ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Animal Nutrition

Growth-Promoting and Antioxidant Effects of Magnolia Bark Extract in Chickens Uninfected or Co-Infected with *Clostridium perfringens* and *Eimeria maxima* as an Experimental Model of Necrotic Enteritis

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Abstract

Background: Magnolia tree bark has been widely used in traditional Asian medicine. However, to our knowledge, no studies have been reported investigating the effects of dietary supplementation with magnolia bark extract in chickens.

Objective: We tested the hypothesis that dietary supplementation of chickens with a *Magnolia officinalis* bark extract would increase growth performance in uninfected and *Eimeria maxima*/*Clostridium perfringens* co-infected chickens.

Methods: A total of 168 chickens were fed from hatch either a standard diet or a diet supplemented with 0.33 mg or 0.56 mg *M. officinalis* bark extract/kg (M/H low or M/H high, respectively) from days 1 to 35. At day 14, half of the chickens were orally infected with *E. maxima*, followed by *C. perfringens* infection at day 18 to induce experimental avian necrotic enteritis. Daily feed intake, feed conversion ratio, body weight gain, and final body weight were measured as indicators of growth performance. Serum α1-acid glycoprotein (AGP) concentrations were measured as an indicator of systemic inflammation, and intestinal lesion scores were determined as a marker of disease progression. Transcript levels for catalase, heme oxygenase 1, and superoxide dismutase in the intestine, liver, spleen, and skeletal muscle were measured as indicators of antioxidant status.

Results: Growth performance increased between days 1 and 35 in uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed M/H-low or M/H-high diets compared with unsupplemented controls. Gut lesion scores were decreased, whereas AGP concentrations were unchanged, in co-infected chickens fed magnolia-supplemented diets compared with unsupplemented controls. In general, transcripts for antioxidant enzymes increased in chickens fed magnolia-supplemented diets compared with unsupplemented controls, and significant interactions between dietary supplementation and co-infection were observed for all antioxidant enzyme transcript levels.

Conclusion: Magnolia bark extract might be useful for future development of dietary strategies to improve poultry health, disease resistance, and productivity without the use of antibiotic growth promoters. *Curr Dev Nutr* 2018;2:nzy009.

Introduction

The bark of *Magnolia officinalis* and *Magnolia obovata* have been used in traditional Chinese, Japanese, and Korean medicine (1). The Chinese name *houpu* refers to the thick (hou) bark and unadorned (pu) part of the plant (2). Historically, houpu has been used in humans to treat anxiety and mood disorders (3) and as an analgesic (1). The major active components of houpu include magnolol, honokiol, 4-*O*-methylhonokiol, and obovatol (1). Both magnolol and its isomer

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Abbreviations used: AFAR, aflatoxin B1 aldehyde reductase; AGP, α1-acid glycoprotein; CAT, catalase; HMOX1, heme oxygenase 1; Keap1, Kelch-like ECH-associated protein 1; MDA, malondialdehyde; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; Nrf2, nuclear factor E2–related factor 2; SOD, superoxide dismutase.

honokiol selectively interact with γ-aminobutyric acid receptors (4). These biphenolic lignans possess in vitro and in vivo anxiolytic, nootropic, antidepressant, anti-inflammatory, antitumorigenic, and antioxidant activities (5–11). However, relatively few studies, if any, have investigated the medicinal effects of magnolia bark extracts, or its active compounds, in veterinary medicine.

Among the most important and economically devastating infectious diseases for the global poultry industry are avian coccidiosis and necrotic enteritis, with estimated annual losses exceeding $6 billion (12–14). These related diseases are caused by 2 distinct enteric pathogens, *Eimeria* (coccidiosis) and *Clostridium perfringens* (necrotic enteritis). Field outbreaks of coccidiosis typically occur simultaneously with necrotic enteritis. Over the past 40 y, prophylactic use of therapeutic antibiotic growth promoters and anticoccidial drugs has been used to control coccidiosis and necrotic enteritis. However, coccidiosis and necrotic enteritis have re-emerged as serious threats facing the commercial poultry industry as a consequence of the international movement to ban antibiotics use in food animal production (15, 16).

A large, diverse array of alternatives to antibiotics for control of avian coccidiosis and necrotic enteritis have been reported (12, 17). Among these are plant-based phytochemicals (18). Phytochemicals that have been shown to enhance protective immunity to coccidiosis and necrotic enteritis include anethole, artemisinin, capsaicin, carvacrol, cinnamaldehyde, curcumin, propyl thiosulfinate, and propyl thiosulfinate oxide (19–24). Plant-based chemical extracts that increase disease resistance in which the active components have not been identified include those from *Aloe vera* (aloe), *Artemisia annua* (sweet wormwood), *Azadirachta indica* (neem), *Bidens pilosa* (Spanish needle), *Capsicum* spp. (pepper), *Carthamus tinctorius* (safflower), *Curcuma longa* (turmeric), *Echinacea purpurea* (purple cone flower), *Gyamopsis tetragonoloba* (guar), *Khaya senegalensis* (African mahogany), *Prunus salicina* (oriental plum), *Punica granatum* (pomegranate), and the mushrooms *Lentinus edodes* (shiitake), *Ganoderma lucidum* (reishi), and *Fomitella fraxinea* (20, 25–38). The current study was undertaken to investigate whether dietary supplementation with magnolia bark extract might promote the growth performance, antioxidant responses, or both in broiler chickens that were

TABLE 1 Diet composition

	Low protein, %	High protein, %
Ingredient		
Corn	69.01	55.78
Soybean meal	23.99	37.03
Soybean oil	2.75	2.97
Dicalcium phosphate	2.00	1.80
Calcium carbonate	1.40	1.51
Salt	0.35	0.38
Poultry vitamin mix[1]	0.20	0.22
Poultry mineral mix[2]	0.15	0.15
dl-Methionine	0.10	0.10
Choline chloride (60%)	0.05	0.06
Total	100	100
Calculated values (dry matter basis)		
Crude protein	18.00	24.00
Calcium	1.19	1.20
Available phosphorus	0.54	0.51
Lysine	1.00	1.40
Methionine	0.42	0.49
Cysteine + methionine	0.65	0.80
True metabolizable energy, kcal/kg	3585	3450

[1]The vitamin mixture provided the following nutrients per kilogram of diet: vitamin A, 2000 IU; vitamin D_3, 22 IU; vitamin E, 16 mg; vitamin K, 0.1 mg; thiamin, 3.4 mg; riboflavin, 1.8 mg; vitamin B-6, 6.4 mg; vitamin B-12, 0.013 mg; biotin, 0.17 mg; pantothenic acid, 8.7 mg; folic acid, 0.8 mg; and niacin, 23.8 mg. [2]The mineral mixture provided the following nutrients per kilogram of diet: Fe, 0.4 mg; Zn, 0.2 mg; Mn, 0.18 mg; Co, 0.0013 mg; Cu, 0.021 mg; and Se, 0.0002 mg.

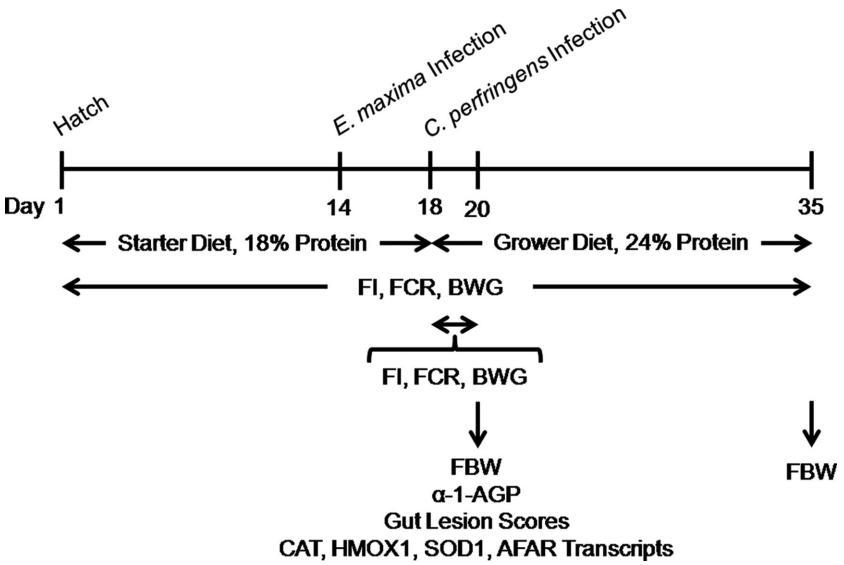

FIGURE 1 Schematic outline of the experimental design. *AFAR*, aflatoxin B1 aldehyde reductase; BWG, body weight gain; *CAT*, catalase; FBW, final body weight; FCR, feed conversion ratio; FI, average daily feed intake; *HMOX1*, heme oxygenase 1, *SOD1*, superoxide dismutase 1; α-1-AGP, α1-acid glycoprotein.

TABLE 2 Primers used for real-time PCR[1]

Target gene	Primer sequence	Annealing temperature	GenBank accession no.
CAT	F 5′-ACTGCAAGGCGAAAGTGTTT-3′ R 5′-GGCTATGGATGAAGGATGGA-3′	58°C	NM001031215.1
HMOX1	F 5′-CTGGAGAAGGGTTGGCTTTCT-3′ R 5′-GAAGCTCTGCCTTTGGCTGTA-3′	60°C	NM205344
SOD1	F 5′-ATTACCGGCTTGTCTGATGG-3′ R 5′-CCTCCCTTTGCAGTCACATT-3′	58°C	NM205064.1
AFAR	F 5′-CAAACTGCAGGGTTCTCTTGG-3 R 5′-GAAGTAGTTGGGGCAGTCGTG-3′	60°C	XM417628.2
GAPDH	F 5′-GGTGGTGCTAAGCGTGTTAT-3′ R 5′-ACCTCTGTCATCTCTCCACA-3′	60°C	K01458

[1] *AFAR*, aflatoxin B1 aldehyde reductase; *CAT*, catalase; F, forward primer; *HMOX1*, heme oxygenase 1; R, reverse primer; *SOD1*, superoxide dismutase 1.

either uninfected or co-infected with *E. maxima* and *C. perfringens* in an experimental model of avian necrotic enteritis.

Methods

Preparation of magnolia extract

An extract of *M. officinalis* bark was obtained from Pancosma (Geneva, Switzerland). Briefly, the crude material was washed, dried at 50°C to a dry matter content of ≥91%, and comminuted. The dried material was extracted with supercritical carbon dioxide at a flow rate of 1200–1400 L/h for 3.5 h at a pressure of 25–30 MPa and a temperature of 35–40°C, and the extract was taken up in ethanol (39).

Chickens and experimental design

All of the experiments were approved by the Beltsville Agricultural Research Center Institutional Animal Care and Use Committee. A total of 168 day-old Ross 708 male broiler chickens (28 chickens/group,

TABLE 3 Effect of magnolia extract dietary supplementation and *E. maxima*/*C. perfringens* co-infection on growth performance in broiler chickens[1]

	Days 1–35				Days 18–20			
	FI[2]	FCR[3]	BWG[4]	FBW[5]	FI[6]	FCR[7]	BWG[8]	FBW[9]
Uninfected								
Basal diet	79.3	1.56	50.9	1827.6	116.5	2.02	57.3	593.4
M/H low	85.8	1.53	56.3	2012.2	109.8	1.78	62.0	688.6
M/H high	84.0	1.49	56.5	2020.0	107.3	1.71	63.0	703.3
Co-infected								
Basal diet	85.5	1.67	51.4	1843.4	138.5	3.96	36.5	572.4
M/H low	87.5	1.55	56.2	2010.9	124.3	2.80	48.8	650.3
M/H high	91.0	1.67	54.9	1963.8	135.8	2.49	50.8	638.8
Pooled SEM	1.50	0.04	1.53	53.5	6.95	0.25	2.61	22.0
Main effect means								
Feed								
Basal	82.4	1.62	51.2	1835.3	127.5	2.99	46.9	582.9
M/H low	86.7	1.54	56.3	2011.6	117.1	2.29	55.4	669.5
M/H high	87.5	1.58	55.7	1991.9	121.6	2.10	56.9	671.1
Pooled SEM	1.06	0.03	1.08	37.79	4.91	0.18	1.85	15.54
Necrotic enteritis								
Uninfected	83.0	1.53	54.6	1953.3	111.2	1.84	60.8	661.8
Co-infected	88.0	1.63	54.2	1939.4	132.9	3.08	45.4	620.5
Pooled SEM	0.86	0.03	0.88	30.86	4.01	0.15	1.51	12.69
Source of variation, *P*								
Feed	0.0067	0.2946	0.0072	0.0074	0.3389	0.0065	0.0025	0.0335
Necrotic enteritis	0.0007	0.0088	0.7574	0.7538	0.0013	<0.0001	<0.0001	0.0009
Feed × necrotic enteritis	0.1943	0.2394	0.7824	0.7831	0.6098	0.0841	0.2329	0.6165

[1] BWG, body weight gain; FBW, final body weight; FCR, feed conversion ratio; FI, feed intake; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg.
[2] Average daily FI between days 1 and 35 in g/chicken.
[3] FCR between days 1 and 35 in g feed/g BWG.
[4] BWG between days 1 and 35 in g/chicken.
[5] FBW at day 35 in g/chicken.
[6] Average daily FI between days 18 and 20 in g/chicken.
[7] FCR between days 18 and 20 in g feed/g BWG.
[8] BWG between days 18 and 20 in g/chicken.
[9] FBW at day 20 in g/chicken.

7 chickens/experimental unit) were obtained from Longenecker's Hatchery (Elizabethtown, PA), randomly housed in Petersime starter brooder cage units, and provided with feed and water ad libitum. The experimental design consisted of a completely randomized 2 × 3 factorial arrangement with uninfected or *E. maxima*/*C. perfringens* co-infected chickens, each provided with an unsupplemented diet or with diets supplemented with 0.33 mg (M/H low) or 0.56 mg (M/H high) *M. officinalis* bark extract/kg.

Experimental necrotic enteritis disease model

Chickens were fed an antibiotic-free starter diet containing 18% dry matter protein (USDA-Feed Mill) from days 1 to 18 of age and a standard grower diet containing 24% dry matter protein from days 19 to 35 (**Table 1**, **Figure 1**). Both diets were either unsupplemented or supplemented with low or high amounts of magnolia bark extract. Chickens were infected by oral gavage on day 14 with 1.0×10^4 oocysts/bird of *E. maxima* Beltsville strain 41A, as described (40). At day 18, *E. maxima*–infected chickens were infected by oral gavage with 1.0×10^9 CFUs/bird of *C. perfringens* strain Del-1 (40). Uninfected chickens received an equal volume of PBS by oral gavage.

Measurement of chicken growth performance, serum α1-acid glycoprotein, and intestinal lesion scores

Average daily feed intake, feed conversion ratio, body weight gain, and final body weight were measured as variables of growth performance, as described (40). Each variable was measured during 2 time periods: the entire experimental period (days 1–35) and in the 2-d period immediately after *C. perfringens* infection in co-infected chickens (days 18–20) when intestinal lesions are at maximum size (12). Serum samples were collected at day 20 and α1-acid glycoprotein (AGP) concentrations were determined by ELISA, as described (41). Intestinal lesion scores were determined at day 20 on a scale from 0 (none) to 4 (high) by 5 independent observers in a blinded fashion, as described (42).

Antioxidant and detoxifying gene transcript level measurements

Chickens were killed on day 20 by cervical dislocation; the intestinal jejunum, liver, spleen, and breast muscle were collected and homogenized by using a hand-held rotor-stator homogenizer (TissueRuptor; Qiagen); and tissue homogenates processed by qRT-PCR for levels of transcripts encoding the antioxidant enzymes catalase (*CAT*), heme oxygenase 1 (*HMOX1*), and superoxide dismutase 1 (*SOD*) 1, and the detoxifying enzyme aflatoxin B1 aldehyde reductase (*AFAR*), as described (43). Briefly, total RNA was extracted by using TRIzol (Invitrogen) and purified with the RNeasy Mini RNA purification kit (Qiagen), and 5.0 μg total RNA were treated with 1.0 U DNase I (Sigma) for 15 min at 22°C followed by 10 min at 70°C. The RNA was reverse-transcribed by using the StrataScript first-strand synthesis system (Stratagene) according to the manufacturer's recommendations. cDNA was amplified by using the Mx3000P QPCR system (Agilent Technologies) and Brilliant SYBR Green qPCR master mix (Stratagene) for 40 cycles at 72°C for 1 min with oligonucleotide primers for chicken *CAT, HMOX1, SOD1, AFAR*, and *GAPDH* (**Table 2**). The levels of individual transcripts were normalized to those of *GAPDH* by the Q-gene program (44). To normalize individual replicates, the logarithmic-scaled threshold cycle (C_t) values were transformed to linear units of normalized expression before calculating the means and SEMs for the references and individual targets, followed by determination of mean normalized expression (44).

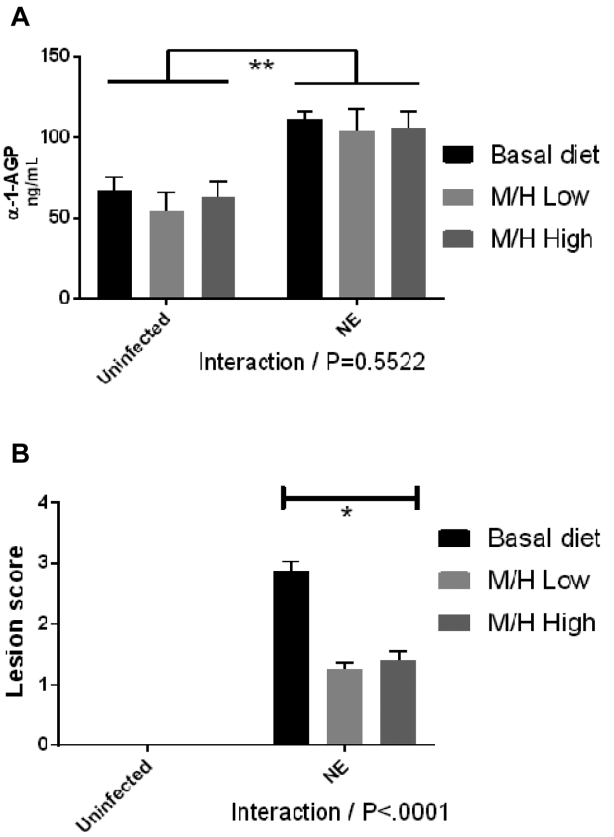

FIGURE 2 Effects of dietary supplementation with magnolia bark extract on serum α-1-AGP concentrations (A) and intestinal lesion scores (B). Chickens were fed unsupplemented or magnolia extract–supplemented diets and were uninfected or co-infected with *E. maxima* and *C. perfringens*. Serum α-1-AGP concentrations and intestinal lesion scores were measured on day 20. Values are means ± SEMs, $n = 4$. *Difference between chickens fed the magnolia extract–supplemented diet compared with unsupplemented controls, $P < 0.05$. *E. maxima*/*C. perfringens* co-infected chickens compared with uninfected controls, $P < 0.05$. *P* values for the interaction between dietary magnolia extract supplementation and *E. maxima*/*C. perfringens* co-infection are listed below each panel. M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; NE, Necrotic enteritis; α-1-AGP, α1-acid glycoprotein.

Statistical analysis

Each cage was considered as an experimental unit. All of the treatments were conducted in quadruplicate and data were expressed as mean ± SEM values for each treatment group. Mean separations were carried out by using Duncan's multiple range test. Statistical analysis was carried out by 2-factor ANOVA by using SAS software (version 9.4; SAS Institute) with magnolia extract supplementation and variables of necrotic enteritis as main factors and interaction of main effects. *P* values ≤0.05 were considered significant.

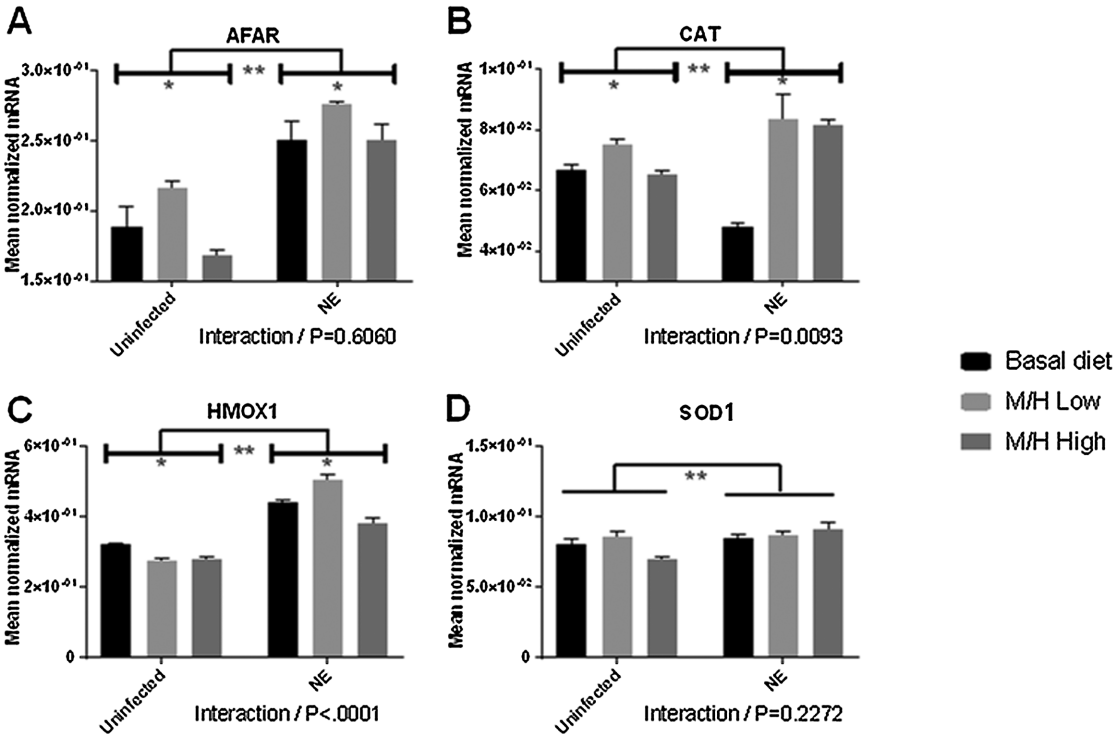

FIGURE 3 Effects of dietary supplementation with magnolia bark extract on antioxidant and detoxifying gene transcript levels in the intestine. Chickens were fed unsupplemented or magnolia extract–supplemented diets and were uninfected or co-infected with *E. maxima* and *C. perfringens*. The levels of transcripts for *AFAR* (A), *CAT* (B), *HMOX1* (C), and *SOD1* (D) in the intestinal jejunum were measured at day 20. Values are means ± SEMs, $n = 4$. *Difference between chickens fed the magnolia extract–supplemented diet compared with unsupplemented controls, $P < 0.05$. **Difference between *E. maxima*/*C. perfringens* co-infected chickens compared with uninfected controls, $P < 0.05$. *P* values for the interaction between dietary magnolia extract supplementation and *E. maxima*/*C. perfringens* co-infection are listed below each panel. *AFAR*, aflatoxin B1 aldehyde reductase; *CAT*, catalase; *HMOX1*, heme oxygenase 1; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; NE, Necrotic enteritis; *SOD1*, superoxide dismutase 1.

Results

Growth performance

The effect of dietary supplementation with magnolia bark extract on chicken growth performance is presented in **Table 3**. Growth performance was measured over the entire experimental period (days 1–35) and in the 2-d period immediately after *C. perfringens* infection in co-infected chickens [days 18–20 when intestinal lesions are maximum (12)]. Between days 1 and 35, both uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed the magnolia extract–supplemented diet showed increased daily feed intakes, body weight gains, and final body weights compared with birds given the unsupplemented diet. Between days 18 and 20, uninfected and co-infected chickens fed the magnolia-supplemented diet showed increased body weight gains and final body weights, but decreased feed conversion ratios, compared with unsupplemented controls. Between days 1 and 35, *E. maxima*/*C. perfringens* co-infected chickens fed both the unsupplemented and magnolia-supplemented diets showed increased daily feed intakes and feed conversion ratios compared with uninfected birds. Between days 18 and 20, co-infected chickens fed the unsupplemented and magnolia-supplemented diets showed increased daily feed intakes and feed conversion ratios, but decreased body weight gains and final body weights, compared with uninfected controls. No significant interactions between dietary magnolia supplementation and *E. maxima*/*C. perfringens* co-infection either at days 1–35 or days 18–20 were observed on daily feed intakes, feed conversion ratios, body weight gains, or final body weights.

Serum AGP concentrations

At day 20, serum AGP concentrations were unchanged in both uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed the magnolia extract–supplemented diet compared with chickens fed the unsupplemented diet (**Figure 2**A). At the same time, AGP concentrations were increased in *E. maxima*/*C. perfringens* co-infected chickens fed both the unsupplemented and magnolia-supplemented diets compared with uninfected chickens ($P = 0.0002$). No significant interaction between dietary magnolia supplementation and *E. maxima*/*C. perfringens* co-infection was observed on AGP concentrations.

Gut lesion scores

At day 20, gut lesion scores were decreased in *E. maxima*/*C. perfringens* co-infected chickens fed the magnolia extract–supplemented diets compared with co-infected chickens fed the unsupplemented diet (Figure 2B) ($P < 0.0001$).

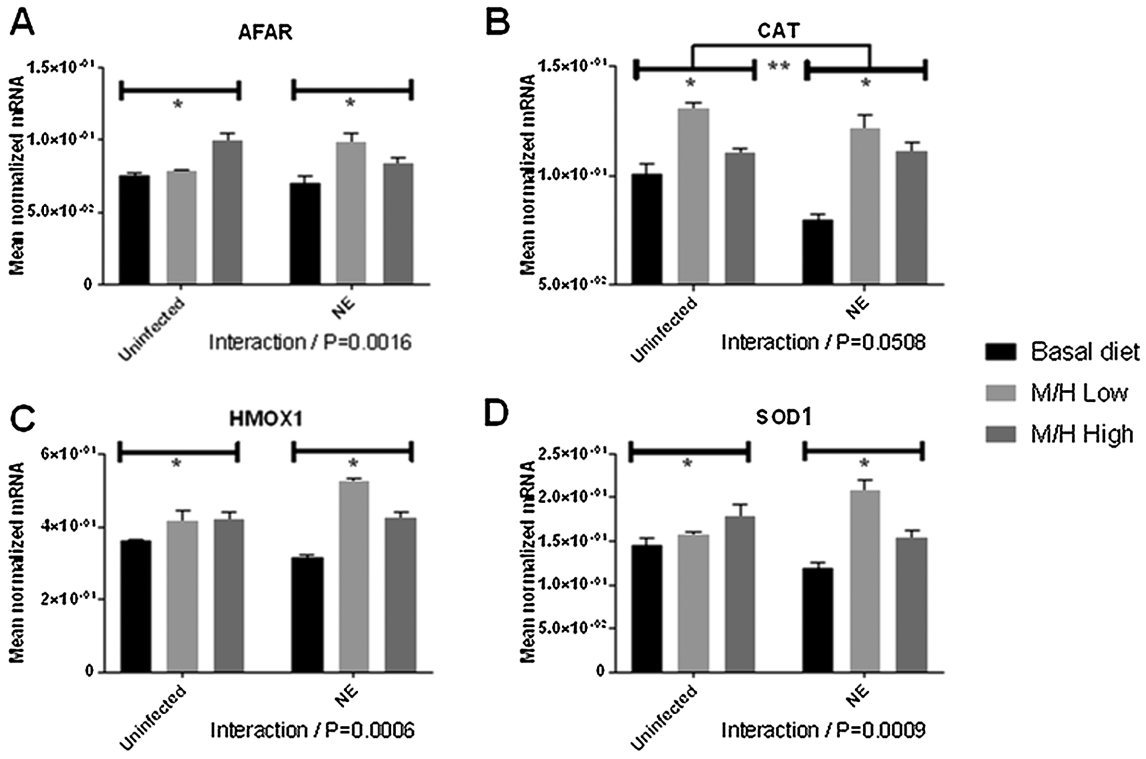

FIGURE 4 Effects of dietary supplementation with magnolia bark extract on antioxidant and detoxifying gene transcript levels in the liver. Chickens were fed unsupplemented or magnolia extract–supplemented diets and were uninfected or co-infected with *E. maxima* and *C. perfringens*. The levels of transcripts for *AFAR* (A), *CAT* (B), *HMOX1* (C), and *SOD1* (D) in the liver were measured at day 20. Values are means ± SEMs, $n = 4$. *Difference between chickens fed the magnolia extract–supplemented diet compared with unsupplemented controls, $P < 0.05$. **Difference between *E. maxima*/*C. perfringens* co-infected chickens compared with uninfected controls, $P < 0.05$. *P* values for the interaction between dietary magnolia extract supplementation and *E. maxima*/*C. perfringens* co-infection are listed below each panel. *AFAR*, aflatoxin B1 aldehyde reductase; *CAT*, catalase; *HMOX1*, heme oxygenase 1; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; NE, Necrotic enteritis; *SOD1*, superoxide dismutase 1.

Antioxidant and detoxifying transcript levels

The expression levels of the antioxidant enzymes *CAT, HMOX1, SOD1*, and the detoxifying enzyme *AFAR*, in the intestinal jejunum (**Figure 3**), liver (**Figure 4**), spleen (**Figure 5**), and breast muscle (**Figure 6**) were measured at day 20 (2 d after *C. perfringens* infection). Previous studies have established that these 4 enzymes are transcriptionally regulated by phytonutrients through the nuclear factor E2–related factor 2 (Nrf2)–Kelch-like ECH-associated protein 1 (Keap1) pathway (45). In the jejunum, *AFAR* and *CAT* transcript levels generally increased in both uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed the magnolia-supplemented diet compared with unsupplemented controls (Figure 3). *HMOX1* transcript levels generally decreased in magnolia-supplemented chickens compared with unsupplemented controls, except for the M/H-low group in co-infected chickens in which *HMOX1* transcripts were increased in the supplemented compared with the unsupplemented groups. All 4 transcript levels increased in the magnolia-supplemented, co-infected group compared with the magnolia-supplemented, uninfected group. Significant interactions between magnolia supplementation and *E. maxima*/*C. perfringens* co-infection were observed for CAT ($P = 0.0093$) and *HMOX1* ($P < 0.001$) transcript levels.

In the liver, the levels of all 4 antioxidant enzyme transcripts increased in both uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed the magnolia-supplemented diet compared with unsupplemented controls (Figure 4). In general, all 4 transcript levels increased in co-infected chickens fed the M/H-low diet compared with the uninfected, M/H-low group, but decreased in chickens fed the M/H high diet compared with the uninfected, M/H-high group. Significant interactions between magnolia supplementation and *E. maxima*/*C. perfringens* co-infection were observed for *AFAR* ($P = 0.0016$), *HMOX1* ($P = 0.0006$), and *SOD1* ($P = 0.0009$).

In the spleen, the levels of *AFAR*, *HMOX1*, and *SOD1* transcripts increased in the magnolia-supplemented, *E. maxima*/*C. perfringens* co-infected group compared with the unsupplemented, co-infected group (Figure 5). *HMOX1* transcripts increased, whereas *AFAR* and *SOD1* transcripts decreased, in the magnolia-supplemented, uninfected group compared with the unsupplemented, uninfected group. *AFAR*, *MHOX1*, and *SOD1* transcripts increased in magnolia-supplemented, co-infected group compared with the magnolia-supplemented, uninfected group. Significant interactions between magnolia supplementation and *E. maxima*/*C. perfringens* co-infection were observed for *AFAR*

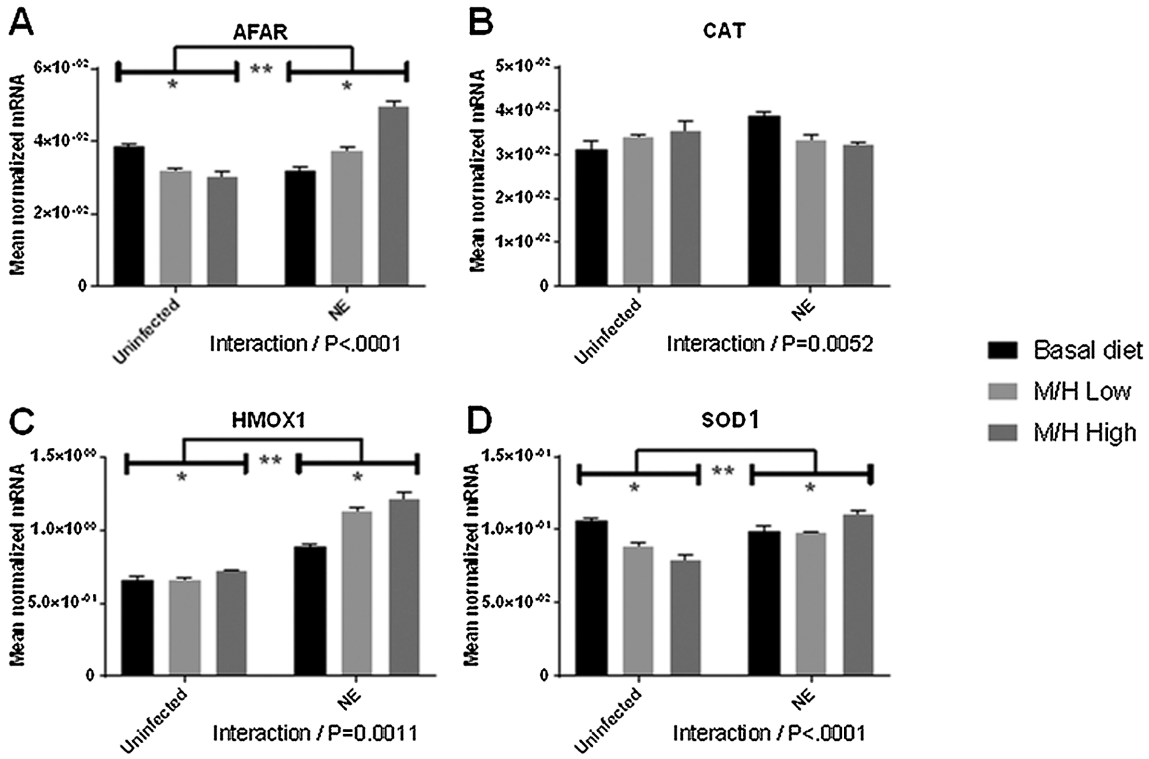

FIGURE 5 Effects of dietary supplementation with magnolia bark extract on antioxidant and detoxifying gene transcript levels in the spleen. Chickens were fed unsupplemented or magnolia extract–supplemented diets and were uninfected or co-infected with *E. maxima* and *C. perfringens*. The levels of transcripts for *AFAR* (A), *CAT* (B), *HMOX1* (C), and *SOD1* (D) in the spleen were measured at day 20. Values are means ± SEMs, $n = 4$. *Difference between chickens fed the magnolia extract–supplemented diet compared with unsupplemented controls, $P < 0.05$. **Difference between *E. maxima*/*C. perfringens* co-infected chickens compared with uninfected controls, $P < 0.05$. *P* values for the interaction between dietary magnolia extract supplementation and *E. maxima*/*C. perfringens* co-infection are listed below each panel. *AFAR*, aflatoxin B1 aldehyde reductase; *CAT*, catalase; *HMOX1*, heme oxygenase 1; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; NE, Necrotic enteritis; *SOD1*, superoxide dismutase 1.

($P < 0.0001$), *CAT* ($P = 0.0052$), *HMOX1* ($P = 0.0011$), and *SOD1* ($P < 0.0001$).

In breast muscle, transcript levels for all 4 enzymes generally increased in the magnolia-supplemented, co-infected and supplemented, uninfected groups compared with the corresponding unsupplemented groups, with the exception of *CAT* and *HMOX1*, in which decreased transcript levels were seen (Figure 6). In this same tissue, all 4 transcript levels generally decreased in the unsupplemented or magnolia-supplemented, *E. maxima*/*C. perfringens* co-infected groups compared with the corresponding uninfected groups'. Significant interactions between magnolia supplementation and *E. maxima*/*C. perfringens* co-infection were observed for *CAT* ($P = 0.0003$), *HMOX1* ($P < 0.0001$), and *SOD1* ($P = 0.0042$).

Discussion

The present study was conducted to investigate the effects of dietary supplementation of newly hatched broiler chickens with magnolia bark extract on growth performance, antioxidant status, and resistance to necrotic enteritis. During the normal growth period (days 1–35), feed intake, body weight gain, and final body weight increased in uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed magnolia-supplemented diets compared with unsupplemented controls. During the time of maximum gut pathology in *E. maxima*/*C. perfringens* co-infected chickens (days 18–20; 2 d after *C. perfringens* infection), body weight gain and final body weight increased, and the feed conversion ratio decreased, in uninfected and co-infected chickens fed magnolia-supplemented diets compared with unsupplemented controls. At day 20, gut lesion scores decreased, whereas AGP concentrations were unchanged, in co-infected chickens fed magnolia-supplemented diets compared with unsupplemented controls. Also at day 20, transcripts for antioxidant enzymes generally increased in chickens fed magnolia-supplemented diets compared with unsupplemented controls, and significant interactions between dietary supplementation and co-infection were observed for all antioxidant enzyme transcript levels.

Growth performance is among the most important outcomes for commercial broiler production. Currently, a typical US commercial broiler consumes a total of ~9 pounds (~4082 g) of feed and reaches 5 pounds (~2268 g) of body weight in 35–40 d, with a live-weight feed conversion ratio of 1.6 kg feed/kg body weight gain (46). Over

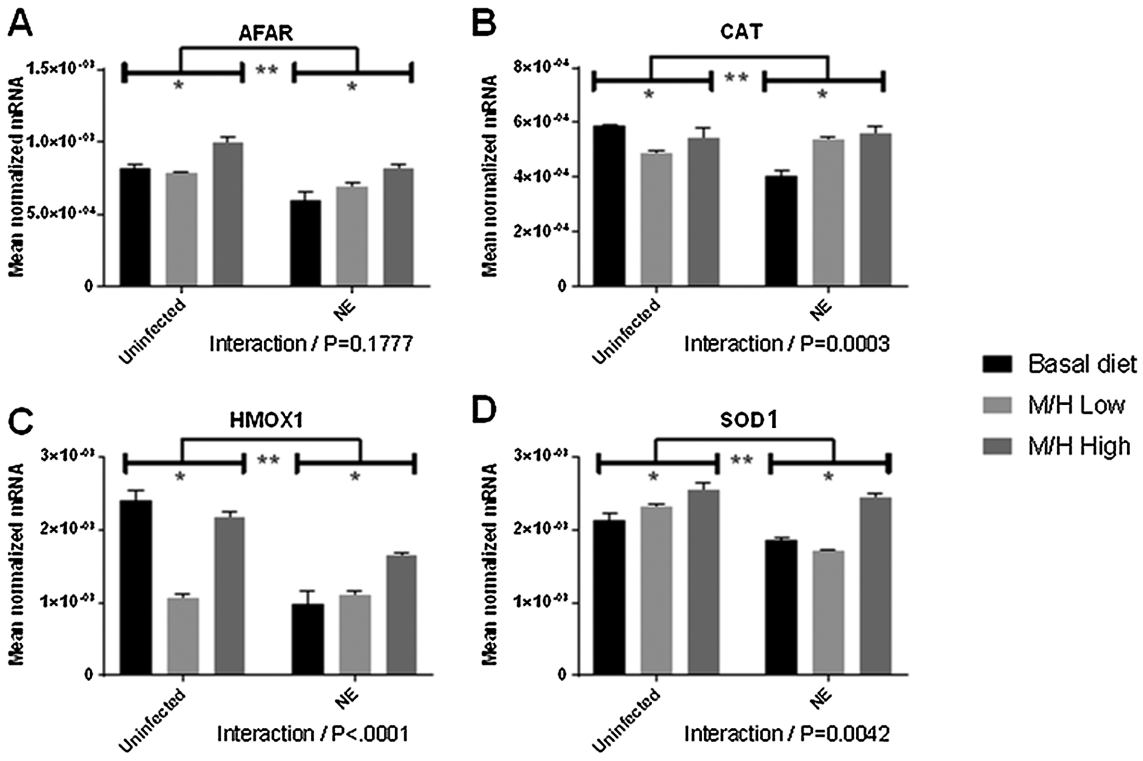

FIGURE 6 Effects of dietary supplementation with magnolia bark extract on antioxidant and detoxifying gene transcript levels in breast muscle. Chickens were fed unsupplemented or magnolia extract–supplemented diets and were uninfected or co-infected with *E. maxima* and *C. perfringens*. The levels of transcripts for *AFAR* (A), *CAT* (B), *HMOX1* (C), and *SOD1* (D) in breast muscle were measured at day 20. Values are means ± SEMs, $n = 4$. *Difference between chickens fed the magnolia extract–supplemented diet compared with unsupplemented controls, $P < 0.05$. **Difference between *E. maxima*/*C. perfringens* co-infected chickens compared with uninfected controls, $P < 0.05$. *P* values for the interaction between dietary magnolia extract supplementation and *E. maxima*/*C. perfringens* co-infection are listed below each panel. *AFAR*, aflatoxin B1 aldehyde reductase; *CAT*, catalase; *HMOX1*, heme oxygenase 1; M/H high, 0.56 mg *M. officinalis* bark extract/kg; M/H low, 0.33 mg *M. officinalis* bark extract/kg; NE, Necrotic enteritis; *SOD1*, superoxide dismutase 1.

the past 50 y, US broiler growth rates have doubled and corresponding feed conversion rates have been reduced by ∼50%, primarily as a consequence of improved breeding strategies (47). A number of factors, however, threaten the continuing trend toward more-efficient poultry production, including discontinuation of the use of antibiotic growth promoters and, relatedly, the re-emergence of infectious diseases (12, 14, 17). Dietary supplementation with phytochemicals and micronutrients has recently emerged as an attractive alternative to antibiotic growth promoters to increase chicken growth and enhance resistance to infectious pathogens (48–50). To the best of our knowledge, the current study is the first to describe the beneficial effects of *M. officinalis* extract on broiler growth performance and resistance to necrotic enteritis.

To begin to elucidate the molecular mechanisms through which magnolia bark extract might promote resistance to *E. maxima*/*C. perfringens* infection, we evaluated antioxidant enzymes whose catalytic activities, expression levels, or both are altered in experimental necrotic enteritis. Georgieva et al. (51) reported that serum concentrations of malondialdehyde (MDA), a lipid peroxidation product and in vivo biomarker of oxidative stress, increased in chickens infected with *Eimeria tenella* compared with uninfected controls. Lee et al. (52, 53) and Xu et al. (54) reported that serum MDA concentrations, CAT and SOD1 activities, and glutathione peroxidase 7 (*GPX7*) transcript levels increased in broiler chickens co-infected with *E. maxima* and *C. perfringens* compared with uninfected controls. In contrast, transcripts encoding peroxiredoxin 6 (*PRDX6*), a thiol-specific antioxidant enzyme involved in intracellular redox regulation, decreased in co-infected birds compared with uninfected controls. In further studies, providing chickens with the micronutrient selenium, either through dietary supplementation or in ovo injection, reversed the effects of experimental necrotic enteritis (i.e., MDA concentrations, CAT and SOD1 activities, and *GPX7* transcript levels decreased, whereas *PRDX* transcript levels increased, compared with unsupplemented controls) (52–54).

Many dietary phytochemicals induce the expression of enzymes involved in cellular antioxidant response (e.g., CAT, HMOX1, SOD1) and the detoxification of carcinogens (e.g., AFAR). The antioxidant defense system is composed of endogenous enzymatic, endogenous nonenzymatic, and exogenous mediators (55). SOD enzymes (SOD1, SOD2, and SOD3) catalyze the conversion of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (56). SOD1, or Cu,Zn-SOD, utilizes copper and zinc ions for both structural and catalytic roles. CAT catalyzes the reaction of hydrogen peroxide (H_2O_2) to

H_2O and O_2 (57). HMOX1 is a rate-limiting enzyme in the catabolism of heme to biliverdin, free iron, and carbon monoxide (58). AFAR catalyzes the NAD(P)H-dependent reduction and detoxification of a dialdehydic metabolite of the hepatocarcinogen aflatoxin B_1 (59). Nrf2 is a transcription factor that regulates the induction of these and other detoxifying and antioxidant genes through the antioxidant response elements, or electrophile response elements, located in their promoter regions (45). Normally, Nrf2 is localized in the cytoplasm in association with its repressor protein, Keap1, where it silences Nrf2 activity through its ubiquitination and proteolysis. Oxidation of cysteine residues within Keap1 by phytochemicals, or phytochemical-induced Nrf2 phosphorylation, have been proposed to dissociate Keap1 from Nrf2, allowing the transcription factor to enter the nuclease and bind to antioxidant response elements of antioxidant and detoxifying enzyme genes, thereby driving their expression (45). On the basis of the results of the current study, a similar mechanism may be operative in the case of magnolia bark extract–induced upregulation of *AFAR*, *CAT*, *HMOX1*, and *SOD1* genes. Of interest, Pang et al. (2) reported that CAT and SOD1 activities in plasma and liver increased after in vivo treatment of mice with magnolol and honokiol, the major active components of *M. officinalis*, whereas Rajgopal et al. (60) showed that magnolia bark extract activated Nrf2-dependent *HMOX1* gene expression in vitro in murine hepatocytes.

In summary, this study shows that dietary supplementation of broiler chickens experimentally co-infected with *E. maxima* and *C. perfringens* with a magnolia bark extract between days 1 and 35 of age improves growth performance and reduces intestinal lesions compared with unsupplemented controls. In general, transcripts for the antioxidant enzymes *CAT*, *HMOX1*, and *SOD1*, and the detoxifying enzyme *AFAR*, increased in uninfected and *E. maxima*/*C. perfringens* co-infected chickens fed magnolia-supplemented diets compared with unsupplemented controls, and significant interactions between dietary supplementation and co-infection were observed for all enzyme transcript levels depending on the tissue of origin. These findings might be of benefit for the future development of dietary strategies to improve poultry health, disease resistance, and growth productivity without the use of in-feed antibiotic growth promoters.

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The authors' responsibilities were as follows—SO, UDG, and HSL: conducted the research; SO: analyzed the data and performed the statistical analysis; SO and EPL: wrote the manuscript; HSL: had responsibility for the content; and all authors: designed the research, and read and approved the final manuscript.

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