Protective effects of *Fagopyrum dibotrys* on oxidized oil-induced oxidative stress, intestinal barrier impairment, and altered cecal microbiota in broiler chickens

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ABSTRACT The objective of this study was to evaluate protective effects of *Fagopyrum dibotrys* on antioxidant ability, intestinal barrier functions, and cecal microbiota in broiler chickens fed oxidized soybean oil. A total of 640 male Tiejiaoma broilers were randomly assigned to 8 treatments with 8 cages (10 birds per cage), as follows: birds fed basal diets containing fresh soybean oil and 0, 0.5, 1, or 2% *F. dibotrys* (FSCON, FSFAL, FSFAM, and FSFAH, respectively), and birds fed basal diets containing oxidized oil and 0, 0.5, 1, or 2% *F. dibotrys* (OSCON, OSFAL, OSFAM, and OSFAH). Oxidized oil significantly decreased transcription of Nrf2 and its downstream genes, including CAT and SOD1 in the jejunal mucosa, increased jejunal mucosa IL-6 mRNA expression, and decreased jejunal mucosa IL-22 mRNA expression and downregulated Claudin-1 and ZO-1; however, all these effects were reversed by *F. dibotrys*. Either 1 or 2% *F. dibotrys* alleviated the decreased liver SOD induced by oxidized oil on d 42. The decreased SOD and GPX, and increased MDA induced by oxidized oil were reversed by adding 1 or 2% *F. dibotrys* in jejunal mucosa. In addition, based on 16S rDNA, 2% *F. dibotrys* promoted the *Firmicutes* phylum and *Candidatus_Arthromitus* genera, but suppressed the *Proteobacteria* phylum and *Streptococcus, Enterococcus*, and *Escherichia* genera. In summary, oxidative stress induced by oxidized oil was ameliorated by *F. dibotrys* upregulating transcription of Nrf2 and its downstream genes to restore redox balance, reinforcing the intestinal barrier via higher expression of Claudin-1/ZO-1, ameliorating the inflammatory response by regulating expression of IL-6 and IL-22, and facilitating growth of *Candidatus_arthromitus* in the cecum. Therefore, *F. dibotrys* has potential as a feed additive for poultry by ameliorating oxidative stress caused by oxidized oil, enhancing barrier function, and improving gut microbiome composition.

Key words: broiler, *F. dibotrys*, oxidized oil, antioxidant status, cecal microbiota

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INTRODUCTION

In poultry production, oxidative stress is common and caused by climate, dietary antinutritional factors, debeaking, and breeding environment (Kpomasse et al., 2021). In fast-growing broilers, the intestinal tract is critical for feed digestion and nutrient absorption (Adedokun and Olojede, 2019). Furthermore, the intestinal epithelium is directly exposed to the external environment and to compounds that produce considerable reactive oxygen species (**ROS**), causing oxidative stress (Vancamelbeke and Vermeire, 2017) that may impair intestinal mucosal barrier function (Dong et al., 2020), liver function (Tan et al., 2018), the immune system (Zhang et al., 2022), and antioxidant enzyme activities (Tan et al., 2019a).

Adding oils to poultry diets can provide energy, essential fatty acids, fat-soluble substances (e.g., vitamins and carotenoids), alleviate acute heat stress (Abdel-Wareth et al., 2022), and reduce feed dustiness (Lauridsen, 2020). However, high concentrations of unsaturated fatty acids in soybean oil make them prone to oxidation in hot climates or during feed processing (Al-Khalaifah and Al-Nasser, 2021). Oxidized oils contain varying amounts of peroxidation products, including lipid hydroperoxides, secondary oxidation products, aldehydes, hydrocarbons, carbonyls, 4-hydroxynonenal, and

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malondialdehydes (Grootveld et al., 2022). The lipid peroxidation product 4-hydroxynonenal can induce chronic inflammation and endothelial cell dysfunction, and activate the Nrf2/ARE transcription pathway (Siow et al., 2007). Supplementation of moderately oxidized oil to the diet of broilers caused mild oxidative stress due to slightly increased plasma triglyceride and cholesterol concentrations and significantly decreased SOD activity (Acikgoz et al., 2011). Furthermore, oxidized soybean oil increased serum malondialdehyde (**MDA**) concentrations, decreased tight junction protein 1 and mucin 2, and caused intestinal microbial disorders in broiler chickens (Dong et al., 2020). Therefore, oxidized oil in broiler diets negatively affected nutrient balance and antioxidative status (Engberg et al., 1996).

Fagopyrum dibotrys (***F. dibotrys***) (D. Don) Hara, an important perennial buckwheat plant, is widespread in China, India, Nepal, Kashmir, Vietnam, and Thailand. This plant, which is also called *F. cymosum* (Trev.) Meisn, has been well explored due to its long tradition of both edible and medicinal use. In China, its rhizome was regarded as folk medicine for clearing away heat and toxic materials, removing blood stasis and expelling pus; it was used to treat lung diseases, rheumatism, cancer, dysmenorrhea, inflammation, lumbago, snakebite, and traumatic injuries, and considered especially effective for lung cancer (Chan, 2003). More than 100 compounds have been isolated from *F. dibotrys,* including flavonoids, phenols, terpenes, steroids, and fatty acids. Proanthocyanidins extracted from *F. dibotrys* rhizome had antioxidant and antidiabetic activities (Li et al., 2021). Furthermore, *F. dibotrys* can alleviate intestinal inflammation and enhance mucosal epithelial function by increasing expression of Claudin-1, Claudin and ZO-1 tight junction proteins in colonic epithelial cells affected by irritable bowel syndrome (Liu et al., 2012). In addition, *F. dibotrys* prevented development of experimental arthritis by reducing production of IL-1 and TNF-α, and thereby suppressing the inflammatory response (Shen et al., 2013).

Most studies have focused on the influence of oxidized oil on broilers (Tan et al., 2019a) or physiological functions of *F. dibotrys* in cancer cells (Chan, 2003) or animal models (Shen et al., 2013). However, effects of *F. dibotrys* on broilers fed oxidized oil have apparently not been reported. Here, we studied effects of *F. dibotrys* on growth performance, antioxidant status, intestinal barrier, and intestinal microbiota of broiler chickens under oxidative stress induced by oxidized oil.

MATERIALS AND METHODS

Bird, Diets, and Experimental Design

All experimental procedures were reviewed and approved by the Animal Care and Use Committee of Guizhou Academy of Agricultural Sciences, Guiyang, China.

One day old male Tiejiaoma broilers (640) were obtained from a commercial hatchery (Guiyang, Guizhou, China) and assigned to 8 treatments with 8 replicate cages of 10 birds each, according to a completely randomized design. The body weight distributions of each cage had no significant difference on d 1. The amount of *F. dibotrys* was added by replacing wheat bran in the basal diet. Feed and water were offered ad libitum. The basal diet was formulated to meet or exceed the recommended nutritional requirements of broilers (Hintz et al., 1994). Corn and soybean-based diets for starter (1−21d) and grower (22−42d) phases are shown in Table 1. The crude protein (CP), calcium (Ca), total phosphorus (P), and crude fat contents in diets were analyzed following the classical procedures according to the Association of Official Analytical Chemists (AOAC, 1990). No antibiotic growth promoters or antioxidants were added to the basal diet. Experimental diets were produced by adding 4% fresh or oxidized soybean oil. Birds were maintained at 34 to 35°C during the first 3 d, followed by a reduction to 28 to 30°C during the next 2 wk and 24 to 25°C for the remainder of the trial. Lights were on continuously throughout the trial.

Preparation of Oxidized Oil and Fagopyrum Dibotrys

Fresh soybean oil was purchased from the Gufeng Edible Oil Company (Guiyang, China). Oxidized oil was prepared as described (Tan et al., 2018); in brief, the oil was put in an open container and heated to 200°C for 30 h to produce oxidized oil, and subsequently cooled to room temperature. The oil was heated at 200°C for no more than 3 h at each time. The peroxide value was 84 meq/kg, compared to 2.5 meq/kg for fresh oil.

The two years old *Fagopyrum dibotrys* rhizoma was dug from experimental field of Guizhou Animal Husbandry and Veterinary Research Institute and washed with water. The dried *Fagopyrum dibotrys* rhizoma was crushed and strained using a 150-mesh sieve. The dimeric procyanidin content was 7.3% in *Fagopyrum dibotrys* rhizoma according to the report of Deng et al. (2017). A total of 0, 365, 730, 1,460 ppm dimeric procyanidin were calculated in containing 0, 0.5, 1, and 2% *F. dibotrys,* respectively.

Growth Performance Measurement

Chickens and feed were weighed on a per-cage basis on day of hatch, and on d 7, 14, 21, and 42. Feed intakes (**FI**), body weight gain (**BWG**), and feed conversion ratio (**FCR**) were calculated for d 1−7, 7−21, and 21−42.

Sample Collection

On d 21 and 42, 16 birds (2 per cage) per treatment were randomly selected and killed by intravenous administration of sodium pentobarbital (30 mg/kg of body weight). Blood samples were collected before kill, and serum was isolated by centrifugation at 3,000 × g for 10 min at 4°C and stored at −20°C. The intestines were removed, digesta flushed with 4% saline, and

Table 1. Ingredients and nutrient composition of the basal diet fed to broilers.

Ingredient (%)	1−21 d				21−42 d			
Corn	51.49	51.49	51.49	51.49	57.0	57.0	57.0	57.0
Soybean meal	38.0	38.0	38.0	38.0	25.2	25.2	25.2	25.2
Wheat bran	2.0	1.5	1.0	0	2.0	1.5	1.0	0
Fagopyrum dibotrys	0	0.5	1.0	2.0	0	0.5	1.0	2.0
Soybean oil	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Choline chloride (50%)	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.2
Vitamin premix[1]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Trace mineral premix[2]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine	0.08	0.08	0.08	0.08	0.15	0.15	0.15	0.15
DL-Methionine	0.3	0.3	0.3	0.3	0.07	0.07	0.07	0.07
Dicalcium phosphate	2.0	2.0	2.0	2.0	1.65	1.65	1.65	1.65
Limestone	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Corn gluten meal	0	0	0	0	7.9	7.9	7.9	7.9
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nutrient composition, %[3]								
ME, kcal/kg	3,004	2,996	2,988	2,980	3,110	3,106	3,098	3,090
CP, %	22.5	22.5	22.5	22.4	20.5	20.4	20.4	20.4
Calcium, %	1.01	1.02	1.02	1.02	0.90	0.90	0.91	0.91
Total phosphorus, %	0.71	0.71	0.70	0.70	0.62	0.62	0.62	0.61
Available phosphorus, %	0.48	0.48	0.48	0.48	0.41	0.40	0.40	0.40
Crude fat	3.5	3.5	3.4	3.4	3.4	3.4	3.4	3.3
Lysine, %	1.32	1.32	1.31	1.31	1.00	0.99	0.99	0.98
Methionine, %	0.64	0.64	0.64	0.64	0.43	0.41	0.41	0.40

[1]The vitamin premix provided the following per kg of diets: vitamin A, 10,000 IU; vitamin D3, 2,400 IU; vitamin E, 20 mg; vitamin K3, 2 mg; vitamin B1, 2 mg; vitamin B2, 6.4 mg; VB6, 3 mg;VB12, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; pantothenic acid,10 mg; nicotinamide, 30 mg.
[2]The trace mineral premix provided the following per kg of diets: Cu,16 mg (as CuSO4•5H2O); Zn, 110 mg (as ZnSO4); Fe, 80 mg (as FeSO4•H2O); Mn, 120 mg (as MnO); Se, 0.3 mg (as Na2SeO3); I, 1.5 mg (as KI); Co, 0.5mg.
[3]The CP, calcium, total phosphorus, and crude fat value is analyzed concentrations. The other nutrient levels are calculated values.

mucosal membranes gently scraped to obtain samples. A portion of jejunal mucosal samples were immediately frozen and stored at −80°C for mRNA determination, whereas another portion of jejunal mucosal samples were immediately frozen and stored at −20°C for assessing antioxidant enzymes. Cecal contents were collected aseptically, snap frozen, and stored at −80°C for 16S rDNA sequencing. The liver was extracted and frozen in liquid nitrogen and stored at −20°C for analyzing antioxidant enzyme activities.

Antioxidant Enzyme Analysis

The jejunal mucosa and liver samples (∼0.1 g) were homogenized in 0.9% saline and centrifuged at 3,000 × *g* for 10 min at 4°C, with supernatant and serum preserved at −20°C. Total antioxidant capacity (**T-AOC**) and MDA concentrations were assessed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Superoxide dismutase (**SOD**) was analyzed by the method of WST-8 (Beyotime Biotechnology Institute, Shanghai, China); and glutathione peroxidase (**GPX**) activity was measured by the 5,5'-dithiobis-(2-nitrobenzoic acid) method (Geruisi Bioengineering Institute, Suzhou, China). Protein concentrations in the supernatant were assayed using BCA kits (Beijing Solarbio Science & Technology Company, Beijing, China).

Measurement of Secretory IgA in Mucosa

Jejunal mucosa samples (∼0.1 g) were placed in tubes containing 0.9 mL of saline, and the mixtures were homogenized. The supernatants were collected by centrifugation at 4,000 × *g* for 10 min at 4°C. Total sIgA concentration was measured using a chicken secretory IgA ELISA Quantification Set (Shanghai Jianglai Biotechnology Company, Shanghai, China), according to the manufacturer's instructions. Protein concentration in the supernatant was measured using a BCA protein quantification kit (Beijing Solarbio Science & Technology Company), and results expressed as the IgA level per gram of protein.

Measurement of Serum Diamine Oxidase

The serum diamine oxidase (**DAO**) was determined by the 4-aminoantipyrine method (Geruisi Bioengineering Institute).

RNA Isolation and qRT-PCR Analysis

Total RNA was extracted from jejunal mucosa samples using Trizol, in accordance with the manufacturer's instructions. The RNA was reversed to cDNA with RevertAid First Strand cDNA Synthesis Kit (Thermo, Waltham, MA). GAPDH were used as a reference gene and gene expression was determined using the SYBR PremixEx Taq Tli RNaseH Plus (Takara Biomedical Technology, Dalian, China) according to the product protocols and calculated using the $2^{-\triangle\triangle Ct}$ method and qRT-PCR primers (Table 2). Birds fed basal diets containing fresh soybean oil and 0% *F. dibotrys* (FSCON) was the control group.

Table 2. Oligonucleotide primers used for quantitative real-time PCR.

Gene	Primer fragment (5'→3')	Accession number	Length (bp)	Tem (°C)
CAT	F:CCTGACACGCATAGACATCG R:CATTGGCCCGTCCCTC	NM_001031215.2	102	60
SOD1	F:GTGGGTGACCTCGGCAATG R:CGGAAGAGCAAGTACAGCAATC	NM_205064.1	299	60
Nrf2	F:ACACCAAAGAAAGACCCTCC R:GAACTGCTCCTTCGACATCA	NM_205117.1	198	60
IL-6	F:GAAATCCCTCCTCGCCAATC R:CCTCACGGTCTTCTCCATAAAC	NM_204628.1	106	60
IL-22	F:CTTCTCAGGATGGGTTGTCTTC R:GGCTTGATGGGCATTGGA	NM_001199614.1	106	60
Claudin-1	F:CATACTCCTGGGTCTGGTTGGT R:GACAGCCATCCGCATCTTCT	NM_001013611.2	100	60
Occludin	F: TGCTGTCTGTGGGTTCCTC R: CCAGTAGATGTTGGCTTTGC	NM_205128.1	106	60
ZO-1	F:TTGCCACAAGTGCGGTAT R:GTGGTGTAGGCAGTGGTTTACT	XM_015278975.3	298	60
GAPDH	F: TGAAAGTCGGAGTCAACGG R: GGTCACGCTCCTGGAAGATA	NM_204305.1	234	60

Abbreviations: CAT, catalase; F, forward; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Nrf2, nuclear factor erythroid 2-related factor 2; IL-6, interleukin 6; IL-22, interleukin 22; R, reverse; SOD1, superoxide dismutase 1; ZO-1, tight junction protein 1.

16S rDNA Sequencing of Cecal Microbiota

Six samples of cecal digesta per group were randomly selected for 16S rDNA sequencing on d 21. Multiplexed 16S rDNA libraries were prepared using standard 16S metagenomic sequencing library protocols from Illumina (San Diego, CA), which used the subthreads to obtain high quality CCS sequences by lima (v1.7.1). Sequences after processing were clustered at 97% sequence identity to generate operational taxonomic unit and each operational taxonomic unit was annotated using the Silva128 16S rDNA database (bacteria). The alpha diversity indices (ACE index, Chao1 index, Shannon index, and Simpson index) were measured by using ASV table in QIIME2. The beta diversity analysis was visualized by principal coordinate analysis (**PCoA**). Permutational multivariate analysis of variance was used to assess the significance of differentiation of microbiota structure among groups.

Statistical Analyses

Data were analyzed using the General Linear Model procedure in SPSS version 18.0 software (SPSS Inc. Chicago, IL), and subjected to two-way ANOVA in a 4 × 2 factorial arrangement to analyze the main effects of dietary treatments and oxidized oil, and their interaction. We analyzed 0, 0.5, 1, and 2% levels of *Fagopyrum dibotrys* in diet to give linear or quadratic responses. The significant differences among groups were determined using Tukey's multiple range tests when a significant interaction was observed. Differences were considered significant at $P < 0.05$.

RESULTS

Growth Performance

For 21 to 42 d, there was an interaction ($P<0.05$) of oils and diet for FCR (Table 3). Oxidized oil significantly increased FCR on d 21 to 42. FCR were not significantly changed with *Fagopyrum dibotrys* compared to the control group. Diet had a significant effect on FI from d 21 to 42 ($P<0.05$) and FI was linearly decreased with increasing *Fagopyrum dibotrys* ($P < 0.05$). Oxidized oil significantly increased FI on d 1 to 7 and 7 to 21 and BWG on d 1 to 7 and increased FCR on d 21 to 42. Furthermore, FI was lower in the *Fagopyrum dibotry* group than in the CON group on d 21 to 42, irrespective of oil ($P < 0.05$).

Antioxidant Status

Oxidized oil increased MDA concentrations ($P < 0.001$) and decreased SOD activity ($P = 0.049$) in the serum compared to the control group (Table 4). On the other hand, supplementation of *F. dibotry* to broiler diets did not affect the activities of T-AOC in the serum, irrespective of oil ($P > 0.05$).

The MDA concentration was significantly higher due to oxidized oil in the liver, but *F. dibotry* did not affect antioxidant activities on d 21 (Table 5). The liver SOD activity was significantly lower on d 42 due to oxidized oil, whereas 0.5, 1 and 2% *F. dibotry* in diets showed significant improvement liver SOD activity by 7.06%, 24.71%, and 18.82%, respectively. On d 42, 2% *F. dibotry* alleviated the liver increased MDA concentrations induced by oxidized oil ($P < 0.05$), whereas 1% *F. dibotry* decreased MDA concentrations in fresh oil groups. The liver T-AOC activity was significantly increased in chickens fed 0.5, 1, and 2% *F. dibotry* in a dose-dependent manner, regardless of oxidized or fresh oil.

The content of MDA in the jejunal mucosa of broilers eating oxidized oils was linearly decreased ($P < 0.001$) on d 21 and 42 by adding *F. dibotry* (Table 6). Oxidized oil significantly decreased jejunal mucosa SOD activity on d 21, but *F. dibotry* increased SOD activity ($P = 0.014$), whereas in broilers fed fresh oil, *F. dibotry* promoted SOD activity on d 42 ($P = 0.007$).

Table 3. Effects of dietary *Fagopyrum dibotry* on growth performance of broiler chickens fed oxidized oil.

Treatment		D 1–7			D 7–21			D 21–42		
Oils	*Fag* (%)	BWG (g/bird)	FI (g/bird)	FCR (g/g)	BWG (g/bird)	FI (g/bird)	FCR (g/g)	BWG (g/bird)	FI (kg/bird)	FCR (g/g)
OS	0	53.79	55.60	1.03	250	416	1.67	677	1.55	2.31[a]
	0.5	51.97	55.30	1.07	268	438	1.64	680	1.55	2.28[a]
	1	50.86	53.00	1.04	247	415	1.68	646	1.49	2.32[a]
	2	52.03	54.00	1.04	243	417	1.72	606	1.37	2.26[a]
FS	0	42.9	44.79	1.04	244	397	1.63	651	1.41	2.18[b]
	0.5	43.72	45.00	1.04	245	401	1.63	664	1.52	2.28[a]
	1	45.60	40.80	0.89	249	411	1.65	682	1.39	2.03[b]
	2	44.70	47.80	1.07	252	411	1.64	664	1.44	2.16[b]
SEM		0.741	0.751	0.013	2.497	3.99	0.015	7.759	0.014	0.018
Main effects										
Oils	OS	52.16	54.48	1.05	252	421	1.677	652	1.49	2.29[a]
	FS	44.23	44.60	1.01	247	405	1.638	665	1.44	2.16[b]
Fag	0	48.35	50.20	1.04	247	407	1.651	663	1.48	2.24
	0.5	47.85	50.15	1.05	257	420	1.633	672	1.53	2.28
	1	48.23	46.90	0.97	248	413	1.668	664	1.44	2.17
	2	48.36	50.90	1.06	248	414	1.679	635	1.40	2.21
P values										
Linear		0.962	0.915	0.779	0.771	0.697	0.379	0.184	0.020	0.284
Quadratic		0.879	0.400	0.212	0.321	0.471	0.633	0.240	0.142	1.000
Fag		0.994	0.261	0.099	0.463	0.728	0.731	0.354	0.013	0.182
Oil		< 0.001	<0.001	0.210	0.371	0.043	0.200	0.403	0.072	0.001
Fag × Oil		0.608	0.540	0.104	0.135	0.439	0.827	0.172	0.064	0.039

Abbreviations: BWG, body weight gain; FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; FI, feed intakes; FCR, feed conversion ratio; OS, oxidized soybean oil.

[ab]Different letters in the same row indicate significant differences ($P < 0.05$), and the same letter means no significant difference ($P > 0.05$).

Furthermore, 0.5, 1, and 2% *F. dibotry* alleviated decreased jejunal mucosal GPX activities induced by oxidized oil in a dose-dependent manner ($P < 0.001$) on d 21, whereas 2% *F. dibotry* increased GPX activity compared to the oxidized oil treatment ($P < 0.001$).

Intestinal Secretory IgA Concentration

Jejunal sIgA concentrations on d 42 were higher ($P < 0.001$) in the *Fagopyrum dibotry* group than in control groups (Figure 1).

Serum Diamine Oxidase Activity

The serum DAO activity was significantly higher ($P < 0.05$) in the oxidized oil groups than in the fresh oil groups on d 21, and the similar result was observed on d 42 ($P < 0.05$; Figure 2). Furthermore, DAO activity was lower ($P < 0.05$) in the oxidized oil group with treatment of *F. dibotrys* than in the control group on d 21 and 42. There was an interaction ($P < 0.05$) between oils and dietary treatments on DAO activity on d 42. The serum DAO activity significant linearly decreased with *F. dibotry* on d 21 and 42 ($P < 0.05$).

Table 4. The T-AOC, MDA, and SOD activity in the serum of broiler chickens fed with oxidized oil and various concentrations of *Fagopyrum dibotry*.

Treatment		D 21			D 42		
Oils	*Fag* (%)	MDA (U/mL)	SOD (U/mL)	AOC (mmol/mL)	MDA (U/mL)	SOD (U/mL)	AOC (mmol/mL)
OS	0	4.22	1,128	1.04	4.73	908	0.85
	0.5	3.34	1,188	1.01	4.48	840	0.89
	1	3.97	1,084	1.08	4.73	932	0.88
	2	3.54	1,162	0.99	5.22	890	0.87
FS	0	2.74	1,090	1.03	4.97	996	0.89
	0.5	2.88	1,126	0.98	4.43	1017	0.89
	1	2.95	1,109	1.03	6.20	987	0.90
	2	2.32	1,130	0.99	5.00	955	0.91
SEM		0.108	28.713	0.013	0.157	23.863	0.013
Main effects							
Oils	OS	3.76	1,140	1.03	4.79	893	0.87
	FS	2.72	1,113	1.01	5.15	989	0.90
Fag	0	3.48	1,109	1.04	4.85	952	0.87
	0.5	3.11	1,157	1.00	4.46	929	0.89
	1	3.46	1,097	1.06	5.46	960	0.89
	2	2.93	1,146	0.99	5.11	923	0.89
P values							
Linear		0.568	0.829	0.494	0.215	0.795	0.640
Quadratic		0.926	0.986	0.641	0.949	0.890	0.630
Fag		0.243	0.857	0.235	0.151	0.935	0.927
Oil		<0.001	0.641	0.414	0.255	0.049	0.287
Fag × Oil		0.323	0.957	0.935	0.229	0.796	0.938

Abbreviations: FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; MDA, malonaldehyde; OS, oxidized soybean oil; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

Table 5. Antioxidant enzyme activities in the liver of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry.*

Treatment		D 21				D 42			
Oils	*Fag* (%)	MDA (nmol/mgprot)	SOD (U/mgprot)	AOC (mmol/mgprot)	GPX (nmol/min/g)	MDA (nmol/mgprot)	SOD (U/mgprot)	AOC (mmol/mgprot)	GPX (nmol/min/g)
OS	0	1.46	112	0.86	2,605	0.61	85	0.78	3,110
	0.5	1.17	117	0.88	2,959	0.43	91	0.97	3,147
	1	1.06	104	0.94	2,765	0.45	106	0.95	2,807
	2	1.21	100	0.96	2,765	0.39	101	1.09	3,128
FS	0	1.02	117	0.82	2,817	0.59	97	0.84	2,997
	0.5	0.89	102	0.86	2,895	0.49	99	0.86	2,921
	1	1.10	114	0.97	2,904	0.39	107	1.01	2,900
	2	1.00	99	0.77	2839	0.45	107	1.13	2,944
SEM		0.046	1.957	0.024	36.137	0.019	1.622	0.024	32.311
Main effects									
Oils	OS	1.23	108	0.91	2,773	0.47	96	0.95	3,048
	FS	1.00	108	0.85	2,864	0.48	102	0.96	2,941
Fag	0	1.24	115	0.84	2,711	0.60	91	0.81	3,054
	0.5	1.03	110	0.87	2,927	0.46	95	0.92	3,034
	1	1.08	109	0.95	2,835	0.42	106	0.98	2,854
	2	1.11	99	0.86	2,802	0.42	104	1.11	3,036
P values									
Linear		0.550	0.021	0.387	0.576	0.002	0.002	<0.001	0.471
Quadratic		0.265	0.586	0.260	0.091	0.074	0.390	0.769	0.145
Fag		0.449	0.062	0.391	0.224	0.004	0.005	0.001	0.11
Oil		0.02	0.981	0.26	0.219	0.787	0.049	0.769	0.105
Fag × Oil		0.357	0.167	0.419	0.580	0.659	0.701	0.524	0.311

Abbreviations: FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; GPX, glutathione peroxidase; MDA, malonaldehyde; OS, oxidized soybean oil; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

Relative mRNA Expression of Antioxidant, Inflammation, and Barrier Function-Related Genes in the Chicken Jejunal Mucosa

On d 21, oxidized oil down-regulated genes related to intestinal barriers such as Claudin-1 ($P < 0.001$) and ZO-1 ($P = 0.003$) in the jejunal mucosa (Table 7). However, 2% *F. dibotry* reversed downregulation of ZO-1 ($P = 0.009$) and *F. dibotry* improved ($P = 0.042$) downregulation of Claudin-1. Oxidized oil decreased ($P = 0.012$) the mRNA expression of antioxidant related genes Nrf2 and mRNA expression of Nrf2 was elevated with 1 or 2% *F. dibotry,* regardless of oil treatment ($P < 0.05$). In the oxidized oil groups, the mRNA expression of CAT was elevated with *F. dibotry* supplementation. Furthermore, interactions of *F. dibotry* and oil challenge significantly affected jejunal mucosa Nrf2 and CAT expression on d 21.

There was an interaction ($P < 0.05$) between *F. dibotry* and oil on the mRNA expression of CAT, SOD1, and IL-22 in the jejunum on d 42 (Table 8). The mRNA expression of CAT, SOD1, IL-22, Claudin-1, and ZO-1 in oxidized oil groups was increased ($P < 0.05$) with *F. dibotry* supplementation.

Cecal Microbiome

In this study, 734,737 effective and high-quality CCS were obtained from all samples after processing and filtering. The alpha diversity of Chao1 index differed between oxidized oil and fresh oil groups ($P = 0.004$, Table 9). With the addition of *F. dibotry*, the alpha diversity of ACE index differed ($P = 0.026$, Table 9). There was an interaction ($P < 0.05$) between oils and dietary treatments on the ACE, Chao1, Simpson and Shannon indices (Table 9). Beta diversity was analyzed by Principal coordinates analysis (**PCoA**), with clear differentiation ($P < 0.05$) of the microbial community among the 8 groups (Figure 3). The oxidized oil groups were separated, whereas groups treated with 2% *F. dibotry* and oxidized oil were close to FS-FAM, FS-FAL, and FS-CON groups.

At the phylum level, *Firmicutes* (86.13−96.62%), *Proteobacteria* (1.74−19.89%), *Bacteroidetes* (0.69−1.64%), and *Fusobacteria* (0.16−1.02%) accounted for > 90% of the total cecal microbiota (Figure 4A). The relative abundance of *Firmicutes* was higher in treatment with *F. dibotry* group, regardless of oxidized oil challenge, whereas relative abundance of *Proteobacteria* was opposite. In Figure 4B, the top 10 genera are listed. The relative abundance of *Streptococcus, Enterococcus*, and *Escherichia* were lower in treatment with 2% *F. dibotry* group, regardless of oxidized oil challenge. However, the relative abundance of *Candidatus_Arthromitus* was higher in treatment with *F. dibotry* group, regardless of oxidized oil challenge.

DISCUSSION

Oxidative stress, defined as an imbalance between pro-oxidant and antioxidant systems, is involved in common enteric diseases of broilers (Lauridsen, 2019). Oxidized lipids can reduce palatability, produce harmful peroxidation products, and reduce energy value of feed materials (Grootveld et al., 2022). Tiejiaoma broilers are a heritage breed that is largely restricted to the Guizhou, Guangxi, and Sichuan province. Their meat is high-quality, while the growth rate and weight are smaller

Table 6. Antioxidant enzyme activities in the jejunal mucosa of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry*.

Treatment		D 21				D 42			
Oils	*Fag* (%)	MDA (nmol/mgprot)	SOD (U/mgprot)	T-AOC (mmol/mgprot)	GPX (nmol/min/g)	MDA (nmol/mgprot)	SOD (U/mgprot)	T-AOC (mmol/mgprot)	GPX (nmol/min/g)
OS	0	0.32^{a}	113^{b}	1.74	1,154^{e}	0.25^{a}	151^{c}	1.19	2,071^{b}
	0.5	0.25^{b}	131^{a}	1.76	1,363^{d}	0.19^{b}	152^{c}	1.19	2,168^{b}
	1	0.25^{b}	129^{a}	1.83	1,671^{bc}	0.15^{c}	141^{c}	1.20	2,050^{b}
	2	0.20^{cd}	125^{ab}	1.80	1,702^{abc}	0.10^{d}	157^{bc}	1.19	2,602^{a}
FS	0	0.21^{bc}	122^{ab}	1.77	1,642^{c}	0.13^{cd}	157^{bc}	1.19	2,041^{b}
	0.5	0.21^{bc}	122^{ab}	1.80	1,614^{c}	0.13^{cd}	170^{ab}	1.20	2,084^{b}
	1	0.16^{d}	125^{ab}	1.77	1,874^{a}	0.13^{cd}	175^{a}	1.19	2,125^{b}
	2	0.20^{cd}	123^{ab}	1.76	1,835^{ab}	0.14^{cd}	181^{a}	1.20	2,094^{b}
SEM		0.004	1.041	0.012	15.362	0.003	1.429	0.001	19.985
Main effects									
Oils	OS	0.26^{a}	125	1.78	1,473^{b}	0.17^{a}	149^{b}	1.19	2,223^{a}
	FS	0.19^{b}	123	1.78	1,741^{a}	0.13^{b}	170^{a}	1.20	2,086^{b}
Fag	0	0.26^{a}	118^{b}	1.76	1,399^{b}	0.19^{a}	154^{b}	1.19	2,056^{b}
	0.5	0.23^{ab}	127^{a}	1.78	1,488^{b}	0.16^{bc}	161^{ab}	1.19	2,126^{b}
	1	0.21^{b}	127^{a}	1.80	1,773^{a}	0.14^{bc}	158^{ab}	1.20	2,087^{b}
	2	0.20^{b}	124^{ab}	1.78	1,769^{a}	0.12^{c}	169^{a}	1.20	2,348^{a}
P values									
Linear		<0.001	0.714	0.824	<0.001	<0.001	<0.001	0.258	0.490
Quadratic			0.411	0.057	0.104	<0.001	<0.001	0.011	0.950	0.005
Fag			<0.001	0.014	0.608	<0.001	<0.001	0.007	0.425	<0.001
Oil			<0.001	0.519	0.804	<0.001	<0.001	<0.001	0.525	0.002
Fag × Oil			<0.001	0.027	0.361	0.001	<0.001	0.016	0.123	<0.001

Abbreviations: FS, fresh soybean oil; *Fag*, *Fagopyrum dibotry*; GPX, glutathione peroxidase; MDA, malonaldehyde; OS, oxidized soybean oil; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.
^{abcd}Different letters in the same row indicate significant differences ($P < 0.05$), and the same letter means no significant difference ($P > 0.05$).

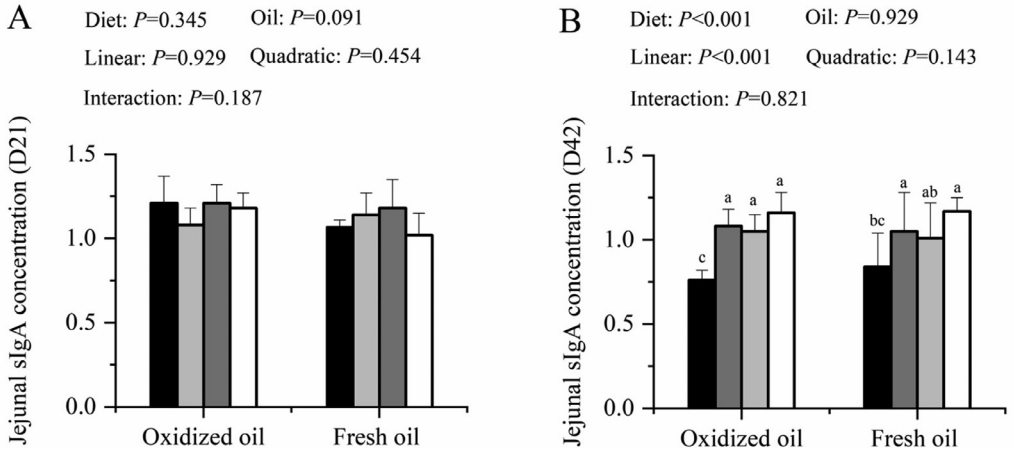

Figure 1. The secretory IgA (sIgA) concentration (mg/g protein) of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry* on d 21 (A) and 42 (B). Oxidized oil, chickens with oxidized soybean oil diet; Fresh oil, chickens with fresh soybean oil diet; □, basal diet with 2% *Fagopyrum dibotry*; ▨ basal diet with 1% *Fagopyrum dibotry*; ▨ basal diet with 0.5% *Fagopyrum dibotry*; ■, basal diet without *Fagopyrum dibotry*. [a,b,c]Mean values with unlike letters were significantly different ($P < 0.05$).

(Tan et al., 2017). Therefore, the growth performance in this experiment was lower than that of white-feather broilers (Tan et al., 2019b). In the current study, oxidized oil significantly increased BWG on d 1 to 7, FI on d 1 to 7 and 7 to 21, and FCR on d 21 to 42, similar to other research (Zhang et al., 2022). Livestock resist oxidative stress by accelerating respiration, promoting glucose metabolic activity, and accelerating decomposition (Sies, 2015). Therefore, the increased FI on d 1 to 7 and 7 to 21 with oxidized oil diets might be due to increased metabolic rate in broilers in response to oxidative stress. On the other hand, by increasing feed intake to resist oxidative stress, perhaps some of the remaining nutrients leaded to increased BWG on d 1 to 7 (Tavarez et al., 2011). Oxidation products such as aldehydes, ketones, esters, and polymerized oils, when present in oxidized oil, may also reduce fat retention and energy value of the diet, leading to a detrimental effect on growth performance (Zhang et al., 2011). Therefore, oxidized oil increased FCR on d 21 to 42, perhaps due to deleterious effects on nutrient absorption and utilization.

Regarding effects of *F. dibotrys* supplementation to protect broilers consuming oxidized oil, 2% *F. dibotrys* reduced FI compared to other treatments on d 21 to 42, similar to a previous study involving 1% *F. dibotrys* (Tan et al., 2017). As a traditional Chinese medicine, *F. dibotrys* was fed to broilers, and in some cases reduced palatability and feed intake (Zhang and Chen, 2018). However, BWG and FCR were not significantly changed

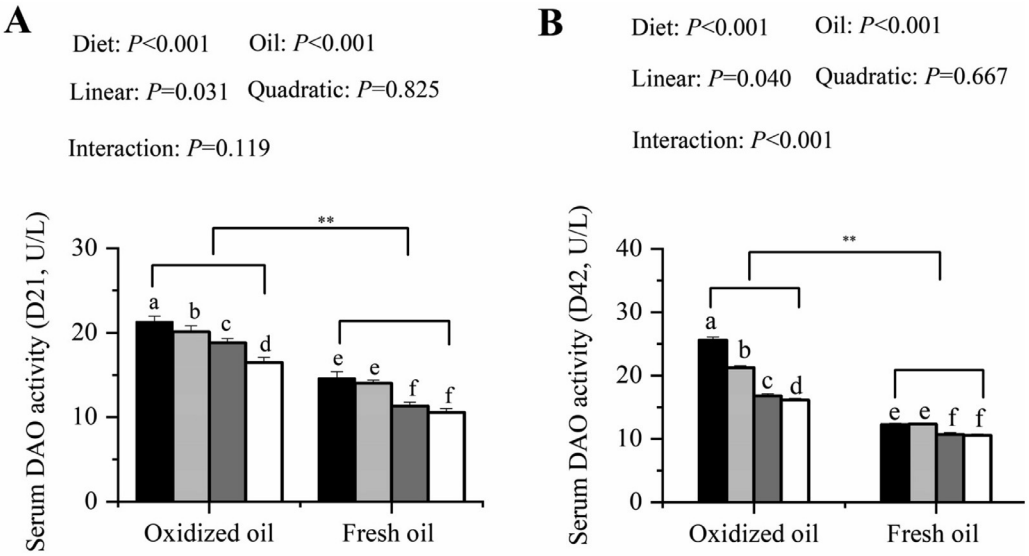

Figure 2. The serum diamine oxidase (DAO) activity (U/L) of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry* on d 21 (A) and 42 (B). Oxidized oil, chickens with oxidized soybean oil diet; Fresh oil, chickens with fresh soybean oil diet; □, basal diet with 2% *Fagopyrum dibotry*; ▨ basal diet with 1% *Fagopyrum dibotry*; ▨ basal diet with 0.5% *Fagopyrum dibotry*; ■, basal diet without *Fagopyrum dibotry*. [a,b,c,d,e,f]Mean values with unlike letters were significantly different ($P < 0.05$). **Significat main effect ($P < 0.05$) of oils.

Table 7. The relative mRNA expression of jejunal antioxidant, inflammation and tight junction of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry* on d 21.

Treatment		D 21							
Oils	*Fag* (%)	CAT	SOD1	Nrf2	IL-6	IL-22	Claudin-1	Occludin	ZO-1
OS	0	0.95^{b}	1.12	0.76^{d}	1.80	0.75	0.73	0.97	0.57
	0.5	1.39^{ab}	1.19	0.92^{cd}	1.69	0.93	0.86	1.00	0.67
	1	1.15^{b}	1.16	1.83^{a}	1.57	1.10	1.00	0.96	1.06
	2	1.85^{a}	1.17	1.93^{a}	1.46	1.00	0.94	1.09	2.29
FS	0	1.00^{b}	0.98	1.00^{cd}	1.01	1.10	1.01	1.08	1.01
	0.5	0.96^{b}	1.17	1.14^{bc}	0.92	1.19	1.21	0.93	1.37
	1	1.21^{b}	1.14	1.39^{b}	1.13	1.25	1.27	0.93	2.12
	2	0.93^{b}	1.15	1.36^{b}	0.95	1.47	1.35	0.88	2.33
SEM		0.050	0.026	0.024	0.032	0.040	0.035	0.019	0.080
Main effects									
Oils	OS	1.34^{a}	1.16	1.36^{a}	1.63	0.95	0.88	1.01	1.15
	FS	1.03^{b}	1.11	1.22^{b}	1.01	1.25	1.21	0.95	1.70
Fag	0	0.98	1.05	0.88^{b}	1.40	0.92	0.87	1.02	0.79
	0.5	1.17	1.18	1.03^{b}	1.30	1.06	1.03	0.97	1.02
	1	1.18	1.15	1.61^{a}	1.35	1.17	1.14	0.94	1.59
	2	1.39	1.16	1.65^{a}	1.21	1.24	1.15	0.98	2.31
P values									
Linear		0.067	0.195	<0.001	0.435	0.033	0.043	0.482	<0.001
Quadratic		0.953	0.251	0.590	0.885	0.719	0.439	0.293	0.249
Fag		0.070	0.324	<0.001	0.191	0.064	0.042	0.534	<0.001
Oil		0.007	0.336	0.012	<0.001	0.001	<0.01	0.195	0.003
Fag × Oil		0.010	0.818	<0.001	0.163	0.571	0.852	0.065	0.183

Abbreviations: CAT, catalase; FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-6, interleukin 6; IL-22, interleukin 22; OS, oxidized soybean oil; SOD1, superoxide dismutase 1; Nrf2, nuclear factor erythroid 2-related factor 2; ZO-1, tight junction protein 1.

^{abcd}Different letters in the same row indicate significant differences ($P < 0.05$), and the same letter means no significant difference ($P > 0.05$).

with 2% *F. dibotrys*, perhaps due to the high flavonoid and phenol concentrations that would be expected to improve health (Wang et al., 2005).

Oxidized lipids in diets are absorbed by the small intestine, incorporated into chylomicrons, appear in the bloodstream, and contribute to the total body pool of oxidized lipid (Staprans et al., 1994). A major product of lipid peroxidation is MDA; increasing concentrations are related to lipid peroxidation and oxidative stress (Tan et al., 2018). In the present study, oxidized oil significantly increased serum MDA concentrations on d 21, indicating lipid peroxidation. Moreover, oxidized oil significantly decreased SOD activity on d 42. Interestingly, on d 21 and 42, *F. dibotrys* tended to improve

Table 8. The relative mRNA expression of jejunal antioxidant, inflammation and tight junction of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry* on d 42.

Treatment		D 42							
Oils	*Fag* (%)	CAT	SOD1	Nrf2	IL-6	IL-22	Claudin-1	Occludin	ZO-1
OS	0	1.90^{b}	0.82^{c}	0.85	1.12	0.87^{d}	0.56	0.74	0.34
	0.5	2.07^{b}	0.86^{bc}	0.91	1.13	1.04^{cd}	1.12	0.94	0.75
	1	7.30^{a}	0.91^{bc}	1.05	1.08	1.08^{bcd}	1.75	0.99	1.04
	2	7.55^{a}	1.12^{a}	1.04	1.07	1.10^{bcd}	3.74	0.99	1.49
FS	0	1.03^{b}	1.02^{ab}	1.00	1.01	1.22^{bc}	1.00	1.01	1.02
	0.5	1.61^{b}	1.20^{a}	1.06	1.04	1.36^{b}	1.31	1.04	1.34
	1	1.55^{b}	1.18^{a}	0.94	0.84	1.37^{b}	1.72	0.98	1.39
	2	2.39^{b}	1.15^{a}	1.02	0.87	1.83^{a}	4.99	1.04	1.37
SEM		0.209	0.015	0.021	0.017	0.023	0.130	0.023	0.068
Main effects									
Oils	OS	4.71^{a}	0.93^{b}	0.96	1.10	1.02^{b}	1.79	0.91	0.90
	FS	1.65^{b}	1.14^{a}	1.01	0.94	1.45^{a}	2.26	1.02	1.28
Fag	0	1.47^{b}	0.92^{b}	0.93	1.06	1.04^{c}	0.78	0.87	0.68
	0.5	1.84^{b}	1.03^{a}	0.98	1.08	1.20^{bc}	1.22	0.99	1.04
	1	4.43^{a}	1.05^{a}	1.00	0.96	1.22^{b}	1.73	0.98	1.22
	2	4.97^{a}	1.14^{a}	1.03	0.97	1.46^{a}	4.37	1.02	1.43
P values									
Linear		0.006	0.024	0.134	0.081	0.018	<0.001	0.088	0.048
Quadratic		0.929	0.866	0.832	0.875	0.719	0.001	0.426	0.328
Fag		<0.001	0.001	0.413	0.047	<0.001	<0.001	0.166	0.009
Oil		<0.001	<0.001	0.313	<0.001	<0.001	0.094	0.038	0.013
Fag × Oil		<0.001	0.013	0.131	0.357	0.013	0.355	0.219	0.197

Abbreviations: FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; CAT, catalase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-6, interleukin 6; IL-22, interleukin 22; Nrf2, nuclear factor erythroid 2-related factor 2; OS, oxidized soybean oil; SOD1, superoxide dismutase 1; ZO-1, tight junction protein 1.

^{abcd}Different letters in the same row indicate significant differences ($P < 0.05$), and the same letter means no significant difference ($P > 0.05$)

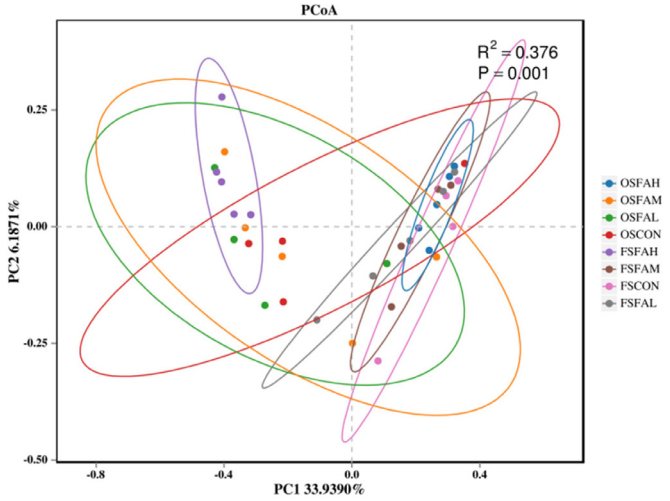

Figure 3. The β-diversity of cecal microbiota of broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry*. Abbreviations: FSFAH, basal diet with fresh soybean oil and 2% *Fagopyrum dibotry*; FSFAM, basal diet with fresh soybean oil and 1% *Fagopyrum dibotry*; FSFAL, basal diet with fresh soybean oil and 0.5% *Fagopyrum dibotry*; FSCON, basal diet with fresh soybean oil and 0% *Fagopyrum dibotry*; OSFAH, basal diet with oxidized soybean oil and 2% *Fagopyrum dibotry*; OSFAM, basal diet with oxidized soybean oil and 1% *Fagopyrum dibotry*; OSFAL, basal diet with oxidized soybean oil and 0.5% *Fagopyrum dibotry*; OSCON, basal diet with oxidized soybean oil and 0% *Fagopyrum dibotry*.

parameters related to oxidation in the serum. Ruan et al. (2017) also reported that 50 to 200 mg/kg *F. dibotrys* rhizoma had antidiabetic activity in mice via antioxidative effects.

The liver is involved in redox balance (Sadasivam et al., 2022) and undergoes oxidative stress following consumption of oxidized soybean oil (Ammouche et al., 2002). Oxidized oils can damage oxidative status of liver in rat (Zalejska-Fiolka et al., 2010), finishing barrows (Boler et al., 2012), and juvenile largemouth bass (Chen et al., 2013). In the present study, oxidized oil significantly increased MDA concentrations on d 21 and decreased SOD concentration on d 42. However, as expected, dietary *F. dibotrys* (1 and 2%) alleviated the decrease in hepatic SOD activity on d 42, with a tendency for a downregulation of MDA in the 1% *F. dibotrys* treatment compared to the oxidized oil treatment on d 21. Hepatic T-AOC capacity was significantly improved by 1 or 2% *F. dibotrys* on d 42. Although the oxidized oil was toxic, confirmed by increased hepatic MDA concentrations and altered enzymatic activities, feeding *F. dibotrys* mitigated oxidized oil-induced oxidative stress.

The intestinal epithelial barrier has key roles in nutrient digestion and absorption, and preventing invasion of pathogenic bacteria. As an intracellular enzyme particularly abundant in the small intestinal epithelia, DAO is released into circulation following intestinal villi damage, making it a marker of intestinal mucosal injury (Luk et al., 1980). The serum DAO serves as a marker of injury and integrity of the intestinal mucosa (Chen et al., 2022). In this study, the serum DAO activity of broilers was decreased by *F. dibotrys* at 21 days in oxidized oil group. In the fresh oil group, 1% and 2% of *F. dibotrys* also could remarkably decrease the DAO activity. The results showed that the addition of *F. dibotrys* reduced the gut damage. Serum DAO concentrations of *F. dibotry* groups were significantly decreased, consistent with preservation of small intestinal mucosal integrity (Chen et al., 2022). A possible reason is the pharmacological component of *F. dibotrys,* including flavonoids, phenols, terpenes, steroids, and fatty acids (Zhang et al., 2016).

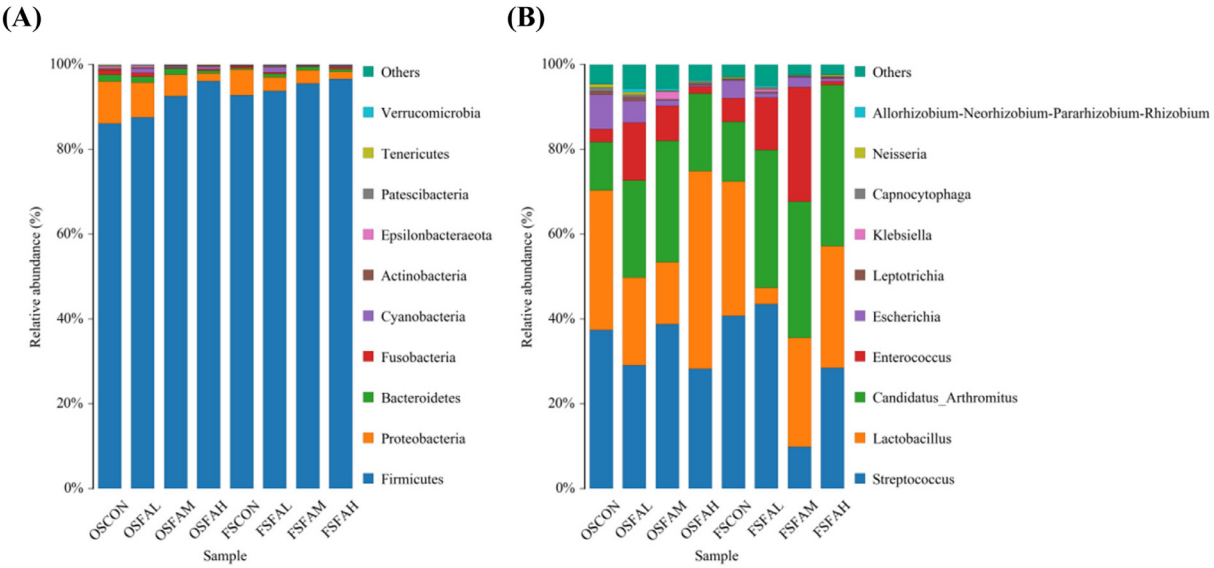

Figure 4. Microbial community bar plot at the (A) phylum and (B) genus level in broiler chickens fed oxidized oil and various concentrations of Fagopyrum dibotry. Abbreviations: FSFAH, basal diet with fresh soybean oil and 2% *Fagopyrum dibotry*; FSFAM, basal diet with fresh soybean oil and 1% *Fagopyrum dibotry*; FSFAL, basal diet with fresh soybean oil and 0.5% *Fagopyrum dibotry*; FSCON, basal diet with fresh soybean oil and 0% *Fagopyrum dibotry*; OSFAH, basal diet with oxidized soybean oil and 2% *Fagopyrum dibotry*; OSFAM, basal diet with oxidized soybean oil and 1% *Fagopyrum dibotry*; OSFAL, basal diet with oxidized soybean oil and 0.5% *Fagopyrum dibotry*; OSCON, basal diet with oxidized soybean oil and 0% *Fagopyrum dibotry*.

Table 9. The α-diversity of cecal microbiota in broiler chickens fed oxidized oil and various concentrations of *Fagopyrum dibotry* on d 21.

Treatment		D 21			
Oils	*Fag* (%)	ACE	Chao1	Shannon	Simpson
OS	0	98.53^{ab}	120.00^{a}	2.06^{ab}	0.71^{a}
	0.5	127.39^{a}	124.66^{a}	1.62^{b}	0.62^{a}
	1	89.89^{ab}	124.60^{a}	1.57^{b}	0.60^{a}
	2	128.85^{a}	146.62^{a}	2.38^{ab}	0.63^{a}
FS	0	118.71^{a}	114.38^{a}	1.26^{b}	0.32^{b}
	0.5	135.36^{a}	136.65^{a}	3.12^{a}	0.72^{a}
	1	119.23^{a}	99.20^{ab}	2.43^{ab}	0.60^{a}
	2	57.69^{b}	54.37^{b}	1.88^{ab}	0.60^{a}
SEM		4.328	4.545	0.118	0.021
Main effects					
Oils	OS	111.17	128.97^{a}	1.907	0.642
	FS	107.75	101.15^{b}	2.173	0.555
Fag	0	108.62^{ab}	117.19	1.66	0.515
	0.5	131.38^{a}	130.65	2.37	0.670
	1	104.56^{ab}	111.90	2.00	0.601
	2	93.27^{c}	100.50	2.13	0.608
P values					
Linear		0.118	0.177	0.390	0.365
Quadratic		0.103	0.272	0.288	0.156
Fag		0.026	0.154	0.213	0.104
Oil		0.695	0.004	0.268	0.052
Fag × Oil		0.001	0.001	0.003	0.001

Abbreviations: FS, fresh soybean oil; *Fag, Fagopyrum dibotry*; OS, oxidized soybean oil.

^{abc}Different letters in the same row indicate significant differences ($P < 0.05$), and the same letter means no significant difference ($P > 0.05$).

The secretory IgA is abundant at all mucosal sites, including the intestinal mucosa, and can prevent translocation of pathogenic bacteria across the epithelium and maintain homoeostasis with commensal microbiota (Donald et al., 2022). Stimulation of mucosal IgA requires large doses of bacteria, the extent of which depends on the total bacterial dose (Han et al., 2022). Moderately oxidized oil stimulated IgA secretion, whereas severely oxidized oil decreased IgA secretion into serum (Zhang et al., 2022). In our study, oxidized oil increased did not alter jejunal mucosa sIgA concentrations, similar to other reports (Huang et al., 2016). However, limited studies have been conducted to evaluate effects of *F. dibotrys* on broiler intestinal mucosa sIgA content. In the present study, greater sIgA concentrations in birds fed *F. dibotrys* indicated enhanced intestinal mucosal immunity protecting the intestine against pathogen adherence (Tan et al., 2017). The gut microbiota interacts directly or indirectly with the host immune system (Donald et al., 2022). Perhaps *F. dibotrys* altered the intestinal microbial composition and stimulated antibody production, protecting villi from damage and promoting intestinal health and function.

The intestinal barrier includes an extensive enzyme and non-enzyme antioxidant system (Jang et al., 2022) that was easily damaged by oxidized oil (Tan et al., 2019a). As the antioxidant activity of *F. dibotrys*, the intestinal mucosa was undoubtedly the target of this herbal medicine (Li et al., 2015). In this experiment, 2% *F. dibotrys* alleviated the increase in MDA concentration in the jejunal mucosa on d 21 and 42, with upregulation of GPX and SOD in the 2% *F. dibotrys* treatment compared to oxidized oil. In another study, *F. dibotrys* supplemented broilers had lower MDA concentrations and higher SOD activity in the intestine mucosa than those in the control group (Tan et al., 2019b), consistent with our results. The rhizome of *F. dibotrys* has a long history of being used as an anticancer and anti-inflammatory herb in China (Chan, 2003). This compound contains flavonoids, phenols, fagopyritols, triterpenoids, fatty acids, and steroids, which have bioactivities such as antitumor, antioxidation, antidiabetes, etc. (Li et al., 2021). In the present study, *F. dibotrys* effectively alleviated oxidant stress caused by oxidative oils.

Nrf2, the key regulator of oxidative stress and a member of leucine zipper transcription factors, counteracts induced reactive oxygen species (ROS) and reactive nitrogen species (**RNS**) by activation of antioxidant enzymes such as SOD, CAT, GSH, GPX, HO-1 (Salehabadi et al., 2022). Dietary oxidized oil activates the Nrf2 signaling pathway in the liver of pigs (Varady et al., 2012) and in intestinal mucosa of mice (Varady et al., 2011), likely an adaptive mechanism to prevent cellular oxidative damage. The present findings were similar to a report that dietary oxidized oil in broilers damaged antioxidant enzyme activity through the Nrf2 pathway (Dong et al., 2020). *F. dibotrys* induced upregulation and nuclear translocation of Nrf2 in several cells in vitro and in vivo (Li et al., 2015). Similarly, in our study, *F. dibotrys* (1 and 2%) significantly improved antioxidant indices by significantly increasing expression of the antioxidant gene Nrf2 and downstream genes such as CAT, superoxide dismutase 1 compared to the oxidized oil group. Interleukin 6 (**IL-6**), promptly and transiently produced in response to infections and tissue injuries, has pathological effects in chronic inflammation and autoimmunity (Tanaka et al., 2014). In response to oxidized oil, the proinflammatory cytokine IL-6 was significantly increased (Rose-John, 2018). However, mRNA expression of IL-6 in jejunal mucosa treated with oxidized oils were markedly reduced after treatment with *F. dibotrys;* this compound had anti-inflammatory protection for ulcerative colitis (Ge et al., 2017) and it stimulated expression of IL-22 in jejunal mucosa, which could alleviate intestinal epithelial oxidative stress and promote repair (Wan and Pan, 2016).

Tight junction (**TJ**) proteins are vital for regulation of gut barrier function. During oxidative stress, disrupted TJ may increase mucosal permeability and promote pathogen invasion (Heinemann and Schuetz, 2019). The intestinal TJ is mainly composed of claudins, occludin, and zonula occluden-1 (**ZO-1**). In this study, although mRNA expression of Claudin-1, occludin and ZO-1 in jejunal mucosa in oxidized oil group were significantly lower than the nonoxidized oil group, this was reversed by *F. dibotrys*. Although oxidized oil would increase the intestinal permeability of broilers, *F. dibotrys* could alleviate this by regulating expression of tight junction proteins. In rats with irritable bowel syndrome, *F. dibotrys* alleviated hyperalgesia by reducing intestinal inflammation and enhancing mucosal epithelial function after regulating the structure and function of TJs (Liu et al., 2012).

Intestinal ROS are generated by gastrointestinal tract epithelial cells as a result of oxygen metabolism or by enteric commensal bacteria (Jones et al., 2012), but the antioxidant system maintains the microbiota (Direito et al., 2021). The cecal microbiota of poultry has essential roles in mediating the manipulation of intestinal barrier by dietary intervention (Kieronczyk et al., 2020). Based on alpha diversity, *F. dibotrys* increased cecal microbial richness in broilers fed oxidized oil, which reflected a more stable microbiota community that resisted colonization by pathogens and promoting productivity (Zhang et al., 2018). In addition, beta diversity had significant clustering according to experimental groups, demonstrating that cecal microbial community structure was affected by *F. dibotrys* addition. *Firmicutes* is the most prominent phylum and includes many probiotics, for example, *Clostridia, Negativicutes, Thermolithobacteria*, and *Tissierellia* (Chandrangsu et al., 2018). Perhaps the amount of *F. dibotrys* affects growth of *Firmicutes* in broilers. *Proteobacteria* are commonly involved in metabolic disorders, inflammatory, asthma, and chronic obstructive pulmonary disease (Rizzatti et al., 2017). Decreased *Proteobacteria* indicated that *F. dibotrys* improved gut health and reduced infection with harmful bacteria.

In conclusion, *F. dibotry* alleviated intestinal mucosal impairment in broiler chickens challenged with oxidized oil by ameliorating oxidative stress, supporting the intestinal mucosal barrier and modulating gut microbiota. These results provided new evidence regarding dietary *F. dibotry* as an intervention strategy to control oxidative damage in broiler production.

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DISCLOSURES

The authors declare no competing financial interest.

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