The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with *Eimeria* species

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ABSTRACT Supplementation of broiler diets with feed additives such as chemotherapeutic drugs and antibiotics has side effects, meat residues, and antibiotics resistance complications. Plant-derived natural compounds could be safe and easy substitutes for chemical additives. One of the natural compounds is curcumin, the extract from herbal plant *Curcuma longa*, known for its antioxidant and antimicrobial properties which may be effective in reducing coccidia infection in poultry. The objective of this study was to evaluate the effects of curcumin on *Eimeria* challenged (**C**) and nonchallenged (**NC**) Cobb 500 broilers. A total of 360 12-day-old male chicks were housed in 36 cages in a completely randomized design with 6 replicates per treatment of 10 birds each cage. The six corn–soybean meal–based treatment diets were fed from day 12 to 20 to C and NC birds in 3-by-two factorial arrangement: nonchallenged control (**NCC**), NC + 100 mg/kg curcumin, NC + 200 mg/kg curcumin, challenged control (**CC**), C + 100 mg/kg curcumin, and C + 200 mg/kg curcumin. Broilers in C groups were inoculated orally with 50,000 oocysts of *Eimeria maxima*, 50,000 oocysts of *Eimeria tenella*, and 250,000 oocysts of *Eimeria acervulina* on day 14. The intestinal permeability (day 19), growth performance parameters, and intestinal lesion scoring were measured and recorded on day 20. The means were subjected to two-way ANOVA, and main factors effect and their interactions were considered. The growth performance and permeability were higher ($P < 0.001$) in the NC and C groups, respectively. However, no interaction was observed between curcumin dose and cocci challenge on both of these parameters. Results from lesion scores and oocyst shedding showed reduction ($P < 0.050$) in birds fed C + 200 mg/kg curcumin compared with those fed C + 100 mg/kg curcumin or CC. Curcumin treatment showed higher production of GSH ($P = 0.002$) and total glutathione (GSH+2GSSG) ($P = 0.002$) but lower GSH/GSSG ratio ($P < 0.001$) than the NCC group. Curcumin exhibited some positive responses on antioxidant capacity, lesion score, and oocyst shedding in the present study, suggesting that curcumin alone or a combination with other feed additives could be a dietary strategy to improve gut health in broilers.

Key words: broiler, curcumin, *Eimeria* spp., glutathione, intestinal health

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INTRODUCTION

Coccidiosis caused by genus *Eimeria* is a serious parasitic disease in the poultry industry because of its high morbidity, mortality, and economic burden associated with it during severe infection (Pop et al., 2019). Worldwide coccidiosis is responsible for annual loss of around $ 2.4 billion in poultry business (Santos et al., 2020). Seven species of *Eimeria* are involved, of which commonly found and infective are *Eimeria maxima*, *Eimeria tenella*, and *Eimeria acervulina* (Haug et al., 2008; Kim et al., 2011). These three species have their own localization sites within the gut, where *E. acervulina* is found in the upper gastrointestinal tract (**GIT**) around the duodenum and jejunum, *E. maxima* is found around middle GIT in the jejunum and ileum, and *E. tenella* is mostly colonized in the ceca (Chapman, 2014). Coccidia can cause tissue damage in the GIT, which allows secondary bacterial infections by

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Clostridium perfringens (Helmbolt and Bryant, 1971), or *Salmonella* typhimurium (Arakawa et al., 1981). Different strategies have been used to control coccidiosis in the farms, such as vaccination for day-old chicks with low dose of *Eimeria* spp. To improve long-term sustainability of coccidiosis control in poultry, the rotational program of a vaccine and drugs is a common strategy (Hafez, 2008). In addition, supplementation with ionophores as coccidiostats and feed additives such as nitro compounds have been used to reduce coccidiosis and intestinal damage in poultry (Yun et al., 2000; Chapman et al., 2010; Teng et al., 2020a). Increasing problems of drug resistance, side effects of chemotherapeutic drugs, susceptibility of poultry to ionophore toxicity, and recent trend of "no antibiotics ever" with increased consumer demands to decrease the use of antibiotics in poultry result in immense interest of poultry producers to use natural antimicrobials in animal feed (Lillehoj et al., 2018). At the same time, chemotherapeutic drugs and antibiotics have side effects, meat residue, and resistance complication, leading to costly chicken farming in the long run (Mehdi et al., 2018; Yadav and Jha, 2019). The use of these antibiotics as growth promoters at prophylactic doses is also discouraged worldwide, leading to strict regulation in some countries; thus, other feed additives are being explored as alternatives (Yadav et al., 2019). Natural compounds produced from various plant sources are good substitutes to chemical additives (Durmic and Blache, 2012).

Natural compounds have been used in poultry feeding with promising effects on parasites control by altering oocyst's wall formation or by destroying sporozoites (Kim et al., 2013; Fatemi et al., 2015). These compounds not only affect parasites directly but also indirectly improve efficiency and overall performance by their beneficial effects such as immunomodulation, antioxidative, and anti-inflammatory mechanism to defend against coccidia (Pop et al., 2019). Some other characteristic functions of these natural compounds are to help in digestion and absorption of feed, flourishing beneficial gut microbiota, and maintaining healthy gut structure (Yadav and Jha, 2019). One of the studied natural compounds is curcumin which is known for its antioxidant (Tilak et al., 2004) and antimicrobial property (Naz et al., 2010). Curcumin is extracted from herbal plant *Curcuma longa* also known as turmeric (Kocaadam and Sanlier, 2017). It is a native plant of Asia, and its rhizome is used as a dye and in food condiment as it eliminates free radicals and protects cells against lipid peroxidation (Khan et al., 2012). This bioactive compound, curcumin, is also known to have pharmacological effects such as anti-inflammatory, gastroprotective, antiproliferative, antiarthritic, and neuroprotective well-being of both humans and animals (Prasad et al., 2014). Rajput et al. (2013) reported role of curcumin in increasing nutrient digestibility and found that 200 mg/kg supplementation of curcumin improved performance and fat metabolism (Rajput et al., 2013). Another study also found that curcumin improved nutrient metabolism by enhancing the production of bile acids and activity of gastric enzymes to accelerate digestion and absorption (Platel and Srinivasan, 2000). Furthermore, it has been shown that curcumin has the potential to destroy sporozoites of *E. tenella* and reduce the oocyst shedding and gut lesions (Khalafalla et al., 2011). Curcumin is also proven to be protective to maintain gut integrity by boosting humoral immunity of the host (Kim et al., 2013). However, the information regarding use of curcumin in mixed *Eimeria* challenge setup for broiler chicken is limited. The hypothesis of this study is that dietary supplementation of curcumin will provide beneficial effects on growth performance, body antioxidant system, and intestinal integrity in *Eimeria* challenged broilers. Therefore, the objective of this study was to determine the effects of curcumin compound on growth performance, antioxidant status, oocyst shedding, and gut health parameters of broiler chickens in *Eimeria* challenged and nonchallenged conditions.

MATERIALS AND METHODS

Bird Husbandry and Dietary Treatments

This study was conducted after the approval of the International Animal Care and Use Committee at the University of Georgia (Athens, GA). Curcumin used in this study was 95% natural turmeric extract acquired from Hard Eight Nutrition (BulkSupplements.com; 7511 Eastgate Road, Henderson, NV). A total of 360 12-day-old male Cobb 500 breed broiler chicks were used in the study. The birds were distributed in a completely randomized design with a factorial arrangement of 3 by two, with 10 birds per cage replicate and 6 replicate cages per treatment. The main factors were diets (3 levels of curcumin; 0, 100, and 200 mg/kg) and 2 doses of *Eimeria* either challenged (**C**) or nonchallenged (**NC**). In treatment diets, curcumin was mixed at concentrations of 0 (no curcumin added), 100, and 200 mg/kg for both C and NC birds as shown in Table 1. The treatment diets were fed from day 12 to the final day of the trial (day 20). For *Eimeria* doses, each bird in the C group was given an oral gavage of a mixture of 50,000 oocyst/bird of *E. maxima*, 50,000 oocyst/bird of *E. tenella*, and 250,000 oocysts/birds of *E. acervulina* on day 14 using a repeated pipette (Eppendorf Multipette M4, Millipore Sigma, St. Louis, MO), and birds in the NC groups were orally provided with the same amount of phosphate buffer saline (**PBS** 1%; Thermo Fischer Scientific, Waltham, MA) (Santos et al., 2020). The chicks were provided feed and water *ad libitum* throughout the study and kept in controlled environment as per recommendation of Cobb Broiler Management Guide (Cobb 2018a,b).

Sample Collection and Analysis

Growth Performance The body weight of all the birds in a cage and feed weight were recorded on day 12 and 20 to obtain the average body weight (**BW**), body weight gain (**BWG**), feed intake (**FI**), and feed conversion rate (**FCR**) as shown in Table 2.

Table 1. Composition and calculated nutrient contents of control and treatment diets, as-is basis.

Items[1]	Control[2]	100 mg/kg[2] curcumin	200 mg/kg[2] curcumin
Ingredients %			
Corn, grain	60.01	60.01	60.01
Soybean meal (48%)	34.15	34.15	34.15
Dicalcium phosphate	1.58	1.58	1.58
Soybean oil	1.53	1.53	1.53
Limestone	1.17	1.17	1.17
Common salt	0.35	0.35	0.35
DL-methionine	0.29	0.29	0.29
Vitamin Premix[3]	0.25	0.25	0.25
L-lysine-HCL	0.22	0.22	0.22
Mineral premix[4]	0.08	0.08	0.08
L-threonine	0.07	0.07	0.07
Cr_2O_3	0.30	0.30	0.30
Curcumin, mg/kg	0	100	200
Nutrients			
ME, kcal/kg	3,010	3,010	3,010
Crude protein	21.25	21.25	21.25
Lysine %	1.32	1.32	1.32
Methionine %	0.63	0.63	0.63
TSAA %	0.98	0.98	0.98
Threonine %	0.86	0.86	0.86
Calcium %	0.90	0.90	0.90
Available phosphorus %	0.45	0.45	0.45

[1]Control, 100 mg/kg curcumin, 200 mg/kg curcumin.
[2]All 3 diets: control, 100 mg/kg curcumin, 200 mg/kg curcumin were from same basal mix.
[3]Provided per kg of DSM Vitamin premix: Vit. A 2,204,586 IU, Vit. D_3 200,000 ICU, Vit. E 2,000 IU, Vit. B12 2 mg, biotin 20 mg, menadione 200 mg, thiamine 400 mg, riboflavin 800 mg, d-pantothenic acid 2,000 mg, Vit. B6 400 mg, niacin 8,000 mg, folic acid 100 mg, choline 34,720 mg.
[4]Provided per kg of mineral premix: Ca 0.72 g, Mn 3.04 g, Zn 2.43 g, Mg 0.61 g, Fe 0.59 g, Cu 22.68 g, I 22.68 g, and Se 9.07 g.

Lesion Score The four-score scale method was used as proposed by Johnson and Reid (1970) for the upper intestinal tract (duodenum), middle intestinal tract (jejunum and ileum), and lower intestinal tract (ceca) lesion scoring (8 birds/cage). Data were analyzed in a nonparametric way to determine average score and percentage of each score (Figure 1). The score ranges from 0 to 4, where 0 is no appearance of gross lesions and 4 is severe lesions. The lesions were compared for typical lesion of 3 of the Eimeria spp. used in this study as elaborated in poultry coccidiosis diagnostic and testing procedures (Conway and McKenzie, 2007).

Intestinal Permeability On day 19 that is 5 d post infection (**dpi**), the fluorescein isothiocyanate dextran (**FITC-d**; 100 mg, MW 4,000; Sigma-Aldrich, St. Louis, MO) was orally administered with 1 mL/bird (6 birds/treatment) to determine gut permeability. Ten birds from an extra NC group were also gavaged with FITC-d to make standard curve for comparing NC and C groups. Birds were kept in new cage for 2 h after gavage without feed. Immediately after birds were euthanized by cervical dislocation, 3 mL of blood samples were collected from heart and then centrifuged (Eppendorf Centrifuge 5430R; Eppendorf, Hamburg, Germany) at 1,000 g for 15 min to obtain serum. The serum samples and standards were transferred to a black 96-well microplate (Ref. 655077; Greiner Bio-One, Monroe, NC) and measured using a spectrophotometer (Spectramax, M5; Molecular Devices, San Jose, CA) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Data is presented in Table 3. The FITC-d gavage solution and serum samples were kept in a dark place to avoid direct light exposure.

Oocyst Shedding For the oocyst shedding, fecal samples were collected in the cages from the C groups and later processed in the laboratory. Five grams of the fecal sample was used, and 45 mL of saturated NaCl was added. The samples were mixed well and allowed to stand for 15-30 s for solid fecal samples to settle. The diluted fecal samples were obtained with a Pasteur pipette to fill McMaster chamber slides (McMaster Egg Counting Chamber, Vetlab Supply, Palmetto Bay, FL). Approximately 5-min time lapse was given to allow the oocysts float to the top of the solution. The total number was counted by the formula as shown in the following to obtain the number of oocysts per gram of feces as presented in Table 4.

$$\text{Number of oocysts per gram of feces} = (\text{Number of oocysts counted} / 0.15) \times \text{Dilution}$$

where, 0.15 = volume of McMaster counting chamber.

Glutathione

Liver samples (one bird/cage) were collected from one bird per cage at 20 d of age, rinsed with ice-cold PBS and snap-frozen in liquid nitrogen. The samples were

Table 2. Body weight (BW), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) from 12 to 20 d of age in accordance with the treatments.

Item						
Treatment	Dosage (mg/kg)	Challenge	BW, g	BWG, g	FI, g	FCR
NCC	0	No	784.20	484.05	680.51	1.419
NC + curcumin	100	No	806.56	506.34	673.54	1.331
NC + curcumin	200	No	806.33	505.78	684.54	1.354
CC	0	Yes	658.11	357.14	600.41	1.687
C + curcumin	100	Yes	635.67	335.27	571.76	1.715
C + curcumin	200	Yes	639.37	339.72	577.26	1.703
Main effect	Dosage					
	Control		721	421	641	1.55
	Curcumin 100		721	421	623	1.52
	Curcumin 200		723	423	631	1.53
		Challenge				
		No	799[a]	499[a]	680[a]	1.37[b]
		Yes	644[b]	344[b]	583[b]	1.7[a]
Source of variance			*P*-value	*P*-value	*P*-value	*P*-value
Dosage effect			0.987	0.983	0.245	0.745
Challenge effect			<0.001	<0.001	<0.001	<0.001
Dose × challenge			0.150	0.167	0.393	0.363
SEM			13.989	13.996	9.189	0.033

[ab]Means in same column followed by different letters differ by Duncan's test ($P < 0.05$).

Challenged group inoculated with 50,000 oocyst/bird of *Eimeria maxima*, 50,000 oocyst/bird of *E. tenella*, and 250,000 oocysts/birds of *E. acervulina* on day 14 after hatch.

Abbreviations: CC, challenged control; C + curcumin 100, challenged +100 mg/kg curcumin; C + curcumin 200, challenged +200 mg/kg curcumin; NCC, nonchallenged control; NC + curcumin 100, nonchallenged +100 mg/kg curcumin; NC + curcumin 200, nonchallenged +200 mg/kg curcumin.

processed for glutathione (**GSH**) and glutathione disulfide (**GSSG**) quantification within 24 h of collection. For this procedure, 70 mg of liver samples were homogenized in PBS containing 10 mM diethylenetriaminepentaacetic acid (DTPA, Sigma-Aldrich, St. Louis, MO). Immediately, 10% perchloric acid with 1 mM DTPA was added and centrifuged. The supernatants were collected and frozen at −80°C until further processing. Glutathione and GSSG were quantified using high-performance liquid chromatography (Dionex UltiMate 3000; Thermo Fisher Scientific, Waltham, MA). Peaks were quantified using external GSH and GSSG standards and the Chromeleon Chromatography Data System software (Dionex Version 7.2; Thermo Fisher Scientific). Total glutathione was calculated by the formula GSH + 2GSSG, and glutathione redox status was assessed by the ratio GSH/GSSG. Concentrations of GSH and GSSG were standardized to total protein that was quantified by a Pierce BCA Protein Assay (Thermo Fisher Scientific).

Statistical Analysis

Battery cage was the experimental unit for all the measurements. The replicates of experimental units were grouped in a completely randomized design, where treatments were grouped in a factorial design. The means of data were subjected to two-way ANOVA, and main factor effects and their interactions were evaluated. The significant means were further compared by Duncan's multiple range test (Teng et al., 2020a). Analysis was performed using PROC GLM procedure of SAS (SAS Institute, 2010). The level of significance was set at $P < 0.05$. To compare the lesion scores between treatments for different parts of GIT, the post hoc of Kruskal–Wallis nonparametric statistic was used (Elliott and Hynan, 2011). Both the average value and percentage of scores were included and presented in the result section as previously done by Teng et al. (2020a).

RESULTS

Growth Performance

The growth performance was measured from day 12 to 20 because the chicks were fed treatment diets from day 12 and *Eimeria* challenged on day 14, providing 2 d for the birds to acclimatize to the treatment diets. The study was concluded on day 20 that was 6 dpi. During this period, although there was no interaction between curcumin dose and challenge, the growth performance was influenced by *Eimeria* challenge ($P < 0.001$) (Table 2). The C groups had significantly reduced BW, BWG, FI, and increased FCR compared with the NC groups. Although, the growth parameters were not significant between the treatment diets; however, there is numerical improvement in the FCR by curcumin inclusion at 100 and 200 mg/kg ($P = 0.745$) in the NC groups.

Gut Health

Lesion Score The lesion scoring was performed for the duodenum, ileum, and jejunum, and the ceca sections of the gut based on different infection sites for different *Eimeria* spp. included in this study as presented in Figure 1. The results showed that the NC birds had score 0 (100%) for all the 3 locations in the gut tested for lesions, indicating that no lesions were observed in these birds and verifying that there was no cross

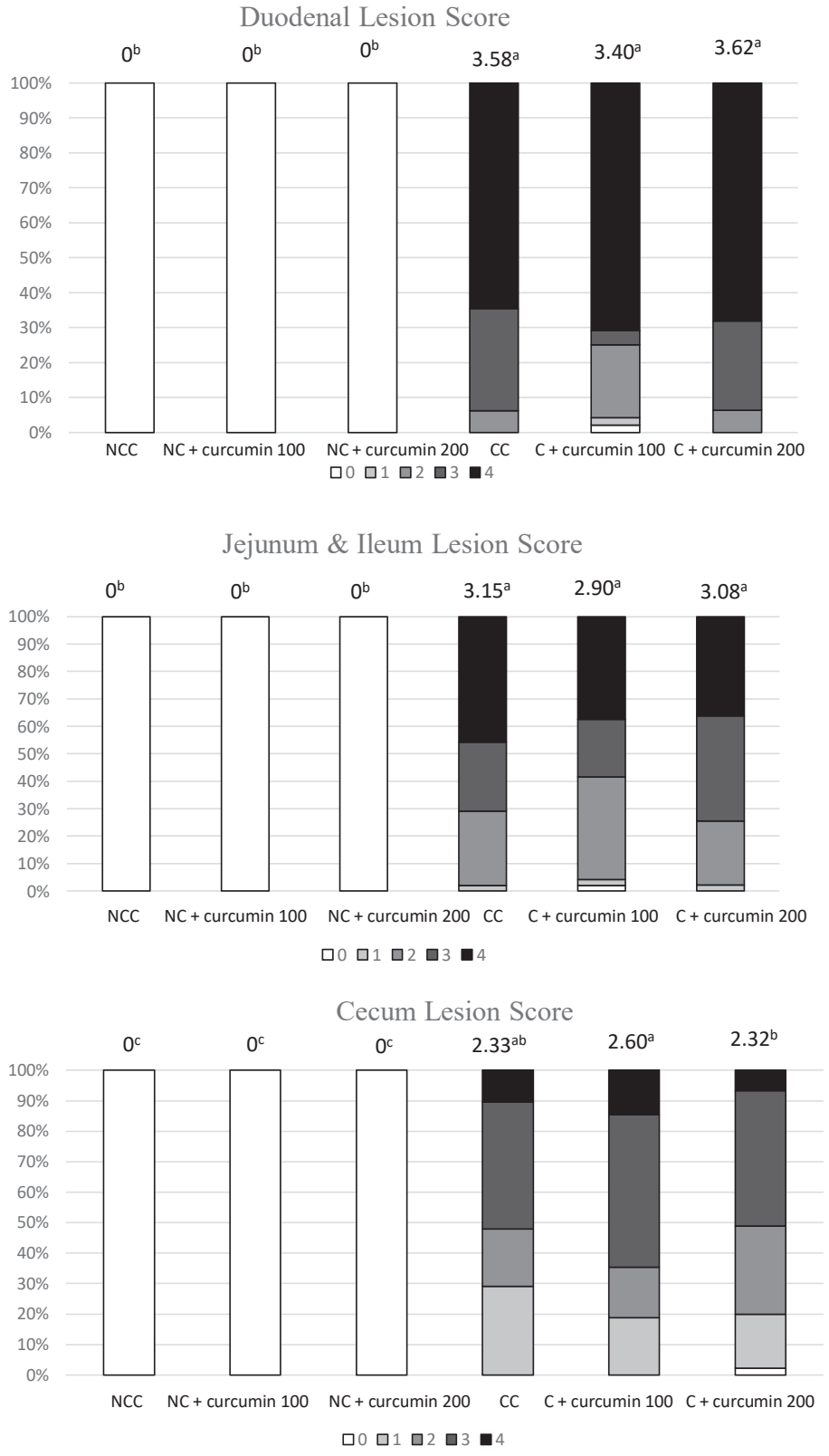

Figure 1. Effects of curcumin supplementation on intestinal lesion scores of *Eimeria* spp. nonchallenged and challenged broiler birds. [ab]Means followed by different letters in superscript differ by Duncan's test ($P < 0.05$). Challenged group inoculated with 50,000 oocyst/bird of *Eimeria maxima*, 50,000 oocyst/bird of *E. tenella* and 250,000 oocysts/birds of *E. acervulina* on day 14 post hatch. Intestinal lesion scoring was done 6 dpi for 48 birds/treatment. The average of lesion scores is presented in the figures. Abbreviations: C control, challenged control; curcumin 100, 100 mg/kg curcumin; curcumin 200, 200 mg/kg curcumin; NC control, nonchallenged control.

Table 3. FITC-d μg/mL concentration in serum of broiler chicken at 20 d of age.

Permeability			
Treatment	Dosage (mg/kg)	Challenge	FITC-d (μg/mL)
NCC	0	No	−0.00309
NC + curcumin	100	No	<0.0001
NC + curcumin	200	No	−0.00875
CC	0	Yes	0.084775
C + curcumin	100	Yes	0.074074
C + curcumin	200	Yes	0.106599
Main effect	Dose		
	Control		0.0408
	Curcumin 100		0.0370
	Curcumin 200		0.0489
		Challenge	
		No	−0.0039[b]
		Yes	0.0884[a]
Source of variance			*P*- value
Dosage effect			0.543
Challenge effect			<0.001
Dose ×challenge			0.170
SEM			0.009

[ab]Means in same column followed by different letters differ by Duncan's test ($P < 0.05$).

Challenged group inoculated with 50,000 oocyst/bird of *Eimeria maxima*, 50,000 oocyst/bird of *E. tenella* and 250,000 oocysts/birds of *E. acervulina* on day 14 post hatch.

Abbreviations: CC, challenged control; C + curcumin 100, challenged +100 mg/kg curcumin; C + curcumin 200, challenged +200 mg/kg curcumin; NCC, nonchallenged control; NC + curcumin 100, nonchallenged +100 mg/kg curcumin; NC + curcumin 200, nonchallenged +200 mg/kg curcumin.

contamination between the groups. For the duodenum, all three NC groups had score 0 (100% of the birds). In the C groups, control birds had score 2 (6%), score 3 (29%), and score 4 (65%). Similarly, 100 mg/kg curcumin group got score 0 (2%), 1 (2%), 2 (21%), 3 (4%), and 4 (71%). In addition, 200 mg/kg curcumin–fed group got score 2 (6%), 3 (26%), and 4 (68%).

Lesion score for the jejunum and ileum in the C group: for control treatment diet–fed birds had score 1 (2%), 2 (27%), 3 (25%), and 4 (46%); 100 mg/kg curcumin–fed birds scored 0 (2%), 1 (2%), 2 (38%), 3 (21%), and 4 (38%); and 200 mg/kg curcumin group are scored 1 (2%), 2 (23%), 3 (38%), and 4 (36%).

Lesion score for the ceca in the C group: control treatment birds had score 1 (29%), 2 (19%), 3 (42%) and 4 (10%); 100 mg/kg curcumin–fed birds scored 1 (19%), 2 (17%), 3 (50%), and 4 (15%); and 200 mg/kg curcumin group are scored 0 (2%) 1 (18%), 2 (29%), 3 (44%), and 4 (7%).

Intestinal Permeability The effects of *Eimeria* challenge were observed where the NC groups had significantly lower permeability of the gut than the C groups ($P < 0.001$) (Table 3), which means *Eimeria*-challenged birds had a leaky gut due to damage caused by the *Eimeria* spp. There were no differences due to doses in the permeability ($P = 0.543$), and no interaction between main effects of dose and challenge were observed ($P = 0.170$). However, 100 mg/kg curcumin–fed treatment had numerically lower permeability than the control and 200 mg/kg curcumin–fed birds.

Oocyst Shedding Supplementation of 200 mg/kg curcumin in the C group (C + 200 mg/kg curcumin) had significantly reduced the oocyst count of *E. maxima* compared with the challenged control (CC) group ($P = 0.044$). However, there were no differences in the oocyst count for *E. tenella* ($P = 0.466$), *E. acervulina* ($P = 0.190$), and the overall oocyst count ($P = 0.206$) as presented in Table 4.

Glutathione Curcumin dose and *Eimeria* challenge along with their interactions were observed in the levels of GSH, GSH + 2GSSG, and GSH/GSSG as shown in Table 5. For GSH, 100 and 200 mg/kg curcumin in the NC group (NC + 100 and NC + 200 mg/kg) had significantly higher production than the nonchallenged control (NCC), the CC, and C + 200 mg/kg curcumin groups ($P = 0.003$), whereas in the challenged case, C + 100 mg/kg curcumin significantly increased GSH compared with the CC and C + 200 mg/kg curcumin ($P = 0.002$). For total glutathione (GSH + 2GSSG), the NC + 100 or 200 mg/kg curcumin showed higher production than the NCC ($P = 0.002$), where NC + 200 mg/kg curcumin showed the highest GSH + 2GSSG. The C + 100 mg/kg curcumin had significantly higher GSH + 2GSSG than the NCC and C + 200 mg/kg curcumin ($P = 0.003$). For GSH/GSSG ratio, the NCC group had the highest among the treatments ($P < 0.001$). The curcumin treatments in the C groups (C + 100 and C + 200 mg/kg curcumin) significantly reduced the GSH/GSSG ratio compared with the NCC group, whereas there was no change among the treatments within the C groups.

For GSSG levels, no interaction was observed among the treatments ($P = 0.097$). However, there was a curcumin dose effect ($P = 0.007$); the curcumin treatments (100 and 200 mg/kg) significantly increased GSSG levels compared with the control.

Table 4. Oocyst shedding (oocyst/gram) at 6 dpi.

Treatment	*Eimeria maxima*	*Eimeria tenella*	*Eimeria acervulina*	Total
CC	12,784[a]	24,012	1,236,173	1,272,970
C + curcumin 100	10,783[a,b]	20,232	1,525,207	1,556,222
C + curcumin 200	7,004[b]	16,786	1,339,781	1,363,570
P value	0.044	0.466	0.190	0.206
SEM	995.48	2,301.26	65,022	65,759

[ab]Means in same column followed by different letters in superscript differ by Duncan's test ($P < 0.05$).

Challenged group inoculated with 50,000 oocyst/bird of *E. maxima*, 50,000 oocyst/bird of *E. tenella*, and 250,000 oocysts/birds of *E. acervulina* on day 14 after hatch.

Abbreviations: CC, challenged control; C + curcumin 100, challenged +100 mg/kg curcumin; C + curcumin 200, challenged +200 mg/kg curcumin.

DISCUSSION

Although there are several studies performed in the past to study curcumin or turmeric (Eevuri and Putturu, 2013), limited studies were conducted in the cocci challenge models. The present study evaluated the growth performance and gut health parameters with and without cocci challenge for the birds fed a control diet and 2 curcumin diets at 100 and 200 mg/kg. The growth performance in challenged birds was significantly decreased even in few days after the challenge, and FCR increased significantly compared with nonchallenged birds. Similarly, significant reduction in growth performance along with increased lesion scores, gut permeability, and decreased metabolizable energy and intestinal morphology were obtained by Teng et al. (2020a,b). Dalloul and Lillehoj (2006) also reported increased mortality and decreased performance in severe coccidiosis. Although the birds were not fed curcumin diets for a longer period in the present study, it might be beneficial if curcumin is fed in broilers for a 42-d period. A study by Rajput et al. (2013) showed no significant difference in growth performance during a starter phase (0–21 d) among the curcumin treatments at 100, 150, and 200 mg/kg. However, birds fed curcumin-supplemented diets for 42 d had significant increase in the BW and feed efficiency during a finisher stage (22–42 d). This could be due to larger villus area induced by curcumin feeding for a longer period, improving nutrient absorption in the later phase (Rajput et al., 2013). The improvement in FCR was also observed in the birds fed *C. longa* which is a source of curcumin and used as a food preservative and coloring material with medical value and biological action (Abou-Elkhair et al., 2014). The improved FCR could be due to the upregulation of amylase, trypsin, chymotrypsin, and lipase secretion as shown in laboratory animals (Platel and Srinivasan, 2000; Abou-Elkhair et al., 2014). However, the present study showed that curcumin supplementation did numerically but not significantly increased growth parameters, which might be attributed to a short study period (20 d) and shorter duration of curcumin inclusion in treatment diets (12–20 d).

Beside growth performance, the lesion score parameter in the present study showed that NC group had minimum to no lesions on different locations (duodenum, jejunum, and ileum, and ceca) in the GIT. Likewise, Teng et al. (2020b) also observed similar results with least intestinal lesions in NC compared with C groups. However, unlike the present study, Scheurer et al. (2013) reported a score higher than 0 for nonchallenged birds, which may be due to pre-existing oocysts or possible cross contamination from the C group. In general, the mean lesion scores in the challenged birds were higher in the present study than in the study by Scheurer et al. (2013), which could be due to higher doses of mixed *Eimeria* spp. used in the present study. Another study by Duffy et al. (2005) had similar mean lesion score values to the present study, suggesting that the challenge doses used in the present study were appropriate. The higher inclusion of curcumin (200 mg/kg) had significantly lower cecal lesion score than 100 mg/kg curcumin during the challenge phase, which might be attributed to improved resistance to *Eimeria* spp. by the higher dose of curcumin. In a study by Kim et al. (2013), feeding an extract of *C. longa* known to contain curcumin showed improved resistance

Table 5. Activity of glutathione (GSH), glutathione disulfide (GSSG), total glutathione (GSH+2GSSG), glutathione redox status (GSH/GSSG).

Item						
Treatment	Dosage (mg/kg)	Challenge	GSH (nmol/mg)	GSSG (nmol/mg)	Total glutathione (GSH+2GSSG) (nmol/mg)	GSH/GSSG
NCC	0	No	25.59^{c}	0.07	25.79^{c}	357.49^{a}
NC + curcumin	100	No	44.62^{a,b}	0.38	45.39^{a,b}	126.39^{b}
NC + curcumin	200	No	54.41^{a}	0.47	55.37^{a}	119.98^{b}
CC	0	Yes	34.67^{b,c}	0.38	35.42^{b,c}	96.82^{b}
C + curcumin	100	Yes	37.76^{b}	0.45	38.66^{b}	85.35^{b}
C + curcumin	200	Yes	34.79^{b,c}	0.41	35.60^{b,c}	92.10^{b}
Main effect	Dose					
	Control		30.13	0.22^{b}	30.61	227.16
	Curcumin 100		41.19	0.42^{a}	42.03	105.87
	Curcumin 200		44.60	0.44^{a}	45.49	106.04
		Challenge				
		No	40.79	0.33	41.42	192.09^{a}
		Yes	35.74	0.40	36.56	91.42^{b}
Source of variance			*P*-value	*P*-value	*P*-value	*P*-value
Dose effect			0.002	0.007	0.002	<0.001
Challenge effect			0.071	0.139	0.084	<0.001
Dose × challenge			0.003	0.097	0.003	<0.001
SEM			2.064	0.032	2.110	16.650

[ab]Means followed by different letters in superscript differ by Duncan's test ($P < 0.05$).

Challenged group inoculated with 50,000 oocyst/bird of *Eimeria maxima*, 50,000 oocyst/bird of *E. tenella*, and 250,000 oocysts/birds of *E. acervulina* on day 14 after hatch.

Abbreviations: CC, challenged control; C + curcumin 100, challenged +100 mg/kg curcumin; C + curcumin 200, challenged +200 mg/kg curcumin; NCC, nonchallenged control; NC + curcumin 100, nonchallenged +100 mg/kg curcumin; NC + curcumin 200, nonchallenged +200 mg/kg curcumin.

to inflammation caused by *E. maxima* and *E. tenella* infection. This resistance causes decrease in lesion scores which is in accordance with the present study, although the cecal lesion caused by *E. maxima* was numerically less with curcumin supplementation compared with the challenged control.

The gut permeability in the present study was overall increased in the *Eimeria* challenged birds compared with the nonchallenged birds ($P < 0.001$). A similar result was found by a study where gut leakiness was compared between the 2 groups (Teng et al., 2020a). A study by Lee et al. (2013) had shown that a combination of *Capsicum* oleoresin (concentrated extract from peppers) and turmeric oleoresin (extract of *C. longa*) was effective in reducing necrotic lesion in the gut by boosting immunity against avian necrotic enteritis. Another study using 0.1% mixture of ascorbic acid, solid dispersion of curcumin, polyvinylpyrrolidone, and boric acid showed an increase in the gut barrier integrity compared with a control challenged with *S*almonella enteritidis (Hernandez- Patlan et al., 2019). However, the present study did not find any significant difference by feeding different levels of curcumin solely although 100 mg/kg supplementation decreased the permeability numerically. The logic behind not having significant difference in our study could be the sole use of curcumin, higher doses of cocci challenge, or a short period of feeding curcumin to broiler chickens.

Oocyst shedding count was one of the parameters used to find the effect of curcumin in cocci challenge models (Chasser et al., 2020). There was no oocyst shedding in the NC groups in the present study as the birds were not infected with *Eimeria* spp. (data not shown). In the present study, significant reduction in *E. maxima* oocyst output was found when 200 mg/kg curcumin was fed compared with the CC group. By contrast, oocyst shedding of *E. maxima* was unaffected when *C. longa* was fed (Kim et al., 2013). This contradiction could be due to sample collection day after infection as fecal samples were collected on 6 dpi in the present study, whereas Kim et al. (2013) enumerated oocyst 10 dpi. This indicates that fecal sampling time points are important to observe positive effects by curcumin treatments.

Similar to the present study, another study found no significant difference in oocyst shedding of *E. acervulina* when only *C. longa* (turmeric) was used (Lee et al., 2010), although the differences were found when curcumin was used in combination with *Capsicum* and *Lentinus* (shiitake mushroom—known for its antitumor and antiviral properties) (Lee et al., 2010). Thus, the variation in oocyst shedding between different studies suggests that curcumin may exert differential cytotoxicity and effectiveness against different *Eimeria* spp.

In the present study, the antioxidant parameters such as GSH, total glutathione, and GSH/GSSG were affected by curcumin dose and *Eimeria* challenge as well as their interaction, whereas GSSG was influenced only by curcumin dose. These results also indicate that GSH and GSSG productions were upregulated with rapid conversion of GSH to oxidized form GSSG as a result of *Eimeria* infection as well as curcumin supplementation. Alteration in the antioxidant enzymes and reduction of nonenzymatic antioxidants are evident during the stress because of *Eimeria* challenge (Georgieva et al., 2011). A study by Zhang et al. (2018) showed that curcumin increased the glutathione peroxidase in poultry to scavenge reactive oxygen species which caused oxidative stress. In the present study, the increased ratio of GSH to GSSG in NCC group is the indication of lower level of oxidative stress which is expected in normal birds, where limited GSH is produced and less oxidized to form GSSG. However, *Eimeria* spp. infection increased GSSG levels and significantly decreased the GSH/GSSG ($P < 0.001$). This study found interaction between 2 main factors, curcumin dose and *Eimeria* challenge for GSH and total glutathione, and decreased GSH/GSSG, as well as a trend of interaction for GSSG ($P < 0.001$). This finding suggested that GSH was increased in the nonchallenged birds because of the dietary supplementation of curcumin, whereas the additional GSH has been consumed to GSSG during coccidiosis. This is an indirect piece of evidence showing that curcumin reduced antioxidative stress caused by the *Eimeria* infection. The increase in GSH level in a concentration-dependent manner with inclusion of curcumin in diet was reported previously in several studies (Banerjee et al., 2008; Zhang et al., 2018). Interestingly, curcumin supplemented in the NC groups at both 100 and 200 mg/kg levels also increased the oxidized form of glutathione (GSSG) and decreased GSH/GSSG ratio compared with the NCC group ($P < 0.001$). The mechanism behind increasing GSSG with curcumin feeding could be due to the ability of curcumin to elevate the level of cellular GSH by *de novo* GSH synthesis catalyzed by the enzyme glutamate–cysteine ligase as well as turnover of the GSH/GSSG redox cycle (Zheng et al., 2007; Zhang et al., 2018). Another reason for increased GSSG and decreased ratio of GSH/GSSG could be due to the capability of curcumin to increase activity of glutathione S-transferase enzyme which causes conjugation reaction to convert GSH to GSSG (Nishinaka et al., 2007). This indicates that curcumin appears to have biphasic effects on the GSH metabolism and acts as an antioxidant as well as a prooxidant, observed in this study which is in accordance with the study by Zhang et al. (2018). This study also found that 100 mg/kg of curcumin supplementation may have reached the plateau for its maximal effects on the redox reaction.

In conclusion, the results indicate that curcumin supplementation did not completely mitigate the negative effects of coccidiosis on growth performance but improved the capacity against *Eimeria* infection by increasing antioxidant activities, mostly GSH synthesis to scavenge reactive oxygen species, decreasing lesion score of the ceca when included at 200 mg/kg and significantly decreasing oocyst shedding of *E. maxima*, as well as *E. tenella* to some extent. Curcumin did not show any significance in gut permeability which may be due to higher challenge dose of mixed *Eimeria* spp. or short duration of curcumin feeding. Thus,

further studies are warranted to determine curcumin feeding from day one to recovery period of cocci challenge, effects on intestinal integrity, immunity, and the gut microbiome. Furthermore, long-term effects of curcumin feeding alone or in combination with other feed additives such as organic acids, phytogenic extracts, essential oils, or probiotics on the gut health in *Eimeria*-challenged broilers would provide potential benefits for the poultry industry. On the verse of exploring antibiotic alternatives, a naturally available phytochemical with known pharmacological effects, curcumin, could be a potential alternative for the poultry farmers and industry.

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