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Evaluation of a *dacitic* (*rhyolitic*) *tuff breccia* use on performance, inflammatory, and antioxidant responses in broilers mildly challenged with *Eimeria* spp.

P.-Y. Teng ,* J. Choi ,* S. Yadav,* B. Marshall,* F. L. S. Castro,† J. Ferrel ,† and W. K. Kim *,[1]

*Department of Poultry Science, University of Georgia, Athens, GA 30602, USA; and †AZOMITE Mineral Products Inc., Nephi, UT 84648, USA

ABSTRACT The objective of the study was to investigate the effects of a dacitic tuff breccia (**DTB**) on *Eimeria*-infected broilers. A total of 600 one-day-old Cobb 500 male chickens were randomly assigned to 5 treatments with 10 replicates of 12 birds. Treatments were: an unchallenged control (**UC**), a challenged (**CC**) control (0% DTB), and 3 challenged groups with 0.125, 0.25, or 0.5% DTB. At d 14, birds in the CC and DTB groups were orally gavaged with mixed *Eimeria* spp., while the UC received water. Growth performance was evaluated during prechallenge, challenge, and postchallenge periods (0−14 d; 14−20 d; and 20−26 d, respectively). Gastrointestinal permeability was measured at 5 days postinfection (**dpi**). Intestinal histology and nutrient digestibility of dry matter (**DM**), crude protein (**CP**), and ileal digestible energy (**IDE**) were measured at 6 dpi. Liver activity of glutathione peroxidase (**GSH-Px**) was determined at 6 dpi, and concentrations of reduced (**GSH**) and oxidized glutathione (**GSSG**) were analyzed at 6 and 12 dpi. Data were analyzed using a linear mixed model analysis and Tukey's test ($P \leq 0.05$). From 0 to 14 d, similar average daily gain (**ADG**) and average daily feed intake (**ADFI**, $P > 0.05$) were observed. Gain:feed ratio (**GF**) was higher in 0.125, 0.25, and 0.5% of DTB than the CC and UC ($P < 0.001$). From 14 to 20 d, the UC had the highest ADG, ADFI, and GF ($P < 0.001$). At 5 dpi, intestinal permeability was higher in the challenged groups than the UC. Additionally, the UC showed the highest apparent ileal digestibility of CP, whereas 0.125% DTB had higher CP digestibility than the CC and 0.5% DTB ($P < 0.001$). At 6 dpi, 0.125% DTB increased GSH-Px activity compared to the CC, 0.5% DTB, and UC ($P < 0.001$). At 12 dpi, 0.125% DTB showed increased GSH concentration compared to the CC, 0.25% DTB, and 0.5% DTB ($P < 0.01$). The mild coccidia infection negatively impacted growth performance, apparent ileal nutrient digestibility, intestinal histology, and gastrointestinal integrity in broilers. The use of 0.125% DTB exhibited potential in improving antioxidant responses, apparent ileal digestibility of CP, and growth performance.

Key words: broiler, dacitic tuff breccia, *Eimeria*, oxidative stress, performance

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INTRODUCTION

Coccidiosis, an enteric disease caused by protozoan of the genus *Eimeria*, is a worldwide economic and welfare issue for the poultry industry (Blake et al., 2020). *Eimeria* species are transmitted through the ingestion of sporulated oocysts shed in the feces of infected birds. Upon ingestion, the oocysts release sporozoites that invade the intestinal epithelial cells and undergo several rounds of replication, causing damage to the intestinal cells (McDougald, 2013). This damage can lead to decreased nutrient digestibility, increased inflammation, and oxidative stress, ultimately resulting in deteriorated growth performance and even mortality (Georgieva et al., 2006; Yegani and Korver, 2008; Perez-Carbajal et al., 2010; Sharma et al., 2022; Teng et al, 2023; Tompkins et al., 2023). Furthermore, coccidiosis is recognized as the main risk factor for necrotic enteritis by contributing to enteric dysbiosis (McDougald, 2013). Therefore, preventing and controlling coccidiosis is essential for maintaining the health and productivity of poultry flocks. While anticoccidial drugs are the most commonly used approach for preventing coccidiosis, there has been a growing interest in the use of nutritional strategies to alleviate the negative effects of coccidiosis due to public concerns regarding the use of antibiotics in animal production (Cervantes, 2015). Several nutritional strategies have been proposed including mineral supplementation (Georgieva et al., 2011; Bortoluzzi et al., 2020; Santos et al., 2020; Alagawany et al., 2021).

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[1]Corresponding author: wkkim@uga.edu

AZOMITE, a *dacitic* (*rhyolitic*) *tuff breccia* (**DTB**), is a naturally occurring mineral product of volcanic origination and classified as a hydrated sodium calcium aluminosilicate. It has been reported to contain over 70 minerals and trace minerals in measurable amounts, such as Fe, Mg, Mn, Se, Zn, Cu, and rare earth elements (**REE**), including lanthanides (AZOMITE International, 2021). Previous studies have demonstrated a potential of DTB in improving feed efficiency in broilers (Pirzado et al., 2020, 2021; Juzaitis-Boelter et al., 2021), bone characteristics in broilers (Pirzado et al., 2020, 2021; White et al., 2022) and laying hens (Juzaitis-Boelter et al., 2021), egg production of laying hens and broiler breeders (Juzaitis-Boelter et al., 2021), and nutrient digestibility (Pirzado et al., 2020, 2021; Juzaitis-Boelter et al., 2021; White et al., 2022). Moreover, DTB might have beneficial effects on the oxidative system of the broilers because it is rich in various kinds of trace minerals, including selenium, copper, manganese which are key cofactors for important antioxidant enzymes such as glutathione peroxidase (**GPx**) and superoxide dismutase (**SOD**) (Richards et al., 2010). Previous studies have shown that supplementing these minerals in broiler diets could improve their oxidative status (Echeverry et al., 2016; Santos et al., 2020; White et al., 2022). Improvement in oxidative status has also been observed in REE supplementation in broiler diet by Bölükbaşı et al. (2016). Additionally, DTB supplementation in diets has been shown to positively affect the immune response of the birds (Bowen et al., 2022; Pirzado et al., 2022).

Because coccidiosis would cause oxidative stress and disturb immune functions, supplementing DTB in broiler diets might ameliorate the negative impact of coccidiosis by mitigating the *Eimeria*-derived inflammation and oxidative stress, especially during the postchallenge recovery phase. Therefore, the objective of this study was to assess the effect of DTB on performance, antioxidant defense, apparent ileal digestibility (**AID**), intestinal health, and inflammation in broilers challenged with a pool of *Eimeria* spp.

MATERIALS AND METHODS

Bird Husbandry and Challenge

The study was conducted with the approval of the Institutional Animal Care and Use Committee (**IACUC**) of the University of Georgia (Athens, Georgia, USA). A total of 600 one-day-old male broiler chicks (Cobb 500) were individually weighed and evenly distributed in a randomized complete block design (**RCBD**) with 5 treatments, 10 replicates of 12 birds. The treatments consisted of an unchallenged control (**UC**, 0% DTB), a challenged control (**CC**, 0% DTB), and the challenged groups containing either 0.125, 0.25, or 0.50% DTB. On d 14, CC and DTB-fed birds were orally gavaged with approximately 12,500 *E. maxima*, 12,500 *E. tenella*, and 62,500 *E. acervulina* sporulated oocysts in a 1 mL solution (the challenge dose prepared and provided by Dr. S. E. Aggrey's Lab), whereas the UC received the same volume of water. The dosage of sporulated oocysts provided was selected to induce a medium-low severity challenge, according to Teng et al. (2020). The chicks were allocated to 50 cages (0.04 m^2/bird) equipped with a trough feeder and drinker, providing ad libitum water and feed from 1 to 26 d of age. The lighting and temperature programs were set according to Cobb 500 management guide (Cobb Vantress, 2018a).

The starter (0−14 d) and grower (14−26 d) corn-soybean meal-based diets were formulated to meet or exceed the Cobb 500 nutrient specifications (Cobb Vantress, 2018b) for all ingredients (Table 1). The treatments were obtained by adding 0.125, 0.25, or 0.5% of DTB (AZOMITE Feed-grit, AZOMITE Mineral Products, Inc., Nephi, UT) to the control diet (0% of DTB), as a replacement of ground corn. Titanium dioxide (**TiO_2**, 0.3%, Sigma Aldrich, St. Louis, MO) was added

Table 1. Diet formulation according to the treatments (1−26 d, as-fed basis; % diet).

Ingredients	Starter (0−14 d)	Grower (14−26 d)
Corn	61.10	63.66
Soybean meal—48%	25.85	22.87
Soybean oil	0.43	0.90
Corn DDGS	5.00	5.00
Meat and bone meal	5.00	5.00
L-Threonine	0.14	0.06
Limestone	0.64	0.60
Dicalcium phosphate	0.18	0.03
Salt	0.20	0.21
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.08	0.08
DL-Methionine	0.31	0.28
L-Lysine HCl	0.30	0.27
Filler (corn)[3]	0.50	0.50
Titanium dioxide	-	0.30
Calculated nutrient composition		
Metabolizable energy (kcal/g)	2.98	3.03
Crude protein (%)	21.19	19.88
Calcium (%)	0.90	0.84
Available phosphorus (%)	0.45	0.42
Dig. arginine (%)	1.32	1.22
Dig. lysine (%)	1.22	1.12
Dig. methionine (%)	0.63	0.59
Dig. TSAA (%)	0.91	0.85
Dig. threonine (%)	0.83	0.73
Dig. tryptophan (%)	0.24	0.22
Dig. valine (%)	1.09	1.02
Analyzed nutrient value		
Crude protein (%)	22.14	20.41
Energy (kcal/g)	2.91	2.95
Calcium (%)	0.86	0.83
Phosphorus (%)	0.65	0.62
Arginine (%)	1.31	1.29
Methionine (%)	0.63	0.61
Threonine (%)	0.94	0.92

[1]Vitamin premix: Supplemented per kg of diet: thiamin mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 g; pyridoxine HCl, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 g; transretinyl acetate, 1,892 g; α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[2]Mineral premix: Supplemented as per kg of diet: manganese ($MnSO_4 \cdot H_2O$), 60 mg; iron ($FeSO_4 \cdot 7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 5 mg; iodine (ethylene diaminedihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

[3]Corn was replaced by AZOMITE at 0.125, 0.25, or 0.5% according to the treatments.

Table 2. Primer pairs used for RT-qPCR analysis.

Gene	GenBank identification	Forward sequence	Reverse sequence
GAPDH[1]	NM_204305.2	AGCCATTCCTCCACCTTTGAT	AGTCCACAACACGGTTGCTGTAT
Claudin-1	NM_001013611.2	TGGAGGATGACCAGGTGAAGA	CGAGCCACTCTGTTGCCATA
Occludin	NM_205128.1	GTTGGATGAGTCCCAGTATG	GTCGAACTCCTGCTTGTAG
Mucin-2	XM_040673077.2	CCGTTCTCTGGAGAGAGTTGTC	TCCTGGCAGCAGGAACAA
Interleukin-10	NM_001004414.4	AGGGTGAAGTTTGAGGAAAT	GTGTAGAAGCGCAGCAT
Interferon γ	NM_205149.2	GGAGCTCTATACTCTGAAAAACAACC	CGCTGGATTCTCAAGTCGTT
Tumor necrosis factor-α	MF000729.1	CGTGGTTCGAGTCGCTGTAT	CCGTGCAGGTCGAGGTACT
Interleukin -1β	NM_204524.2	GCATCAAGGGCTACAAGCTC	AGATGAAGCGGGTCAGCTC

[1]GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

to the grower diet as an indigestible marker to determine the nutrient digestibility.

Data Collection and Sampling

Growth Performance Birds and feed were weighed by cage at 14, 20, and 26 d of age and used to calculate the average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed ratio (**GF**), and adjusted for mortality.

Gastrointestinal Permeability On d 19 (5 days postinfection, **dpi**), 1 bird per cage (10 birds/treatment) was randomly selected and orally gavaged with fluorescein isothiocyanate dextran (1 mL, 2.2 mg/mL, **FITC-d**, 100 mg, MW 4,000; Sigma-Aldrich, Canada) and kept, without feed for 2 h according to Castro et al. (2020). Blood was collected by cardiac puncture immediately after euthanasia by cervical dislocation, and serum was obtained after centrifugation (Eppendorf Centrifuge 5430R, Eppendorf, Hamburg, Germany). Throughout the assay, the FITC-d solution, blood, and serum samples were protected from direct light exposure. The samples were analyzed at excitation and emission wavelength of 485 and 528 nm, respectively (SpectraMax ABS Plus, Softmax Pro 7 software, Molecular Devices, San Jose, CA).

Gene Expression On d 20 (6 dpi), another bird/replicate (10 birds/treatment) was randomly selected and euthanized, and samples of the proximal jejunum prior to the Meckel's diverticulum and cecal tonsil were collected and kept at −80°C for quantitative reverse-transcriptase polymerase chain reaction (**qRT-PCR**) analysis. Total RNA was extracted using QIAzol Lysis Reagent (Qiagen, Germantown, MD), and RNA purity and quantity were determined by a Nanodrop 1000 (Thermo Fisher Scientific, Pittsburgh, PA). cDNA was obtained from total RNA using high-capacity cDNA reverse transcription kits (Thermo Fisher Scientific, Waltham, MA), and qRT-PCR analysis (Applied Biosystems StepOnePlus, Thermo Fisher Scientific, Waltham, MA) was performed using iTaq Universal SYBR Green Supermix (BioRad, Hercules, CA). Relative gene expression data were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Scmittgen, 2001) with the mean ΔCt of the unchallenged control (0% DTB) being used to obtain the $\Delta\Delta$Ct values. The expression of interleukin-10 (**Il-10**), interferon-γ (**INF-γ**), and interleukin-1β (**Il-1β**) in the cecal tonsil was evaluated at d 20, and Occludin, Claudin-1 (**CLDN-1**), and Mucin-2 (**Muc-2**) in the jejunum samples at 20 and 26 d, using glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) as the control gene. The forward and reverse primers are shown in Table 2.

Intestinal Histomorphology On d 20 (6 dpi), approximately 2 cm long sections from the mid-portion of the duodenum, jejunum, and ileum were collected from the selected bird and were stored in 10% neutral-buffered formalin until processed. Hematoxylin and eosin (**H&E**) staining method was used, and pictures were taken using a light microscope with an attached camera (10× eyepiece and 1.6× magnification; Leica DC500 camera, Leica Mucrosystems Inc., Buffalo Groove, IL). The images were analyzed using ImageJ (Image Processing and Analysis in Java − ImageJ 1.50i, National Institutes of Health) to measure crypt depth and villus height of 3 villi and crypts per slide.

Antioxidant System On d 20 and 26 (6 and 12 dpi, respectively), liver samples were collected from the selected bird and kept at −80°C, and the activities of glutathione peroxidase (**GPx**) and superoxide dismutase (**SOD**) and concentration of reduced and oxidized glutathione (**GSH** and **GSSG**, respectively) were measured. The Glutathione Peroxidase Assay Kits (Cayman Chemical, Glutathione Peroxidase Assay Kits, item No. 703102, Ann Arbor, MI), Superoxide Dismutase Assay Kits (Cayman Chemical, Superoxide Dismutase Assay Kit, item No. 706002, Ann Arbor, MI), and Glutathione Assay Kits (Cayman Chemical, Glutathione Assay Kit, item No. 703002, Ann Arbor, MI) were used. Sample preparation and assay were performed according to the manufacturer's instructions. Additionally, a protein quantification assay kit (Pierce BCA Protein Assay Kit, Ref. 23227, Thermo Scientific, Rockford, IL) was used to standardize the samples. The procedure followed the manufacturer's instructions, and bovine serum albumin (2 mg/mL) was used as the protein standard.

Apparent Ileal Digestibility At d 20 (6 dpi), 4 other birds were randomly selected per replicate cage (40 birds/treatment) and euthanized by cervical dislocation, and the ileal digesta were collected. The digesta samples were pooled within the same replicate, homogenized, oven-dried, and ground. The titanium dioxide content was measured according to Short et al. (1996). Crude protein (**CP**) content was analyzed in the Agricultural Experiment Station Chemical Laboratories at the

University of Missouri-Columbia (N × 6.25, LECO, St. Joseph, MI), and gross energy (**GE**) was measured using a bomb calorimeter (IKA Calorimeter C1, IKA Works Inc., Wilmington, NC). Apparent ileal digestibility of nutrients and ileal digestible energy (**IDE**) were calculated according to the following equations.

$$AID, \% = 1 - \left(\frac{Nutrient_{Digesta}}{Nutrient_{Diet}} \times \frac{TiO_{2\,Diet}}{TiO_{2\,Digesta}} \times 100 \right)$$

$$IDE = GE_{Diet} - \left(GE_{Digesta} \times TiO_{2\,Diet} \right) / TiO_{2\,Digesta}$$

Statistical Analysis

Tests for normality and homogeneity were conducted, and variables were analyzed using a linear mixed model analysis, Proc Mixed SAS v9.4 (SAS Institute, 2017), with fixed effect as treatment and random effect as block for each variable. Least squares means were adjusted for difference determination by Tukey's test. Additionally, linear and quadratic contrasts were performed using calculated coefficients to account for the uneven intervals of AZOMITE inclusions. Significance was considered at $P \leq 0.05$ with a trend tested at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Growth Performance

From 0 to 14 d (prechallenge), no differences in ADG ($P = 0.383$) and ADFI ($P = 0.264$) were observed among the treatments (Table 3). However, GF ratio was significantly higher in the 0.125% DTB compared to the 0.5% DTB and in all DTB treatments compared to both UC and CC ($P < 0.001$). A quadratic effect was found for GF, with its maximum value at 0.125% DTB ($P = 0.004$).

From 14 to 20 d (challenge phase), ADG ($P < 0.001$), FI ($P < 0.001$), and GF ($P < 0.001$) were better in the UC compared to all other treatments, and no differences were seen among the challenged groups. On average, the *Eimeria* challenge reduced ADG, ADFI, and GF by 52.5, 25.0, and 36.9%, respectively, compared to the UC.

During the recovery phase (20−26 d), ADG (trend, $P = 0.057$) and ADFI ($P < 0.001$) were higher in the UC than the other treatments, without differences among the challenged groups. GF ($P = 0.172$) was not different among the treatments. On average, the differences in ADG and ADFI between the UC and challenge groups were reduced to 11.1 and 15.3%, respectively, whereas GF was numerically increased by 5.8% in the challenge groups compared to the UC.

For the overall period (0−26 d), despite the partial recovery observed postchallenge, the ADG ($P < 0.001$) and ADFI ($P < 0.001$) were higher in the UC than the other treatments, without any differences within the challenged groups. An effect of DTB was observed, however, in GF ($P < 0.001$); the UC showed the highest GF value, and the 0.125 and 0.25% DTB had significantly better GF results than the CC ($P < 0.001$), with a quadratic effect ($P = 0.015$). Moreover, overall mortality was, as expected, higher in the challenged groups, and the total numbers of dead birds were 2, 9, 10, 10, and 6, for the UC, CC, 0.125, 0.25, and 0.5% DTB, respectively.

Previous studies with broilers have also demonstrated a potential of DTB in improving the growth performance from 0 to 21 d. Pirzado et al. (2021) have reported an increase in ADG and improvement in feed conversion ratio (**FCR**) when DTB was used at 0.25 or 0.5%. Similarly, Juzaitis-Boelter et al. (2021) observed an increase in BWG when 0.125% DTB was used, and an improvement in feed to gain ratio when 0.125, 0.25, and 0.5% DTB were used compared to the control without DTB.

Table 3. Average daily gain (ADG, kg), average daily feed intake (ADFI, kg), and gain:feed ratio (GF) of unchallenged control (UC, 0% DTB) and broilers fed 0, 0.125, 0.25, or 0.5% DTB challenged with *Eimeria* spp. from 0 to 14, 14−20, 20−26, and 0−26 d of age.

	Treatment						Contrasts		
Variables	Unchallenged control	Challenged control	0.125% DTB	0.25% DTB	0.5% DTB	*P* value	L[1]	Q	SE
0−14 d (prechallenge)									
ADG	0.030	0.030	0.031	0.031	0.031	0.383	0.431	0.201	0.0005
ADFI	0.040	0.040	0.039	0.039	0.040	0.264	0.698	0.366	0.0004
GF	0.755[c]	0.759[c]	0.794[a]	0.787[ab]	0.776[b]	<0.001	0.381	0.004	0.0071
14−20 d (challenge phase)									
ADG	0.070[a]	0.033[b]	0.034[b]	0.034[b]	0.032[b]	<0.001	0.562	0.435	0.0013
ADFI	0.102[a]	0.077[b]	0.077[b]	0.077[b]	0.075[b]	<0.001	0.158	0.606	0.0011
GF	0.686[a]	0.426[b]	0.439[b]	0.438[b]	0.428[b]	<0.001	0.945	0.394	0.0125
20−26 d (recovery phase)									
ADG	0.079[a]	0.071[b]	0.071[b]	0.068[b]	0.071[b]	0.057	0.964	0.473	0.0028
ADFI	0.124[a]	0.107[b]	0.105[b]	0.101[b]	0.107[b]	<0.001	0.974	0.179	0.0032
GF	0.632	0.660	0.681	0.668	0.665	0.172	0.951	0.536	0.0146
0−26 d (overall)									
ADG	0.047[a]	0.035[b]	0.036[b]	0.035[b]	0.035[b]	<0.001	0.897	0.635	0.0007
ADFI	0.067[a]	0.056[b]	0.055[b]	0.055[b]	0.056[b]	<0.001	0.781	0.273	0.0008
GF	0.699[a]	0.621[c]	0.644[b]	0.639[b]	0.632[bc]	<0.001	0.545	0.015	0.0059

[a,b,c]Means followed by superscript letters are different by Tukey's test ($P < 0.05$; $P < 0.10$) within the row.
[1]L, linear effect; Q, quadratic effect.
SE = pooled standard error.

Table 4. Means of fluorescein isothiocyanate dextran (FITC-d, μg/mL), superoxide dismutase (SOD, U/μg) and glutathione peroxidase (GPx, mmol/min/g) activity, reduced glutathione (GSH, mmole/g) and oxidized glutathione (GSSG, mmole/g) concentration and their ratio (GSH:GSSG), and the gene expression of claudin-1 (CLDN-1), occludin, and mucin-2 (Muc-2) of unchallenged control (0% DTB) and broilers fed 0, 0.125, 0.25, or 0.5% DTB challenged with *Eimeria* spp. at 20 or 26 d of age.

		Treatment						Contrasts		
Variables		Unchallenged control	Challenged control	0.125% DTB	0.25% DTB	0.5% DTB	*P* value	L[1]	Q	SE
FITC-d		−0.008[b]	0.241[a]	0.222[a]	0.269[a]	0.282[a]	<0.001	0.423	0.915	0.0424
Gene expression										
20 d (6 dpi)	CLDN-1	1.000	2.775	2.788	1.369	2.074	0.117	0.310	0.336	0.5768
	Occludin	1.000[a]	0.904[b]	0.807[c]	0.882[bc]	0.829[bc]	0.014	0.422	0.720	0.0399
	Muc-2	1.000[a]	0.669[b]	0.550[c]	0.588[bc]	0.610[bc]	<0.001	0.611	0.112	0.0398
26 d (12 dpi)	CLDN-1	1.000	1.298	1.228	1.010	0.869	0.101	0.017	0.791	0.1287
	Occludin	1.000	0.991	0.989	0.960	0.886	0.486	0.109	0.658	0.0525
	Muc-2	1.000[a]	0.884[b]	0.897[ab]	0.752[c]	0.724[c]	0.001	0.011	0.751	0.0512
Antioxidant system										
20 d (6 dpi)	SOD	5.570	5.748	5.502	5.595	5.495	0.742	0.373	0.635	0.1536
	GPx	0.027[c]	0.036[b]	0.042[a]	0.041[ab]	0.037[b]	<0.001	0.715	0.052	0.0022
	GSH	4.271[a]	3.241[b]	2.831[b]	2.836[b]	2.828[b]	<0.001	0.401	0.412	0.2723
	GSSG	0.290	0.514	0.521	0.343	0.277	0.234	0.060	0.895	0.1236
	GSH:GSSG	18.006	9.091	10.959	19.423	15.820	0.689	0.407	0.460	7.7108
26 d (12 dpi)	GSH	3.784[ab]	3.294[bc]	3.966[a]	3.145[c]	2.729[c]	0.005	0.021	0.177	0.2443
	GSSG	0.312	0.212	0.241	0.293	0.356	0.783	0.182	0.959	0.1184
	GSH:GSSG	29.662	40.963	65.709	17.668	7.723	0.110	0.068	0.699	23.3339

[a,b,c]Means followed by superscript letters are different by Tukey's test ($P < 0.05$; $P < 0.10$) within the row.
[1]L, linear effect; Q, quadratic effect.
SE = pooled standard error.

The pronounced decline in growth performance indicates that the *Eimeria* spp. challenge was effective. Moreover, the improvement in performance during the recovery phase compared to the challenge phase demonstrates that the challenge was punctual, as intended. No significant dosage effect of DTB within the challenged treatments might suggest that the challenge was either too severe for nutritional changes to express their beneficial effects or that the challenge impacted the birds' ability to absorb and utilize the elements provided by DTB.

Intestinal Permeability

On 5 dpi, the intestinal permeability result showed that the *Eimeria* challenge groups had significantly higher serum FITC-d than the UC group ($P < 0.001$); however, there were no significant differences among the challenge groups (Table 4). The expression of tight junction proteins (**TJP**), including claudin-1 and occludin, and mucin-2 was evaluated at 20 and 26 d (6 and 12 dpi, respectively; Table 4). On d 20, no differences were observed for claudin-1 ($P = 0.117$). Occludin and mucin-2 expression was the highest in the UC, whereas 0.125% DTB downregulated the expression of occludin ($P = 0.014$) and mucin-2 ($P < 0.001$) compared to the UC and CC. On d 26, no differences were observed for claudin-1 ($P = 0.101$) and occludin ($P = 0.486$) gene expression. Mucin-2 expression was the lowest in the 0.25 and 0.5% DTB, whereas the CC downregulated mucin-2 ($P = 0.001$) compared to the UC. However, there was no significant difference in mucin-2 between the UC and 0.125% DTB; the 0.125% DTB was similar to the UC. Linear effects were observed for claudin-1 ($P = 0.017$) and mucin-2 ($P = 0.011$) at 26 d, with increasing levels of DTB leading to the downregulation of these genes.

The gastrointestinal permeability is considered a measurable feature of the intestinal barrier function, and it can be affected by several factors, such as epithelial damage, and changes in gut microbiota and the mucus layer (Bischoff et al., 2014). These changes can result in the passage of luminal content to the inner layers of the intestinal wall, which is commonly known as "leaky gut." The quantification of FITC-d translocation, tight junction proteins, and mucus production are among the methodologies available to evaluate intestinal permeability (Bischoff et al., 2014; Ducatelle et al., 2018; Gilani et al., 2021; Liu et al., 2021).

In the present study, the increase in FITC-d concentration in the serum, as well as the overall downregulation of occludin and mucin-2 in the challenged groups, are indicators of increased intestinal permeability and loss of the intestinal barrier function integrity. These results were expected, considering that the life cycle of *Eimeria* spp. takes place in the intestinal cells, causing severe damage to the epithelium.

Several studies have shown that minerals such as zinc, copper, and manganese and REE can improve the gastrointestinal environment, promoting health (Bortoluzzi et al., 2020; Santos et al., 2020; Tariq et al., 2020; Broom et al., 2021). The inclusion of DTB, however, had no effect on the FITC-d levels and no or little effect on the expression of the TJP and mucin-2 compared to the CC. Additional studies are needed to evaluate the effect of DTB on the gastrointestinal barrier function.

Antioxidant System and Inflammation Markers

On d 20, no differences were observed for SOD activity ($P = 0.742$, Table 4). However, GPx ($P < 0.001$) was

Table 5. Means of villus height (VH, μm), crypt depth (CD, μm), and their ratio (V:C) in the duodenum, jejunum, and ileum, crude protein (CP, %), dry matter (DM, %), and ileal digestible energy (IDE, kcal/kg) digestibility, and expression of interferon-γ (INF-γ), interleukin-1β (IL-1β), interleukin-10 (Il-10), and tumor necrosis factor-α (TNF-α) from unchallenged control (0% DTB) and broilers fed 0, 0.125, 0.25, or 0.5% DTB challenged with *Eimeria* spp. at 20 d of age.

		Treatment						Contrasts		
Variables		Unchallenged control	Challenged control	0.125% DTB	0.25% DTB	0.5% DTB	*P* value	L[1]	Q	SE
Histomorphology										
Duodenum	VH	2465.728[a]	1527.026[b]	1559.152[b]	1610.108[b]	1536.510[b]	<0.001	0.924	0.354	67.0283
	CD	160.483[c]	260.635[a]	234.129[ab]	223.841[b]	231.482[ab]	<0.001	0.215	0.170	13.9715
	V:C	15.744[a]	6.065[b]	6.976[b]	7.414[b]	7.006[b]	<0.001	0.294	0.171	0.6842
Jejunum	VH	1382.489[a]	858.328[b]	940.929[b]	998.384[b]	902.630[b]	<0.001	0.717	0.123	67.1898
	CD	144.800	162.473	153.324	172.161	144.701	0.245	0.339	0.300	11.1056
	V:C	9.945[a]	5.401[b]	6.338[b]	5.983[b]	6.320[b]	<0.001	0.298	0.498	0.5701
Ileum	VH	788.78	714.538	716.428	752.263	750.636	0.412	0.353	0.747	30.4596
	CD	134.489	138.195	157.568	143.328	138.408	0.515	0.686	0.377	9.9483
	V:C	6.032	5.669	4.610	5.578	5.763	0.263	0.499	0.375	0.4649
Nutrient digestibility										
	CP	79.130[a]	46.508[c]	57.033[b]	52.490[bc]	47.809[c]	<0.001	0.762	0.097	3.4219
	DM	73.639[a]	54.382[b]	54.520[b]	52.436[b]	49.333[b]	<0.001	0.177	0.776	2.7292
	IDE	2998.034[a]	2139.777[b]	2131.854[b]	2117.801[b]	1894.851[b]	<0.001	0.129	0.489	110.880
Inflammation markers										
	INF-γ	1.000[b]	1.983[a]	1.962[a]	2.614[a]	1.981[a]	0.070	0.890	0.337	0.3773
	IL-1β	1.000[c]	1.291[bc]	1.902[a]	1.377[bc]	1.671[ab]	0.035	0.523	0.654	0.2059
	IL-10	1.000[b]	1.644[a]	1.915[a]	1.903[a]	1.874[a]	0.038	0.612	0.522	0.2325
	TNF-α	1.000	0.985	1.007	1.007	1.142	0.830	0.264	0.685	0.1061

[a,b,c]Means followed by superscript letters are different by Tukey's test ($P < 0.05$; $P < 0.10$) within the row.
[1]L, linear effect; Q, quadratic effect.
SE = pooled standard error.

higher in the 0.125% DTB than the CC and 0.5% DTB, and the UC had the lowest activity among all treatments. A quadratic trend was found, with the highest value at 0.125% DTB ($P = 0.052$).

Liver GSH, GSSG, and their ratio (GSH:GSSG) were evaluated at 20 and 26 d (Table 4). At 20 d, GSH concentration was significantly higher in the UC than the other treatments ($P < 0.001$), whereas no differences were observed for GSSG ($P = 0.234$) and GSH:GSSG ($P = 0.689$). A linear trend was seen for GSSG ($P = 0.060$), in which the increase in DTB levels led to a reduction in GSSG concentration. On d 26, GSH concentration ($P = 0.005$) was higher in the 0.125% DTB than the CC, and both UC and 0.125% DTB showed higher values than the 0.25 and 0.5% DTB. No differences were seen for GSSG ($P = 0.783$) and GSH:GSSG ($P = 0.110$). A significant linear effect was observed for GSH ($P = 0.021$), whereas a linear trend was seen for GSG: GSSG ($P = 0.068$).

The current study showed increased IL-10 and IFN-γ in the cecal tonsils of birds infected with *Eimeria* spp. (Table 5). The expression of both INF-γ (trend, $P = 0.070$) and IL-10 ($P = 0.038$) was upregulated in all challenged treatments compared to the UC (Table 5). Similarly, previous studies have reported that coccidia infection enhanced gene expression of IL-10 and IFN-γ (Yun et al., 2000; Haritova and Stanilova, 2012; Arendt et al., 2019; Bremner et al., 2021). Bremner et al. (2021) further suggested that the expression of IL-10 and IFN-γ is strongly correlated to parasite burden. In the current study, IL-1β ($P < 0.001$) was upregulated in the 0.125% DTB and 0.5% DTB compared to the UC, and the 0.125% DTB upregulated its expression compared to the CC and 0.25% DTB. However, there were no differences for the expression of TNF-α ($P = 0.830$). On the contrary, a previous study reported by White et al. (2022) did not observe differences in the expression of IL-10, IL-1β, IFN-γ, among the graded DTB levels and the control group, in the liver of broilers challenged with heat stress, suggesting that potential immune-modulation effects of DTB are different under the challenge conditions induced by environmental temperature and parasites.

Several stressors, including coccidiosis, have been reported to cause an imbalance between the intracellular amounts of enzymatic and nonenzymatic antioxidants and free radical production, leading to oxidative stress (Koinarski et al., 2005; Georgieva et al., 2006, 2011; Mishra and Jha, 2019). Although SOD activity was not affected in the current study, GPx activity was significantly increased in the *Eimeria*-infected birds, especially the ones fed 0.125% DTB. Likewise, the levels of GPx activity in broiler plasma increased more than 2.5-fold in an *Eimeria tenella* challenge (Bun et al., 2011). Unlike the current results, White et al. (2022) reported a linear reduction in GPx activity with increasing DTB levels in broilers raised under heat stress, with 0.25% DTB having lower activity level than the control (0% DTB).

GPx is an important component of the first and second lines of the cellular defense against free radicals, and it has been reported to be an important modulator of metabolic pathways, including cell signaling (Surai et al., 2018a,b). Its main function is to catalyze the reduction of hydroperoxides by GSH, which generates GSSG; therefore, the decrease in GSH concentration in the challenged treatments and the higher (quadratic) level of GSSG in the 0.125% DTB were observed with the higher levels of GPx during the challenge phase in the current study.

During the recovery phase, however, GSH levels were only significantly lower in the 0.25 and 0.5% DTB

compared to the 0% DTB (CC), and both GSH and GSH:GSSG decreased linearly with increasing levels of DTB. These results suggest a re-establishment of the balance between pro- and antioxidant molecules from some of the challenged groups compared to the unchallenged control. However, the lower GSH values obtained with higher dietary levels of DTB is not yet understood. It is possible that elements present in DTB could be acting, at some level, similarly to antioxidants, reducing the amount of GSH required to maintain homeostasis in a nonchallenged situation.

Intestinal Histomorphology and Apparent Ileal Digestibility

On d 20, the duodenum CD ($P < 0.001$) was higher in the CC than the 0.25% DTB, and the UC had the lowest value (Table 5). The VH ($P < 0.001$) and V:C ($P < 0.001$) were the highest for the UC, without differences among the challenged groups. In the jejunum, CD ($P = 0.245$) was similar among the treatments, and the UC had the highest VH ($P < 0.001$) and V:C ($P < 0.001$) among the treatments. No differences were observed for VH ($P = 0.412$), CD ($P = 0.515$), and V:C ($P = 0.263$) in the ileum.

Apparent ileal digestibility of CP ($P < 0.001$) was the highest in the UC, and the 0.125% DTB had higher values than the CC and 0.5% DTB (Table 5). DM digestibility ($P < 0.001$) and IDE ($P < 0.001$) were the highest for the UC, without any differences among the challenged groups. A quadratic trend was found for CP ($P = 0.097$), with the maximum value at 0.125% DTB.

It has been established that the intracellular cycle of *Eimeria* spp. can result in intestinal epithelial cell damage by its direct physical disruption and the induction of inflammation and oxidative stress (López-Osorio et al., 2020; Teng et al., 2020; El-Shall et al., 2022). Consequently, there is an increased loss of the epithelial cells that can be partially compensated by the increase in cell proliferation in the crypts, commonly leading to decreased VH, higher CD, and low V:C values (Ducatelle et al., 2018). Indeed, in the present study, VH and V:C in the duodenum and jejunum were significantly reduced, and CD in the duodenum was increased in all groups challenged with *Eimeria* spp. compared to the UC. Although not statistically significant, a similar numerical pattern was observed in the ileum. Additionally, differences within the challenged groups were only observed in the duodenum, with the 0.25% DTB showing significantly lower CD than the CC. Unlike the present study, White et al. (2022) observed an increase in CD in the ileum of heat stressed broilers when 0.25% DTB was used, compared to the control and 0.125% DTB.

In agreement with the histology results, significant reduction in IDE, AID of DM, and AID of CP were observed in challenged vs. unchallenged birds. As previously mentioned, due to the parasite's life cycle, it is possible that the destruction of villous cells, which are responsible for nutrient absorption, could reduce absorptive surface area (Teng et al., 2021). Moreover, a decrease in endogenous enzyme (Su et al., 2015) and nutrient transporters gene expression (Paris and Wong, 2013; Su et al., 2015; Teng et al., 2021) have been reported during coccidiosis, which might explain the current findings.

Among the challenged groups, the 0.125% DTB had a positive effect on AID of CP compared to the CC and 0.5% DTB. Improvement in AID of CP was reported in broilers fed 0.25% DTB in a low energy diet compared to the low energy control (Pirzado et al., 2020), and in a low protein diet, compared to both control and the low protein control (Pirzado et al., 2021). Additionally, Pirzado et al. (2021) found an increase in trypsin activity with 0.25% DTB (198.66 U/mg) compared to the low protein group (118.88 U/mg, significant) and the control (178.38 U/mg, numerical), which might explain the observed improvement in AID of CP. White et al. (2022) did not find differences in DM and apparent metabolizable energy corrected for nitrogen (**AMEn**), however, AID of CP linearly increased with DTB inclusion, with 0.5% DTB showing the highest value among the treatments.

Improvement in the digestibility of other nutrients has also been reported in poultry fed various levels of DTB. Higher DM, ME, Ca, and P digestibility was obtained in broilers fed 0.25% DTB compared to the low energy (Pirzado et al., 2020) and low protein controls (Pirzado et al., 2021). The AMEn, apparent nitrogen retention (**ANR**), and Ca and P digestibility were improved in laying hens fed 0.25% DTB than the control, at 85 wk. Moreover, apparent Ca and P digestibility was improved when 0.5% DTB was added in broiler diets for 21 d (Juzaitis-Boelter et al., 2021), and P digestibility when 0.125, 0.25, and 0.5% DTB were supplemented in broiler diets under heat stress for 42 d (White et al., 2022). It has been hypothesized that the unique element composition of DTB could contribute, directly, as cofactors for digestive enzymes, or indirectly, in improving enterocyte function and microbiota modulation. However, additional studies are needed to clarify how DTB could influence nutrient utilization and different metabolic pathways that are essential to broiler development and growth. In conclusion of the current study, the use of 0.125% DTB exhibited potential in improving antioxidant responses, AID of CP, and growth performance in broiler chickens.

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DISCLOSURES

There is no conflict of interest.

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