

Effects of dietary quercetin on the antioxidative status and cecal microbiota in broiler chickens fed with oxidized oil

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ABSTRACT This study was conducted to evaluate the effects of quercetin on the antioxidant ability, intestinal barrier functions, and cecal microbiota in broiler chickens fed with oxidized soya oil. Four hundred eighty male Arbor Acres broilers were randomly assigned to 5 treatments, each involving 8 cages (12 birds per cage). The treatment groups were as follows: the control group, birds fed with basal diets containing oxidized oil, and birds fed with basal diets containing oxidized oil and supplemented with 200 ppm of quercetin, 400 ppm of quercetin, and 800 ppm of quercetin. The results showed that dietary supplementation with quercetin at a dose of 400 ppm or 800 ppm alleviated the increased serum malondialdehyde (MDA) level induced by oxidized oil on day 11 ($P = 0.005$) and reversed the increased MDA level in the mucosa on day 11 ($P = 0.021$). Quercetin significantly upregulated the transcription of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream genes such as catalase ($P < 0.001$), superoxide dismutase 1 ($P < 0.001$), glutathione peroxidase 2 ($P = 0.018$),

heme oxygenase-1 (HO-1) ($P = 0.0$), and thioredoxin ($P = 0.002$) and reversed the mRNA expression of HO-1 ($P = 0.007$) in the ileal mucosa. Tight junction protein 1 was only downregulated by oxidized oil ($P = 0.013$). In addition, quercetin (800 ppm) alleviated the decreased mRNA expression of mucin 2 (MUC2), which contributed to the intestinal chemical barrier ($P = 0.039$). The supplemental dose of 400 ppm of quercetin was able to promote *Lactobacillus* in the cecum, which enhanced the gastrointestinal tract health. In summary, these results indicated that quercetin ameliorated the oxidized oil-induced oxidative stress by upregulating the transcription of Nrf2 and its downstream genes to restore redox balance and reinforced the intestinal barrier via higher expression and secretion of MUC2 and facilitating the growth of *Lactobacillus* in the cecum. Therefore, quercetin could be a potential feed additive that can be applied in poultry production for amelioration of oxidative stress caused by oxidized oil and preventing the potential invasion of exogenous pathogens.

Key words: broiler, quercetin, oxidized oil, antioxidant status, cecal microbiota

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INTRODUCTION

Oxidative stress is a major concern in poultry production and derived from nutritional, environmental heat stress, and pathological factors, which negatively impact poultry health and production (Lin et al., 2017; Mishra and Jha, 2019). The great potential of intestinal epithelia for nutrient absorption contributes to fast growth of broilers (Mishra and Jha, 2019). As the intestinal epithelium is located at the interface of its luminal environments and organisms, it is prone to

oxidative damage (Circu and Aw, 2012). Moreover, feed exposes the intestinal epithelia of broilers to a wide variety of exogenous potentially harmful substances, which leads to excessive generation of reactive oxygen species (ROS) and results in oxidative stress (Mishra and Jha, 2019).

Oil is commonly used in poultry feed to meet the energy requirement, especially during summer to alleviate acute heat stress (Tan et al., 2018). However, high contents of unsaturated fatty acids in soybean oils make them prone to oxidation in hot climates. Lipid peroxidation not only damages DNA, protein, and membrane lipids but also decreases feed utilization and growth performance (Liang et al., 2015). Broilers are easily exposed to cumulative oxidative by-products through oxidized oil intake, resulting in an imbalance of redox status especially in the liver, which is the target organ for detoxication (Wang et al., 2016). Oxidized fish oil diet also

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caused microbial disorder and decreased the species richness and intestinal microbial diversity in fish (Peng et al., 2019).

Owing to the commercially related stressor, additional supports for the antioxidant system in poultry are strongly needed (Surai et al., 2019). Quercetin, a flavonoid compound, is widely distributed in plants and reported to possess strong anti-inflammatory, antioxidant, and antibacterial effects (Duenas et al., 2010; Li et al., 2016; Wang et al., 2018). In vitro studies based on DPPH scavenging ability and ferric reducing antioxidant power (FRAP) assays showed that quercetin was the most potent antioxidant among its 6 metabolites and butylated hydroxytoluene (Lesjak et al., 2018). Quercetin could alleviate H₂O₂-induced cell damage, reduce the intracellular ROS level, and reverse the change of malondialdehyde (MDA) levels (Chen et al., 2018). Dietary quercetin reversed the reduced Chao1 and Shannon index and meanwhile counteracted the dextran sulfate sodium-induced increased myeloperoxidase levels and reduced glutathione, MDA, and serum nitrate concentration in mice (Hong and Piao, 2018).

But most studies focused on the influence of oxidized oil on broilers (Liang et al., 2015; Tan et al., 2019) or the modulation function of quercetin alone on broilers, especially the shelf life of poultry meat or oxidative status of hen layers (Goliomytis et al., 2014; Hager-Theodorides et al., 2014; Liu et al., 2014a,b). However, the effects of quercetin on broilers fed with oxidized oil remain to be further investigated. Here, we studied the effect of quercetin on growth performance, antioxidant status, and intestinal microbiota of broiler chickens under oxidative stress induced by oxidized oil.

MATERIALS AND METHODS

Bird, Diets, and Experimental Design

A total of four hundred eighty, 1-day-old, male, Arbor Acres broilers were randomly assigned to 5 treatments, each involving 8 cages (12 birds per cage, about 80 cm [wide] × 60 cm [high] × 200 cm [length]). The broilers were raised on a floor pen and fed with feed, and tap water was provided using a nipple-type drinker. Feed in pellet form and water were provided *ad libitum*. The basal diet was formulated to meet or exceed the nutrient requirement for broilers as recommended by NRC (1994). The ingredient and nutrient compositions of the basal diets for the starter (0 to 21 D) and grower (22 to 42 D) phases are shown in Table 1. The room temperature was maintained at 35°C during the first 3 D, followed by a reduction to 28°C–30°C during the next 2 wk and 25°C for the remainder of the trial. A standard lighting regime was followed: 23 h of light and 1 h of darkness for the first 5 D followed by 20 h of light and 4 h of darkness from day 6 until the end of the trial. The experiment procedures were approved and conducted under the guidelines of the China Agricultural

Table 1. Ingredient and nutrient compositions of the basal diets.

Ingredients, %	Starter diet	Finisher diet
Corn	51.169	56.559
Soybean meal	35.835	31.400
Wheat flour	5.000	3.219
Soy oil ¹	4.000	5.500
Limestone	1.348	0.581
Dicalcium phosphate	1.248	1.533
Salt	0.300	0.300
L-Lysine (78%)	0.356	0.170
DL-Methionine (98%)	0.215	0.208
Choline chloride (50%)	0.150	0.150
Mineral premix ²	0.200	0.200
Enzyme blend ³	0.030	0.030
Vitamin premix ⁴	0.030	0.030
Phytase (5,000 FTU/kg)	0.020	0.020
Additive of Exp ⁵	0.100	0.100
Nutrient composition, %		
ME (kcal/kg)	3,000	3,150
CP, %	21.50	19.50
Lysine, %	1.41	1.16
Methionine, %	0.53	0.50
Methionine + Cysteine, %	0.85	0.80
Calcium, %	1.00	0.79
Available phosphorus, %	0.35	0.39

¹The same percentage of soybean oil was used in different treatment groups. The control group (Ctr) was fed with fresh soybean oil, the Ox group was fed with oxidized oil, and the OxL, OxM, and OxH groups were fed with oxidized oil and different concentrations of quercetin. Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²The mineral premix provided per kg diet: CuSO₄·5H₂O, 8 mg; FeSO₄, 80 mg; MnSO₄·H₂O, 100 mg; Na₂SeO₃, 0.15 mg; KI, 0.35 mg.

³The enzyme provided per kg diet: xylanase, ≥2,250 viscosity unit; β-dextranase, ≥52 AGL U/g.

⁴The vitamin premix provided per kg diet: vitamin A, 9,500 IU; vitamin D₃, 62.5 µg; vitamin E, 300 IU; vitamin K₃, 2.65 mg; vitamin B₆, 6 mg; vitamin B₁₂, 0.025 mg; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; nicotinic acid, 50 mg.

⁵The control group was fed with the basal diet containing 0.100% zeolite powder as the additive of Exp. The other groups were fed with the basal diet supplemented with 200 ppm, 400 ppm, and 800 ppm of quercetin and 800 ppm, 600 ppm, and 200 ppm of zeolite powder as the carrier separately to make 0.100% additive of Exp.

University Animal Care and Use Committee (approval number: AW04129102-1-1).

The treatment groups were as follows: control group (Ctr), birds fed with basal diet containing oxidized oil (Ox), birds fed with basal diet containing oxidized oil and supplemented with 200 ppm of quercetin (OxL), 400 ppm of quercetin (OxM), and 800 ppm of quercetin (OxH). Quercetin (purity >95%) used in this study was obtained from Shaanxi Sciphar Natural Products Co. Ltd. (Shanxi, China).

Preparation of Oxidized Oil

Fresh soybean oil was purchased from the Guchuan Edible Oil Company (Beijing, China). Oxidized oil was prepared according to method of Liang et al., 2015 with some modifications. The oil was put in an open container and heated to 95°C with continuous air flow (6 L/min) for 96 h. Then, the processed oil was cooled down to room temperature before feed preparation, and the peroxide value was 184 meq/kg, with 2.2 meq/kg for the fresh oil.

Performance Parameters

Chickens and feed were weighed by cage (replicate) on the day of hatch, at day 21, and at day 42. Feed intake, body weight gain, and feed conversion ratio were calculated for each period and throughout the whole experiment. Mortalities and health status were observed and recorded daily throughout the experiment.

Sample Collection

On day 11, day 18, and day 42, 8 birds (one bird per cage) of each treatment were randomly selected and killed by intracardial administration of sodium pentobarbital (30 mg/kg of body weight) and jugular exsanguination. Blood from the wing sinus was collected in sterile vacuum blood collection tubes and kept in ice. Serum was obtained after centrifuging the blood at 4°C at 3,000g for 15 min and stored at -80°C until analysis. The liver, spleen, and bursa of Fabricius were obtained in the sterile atmosphere and weighed to calculate the organ index using the following formula: organ index = organ weight (g)/body weight (g) × 100. Then, a piece of the liver was taken and then snap frozen in liquid nitrogen and stored at -80°C for measurements of antioxidant status. Samples (2 cm*2) of the ileum were collected midway between Meckel's diverticulum and the ileocecal junction and were respectively washed with 0.9% physiological saline or 0.1% diethyl pyrocarbonate-treated water before obtaining the mucosa of the intestinal segment and then snap frozen in liquid nitrogen and stored at -80°C. Samples of the ileum (1 cm) were taken proximal to the ileocecal junction and fixed in 4% (m/vol) paraformaldehyde solution for histological examination. The contents of the cecum were collected aseptically, snap frozen, and stored at -80°C for 16S rRNA sequencing. Blood was collected from the wing vein, and the serum was isolated by centrifugation at 3,000 rpm for 10 min at 4°C and stored at -20°C until analysis.

Intestinal Histomorphology

Formalin-fixed ileum samples were prepared using paraffin embedding techniques. Consecutive sections (5 μm) were stained using hematoxylin and eosin for histomorphological observation and using periodic acid-Schiff and alcian blue-periodic acid-Schiff for observation of neutral and acidic mucous cells. For histomorphological observation, the villus height (from the tip of the villus to the crypt opening) and the crypt depth (from the base of the crypt to the crypt opening) were measured from 10 randomly selected villi and the associated crypt, with one section per chicken at 40× magnification. The villus height-to-crypt depth ratio was then calculated from these measurements. The measurements of goblet based on periodic acid-Schiff and alcian blue-periodic acid-Schiff staining were calculated by the ratio of positive cells per villus vs. the area of the associated villus.

RNA Isolation and Quantitative Reverse Transcription Polymerase Chain Reaction

The RNA was extracted from the ileal mucosa using the Eastep Super Total RNA Extraction Kit (Promega Co., Shanghai, China). RNA quantity was measured using the Nanodrop (Thermo Fisher, Waltham, MA) at 260 nm and 280 nm. Then, total RNA was reversed transcribed into cDNA using the PrimeScrip RT reagent Kit with gDNA Eraser Perfect Real Time (Takara Biomedical Technology, Beijing, China), and the gene expression levels were determined using the SYBR Premix Ex Taq Tli RNaseH Plus (Takara Biomedical Technology) according to the product protocols. The primers of glutamate-cysteine ligase modifier subunit (**GCLM**) and β-actin are shown in Table 2, and $2^{-\Delta\Delta C_t}$ was calculated to measure the GCLM expression level.

Antioxidant Status

The intestinal (ileal) mucosa and liver were homogenized in pre-cold 0.9% saline and centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant and serum were preserved at -20°C for further analysis. The activity of total antioxidant capacity (**TAOC**), MDA, catalase (**CAT**), superoxide dismutase (**SOD**), and glutathione peroxidase (**GPX**) was analyzed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). In brief, TAOC activity was determined by the FRAP method. The MDA content was determined by the TBA reaction method. The activity of CAT was determined based on the reaction between ammonium molybdate and hydrogen peroxide under the catalytic action of CAT. Activity of SOD was detected by the WST method. The GPX activity was measured by the 5,5'-dithiobis-(2-nitrobenzoic acid) method. In the tissue homogenate, the values of TAOC, CAT, GPX, and SOD activities were expressed as units per milligram of protein, and MDA activity was expressed as nanomoles per milligram of protein. The cellular ROS level of the liver was measured using the probe 2',7'-dichlorodihydrofluorescein diacetate (Solarbio, Beijing, China) (Pang et al., 2016). In brief, ice-cold liver homogenates were centrifuged (10,000 × g, 15 min, 4°C). Then, the supernatants were incubated with 10 μM 2',7'-dichlorodihydrofluorescein diacetate for 1 h in the dark. Fluorescence was immediately read at an excitation wavelength of 485 nm and an emission wavelength of 525 nm using a spectrophotometer (Molecular Devices SpectraMax i3x plate reader, San Jose, CA). Protein concentrations in the supernatant were assayed using BCA kits.

Cecal Microbiota Populations

The cecum was removed from the chicken and put into the sterile tube before snap freezing in liquid nitrogen and stored at -80°C for further analysis. Multiplexed 16S rDNA libraries were prepared using standard 16S metagenomic sequencing library protocols from Illumina (San Diego, CA), which used the V3-V4 region of 16S

Table 2. Primer used for real-time PCR of the ileal mucosa.

Gene	Forward primer	Reverse primer	Accession number
β -actin	CCACCGCAAATGCTTCTAAAC	AAGACTGCTGCTGACACCTTC	NM_205518.1
MUC2	CCCTCACCCAGCCGACTTC	GCCGTTGGTGGAGGTGTTACAG	JX284122.1
ZO1	CTTCAGGTGTTTCTCTCCTCCTC	CTGTGGTTTCATGGCTGGATC	NM_001301025.3
Claudin-2	TCCTGTGTGTCTCCTCCCTTG	ACTCGGTCCCTGGCTGGTGAG	NM_001277622.1
Nrf2	GGGACGGTGACACAGGAACAAC	GCTCTCCACAGCGGAAAATCAG	NM_205117.1
GCLM	GCCATAGGCACCTCTGACCTTG	CGGCATCACGCAACATGAAGC	NM_001007953.1
CAT	TCAGAGGGACGGGCCAATGTG	CTGAAACGGACATGCGGCTCTC	NM_001031215.2
SOD1	GGCAAGCAGCACGGTGGAC	CTTCTGCCACTCCTCCCTTTGC	NM_205064.1
GPX2	ACGGCACCAACGAGGAGATCC	CTTCCCGTTACCTGGCACTTC	NM_001277854.2
GLRDX	AGCGAACCGTCCCTCGTGTG	GCATCATGGGGAGCTGCTGTTC	NM_205160.1
TXN	GGTGAAGAGCGTGGGCAATC	GGTCCACACCATGTGGCAGA	NM_205453.1
HO-1	GGCAGAGCTTCGCACAAGGAG	CCACCGCACCAGGGGAGAG	NM_205344.1
NOX2	TGCTGATTCTGCTGCCAGTATGTC	GTTCTGTCCAGTTGCCGTCTTAC	NM_001100286.1
IL-8	ATGAACGGCAAGCTTGAGCTG	TCCAAGCACACCTCTCTCCATCC	NM_205018.1

Abbreviations: CAT, catalase; GCLM, glutamate-cysteine ligase modifier subunit; GLRDX, glutaredoxin; GPX2, glutathione peroxidase 2; HO-1, heme oxygenase-1; MUC2, mucin 2; Nrf2, nuclear factor erythroid 2-related factor 2; TXN, thioredoxin; SOD1, superoxide dismutase 1; ZO1, tight junction protein 1.

rDNA for target amplification. The authors used Illumina Miseq (Illumina) to performed paired-end reads (300 bp) to generate ~200,000 sequences per sample. Subsequent analysis was performed in Quantitative Insights Into Microbial Ecology based on barcode sequences, and paired-end reads were merged (minimum score <20, mismatch). Additional quality filter steps were applied to exclude short reads <230 bp and chimeras. To ensure high coverage in all samples, samples producing <35,000 reads were excluded. Sequences after processed were clustered at 97% sequence identity to generate operational taxonomic unit and each operational taxonomic unit was annotated using the Silva128 16S rRNA database (bacteria and archaea). The fastq files generated by Illumina sequencing output were uploaded in the NCBI BioProject database (BioProject ID, PRJNA595453; submission ID, SUB6687114).

Statistical Analysis

Statistical significance was analyzed using SPSS 17.0 (IBM, Armonk, NY). The t-test was used to compare

the Ctr and Ox treatment groups. The Ctr group was used as a positive control, and we just analyzed 0 (Ox), 200 (OxL), 400 (OxM), and 800 (OxH) ppm levels of quercetin in oxidized oil diet to give linear or quadratic responses. When significant differences among the different levels of quercetin in oxidized oil treatments were found, the significance among the groups and oxidized oil treatment was identified using the Duncan test for multiple comparisons.

RESULTS

Performance and Internal Organ Weights

As shown in Table 3, feed intake, body weight gain, and feed conversion ratio were not significantly influenced in the Ox group compared with the Ctr group from day 1 to day 42. On the other hand, quercetin with oxidized oil did not induce significant changes compared with oxidized oil treatment. As shown in Table 4, relative weight of the liver was significantly increased by oxidized oil treatment on day 18 ($P =$

Table 3. Effects of dietary quercetin on growth performance of broiler chickens fed with oxidized oil.

Item		Treatment ¹					SEM	<i>P</i> -value, t-test	Linear	Quadratic	<i>P</i> -value
		Ctr	Ox	OxL	OxM	OxH					
1–21 D	FI, ² g/bird	1,123	1,124	1,141	1,124	1,152	9.7	0.961	0.460	0.783	0.765
	BWG, ³ g/bird	802	795	783	779	785	4.3	0.659	0.571	0.320	0.716
	FCR, ⁴ g/g	1.40	1.41	1.46	1.44	1.47	0.012	0.656	0.275	0.704	0.587
21–42 D	FI, g/bird	2.63	2.70	2.66	2.80	2.70	0.023	0.350	0.619	0.288	0.220
	BWG, g/bird	1,800	1,820	1,770	1,840	1,790	13.0	0.592	0.719	0.821	0.402
	FCR, g/g	1.46	1.48	1.50	1.53	1.51	0.008	0.498	0.125	0.126	0.181
1–42 D	FI, g/bird	3,750	3,840	3,770	3,910	3,850	23.0	0.189	0.536	0.663	0.350
	BWG, g/bird	2,600	2,620	2,550	2,610	2,570	15.0	0.745	0.619	0.904	0.490
	FCR, g/g	1.44	1.47	1.48	1.50	1.50	0.006	0.170	0.056	0.333	0.197
	Mortality, %	1.04	3.12	0.00	1.04	1.04					

Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviation: Ctr, control group.

¹Ctr: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²FI: feed intake, g/bird.

³BWG: body weight gain, g/bird.

⁴FCR: feed conversion ratio.

Table 4. Effects of dietary quercetin on the internal organ index of broiler chickens fed with oxidized oil.

Item	Treatment ¹						SEM	P-value, t-test	Linear	Quadratic	P-value
	Ctrl	Ox	OxL	OxM	OxH						
11 D	Liver	3.920	4.090	4.230	4.430	4.010	0.0560	0.272	0.600	0.022	0.102
	Spleen	0.063	0.059	0.067	0.062	0.061	0.0020	0.631	0.914	0.356	0.493
	Bursa of Fabricius	0.200	0.186	0.196	0.180	0.204	0.0060	0.464	0.490	0.611	0.682
18 D	Liver	2.950	3.380*	3.200	3.380	3.510	0.0510	0.005	0.141	0.297	0.197
	Spleen	0.069	0.066	0.069	0.063	0.071	0.0020	0.625	0.625	0.601	0.704
	Bursa of Fabricius	0.255	0.201	0.239	0.224	0.187	0.0090	0.054	0.359	0.100	0.249
42 D	Liver	2.180	2.410*	2.750	2.460	2.340	0.0590	0.042	0.346	0.193	0.171
	Spleen	0.095	0.079	0.087	0.07	0.087	0.0040	0.145	0.582	0.994	0.800
	Bursa of Fabricius	0.038	0.041	0.046	0.046	0.041	0.0020	0.682	0.727	0.229	0.635

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviation: Ctrl, control group.

¹Ctrl: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

0.005) and day 42 ($P = 0.042$). However, no significant difference in the relative weight of the liver was observed between the group treated with oxidized oil and that cotreated with quercetin and oxidized oil on day 11 ($P = 0.102$), day 18 ($P = 0.197$), and day 42 ($P = 0.171$). The relative weight of the spleen and bursa of Fabricius was not significantly influenced by oxidized oil and quercetin treatment on day 11, day 18, and day 42.

Antioxidant Status

As shown in Table 5, Table 6, and Table 7, the activities of TAOC, MDA, GPX, CAT, SOD, and ROS are examined in the liver, serum, and intestinal mucosa. As

shown in Table 5, oxidized oil significantly increased the ROS ($P = 0.014$) and MDA level ($P < 0.001$) and enhanced the activity of CAT ($P = 0.16$) and SOD ($P = 0.020$) in the liver compared with the Ctrl group, but 200 ppm of quercetin alleviated the increase of ROS activity induced by oxidized oil on day 11 ($P = 0.018$). In addition, higher concentration of quercetin significantly decreased the activity of GPX ($P = 0.001$) and the level of MDA on day 11 ($P = 0.028$). Oxidized oil did not significantly influence the ROS, TAOC, MDA, GPX, CAT, and SOD activities in the liver on day 18 and day 42. However, 200 ppm of quercetin significantly decreased the level of MDA ($P = 0.004$) and the activity of GPX ($P =$

Table 5. ROS, TAOC, MDA, GPX, CAT, and SOD activity in the liver of broiler chickens fed with oxidized oil and different concentrations of quercetin.

Item	Treatment ¹						SEM	P-value, t-test	Linear	Quadratic	P-value
	Ctrl	Ox	OxL	OxM	OxH						
11 D	ROS ²	7.88	7.96 ^{*a}	7.87 ^b	7.91 ^{a,b}	7.92 ^{a,b}	0.018	0.014	0.426	0.016	0.018
	TAOC ³	1.20	1.25	1.18	1.20	1.33	0.024	0.382	0.200	0.161	0.298
	MDA ⁴	0.55	1.15 ^{*a,b}	1.42 ^a	0.80 ^b	0.98 ^b	0.073	<0.001	0.120	0.605	0.028
	GPX ⁵	15.27	13.89 ^a	13.51 ^a	14.96 ^a	10.68 ^b	0.395	0.155	0.003	0.012	0.001
	CAT ⁵	7.24	12.18 [*]	12.31	11.68	11.80	0.595	0.016	0.751	0.926	0.977
	SOD ⁵	145.96	163.56 [*]	150.44	151.5	162.79	2.760	0.020	0.788	0.076	0.313
18 D	ROS ²	8.22	8.19	8.33	8.26	8.21	0.023	0.663	0.759	0.082	0.173
	TAOC ³	1.12	1.19 ^a	1.04 ^b	0.98 ^b	1.02 ^b	0.023	0.369	0.034	0.021	0.028
	MDA ⁴	1.35	1.67 ^a	1.04 ^b	0.99 ^b	1.32 ^{a,b}	0.076	0.255	0.226	0.001	0.004
	GPX ⁵	16.76	15.24 ^{3a,b}	12.86 ^c	13.20 ^{b,c}	16.50 ^a	0.410	0.185	0.062	0.001	0.003
	CAT ⁵	7.51	9.79	10.31	9.95	10.02	0.387	0.101	0.942	0.828	0.974
	SOD ⁵	162.98	176.46 ^a	150.71 ^c	156.38 ^{b,c}	169.83 ^{a,b}	2.772	0.141	0.967	0.001	0.006
42 D	ROS ²	8.40	8.35	8.29	8.08	8.36	0.057	0.346	0.992	0.260	0.624
	TAOC ³	1.30	1.34 ^b	1.72 ^a	1.49 ^{a,b}	1.24 ^b	0.047	0.730	0.115	0.012	0.009
	MDA ⁴	1.25	1.50	1.82	1.46	1.11	0.103	0.385	0.120	0.330	0.238
	GPX ⁵	24.46	23.76	28.22	26.47	22.35	0.762	0.786	0.226	0.026	0.062
	SOD ⁵	209.06	212.23	225.21	205.14	196.59	7.240	0.808	0.060	0.469	0.104

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviations: CAT, catalase; Ctrl, control group; GPX, glutathione peroxidase; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TAOC, total antioxidant capacity.

¹Ctrl: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²ROS: log (fluorescence value of ROS).

³TAOC: U/mg of protein.

⁴MDA: nM/mg of protein.

⁵GPX, SOD, CAT: U/mg of protein.

Table 6. TAOC, MDA, CAT, SOD, and GPX activity in the serum of broiler chickens fed with oxidized oil and different concentrations of quercetin.

Item		Treatment ¹					SEM	<i>P</i> -value, t-test	Linear	Quadratic	<i>P</i> -value
		Ctr	Ox	OxL	OxM	OxH					
11 D	TAOC ²	5.40	6.52	6.17	5.32	7.19	0.372	0.304	0.555	0.173	0.460
	MDA ³	3.40	5.81 ^a	2.85 ^b	2.70 ^b	2.79 ^b	0.316	0.105	0.011	0.014	0.005
	CAT ⁴	1.93	2.81 ^a	2.13 ^{a,b}	0.98 ^b	1.84 ^{a,b}	0.200	0.152	0.067	0.011	0.021
	SOD ⁴	84.23	94.97	84.46	104.02	81.69	3.493	0.152	0.424	0.351	0.224
	GPX ⁴	1,674.11	1,629.20 ^b	1,899.11 ^{a,b}	1,624.79 ^{a,b}	2,158.01 ^a	50.466	0.669	0.001	0.233	0.001
18 D	TAOC ²	10.64	7.52 [*]	10.13	8.9	10.71	0.44	0.049	0.055	0.677	0.116
	MDA ³	1.77	2.70 [*]	2.36	1.88	2.48	0.121	0.001	0.584	0.055	0.211
	CAT ⁴	2.02	2.61	1.96	1.88	1.87	0.151	0.387	0.212	0.270	0.390
	SOD ⁴	91.15	77.80	90.34	91.13	69.88	3.048	0.135	0.258	0.032	0.115
	GPX ⁴	2,139.99	2,197.03	2,168.06	2,145.2	2,127.31	47.347	0.747	0.627	0.886	0.966
42 D	CAT ⁴	1.18	1.69	1.57	1.61	1.92	0.093	0.085	0.110	0.205	0.239
	SOD ⁴	100.92	101.58	105.67	105.05	107.87	2.998	0.956	0.564	0.883	0.934
	GPX ⁴	2,581.29	2,306.27 ^b	2,255.79 ^b	2,743.26 ^a	2,059.50 ^b	123.903	0.156	0.423	0.017	0.017

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviations: CAT, catalase; Ctr, control group; MDA, malondialdehyde; GPX, glutathione peroxidase; SOD, superoxide dismutase; TAOC, total antioxidant capacity.

¹Ctr: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²TAOC: U/mg of protein.

³MDA: nM/mg of protein.

⁴CAT, SOD, GPX: U/mg of protein.

0.003) and SOD ($P = 0.006$) compared with the Ox group on day 18. And 200 ppm of quercetin significantly increased the TAOC ($P = 0.009$) activity compared with the Ox group on day 42.

As shown in Table 6, antioxidant status of serum was examined. The oxidized oil significantly increased the serum MDA concentration ($P = 0.001$) and decreased the TAOC ($P = 0.049$) activity on day 18, and quercetin (200 ppm to 800 ppm) decreased the level of MDA ($P = 0.005$); 800 ppm of quercetin increased the activity of GPX ($P = 0.001$) on day 11. Catalase, SOD, and GPX activities were not significantly influenced by quercetin with oxidized oil on day 18 and day 42 compared with the Ox group, except that treatment with 800 ppm of quercetin increased the activity of GPX compared with the oxidized oil treatment on day 11. On day 42, the only difference detected was in GPX activity between

the OxM and OxL groups ($P = 0.017$), as shown in Table 6.

As shown in Table 7, quercetin at a concentration of 400 and 800 ppm alleviated the increased ileal mucosal MDA levels induced by oxidized oil in a dose-dependent manner ($P = 0.021$). And only treatment with 800 ppm of quercetin increased the activity of GPX compared with the oxidized oil treatment ($P = 0.049$).

Ileum Morphology

The characteristics of ileum morphology at day 11 are listed in Table 8. Compared with the Ctr group, the morphology characteristics of the ileum such as the villus height, crypt depth, and the density of the neutral/acidic goblet were not significantly influenced by

Table 7. TAOC, MDA, CAT, SOD, and GPX activity in the ileal mucosa of broiler chickens fed with oxidized oil and different concentrations of quercetin on day 11.

Item	Treatment ¹					SEM	<i>P</i> -value, t-test	Linear	Quadratic	<i>P</i> -value
	Ctr	Ox	OxL	OxM	OxH					
TAOC ²	1.15	0.93	0.97	1.03	0.95	0.034	0.066	0.837	0.306	0.740
MDA ³	0.51	0.89 ^{*a}	0.74 ^{a,b}	0.47 ^c	0.61 ^{b,c}	0.043	0.008	0.020	0.052	0.021
CAT ⁴	4.49	7.27	8.73	8.05	10.23	0.781	0.128	0.277	0.942	0.690
SOD ⁴	72.37	69.71	68.35	68.31	61.85	2.094	0.662	0.246	0.762	0.677
GPX ⁴	25.28	26.77 ^b	24.89 ^b	24.56 ^b	35.65 ^a	1.417	0.927	0.012	0.218	0.049

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviations: CAT, catalase; Ctr, control group; GPX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase; TAOC, total antioxidant capacity.

¹Ctr: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²TAOC: U/mg of protein.

³MDA: nM/mg of protein.

⁴CAT, SOD, GPX: U/mg of protein.

Table 8. Ileal morphology of broiler chickens fed with oxidized oil and quercetin on day 11.

Item	Treatment ¹					SEM	P-value, t-test	Linear	Quadratic	P-value
	Ctr	Ox	OxL	OxM	OxH					
Villus height, μm	383.82	376.83	343.3	389.9	390.14	8.426	0.785	0.329	0.699	0.316
Crypt depth, μm	77.53	68.78 ^b	84.63 ^a	77.59 ^{a,b}	70.58 ^b	1.970	0.152	0.649	0.022	0.045
V/C ²	5.08	5.52 ^{*a}	4.24 ^b	5.14 ^a	5.58 ^a	0.113	0.019	0.119	0.003	<0.001
Number of neutral mucus goblet/(mm ²)	1,959	1,832	1,727	1,622	1,470	52.9	0.475	0.015	0.822	0.103
Number of acidic mucus goblet/(mm ²)	1,917	171	1,738	1,439	1,401	60.9	0.368	0.043	0.726	0.137

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviation: Ctr, control group.

¹Ctr: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

²V/C: (villus height)/(crypt depth), $\mu\text{m}/\mu\text{m}$.

oxidized oil treatment, but the ratio of villus height and crypt was significantly increased ($P = 0.019$). However, 200 ppm of quercetin increased the crypt depth ($P = 0.045$) and significantly decreased the ratio of villus height and crypt depth compared with Ox group ($P < 0.001$). On the other hand, the density of the neutral and acidic goblet was not significantly influenced by quercetin and oxidized oil.

Relative mRNA Expression of Antioxidant and Barrier Function–Related Genes in the Chicken Ileal Mucosa

As shown in Table 9, oxidized oil significantly downregulated the genes related to intestinal barriers such as tight junction protein 1 ($P = 0.001$) and mucin 2 (MUC2) ($P = 0.013$) compared with the Ctr group in the ileal mucosa. But 800 ppm of quercetin reversed the downregulation of MUC2 ($P < 0.001$) and upregulated the mRNA expression

of Claudin-2 ($P = 0.001$). The concentration of 400 ppm of quercetin also reversed the downregulated transcription of tight junction protein 1 induced by oxidized oil ($P = 0.039$). As for the antioxidant genes, oxidized oil significantly decreased the mRNA expression of thioredoxin (TXN) ($P = 0.037$) and IL-8 ($P = 0.024$). However, 800 ppm of quercetin significantly increased expression of the antioxidant-related gene such as nuclear factor erythroid 2–related factor 2 (Nrf2), GCLM, CAT, superoxide dismutase 1, glutathione peroxidase 2, glutaredoxin, TXN, and heme oxygenase-1 (HO-1) compared with the Ox group (P -values are listed in Table 9). And quercetin decreased the mRNA expression of NOX2 compared with the Ctr group ($P = 0.033$) and reversed the decreased expression of IL-8 induced by oxidized oil ($P = 0.001$).

Cecal Microbiota

As shown in Figure 1, the alpha diversity of cecal microbiota was not significantly different between

Table 9. Relative mRNA expression of antioxidant- and inflammation-related genes in the broiler chicken ileal mucosa on day 11.

Item	Treatment ¹					SEM	P-value, t-test	Linear	Quadratic	P-value
	Ctr	Ox	OxL	OxM	OxH					
MUC2	1.00	0.15 ^{*b}	0.71 ^b	0.42 ^b	2.19 ^a	0.147	0.001	<0.001	0.080	<0.001
ZO1	1.03	0.22 ^{*b}	0.34 ^{a,b}	0.41 ^a	0.27 ^{a,b}	0.063	0.013	0.413	0.006	0.039
Claudin-2	1.00	0.71 ^b	1.53 ^b	0.67 ^b	3.34 ^a	0.23	0.441	0.001	0.059	0.001
Nrf2	1.00	0.37 ^b	0.65 ^b	0.81 ^b	2.10 ^a	0.157	0.102	<0.001	0.270	0.002
GCLM	1.65	0.67 ^b	0.66 ^b	0.57 ^b	1.35 ^a	0.142	0.192	0.002	0.011	0.002
CAT	1.00	0.50 ^b	0.87 ^b	0.96 ^b	2.30 ^a	0.146	0.121	<0.001	0.182	<0.001
SOD1	1.00	0.40 ^b	0.97 ^b	0.52 ^b	1.92 ^a	0.142	0.178	<0.001	0.154	<0.001
GPX2	1.00	1.01 ^b	1.40 ^b	1.28 ^b	2.21 ^a	0.131	0.970	0.003	0.494	0.018
GLRDX	1.15	0.90 ^b	1.55 ^b	1.17 ^b	2.39 ^a	0.150	0.412	0.002	0.525	0.008
TXN	1.14	0.77 ^{*b}	1.48 ^a	1.45 ^a	2.08 ^a	0.118	0.037	<0.001	0.486	0.002
HO-1	1.00	0.45 ^b	0.87 ^{a,b}	0.84 ^{a,b}	1.29 ^a	0.087	0.101	0.001	0.715	0.007
NOX2	1.03	0.90 ^a	1.10 ^a	0.74 ^{a,b}	0.49 ^b	0.068	0.397	0.012	0.372	0.033
IL-8	0.80	0.36 ^{*c}	0.51 ^b	0.47 ^{b,c}	0.65 ^a	0.033	0.024	<0.001	0.996	0.001

*Means were significantly different compared with the control. Means within 4 treatments (Ox, OxL, OxM, and OxH) lacking a common superscript differ ($P < 0.05$).

Abbreviations: CAT, catalase; GCLM, glutamate–cysteine ligase modifier subunit; GLRDX, glutaredoxin; GPX2, glutathione peroxidase 2; HO-1, heme oxygenase-1; MUC2, mucin 2; Nrf2, nuclear factor erythroid 2–related factor 2; TXN, thioredoxin; SOD1, superoxide dismutase 1; ZO1, tight junction protein 1.

¹Ctr: basal diet; Ox: basal diet with oxidized oil; OxL: basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM: basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

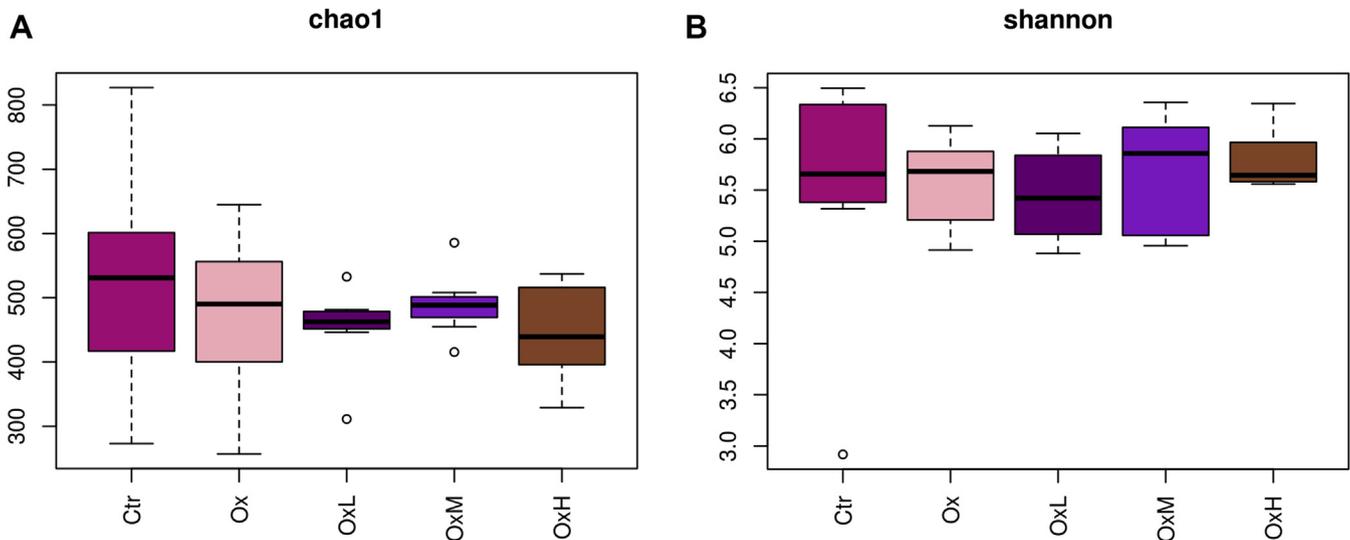


Figure 1. Effects of quercetin on the α -diversity of cecal microbiota. The α -diversity was evaluated by (A) Chao1 index and (B) Shannon index. Abbreviations: Ctr, control group – basal diet; Ox, basal diet with oxidized oil; OxL, basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM, basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH, basal diet supplemented with oxidized oil and 800 ppm of quercetin.

treatments. Beta diversity was analyzed by partial least squares discrimination analysis, as shown in Figure 2, which showed remarkable differentiation of the microbial community among the 5 groups. The Ctr group and oxidized oil group were separated, whereas the groups treated with quercetin and oxidized oil were in the transition status between these 2 groups. As shown in Figure 3A, at the phylum level (relative abundance >0.1%), Firmicutes (96.4–97.6%), Tenericutes (0.7–2.0%), Proteobacteria (0.3–2.4%), and Actinobacteria (0.2–0.5%) made up more than 99.9% of the total cecal microbiota. However, no significant difference was observed among treatments at the phylum level. In Figure 3B, the top 20 genera are listed. Among the 20 genera, the relative abundance of *Lactobacillus* was higher in the oxidized oil treatment group than in the Ctr group, and treatment with quercetin further increased the relative abundance of *Lactobacillus* in the cecum.

DISCUSSION

As mentioned in Introduction, consumption of oxidized oil was reported to induce oxidative damage in broilers. However, dietary oxidized oil was not able to affect broiler growth during the early growth phase in our study. Similar results were found in the study of Tan et al. (2018). Moderately thermo-oxidized oil did not impair the nutrient digestibility of diet, and there was no significant effect of oxidized oil on digestibility coefficients of crude protein and ether extract (Açıkgoz et al., 2011). In addition, treatment with quercetin at a concentration of 200 ppm to 800 ppm with oxidized oil did not induce any significant changes in growth performance from day 1 to day 42. Similarly, dietary quercetin at a concentration of 200 ppm to 600 ppm had no significant effect on growth performance (Yang et al., 2020). In agreement with another study on pigs (Lu et al.,

2014), oxidized oil increased the liver weight and liver index on day 18, which was as a result of the elevated weight of the liver. Oxidized oil could induce a higher rate of hepatic lipid metabolism, and the increase in liver weight may be the result of higher metabolic activity or reflection of an upregulation of mRNA expression for the fatty acid catabolism pathway (Liu et al., 2014a,b).

The liver is an important organ for nutrient metabolism and sensitive indicator of toxicity (Liu et al., 2014a,b). Based on previous studies, animal fed with oxidized oils experienced stress responses (Liu et al., 2014a,b). In our study, oxidized oil significantly increased the MDA and ROS level in the liver on day 11, and quercetin alleviated the increase in the ROS level induced by oxidized oil in the liver. Similar results were shown wherein quercetin prevented the in vitro production of ROS such as peroxides, superoxide, and reactive nitrogen species nitric oxide, induced by a proinflammatory cytokine mixture in liver cells (Crespo et al., 2008). However, the parameters related to oxidation in the liver on day 18 and 42 were less affected by oxidized oil. In this study, the peroxide value was much lower than that in other reports. And under mild oxidative stress, organisms usually block extensive biosynthesis to prevent or neutralize negative ROS effects, gradually leading to the adaptive response (Lushchak, 2014).

Oxidized oil in diets was absorbed by the small intestine, which was incorporated into chylomicrons and then circulated in blood, where they contributed to the total body pool of oxidized lipids (Staprans et al., 1994). Dietary quercetin alleviated the increase of the MDA level in serum on day 11. Most antioxidant enzymes were not significantly upregulated by quercetin except GPX on day 11. Similarly, quercetin was reported to elevate the serum FRAP and the content of small antioxidant molecules such as ascorbic acid and vitamin E in Wistar rats after they were fed with 0.5% quercetin for 2 wk (Zhao et al., 2011). Based on

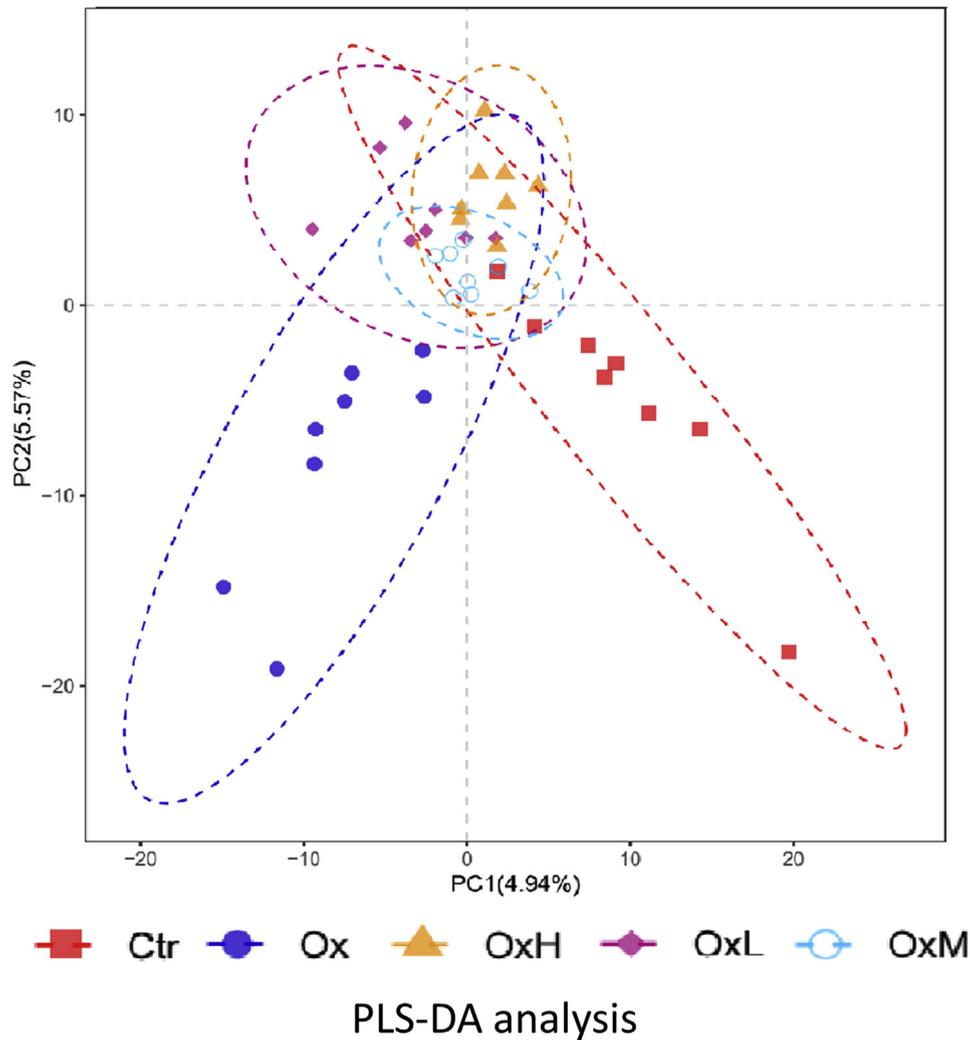


Figure 2. Effects of quercetin on the cecal microbiota: quercetin alleviated the change caused by oxidized oil in the cecum. Abbreviations: Ctr, control group – basal diet; Ox, basal diet with oxidized oil; OxL, basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM, basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH, basal diet supplemented with oxidized oil and 800 ppm of quercetin; PC, principal component; PLS-DA, partial least squares discrimination analysis.

the changes of oxidative status and the protection effects of quercetin against oxidized oil during the whole trial, in this study, we then focused on the early stage after broilers were fed with oxidized oil.

As the first defense line against oxidative stress, the intestinal mucosa contains an extensive enzyme and non-enzyme antioxidant system. Meanwhile, the intestinal mucosa experiences oxidative stress when exposed to oxidized soybean oil after intake of highly oxidized diets (Liang et al., 2015). The redox status and the morphology of the ileum were examined in our studies. Because concentration of dietary quercetin was much higher in the lumen of the intestine than in plasma and tissue, the intestinal mucosa was undoubtedly the target of this flavonoid (Murota et al., 2004). In this experiment, dietary quercetin (400 ppm and 800 ppm) alleviated the increase of the MDA level in the ileal mucosa on day 11, and the tendency of upregulation of GPX was observed in the 800-ppm quercetin treatment compared with the oxidized oil treatment. In another study about transport stress of pigs, quercetin-supplemented pigs showed lower levels of ROS and

MDA in the intestine than the those in the control group (Zou et al., 2016). As for the morphology changes, in an *in vivo* jejunal ischemia–reperfusion injury experiment in rats, the intestinal gland depth was significantly higher in the quercetin group, and deeper intestinal glands contributed to the faster recovery of the intestinal epithelium as it was the site of proliferation of epithelial cells (Curgali et al., 2018). In our study, 200 ppm of quercetin significantly increased the crypt depth and decreased the ratio of villus height and crypt depth, which indicated faster recovery of the epithelium.

Mucus is composed of 95% water, 1% salts, 1–2% lipids, and protein. The thickness of the intestinal mucus layer in the small intestine depends on the diet (Gonzales et al., 2016). However, in this study, the mucus goblets were not significantly influenced by different concentrations of quercetin.

To further elucidate the changes of redox status and mucin, mRNA expression was examined in the ileal mucosa. Mucus in the gastrointestinal tract was composed of both membrane-bound and secreted mucins, among which MUC2 was secreted in the intestine (Gonzales

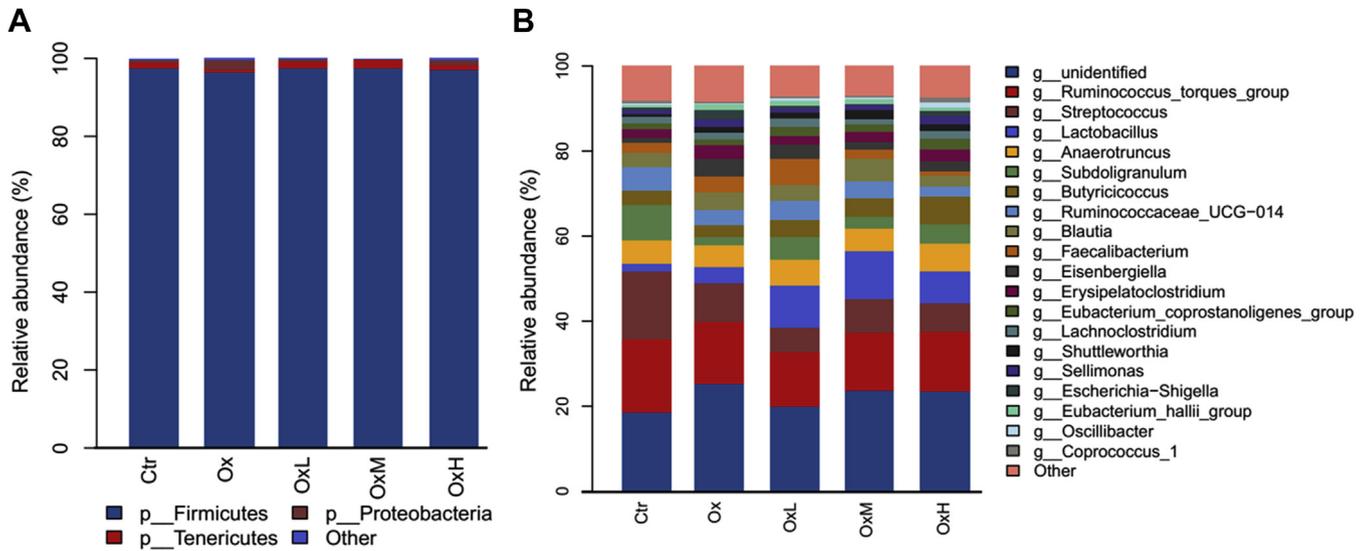


Figure 3. Effects of quercetin on the cecal microbiota. (A) No significant change was observed at phylum level. (B) Quercetin further increased the abundance of Lactobacillaceae induced by oxidized oil. Abbreviations: Ctr, control group – basal diet; Ox, basal diet with oxidized oil; OxL, basal diet supplemented with oxidized oil and 200 ppm of quercetin; OxM, basal diet supplemented with oxidized oil and 400 ppm of quercetin; OxH: basal diet supplemented with oxidized oil and 800 ppm of quercetin.

et al., 2016). Quercetin (800 ppm) significantly alleviated the downregulation of MUC2 mRNA expression induced by oxidized oil. In an in vitro experiment of LS174 T cells, a human intestinal goblet cell-like cell, quercetin could induce mRNA levels and secretion of MUC2 via the protein kinase C alpha/extracellular regulated protein kinases 1-2 pathway, and the same effects of quercetin also observed in the intestinal epithelial Caco-2 cells (Damiano et al., 2018). As a member of leucine zipper transcription factors, Nrf2 was essential for the induction of many stress-responsive or cytoprotective enzymes, such as CAT, SOD, GPX, HO-1, TXN, and glutamate-cysteine ligase (Surh et al., 2008). Quercetin could induce the upregulation and nuclear translocation of Nrf2 in several cells in vitro and in vivo (Tanigawa et al., 2007; Surh et al., 2008). In agreement with these observations, in our study, the group treated with quercetin (800 ppm) showed significantly increased expression of the antioxidant gene such as Nrf2 and downstream genes such as CAT, superoxide dismutase 1, glutathione peroxidase 2, glutaredoxin, and TXN compared with the Ctr group. Heme oxygenase-1 could catalyze the decomposition of heme (a toxic oxidant) into bilirubin (a potent antioxidant), carbon monoxide (a novel gaseous signal messenger), and ferrous efflux. In an in vitro study on human hepatocytes, Nrf2 translocation into nuclei and subsequent upregulation of HO-1 induced by quercetin protected human hepatocytes from ethanol-induced oxidative stress (Yao et al., 2007). In the present study, quercetin reversed the decrease of HO-1 mRNA expression in the ileal mucosa induced by oxidized oil. NADPH oxidases (NOX) were the major sources of superoxide anions and mainly expressed in phagocytes, including chicken heterophils (Katsuyama, 2010; Nambooppha et al., 2018). In our study, the group treated with quercetin (800 ppm) showed significant downregulation in the

mRNA expression of NOX2 compared with the oxidized oil treatment group. Cytosolic components p47^{phox}, p67^{phox}, NOXO1, and NOXA1 were required for NOX2 activation, while quercetin was able to downregulate the expression of the p47^{phox} subunit, leading to the inhibition of NADPH oxidase (Yousefian et al., 2019). Similar results were also observed in the myocardial ischemia-reperfusion injury model in rabbits in vivo; quercetin inhibited myocardial ischemia-reperfusion injury-induced NOX2 mRNA and protein expression (Wan et al., 2009). IL-8 secreted by enterocytes attracted and activated neutrophils during the acute phase of inflammation (Martirosyan et al., 2016). IL-8 could rapidly be upregulated by proinflammatory cytokines, such as bacterial or viral products, and cell stresses (Hoffmann et al., 2002). In response to bacterial infection, heterophils were being secreted as a major source of proinflammatory cytokines such as IL-6 and IL-8 and other chemokines to communicate between innate and adaptive immune systems and affected tissues (Khampeerathuch et al., 2017). In our experiment, quercetin (800 ppm) alleviated the decrease in IL-8 expression levels caused by oxidized oil. In another study, isolated heterophils from hybrid chickens treated with quercetin also showed higher relative expression levels of IL-8, and the increase in proinflammatory gene expression such as that of IL-6 and IL-8 was suggested to be associated with the resistance to extraintestinal *Salmonella enteritidis* (Khampeerathuch et al., 2017).

Physiologically, ROS are generated by gastrointestinal tract epithelial cells as a result of oxygen metabolism or by enteric commensal bacteria and involved in the regulation of gut health (Mishra and Jha, 2019). And the antioxidant system maintains the microbiota in the luminal epithelium (Mishra and Jha, 2019). Enterocytes are able to absorb quercetin glucosides through transport and hydrolysis. However, owing to the highly

hydrophobic property of quercetin and the highly hydrophilic property of the intestinal mucus layer, most of the dietary quercetin was not absorbed by the intestine, and the dietary quercetin could show a beneficial effect in the unabsorbed form in the gut lumen (Murota et al., 2004; Gonzales et al., 2016). In vitro coculture of quercetin and different *Lactobacillus* strains showed that quercetin increased the cell surface hydrophobicity and improved the autoaggregation and coaggregation capacity of the examined *Lactobacillus* strains (Dos Santos et al., 2019). And cell surface hydrophobicity and aggregation were strongly related to the ability of probiotics to adhere to the intestinal mucosa, which supported the beneficial modulatory effects exerted by quercetin on the examined *Lactobacillus* strains (Dos Santos et al., 2019). In this study, quercetin significantly enhanced the relative abundance of *Lactobacillus* in the cecum, which contributed to the gastrointestinal tract health.

Based on these results, quercetin ameliorated the oxidized oil-induced oxidative stress by restoring the redox balance, reinforcing the intestinal barrier, and facilitating the growth of *Lactobacillus* in the cecum. During this oxidative stress process caused by oxidized oil, quercetin at a concentration of 800 ppm enhanced the inherent antioxidant genes Nrf2 and its downstream genes such as SOD, CAT, GPX, TXN, and HO-1 to maintain the redox balance and reinforced the intestinal chemical barrier. The concentration of 400 ppm of quercetin also contributed to restore the redox balance and facilitated the growth of *Lactobacillus* in the cecum. Therefore, quercetin could be a potential feed additive that can be applied in poultry production for amelioration of oxidative stress and preventing the potential invasion of exogenous pathogens.

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