Comparative efficacy of tannin-free grain sorghum varieties for the control of necrotic enteritis caused by *Clostridium perfringens* in broiler chickens

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ABSTRACT A 28-day battery cage study was conducted to test the efficacy of tannin-free grain sorghum varieties fed to Cobb 500 male broiler chickens ($n = 512$) and challenged with *Eimeria maxima* (**EM**) and *Clostridium perfringens* (**CP**). Birds were fed 1 of 8 treatments (corn, red/bronze, white/tan, or U.S. No. 2 sorghum) and were grouped by challenge method (challenged with EM/CP or unchallenged). On d 14, birds in the challenge group were orally inoculated with ∼5,000 oocysts of EM, and on d 19, 20, and 21, birds were given a broth culture of CP with ∼10^8 CFU/mL once daily. On d 21, three birds were scored for the degree/presence of necrotic enteritis (**NE**) lesions. Birds and feed were group weighed (d 0, 14, 21, and 28) to calculate average feed intake (**FI**), body weight gain (**BWG**), and adjusted feed conversion ratio (**AdjFCR**). Intestinal integrity was assessed through histological analysis of intestinal tissues, and change in transcriptome was determined using mRNA-sequencing on intestinal mucosa. Relative concentrations of secondary metabolites in grain sorghum were determined by LC-MS/MS analysis. Data were analyzed as a 2-way ANOVA with factors of treatment, challenge and their interaction. Regardless of challenge from 14 to 21 d, birds on the corn, white/tan, and U.S. No. 2 treatments were more efficient than those fed red/bronze treatment ($P = 0.0026$). From 14 to 28 d, BWG was significantly higher for the white/tan treatment ($P = 0.024$) compared to the red/bronze treatment. At 21 d, a significant interaction was observed for lesion score ($P = 0.0001$) in which, challenged birds fed red/bronze and white/tan treatments had reduced intestinal lesions compared to U.S. No. 2 and corn treatments. No differences among treatments were observed in jejunum morphology, but differential expression analysis showed an upregulation in defense response to bacteria and biotic stress in the challenged red/bronze treatment compared to the challenged corn. This study demonstrated improved gut health and minimal impact on growth and efficiency of broilers fed select grain sorghum varieties when challenged with EM/CP.

Key words: tannin-free grain sorghum, broiler, intestinal health, necrotic enteritis, gene expression

2023 Poultry Science 102:102300
https://doi.org/10.1016/j.psj.2022.102300

INTRODUCTION

Increased feed costs in poultry production are a result of competition among human, animal and industrial users. The pressure on corn production will continue to increase as the demand to meet global food supplies increases. As a result, alternative feedstuffs are of interest to reduce the dependence on corn in poultry diets. Tannin-free grain sorghum for animal feed use is commercially available in the United States and is one alternative feed ingredient due to its nutritional equivalency to corn. In addition, some dietary ingredients may have beneficial secondary metabolites that can have a direct impact on digestion, absorption, and utilization of nutrients in the gastrointestinal tract. It has been demonstrated that a healthy intestinal tract is important for efficient digestion, nutrient absorption, and consequently, optimal performance (Choct, 2009). While limited data are available to support the use of tannin-free varieties of grain sorghum as an alternative feedstuff to corn in broiler diets, there is also limited data surrounding its potential as a functional feedstuff and its efficacy for controlling intestinal diseases in broilers.

Received June 2, 2022.
Accepted October 15, 2022.

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The industry constantly integrates ways to reduce the impact on performance of 2 of the most detrimental intestinal diseases, coccidiosis and necrotic enteritis (**NE**) (Shields et al., 2021). Coccidiosis is among the most ubiquitous enteric disease in commercial poultry production. In fact, coccidiosis is known as the costliest disease to the global poultry industry accounting for 80% of the worldwide cost for disease treatments (Tonda et al., 2018), whereas NE costs the global poultry industry approximately $6 billion annually (Wade et al., 2015). The combination of predisposing factors (e.g., immunosuppression, stress, and dietary factors) due to coccidiosis can lead to NE infection (Paiva and McElroy, 2014; Adhikari et al., 2020). Coccidiosis and NE negatively affect weight gain, feed efficiency, and mortality (Timbermont et al., 2011). Several species of *Eimeria*, protozoan parasites, cause coccidiosis and damage the intestinal epithelium (Vermeulen et al., 2001). Common coccidiosis measures include the use of chemical anticoccidials and ionophore-type feed additives (Oviedo-Rondón, 2019).

However, the industry has transitioned to reducing or eliminating the use of in-feed antibiotics due to increasing concern of antibiotic resistance and residues in poultry products. This rise in the removal of in-feed antibiotics, has led the industry to seek novel ways to mitigate intestinal disruptions while maintaining an optimally functional intestinal tract (Oviedo-Rondón, 2019). Vaccination for coccidiosis and feed additives including plant extracts, organic acids, and probiotics are a few alternative methods used to combat coccidiosis in no antibiotic ever (**NAE**) or antibiotic-free (**ABF**) production systems (Tonda et al., 2018).

Previous studies have shown the role of grain sorghum as a functional feedstuff due to its abundance of secondary metabolites. Some of the compounds found include flavonoids and proanthocyanidins associated with antimicrobial and antioxidative functions (Shen et al., 2018; Ashley et al., 2019). These dietary polyphenols that are most recognized for their antioxidant, anti-inflammatory, and immunomodulatory functions have shown the ability to improve health and productivity (Abdel-Moneim et al., 2020). The mechanism of action of these compounds on the intestinal health of broilers fed tannin-free varieties of grain sorghum is of interest. Polyphenols can protect the host from pathogens by modulating cell signaling pathways, and studies have shown the inhibitory effects of polyphenolic compounds on *Escherichia coli* and *Salmonella enteritidis* (Al-Zoreky, 2009).

The overall aim of this study was to evaluate the efficacy of tannin-free grain sorghum varieties to control NE caused by *Clostridium perfringens* in broiler chickens. Specific objectives directed at identifying the role of grain sorghum as a functional feedstuff included: 1) quantifying the relative concentration of select secondary metabolites in tannin-free grain sorghum; 2) evaluating the effects of grain sorghum on intestinal morphology; and 3) assessing the impact of grain sorghum on gene expression in the intestinal mucosa of broilers challenged with *Eimeria maxima* (**EM**) and *Clostridium perfringens* (**CP**).

MATERIALS AND METHODS

This experiment was conducted in accordance with specific guidelines approved by the interinstitutional agreement with Clemson University—Institutional Animal Care and Use Committee (IACUC) and Southern Poultry Research and Feed, Inc., Animal Use Protocol (AUP) #Clemson NE battery 1220.

Grains

Three tannin-free, animal feed-grade varieties of grain sorghum; red/bronze, U.S. No. 2, and white/tan were sourced from U.S. origin grain sorghums. The red/bronze was sourced from South Carolina, white/tan was sourced from Texas, and the state of origin for the U.S. No. 2 commercial grade sorghum was not determinable. Red/bronze and white/tan grain sorghum varieties were identity-preserved (contained a single variety), whereas U.S. No. 2 was a red/bronze-based variety that may have contained other mixed grain sorghum varieties. The nutrient analyses for each variety compared to corn are shown in Table 1. Red/bronze grain sorghum, commonly known to be a high tannin-type sorghum variety, was tested to ensure zero tannin content using the Adams-Harbertson assay (Harbertson et al., 2003), which indicated no tannins were detected for the red/bronze grain sorghum.

Birds and Husbandry

A 28-day battery cage study was conducted with 512-day-old male byproducts from the female line for Cobb 500 broilers. Birds were housed in an environmentally controlled, insulated room with concrete floor.

Table 1. Nutrient and energy analyses of sources of corn and tannin-free varieties of grain sorghum (red/bronze, white/tan, U. S. No. 2).

		Grain sorghum variety[1]		
	Corn[1]	Red/bronze	White/tan	U.S. No. 2
		%		
Dry matter	88.34	87.70	89.44	84.44
Ash	1.04	1.49	1.49	1.39
Crude fat	3.52	1.97	1.93	2.93
Crude fiber	2.30	1.80	1.60	2.20
Crude protein	7.00	10.25	9.57	8.65
Methionine	0.17	0.24	0.23	0.14
Lysine	0.25	0.27	0.25	0.23
Threonine	0.27	0.34	0.30	0.28
GE, kcal/kg	3,861	3,889	3,838	3,860
ME, kcal/kg[2]	3,384	3,441	3,139	3,157

[1]Proximate analysis was determined using the AOAC (Association of Official Analytical Chemists) method (Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO).

[2]Calculated ME values for grain sorghum varieties were used.

Continuous light was provided throughout the experiment and all birds had ad libitum access to mash feed and water. Birds were grouped, weighed by cage (8 birds per cage, 8 cages per treatment; 8 treatments) and randomly distributed in battery cages (Petersime nv, Zulte, Belgium). Groups of 4 cages were considered blocks for use in later analysis.

Treatments

Each whole grain sorghum was ground through a hammer mill (Premier 1 Supplies, Washington, IA) with a 4-mm sieve. Starter and grower diets were formulated based on a commercial industry standard (Cobb 500, 2018). Experimental treatments were prepared with each respective test grain of corn or grain sorghum. Birds were fed 1 of 8 treatments comprised of the grain types corn, red/bronze sorghum, white/tan sorghum or U.S. No. 2 sorghum according to a phase-feeding program: starter (0−14 d of age; Table 2), and grower (15−28 d of age; Table 3). Treatment groups were either unchallenged (T1, T2, T3, T4; Tables 2 and 3) or challenged with EM at 14 d of age and CP at 19, 20, and 21 d of age (T5, T6, T7, T8; Tables 2 and 3).

Performance

Birds and feed were weighed by cage on d 0, 14, 21, and 28 to calculate average feed intake (**FI**), body weight gain (**BWG**), and adjusted feed conversion ratio (**AdjFCR**). Mortality was recorded daily, and FI and BWG were adjusted for mortality when calculating AdjFCR (Eq. (1)).

$$\text{AdjFCR} = \frac{\text{Total Feed Intake}}{\text{BWG} + \text{mortality BW}} \quad (1)$$

Disease Induction and Lesion Scoring

At 14 d of age, birds in the challenge group (T5, T6, T7, T8) were orally inoculated with ~5,000 oocysts of EM. On d 19, 20, and 21, challenge group birds were orally given a broth culture of CP with ~10^8 cfu/mL once daily. On d 21, three birds from each treatment of the challenge group were randomly selected, euthanized and examined for the presence and degree of NE lesions. Lesion scoring was based on a score of 0 to 3 (0: normal, no necrotic lesions; 1: slight mucus covering small intestine; 2: necrotic small intestinal mucosa; 3: sloughed and bloody small intestinal mucosa and contents per Hofacre et al., 2008).

Relative Concentration of Tannin-Free Grain Sorghum Metabolites and Analysis

An untargeted metabolomic analysis using LC-MS/MS on an Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) identified metabolites found in the tannin-free grain sorghum varieties of this study. Based on the results from this untargeted analysis, 24 metabolites (including isomers) were selected that may be relevant to the effects of grain sorghum in broiler diets. A targeted analysis using LC-MS/MS (Orbitrap Fusion Mass Spectrometer; Thermo Fisher Scientific, Waltham, MA) of the 24 select metabolites determined the relative concentration of each metabolite

Table 2. Ingredient composition and calculated/analyzed nutrient composition of starter diet with respective test ingredient: corn or red/bronze, white/tan, U.S. No. 2 grain sorghum (as-fed) from 1 to 14 d of age.

	Starter dietary treatments[1]			
Ingredients	C T1 & T5	RB T2 & T6	WT T3 & T7	No. 2 T4 & T8
		%		
Corn	50.88	0.00	0.00	0.00
Red/bronze grain sorghum	0.00	56.00	0.00	0.00
White/tan grain sorghum	0.00	0.00	53.87	0.00
U.S. No. 2 grain sorghum	0.00	0.00	0.00	55.14
Soybean meal, 47.5% CP	42.22	37.78	40.82	39.67
Fat, vegetable	4.49	1.00	2.75	2.54
Monodicalcium phosphate	0.79	0.77	0.75	0.74
Limestone	0.36	0.49	0.42	0.46
Sodium chloride	0.53	0.53	0.53	0.53
DL-methionine	0.25	0.31	0.31	0.31
L-threonine	0.01	0.03	0.01	0.02
L-lysine	0.02	0.18	0.08	0.12
Choline chloride, 60%	0.15	0.16	0.15	0.15
Vitamin premix[2]	0.25	0.25	0.25	0.25
Trace minerals[3]	0.08	0.08	0.08	0.08
Phytase[4]	0.01	0.01	0.01	0.01
Filler[5]	0.00	2.43	0.00	0.00
	100.00	100.00	100.00	100.00
Calculated composition		%		
ME, kcal/kg	3,031	3,031	3,031	3,031
Crude protein	22.62	23.31	23.78	23.43
Crude fat	6.57	2.90	4.43	4.38
Calcium	0.90	0.90	0.90	0.90
Available phosphorus	0.43	0.43	0.43	0.43
Sodium	0.21	0.21	0.21	0.21
Dig. lysine	1.24	1.24	1.24	1.24
Dig. methionine	0.57	0.62	0.62	0.62
Dig. threonine	0.83	0.83	0.83	0.83
Dig. methionine + cysteine (SAA)	0.88	0.93	0.93	0.93
Analyzed composition[6]		%		
Crude protein	22.87	20.75	24.36	23.95
Crude fat	6.15	2.54	4.11	3.94
Lysine	1.33	1.20	1.23	1.47
Methionine	0.56	0.61	0.54	0.61
Threonine	0.85	0.76	0.81	1.01
Methionine + cysteine (SAA)	0.90	0.92	0.88	0.99

[1]Treatment abbreviations: C: corn; RB: red/bronze; WT: white/tan; No. 2: U.S. No. 2. Unchallenged treatments: T1, T2, T3, T4. Challenged treatments: T5, T6, T7, T8 ($n = 8$ birds/cage/treatment).

[2]Supplied per kg of diet: thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin), 12.0 μg; 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 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[3]Supplied 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 diamine dihydroiodide), 0.15 mg; selenium ($NaSe0_3$), 0.3 mg.

[4]Quantum Blue phytase (AB Vista, Marlborough, Wiltshire).

[5]Solka-Floc®40 (Solvaira Specialty LP, Urbana, OH) and sand.

[6]Proximate analysis was determined using the AOAC (Association of Official Analytical Chemists) method (University of Missouri Agricultural Experiment Station, Columbia, MO).

to an internal standard (13C6 resveratrol) in grain sorghum varieties: red/bronze, white/tan, and U.S. No. 2. Grain sorghum samples used in the targeted analysis were pooled samples per grain type; therefore, descriptive statistics including the average and standard deviation of the data were used for statistical analysis.

Intestinal Mucosa Preparation and Gene Expression—mRNA-Sequencing Analysis

Group bodyweights were collected at 28 d of age, 1 bird from 4 of the 8 cages/treatment was randomly selected, weighed, and euthanized to collect intestinal mucosa for gene expression analysis. Following euthanasia for the intestinal mucosa sampling, a 10-cm section of the jejunum (anterior to Meckel's diverticulum) was removed, rinsed with ice-cold phosphate-buffered saline, and cut open to expose the mucosal layer. With an RNAse-free slide, the mucosal layer was scraped into a 2-mL tube of 1.5 mL of RNAlater solution, stored at 4°C for 24 h, and transferred to −20°C until total RNA extraction (Chen et al., 2015). Total RNA was extracted using a standard TRIzol method (Rokyta et al., 2012). Library preparation was completed using a NEBNext Ultra II RNA Library Prep Kit (New England Biolabs, Ipswich, MA), and the libraries were sequenced on an Illumina NovaSeq 6000 (Illumina, San Diego, CA). Quality metrics of the raw data were assessed with FastQC (0.11.9) (Andrews, 2010) and summarized using MultiQC (1.11) (Ewels et al., 2016). Quality trimming and adapter removal were performed using TrimGalore (0.6.5) (Krueger, 2015). Due to variation in sequencing depth between samples, downsampling with seqtk (1.3-r106) (Li, 2012) was done for samples with an extremely large number of reads. The reads were aligned to the *Gallus gallus* GRCg6a reference genome using HISAT2 (2.2.1) (Kim et al., 2019). Read counts from genomic features were obtained using Subread featureCounts (2.0.1) [with -P -B -C flags] (Liao et al., 2014). Library normalization and identification of differentially expressed genes across different conditions were performed using edgeR (3.30.3) (Robinson et al., 2010) quasi-likelihood pipeline, with only samples with >5 million read pairs included for analysis. Genes were considered differentially expressed (**DEG**) if the false discovery rate (**FDR**) <0.05 and $|\log_2$Fold Change (**FC**)| >1. ClusterProfiler (3.16.1) (Yu et al., 2012) was used to perform gene set enrichment analysis (**GSEA**) using gene ontology (**GO**). Overrepresentation analysis for enriched Reactome pathways was performed using g:Profiler web server (Raudvere et al., 2019).

Table 3. Ingredient composition and calculated/analyzed nutrient composition of grower diet with respective test ingredient: corn or red/bronze, white/tan, U.S. No. 2 grain sorghum (as-fed) from 15 to 28 d of age.

	Grower dietary treatments[1]			
Ingredients	C T1 & T5	RB T2 & T6	WT T3 & T7	No. 2 T4 & T8
		%		
Corn	59.34	0.00	0.00	0.00
Red/bronze grain sorghum	0.00	65.96	0.00	0.00
White/tan grain sorghum	0.00	0.00	62.96	0.00
U.S. No. 2 grain sorghum	0.00	0.00	0.00	64.45
Soybean meal, 47.5% CP	32.96	27.58	31.30	29.95
Fat, vegetable	5.10	0.85	3.04	2.80
Monodicalcium phosphate	0.73	0.70	0.68	0.66
Limestone	0.50	0.65	0.57	0.61
Sodium chloride	0.56	0.56	0.56	0.56
DL-methionine	0.25	0.25	0.26	0.26
L-threonine	0.02	0.05	0.01	0.03
L-lysine	0.07	0.26	0.14	0.18
Choline chloride, 60%	0.15	0.17	0.15	0.16
Vitamin premix[2]	0.25	0.25	0.25	0.25
Trace minerals[3]	0.08	0.08	0.08	0.08
Phytase[4]	0.01	0.01	0.01	0.01
Filler[5]	0.00	2.63	0.00	0.00
	100.00	100.00	100.00	100.00
Calculated composition		%		
ME (kcal/kg)	3,086	3,086	3,086	3,086
Crude protein	19.04	19.79	20.35	19.94
Crude fat	7.35	2.89	4.83	4.77
Calcium	0.85	0.85	0.85	0.85
Available phosphorus	0.40	0.40	0.40	0.40
Sodium	0.22	0.22	0.22	0.22
Dig. lysine	1.05	1.05	1.05	1.05
Dig. methionine	0.53	0.53	0.53	0.53
Dig. threonine	0.70	0.70	0.70	0.70
Dig. methionine + cysteine (SAA)	0.80	0.80	0.80	0.80
Analyzed composition[6]		%		
Crude protein	18.68	20.36	20.70	19.20
Crude fat	7.01	2.16	4.63	4.33
Lysine	1.13	1.21	1.20	1.14
Methionine	0.47	0.56	0.47	0.53
Threonine	0.70	0.78	0.78	0.73
Methionine + cysteine (SAA)	0.76	0.89	0.79	0.83

[1]Treatment abbreviations: C: corn; RB: red/bronze; WT: white/tan; No. 2: U.S. No. 2. Unchallenged treatments: T1, T2, T3, T4. Challenged treatments: T5, T6, T7, T8; ($n = 8$ birds/cage/treatment).

[2]Supplied per kg of diet: thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin),12.0 μg; 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 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[3]Supplied 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 diamine dihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

[4]Quantum Blue phytase (AB Vista, Marlborough, Wiltshire, UK).

[5]Solka-Floc®40 (Solvaira Specialty LP, Urbana, OH) and sand.

[6]Proximate analysis was determined using the AOAC (Association of Official Analytical Chemists) method (University of Missouri Agricultural Experiment Station, Columbia, MO).

Intestinal Histomorphology and Analysis

After collecting intestinal mucosa for gene sequencing, intestinal tissue samples were collected for histomorphometric measurements. One-centimeter sections ($n = 3$) of the duodenum (distal to the duodenal loop), jejunum (anterior to Meckel's diverticulum), and ileum (anterior to the cecal junction) were removed from each bird, opened longitudinally, rinsed, and fixed with 10% neutralizing buffer formalin for 24 h and transferred to 70% ethanol until processed and embedded in paraffin. Five 10-μm sections of the duodenum, jejunum, and ileum were microtomed (Leica Biosystems, Deer Park, IL), placed on a glass slide and stained with hematoxylin/

eosin (Biloni et al., 2013). Villi height (top of villi to top of submucosa), villi width (middle of each villi), crypt depth (region of transition between crypt and villi), and crypt:villi ratio (ratio of crypt depth to villi height) (Chen et al., 2015) were measured from 10 random villi per sample under 4× magnification with a Zeiss microscope (Carl Zeiss, Heidelberg, Germany) using Lumenera Infinity 2 software (Ottawa, Ontario, CA). The average of 10 replicate measurements per sample/treatment was used for statistical analyses.

Statistical Analysis

Data were analyzed as a 2 × 4 factorial treatment design with factors of challenge and grain types (corn, red/bronze sorghum, white/tan sorghum, or U.S. No. 2 sorghum) defining the treatments and their interaction. Each cage was used as the experimental unit and randomly blocked as groups of 4 cages in the analysis. Two-way analysis of variance (**ANOVA**) followed by Fisher's least significant difference procedure was used to determine if differences existed among the treatment means. All statistical calculations were performed using JMP Pro version 16 (SAS Institute, 2021). Statistical significance was based on a P value <0.05.

RESULTS

Performance and Mortality

Performance was measured from 0 to 14 d (Table 4) before treatments were challenged. During this time, BW, BWG, FI, and AdjFCR were not significantly affected by corn or grain sorghum treatments. When birds were challenged at 14 d, there were no significant differences observed in BW across all treatments (Table 4).

Performance of birds during challenge at 14 to 21 d and 14 to 28 d are shown in Table 5. Regardless of challenge from 14 to 21 d, birds in the corn, white/tan, and U.S. No. 2 treatments were more efficient than those fed the red/bronze treatment ($P = 0.0026$). From 14 to 28 d, which includes the post challenge recovery period (21−28 d), BWG was significantly higher for the white/tan treatment ($P = 0.024$; 0.570 kg) compared to the red/bronze treatment (0.451 kg), whereas corn and U.S. No. 2 treatments were intermediate. At 28 d, AdjFCR was significantly better ($P = 0.026$) in white/tan treatment compared to red/bronze and U.S. No. 2 treatments. U.S. No. 2 treatment had an intermediate AdjFCR to corn and red/bronze treatments.

As expected, mortality was significantly higher in birds challenged with EM/CP from 14 to 28 ($P = 0.0049$), and no significant differences were observed due to the effect of treatment. While mortality from 14 to 28 d was numerically lower in the red/bronze (2.34%), no significant difference was detected for this variable ($P > 0.05$).

NE Lesion Scoring and NE Mortality

At 21 d, a significant interaction was observed for lesion score ($P = 0.0001$; Table 5) in which, challenged birds fed the red/bronze and white/tan treatments had reduced intestinal lesions compared to U.S. No. 2 and corn treatments. Mortality due to NE ($P = 0.0075$; Table 5) was significant in the challenge group as expected. Although no significant differences were observed in NE mortality for main effect of treatment, red/bronze had no mortality (0.00%) and U.S. No. 2 (1.56%) was numerically lower when compared to corn (3.13%) and white/tan (3.91%) treatments.

Table 4. Performance of broiler chickens from 0 to 14 d of age before challenge fed 1 of 8 treatments (corn, red/bronze, white/tan, or U.S. No. 2 grain sorghum).

			Variable				
Grain[1]	Trt[2]	Challenge[2]	BW[3]	BW[3]	BWG[3]	FI[3]	AdjFCR[3]
			Age (d)				
			0	14	0−14		
			kg/bird				kg:kg
C	T1	−	0.046[a]	0.176	0.130	0.284	1.61
RB	T2	−	0.045[a,b,c]	0.178	0.133	0.296	1.69
WT	T3	−	0.046[a,b]	0.198	0.152	0.327	1.68
No. 2	T4	−	0.044[c]	0.192	0.147	0.316	1.66
C	T5	EM/CP	0.044[c]	0.186	0.142	0.293	1.61
RB	T6	EM/CP	0.045[a,b,c]	0.188	0.143	0.318	1.69
WT	T7	EM/CP	0.046[a,b]	0.182	0.137	0.302	1.66
No. 2	T8	EM/CP	0.045[b,c]	0.194	0.149	0.321	1.66
		SEM[4]	0.003	0.009	0.009	0.014	0.074
		Main Effect of Trt[5]	P value				
			0.0305	0.6649	0.6314	0.3343	0.9858

[1]Treatment abbreviations: C: corn; RB: red/bronze; WT: white/tan; No. 2 = U.S. No. 2.
[2]Trt (Treatment): Unchallenged treatments (−) = T1, T2, T3, T4. Challenged treatments (EM/CP) = *E. maxima* (EM) with *C. perfringens* (CP) = T5, T6, T7, T8; ($n = 8$ birds/cage/treatment).
[3]Measurement abbreviations: BW: body weight; BWG: body weight gain; FI: feed intake; AdjFCR: feed conversion ratio, adjusted for mortality.
[4]SEM for $n = 8$.
[5,a,b,c]Means within the same column lacking a common superscript are significantly different at $P < 0.05$.

Table 5. Performance, lesion scores, and mortality of broiler chickens fed 1 of 8 treatments (corn, red/bronze, white/tan, or U.S. No. 2 grain sorghum) challenged with *E. maxima* (14 d; EM) and *C. perfringens* (19, 20, and 21 d; CP).

			Age (d)									
			14–21		Variable (kg)	14–28			21	21	14–28
Grain[1]	Trt[1]	Challenge[2]	BWG[3]	FI[3]	AdjFCR[3]	BWG[3]	FI[3]	AdjFCR[3]	Lesion score	NE[3] Mortality, %	Mortality, %
C	T1	–	0.251	0.396	1.59	0.671	1.26	1.56	0.00[d]	0.00	1.56
RB	T2	–	0.196	0.389	2.17	0.554	1.19	1.85	0.00[d]	0.00	3.13
WT	T3	–	0.235	0.402	1.72	0.713	1.31	1.61	0.00[d]	0.00	1.56
No. 2	T4	–	0.241	0.416	1.75	0.691	1.32	1.63	0.00[d]	0.00	1.56
C	T5	EM/CP	0.178	0.346	1.93	0.376	1.16	2.02	1.21[a]	6.25	9.38
RB	T6	EM/CP	0.160	0.359	2.38	0.348	1.02	2.07	0.71[c]	0.00	1.56
WT	T7	EM/CP	0.175	0.355	2.09	0.426	1.18	1.84	0.63[c]	7.81	10.93
No. 2	T8	EM/CP	0.160	0.354	2.26	0.374	1.13	2.18	0.96[b]	3.12	7.81
SEM[4]			0.016	0.018	0.131	0.042	0.069	0.085	0.064	2.16	2.591
Main effect of Trt[1]											
C			0.215	0.371	1.77[b]	0.523[a,b]	1.21	1.79[b,c]	0.60[a]	3.13	5.47
RB			0.178	0.374	2.28[a]	0.451[b]	1.10	1.96[a]	0.35[b,c]	0.00	2.34
WT			0.205	0.377	1.91[b]	0.570[a]	1.24	1.72[c]	0.31[c]	3.91	6.25
No. 2			0.200	0.385	2.01[b]	0.532[a]	1.22	1.91[a,b]	0.48[a,b]	1.56	4.69
Main effect of challenge[2]											
No EM/CP			0.230	0.401	1.82	0.657	1.26	1.66	0.00	0.00	1.95
EM/CP			0.168	0.353	2.17	0.381	1.12	2.03	4.30	4.30	7.42
Source of Variation								*P* value			
Trt			0.1343	0.8665	0.0026	0.0243	0.2035	0.0258	0.0001	0.2963	0.4846
Challenge			< 0.0001	0.0003	0.0004	< 0.0001	0.0054	< 0.0001	< 0.0001	0.0075	0.0049
Trt x challenge			0.5071	0.8361	0.7354	0.4918	0.9365	0.1334	0.0001	0.2963	0.1759

[1]Grain abbreviations: C: corn; RB: red/bronze; WT: white/tan; No. 2 = U.S. No. 2; Treatments (Trt) Unchallenged treatments (−): T1, T2, T3, T4. Challenged treatments (EM/CP): T5, T6, T7, T8.

[2]Challenge: EM/CP = *E. maxima* (EM) with *C. perfringens* (CP).

[3]Measurement abbreviations: BWG: body weight gain (kg/bird); FI: feed intake (kg/bird); FCR: feed conversion ratio, adjusted for mortality; NE: necrotic enteritis.

[4]SEM for $n = 8$; for lesion score (3 birds/cage).

[a-d]Means within the same column lacking a common superscript are significantly different at $P < 0.05$.

Table 6. Relative concentration of metabolites to internal standard[1] in tannin-free grain sorghum varieties (red/bronze, white/tan, and U.S. No. 2).

					Grain sorghum variety					
					RB	Std dev[6]	WT	Std dev[6]	No. 2	Std dev[6]
Metabolite[2]	Classification[3]	Function[4]	MW[5]	RT[5]	ug/g					
Hydroxycoumarin(s)	Phenolic acid	Anti-inflammatory; anticancer; antioxidative; antiobesity; antidiabetic	162.03	6.92	22.29	4.81	13.17	13.64	33.99	7.22
4-Coumaric acid	Phenolic acid		164.05	7.77	2.78	0.34	3.11	1.47	6.21	1.03
Caffeic acid(s)	Phenolic acid		180.04	6.89	23.87	11.17	17.00	16.47	37.74	9.36
Ferulic acid	Phenolic acid		194.06	7.93	4.72	0.59	7.54	3.72	9.72	1.14
Apigeninidin	Flavonoid	Antioxidative; anti-inflammatory	254.06	7.20	44.80	8.64	0.48	0.59	23.12	6.72
Formononetin	Flavonoid		268.07	7.47	50.33	8.78	0.39	0.31	16.65	8.09
Luteolinidin	Flavonoid		270.05	6.93	11.09	1.67	0.71	0.49	43.55	9.86
Apigenin	Flavonoid		270.05	9.17	35.41	14.36	33.82	20.75	22.90	5.06
Naringenin(s)	Flavonoid		272.07	8.30	5.18	1.29	2.50	1.06	6.16	1.83
Naringenin-arabinosylglucoside	Flavonoid		566.16	8.02	1.93	2.98	0.05	0.03	2.32	1.43
Kaempferol	Flavonoid		286.05	7.08	0.24	0.09	0.24	0.12	0.73	0.13
Luteolin	Flavonoid		286.05	8.67	36.26	14.85	232.89	107.48	58.10	15.22
Formononetin_glucopyranoside	Flavonoid		430.13	6.61	1.64	0.35	0.05	0.03	0.58	0.14
3,5,7,2′,6′-Pentahydroxyflavanone	Flavonoid		304.06	8.10	5.80	2.97	56.52	27.27	5.33	3.27
Methoxybiochanin A	Flavonoid		314.08	10.67	3.26	1.72	15.51	7.49	1.24	0.55
Isorhamnetin(s)	Flavonoid		316.06	8.52	0.25	0.15	4.88	1.93	0.65	0.19
Esculin	Flavonoid		340.08	6.47	0.07	0.01	0.09	0.03	0.15	0.03
Daidzin	Flavonoid		416.11	7.09	1.17	0.96	0.64	0.54	0.19	0.14
Dinitrocarbanilide	Nicarbazin related	Anticoccidial	302.07	10.80	0.17	0.15	0.20	0.19	0.07	0.04

[1]Data analyzed for relative quantification of metabolites to internal standard (IS) (13C6 resveratrol; concentration = 2.5 ug/g grain tissue). Samples were extracted and analyzed using LC-MS/MS by Clemson University-MUAL. To determine the relative concentration of metabolites to the IS, the following equation was used:Relative concentration (13C6 resveratrol equivalents; $\frac{\text{ug}}{\text{g grain}}$) = $\frac{\text{Peak Area}_{\text{metabolite}}}{\text{Peak Area}_{13C6\ \text{resveratrol}}} \times \left(\frac{0.5\ \mu\text{g 13C6 resveratrol}}{1\ \text{mL extract}}\right) \times \frac{1\ \text{ml extract}}{0.2\ \text{g grain}}$

[2]Average metabolite (including isomers) (ug/g) for 5 replicates per grain sorghum variety.

[3]Classification references: Phenolic acid: Hahn et al., 1983; Khoddami et al., 2015; Duodu and Awika, 2019; Flavonoid: Duodu and Awika, 2019; Rao et al., 2018; Khoddami et al., 2015; Nicarbazin related: Long et al., 1988; Bacila et al., 2018.

[4]Function references: Phenolic acid and flavonoid: Duodu and Awika, 2019; Nicarbazin related: Bacila et al., 2018.

[5]MW: molecular weight; RT: run time (minutes).

[6]Std dev: standard deviation.

Table 7. Subset of differentially expressed genes (DEGs) in the jejunum of broilers at 28 d of age fed red/bronze treatment (RB T6) compared to corn treatment (C T5) challenged with *E. maxima* (14 d; EM) and *C. perfringens* (19, 20, and 21 d; CP). See supplementary data for all DEGs.

Ensembl_ID	Gene symbol	Protein name	Log_2FC[1]	LogCPM[2]	FDR[1]
ENSGALG00000016761	*LYG2*	Lysozyme g	6.088	8.695	0.003
ENSGALG00000025945	*AVD*	Avidin	6.939	8.808	0.013
ENSGALG00000043064	*EXFABP*	Extracellular fatty acid binding protein	7.675	3.177	0.013
ENSGALG00000038096	*NOS2*	Nitric oxide synthase	6.302	6.569	0.013

[1]Log_2FC: log fold change; FDR: false discovery rate. Genes were considered differentially expressed (DEG) if the FDR < 0.05 and $|log_2FC| > 1$ using the Quasi-likelihood F test (QLF).
[2]LogCPM = average log counts per million across samples.

Relative Concentration of Tannin-Free Grain Sorghum Metabolites

The relative quantity of 19 target metabolites (including isomers) to the internal standard, 13C6 resveratrol are shown in Table 6 for each grain sorghum variety. The greatest relative quantities of flavonoid and phenolic compounds in red/bronze and U.S. No. 2 grain sorghum were hydroxycoumarin, caffeic acid, apigenindin, formononetin, and luteolinidin compared to the white/tan variety. However, the relative quantities for pentahydroxyflavanone and methoxybiochanin A were higher in white/tan grain sorghum compared to the other varieties.

Intestinal Gene Expression

mRNA-sequencing revealed 152 DEGs (supplementary data table), 123 upregulated and 29 downregulated, in challenged red/bronze when compared to challenged corn. A subset of DEGs associated with defense responses to bacteria are shown in Table 7. GSEA using GO showed an upregulation in defense response to biotic stress in challenged red/bronze when compared to challenged corn (Figure 1). The reactome pathway associated with metal sequestration by antimicrobial proteins was over-represented among genes upregulated under the red/bronze treatment (R-GGA-6799990; Padj = 9.537×10^{-4}).

Intestinal Histomorphology

No differences among treatments were observed in jejunum morphology ($P > 0.05$) with villi height, villi width, crypt depth, and crypt:villi ratio. Representative images of the jejunum for each treatment are shown in Figure 2.

DISCUSSION

A previous study conducted by Moritz et al. (2021, unpublished), evaluated the effect of tannin-free grain sorghum as full-substitution for corn on the growth performance, carcass traits, and intestinal histomorphology and gene expression of broilers in floor pens under no challenge to 42 d. Although performance was evaluated in the present study, the primary objective of this study was to test the efficacy of tannin-free grain sorghum in broilers under coccidia with *C. perfringens* challenge raised in battery cages to 28 d. Based on previous studies, birds fed tannin-free grain sorghum were expected to have similar performance to those fed a corn diet. In fact, Garcia et al. (2013) observed no influence on growth performance or carcass traits when corn was fully replaced by inclusion of grain sorghum. In general, results in the present study show that there were no negative effects on performance. Also, the performance of birds in the prechallenge period (Table 4) indicates that they grew as expected with no significant impact on BW at the start of the induced challenge at 14 d.

Once challenge was induced 14 to 21 d, results indicated that birds in the red/bronze treatment were less efficient than birds in the white/tan treatment. BWG was also higher and feed efficiency was better in the white/tan treatment. These observed differences in BWG and AdjFCR may have been confounded by metabolizable energy values of grain sorghum used in this study (Table 5). It is well known that broilers regulate their feed consumption until their energy requirement for maintenance is met (Sibbald, 1980; Leeson et al., 1996; Gous et al., 2018). Previous studies observed that birds can adapt to low energy diets by increasing feed intake, thereby influencing growth rate (Leeson et al., 1996). Moreover, similar findings where ME values for red/bronze grain sorghum were overestimated and influenced feed intake (A. Moritz, unpublished data) are in accord with findings in the present study. Therefore, using accurate ME values for feed ingredients is important when variations in nutrient composition exist depending on antinutritional factors, and the region or environment the feedstuff is grown and sourced (Scott et al., 1998). The utilization of a feed ingredient in a formulation is dependent on the energy content of the diet (Kleyn, 2013) which can ultimately influence the growth and efficiency (Meloche et al., 2013) of birds.

Similar to corn, grain sorghum can also lend itself to a variety of uses for animals, industry, and humans including, ethanol production and many human food-grade products, like flour and syrup (Selle et al., 2010). Although white/tan grain sorghum performed the best due to higher BWG and better feed efficiency, it may not be a variety of grain sorghum that is as practical or available for animal feed-use. White/tan varieties are more likely to be used for human food-grade products,

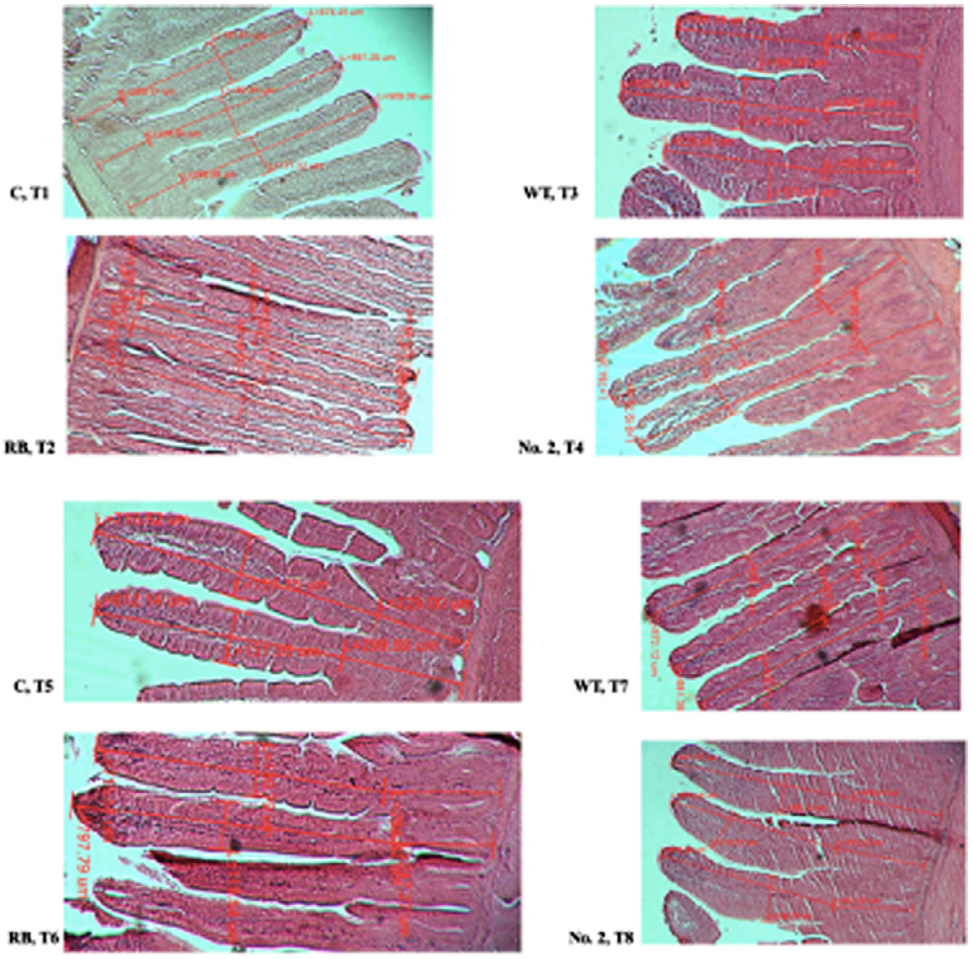

Figure 1. Representative images of jejunum morphology of broilers at 28 d of age fed corn (T1, T5) and select tannin-free grain sorghum (T2, T3, T4, T6, T7, T8) dietary treatments challenged with *E. maxima* (14 d) and *C. perfringens* (19, 20, and 21 d). Abbreviations: C, corn; RB, red/bronze; No. 2, U.S. No. 2; WT, white/tan. Treatments (Trt), Unchallenged treatments = T1, T2, T3, T4. Challenged treatments = T5, T6, T7, T8.

whereas, red/bronze and U.S. No. 2 grain sorghum varieties are the most typical commodity grain sorghum used for animal feed use (Personal communication, 2021; Brent Crafton, United Sorghum Checkoff Program, Lubbock, TX).

Among the tannin-free grain sorghum varieties used in this study, red/bronze and U.S. No. 2 have the most pigmented seeds which are known to have a higher content of polyphenolic compounds (Khoddami et al., 2015). Fagundes et al. (2017) evaluated the effect of grain sorghum on the intestinal microbiota of chickens fed sorghum-based diets and showed that altered microbiota due to hydrolysable tannins decreased *C. perfringens* without affecting performance. The major classes of phenolic compounds include tannins, phenolic acids, and flavonoids (Cardoso et al., 2017), but the grain sorghum used in this present study came from varieties selected to be tannin-free. Therefore, compounds discussed from relative quantitative analysis (Table 6) are nontannin polyphenolics. Apigenindin is a type of flavonoid polyphenol related to pigmentation and plays an active role in regulating intestinal inflammation (Makanjuola et al., 2018). In some instances, dietary polyphenols have been shown to reduce pathogens in poultry as a result of reducing inflammation (Abdel-Moneim et al., 2020). Results in the present study show a higher abundance of apigenindin in red/bronze grain sorghum and its function in decreasing inflammation may explain the observed reduction in lesions for the red/bronze treatment. Similarly, caffeic acid, classified as a nonflavonoid polyphenol, is most commonly associated with antioxidant activity and reducing oxidative stress (Abdel-Moneim et al., 2020). The high relative abundance of caffeic acid in red/bronze and U.S. No. 2 grain sorghum may have contributed to mitigating NE shown in reduced lesion score and NE mortality.

Similarly, the flavonoid content in grain sorghum is a result of genetic selection in which, Awika et al. (2011) described tan-based grain sorghum varieties to have high levels of flavonoids. Reduced lesions were also observed in the white/tan treatment and may be a result of the higher levels of flavonoids in grain sorghum varieties compared to corn. In general, grain sorghum is known to contain a diverse group of secondary metabolites especially, flavonoids, that are not found in other cereal grains like corn and wheat (Awika et al., 2011). In addition, dinitrocarbanilide was found to be higher in red/bronze and white/tan grain sorghum varieties. In fact, nicarbazin is an equimolar complex of dinitrocarbanilide and a common broad spectrum chemical anticoccidial used against *Eimeria* (Long et al., 1988; Da Costa et al., 2017; Bacila et al., 2018). Reduced lesions in red/

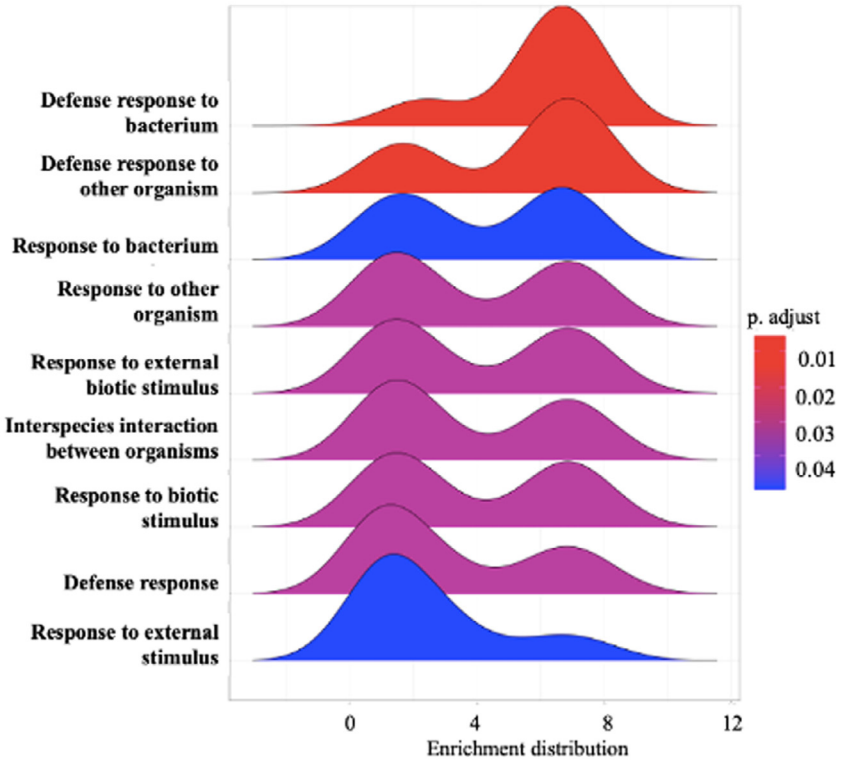

Figure 2. Gene set enrichment analysis (GSEA) using gene ontology (GO) and gene expression values in the jejunum of broilers at 28 d of age fed red/bronze treatment compared to corn treatment challenged with *E. maxima* (14 d; EM) and *C. perfringens* (19, 20, and 21 d; CP).

bronze and white/tan treatments may be a result of dinitrocarbanilide content and may be of interest to further investigate its relation to nicarbazin and its mode of action. Yet, Abdel-Moneim et al. (2020) noted that the antioxidant properties of polyphenolic compounds need to be studied further to understand the mechanisms that prevent disease.

Common intestinal pathogens affecting poultry like *Clostridium perfringens* induce an inflammatory response, and Sobhani et al. (2021) observed the ability of polyphenols to alter specific cell signaling pathways and regulate immune response. Moreover, Zhong et al. (2014) found that polyphenols can mitigate the inflammatory response in challenged chickens by reducing the expression of toll-like receptor genes involved in the innate immune response. Previous research by Selle et al. (2010) found that white grain sorghum varieties fed to broilers had better growth performance than red grain sorghum varieties due to the differences in phytate and phenolic compounds (nontannin phenols). However, studies on polyphenols as antimicrobial agents suggest that a specific subset of phenolic compounds such as, flavon-3-ols may be linked to antimicrobial activity inhibiting the growth of *C. perfringens* (Daglia, 2012; Shields et al., 2021). These differences between grain sorghum varieties may have implications on how specific metabolites affect intestinal health. Therefore, feed ingredients like grain sorghum may be an alternative or supplemental method to mitigate certain intestinal diseases due to its known antimicrobial and antioxidative properties that could improve nutrient absorption and utilization.

The major polyphenols identified in this present study are well within the findings by Khoddami et al. (2015) for red grain sorghum varieties. Relative concentrations of flavonoid and phenolic compounds were greatest in red/bronze and U.S. No. 2 grain sorghum. In fact, some polyphenolic compounds can have antinutritional factors with antagonistic effects on the gut, nutrient digestibility, and absorption that can compromise performance (Khoddami et al., 2015). In the study by Khoddami et al. (2015), results showed a negative correlation between phenolic acid, coumaric acid, and energy utilization. Interestingly, high levels of hydroxycoumarin, a polyphenol related to coumaric acid, in red/bronze grain sorghum may have negatively impacted the energy utilization affecting performance in combination with the variable ME value mentioned previously. Further studies according to Cardoso et al. (2017) are needed to understand the effect of dietary factors on the bioavailability of phenolic compounds and their functional role in health. Overall, results from the present study show the potential functional role of select metabolites in grain sorghum and their influence on nonenteric disease and enteric disease-induced groups of birds.

In this study, the intestinal morphology was analyzed in the jejunum (Figure 2) because this is a primary site for nutrient absorption (Liu et al., 2016), and any changes in epithelial integrity that could affect nutrient digestion and absorption as a result of diet would be observed in the intestinal lumen (Torres et al., 2013). Chen et al. (2015) reported that an increase in crypt depth and crypt:villi ratio indicates increased demand for cell proliferation to maintain optimal gut function.

Chen et al. (2015) also reported the differences between narrow and widening villi and how they indicate compromised gut integrity. Narrow villi have a greater surface area for nutrient absorption compared to widening villi because widening villi have increased gut-associated tissue proliferation in response to a compromised gut (Chen et al., 2015). No differences were observed in the jejunum morphology of the present study, and these results are in agreement with previous findings by Torres et al. (2013) and Fagundes et al. (2017) showing no effect on villus height and crypt depth in low-tannin grain sorghum diets.

In general, the measurements for each morphological parameter were taken at 28 d during the recovery time which could be a reason for the lack of differences observed. However, findings by Star et al. (2010) suggest that intestinal cells in subclinical cases of NE are persistently inflamed and in recovery mode. As a result, Xu et al. (2003) explained that villi would generally be shorter, and crypts deeper in response to inflammation. Therefore, since challenged treatments in the present study were all affected to some degree based on their lesion scores, it would have been expected to see significant differences in the histomorphology. Nevertheless, evaluating the intestinal morphology can provide more information on the size of villi compared to cell turnover and tissue repair when intestinal tissue is damaged by enteric pathogens.

Differential expression analysis following mRNA-sequencing provided further insight on the changes in the intestinal mucosa when birds were infected with EM/CP. As a result, the efficacy of red/bronze grain sorghum could be determined by evaluating the genes expressed and relate this to its ability to respond to enteric pathogens. Results indicated that red/bronze-EM/CP had upregulated DEGs predominantly associated with defense responses to bacteria and other organisms compared to corn-EM/CP. These findings support that red/bronze grain sorghum was effective in protecting the host from pathogen invasion of the bacteria causing NE, *C. perfringens*, due to its response in upregulating defense receptors. The mucus layer secretes a significant number of antibacterial agents in the intestinal epithelium and plays a critical role in microbial balance, nutrient transport, and regulating immune response (Lan et al., 2005; Duangnumsawang et al., 2021). More studies have reported that the gram-positive bacteria, *C. perfringens*, which causes NE and the severity may be linked to the differential expression of specific intestinal genes (Coursodon et al., 2012; Zhou et al., 2017).

Related to the regulation of immune response, the DEG gene *LYG2*, which is associated with lysozyme activity, was significantly upregulated with a 6.09-fold increase (Table 7). Lysozyme proteins are commonly known for activating the innate immune response and having a protective effect on the host from enteric pathogens (Bar Shira et al., 2018). The red/bronze challenged treatment also had a 6.94-fold increase in avidin (***AVD***) (Table 7), an acute phase protein commonly expressed in the intestine when the gut has been compromised by injury and facilitates tissue repair (Elo et al., 1979). Interestingly, an extracellular fatty acid binding protein (***EFABP***) was upregulated with a 7.68-fold increase (Table 7). The intestinal mucosa contains several types of FABPs involved in fatty acid transport and metabolism. A study by Katongole and March (1979) evaluated FABP in the intestine of chickens and found that low-fat diets resulted in higher expression of FABP in the intestine than high-fat diets. The higher expression of *EFABP* may be explained by the lower % of analyzed crude fat (Tables 2 and 3) in the red/bronze treatment compared to its counterparts. Conversely, a previous study showed a downregulation of FABP when birds were challenged with coccidiosis vaccine which may have been attributed to the structural damage from a compromised gut barrier; thus, a decrease in fatty acid utilization (Chen et al., 2015). At last, *NOS2*, associated with arginine catabolism and nitric oxide activity, was upregulated with a 6.30-fold increase. Studies have observed the effect of arginine and how coccidia use nitric oxide as a substrate resulting in decreased available arginine (Allen and Fetterer, 2000). However, according to a study by Dominguez et al. (2015) a higher production of nitric oxide resulted in a higher antioxidant capacity reducing oxidative stress to combat *Eimeria* spp. and reduced lesions in the jejunum and cecum. These same findings are consistent with the increased expression of NOS2 and the reduced lesions in the red/bronze treatment.

Comparisons between unchallenged and challenged treatment groups other than red/bronze and corn were analyzed for differential gene expression; however, many of these other comparisons showed variability between replicates. As a result, fewer observed treatment comparisons of DEGs may have been affected by the quality and quantity of the mucosa sample collected from 28-d broilers as mucosa production is greatest at the first week of age and decreases with age (Duangnumsawang et al., 2021).

In terms of practicality for a poultry nutritionist, gene expression analysis may be of use if considering using an inclusion of grain sorghum in diet formulation with other feed additives to combat coccidiosis. There is an abundance of literature that explains how multifaceted gut health is and nutrition is only a single factor among many others that can influence the intestinal epithelium (Yegani et al., 2008). As a result, it may be more practical to supplement grain sorghum with a combination of feed additives to enable several modes of action and synergistic effects in mitigating coccidiosis. A nutritionist would also want to be aware of antagonistic effects that grain sorghum may have with other feed additives (e.g., enzymes, organic acids, probiotics, plant extracts) in a diet. Overall, gene expression can provide the insight on what genes are up- or downregulated which may relate to beneficial or detrimental effects on intestinal integrity and immune activity. When considering grain sorghum as a functional feedstuff, it is of interest to know if there are any observed protective effects against enteric pathogen invasion. Similarly, data from the relative concentration of polyphenolic compounds in grain sorghum can give insight on synergistic or antagonistic effects and

any interactions between specific compounds in a grain sorghum-based diet supplemented with other feed additives and ingredients.

Current findings and previous studies indicate that tannin-free grain sorghum did not negatively affect performance. Red/bronze grain sorghum reduced lesions when birds were challenged, and intestinal morphology was unaffected. Gene expression and relative concentration of select polyphenolic compounds aided in reducing the severity of NE when birds were fed grain sorghum treatments. Gene expression and metabolite results provide future direction for analysis on specific genes of interest and polyphenolic compounds that influence the intestinal integrity. While feeding identity preserved white/tan and red/bronze grain sorghum showed advantages in feed efficiency or reduced lesions, current availability and prices could be significant constraints to be used as common poultry feed ingredients. Therefore, U.S. No. 2 might be an intermediate to the other grain sorghum treatments for performance and efficacy on intestinal health in challenged birds. Furthermore, the efficacy of grain sorghum and how these results compare to the cost and availability of selected grain sorghum varieties will influence the nutritionist to consider using it as a functional feedstuff. In the end, growth rate and feed efficiency are the most important targets in poultry production to evaluate bird performance (Sugiharto et al., 2016).

ACKNOWLEDGMENTS

The authors sincerely thank Southern Poultry Feed and Research, Inc., University of Georgia feed mill, University of Missouri Agricultural Experiment Station, and Clemson University Multi-user Analytical Lab (MUAL) for project support, facilities, and sample analyses. The authors would also like to thank Dr. Guillermo Rimoldi of the Clemson Veterinary Diagnostic Center for his expertise provided on processes involved with intestinal histomorphology. We acknowledge the assistance of Dr. Rooksana Noorai and Kaitlyn Williams of the Clemson University Genomics and Bioinformatics Facility for services and facilities provided. The facility is supported by Grants P20GM109094 and P20GM139767 Institutional Development Awards (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health.

DISCLOSURES

Mireille Arguelles-Ramos reports a relationship with US Grains Council that includes: consulting or advisory and speaking and lecture fees. Other authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102300.

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