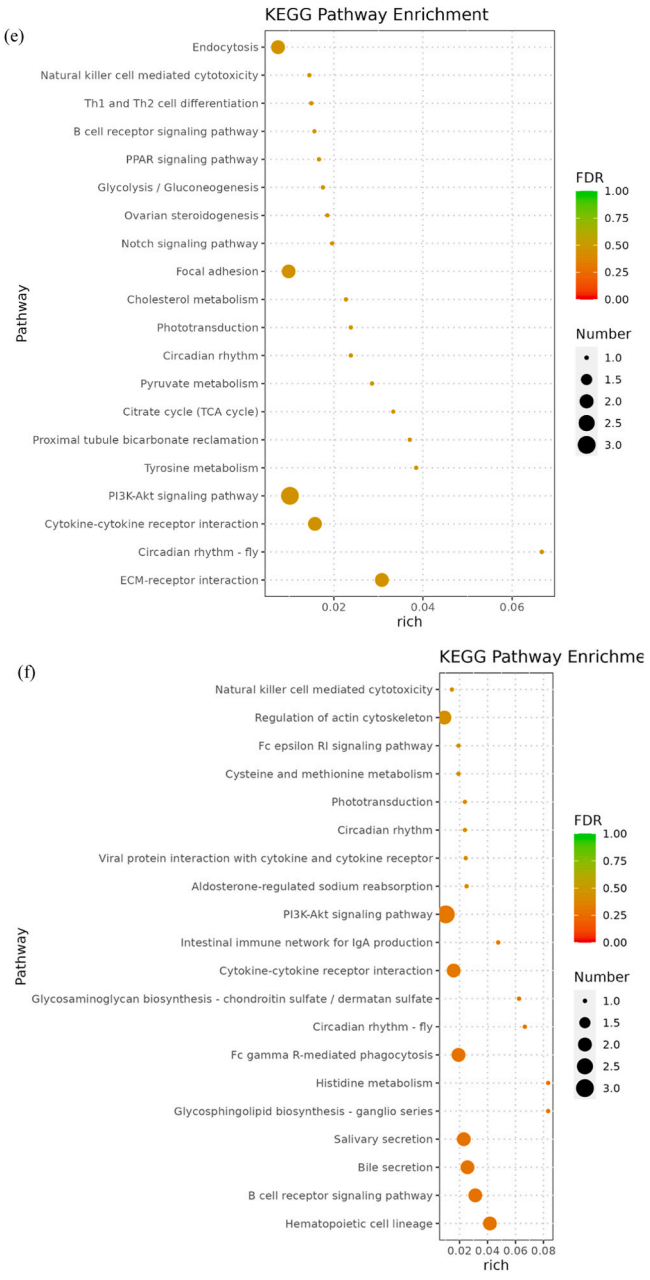

Fig. 7. (*continued*).

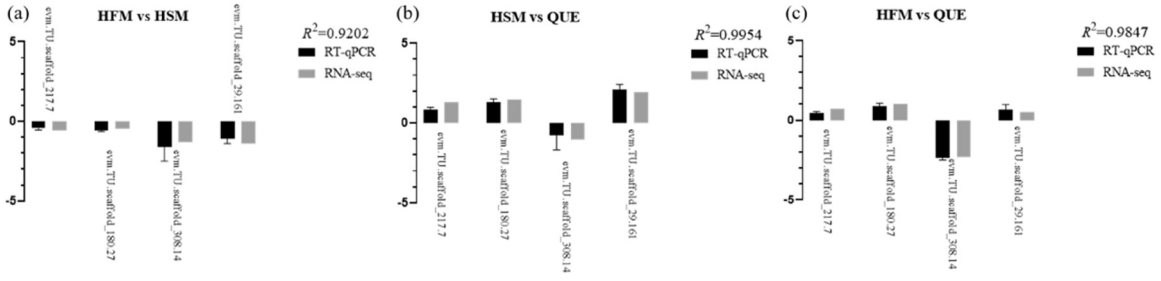

Fig. 8. Relative expression levels (log2 fold change). Note: Letters in corner marks a to c represent HFM vs HSM (a), HSM vs QUE (b), HFM vs QUE (c), respectively.

above studies showed that replacing fish meal with soybean meal and other plant proteins would change the metabolic pathway of farmed animals and produce a series of negative effects, which is consistent with the results observed in this study.

As mentioned in the introduction, QUE has good antioxidant activity. In this study, the addition of QUE significantly improved the activities of serum T-AOC, intestinal GSH, hepatic GSH, CAT, and T-AOC and showed no significant differences from those of the whole fish meal diet.

In addition, when the QUE supplemental level was 0.25 g/kg, liver MAD activity was significantly reduced. These results suggested that QUE could enhance the antioxidant capacity of spotted sea bass and slow down or repair the oxidative damage caused by high soybean meal to the level of whole fish meal. Pes et al. (2016, 2018) for silver catfish (*Rhamdia quelen*) of the two studies show that QUE can reduce lipid peroxidation in fish tissue, improve the defense of the antioxidant system composed of CAT and GSH, and protect against oxytetracycline induced oxidative stress. Khan et al. (2023) reported that the antioxidant enzyme activity in rohu (*Labeo rohita*) increased with an increase in QUE level. These findings are consistent with the results of this study. The prominent antioxidant effect of QUE can be attributed to the chain-breaking effect of the compound in the formation of free radicals, thereby preventing membrane oxidation (Bischoff, 2008). QUE has been reported to stimulate endogenous antioxidant enzymes and prevent cell apoptosis (Aluani et al., 2017). In this study, it was found that the addition of QUE led to the up-regulation of PI3K-Akt signaling pathway, Foxo signaling pathway, and PPAR signaling pathway related genes. The PI3K-Akt signaling pathway is a key signaling pathway that plays an important role in a variety of cellular functions, including cell growth, differentiation, and apoptosis. Moreover, Akt signal transduction can also reduce cellular oxidative stress and alleviate cell damage (He et al., 2020; Wang et al., 2019). Further, it has been reported that the PI3K-Akt signaling pathway can activate the Nrf2 pathway and promote the expression of HO-1 and NQO-1, thereby secreting multiple cellular antioxidant enzymes to protect tissues from oxidative stress (Zhang et al., 2021). The Foxo signaling pathway regulates a variety of cellular programs, including cell cycle arrest, ROS clearance, and apoptosis (Kim, Koh, 2017). PPAR is a nutritional sensor for multiple homeostatic functions and can further regulate multiple signaling pathways (Kvandová, Majzúnová, Dovinová, 2016). PEPCK, as an important kinase and gluconeogenic enzyme, plays an important role in the regulation of glucose metabolism (Kobylinska, Borek, Posmyk, 2018). In addition, Foxo activity is tightly controlled by insulin signaling pathways and multiple ROS-induced post-translational modifications, and in cells, ROS levels can be perceived by stimulating cysteine residues in proteins that control various signaling cascades and inhibiting oxidative modifications (de Keizer, Burgering, Dansen, 2011). This suggests that ROS clearance and antioxidant regulation are closely related to protein and amino acid metabolism, glucose metabolism, and other apoptotic pathways.

In this study, glutathione S-transferase (GST) expression was up-regulated in the glutathione metabolism pathway in the high-soybean meal diet of spotted sea bass. However, the gene expression of the glutathione metabolism pathway and related GSH enzymes did not change significantly after the addition of QUE, which was not significantly different from that of the whole fish meal diet. This suggests that the large amount of soybean meal may be the cause of the up-regulation of GST expression. GST catalyzes reactions between GSH and aliphatic groups of aromatic epoxy compounds and halides, forms glutathione complexes in the liver and is excreted through bile, or converts GSH in the kidneys to mercaptoacids, which are highly water-soluble and excreted in the urine. In addition, GSH catalyzes the reaction of organic nitrates with glutathione to produce nitrites, which interact with amines and lead to the formation of carcinogenic nitrosamines (Gupta, 2016; Testa, Clement, 2015). The above GST regulatory pathways are GSH consumption, and excessive GSH consumption is easy to cause liver toxicity, reactive oxygen species outbreaks, and the body's antioxidant capacity is weakened (Wang, Zhang, 2023). Therefore, the down-regulation of GST gene expression may be the cause of the decrease of GSH level in spotted sea bass, which is also consistent with the results of enzyme activity in this study. However, no up-regulated expression of GSH gene was observed in the intestinal transcriptome results of this study. Compared with the high-soybean meal diet of spotted sea bass, the enzyme activity of the QUE supplementation group showed increased intestinal GSH level, which was obviously not the result of upregulated intestinal GSH gene expression. GSH is a tripeptide composed of glutamic acid, cysteine, and glycine, and its transmembrane outflow depends on various transporters (Bray, Taylor, 1993; Papet et al., 2019). This study found that QUE promoted the intestinal protein and amino acid metabolism of sea bass. Therefore, the concentration of precursor amino acids for the synthesis of GSH and the source of amino acids for the synthesis of transmembrane proteins may be correspondingly increased, thereby promoting GSH synthesis or accelerating the outflow of liver and serum GSH into the intestine. In this study, the serum and liver GSH levels of the QUE supplementation group were significantly increased.

In this study, QUE was found to promote the expression of PEPCK, an important gene in the gluconeogenic pathway, and up-regulate DEGs, a pathway related to energy metabolism. The author speculated that this may be related to the unique way quercetin is absorbed. Before entering the intestinal cells, quercetin needs to be processed into quercetin glycoside by microorganisms or digestive fluid. Quercetin glycoside is absorbed by intestinal cells in two ways: 1) It is absorbed by intracellular transporters and then deglycosylated by glycosidase. 2) Deglycosylation is carried out through luminal hydrolases, and then glycoside ligands are transported to intestinal cells through transporter transport or passive diffusion (Day et al., 2003; Wolffram, Blöck, Ader, 2002; Ziberna et al., 2014). In addition, further biotransformation of quercetin aglycones is associated with glucosylation, sulfation, and methylation of hydroxyl groups (Lesjak et al., 2018). The absorption and utilization of quercetin and further biotransformation of intestinal cells are supported by energy metabolism through transporters. Therefore, it leads to a series of corresponding changes in related pathways and genes.

In the results of this study, soybean meal can reduce the antioxidant capacity of sea bass to a certain extent, and the addition of QUE can alleviate this adverse effect to a certain extent. Therefore, it is recommended to widely promote the use of QUE in the aquaculture industry to improve the choices for green and safe aquaculture. In addition, it is unclear whether soybean meal can improve the bioavailability of QUE. But there is no doubt that the combination of soybean meal and QUE is not only a topic worthy of in-depth research, but also a bold innovation to support green and healthy farming and solve the problem of fishmeal shortage. On the other hand, due to the body's special use of QUE, we can further study and explore the interaction and mechanism of QUE, the intestinal microbes, and intestinal tract through high-throughput 16 s rRNA sequencing and intestinal metabolomics analysis. Studies have shown that structural modification of QUE can improve its bioavailability to a certain extent, and different forms of QUE will also affect its bioavailability (Mullen, Edwards, Crozier, 2006; Wiczkowski et al., 2014; Zheng et al., 2017). Therefore, the study of the synthesis and structure-activity relationship of QUE and its derivatives can accelerate the development and use of QUE. In addition, studies have reported that quercetin may also support the production of free radicals through REDOX reactions, and even produce pro-oxidation effects (Carrillo-Garmendia et al., 2023). There is no doubt that the negative effects of QUE limit its application in practice, which should be studied and discussed in the future.

5. Conclusion

In summary, QUE can increase the antioxidant capacity and alleviate the adverse reactions caused by high soybean meal on the spotted sea bass. Transcriptomic analysis showed that QUE may affect the body's antioxidant capacity, apoptosis, and cell growth through the PI3K-Akt signaling pathway and Foxo signaling pathway-related pathways. On the other hand, QUE may also enhance its bioavailability by up-regulating phosphoenolpyruvate carboxykinase (PEPCK) and other genes related to energy metabolism, thus better exerting its antioxidant effect. These results reveal the potential of QUE in improving the antioxidant capacity of the body, and provide a theoretical basis for alleviating the oxidative stress caused by soybean meal on cultured animals.

Ethical approval

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

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

Lumin Kong: Methodology. **Zhongyin Long:** Methodology, Conceptualization. **Huihui Qin:** Methodology. **Hao Lin:** Methodology. **Sishun Zhou:** Methodology. **Zhongbao Li:** Supervision, Software, Project administration, Methodology, Conceptualization. **Longhui Liu:** Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Yanbo Zhao:** Methodology, Conceptualization. **Zhangfan Huang:** Methodology, Conceptualization. **Jianrong Ma:** Conceptualization. **Yi Lin:** Methodology.

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.

Acknowledgments

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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102429.

Data availability

Data will be made available on request.

References

Ai, Q.H., Mai, K.S., Li, H.T., Zhang, C.X., Zhang, L., Duan, Q.Y., Tan, B.P., Xu, W., Ma, H. M., Zhang, W.B., Liufu, Z.G., 2004. Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, *Lateolabrax japonicus*. Aquaculture 230, 507–516.

Aluani, D., Tzankova, V., Kondeva-Burdina, M., Yordanov, Y., Nikolova, E., Odzhakov, F., Apostolov, A., Markova, T., Yoncheva, K., 2017. Evaluation of biocompatibility and antioxidant efficiency of chitosan-alginate nanoparticles loaded with quercetin. Int J. Biol. Macromol. 103, 771–782.

Antonopoulou, E., Chouri, E., Feidantsis, K., Lazou, A., Chatzifotis, S., 2017. Effects of partial dietary supplementation of fish meal with soymeal on the stress and apoptosis response in the digestive system of common dentex (*Dentex dentex*). J. Biol. Res. Thessal. 24.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S., Crowe, S.E., 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev. 94, 329–354.

Bischoff, S.C., 2008. Quercetin: potentials in the prevention and therapy of disease. Curr. Opin. Clin. Nutr. 11, 733–740.

Bray, T.M., Taylor, C.G., 1993. Tissue glutathione, nutrition, and oxidative stress. Can. J. Physiol. Pharm. 71, 746–751.

Bruno-Bárcena, J.M., Andrus, J.M., Libby, S.L., Klaenhammer, T.R., Hassan, H.M., 2004. Expression of a heterologous manganese superoxide dismutase gene in intestinal lactobacilli provides protection against hydrogen peroxide toxicity. Appl. Environ. Micro 70, 4702–4710.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Method Enzym. 52, 302–310.

Carrillo-Garmendia, A., Madrigal-Perez, L.A., Regalado-Gonzalez, C., 2023. The multifaceted role of quercetin derived from its mitochondrial mechanism. Mol. Cell Biochem.

Chen, Q., Wang, C.C., Sun, Y.L., Chen, S.M., Zhou, J.Q., Han, T., et al., 2024. An integrated analysis of transcriptome and metabolome reveals three bcaas relieve lipid accumulation by inhibiting lipid synthesis and promoting lipid oxidation in the liver of largemouth bass (Micropterus salmoides). Aquaculture 581. https://doi.org/10.1016/j.aquaculture.2023.740384).

Chen, Z.C., Zhao, S.F., Liu, Y., Yang, P., Ai, Q.H., Zhang, W.B., Xu, W.Q., Zhang, Y.A., Zhang, Y.J., Mai, K.S., 2018. Dietary citric acid supplementation alleviates soybean meal-induced intestinal oxidative damage and micro-ecological imbalance in juvenile turbot, *Scophthalmus maximus* L. Aquac. Res. 49, 3804–3816.

Day, A.J., Gee, J.M., DuPont, M.S., Johnson, I.T., Williamson, G., 2003. 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. Pharm. 65, 1199–1206.

Dong, G.F., Yang, Y.O., Song, X.M., Yu, L., Zhao, T.T., Huang, G.L., Hu, Z.J., Zhang, J.L., 2013. Comparative effects of dietary supplementation with maggot meal and soybean meal in gibel carp (*Carassius auratus gibelio*) and darkbarbel catfish (*Pelteobagrus vachelli*): growth performance and antioxidant responses. Aquacult Nutr. 19, 543–554.

Duan, Y.F., Zhang, Y., Dong, H.B., Zheng, X.T., Wang, Y., Li, H., Liu, Q.S., Zhang, J.S., 2017. Effect of dietary poly-β-hydroxybutyrate (PHB) on growth performance, intestinal health status and body composition of Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931). Fish. Shellfish Immun. 60, 520–528.

Fehrmann-Cartes, K., Coronado, M., Hernández, A.J., Allende, M.L., Feijoo, C.G., 2019. Anti-inflammatory effects of Aloe vera on soy meal-induced intestinal inflammation in zebrafish. Fish. Shellfish Immun. 95, 564–573.

Gao, S.Y., Chen, W.J., Cao, S.P., Sun, P., Gao, X.C., 2024. Microalgae as fishmeal alternatives in aquaculture: current status, existing problems, and possible solutions. Environ. Sci. Pollut. Res. 31, 16309–16327.

Gephart, J.A., Golden, C.D., Asche, F., Belton, B., Brugere, C., Froehlich, H.E., Fry, J.P., Halpern, B.S., Hicks, C.C., Jones, R.C., Klinger, D.H., Little, D.C., McCauley, D.J., Thilsted, S.H., Troell, M., Allison, E.H., 2020. Scenarios for global aquaculture and its role in human nutrition. Rev. Fish. Sci. Aquac. 29, 122–138.

Guo, Y., Bruno, R.S., 2015. Endogenous and exogenous mediators of quercetin bioavailability. J. Nutr. Biochem. 26, 201–210.

Gupta, P.K., 2016. In: gupta, pk (Ed.), Chapter 8 - Biotransformation. fundamentals of toxicology. Academic Press, pp. 73–85.

Hamza, T.A., Hadwan, M.H., 2020. New spectrophotometric method for the assessment of catalase enzyme activity in biological tissues. Curr. Anal. Chem. 16, 1054–1062.

Harrap, K.R., 1967. Application of 5,5′ dithiobis-2-nitrobenzoic acid to determination of acid-soluble disulphide content of blood. Biochem. Pharm. 16, 725.

He, S.S., Li, A.P., Zhang, W.N., Zhang, L.C., Liu, Y.T., Li, K., Qin, X.M., 2020. An integrated transcriptomics and network pharmacology approach to exploring the mechanism of adriamycin-induced kidney injury. Chem. Biol. Inter. 325.

Holloway, A.C., Keene, J.L., Noakes, D.G., Moccia, R.D., 2004. Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquac. Res 35, 1025–1030.

Hsieh, M.C., Huang, C.H., Yang, H.W., Chen, J.C., Hsu, C.K., 2017. Black soybean seed coat containing diet enhancing the growth performance of juvenile hybrid tilapia *Orechromis nitoticus* x *O-aureus*. Aquac. Res. 48, 2593–2601.

Huang, Z., Zhou, C.P., Lin, H.Z., Wang, J., Wang, Y., Yu, W., 2022. Effect of dietary carbohydrate on glycometabolism of juvenile golden pompano *(Trachinotus ovatus)* related to growth performance, hepatic histopathology, transcriptome profiles and identification of differentially expressed genes. Aquac. Rep. 27. https://doi.org/10.1016/j.aqrep.2022.101362.

Jia, Y., Yan, X., Huang, Y., Zhu, H., Qi, B., Li, Y., 2022. 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, 133685.

Jiang, W.D., Hu, K., Zhang, J.X., Liu, Y., Jiang, J., Wu, P., Zhao, J., Kuang, S.Y., Tang, L., Tang, W.N., Zhang, Y.A., Zhou, X.Q., Feng, L., 2015. Soyabean glycinin depresses intestinal growth and function in juvenile Jian carp (*Cyprinus carpio* var Jian): protective effects of glutamine. Brit J. Nutr. 114, 1569–1583.

Jiang, J., Wu, X.Y., Zhou, X.Q., Feng, L., Liu, Y., Jiang, W.D., Wu, P., Zhao, Y., 2016. Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. Aquaculture 463, 174–180.

Jiang, J., Xu, S., Feng, L., Liu, Y., Jiang, W., Wu, P., Wang, Y., Zhao, Y., Zhou, X., 2018. Lysine and methionine supplementation ameliorates high inclusion of soybean meal inducing intestinal oxidative injury and digestive and antioxidant capacity decrease of yellow catfish. Fish. Physiol. Biochem 44, 319–328.

Jin, G., Zhang, L., Mai, K., Chen, X., Xu, S., Ai, Q., 2023. Effects of different dietary lipid sources on growth performance, hepatic lipid deposition and transcriptome response in spotted sea bass (lateolabrax maculatus). Aquaculture 566, 2023. https://doi.org/10.1016/j.aquaculture.2022.739143.

de Keizer, P., Burgering, B., Dansen, T.B., 2011. Forkhead box O as a sensor, mediator, and regulator of redox signaling. Antioxid. Redox Sign 14, 1093–1106.

Khan, T., Fatima, M., Shah, S., Khan, N., Maryam, Ali, W., Sabir, A., Tahir, I., Laraib, D., 2023. Quercetin supplementation in the diet of Labeo rohita: effects on growth, proximate composition, antioxidative indices and immunity. Anim. Feed Sci. Tech. 303.

Kim, S., Koh, H., 2017. Role of FOXO transcription factors in crosstalk between mitochondria and the nucleus. J. Bioenerg. Biomembr. 49, 335–341.

Kiron, V., Park, Y., Siriyappagouder, P., Dahle, D., Vasanth, G.K., Dias, J., Fernandes, J., Sorensen, M., Trichet, V.V., 2020. Intestinal transcriptome analysis reveals soy derivative-linked changes in atlantic salmon. Front. ImmunoL. 11.
Kobori, M., Takahashi, Y., Akimoto, Y., Sakurai, M., Matsunaga, I., Nishimuro, H., Ippoushi, K., Oike, H., Ohnishi-Kameyama, M., 2015. Chronic high intake of quercetin reduces oxidative stress and induces expression of the antioxidant enzymes in the liver and visceral adipose tissues in mice. J. Funct. Foods 15, 551–560.
Kobylinska, A., Borek, S., Posmyk, M.M., 2018. Melatonin redirects carbohydrates metabolism during sugar starvation in plant cells. J. Pineal Res 64.
Kvandová, M., Majzúnová, M., Dovinová, I., 2016. The role of PPARγ in cardiovascular diseases. Physiol. Res. 65, S343–S363.
Leggatt, R.A., Iwama, G.K., 2009. Exogenous glutathione can increase glutathione levels in tissues of rainbow trout (*Oncorhynchus mykiss*) through extracellular breakdown and intracellular synthesis. Comp. Biochem. Phys. C. 150, 322–328.
Lesjak, M., Beara, I., Simin, N., Pintać, D., Majkić, T., Bekvalac, K., Orčić, D., Mimica-Dukić, N., 2018. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J. Funct. Foods 40, 68–75.
Lin, Y.H., Mui, J.J., 2017. Comparison of dietary inclusion of commercial and fermented soybean meal on oxidative status and non-specific immune responses in white shrimp, *Litopenaeus vannamei*. Fish. Shellfish Immun. 63, 208–212.
Liu, S.Y., Xu, M., Chen, B.P., Li, F.L., Deng, Y.Q., Zhang, Y.F., Lin, G., Chen, D.F., Geng, Y., Ou, Y.P., Huang, X.L., 2024. The potential mechanism of concentrated mannan-oligosaccharide promoting goldfish's (*Carassius auratus Linnaeus*) resistance to Ichthyophthirius multifiliis invasion. Fish. Shellfish Immunol. 144.
Macusi, E.D., Cayacay, M.A., Borazon, E.Q., Sales, A.C., Habib, A., Fadli, N., Santos, M. D., 2023. protein fishmeal replacement in aquaculture: a systematic review and implications on growth and adoption viability. Sustainability 15.
Mullen, W., Edwards, C.A., Crozier, A., 2006. Absorption, excretion and metabolite profiling of methyl-, glucuronyl-, glucosyl- and sulpho-conjugates of quercetin in human plasma and urine after ingestion of onions. Brit J. Nutr. 96, 107–116.
Papet, I., Rémond, D., Dardevet, D., Mosoni, L., Polakof, S., Peyron, M., Savary-Auzeloux, I., 2019. Chapter 21 - sulfur amino acids and skeletal muscle. In: walrand, s (Ed.), Nutrition and Skeletal Muscle. Academic Press, pp. 335–363.
Peng, K., Lv, X.H., Zhao, H.X., Chen, B., Chen, X.Y., Huang, W., 2022. Antioxidant and intestinal recovery function of condensed tannins in Lateolabrax maculatus responded to in vivo and in vitro oxidative stress. Aquaculture 547.
Pês, T.S., Saccol, E.M.H., Londero, É.P., Bressan, C.A., Ourique, G.M., Rizzetti, T.M., Prestes, O.D., Zanella, R., Baldisserotto, B., Pavanato, M.A., 2018. Protective effect of quercetin against oxidative stress induced by oxytetracycline in muscle of silver catfish. Aquaculture 484, 120–125.
Pês, T.S., Saccol, E., Ourique, G.M., Londero, É.P., Gressler, L.T., Golombieski, J.I., Glanzner, W.G., Llesuy, S.F., Gonçalves, P., Neto, J.R., Baldisserotto, B., Pavanato, M.A., 2016. Quercetin in the diet of silver catfish: Effects on antioxidant status, blood parameters and pituitary hormone expression. Aquaculture 458, 100–106.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Bio Med 26, 1231–1237.
Sheikhzadeh, N., Tayefi-Nasrabadi, H., Oushani, A.K., Enferadi, M., 2012. Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rainbow trout (*Oncorhynchus mykiss*). Fish. Physiol. Biochem. 38, 413–419.
Testa, B., Clement, B., 2015. Chapter 24 - biotransformation reactions and their enzymes. In: Wermuth, C.G., Aldous, D., Raboisson, P., Rognan, D. (Eds.), The Practice of Medicinal Chemistry (Fourth Edition). Academic Press, San Diego, pp. 561–584.
Trakooncharoenvit, A., Tanaka, S., Mizuta, E., Hira, T., Hara, H., 2020. 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, 1389–1398.
Wang, L., Cui, Z.H., Ren, X., Li, P., Wang, Y., 2021. Growth performance, feed cost and environmental impact of largemouth bass Micropterus salmoides fed low fish meal diets. Aquacult Rep. 20.
Wang, D.Z., Jin, M.Y., Zhao, X.Y., Zhao, T.Y., Lin, W., He, Z.L., Fan, M.J., Jin, W., Zhou, J., Jin, L.W., Zheng, C., Jin, H., Zhao, Y.S., Li, X.K., Ying, L., Wang, Y., Zhu, G. H., Huang, Z.F., 2019. FGF1$^{\Delta HBS}$ ameliorates chronic kidney disease via PI3K/AKT mediated suppression of oxidative stress and inflammation. Cell Death Dis. 10.
Wang, P., Powell, S.R., 2010. Decreased sensitivity associated with an altered formulation of a commercially available kit for detection of protein carbonyls. Free Radic. Bio Med. 49, 119–121.
Wang, W.J., Zhang, N., 2023. Oridonin inhibits Hela cell proliferation via downregulation of glutathione metabolism: a new insight from metabolomics. J. Pharm. Pharm. 75, 837–845.
Wang, T., Zhou, N.N., He, J.Y., Hao, Z.Z., Zhou, C.T., Du, Y.D., Du, Z.Y., Su, X.Y., Zhang, M.L., 2023. Xylanase improves the intestinal barrier function of Nile tilapia (*Oreochromis niloticus*) fed with soybean (*Glycine max*) meal. J. Anim. Sci. Biotechnol. 14.
Wiczkowski, W., Szawara-Nowak, D., Topolska, J., Olejarz, K., Zielinski, H., Piskula, M. K., 2014. Metabolites of dietary quercetin: Profile, isolation, identification, and antioxidant capacity. J. Funct. Foods 11, 121–129.
Wolffram, S., Blöck, M., Ader, P., 2002. Quercetin-3-glucoside is transported by the glucose carrier sglt1 across the brush border membrane of rat small intestine. J. Nutr. 132, 630–635.
Yan, X.B., Huang, W.B., Suo, X.X., Pan, S.M., Li, T., Liu, H., Tan, B.P., Zhang, S., Yang, Y. Z., Dong, X.H., 2023. Integrated analysis of microbiome and host transcriptome reveals the damage/protective mechanism of corn oil and olive oil on the gut health of grouper (9 Epinephelus fuscoguttatus x ♂ E. lanceolatu). Int. J. Biol. Macromol. 253.
Yoshinaga, H., Yasuike, M., Mekuchi, M., Soma, S., Yamamoto, T., Murashita, K., Matsunari, H., Oku, H., Furuita, H., 2023. Multi-omics analysis of hepatopancreas of red seabream (Pagrus major) fed a soybean meal-based diet. Aquaculture 574.
Yu, G., Liu, Y., Ou, W., Dai, J., Ai, Q., Zhang, W., Mai, K., Zhang, Y., 2021. The protective role of daidzein in intestinal health of turbot (Scophthalmus maximus L.) fed soybean meal-based diets. Sci. Rep. -Uk 11, 3352.
Zhang, Q.Q., Feng, A.Z., Zeng, M.N., Zhang, B.B., Shi, J.Y., Lv, Y.X., Cao, B., Zhao, C.X., Wang, M.Y., Ding, Y.F., Zheng, X.K., 2021. Chrysosplenol D protects mice against LPS-induced acute lung injury by inhibiting oxidative stress, inflammation, and apoptosis via TLR4-MAPKs/NF-κB signaling pathways. Innate Immun. -Lond. 27, 514–524.
Zhang, J.X., Guo, L.Y., Feng, L., Jiang, W.D., Kuang, S.Y., Liu, Y., Hu, K., Jiang, J., Li, S. H., Tang, L., Zhou, X.Q., 2013. Soybean β-conglycinin induces inflammation and oxidation and causes dysfunction of intestinal digestion and absorption in fish. Plos One 8.
Zhang, C.X., Rahimnejad, S., Wang, Y.R., Lu, K.L., Song, K., Wang, L., Mai, K.S., 2018. 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, 173–182.
Zheng, Y.Z., Deng, G., Liang, Q., Chen, D.F., Guo, R., Lai, R.C., 2017. Antioxidant activity of quercetin and its glucosides from propolis: a theoretical study. Sci. Rep. Uk 7.
Zhou, C., Ge, X., Lin, H., Niu, J., 2014. Effect of dietary carbohydrate on non-specific immune response, hepatic antioxidative abilities and disease resistance of juvenile golden pompano (Trachinotus ovatus). Fish. Shellfish Immun. 41, 183–190.
Ziberna, L., Fornasaro, S., Čvorović, J., Tramer, F., Passamonti, S., 2014. Chapter 37 - bioavailability of flavonoids: the role of cell membrane transporters. In: Watson, R. R., Preedy, V.R., Zibadi, S. (Eds.), Polyphenols in Human Health and Disease. Academic Press, San Diego, pp. 489–511.