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Effects of *Artemisia ordosica* aqueous extract on intestinal inflammation and antioxidant-related gene expression in lipopolysaccharide-challenged broilers

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ABSTRACT. The purpose of this study was to assess the impact of supplementing broiler diets with *Artemisia ordosica* aqueous extract (AOE) on small intestine inflammation and the expression of antioxidant-related genes in broilers subjected to lipopolysaccharide (LPS) challenge. A total of 96 one-day-old Arbor Acres broilers (half males and half females, with an initial body weight of 50 ± 4.5 g) were assigned into four treatment groups. These groups included broilers fed a basal diet (control), broilers fed a diet containing 1000 mg/kg AOE, broilers on a basal diet subjected to LPS challenge, and broilers on a diet with 1000 mg/kg AOE subjected to LPS challenge. Each treatment consisted of six replicates, with four chicks per replicate. On days 14, 16, 18 and 20, broilers were injected with LPS or an equivalent amount of saline. The results demonstrated that dietary AOE significantly decreased the expression of inflammation-related genes in the small intestine of broilers challenged with LPS. Specifically, there were significant reductions ($P < 0.05$) in the levels of toll like receptor 4 (*TLR4*), lipopolysaccharide-induced TNF factor (*LITAF*) and nitric oxide synthase 2 (*NOS2*) in the duodenum, *TLR4*, nuclear apoptosis-inducing factor 1 (*NAIF1*), interleukin (*IL*)-*1B, IL-6*, *LITAF* and *NOS2* in the jejunum, and RELA proto-oncogene nuclear factor kappa B (NF-κB) subunit, *IL-1B* and NOS2 in the ileum. Moreover, catalase gene expression was increased in the duodenum and jejunum, while glutathione peroxidase was upregulated in the ileum ($P < 0.05$) of LPS-challenged broilers. In conclusion, dietary AOE supplementation could partly alleviate the adverse effects induced by LPS by improving the expression of genes encoding antioxidant enzymes and reducing the expression of inflammation-related genes in the small intestine of broilers.

Introduction

The gastrointestinal tract of broilers is sensitive to various external antigens, and the intestinal mucosa plays an important role in the functioning of the intestinal barrier (Duangnumsawang et al., 2021). In poultry production, intestinal mucosal damage is a common condition, often attributed to a range of pathogenic factors, including environmental stress, bacterial infection, immunological and oxidative stress (Li et al., 2018; Yang et al., 2019; Wang et al., 2022). Lipopolysaccharides (LPS) are bacterial surface glycolipids produced by Gram-negative bacteria. Intraperitoneal or intravenous injection of LPS can induce infection and cause inflammation in broiler chickens, and is therefore a widely used model for studying animal immune stress (Candelli et al., 2021). This LPS-induced stress condition is

characterised by symptoms such as anorexia, fever, impaired growth performance or increased proinflammatory cytokine production (Zhang et al., 2020; 2021). Notably, LPS functions by activating toll-like receptor 4 (TLR4), and it is produced by avian pathogenic *Escherichia coli* (Wang et al., 2022). TLR4 activation triggers phosphorylation of nuclear factor kappa B (NF-κB) and ultimately promotes the synthesis of pro-inflammatory cytokines (such as IL-1B, IL-6 and TNF-α) and induces oxidative stress (Han et al., 2020; Xing et al., 2021).

Artemisia ordosica is a typical subshrub growing in arid and semi-arid areas of northern China, including Inner Mongolia, Ningxia, Gansu and Shaanxi. *A. ordosica* contains many bioactive compounds, which encompass polyphenols, flavonoids, terpenoids, essential oil and polyacetylenes (Zhang et al., 2017; Wang et al., 2019; Zhou et al., 2019). Numerous studies have demonstrated that *A. ordosica* has anti-inflammatory, antimicrobial (Xiao et al., 2020; Tang et al., 2021), antioxidant (Wu et al., 2019) and immunoregulatory (Xing et al., 2019) properties. Previous reports have indicated the potential benefits of *A. ordosica* aqueous extract (AOE) as a natural phytogenic feed additive with antioxidant potential. Studies, such as the one conducted by Xing et al. (2019), have shown that AOE can be incorporated into piglet diets, leading to increased cytokine concentrations and antioxidant enzyme activity in serum, while reducing the malondialdehyde content (Xing et al., 2019). Moreover, it was found that polysaccharides in *A. ordosica* significantly improved the antioxidant function of rats (Xing et al., 2020). It has also been reported that *Artemisia* plants exert no adverse effects on growth performance of broilers and can improve the development of the immune system (Gharetappe et al., 2015; Habibi et al., 2016). In addition, this plant extract was also shown to reduce the cholesterol content in egg yolks (Baghban-Kanani et al., 2019). Furthermore, it was demonstrated that plant extracts (*Astragalus, Artemisia argyi, Caulis Spatholobi*) could mitigate the intestinal inflammatory response in poultry as feed additives (Luo et al., 2021; Yang et al., 2021a; Cui et al., 2022). Our previous study found that AOE exerted a beneficial effect on broilers challenged with LPS. This included reductions in the levels of inflammatory cytokines and stress hormones, as well as the promotion of growth hormone production, ultimately leading to improved growth performance (Li et al., 2017). However, despite these positive findings, the precise mechanism of AOE action remains elusive, especially regarding its molecular regulatory role in alleviating intestinal inflammation. Therefore, in the present study, we further investigated the effects of AOE on gut inflammation and antioxidant-related gene expression in LPS-challenged broilers.

Material and methods

The animal experiment was conducted with the approval of the Experimental Animal Welfare and Ethics Committee of the Inner Mongolia Agricultural University and conducted in accordance with the National Standard Guidelines for Ethical Review of Animal Welfare (GB/T 35892-2018). Every possible measure was taken to minimise any distress or suffering experienced by the animals involved in the study.

Preparation of *Artemisia ordosica* aqueous extract

Artemisia ordosica was harvested from Erdos (Inner Mongolia, China). The freshly collected plant material was first washed with distilled water and subsequently air-dried in a shaded area at a room temperature. Once dried, the plant material underwent a series of processing steps. It was subjected to three rounds of extraction with distilled water at 100 °C, with each extraction lasting 30 min. The resulting extract was then concentrated and subsequently freeze-dried to prepare the powder, and stored in a freezer until analysis.

Experimental animals and diet

A total of 96 one-day-old Arbor Acres broilers (48 males and 48 females, 50 ± 4.5 g initial body weight) were randomly allocated to four treatment groups with six replicates for each treatment and four broilers in each replicate, using a completely randomised experimental design: (1) control group – non-challenged broilers fed a basal diet; (2) AOE group – non-challenged broilers fed a basal diet supplemented with 1000 mg/kg AOE; (3) LPS group – LPS-challenged broilers fed a basal diet; (4) LPS+AOE group – LPS-challenged broilers fed a basal diet supplemented with 1000 mg/kg AOE. All broilers had unrestricted access to water and feed throughout the entire experimental period. On days 14, 16, 18 and 20, broilers in treatment groups 3 and 4 were injected intraperitoneally with LPS solution at a concentration of 500 μg LPS/kg basal body weight. LPS was dissolved in sterile saline to a concentration of 100 μg/ml, the dosage and time

course of treatments with LPS were determined based on our previous studies (Zhang et al., 2017a; Xing et al., 2021). Broilers in treatment groups 1 and 2 were injected intraperitoneally with an equivalent volume of 0.9% sterile saline. LPS (O55:B5) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The basal diet based on maize and soybean (Table 1) was formulated with reference to the nutrient requirements of broilers according to the National Research Council (NRC, 1994).

Table 1. Composition and nutrient levels of the basal diet (air-dry basis, %)

Ingredients	Days	
	1–14	15–28
Maize	51.7	58.5
Soybean meal	41.0	34.3
Soybean oil	3.00	3.00
Dicalcium phosphate	1.90	1.80
Limestone	1.10	1.20
Salt	0.370	0.370
Lysine (98%)	0.0500	0.0300
Methionine	0.190	0.100
Premix[a]	0.710	0.710
Total	100	100
Nutrients levels[b]		
metabolizable energy, MJ/kg	12.6	12.9
crude protein	21.8	20.0
calcium	1.00	1.00
available phosphorus	0.480	0.460
lysine	1.40	1.20
methionine	0.560	0.440
L-cystine	0.400	0.370

[a] provided per kg of diet: IU: vit. A 6141.5, vit. D_3 1789.2; mg: vit. E 7.99, vit. K 1.82, vit. B_1 0.65, vit. B_2 3.93, vit. B_6 2.08, vit. B_{12} 0.01, niacin 18.06, calcium pantothenate 6.65, folic acid 0.59, biotin 0.07, choline 332.28, Fe 60.91, Cu 6.01, Zn 65.75, Mn 62.3, I 0.9, Se 0.21. [b] crude protein was the measured value, while others were the calculated ones

Sample collection

On day 21, one bird was randomly selected from each replicate group, 6 birds per treatment group. Chickens were sacrificed by cervical dislocation, the abdominal cavity was opened, the intestinal tract was extracted, and the duodenum, jejunum, and ileum were separated. Approximately 7 cm of the middle portions from each of these intestinal segments were collected. The chyme was rinsed with normal saline solution, and then the intestinal segments were placed in cryogenic vials. All samples were stored at −80 °C until determination of gene expression levels.

Real-time quantitative PCR

Total RNA from the collected samples was isolated using RNAiso Plus reagent (TaKaRa Biotechnology Co. Ltd., Dalian, China), according to the manufacturer's instructions. Intestinal tissue samples (duodenum, jejunum and ileum) were taken from ultra-low temperature freezer, and 50–100 mg was transferred to a mortar pre-cooled with liquid nitrogen, ground to a powder, the appropriate amount of RNAiso Plus reagent (TaKaRa Biotechnology Co. Ltd., Dalian, China) was added and completely homogenised. Next, a homogenised sample was transferred to a centrifuge tube and allowed to equilibrate to room temperature (15–30 °C) for 5 min. The tube was centrifuged at 12 000 × *g* for 5 min at 4 °C. Subsequently, chloroform (China National Pharmaceutical Group Limited, Beijing, China) (at a ratio of 0.2 ml of chloroform per 1 ml of RNAiso Plus solution) was added to the solution and mixed until it became milky in appearance. The solution was then allowed to stand at room temperature for 5 min before being centrifuged again at 12 000 × *g* for 15 min at 4 °C. The top liquid layer was transferred to a new centrifuge tube without touching the middle layer and mixed with 0.5–1 ml of isopropanol per 1 ml of RNAiso Plus used for homogenisation. The mixture was incubated at room temperature for 10 min and centrifuged at 12 000 × *g* for 10 min at 4 °C to precipitate RNA. The supernatant was carefully removed and 75% cold ethanol equivalent was added to the supernatant. The solution was centrifuged again at 12 000 *g* for 5 min at 4 °C and the supernatant was discarded. The precipitate was dried by leaving the tube open for several minutes. Once dried, the RNA pellet was reconstituted by dissolving it in an appropriate amount of RNase-free water (Biosharp, Hefei, China). To assess the quantity and quality of total RNA, the sample were analysed at 260 nm and 280 nm using a nucleic acid quantifier (Pultton P200CM, San Jose, CA, USA). Total RNA was reverse-transcribed into complementary DNA (cDNA) using the Prime Script RT Reagent Kit with gDNA Eraser (TaKaRa Biotechnology Co. Ltd., Dalian, China). Each sample was added to the RT reaction mixture (10 µl), which contained 0.5 µl PrimeScript™ RT Enzyme Mix I, 2 µl 5 × PrimeScript Buffer, 0.5 µl Random hexamer primers, 0.5 µl Oligo-dT Primer, and RNase-free water. The reaction mixture was incubated for 15 min at 37 °C, followed by 5 s at 85 °C. Real-time quantitative PCR was performed using an ABI StepOnePlus Real-Time PCR system (Applied Biosystems, Grand Island, NY, USA) in

Table 2. Target genes and their primer sequences

Gene name	GenBank No.	Primer sequence	Fragment size, bp	PCR efficiency, %
ACTB	NM_205518	F-GCCAACAGAGAGAAGATGACAC	118	105
		R-GTAACACCATCACCAGAGTCCA		
TLR4	NM_001030693	F-TTCAGAACGGACTCTTGAGTGG	131	94
		R-CAACCGAATAGTGGTGACGTTG		
NAIF1	NM_001030962	F-CCTGGCTGTGCCTTCGGA	198	117
		R-TCACCAAGTGCTGGATGCTA		
RELA	D13721	F-CAGCCCATCTATGACAACCG	151	96
		R-CAGCCCAGAAACGAACCTC		
IL-1B	NM_204524	F-CAGCCTCAGCGAAGAGACCTT	84	103
		R-ACTGTGGTGTGCTCAGAATCC		
IL-6	HM179640	F-AAATCCCTCCTCGCCAATCT	106	99
		R-CCCTCACGGTCTTCTCCATAAA		
LITAF	NM_204267	F-TGTGTATGTGCAGCAACCCGTAGT	229	112
		R-GGCATTGCAATTTGGACAGAAGT		
NOS2	NM_204961.1	F-GCAGCACGTGGCTGAACAA	165	100
		R-CATAGAGACGCTGCTGCCAGA		
SOD1	NM_205064.1	F-TTGTCTGATGGAGATCATGGCTTC	98	95
		R-TGCTTGCCTTCAGGATTAAAGTGA		
CAT	NM_001031215.1	F-GTTGGCGGTAGGAGTCTGGTCT	182	101
		R-GTGGTCAAGGCATCTGGCTTCTG		
GPX7	NM_001163245.1	F-CAAAGTTGCGGTCAGTGGA	136	98
		R-AGAGTCCCAGGCCTTTACTACTTTC		

ACTB – actin beta, *TLR4* – toll-like receptor 4, *NAIF1* – nuclear apoptosis inducing factor 1, *RELA* – RELA proto-oncogene NF-kB subunit, *NOS2* – nitric oxide synthase 2, *IL-1B* – interleukin 1 beta, *IL-6* – interleukin-6, *LITAF* – lipopolysaccharide induced TNF factor, *CAT* – catalase, *SOD1* – superoxide dismutase 1, *GPX7* – glutathione peroxidase 7; F – forward primer, R – reverse primer, PCR - Polymerase Chain Reaction

a 96-well plate and a total reaction volume of 20 µl, including 10 µl of SYBR Premix Ex Taq II Reagent Kit (TaKaRa Biotechnology Co. Ltd., Dalian, China), 0.4 µl of forward primer (10 pmol), 0.4 µl of reverse primer (10 pmol), 2 µl of cDNA, and 7.2 µl of nuclease-free water. Reactions were run in duplicate using the following cycle conditions: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s and extension at 72 °C for 20 s (PCR stage), followed by an additional extension step at 95 °C for 15 s, cooling at 60 °C for 1 min, and another extension step at 95 °C for 15 s (melt-curve stage). Sequences of target genes and primers are shown in Table 2. *β-actin* (*ACTB*) was used as a housekeeping gene and relative expression levels of individual treatment groups were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Statistical analysis

All data were analysed using the general linear model procedure implemented in the SAS 9.0 software (SAS Institute, Inc., Cary, NC, 2003) as a 2 × 2 factorial arrangement, with dietary treatments with AOE and LPS challenge as the main effects. If a significant treatment effect was observed, the significance of differences between treatments was assessed using Tukey's honest significant difference test, at a predetermined significance level of $P < 0.05$.

Results

Expression of inflammation and antioxidant-related genes in the duodenum

Table 3 shows the effect of AOE on the expression of related genes in the duodenum of broilers challenged with LPS. As a main factor, LPS injection into broilers significantly increased the expression of the toll like receptor 4 (*TLR4*), nuclear apoptosis-inducing factor 1 (*NAIF1*), *RELA*, interleukin (*IL*)-*1B*, *IL-6,* lipopolysaccharide-induced TNF factor (*LITAF*) and nitric oxide synthase 2 (*NOS2*) genes, while reducing expression levels of the superoxide dismutase 1 (*SOD1)* and glutathione peroxidase 7 (*GPX7)* genes in the duodenum of broilers ($P < 0.05$). The AOE diet significantly decreased *NOS2* gene expression in the duodenum ($P < 0.05$), significantly increased catalase (*CAT*)

Table 3. Effect of *Artemisia ordosica* aqueous extract (AOE) on the expression of inflammation- and antioxidantrelated genes in the duodenum of lipopolysaccharide (LPS) challenged broilers

Item	LPS (-)		LPS (+)		SEM	*P*–value		
	AOE (-)	AOE (+)	AOE (-)	AOE (+)		AOE	LPS	AOE × LPS
TLR4	0.884^{c}	1.15^{bc}	1.53^{a}	1.24^{b}	0.126	0.953	0.0010	0.0087
NAIF1	0.878^{b}	1.13^{ab}	1.48^{a}	1.24^{ab}	0.129	0.936	0.0061	0.0453
RELA	1.01	1.13	1.52	1.33	0.127	0.809	0.0424	0.325
IL1B	0.973^{b}	1.22^{ab}	1.52^{a}	1.30^{ab}	0.137	0.926	0.0508	0.133
IL6	1.01^{b}	1.83^{ab}	2.51^{a}	2.09^{a}	0.337	0.467	0.0062	0.0393
LITAF	1.02^{b}	1.16^{b}	2.46^{a}	1.35^{b}	0.295	0.0971	0.0076	0.0359
NOS2	1.01^{b}	1.19^{b}	1.68^{a}	1.20^{b}	0.125	0.0493	<0.001	<0.001
CAT	1.03^{b}	1.23^{a}	0.972^{b}	1.25^{a}	0.0672	0.0007	0.719	0.515
SOD1	1.06^{ab}	1.23^{a}	0.776^{b}	0.992^{ab}	0.0954	0.0566	0.0123	0.892
GPX7	1.07^{a}	1.26^{a}	0.587^{b}	0.917^{ab}	0.1503	0.0782	0.0091	0.608

TLR4 – toll-like receptor 4, *NAIF1* – nuclear apoptosis inducing factor 1, *RELA* – RELA proto-oncogene NF-kB subunit, *NOS2* – nitric oxide synthase 2, *IL1B* – interleukin 1 beta, *IL-6* – interleukin-6, *LITAF* – lipopolysaccharide induced TNF factor, *CAT* – catalase, *SOD1* – superoxide dismutase 1, *GPX7* – glutathione peroxidase 7; ^{abc} means within the same row without a common superscript are significantly different at $P < 0.05$ and it represents the significance of the difference between individual treatments; SEM – standard error of the mean

expression ($P < 0.05$), and *SOD1* ($P = 0.057$) and *GPX7* ($P = 0.078$) showed a significant increasing trend. LPS challenge and dietary AOE exhibited a significant interaction effect on expression levels of the *TLR4*, *NAIF1*, *IL-6*, *LITAF* and *NOS2* genes in the duodenum of broilers ($P < 0.05$).

Based on the results of Tukey's honest significant difference test for the four treatments, feeding AOE significantly increased *CAT* gene expression in the duodenum ($P < 0.05$) of broilers injected with normal saline in the LPS-unchallenged group, while birds from the LPS-challenged group showed significantly decreased expression levels of *TLR4*, *LITAF* and *NOS2* in the duodenum ($P < 0.05$), and significantly increased *CAT* gene expression ($P < 0.05$).

Expression of inflammation- and antioxidant-related genes in the jejunum

Table 4 shows the effect of AOE on the expression of inflammation- and antioxidant-related genes in the jejunum of LPS-challenged broilers. As a main factor, LPS injection to broilers significantly increased the expression levels of the *TLR4, NAIF1, RELA, IL-1B, IL-6, LITAF* and *NOS2* genes in the jejunum of broilers ($P < 0.05$) and significantly

Table 4. Effect of *Artemisia ordosica* aqueous extract (AOE) on the expression of inflammation- and antioxidant-related genes in the jejunum of lipopolysaccharide (LPS)-challenged broilers

Item	LPS (-)		LPS (+)		SEM	*P*–value		
	AOE (-)	AOE (+)	AOE (-)	AOE (+)		AOE	LPS	AOE × LPS
TLR4	0.948^{c}	1.17^{cb}	1.64^{a}	1.32^{b}	0.136	0.654	0.0010	0.0189
NAIF1	0.934^{b}	1.07^{b}	1.61^{a}	1.17^{b}	0.150	0.258	0.0102	0.0402
RELA	0.987	0.972	1.38	1.12	0.113	0.288	0.0486	0.354
IL1B	0.893^{b}	0.97^{b}	1.59^{a}	0.941^{b}	0.171	0.0825	0.0448	0.0318
IL6	1.13^{b}	1.41^{b}	2.42^{a}	1.57^{b}	0.275	0.103	0.0021	0.0224
LITAF	0.885^{b}	1.04^{b}	1.61^{a}	1.23^{b}	0.145	0.329	0.0009	0.0436
NOS2	1.01^{b}	1.09^{b}	1.64^{a}	1.10^{b}	0.133	0.0324	0.0051	0.0063
CAT	1.22^{ab}	1.43^{a}	0.957^{b}	1.27^{a}	0.0971	0.0161	0.0392	0.565
SOD1	1.12	1.22	0.902	1.11	0.108	0.283	0.261	0.684
GPX7	1.15	1.32	0.914	0.917	0.132	0.523	0.0387	0.557

TLR4 – toll-like receptor 4, *NAIF1* – nuclear apoptosis inducing factor 1, *RELA* – RELA proto-oncogene NF-kB subunit, *NOS2* – nitric oxide synthase 2, *IL1B* – interleukin 1 beta, *IL-6* – interleukin-6, *LITAF* – lipopolysaccharide induced TNF factor, *CAT* – catalase, *SOD1* – superoxide dismutase 1, *GPX7* – glutathione peroxidase 7; ^{abc} means within the same row without a common superscript are significantly different at $P < 0.05$ and it represents the significance of the difference between individual treatments; SEM – standard error of the mean

decreased the expression levels of the *CAT* and *GPX7* genes ($P < 0.05$). When birds were fed the AOE diet, *NOS2* gene expression in the broiler jejunum decreased significantly ($P < 0.05$) and *IL-1B* gene expression tended to decrease ($P = 0.083$), meanwhile *CAT* gene expression increased significantly ($P < 0.05$). In addition, LPS challenge and dietary AOE also exerted significant interaction effects on the expression of the *TLR4*, *NAIF1*, *IL-1B*, *IL-6*, *LITAF* and *NOS2* genes in the jejunum of broilers ($P < 0.05$).

On the basis of the results of Tukey's honest significant difference test for the four treatments, feeding AOE had no significant effect on the expression of the above genes in the jejunum of broilers from the LPS-unchallenged group injected with normal saline; however, feeding AOE significantly decreased the expression of the *TLR4*, *NAIF1*, *IL-1B*, *IL-6*, *LITAF* and *NOS2* genes, while significantly increasing *CAT* expression ($P < 0.05$) in the jejunum of broilers from the LPS-challenged group ($P < 0.05$).

Expression of inflammation- and antioxidant-related genes in the ileum

Table 5 shows the effect of AOE on the expression of inflammation- and antioxidant-related genes in the ileum of broilers challenged with LPS. As a main factor, injection of LPS into broilers significantly increased the expression levels of the *NAIF1*, *RELA*, *IL-1B*, *IL-6* and *NOS2* genes ($P < 0.05$), and significantly decreased *CAT* and *GPX7* gene expression ($P < 0.05$) in the ileum. When birds were fed the AOE diet, expression levels of the *RELA*, *IL-1B* and *NOS2* genes significantly decreased ($P < 0.05$), while *CAT* gene expression significantly increased in the ileum ($P < 0.05$). Moreover, LPS challenge and dietary AOE interaction had a significant effect on *RELA* and *NOS2* gene expression in the ileum ($P < 0.05$), and caused a trend in the expression of *IL-1B* ($P = 0.066$), *IL-6* ($P = 0.064$) and *GPX7* ($P = 0.079$).

The results of Tukey's honest significant difference test showed that feeding AOE significantly increased the expression of the *CAT* gene in the ileum ($P < 0.05$) in broilers from the LPS-unchallenged group injected with normal saline; however, feeding AOE significantly decreased *RELA*, *IL-1B* and *NOS2* gene expression ($P < 0.05$), while significantly increasing *GPX7* gene expression in the ileum ($P < 0.05$) of birds from the LPS-challenged group.

Discussion

Several studies have found that LPS exerted negative effects on growth performance, blood biochemical parameters, as well as immune and antioxidant function in broilers (Zhang et al., 2020; Yang et al., 2021a; b; Xing et al., 2021). In line with this body of research, the present study also demonstrated that LPS increased the expression of proinflammatory genes such as *IL-1B*, *IL-6*, and *LITAF* in the small intestine, suggesting the role of LPS in inducing inflammation in broilers. This was consistent with the results of our previous study, which revealed that LPS challenge increased serum levels of IL-1B and IL-6 cytokines in broilers on day 21 (Li et al., 2017). This proinflammatory response can be attributed to the recognition of

Table 5. Effect of *Artemisia ordosica* aqueous extract (AOE) on the expression of inflammation- and antioxidant-related genes in the ileum of lipopolysaccharide (LPS)-challenged broilers

Item	LPS (-)		LPS (+)		SEM	*P* - value		
	AOE (-)	AOE (+)	AOE (-)	AOE (+)		AOE	LPS	AOE × LPS
TLR4	0.970	0.912	1.21	0.927	0.0864	0.103	0.203	0.279
NAIF1	0.775	0.939	1.31	1.12	0.126	0.731	0.0214	0.199
RELA	0.972^{b}	0.948^{b}	1.38^{a}	0.965^{b}	0.0916	0.0108	0.0122	0.0181
IL1B	1.05^{b}	1.03^{b}	1.63^{a}	1.15^{b}	0.143	0.0483	0.0076	0.0657
IL6	1.07^{b}	1.27^{b}	1.96^{a}	1.43^{ab}	0.197	0.358	0.0130	0.0642
LITAF	0.869	1.01	0.930	1.02	0.111	0.408	0.787	0.859
NOS2	0.853^{b}	0.877^{b}	1.52^{a}	0.902^{b}	0.131	0.0028	0.0012	0.0023
CAT	0.963^{b}	1.27^{a}	0.729^{b}	0.868^{b}	0.102	0.0231	0.0034	0.361
SOD1	0.932	0.906	0.901	0.928	0.0412	0.998	0.981	0.663
GPX7	0.986^{a}	0.973^{a}	0.614^{c}	0.804^{b}	0.0778	0.124	<0.001	0.0776

TLR4 – toll-like receptor 4, *NAIF1* – nuclear apoptosis inducing factor 1, *RELA* – RELA proto-oncogene NF-kB subunit, *NOS2* – nitric oxide synthase 2, *IL-1B* – interleukin 1 beta, *IL-6* – interleukin-6, *LITAF* – lipopolysaccharide induced TNF factor, *CAT* – catalase, *SOD1* – superoxide dismutase 1, *GPX7* – glutathione peroxidase 7; ^{abc} means within the same row without a common superscript are significantly different at $P < 0.05$ and it represents the significance of the difference between individual treatments; SEM – standard error of the mean

LPS by TLR4, widely recognised as the principal pattern recognition receptor for LPS. Once LPS is recognised, TLR4 activates the NF-κB signalling pathway and upregulates pro-inflammatory cytokines, including *IL-1B*, *IL-6*, *LITAF* (Wang et al., 2022). However, we observed that dietary supplementation with AOE showed a significant alleviating effect on the above responses. Previous studies showed that dietary AOE significantly reduced excessive serum IL-6 concentrations in broilers challenged with LPS (Li et al., 2017). Here, AOE administration reduced the expression of *TLR4*, *NAIF1*, *RELA*, and *NOS2* in the small intestine, thereby inhibiting the activation of the TLR4/MyD88/NF-κB signalling pathway. These findings aligned with those reported by Xing et al. (2021), who found that dietary *A. ordosica* polysaccharide supplementation decreased the excessive production of IL-1B and IL-6 in the liver of LPS-challenged broilers by downregulating mRNA expression of key genes such as *TLR4*, *NAIF1*, *RELA*, *IL-1B* and *IL-6*. Additionally, the supplementation reduced the high expression of TLR4, I-kappaB kinase beta (IKKβ), NF-κB P65, IL-1B, IL-6 proteins, and decreased IkBα protein expression. Similarly, in other studies involving *Artemisia argyi* extracts, it was observed that they significantly attenuated LPS-induced increases in intestinal interleukin (IL-1B and IL-6) concentrations, and mRNA abundance of the *TLR4*, *NAIF1*, *RELA, IL-1B* and *IL-6* genes in the small intestine of LPS-challenged broilers (Zhang et al., 2020; Yang et al., 2021a). It is speculated that AOE can effectively attenuate the expression of LPS-induced intestinal inflammation-related genes through TLR4/NF-κB signalling pathways in broilers. Moreover, it has been reported that the regulation of the intestinal microbiota can decrease TLR expression, and thus reduce pro-inflammatory responses (Mohr et al., 2022). This may also be one of the reasons why AOE alleviates LPS-induced inflammation, but further studies on the gut microbiota are needed to confirm this.

Inflammation and oxidative stress are intricately interconnected, as overexpression of pro-inflammatory cytokines leads to the production of reactive oxygen species, ultimately inflicting oxidative injury in multiple organ systems (Han et al., 2020). Moreover, LPS challenge not only triggers inflammation, but also induces oxidative stress (Jiang et al., 2019). Evidence from LPS-challenged broilers revealed elevated levels of oxidative stress markers, including malondialdehyde, protein carbonyl, and 8 hydroxy-2'-deoxyguanosine, indicating oxidative stress in both the jejunum and ileum (Zhang et al., 2021). Moreover, Zhang et al. (2020) reported that serum CAT, SOD, and plasma glutathione peroxidase (GSH-Px) activities were reduced by LPS in broilers. In the present study, dietary AOE supplementation increased *CAT*, *SOD1* and *GPX7* gene expression in the small intestine of birds treated with LPS. This was consistent with a study by Xing et al. (2021), who found that the activity of antioxidant enzymes, such as CAT, SOD, and GSH-Px, and the expression of related genes/proteins were critical for suppressing oxidative stress, and could be significantly enhanced by *A. ordosica* polysaccharides. Furthermore, it was established that treatment with *A. ordosica* polysaccharides could activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and facilitate the translocation of Nrf2 into the nucleus. Previous studies have shown that *A. ordosica* polysaccharides can partially mitigate oxidative stress through the Nrf2/ Kelch-like ECH-associated protein 1 (Keap1) pathway, leading to increased activity of antioxidant enzymes in broilers challenged with LPS (Xing et al., 2023). Therefore, based on our study results, it is plausible to suggest that AOE exerts a mitigating effect through the Nrf2/Keap1 signalling pathway; however, further research is needed to validate this hypothesis conclusively. Furthermore, it has been proven that LPS can disrupt the integrity of the intestinal barrier by reducing the synthesis of tight junction proteins, thereby increasing intestinal epithelial permeability and causing damage to the intestinal barrier (Bian et al., 2020). We also hypothesised that AOE would alleviate LPS-induced expression of inflammatory factors and genes related to antioxidant enzymes. Notably, this was confirmed by Xing et al. (2023), who found that dietary supplementation with *A. ordosica* polysaccharides attenuated intestinal damage caused by LPS challenge in broilers.

Conclusions

Dietary *Artemisia ordosica* aqueous extract (AOE) supplementation could partially mitigate the lipopolysaccharide-induced detrimental effects by enhancing the expression of antioxidant enzyme genes and suppressing the expression of inflammatory factor genes in the small intestine of broilers. This suggests that AOE may serve as a valuable feed additive to alleviate inflammatory responses and enhance antioxidant capacity in broilers.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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