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Quercetin Attenuates Oxidative Stress and Immune Inflammation via Modulating Heme and ROS Pathways in Rats Fed Protein-Oxidized Soybean Meal

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Abstract

Protein-oxidized soybean meal (OS) is soybean meal whose proteins have undergone oxidative damage during storage, causing oxidative stress and inflammatory injury in animals. Quercetin (Q) has been shown to possess antioxidant and anti-inflammatory effects. Therefore, to explore these effects of Q on rats fed OS, a 2 × 2 two-factor completely randomized design was adopted. 48 SD rats were randomly divided into four groups: fresh soybean meal (FS) group, FS + Q group, OS group, and OS + Q group, and fed for 28 days. The results showed that compared with the FS diet, the OS diet significantly increased serum levels of glucose and urea nitrogen ($P < 0.05$), and induced inflammatory lesions in the duodenum, jejunum, and liver. The OS diet reduced total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px) activity while increasing reactive oxygen species (ROS) content in the jejunum ($P < 0.05$); In the liver, it increased malondialdehyde (MDA) content but decreased glutathione (GSH) levels and GSH-Px activity ($P < 0.05$); the OS diet elevated the contents of the IgG and interleukin-6 (IL-6) in both jejunum and liver ($P < 0.05$), and up-regulated jejunal and hepatic pathways related to heme biosynthesis and ROS synthesis ($P < 0.05$). In contrast, compared with the diet without Q, Q supplementation significantly reduced serum globulin levels ($P < 0.05$), and improved intestinal and liver inflammatory lesions. In the jejunum, Q supplementation decreased ROS content and increased catalase (CAT) activity and T-AOC ($P < 0.05$), and downregulated the "heme biosynthesis" pathway ($P < 0.05$); In the liver, it increased T-AOC and decreased MDA content ($P < 0.05$), downregulated the "ROS metabolic process" and related molecular pathways ($P < 0.05$), and normalized the expression of key genes (*Ccl20, Duox1, Cyp4a2*) and proteins (*Ccl20, Duox1*) in jejunum and liver ($P < 0.05$). These findings indicate that Q alleviates OS-induced oxidative stress, inflammation, and tissue damage in rats by regulating heme and ROS pathways.

Keywords: quercetin; protein oxidation; soybean meal; oxidative stress; immune inflammation

1. Introduction

Soybean meal is widely used in the diets of laying hens, broilers, swine and aquatic species because of its balanced amino-acid profile. However, during processing and storage, the proteins of soybean meal inevitably undergo oxidative modification[1]. This oxidative modification alters the protein's structure and properties, which is characterized by an increase in carbonyl content and a decrease in free sulfhydryl content[2], leading to a loss in nutritional value and the generation of potentially harmful substances[3]. Consumption of diets containing excessively oxidized proteins disrupts redox homeostasis and elicits systemic oxidative stress in animals[4,5]. Long-term intake of highly oxidized proteins leads to the systemic accumulation of reactive carbonyl species and provokes a sustained inflammatory response, severely compromising animal health and productivity[6]. Consequently, the impairment of antioxidant and anti-inflammatory functions in animals caused by protein-oxidized soybean meal (OS) warrants urgent investigation. Quercetin (Q), a natural flavonol with well-documented in-vivo antioxidant and anti-inflammatory properties[7], is extensively used in animal feeds[8,9].

We hypothesized that dietary supplementation with Q would alleviate the adverse effects of OS on animals. To test this hypothesis, we conducted an experiment using growing rats to assess the interactive effects of Q and OS on antioxidant capacity and inflammatory status. Furthermore, to elucidate the molecular mechanisms by which Q ameliorates OS-induced oxidative and inflammatory insults, jejunal and hepatic transcriptome profiling was performed[10]. The study aimed to provide fundamental data for the future application of Q to alleviate oxidative and inflammatory damage induced by OS in animal production.

2. Materials and Methods

2.1. Tested Compounds

The FS, sourced from Yihai Cereal & Oil Industry Co., Ltd. (Lianyungang, China), was stored at –20 ℃ from the date of production. Q (95% purity) was purchased from Hunan E.K. Herb Co., Ltd. (Changsha, China). The preparation method for OS was to place FS on clean, sterilized 35 ℃ constant temperature and humidity chamber for 56 days. The humidity of the chamber set at 60% to prevent moisture loss from the soybean meal. During storage, the soybean meal samples were turned over every 7 days, and the chamber was disinfected with an ultraviolet (UV) lamp for 30 minutes each time to prevent the soybean meal from mold growth. Characteristics of the FS and OS are given in Table 1.

Table 1. Characteristics of the FS and OS.

Items	FS	OS
Crude protein content (%)	43.13	43.14
Fat content (%)	0.88	0.88
Moisture (%)	12.99	12.96
Protein carbonyl (nmol/mg of protein)	7.09	10.32
Free sulfhydryl (nmol/mg of protein)	12.38	5.56
Mold situation	Not moldy	Not moldy
Aflatoxin B1 content (μg/kg)	≤ 30	≤ 30

2.2. Animals and Study Design

A total of 48 three-week-old SPF male Sprague-Dawley (SD) rats (initial weight 55 ± 5 g, purchased from Jiangsu Wukong Biotechnology Co., Ltd., Nanjing, China) were randomly divided into 4 groups, with 12 replicates per group and one rat per replicate. The experiment used a 2 × 2 factorial design: the FS group was fed a corn-soybean meal-based basal diet; the FS + Q group was fed the basal diet supplemented with 400 mg/kg Q; the OS group was fed a basal diet in which OS replaced the FS; and the OS + Q group was fed a basal diet in which OS replaced FS and supplemented with 400 mg/kg Q. The composition and calculated nutrient levels of the basal diets are listed in Table 2. Before diet formulation, the dry matter of soybean meal in each group was adjusted to the same level.

Table 2. The formulation and calculated nutrient levels of diets (%, as-fed basis).

Items	Content (%)	
	FS diet	OS diet
Corn	37.58	37.58

FS	29.91	–
OS	–	29.91
Subflour	1.99	1.99
Wheat	19.94	19.94
Wheat bran	1.00	1.00
Soybean oil	1.99	1.99
Limestone	1.20	1.20
Dicalcium phosphate	1.50	1.50
Sodium chloride	0.25	0.25
Magnesium oxide	0.15	0.15
Choline chloride	0.20	0.20
Cr_2O_3	0.30	0.30
Premix[1]	3.99	3.99
Calculated nutrient levels		
Metabolizable energy (MJ/kg)	13.38	13.38
Crude protein	20.94	20.94
Ether extract	4.49	4.49
Crude fiber	3.99	3.99
Crude ash	5.78	5.78
Calcium	1.10	1.10
Total phosphorus	0.75	0.75
Lysine	1.30	1.30
Methionine + cystine	0.86	0.86

[1] The premix was provided by Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd. (Nanjing, China), and provided the following per kilogram: arginine, 1.10 g; histidine, 0.50 g; isoleucine, 49.52 g; leucine, 30.98 g; lysine, 101.45 g; methionine, 59.85 g; cystine, 0.50 g; phenylalanine, 0.80 g; tyrosine, 0.60 g; threonine, 42.31 g; tryptophan, 20.15 g; valine, 40.90 g; Na, 20.50 g; Cl, 29.50 g; Mg, 0.50 g; K, 0.70 g; Fe, 2496.29 mg; Cu, 180.23 mg; Mn, 1097.03 mg; Zn, 1496.90 mg; I, 16.75 mg; Se, 5.10 mg; carotene, 0.37 mg; vitamin E, 2582.60 IU; thiamine, 1035.91 mg; riboflavin, 466.32 mg; pantothenic acid, 929.74 mg; nicotinic acid, 2308.88 mg; biotin, 8.12 mg; folic acid, 251.29 mg; choline, 146.56 mg; pyridoxine hydrochloride, 481.10 mg; cyanocobalamin, 1.01 mg; linoleic acid, 0.02 g; vitamin A, 542,633.19 IU; vitamin D, 78,932.72 IU; vitamin K, 215.94 mg.

2.3. Breeding Management

All the experimental procedures applied in this study were reviewed and approved by the Nanjing Agricultural University's Animal Care and Use Committee (permit number: NJAU.No20230606094). Rats were housed in SPF-level clean negative pressure cages, with free access to food and water, a 12-hour light/12-hour dark cycle, room temperature and humidity maintained at 20 ± 2 ℃, and at 50% ~ 70%, respectively. The acclimation period lasted 1 week, followed by a formal experimental period of 4 weeks.

2.4. Sampling and Analysis

On the last day of the 5th week of the experiment, all rats were anesthetized with ether, followed by blood collection from the orbit and cervical dislocation for euthanasia. The collected rat blood was left at room temperature for 30 minutes, then centrifuged at 3,000 × g for 15 minutes at 4 ℃ to collect the serum. The serum was aliquoted into centrifuge tubes and stored at −20 ℃ for later analysis. Immediately after euthanasia, the rats were dissected to collect their liver, duodenum, jejunum, and ileum. Part of the liver and intestinal tissues was placed in 4% paraformaldehyde solution for hematoxylin and eosin (H&E) staining, while the remaining tissue was aliquoted into cryogenic tubes, rapidly frozen in liquid nitrogen for 6 hours, and then stored at −20 ℃ for later analysis.

2.5. Serum Biochemical Indicators

The levels of glucose (GLU), total protein (TP), urea nitrogen (UN), triglycerides (TG), total serum cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin (ALB) in serum were measured using an automated biochemical analyzer (BX-4000, Himeliskang Co., Tokyo, Japan). Each group includes 4 replicates.

2.6. Intestinal Histopathology

Intestinal and hepatic tissues fixed in 4% paraformaldehyde were processed for paraffin embedding. Embedded blocks were sectioned at 5 μm, mounted on glass slides, and stained with H&E. The stained sections were photographed using a Nikon microscope (Eclipse E100, Nikon, Tokyo, Japan), and the histomorphological and pathological observations were performed using Scope Image 9.0 software (Bioimager Inc., Richmond Hill, ON, Canada). Each group includes 12 replicates.

2.7. Determination of Antioxidant Indicators

All measurement steps were carried out according to the instructions provided by Nanjing Jiancheng Bioengineering Institute. Precisely weighed 0.1 g of rat jejunal and liver tissues were separately homogenized with cold physiological saline at an appropriate ratio. The homogenates of the jejunal and liver tissues were then centrifuged under the conditions described in the kit instructions. The total protein concentration in the supernatant was measured using a Total Protein (TP) Assay Kit (Catalog No.: A045-2-2). Subsequently, the following indicators were measured using the respective kits: Total Antioxidant Capacity (T-AOC) Assay Kit (Catalog No.: A015-1-2), Malondialdehyde (MDA) Assay Kit (Catalog No.: A003-1-2), Reactive Oxygen Species (ROS) Assay Kit (Catalog No.: E004-1-1), Total Superoxide Dismutase (T-SOD) Assay Kit (Catalog No.: A001-1-2), Catalase (CAT) Assay Kit (Catalog No.: A007-1-1), Glutathione Peroxidase (GSH-Px) Assay Kit (Catalog No.: A005-1-2), and Reduced Glutathione (GSH) Assay Kit (Catalog No.: A006-1-1). Each group included 4 replicates.

2.8. Assessment of Immunological Parameters

All measurement steps were carried out according to the instructions provided by Nanjing Hongsheng Biotechnology Co., Ltd. Accurately weigh 0.1 g each of rat jejunal and liver tissues were homogenized with ice-cold physiological saline at an appropriate ratio, and then centrifuged to obtain the homogenates of the jejunal and liver tissues under the conditions described in the reagent kit instructions. The supernatant total protein concentration was then measured using the Total Protein (TP) Assay Kit (Cat. No.: A045-2-2) from Nanjing Jiancheng Bioengineering Institute. Finally, the parameters were measured using the following ELISA kits: rat immunoglobulin G (IgG) ELISA Kit (Cat. No.: CKE33921-1), rat immunoglobulin M (IgM) ELISA Kit (Cat. No.: CKE30629-1), rat interleukin 6 (IL-6) ELISA Kit (Cat. No.: CKE33628-1), rat interleukin 1β (IL-1β) ELISA Kit (Cat. No.: CKE30206-1), rat tumor necrosis factor α (TNF-α) ELISA Kit (Cat. No.: CKE33827-1), rat intercellular adhesion molecule 1 (ICAM-1; CD54) ELISA Kit (Cat. No.: CKE30356-1), and rat myeloperoxidase (MPO) ELISA Kit (Cat. No.: CKE31928-1). Each group included 4 replicates.

2.9. Transcriptomics Analysis

Transcriptomic analysis procedures for jejunal and liver tissues (0.1 g each) followed our published protocol[11]. The final sequencing libraries were sequenced on the NovaSeq 6000 platform (Illumina, USA) at Paiseno Biotech Co., Ltd. (Shanghai, China). All sequence data have been submitted to the NCBI database (BioProject ID: PRJNA1121032). Each group included 4 replicates.

2.10. Reverse Transcription Quantitative PCR (RT-qPCR)

mRNA expression levels of differentially expressed genes screened by transcriptomics were measured using RT-qPCR. Total RNA was extracted from jejunal and liver tissues (0.05 g each) using the TRIzol Reagent kit (Invitrogen, USA, Cat. No.: 15596-026) according to the kit instructions, and RNA concentration and purity were determined with a NanoDrop 2000 spectrophotometer (Nanodrop, USA), ensuring A260/A280 ratios between 1.8 and 2.0. Reverse transcription was carried out using the All-In-One 5X RT MasterMix kit (Nanjing Hongsheng Biotech Co., Ltd., Cat. No.: G592), followed by qPCR quantification of target genes using the BlasTaq 2X qPCR MasterMix kit (Nanjing Hongsheng Biotech Co., Ltd., Cat. No.: G892). Primer sequences for jejunal antioxidant or immune-inflammation-related genes IRF7, Ccl20, and RT1-M2, liver antioxidant or immune-inflammation-related genes Duox1, Cyp4a2, and Tcf19, as well as the reference gene GAPDH, are shown in Table 3. Primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Gene expression in the liver and intestine of the FS group rats was set to 1, and relative expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method[12]. Each group included 4 replicates, and each replicate included 2 technical repeats.

Table 3. Primer sequences for PCR.

Gene name	Gene bank ID	Primer	Sequences
GAPDH	NM_017008.4	F	GACATGCCGCCTGGAGAAAC
		R	AGCCCAGGATGCCCTTTAGT
IRF7	NM_001033691.1	F	GGACGCTGGATCAACACCTGTG
		R	ACGGGCAGTCTGGGAGAAAGTAG
Ccl20	NM_019233.2	F	TTCACAACACAGATGGCCGA
		R	GGTTCTTAGGCTGAGGAGGTG
RT1-M2	NM_001001717.2	F	GGCCCTGACCCAGTCCTTT
		R	CTGCGCGCAGTAGAGTCTC
Duox1	NM_153739.3	F	AACCCTACCTGCCTAACCC
		R	CTGTCCAGTGCTGCGGTC
Cyp4a2	NM_001044770.2	F	ACCAGATTCTCCTCGCCATAGCC
		R	GCTTCTTGAGACGCAGGTGGATC
Tcf19	NM_213561.3	F	ATCACGGTCCCTCGGTCCAAG
		R	TCTCATCATCCAGTTCTGCCAACAC

2.11. Western Blot (WB)

WB technology was used to further measure the protein expression levels of differentially expressed genes screened from transcriptomics, based on RT-qPCR. Protein extraction was performed using T-PER Tissue Protein Extraction Reagent (Thermo Scientific, USA, Cat. No. 78510) following the manufacturer's instructions to extract total protein from jejunal tissue (0.2 g) and liver tissue (0.2 g) samples, with the addition of Halt Protease and Phosphatase Inhibitor (100X) (Thermo Fisher, USA, Cat. No. 78440) during extraction to inhibit protease and phosphatase activity. Protein concentration was determined using the BCA Protein Assay Kit (Shanghai Beyotime, Cat. No. P0010) and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Electrophoresis was carried out using the Mini-PROTEAN electrophoresis system and Mini Trans-Blot transfer system (Bio-Rad, USA). Protein samples were separated by SDS-PAGE, with 8%-12% separation gel and 5% stacking gel prepared, loading 60 μg of total protein per lane. Electrophoresis conditions were 60 V for pre-running and 80 V for separation for 2 hours. After electrophoresis, proteins were transferred onto a PVDF membrane (Millipore, USA, Cat. No. IPVH00010), which was pre-soaked in methanol for 20 s and equilibrated in Tris-Glycine transfer buffer (containing 5% methanol) for 5 min. Transfer conditions were 100 V constant voltage, wet transfer for 2 hours. After transfer, the PVDF membrane was blocked in T-TBS buffer (containing 3% skim milk) at room temperature for 1 hour, then washed with T-TBS for 5 min × 3 times. Primary antibodies for target proteins Ccl20 in jejunal tissue and Duox1 in liver tissue, as well as the internal reference protein GAPDH (specific information in Table 4), were diluted in T-TBS and incubated overnight at 4℃. The next day, membranes were washed with T-TBS for 5 min × 4 times. Subsequently, Goat anti-rabbit IgG (H + L) secondary antibody

(Thermo Fisher Scientific, USA, Cat. No. 31431) was diluted 1:5000 in T-TBS (containing 2% skim milk), incubated at room temperature for 1 hour, and washed with T-TBS for 5 min × 5 times. Protein band signals were detected using SuperSignal Enhanced Chemiluminescence (ECL) substrate (Thermo Fisher Scientific, USA, Cat. No. 34075), and ECL DualVue Western Markers (Merck, Germany, Cat. No. RPN810) were prepared according to kit instructions. The membrane was incubated at room temperature for 1 min, excess ECL reagent was removed, sealed with plastic wrap, and exposed to X-ray film (Hangzhou Huadong Medicine Co., Ltd.) for 5 ~ 10 min before development and fixation. Results were analyzed using Quantity One software (Bio-Rad, USA) for grayscale analysis. Each group included 3 replicates, with each replicate measured 3 times. Relative expression of target proteins = [(target protein optical density) / (internal reference optical density)] × 10.

Table 4. Primary antibody information.

Primary antibody name	Brand	Catalog number	Dilution	Molecular weight (kDa)
Ccl20	ABCAM	# Ab9829	1:1000	10
Duox1	Proteintech	67226-1-AP	1:600	138
GAPDH	ABCAM	# Ab181602	1:2000	36

2.12. Data Analysis

Data were analyzed using IBM SPSS Statistics software (OEM version, 26.0, IBM Corp., Armonk, NY, USA) with a 2 × 2 two-way analysis of variance (ANOVA). Each measured parameter served as the dependent variable; “soybean meal type (S),” “Q level (Q),” and their interaction (S × Q) were treated as fixed effects to evaluate the significance of main and interaction effects. When the interaction was significant, Tukey’s test was applied for pairwise comparisons. Results are presented as means ± standard error of the mean (SEM). Differentially expressed genes (DEGs) in transcriptomics were screened by DESeq analysis, with criteria of |fold change| > 2 and $P < 0.05$. Differential proteins detected by WB were analyzed using one-way ANOVA followed by Tukey’s test. $P < 0.05$ was considered significant.

3. Results

3.1. Serum Biochemical Indicators

As shown in Table 5, compared with the FS diet, the OS diet significantly increased GLU and UN levels ($P < 0.05$). A significant interaction effect of S × Q on GLB levels was observed ($P < 0.05$), indicating that the addition of Q had a significant mitigating effect on the abnormal elevation of GLB levels in the blood of rats fed the OS diet.

Table 5. Effects of OS and Q on serum biochemical indicators in rats.

Items	**GLU (mmol/L)**	**TP (g/L)**	**ALB (g/L)**	**GLB (g/L)**	**GPT (U/L)**	**GOT (U/L)**	**UN (mmol/L)**	**TC (mmol/L)**
FS	2.23	68.28	44.13	24.15^{xy}	70.18	338.70	5.11	2.64
FS+Q	2.64	69.50	43.10	26.40^{xy}	67.60	289.05	5.21	2.39
OS	3.62	72.45	40.95	31.50^{x}	63.00	269.55	6.35	2.24
OS+Q	3.44	65.10	44.45	20.65^{y}	60.80	190.40	5.82	2.03
SEM	0.18	1.22	0.83	1.43	2.52	33.32	0.15	0.24

Main effects								
Soybean meal type (S)								
FS	2.44^{b}	68.89	43.62	25.28	68.89	313.88	5.16^{b}	2.52
OS	3.53^{a}	68.78	42.70	26.08	61.90	229.98	6.09^{a}	2.14
Q level (Q)								
−	2.93	70.37	42.54	27.83	66.59	304.13	5.73	2.44
+	3.04	67.30	43.78	23.53	64.20	239.73	5.52	2.21
P value								
S	<0.001	0.961	0.597	0.728	0.206	0.240	<0.001	0.487
Q	0.617	0.203	0.476	0.080	0.656	0.361	0.252	0.666
S×Q	0.215	0.084	0.204	0.013	0.972	0.832	0.100	0.965

^{a,b,A,B,x,y} within a column, values with no common superscripts differ significantly ($P < 0.05$).

3.2. Intestinal Histopathology

Fig. 1 shows histological sections of the duodenum, jejunum, and ileum of rats. Compared to the FS diet, rats fed the OS diet exhibited inflammatory lesions of varying sizes, with some areas of the ileal epithelium showing defects and a reduction in goblet cells. In contrast, compared with the diets not supplemented with Q, those groups supplemented with Q showed intact intestinal architecture and markedly reduced inflammation.

Fig. 2 shows histopathological sections of rat liver tissue. Compared to the FS diet, the rats fed the OS diet exhibited blood stasis, hemorrhage, and lymphocyte infiltration around the central vein. In contrast, compared with the diet without Q supplementation, those groups supplemented with Q showed normal liver tissue morphology, with reduced hemorrhage and inflammatory responses.

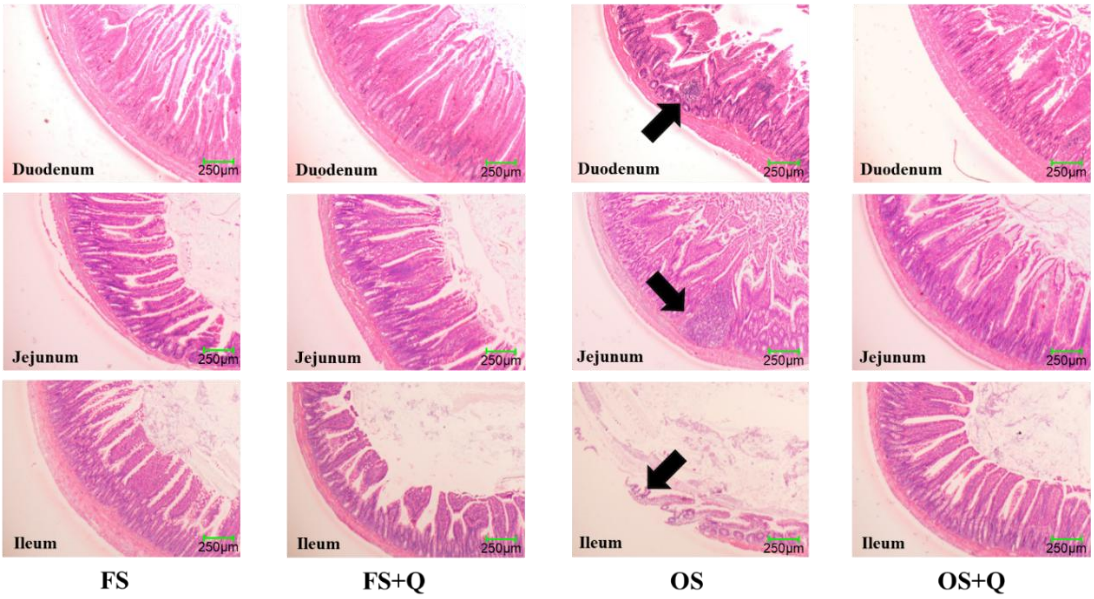


Figure 1. Effect of OS and Q on intestinal histopathology in rat.

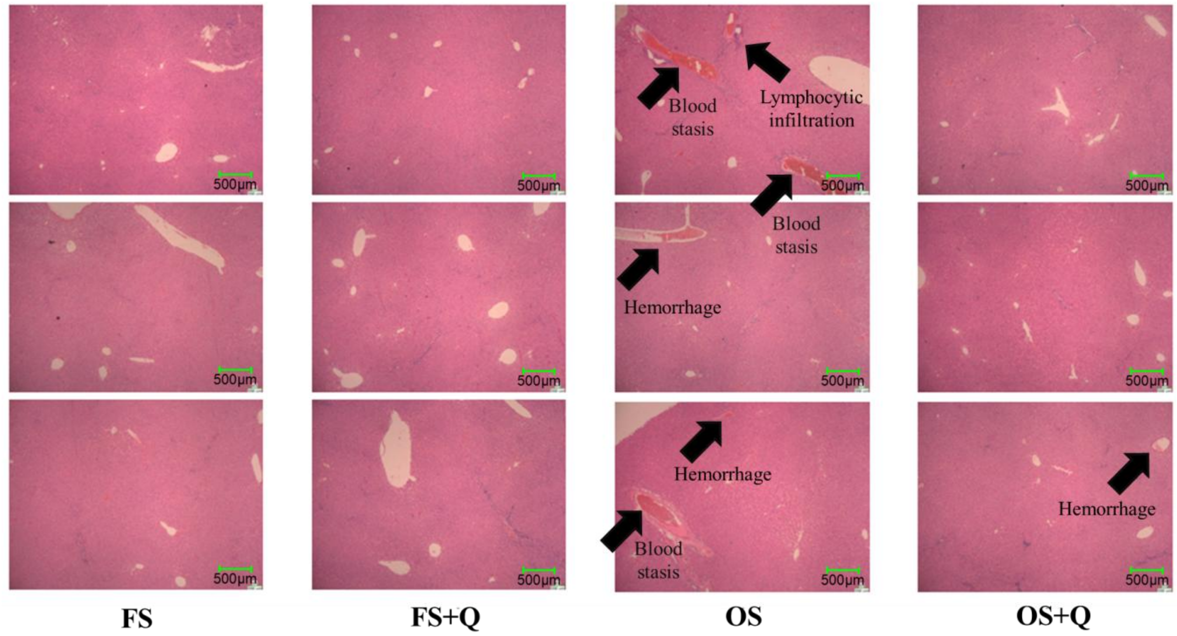


Figure 2. Effect of OS and Q on liver histopathology in rats.

3.3. Antioxidant Indicators

As presented in Table 6, compared with the FS diet, the OS diet significantly decreased jejunal T-AOC and GSH-Px activity ($P < 0.05$), while significantly increasing jejunal ROS levels and CAT activity ($P < 0.05$); compared with no added Q, supplementation with Q significantly reduced jejunal ROS levels and increased CAT activity ($P < 0.05$); there was a significant S × Q interaction effect on jejunal T-AOC ($P < 0.05$), indicating that Q supplementation had a significant enhancing effect on the reduction of jejunal T-AOC in rats fed the OS diet.

Table 6. Effects of OS and Q on jejunal antioxidant indicators in rats.

Items	T-AOC (U/mg prot.)	MDA (nmol/mg prot.)	ROS (a.u./mg prot.)	T-SOD (U/mg prot.)	CAT (U/mg prot.)	GSH (μmol/g prot.)	GSH-Px (U/mg prot.)
FS	4.91^{x}	3.76	185.91	28.13	1.19	8.40	85.50
FS+Q	4.68^{x}	3.69	179.88	29.23	1.52	8.91	89.93
OS	3.69^{y}	4.47	210.23	29.63	1.28	7.83	67.61
OS+Q	4.45^{xy}	3.51	186.45	28.04	2.11	8.11	74.43
SEM	0.16	0.18	4.37	0.52	0.12	0.20	3.51
Main effects							
Soybean meal type (S)							
FS	4.80^{a}	3.73	182.90^{b}	28.68	1.36^{b}	8.66	87.72^{a}
OS	4.07^{b}	3.99	198.34^{a}	28.84	1.70^{a}	7.97	71.02^{b}
Q level (Q)							
−	4.30	4.12	198.07^{A}	28.88	1.24^{B}	8.12	76.56
+	4.57	3.60	183.17^{B}	28.64	1.82^{A}	8.51	82.18

P value							
S	0.007	0.463	0.036	0.893	0.034	0.115	0.014
Q	0.233	0.172	0.041	0.832	0.002	0.338	0.326
S×Q	0.040	0.239	0.186	0.265	0.098	0.770	0.829

^{a,b,A,B,x,y} within a column, values with no common superscripts differ significantly ($P < 0.05$).

As shown in Table 7, the OS diet significantly increased hepatic MDA content while decreasing GSH and GSH-Px levels compared to the FS diet ($P < 0.05$). In contrast, Q supplementation significantly increased the T-AOC level and decreased the MDA content compared to the non-supplemented groups ($P < 0.05$).

Table 7. Effects of OS and Q on liver antioxidant indicators in rats.

Items	T-AOC (U/mg prot.)	MDA (nmol/mg prot.)	ROS (a.u./mg prot.)	T-SOD (U/mg prot.)	CAT (U/mg prot.)	GSH (μmol/g prot.)	GSH-Px (U/mg prot.)
FS	1.52	4.08	159.69	20.84	7.69	15.44	424.51
FS+Q	1.65	3.57	158.80	23.88	8.61	15.54	429.68
OS	1.26	5.54	169.82	21.79	8.08	13.55	364.93
OS+Q	1.64	4.26	159.81	21.98	8.24	15.07	399.46
SEM	0.06	0.25	3.70	0.55	0.24	0.31	10.27
Main effects							
Soybean meal type (S)							
FS	1.59	3.83^{b}	159.25	22.36	8.15	15.49^{a}	427.10^{a}
OS	1.45	4.90^{a}	164.82	21.89	8.16	14.31^{b}	382.20^{b}
Q level (Q)							
−	1.39^{B}	4.81^{A}	164.76	21.32	7.89	14.50	394.72
+	1.65^{A}	3.92^{B}	159.31	22.93	8.43	15.31	414.57
P value							
S	0.204	0.003	0.509	0.654	0.987	0.036	0.022
Q	0.032	0.009	0.518	0.157	0.324	0.123	0.246
S×Q	0.224	0.182	0.588	0.205	0.478	0.171	0.382

^{a,b,A,B,x,y} within a column, values with no common superscripts differ significantly ($P < 0.05$).

3.4. Immunological Parameters

According to the data in Table 8, compared with the FS diet, the OS diet significantly increased the levels of IgG and IL-6 in the jejunal tissue ($P < 0.05$).

Table 8. Effects of OS and Q on jejunal immune inflammatory indicators in rats.

Items	IgG (mg/mg prot.)	IgM (μg/mg prot.)	IL-6 (pg/mg prot.)	IL-1β (pg/mg prot.)	TNF-α (pg/mg prot.)	ICAM (ng/mg prot.)	MPO (ng/mg prot.)

FS	0.28	38.62	2.22	0.95	6.72	1.87	5.78
FS+Q	0.26	39.82	2.11	1.18	5.93	1.88	5.57
OS	0.43	47.48	3.34	1.02	6.38	1.90	5.97
OS+Q	0.43	48.42	3.01	0.98	6.24	1.90	5.79
SEM	0.03	2.79	0.19	0.06	0.27	0.11	0.21
Main effects							
Soybean meal type (S)							
FS	0.27^{b}	39.22	2.17^{b}	1.07	6.33	1.88	5.68
OS	0.43^{a}	47.95	3.18^{a}	1.00	6.31	1.90	5.88
Q level (Q)							
−	0.36	43.05	2.78	0.99	6.55	1.89	5.88
+	0.35	44.12	2.56	1.08	6.09	1.89	5.68
P value							
S	<0.001	0.168	0.003	0.626	0.985	0.928	0.668
Q	0.680	0.858	0.385	0.455	0.457	0.993	0.693
S×Q	0.629	0.983	0.646	0.298	0.597	0.999	0.974

^{a,b,A,B,x,y} within a column, values with no common superscripts differ significantly ($P < 0.05$).

As can be seen from Table 9, compared with the FS diet, the OS diet significantly increased liver IgG levels and significantly decreased IL-1β levels ($P < 0.05$); compared with no Q supplementation, adding Q significantly reduced liver IgG levels ($P < 0.05$); there was a significant interaction effect of S × Q on liver IgG levels ($P < 0.05$), meaning that Q supplementation had a significant mitigating effect on the abnormally elevated liver IgG levels in rats fed the OS diet.

Table 9. Effects of OS and Q on liver immune inflammatory indicators in rats.

Items	**IgG (mg/mg prot.)**	**IgM (μg/mg prot.)**	**IL-6 (pg/mg prot.)**	**IL-1β (pg/mg prot.)**	**TNF-α (pg/mg prot.)**	**ICAM (ng/mg prot.)**	**MPO (ng/mg prot.)**
FS	0.19^{y}	23.76	1.74	0.60	6.34	0.84	3.81
FS+Q	0.19^{y}	21.57	1.74	0.47	4.97	0.82	4.07
OS	0.27^{x}	24.65	1.86	0.43	4.44	0.72	4.12
OS+Q	0.20^{y}	23.09	1.64	0.39	4.51	0.78	3.81
SEM	0.01	1.06	0.10	0.03	0.36	0.03	0.12
Main effects							
Soybean meal type (S)							
FS	0.19^{b}	22.67	1.74	0.54^{a}	5.66	0.83	3.94
OS	0.24^{a}	23.87	1.75	0.41^{b}	4.48	0.75	3.97
Q level (Q)							
−	0.23^{A}	24.21	1.80	0.52	5.39	0.78	3.97

+	0.20^{B}	22.33	1.69	0.43	4.74	0.80	3.94
P value							
S	0.002	0.625	0.958	0.046	0.109	0.267	0.931
Q	0.006	0.450	0.633	0.170	0.347	0.775	0.930
S×Q	0.006	0.897	0.642	0.476	0.302	0.542	0.328

^{a,b,A,B,x,y} within a column, values with no common superscripts differ significantly ($P < 0.05$).

3.5. Transcriptomics

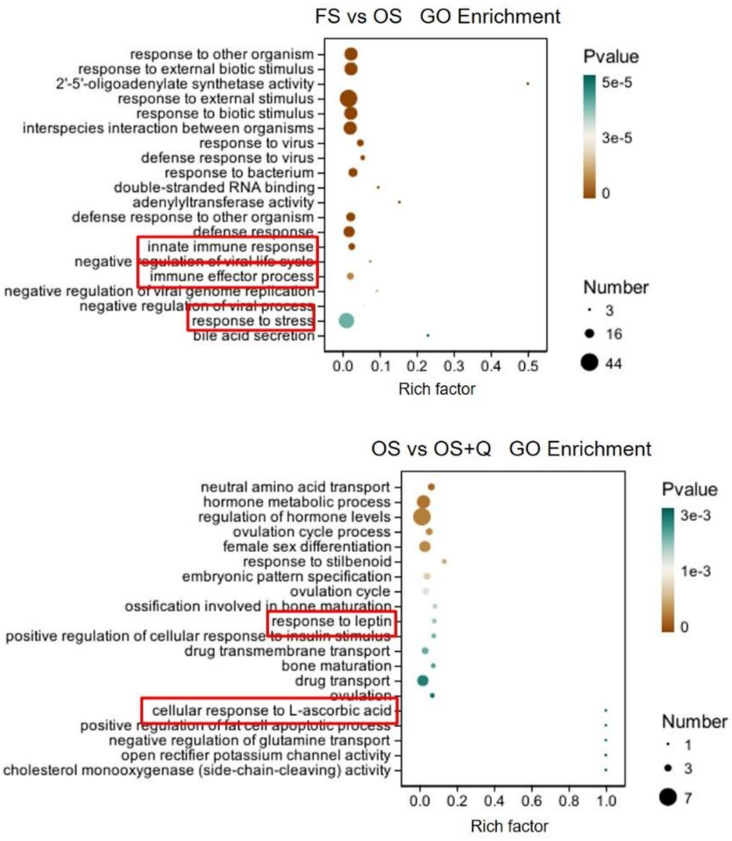
Among the top 20 GO items enriched by gene ontology (GO) analysis, “innate immune response, immune effect process, response to stress” between FS vs OS group, and “response to leptin, cellular response to L-ascorbic acid” between OS vs OS + Q group were related to jejunal antioxidant or immune inflammation (Fig. 3A).

Among the top 20 signaling pathways enriched by the Kyoto Encyclopedia of Genomes and Genomes (KEGG), the following pathways were related to jejunal antioxidant or immune inflammation: “NOD-like receptor signaling pathway, RIG-I receptor signaling pathway, complement and coagulation cascades, glycosphingolipid biosynthesis-globo and iso globo series, PPAR signaling pathway, IL-17 signaling pathway” between FS vs OS group, and “glutathione metabolism, chemical carcinogenesis-ROS” between OS vs OS+Q groups (Fig. 3B).

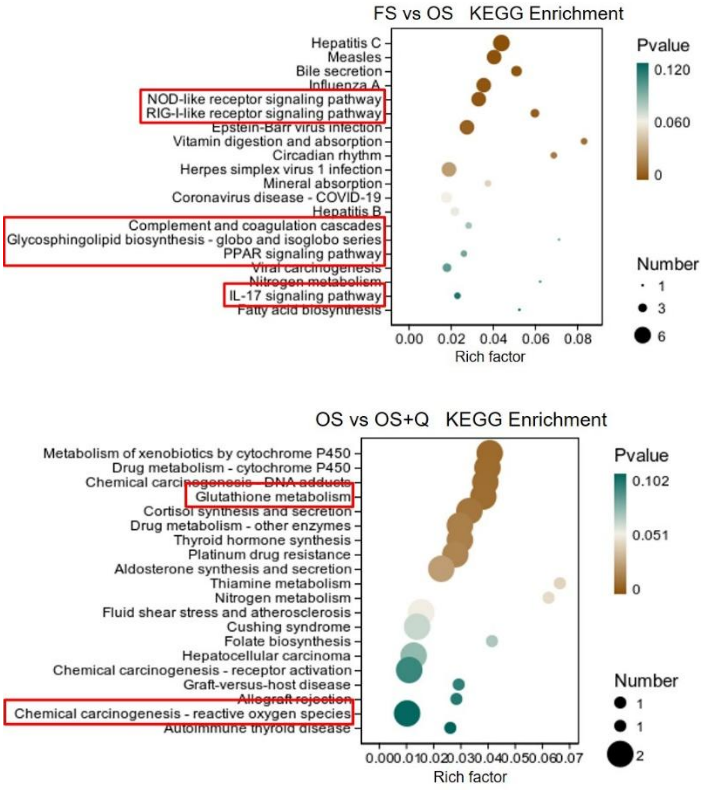
To integrate gene expression results systematically, we performed gene set enrichment analysis (GSEA) based on all annotated genes to clarify the regulatory effect of OS treatment and Q supplementation on key jejunal pathways in rats. Compared with the FS group, the “heme biosynthetic process (GO_0006783)” and the “reactive oxygen species biosynthetic process (GO_1903409)” were up-regulated in the OS group, while the “NOD-like receptor signaling pathway (RNO04621)” and the “RIG-I-like receptor signaling pathway (RNO04622)” were down-regulated in the OS group (P <0.05) (Fig. 3C).

However, compared with the OS group, the “heme biosynthesis process (GO_0006783)” was down-regulated in the OS + Q group ($P < 0.05$), while the “hydrogen peroxide catabolic process (GO_0042744)”, the “B-cell receptor signaling pathway (RNO04662)”, and the “T-cell receptor signaling pathway (RNO04660)” were up-regulated in the OS + Q group ($P < 0.05$) (Fig. 3D).

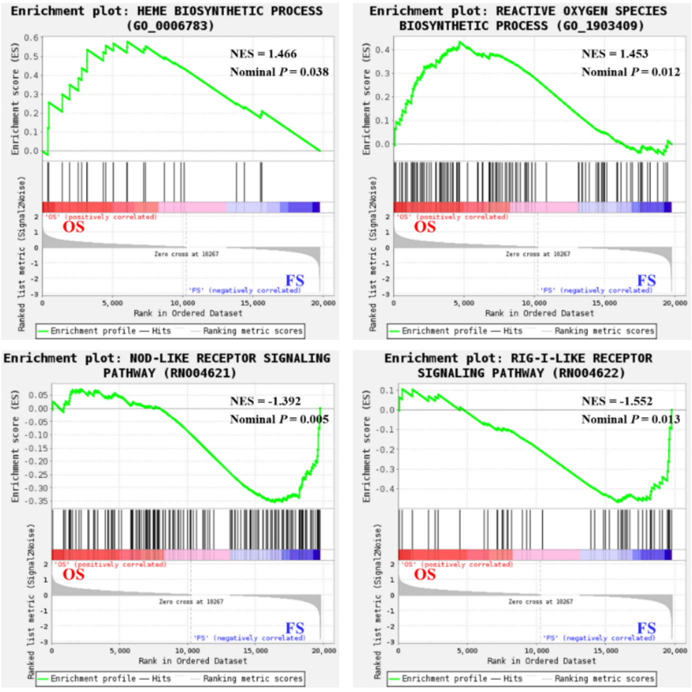
A


FS vs OS GO Enrichment
response to other organism
response to external biotic stimulus
2'-5'-oligoadenylate synthetase activity
response to external stimulus
response to biotic stimulus
interspecies interaction between organisms
response to virus
defense response to virus
response to bacterium
double-stranded RNA binding
adenylyltransferase activity
defense response to other organism
defense response
innate immune response
negative regulation of viral life cycle
immune effector process
negative regulation of viral genome replication
negative regulation of viral process
response to stress
bile acid secretion
0.0 0.1 0.2 0.3 0.4 0.5
Rich factor
Pvalue
5e-5
3e-5
0
Number
3
16
44
OS vs OS+Q GO Enrichment
neutral amino acid transport
hormone metabolic process
regulation of hormone levels
ovulation cycle process
female sex differentiation
response to stilbenoid
embryonic pattern specification
ovulation cycle
ossification involved in bone maturation
response to leptin
positive regulation of cellular response to insulin stimulus
drug transmembrane transport
bone maturation
drug transport
ovulation
cellular response to L-ascorbic acid
positive regulation of fat cell apoptotic process
negative regulation of glutamine transport
open rectifier potassium channel activity
cholesterol monooxygenase (side-chain-cleaving) activity
0.0 0.2 0.4 0.6 0.8 1.0
Rich factor
Pvalue
3e-3
1e-3
0
Number
1
3
7

B


FS vs OS KEGG Enrichment
Hepatitis C
Measles
Bile secretion
Influenza A
NOD-like receptor signaling pathway
RIG-I-like receptor signaling pathway
Epstein-Barr virus infection
Vitamin digestion and absorption
Circadian rhythm
Herpes simplex virus 1 infection
Mineral absorption
Coronavirus disease - COVID-19
Hepatitis B
Complement and coagulation cascades
Glycosphingolipid biosynthesis - globo and isoglobo series
PPAR signaling pathway
Viral carcinogenesis
Nitrogen metabolism
IL-17 signaling pathway
Fatty acid biosynthesis
0.00 0.02 0.04 0.06 0.08
Rich factor
Pvalue
0.120
0.060
0
Number
1
3
6
OS vs OS+Q KEGG Enrichment
Metabolism of xenobiotics by cytochrome P450
Drug metabolism - cytochrome P450
Chemical carcinogenesis - DNA adducts
Glutathione metabolism
Cortisol synthesis and secretion
Drug metabolism - other enzymes
Thyroid hormone synthesis
Platinum drug resistance
Aldosterone synthesis and secretion
Thiamine metabolism
Nitrogen metabolism
Fluid shear stress and atherosclerosis
Cushing syndrome
Folate biosynthesis
Hepatocellular carcinoma
Chemical carcinogenesis - receptor activation
Graft-versus-host disease
Allograft rejection
Chemical carcinogenesis - reactive oxygen species
Autoimmune thyroid disease
0.00 0.01 0.02 0.03 0.04 0.05 0.06 0.07
Rich factor
Pvalue
0.102
0.051
0
Number
1
1
2

C



D

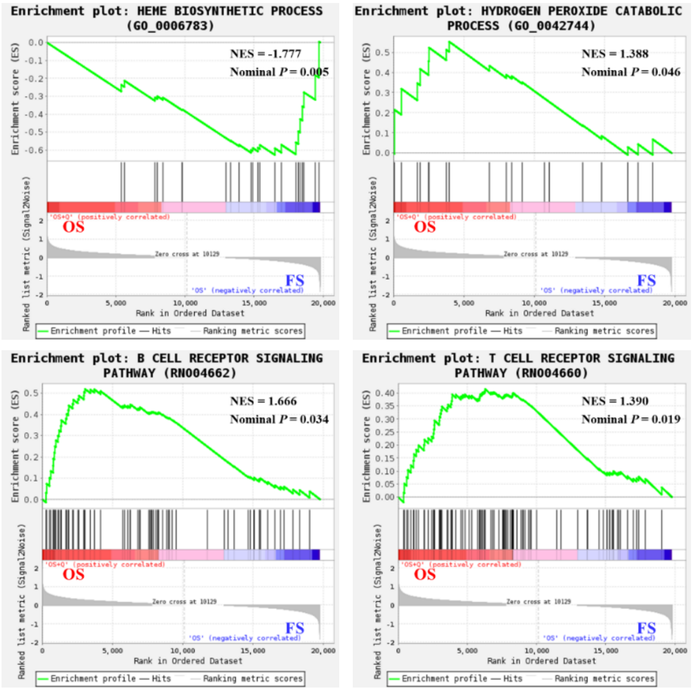


Figure 3. Results of transcriptomic analysis of jejunum. **(A)** GO pathway enrichment analysis of top 20 pathways. **(B)** KEGG pathway enrichment analysis of top 20 pathways. **(C)** Significant pathways of GSEA analysis results in FS vs OS comparison groups (Nominal $P < 0.05$), NES > 1 indicates positive enrichment, while NES < –1 indicates negative enrichment. **(D)** Significant pathways of GSEA analysis results in OS vs OS + Q comparison groups, NES > 1 indicates positive enrichment, while NES < –1 indicates negative enrichment (Nominal $P < 0.05$).

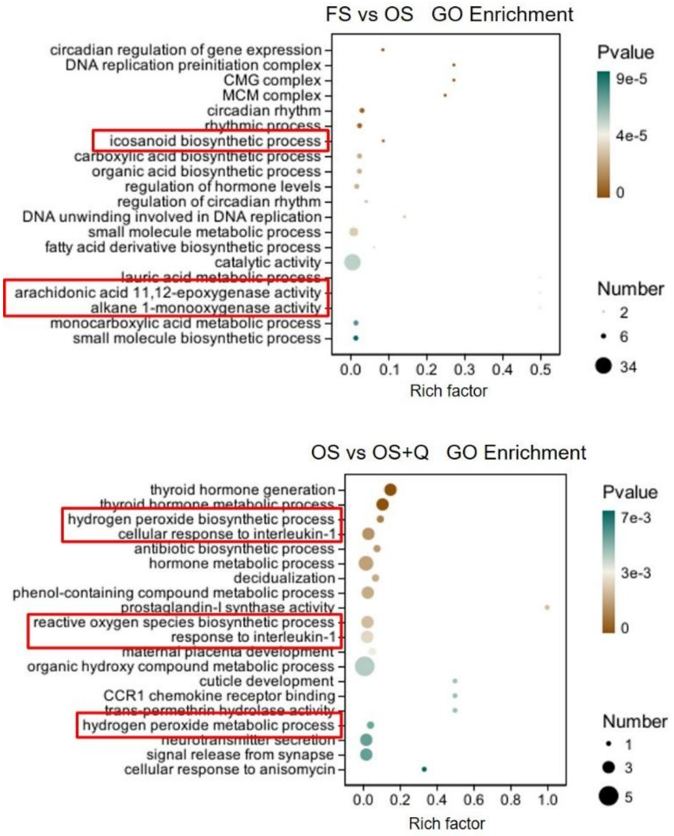
Among the top 20 GO items enriched by GO analysis, “icosanoid biosynthetic process, arachidonic acid 11,12-cyclooxygenase activity, alkane 1-monooxygenase activity” between FS vs OS groups, and “hydrogen peroxide biosynthesis process, cellular response to interleukin-1, ROS biosynthesis process, response to interleukin-1, hydrogen peroxide biosynthesis process” between OS vs OS + Q group were related to liver antioxidant or immune inflammation (Fig. 4A).

Among the top 20 significant pathways enriched by KEGG analysis, the “calcium signaling pathway, inflammatory mediator regulation of TRP channels” in the FS vs OS group, and the “IL-17 signaling pathway, FoxO signaling pathway, JAK-STAT signaling pathway, RIG-I-like receptor signaling pathway, VEGF signaling pathway” in the OS vs OS + Q group were related to liver antioxidant or immune inflammation (Fig. 4B).

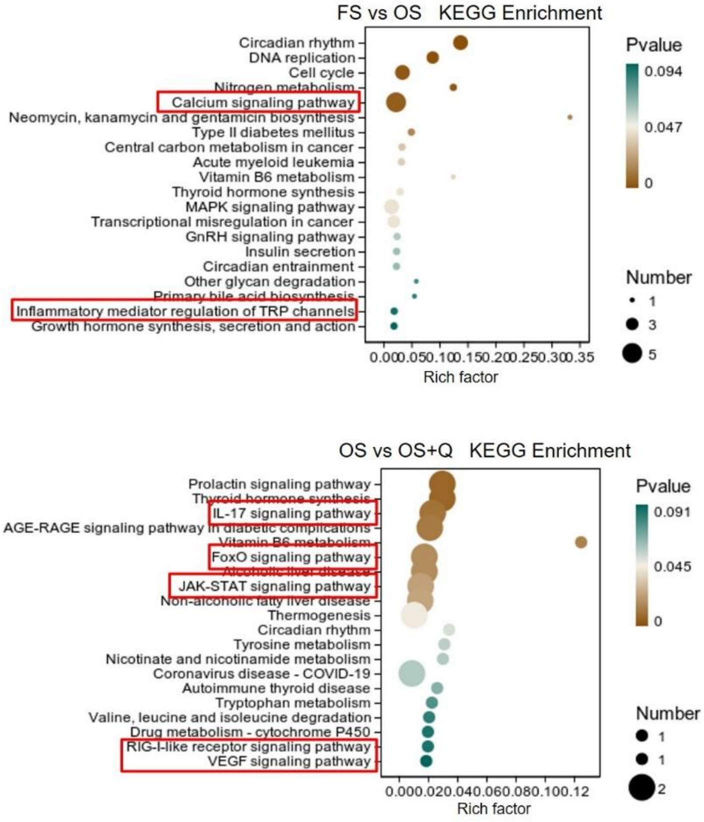
GSEA showed that compared with the FS group, the “cellular response to oxidative stress (GO_0034599)”, “cellular response to ROS (GO_0034614)”, “I-κB kinase signaling pathway (GO_0007249)” and “regulation of cytokine production (GO_0001817)” in the OS group were all significantly up-regulated ($P < 0.05$) (Fig. 4C).

However, compared with the OS group, the “ROS biosynthesis process (GO_1903409)”, “glutathione metabolism (RNO00480)”, “cytokine activity (GO_0005125)” and “acute inflammatory response (GO_0002526)” in the OS + Q group were all significantly down-regulated ($P < 0.05$) (Fig. 4D).

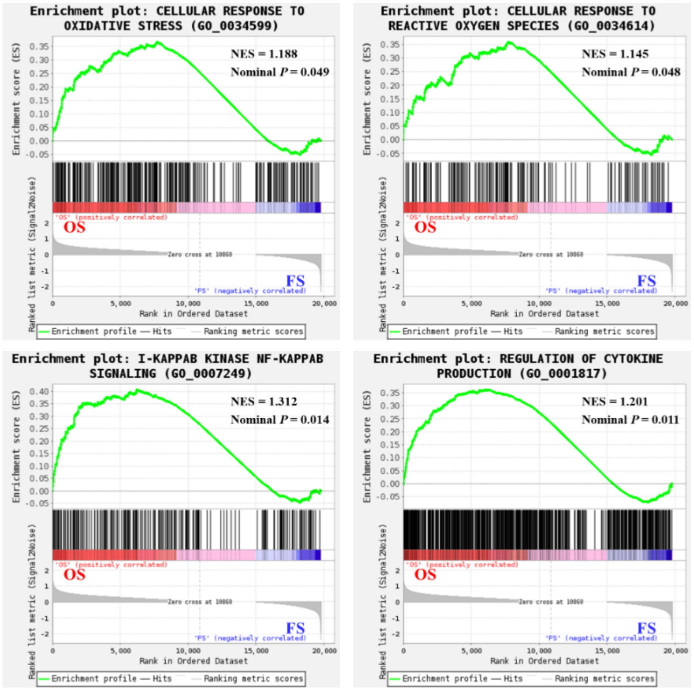
A



B


FS vs OS KEGG Enrichment
Circadian rhythm
DNA replication
Cell cycle
Nitrogen metabolism
Calcium signaling pathway
Neomycin, kanamycin and gentamicin biosynthesis
Type II diabetes mellitus
Central carbon metabolism in cancer
Acute myeloid leukemia
Vitamin B6 metabolism
Thyroid hormone synthesis
MAPK signaling pathway
Transcriptional misregulation in cancer
GnRH signaling pathway
Insulin secretion
Circadian entrainment
Other glycan degradation
Primary bile acid biosynthesis
Inflammatory mediator regulation of TRP channels
Growth hormone synthesis, secretion and action
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35
Rich factor
Pvalue
0.094
0.047
0
Number
1
3
5
OS vs OS+Q KEGG Enrichment
Prolactin signaling pathway
Thyroid hormone synthesis
IL-17 signaling pathway
AGE-RAGE signaling pathway in diabetic complications
Vitamin B6 metabolism
FoxO signaling pathway
Alcoholic liver disease
JAK-STAT signaling pathway
Non-alcoholic fatty liver disease
Thermogenesis
Circadian rhythm
Tyrosine metabolism
Nicotinate and nicotinamide metabolism
Coronavirus disease - COVID-19
Autoimmune thyroid disease
Tryptophan metabolism
Valine, leucine and isoleucine degradation
Drug metabolism - cytochrome P450
RIG-I-like receptor signaling pathway
VEGF signaling pathway
0.00 0.02 0.04 0.06 0.08 0.10 0.12
Rich factor
Pvalue
0.091
0.045
0
Number
1
1
2

C


Enrichment plot: CELLULAR RESPONSE TO OXIDATIVE STRESS (GO_0034599)
NES = 1.188
Nominal P = 0.049
Enrichment plot: CELLULAR RESPONSE TO REACTIVE OXYGEN SPECIES (GO_0034614)
NES = 1.145
Nominal P = 0.048
Enrichment plot: I-KAPPAB KINASE NF-KAPPAB SIGNALING (GO_0007249)
NES = 1.312
Nominal P = 0.014
Enrichment plot: REGULATION OF CYTOKINE PRODUCTION (GO_0001817)
NES = 1.201
Nominal P = 0.011
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'OS' (positively correlated)
OS
Zero cross at 10860
FS
'FS' (negatively correlated)
Rank in Ordered Dataset
0 5,000 10,000 15,000 20,000
Enrichment profile — Hits — Ranking metric scores

D

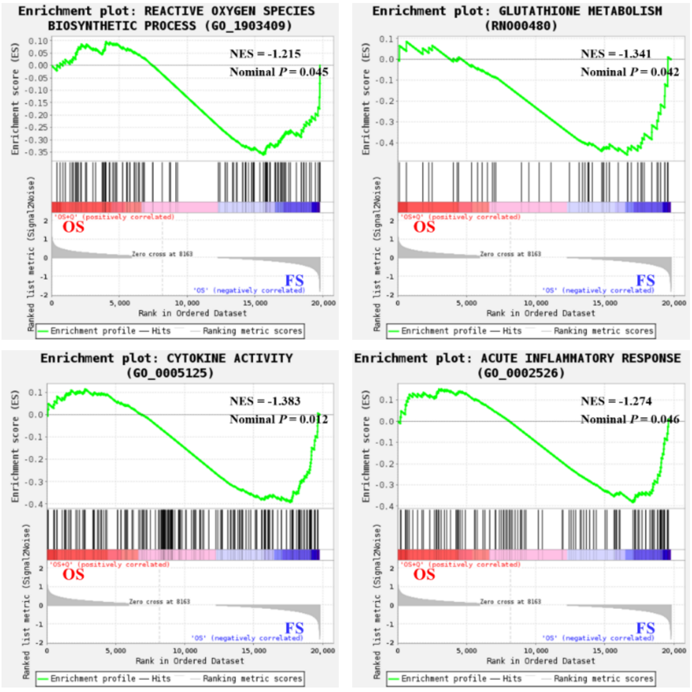


Figure 4. Results of transcriptomic analysis of liver. **(A)** GO pathway enrichment analysis of top 20 pathways. **(B)** KEGG pathway enrichment analysis of top 20 pathways. **(C)** Significant pathways of GSEA analysis results in FS vs OS comparison groups (Nominal $P < 0.05$), NES > 1 indicates positive enrichment, while NES < –1 indicates negative enrichment. **(D)** Significant pathways of GSEA analysis results in OS vs OS+Q comparison groups, NES > 1 indicates positive enrichment, while NES < –1 indicates negative enrichment (Nominal $P < 0.05$).

3.6. RT-qPCR

As illustrated in Fig. 5, compared with the FS diet, the mRNA expression level of the gene *Ccl20*, which is related to antioxidant or immune inflammation, in the jejunal tissue of rats fed the OS diet was significantly increased ($P < 0.05$); compared with no added Q, adding Q significantly increased the mRNA expression level of the gene *RT1-M2* ($P < 0.05$); there was a significant interaction effect of S × Q on the mRNA expression level of *RT1-M2* ($P < 0.05$), indicating that adding Q significantly enhanced the expression of the *RT1-M2* gene in the jejunal tissue of rats fed the OS diet, which otherwise would be decreased.

As shown in Fig. 6, compared with the FS diet, the mRNA expression level of the gene *Cyp4a2*, which is related to antioxidant or immune inflammation, in the liver tissue of rats fed the OS diet was significantly increased ($P < 0.05$), while the mRNA expression level of *Tcf19* was significantly decreased ($P < 0.05$); compared with no added Q, adding Q significantly reduced the mRNA expression level of *Duox1* ($P < 0.05$); there was a significant interaction effect of S×Q on the mRNA expression levels of *Duox1* and *Cyp4a2* ($P < 0.05$), indicating that adding Q significantly reduced the abnormally elevated expression of *Duox1* and *Cyp4a2* genes in the liver of rats fed the OS diet.

As presented in Fig. 7, the mRNA expression levels of some differential genes in the jejunal and liver transcriptomes related to antioxidant or immune inflammation were highly consistent with their RT-qPCR results. The correlation coefficient of these genes in the jejunal tissue was $R^2 = 0.9738$ in the FS vs OS comparison group and $R^2 = 0.9794$ in the OS vs OS Q comparison group; in the liver, the correlation coefficient was $R^2 = 0.9810$ in the FS vs OS comparison group and $R^2 = 0.9708$ in the OS vs OS Q comparison group. This indicates that the transcriptome results of the jejunal and liver tissues are highly reliable.

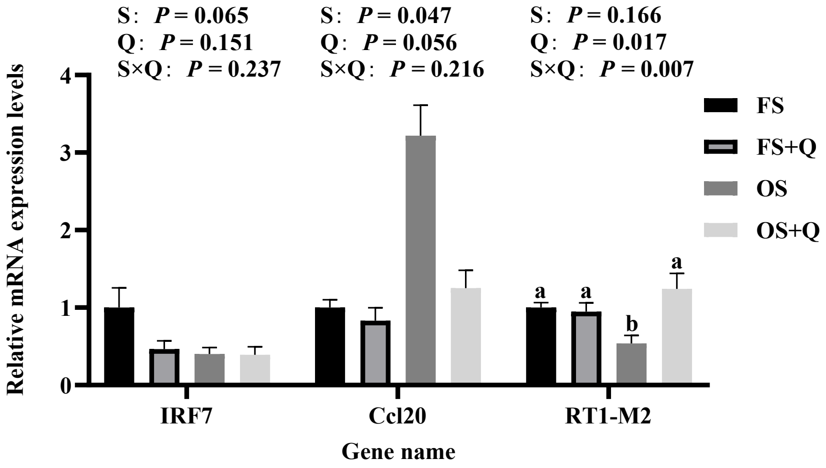


Figure 5. The mRNA expression levels of selected differential genes in rat jejunal tissue. $^{a, b}$ Values in a row with no common letters differ significantly ($P < 0.05$).

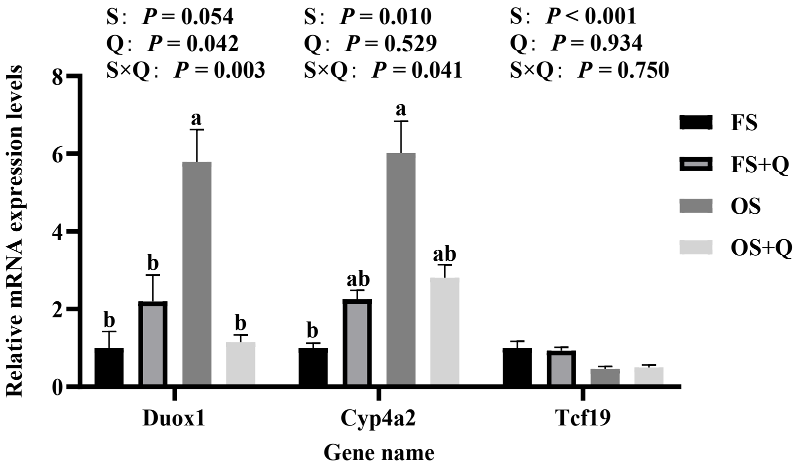


Figure 6. The mRNA expression levels of selected differential genes in rat liver tissue. $^{a, b}$ Values in a row with no common letters differ significantly ($P < 0.05$).

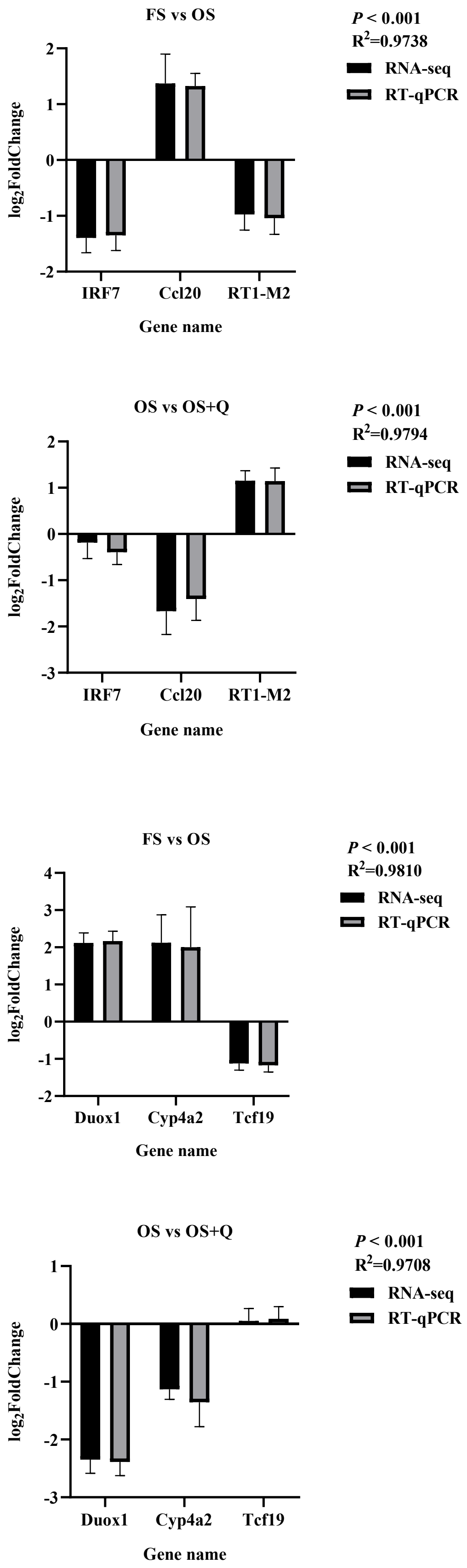


Figure 7. The results of correlation analysis between transcriptome expression and RT-qPCR expression of mRNA of some differential genes.

3.7. WB

As shown in Fig. 8, compared with FS, the expression of the inflammation-related protein Ccl20 in the jejunal tissue of rats fed an OS diet was significantly increased ($P < 0.05$), while the addition of Q to the OS diet significantly reduced the expression of Ccl20 ($P < 0.05$); compared with FS, the expression of the ROS-related protein Duox1 in the liver tissue of rats fed an OS diet was significantly increased ($P < 0.05$), while the addition of Q to the OS diet significantly reduced the expression of Duox1 ($P < 0.05$).

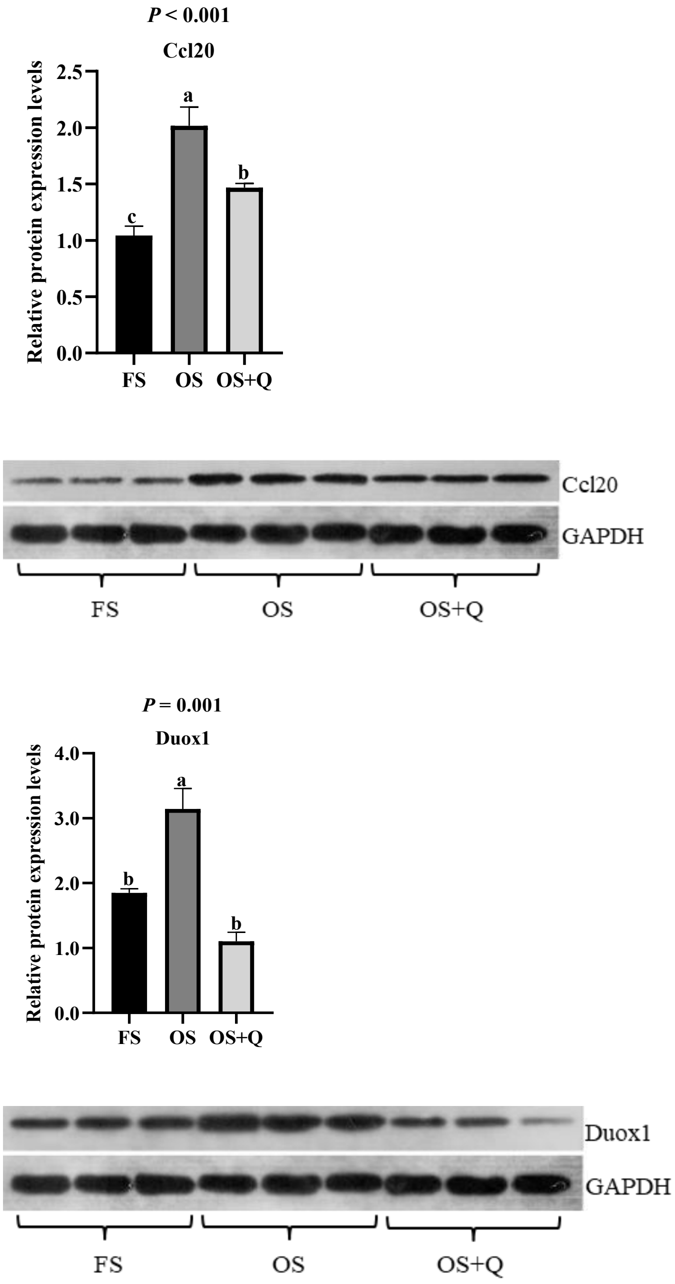


Figure 8. WB results of protein expression corresponding to some differential genes. $^{a-c}$ Values in a row with no common letters differ significantly ($P < 0.05$).

4. Discussion

4.1. Effects of Q on Blood Biochemical Indicators in Rats Fed OS

According to the table 1, compared with FS, the content of protein carbonyl in OS was significantly increased, but the content of protein free sulfhydryl was significantly decreased.

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Consequently, the antioxidant status of rats fed an OS diet is inevitably affected. Some literature points out that long-term consumption of overly oxidized protein foods can lead to oxidative stress in animals[4,5]. Blood biochemical indicators can partially reflect the status of oxidative stress in animals[13]. Research has shown that when animals experience oxidative stress, their glucose metabolism accelerates, insulin resistance increases, and the balance of glucose synthesis and degradation is disrupted, leading to elevated blood GLU levels[14]. The results of this experiment showed that, compared with the FS diet, the OS diet significantly increased serum GLU levels in rats, which aligns with the conclusions of the aforementioned studies. Similarly, compared with the FS diet, the OS diet significantly increased serum UN levels in rats, which was consistent with previous experimental results finding that heat-induced OS diet remarkably raised serum UN levels in broilers[15]. Literature also indicated that the nitrogen metabolism of animal was accelerated under oxidative stress[16]. This phenomenon suggests that OS affects the synthesis and degradation metabolism of amino acids and proteins in rats[11], and may have adverse effects on their liver and renal function by increasing the nitrogen metabolic load[17]. In addition, OS and Q showed a significant interaction on GLB levels, which indicates that Q can significantly reverse the OS-induced elevation of immunoglobulin levels in rats. This is related to Q's ability to suppress inflammatory responses and restore humoral immunity and antibody levels in animals to normal[18,19].

4.2. Effects of Q on Tissue Pathological Changes in rats Fed OS

The accumulation of chronic oxidative stress precipitated significant structural damage to the intestinal mucosa, as evidenced by villus shortening and inflammatory cell infiltration, ultimately compromising gut barrier integrity[20]. Histopathological sections of intestinal tissue showed that, compared with the FS group, the OS group exhibited inflammatory lesions of varying sizes in the intestines, with some areas of ileal epithelium showing defects, reduced goblet cells, and other pathological changes. This is consistent with previous studies reporting that OS induced intestinal inflammation and epithelial damage in bluntnose bream[5], indicating that feeding OS led to substantial organ damage in rats. However, compared with the OS group, the intestinal tissue structure of the OS + Q group was intact, and the inflammatory lesions caused by oxidative stress were effectively alleviated. This is related to Q’s ability to inhibit oxidative damage, reduce inflammatory responses, and protect intestinal health[7,21]. These results indicate that adding Q has a significant mitigating effect on organ lesions in the intestines of rats induced by OS diet-related oxidative stress and enteritis.

Histopathological sections of liver tissue showed that, compared with the FS group, the OS group exhibited hepatic blood stasis, hemorrhage, and lymphocyte infiltration, indicating that OS caused oxidative stress damage and inflammatory responses in rat liver[22]. This result is similar to previous studies showing the negative effects of photo-oxidized milk protein on mouse liver histopathology[23], suggesting that oxidized protein feed not only causes intestinal damage but also harms liver health. However, compared with the OS group, the liver tissue of the OS + Q group appeared normal, and hemorrhage and inflammation were reduced. This is in line with Q’s antioxidant, anti-inflammatory, and hepatoprotective effects[24]. These results suggest that Q significantly alleviates oxidative stress damage and inflammatory responses in liver tissues caused by the OS diet in rats.

4.3. Effects of Q on Oxidative Stress and Immune Inflammation in the Jejunal and Liver Tissues of Rats Fed OS

Oxidative stress refers to the imbalance between oxidation and antioxidation in the animal body, usually triggered by ROS, ultimately leading to oxidative damage within the animal[25]. The results of this study showed that compared to the FS diet, the OS diet significantly reduced the T-AOC and GSH-Px levels in the jejunal tissue of rats and significantly increased ROS levels, which is generally consistent with previous findings that OS induces oxidative stress in broilers[4]. This result further confirms that oxidative stress occurred in rats fed the OS diet. However, compared to not adding Q, supplementation with Q significantly reduced ROS levels and increased CAT activity in the jejunal tissue of rats. This result is consistent with other studies showing that Q, as a strong antioxidant, can scavenge ROS in animals and thus enhance antioxidant capacity[26]. It also aligns with previous literature indicating that Q can enhance the antioxidant defense capacity of the body by elevating cardiac CAT activity in LPS-challenged rats[27]. In addition, OS and Q exhibited a significant interaction effect on T-AOC, indicating that Q can significantly reverse the OS-induced reduction of T-AOC levels in the rat liver, similar to previous experimental results[8].

The results of this study indicate that in the liver, compared to the FS diet, the OS diet significantly increased MDA content and significantly decreased GSH and GSH-Px activity. This suggests that oxidative stress occurred in the rat liver, consistent with previous results from feeding OS to broilers[4]. MDA is a product of lipid peroxidation and an important indicator of oxidative stress, which can cause liver cell damage[28]. GSH and GSH-Px are important antioxidant molecules representing the liver’s reserve antioxidant capacity[29]; their decreased activity indicates severe impairment of the liver’s antioxidant capacity in rats. However, compared to not adding Q, supplementation with Q significantly increased T-AOC levels and decreased MDA content. This result is similar to previous findings that Q can increase T-AOC levels in rat liver tissue[8] and is also comparable to its role in alleviating oxidative stress-induced renal tubular epithelial cell damage in hyperoxaluric model rats by reducing MDA levels[26]. Overall, these results indicate that Q has an effect in alleviating oxidative stress in the liver of rats fed an OS diet.

Research shows that persistent oxidative stress can activate the immune system, promoting the production of antibodies and cytokines[30]. Oxidative stress can also damage intestinal barrier function, leading to increased intestinal mucosal permeability, which allows pathogens and antigens to more easily penetrate the intestinal mucosa, thereby triggering immune responses and inflammation[31]. The results of this study indicate that in the jejunum, compared to the FS diet, the OS diet significantly increased IgG and IL-6 levels. This abnormal increase in IgG suggests that OS induces chronic inflammatory responses in the rat intestine[32]. The abnormal elevation of IL-6 levels suggests that OS triggers the expression of pro-inflammatory cytokines, a phenomenon similar to earlier studies showing that feeding laying hens stored soybean meal led to increased IL-1β and interleukin-4 (IL-4) mRNA expression in the jejunum[33].

Regarding immune inflammation in rat liver, the results of this study show that compared to the FS diet, the OS diet significantly increased liver IgG content and significantly reduced IL-1β content. IgG in liver tissue mainly originates from two sources: first, it is directly secreted by plasma cells infiltrating the liver[34]; second, it is transported from the mesenteric circulation. In this study, simultaneous increases in IgG in both the intestine and liver were observed, suggesting the possible abnormal activation of an “intestinal-liver axis immune dialogue”[35]. Literature indicates that IL-1β is a pro-inflammatory cytokine of the IL-1 family and plays a crucial role in antibacterial host defense. Its reduction suggests immune abnormalities caused by oxidative stress accumulation in the liver due to the OS diet[36]. Furthermore, compared to the group without Q, supplementation with Q significantly decreased IgG content, and OS and Q had a significant interactive effect on IgG levels. This indicates that Q has regulatory effects on liver antioxidant and immune responses, alleviating oxidative stress and inflammatory responses in rat liver, thereby significantly reversing the OS-induced increase in liver IgG levels in rats[37].

4.4. Effects of Q on the Transcription of Oxidative Stress and Immune Inflammation-Related Genes in Rats Fed OS

As the largest digestive organ in animals, the intestine is inevitably exposed to foreign substances and microbial metabolites, which are key factors that trigger the production of ROS in the animal intestine. Disproportionate production and long-term exposure to ROS will lead to the development of various intestinal diseases, such as inflammatory bowel disease[38]. The GSEA results of jejunal gene transcriptome showed that compared with FS diet, OS diet significantly up-regulated jejunal heme biosynthesis and ROS biosynthesis, and down-regulated NOD-like receptor signaling pathways and RIG-I-like receptor signaling pathways. This result suggests that OS may trigger a stress response in the intestine by triggering ROS production in the rat gut, and long-term ROS accumulation further triggers intestinal inflammation in rats[38]. However, the addition of Q to the OS diet significantly down-regulated the heme biosynthesis process, and significantly up-regulated the hydrogen peroxide catabolism process, B-cell receptor signaling pathway, and T-cell receptor signaling pathway, which is similar to the results of previous studies[39]. The above jejunal transcriptome results are basically consistent with the results of ROS content, CAT activity, antibody level and pro-inflammatory cytokine content in each group of jejunal tissue in this study.

The GSEA results of rat liver gene transcriptome showed that compared with FS diet, OS diet significantly up-regulated the response of rat liver cells to oxidative stress and ROS, I-κB kinase signaling pathway, and related signaling pathways that regulate cytokine production. This result suggests that the role of OS in liver transcription may be similar to that in the jejunum, both by triggering ROS production in rats, thereby triggering stress responses in the gut and liver, while long-term ROS accumulation further triggers inflammatory responses in the rat gut and liver[38]. The difference is that ROS mainly down-regulates NOD-like receptor signaling pathways and RIG-I-like receptor signaling pathways in jejunal inflammation, while mainly up-regulates NF-κB and related signaling pathways that regulate cytokine production in liver inflammation, which may be related to the influence of ROS on different inflammatory pathways in different tissues of rats[40]. However, the addition of Q based on OS diet significantly down-regulated ROS synthesis signaling pathway, glutathione metabolism pathway, cytokine activity pathway and acute inflammatory response in rat liver. This result indicates that oxidative stress and inflammatory responses in the rat liver are alleviated, resulting in a significant decrease in transcription of related genes, which is consistent with the results of the literature suggesting that Q can regulate the inflammatory signaling pathway[39]. The above liver transcriptome results are basically consistent with the results of ROS content, GSH activity, antibody level and pro-inflammatory cytokine content in liver tissue in this study.

RT-qPCR and WB analyses were performed to quantify the relative expression levels of genes and proteins associated with jejunal and hepatic differential signaling pathways. The results demonstrated that Q significantly reversed the OS diet-induced downregulation of jejunal *RT1-M2* gene expression and the upregulation of hepatic *Duox1* and *Cyp4a2* gene expression. Furthermore, Q significantly normalized the OS diet-induced upregulation of jejunal Ccl20 and hepatic Duox1 protein expression to baseline levels. Collectively, the above findings suggested that Q exerted a beneficial effect on alleviating oxidative stress and inflammatory responses in OS diet-fed rats.

5. Conclusions

Compared to the FS diet, the OS diet induced significant oxidative stress and pro-inflammatory responses in the jejunal and hepatic tissues of rats. Dietary supplementation with Q effectively enhanced systemic antioxidant capacity, attenuated oxidative damage, and modulated immunological function. Transcriptomic profiling revealed that the OS diet triggered ROS biosynthetic process and immunoinflammatory responses in both the jejunum and liver. Q supplementation scavenged excess ROS, downregulated jejunal heme biosynthesis, activated B-cell and T-cell signaling pathways. In the liver, Q restored glutathione metabolism, suppressed cytokine activity and acute inflammatory responses. Thus, Q could be considered as a potential natural supplement for attenuating oxidative stress and immune inflammation in rats fed OS.

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