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Effects of select tannin-free grain sorghum varieties on the performance, carcass traits, intestinal morphology, and gene expression of jejunal mucosa of broiler chickens

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ABSTRACT

Objective: The objective of this study was to evaluate the effects of tannin-free grain sorghum varieties on the performance, carcass traits, intestinal morphology, and gene expression of broiler chickens.

Materials and Methods: Cobb 500 × Hubbard male broilers (n = 640, 20 birds per pen, 8 pens per treatment) were fed diets based on corn, red/bronze, white/tan, or US No. 2 in crumble/pellet presentation fed in starter, grower, and finisher phases. Group BW and feed intake were recorded weekly. Mortality was recorded daily for the calculation of adjusted feed conversion ratio. At 41 d, two birds per pen were selected for the average pen weight for carcass yield and breast yield values. The intestinal morphology using histology and change in transcription using mRNA-seq was compared among birds fed corn and those fed grain sorghum (1 bird per pen). Pen was considered the experimental unit with model effects assessed with ANOVA and Fisher's LSD procedure.

Results and Discussion: Birds fed the corn treatment had greater BW gain ($P = 0.009$; 3,622, 3,479, 3,518, and 3,483 g for corn, red/bronze, white/tan, and US No. 2 sorghum diets, respectively) at 41 d. Feed intake was greatest for birds fed corn and red/bronze diets (5,495 and 5,599 g, respectively) when compared with the white/tan diet (5,357 g), whereas US No. 2 sorghum-fed birds were intermediate (5,346 g; $P = 0.005$). Birds had improved adjusted feed conversion ratio in all treatments ($P < 0.001$; 1.52, 1.51, and 1.53 g:g for corn, white/tan, and US No. 2 sorghum diets, respectively) compared with red/bronze (1.60 g:g) at 41 d. No effects of grain sorghum treatments were observed on carcass traits and intestinal morphology. The mRNA-seq revealed 46 differentially expressed genes. Birds fed the corn-based diet performed better compared with those fed the tannin-free grain sorghum treatments. However, feeding certain grain sorghum varieties could result in similar feed efficiency to birds fed corn diets.

Implications and Applications: This study demonstrates that tannin-free grain sorghum can be a feasible alternative to corn depending on the variety used, cost, and availability. It may also have implications to improve gut health upon further investigation of its mode of action. Overall, results may allow nutritionists in the commercial poultry industry to consider grain sorghum as an alternative to corn.

Key words: alternative feedstuff, tannin-free grain sorghum, carcass traits, growth performance, intestinal health

INTRODUCTION

Increasing feed costs and demand to meet the global food supply have increased interest within the poultry industry to reduce the heavy dependence on corn and seek alternative feedstuffs (Alshelmani et al., 2021). All commercially available US grain sorghum is tannin free for animal feed use and may be a viable alternative to corn due to its similar ME and nutritional profile. Assessment of growth performance parameters when feeding tannin-free grain sorghum may elucidate perceived issues of tannin-containing grain sorghum.

Gualtieri and Rapaccini (1990) demonstrated that grain sorghum as a partial or full replacement for corn in broiler

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diets resulted in growth inconsistencies due to its high tannin content. These inconsistencies associated with tannins have invoked a negative perception of using grain sorghum to replace corn; however, evaluation of low-tannin grain sorghum have shown it to be an effective substitution without affecting growth in broilers (Hulan and Proudfoot, 1982). Studies by Garcia et al. (2013) reported no differences in feed intake, weight gain, and efficiency when replacing corn with low-tannin cultivars of grain sorghum. However, there are limited data and knowledge to support the use of tannin-free varieties as an alternative to corn.

In addition, the transition to antibiotic-free poultry due to increasing concern of antibiotic resistance and residues has led the industry to seek novel ways to mitigate and control intestinal disruptions (Oviedo-Rondón, 2019). Grain sorghum might be a functional feedstuff due to its beneficial antimicrobial and antioxidant properties. Some of the secondary metabolites found in grain sorghum include flavonoids and proanthocyanidins associated with antimicrobial and antioxidative functions (Shen et al., 2018; Ashley et al., 2019). These polyphenols that are most recognized for these functions have been shown to have the ability to improve health (Abdel-Moneim et al., 2020). Studies have indicated that the concentration of polyphenols and their interaction with each other can protect the host from pathogens and influence the intestinal mucosa (Al-Zoreky, 2009; Fagundes et al., 2017; Abdel-Moneim et al., 2020). Shields et al. (2021) showed that polyphenolic compounds of grain sorghum were associated with antimicrobial activity and inhibited the growth of *Clostridium perfringens*. Furthermore, the objective of this study was to evaluate the effect of tannin-free grain sorghum varieties on broiler growth, carcass traits, intestinal morphology, and gene expression as complete substitutions of corn.

MATERIALS AND METHODS

Animal Care and Use

This experiment was conducted in accordance with principles and specific guidelines approved by the Clemson University Institutional Animal Care and Use Committee (IACUC), Animal Use Protocol (AUP) #2020-021.

Bird Husbandry and Housing

A total of 640 one-day-old Cobb 500 × Hubbard male broiler chicks were housed in a curtain-sided broiler house (20 birds per pen). Birds were randomly distributed in floor pens with dividers (137 cm × 175 cm; 8 pens per treatment) to adhere to commercial stocking density requirements and maintain uniformity across all birds. A brooder lamp was provided per pen from 1 to 21 d of age, and the temperature of the house was 35°C at placement and gradually decreased to reach 24°C with a daily lighting program of 18 h of light to 6 h of dark throughout the study. Each pen was the experimental unit with birds having access to hanging feeders and an automatic drinking system after 21 d of age.

Ingredients

The 3 tannin-free, animal-feed-grade varieties of grain sorghum, red/bronze, US No. 2, and white/tan, were of US origin. The red/bronze variety was sourced from South Carolina, the white/tan variety from Texas, and the US No. 2 variety state of origin was unknown. Red/bronze and white/tan grain sorghum varieties were identity preserved (contained a single variety), whereas US No. 2 was a red/bronze-based variety that may have contained other mixed grain sorghum varieties; nutrient and energy analyses for each variety compared with corn are shown in Table 1. Red/bronze grain sorghum was tested to ensure zero tannin content using the Adams-Harbertson assay (Harbertson et al., 2003).

Dietary Treatments

Each whole grain sorghum variety was ground through a hammermill with a 4-mm sieve. Treatments were formulated based on industry-standard diets and prepared as a basal crumbled starter, pelleted grower, and pelleted finisher diet with each respective test ingredient of corn or grain sorghum. Birds were fed 1 of 4 treatments *ad libitum* of crumble or pellet feed according to a 3-phase feeding program: starter (1 to 14 d of age; Table 2), grower (15

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

Item, %	Corn	Red/bronze[1]	White/tan[2]	US No. 2[3]
DM[4]	88.34	82.75	88.97	87.86
Ash[4]	1.04	1.01	1.04	1.15
Crude fat[4]	3.52	2.46	2.36	2.60
Crude fiber[4]	2.30	1.90	2.40	2.90
CP[4]	7.00	8.94	9.38	9.38
Methionine[4]	0.17	0.16	0.20	0.17
Lysine[4]	0.25	0.20	0.22	0.22
Threonine[4]	0.27	0.30	0.32	0.32
GE, kcal/kg	3,861	3,889	3,838	3,860
ME,[5] kcal/kg	3,384	3,441	3,139	3,157

[1]Tannin-free red/bronze sourced from South Carolina.

[2]Tannin-free white/tan sourced from Texas.

[3]Tannin-free US No. 2 had an unknown origin.

[4]Proximate and AA analyses were determined using the AOAC International method (Novus International Inc. Laboratory Services).

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

to 28 d of age; Table 3), and finisher (29 to 42 d of age; Table 4).

Performance and Carcass Trait Measurements

Group BW (by pen) and feed intake (by pen) were recorded at the start of the experiment and weekly thereafter on d 7, 14, 21, 28, 35, and 41 to determine cumulative BW gain (**BWG**), feed intake (**FI**), and feed conversion ratio. Daily mortality was weighed and recorded for adjusted feed conversion ratio (**AdjFCR**) calculations (Equation [1]). On d 41, two birds from each pen were selected that were similar to the average pen weight for further processing to obtain carcass yield (**CY**) and breast yield (**BY**). Feed was removed from all pens 12 h before the time of processing. Birds were euthanized via electrical stunning before being exsanguinated, scalded, defeathered, and eviscerated. Eviscerated whole carcasses (feet, shanks, and neck removed) were individually weighed to measure hot carcass weight (**HCW**). The front half of each carcass with pectoral major and minor muscles (bone in, skin on) were removed and individually weighed as the breast weight (**BrW**) to determine BY. The CY and BY (Equation [2]; Equation [3]) were calculated as a percentage of the initial live weight (**LBW**) and HCW, respectively.

$$\text{AdjFCR} = \text{total feed intake}/(\text{BWG} + \text{mortality BW}) \quad [1]$$

$$\text{CY} = \text{HCW}/\text{LBW} \times 100 \quad [2]$$

$$\text{BY} = \text{BrW}/\text{HCW} \times 100 \quad [3]$$

Intestinal Mucosa Preparation and RNA Sequencing Analysis

One bird per pen was randomly selected, weighed, and euthanized to collect intestinal mucosa for gene expression analysis in the red/bronze and corn treatments. 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 (ThermoFisher Scientific), 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), and the libraries were sequenced on an Illumina NovaSeq 6000 (Illumina). Quality metrics of the raw data were assessed with FastQC (Andrews, 2010; 0.11.9) and summarized using MultiQC (Ewels et al., 2016; 1.11). Quality trimming and adapter removal were performed using fastp (Ollion et al., 2013; 0.23.2). The reads were aligned to the *Gallus gallus* GRCg6a reference genome using HISAT2 (Kim et al., 2019; 2.2.1). Read counts from genomic features were obtained using Subread featureCounts (Liao et al., 2014; 2.0.2) (with -p -countReadPairs -C flags). Library normalization and identification of differentially expressed genes (**DEG**) across different conditions were performed using edgeR (3.36.0) likelihood ratio test pipeline, with only samples with library size ≥8 million included for analysis (Robinson et al., 2010). Genes were considered DEG if the false discovery rate was <0.05 and $|\log_2\text{Fold Change}| > 1$. ClusterProfiler (4.2.2) was used to perform gene set enrichment analysis (**GSEA**) using gene ontology (Yu et al., 2012). Overrepresentation analysis for enriched Reactome pathways was performed using g:Profiler web server (Raudvere et al., 2019).

Intestinal Histomorphology and Analysis

After collecting intestinal mucosa for gene sequencing, intestinal tissue samples were collected for histomorphometric measurements. Three 1-cm sections from 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 for 24 h and transferred to 70% ethanol until being processed and embedded in paraffin. Five 10-μm sections of the duodenum, jejunum, and ileum were sectioned with a microtome, 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) using Infinity 2 software (Lumenera). The average of 10 replicate measurements per sample/treatment were used for statistical analyses.

Statistical Analysis

Data were analyzed as a completely randomized block design with effects of treatment and pen (4 pens defined each block). One-way ANOVA followed by Fisher's protected LSD procedure was used to determine whether differences existed among the treatment means. Regression imputation (Buck, 1960) was used for weekly FI on d 21 and weekly AdjFCR on d 41 because these observations were clearly outliers indicated by repeated measure analysis on weekly FI and AdjFCR parameters. All statistical calculations were performed using JMP Pro version 16 (SAS Institute Inc.). Statistical significance was based on a $P < 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

Performance

BW and Mortality. Birds fed the corn treatment had heavier BW (Table 5; 212 g) at 7 d than the US No. 2

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

	Starter dietary treatment[1]			
Item	**CN**	**RB**	**WT**	**No. 2**
Ingredient, %				
Corn	56.85	0.00	0.00	0.00
Red/bronze grain sorghum	0.00	60.39	0.00	0.00
White/tan grain sorghum	0.00	0.00	56.12	0.00
US No. 2 grain sorghum	0.00	0.00	0.00	56.32
Soybean meal, 47.5% CP	38.25	35.60	36.52	36.51
Fat, vegetable	2.32	1.29	4.63	4.45
Defluorinated phosphate	0.90	0.84	0.87	0.85
Limestone	0.14	0.25	0.20	0.22
Sodium chloride	0.43	0.43	0.43	0.43
DL-methionine	0.33	0.31	0.35	0.34
L-threonine	0.05	0.05	0.06	0.06
L-lysine	0.13	0.24	0.21	0.22
Choline chloride, 60%	0.16	0.16	0.16	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
Sacox 60[5]	0.05	0.05	0.05	0.05
BMD 50[6]	0.05	0.05	0.05	0.05
Calculated composition, %				
ME, kcal/kg	3,031	3,031	3,031	3,031
CP	22.38	23.55	22.95	22.96
Crude fat	4.83	3.35	6.40	6.37
Calcium	0.90	0.90	0.90	0.90
Sodium	0.21	0.21	0.21	0.21
Digestible lysine	1.24	1.24	1.24	1.24
Digestible methionine	0.63	0.62	0.64	0.64
Digestible threonine	0.83	0.83	0.83	0.83
Digestible methionine + cysteine (sulfur AA)	0.93	0.93	0.93	0.93
Total phosphorus	0.58	0.57	0.56	0.57
Available phosphorus	0.43	0.43	0.43	0.43
Analyzed composition,[7] %				
CP	24.56	22.06	22.81	22.00
Crude fat	4.47	2.72	6.12	6.26
Calcium	0.90	0.82	0.81	0.88
Sodium	0.25	0.22	0.24	0.26
Lysine	1.35	1.39	1.25	1.22
Methionine	0.66	0.68	0.74	0.69
Methionine + cysteine (sulfur AA)	1.04	1.05	1.06	1.02
Glycine	0.95	0.92	0.80	0.81
Proline	1.12	1.13	0.85	0.90
Total phosphorus	0.87	0.80	0.88	0.84

[1]There were 8 replicates for dietary treatments: CN = corn; RB = red/bronze; WT = white/tan; No. 2 = US No. 2.

[2]Supplied per kilogram 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 µg; *trans*-retinyl acetate, 1,892 µg; all-rac α tocopheryl acetate, 11 mg; and ethoxyquin, 125 mg.

[3]Supplied per kilogram of diet: manganese ($MnSO_4$•H_2O), 60 mg; iron ($FeSO_4$•$7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4$•$5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

[4]Quantum Blue phytase (AB Vista); added at 0.011% of the diet with 1,650% Ca and 1,500% available P.

[5]Bacitracin methylene disalicylate, BMD 50 (Zoetis).

[6]Salinomycin sodium, Sacox 60 (Huvepharma).

[7]Proximate, mineral, and AA analyses were determined using the AOAC International method (Novus International Inc. Laboratory Services).

treatment ($P = 0.002$; 194 g). After 7 d, BW in each treatment separated, and birds fed corn and white/tan treatments were heavier than those fed red/bronze and US No. 2 at 14, 21, 38, and 35 d (Table 5). However, by 41 d, birds fed the corn treatment were heavier compared with those fed each of the grain sorghum treatments ($P = 0.009$). With respect to overall mortality from 0 to 41 d, there were no significant differences, but it was observed that mortality was numerically greatest in the corn treatment (20.45%; Table 5).

Cumulative BWG, FI, and AdjFCR. Body weight gain was significantly less for birds in the US No. 2 (Table 6; $P = 0.003$) treatment at wk 1, 0 to 7 d, and FI was less compared with all other treatments ($P = 0.012$). Thereafter, from wk 2 (0–14 d) to wk 5 (0–35 d), BWG ($P < 0.05$) was greatest in the corn and white/tan treatments compared with the red/bronze and US No. 2 treatments. As a result of greater BWG in corn and white/tan treatments, FI was greater for these birds than for those fed the red/bronze and US No. 2 treatments (Table 6). At wk 6 (0–41 d), BWG was significantly greater in the corn treatment ($P = 0.009$; 3,622.49 g) than in the grain sorghum treatments. The red/bronze treatment had significantly greater FI ($P = 0.005$) at wk 6 than the white/tan and US No. 2 treatments. Birds in the corn, white/tan, and US No. 2 treatments showed a significantly improved AdjFCR than those fed the red/bronze treatment from wk 3 (0–21 d) to wk 6 (0–41 d) ($P < 0.05$).

In this study, all birds performed better than typical Cobb 500 × Hubbard male broiler performance guidelines. Also, when compared with the performance of birds at 42 d of age, industry results were reported to have an average live BW of 2,558 g and an actual feed efficiency of 1.65 (AgriStats, 2022). Birds fed tannin-free grain sorghum were expected to have similar performance to those fed a corn diet due to their similar nutritional values. In general, we found that BW and FI were reduced in the grain sorghum treatments, but AdjFCR was improved in all treatments except for with birds fed the red/bronze variety at 41 d. There were no significant differences in carcass traits or intestinal morphology, indicating that tannin-free grain sorghum may be an alternative to corn with no effects on these parameters. Similar to our findings, Hulan and Proudfoot (1982) observed that low-tannin grain sorghum can be included up to 45 and 58% of the total ration in broiler starter and finisher diets, respectively, without adverse effects on feed conversion and CY. Additionally, Garcia et al. (2013) observed no influence on growth performance or carcass traits when corn was fully replaced by the inclusion of low-tannin grain sorghum. Total phenolic compound analysis in the present study indicated no tannins detected for red/bronze grain sorghum.

Results in the present study showed that the corn treatment had the highest BWG and FI throughout, but white/tan (1.51) and US No. 2 (1.53) treatments proved to be similarly efficient to the corn treatment (1.52) with no differences in cumulative AdjFCR (0–41 d). These results are consistent with Liu et al. (2016), who reported an average feed conversion ratio of 1.63 in broilers fed red and white grain sorghum varieties. The greater analyzed CP content in the starter diet of the corn treatment (1.75 – 2.6% CP difference; Table 2) may have given birds an early advantage resulting in greater BW throughout grow-out. This observation is in accord with previous findings first shown by Fraps (1943) studying the relationship between protein content in the diet and body composition of broilers. Jackson et al. (1982) and Smith and Pesti (1998) have also demonstrated that increasing dietary protein resulted in heavier birds. In addition, studies on the concept of early nutrition and increasing AA density have reported them to influence performance through the later life of broilers (Noy and Sklan, 1998; Kidd et al., 2004; Corzo et al., 2010). The corn treatment had an overall greater BW and, therefore, outperformed the grain sorghum treatments, but it is important to consider that growth rate is dependent on AA and not dietary protein (Ferket and Gernat, 2006).

Based on results for weekly BWG, there was improvement in BWG as it was not significantly different for corn, US No. 2, and white/tan treatments from 14 to 21 d (Table 7) and from 21 to 28 d for corn and white/tan treatments. The grower phase diets for grain sorghum treatments contained 20 to 21% CP, meeting or exceeding the calculated CP (Table 3). In the finisher phase diets, white/tan and US No. 2 contained 19% CP, red/bronze had 17.2% CP, and corn had the least with 16.6% CP, and no significant differences in BWG were observed from 28 to 35 d and 35 to 41 d (Table 7). Results from this study demonstrate that marginal differences in CP and other EAA such as glycine and proline could have an effect on BWG. In fact, soybean meal is a major source of glycine in poultry diets and plays both metabolic and physiologic roles in the body of broiler chickens (Wu, 2013; He et al., 2021). Therefore, as a result of the greater inclusion of soybean meal (38%) in the starter phase corn treatments compared with soybean meal inclusion for the grain sorghum treatments (36–37%), greater concentrations of glycine and proline may have given a nutritional advantage to the corn-fed birds (Table 2).

In this study, the nutrient analysis revealed that the corn diet was the only one meeting the Ca requirement in the starter phase (0.90% Ca), whereas the other treatments were marginally deficient (0.82, 0.81, and 0.88% for red/bronze, white/tan, and US No. 2 diets; Table 2). Furthermore, a Ca deficiency was present for most diets in the grower and finisher phases (except the red/bronze grower diet). This unintended Ca deficiency across treatments was observed due to a mineral analysis error on the Ca matrix value for soybean meal. As a result, the lesser inclusion of limestone contributed to the lower analyzed Ca levels from calculated levels in phase-fed diets (Table 2, Table 3, and Table 4). A study by Yan and others re-

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

	Grower dietary treatment[1]			
Item	**CN**	**RB**	**WT**	**No. 2**
Ingredient, %				
Corn	65.36	0.00	0.00	0.00
Red/bronze grain sorghum	0.00	67.87	0.00	0.00
White/tan grain sorghum	0.00	0.00	63.08	0.00
US No. 2 grain sorghum	0.00	0.00	0.00	63.30
Soybean meal, 47.5% CP	30.18	28.57	29.61	29.60
Fat, vegetable	1.84	0.87	4.62	4.42
Defluorinated phosphate	0.81	0.74	0.77	0.74
Limestone	0.28	0.39	0.33	0.36
Sodium chloride	0.46	0.47	0.47	0.47
DL-methionine	0.27	0.25	0.28	0.28
L-threonine	0.05	0.02	0.04	0.04
L-lysine	0.14	0.22	0.25	0.20
Choline chloride, 60%	0.16	0.16	0.16	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
Sacox 60[5]	0.05	0.05	0.05	0.05
BMD 50[6]	0.05	0.05	0.05	0.05
Calculated composition, %				
ME, kcal/kg	3,086	3,086	3,086	3,086
CP	19.14	20.94	20.26	20.28
Crude fat	4.54	3.03	6.46	6.43
Calcium	0.85	0.85	0.85	0.85
Sodium	0.22	0.22	0.22	0.22
Digestible lysine	1.05	1.05	1.05	1.05
Digestible methionine	0.55	0.53	0.55	0.54
Digestible threonine	0.70	0.70	0.70	0.70
Digestible methionine + cysteine (sulfur AA)	0.81	0.81	0.81	0.81
Total phosphorus	0.53	0.53	0.52	0.53
Available phosphorus	0.40	0.40	0.40	0.40
Analyzed composition,[7] %				
CP	20.12	19.00	20.94	20.38
Crude fat	4.30	3.05	6.26	5.52
Calcium	0.74	0.86	0.78	0.74
Sodium	0.26	0.27	0.26	0.22
Lysine	1.05	1.05	1.16	1.24
Methionine	0.57	0.60	0.65	0.65
Methionine + cysteine (sulfur AA)	0.90	0.89	0.97	0.99
Glycine	0.75	0.75	0.78	0.84
Proline	0.93	0.83	0.95	1.01
Total phosphorus	0.73	0.86	0.76	0.71

[1]There were 8 replicates for dietary treatments: CN = corn; RB = red/bronze; WT = white/tan; No. 2 = US No. 2.

[2]Supplied per kilogram 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 µg; *trans*-retinyl acetate, 1,892 µg; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[3]Supplied per kilogram of diet: manganese ($MnSO_4$•H_2O), 60 mg; iron ($FeSO_4$•$7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4$•$5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

[4]Quantum Blue phytase (AB Vista); added at 0.011% of the diet with 1,650% Ca and 1,500% available P.

[5]Bacitracin methylene disalicylate, BMD 50 (Zoetis).

[6]Salinomycin sodium, Sacox 60 (Huvepharma).

[7]Proximate, mineral, and AA analyses were determined using the AOAC International method (Novus International Inc. Laboratory Services).

Table 4. Ingredient composition and calculated or analyzed energy and nutrient composition of finisher diet with respective test ingredient: corn or red/bronze, white/tan, or US No. 2 grain sorghum (as fed) from 29 to 42 d of age

	Finisher dietary treatment[1]			
Item	**CN**	**RB**	**WT**	**No. 2**
Ingredient, %				
Corn	69.49	0.00	0.00	0.00
Red/bronze grain sorghum	0.00	71.59	0.00	0.00
White/tan grain sorghum	0.00	0.00	66.54	0.00
US No. 2 grain sorghum	0.00	0.00	0.00	66.76
Soybean meal, 47.5% CP	25.75	24.56	25.66	25.64
Fat, vegetable	2.32	1.37	5.32	5.11
Defluorinated phosphate	0.70	0.62	0.65	0.62
Limestone	0.37	0.47	0.41	0.44
Sodium chloride	0.48	0.49	0.48	0.49
DL-methionine	0.23	0.20	0.24	0.24
L-threonine	0.05	0.02	0.03	0.03
L-lysine	0.14	0.21	0.18	0.18
Choline chloride, 60%	0.10	0.10	0.10	0.10
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
BMD 50[5]	0.05	0.05	0.05	0.05
Calculated composition, %				
ME, kcal/kg	3,164	3,164	3,164	3,164
CP	17.32	19.39	18.68	18.70
Crude fat	5.10	3.57	7.18	7.15
Calcium	0.80	0.80	0.80	0.80
Sodium	0.22	0.22	0.22	0.22
Digestible lysine	0.94	0.94	0.94	0.94
Digestible methionine	0.49	0.47	0.49	0.48
Digestible threonine	0.64	0.64	0.64	0.64
Digestible methionine + cysteine (sulfur AA)	0.73	0.73	0.73	0.73
Total phosphorus	0.49	0.49	0.48	0.49
Available phosphorus	0.38	0.38	0.38	0.38
Analyzed composition,[6] %				
CP	16.62	17.19	19.31	19.25
Crude fat	4.59	3.54	6.46	6.53
Calcium	0.73	0.73	0.69	0.62
Sodium	0.24	0.25	0.25	0.18
Lysine	0.92	0.95	1.01	0.94
Methionine	0.55	0.51	0.54	0.54
Methionine + cysteine (sulfur AA)	0.83	0.78	0.85	0.82
Glycine	0.65	0.62	0.70	0.68
Proline	0.78	0.70	0.90	0.79
Total phosphorus	0.59	0.67	0.69	0.71

[1]There were 8 replicates for dietary treatments: CN = corn; RB = red/bronze; WT = white/tan; No. 2 = US No. 2.

[2]Supplied per kilogram 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 µg; *trans*-retinyl acetate, 1,892 µg; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[3]Supplied per kilogram of diet: manganese ($MnSO_4$•H_2O), 60 mg; iron ($FeSO_4$•$7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4$•$5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

[4]Quantum Blue phytase (AB Vista); added at 0.011% of the diet with 1,650% Ca and 1,500% available P.

[5]Bacitracin methylene disalicylate, BMD 50 (Zoetis).

[6]Proximate, mineral, and AA analyses were determined using the AOAC International method (Novus International Inc. Laboratory Services).

Table 5. Effect of dietary treatment on broiler BW and percent mortality from 0 to 41 d of age

	Dietary treatment				SEM	
Variable and age	Corn	Red/bronze	White/tan	US No. 2	(n = 8)	*P*-value
BW, g						
0 d	44.9	44.3	44.7	44.8	0.24	NS
0–41 d	20.45	11.04	12.87	15.11	3.38	NS
14 d	596[a]	570[bc]	586[ab]	554[c]	7.50	0.005
21 d	1,210[a]	1,153[b]	1,213[a]	1,165[b]	10.16	0.001
28 d	2,077[a]	1,966[b]	2,042[a]	1,982[b]	17.85	0.001
35 d	3,051[a]	2,895[b]	2,958[ab]	2,901[b]	31.10	0.009
41 d	3,667[a]	3,523[b]	3,563[b]	3,528[b]	28.80	0.009
Mortality, %						
0–41 d	20.45	11.04	12.87	15.11	3.38	NS
Birds culled (due to lameness), %	8.75	3.75	1.50	6.25	—	—

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

ported that birds have the ability to adapt to early Ca deficiency by way of increasing levels of circulating 1, 25 dihydroxycholecalciferol and calbindin (Yan et al., 2005). Several other studies have shown that Ca depletion has a greater effect on bone mineralization than growth performance (Venäläinen et al., 2006; Valable et al., 2018; Li et al., 2020). Therefore, the effect of Ca deficiency may also explain the high mortality in the corn treatment where a greater incidence of lameness was observed, and more birds were culled in this specific treatment (8.75% for corn) because of leg problems than in grain sorghum–fed birds (3.75% for red/bronze, 1.5% for white/tan, and 6.25% for US No. 2; Table 5). Birds in the corn treatment grew faster as seen with greater BWG and FI (Table 6), but they also had greater bone-related issues contributing to high mortality. Greater FI in Ca-deficient diets is consistent with previous findings where birds fed lesser-Ca diets consumed more feed to meet Ca requirements (Bradbury et al., 2014). It is well-documented that increased bone growth occurs during the first 3 wk of age in which Ca is the most important mineral to lay the foundation for skeletal support and strength; as the bird ages, Ca and P required concentrations in the ration decrease (Yan et al., 2005). This situation stresses that ingredient quality and accurate proximate and mineral analyses are important for properly formulating diets to avoid nutritional deficiencies.

Additionally, BWG and FI in grain sorghum treatments may have been confounded by variable ME values of grain sorghum used in this study. The lower BWG and FI for the first 3 wk of grow-out in the US No. 2 treatment may be a result of an underestimated ME value for US No. 2 grain sorghum (Table 1). Broilers consume feed until their energy requirement for maintenance is met (Sibbald, 1980; Leeson et al., 1996; Gous et al., 2018). Birds in the US No. 2 treatment in the present study consumed the amount of feed needed to meet their energy requirement, but this could restrict muscle and fat accretion seen in the lesser BWG. This concept is known as the protein- and energy-dependent phase and has been well studied, with studies describing how body protein is deposited until energy becomes limited, during which energy requirements for maintenance take precedence over AA requirements for growth (Leeson et al., 1996).

On the other hand, in previous studies with low energy diets, birds can adapt by increasing feed intake, thereby influencing growth rate (Leeson et al., 1996). Greater FI for the red/bronze treatment indicates there may have been an overestimated ME value for red/bronze grain sorghum affecting the overall efficiency observed as significantly poorer AdjFCR at 0 to 41 d (1.60) compared with the other treatments (Table 6). Previously reported data by Moritz et al. (2022) determined the nitrogen-corrected apparent ME ($\mathbf{AME_n}$) of tannin-free grain sorghum varieties, showing that values varied by feed phase. Mean AME_n values of modern grain sorghum varieties for broilers in the grower-diet phase were determined as 3,336 (red/bronze), 4,000 (white/tan), and 3,341 (US No. 2 grain sorghum) kcal/kg (as fed), respectively. In the finisher-diet phase, average AME_n values were determined as 3,001 (red/bronze), 3,599 (white/tan), and 3,705 (US No. 2 grain sorghum) kcal/kg (as fed), respectively. Determination of ME for feed ingredients is essential to adequately formulate a diet and properly satisfy the energy requirements of the bird (Sibbald, 1980).

The variable ME values for tannin-free grain sorghum used in this present study demonstrate the importance of obtaining validated ME values. Typically, previously

Table 6. Effect of dietary treatment on cumulative broiler BW gain, feed intake, and adjusted feed conversion ratio

Variable and phase	Dietary treatment				SEM (n = 8)	*P*-value
	Corn	Red/bronze	White/tan	US No. 2		
BW gain, g						
Starter						
0–7 d	166[a]	162[a]	164[a]	149[b]	3.00	0.003
0–14 d	551[a]	526[bc]	541[ab]	509[c]	7.40	0.004
Grower						
0–21 d	1,165[a]	1,109[b]	1,168[a]	1,120[b]	10.09	0.001
0–28 d	2,033[a]	1,922[b]	1,997[a]	1,938[b]	17.81	0.001
Finisher						
0–35 d	3,006[a]	2,851[b]	2,914[ab]	2,856[b]	31.10	0.001
0–41 d	3,622[a]	3,479[b]	3,518[b]	3,484[b]	28.82	0.009
Feed intake, g						
Starter						
0–7 d	185[a]	185[a]	181[a]	163[b]	4.60	0.012
0–14 d	644	641	637	605	18.00	NS
Grower						
0–21 d	1,517[a]	1,522[a]	1,484[ab]	1,449[b]	21.47	0.019
0–28 d	2,776[a]	2,770[a]	2,696[b]	2,650[b]	24.59	0.003
Finisher						
0–35 d	4,365[a]	4,402[a]	4,234[b]	4,223[b]	41.21	0.015
0–41 d	5,495[ab]	5,599[a]	5,357[c]	5,346[bc]	50.85	0.005
Adjusted feed conversion ratio, g						
Starter						
0–7 d	1.11	1.14	1.10	1.09	0.04	NS
0–14 d	1.12	1.17	1.13	1.14	0.02	NS
Grower						
0–21 d	1.28[b]	1.36[a]	1.26[b]	1.28[b]	0.01	<0.001
0–28 d	1.47[b]	1.52[a]	1.46[b]	1.46[b]	0.02	0.051
Finisher						
0–35 d	1.52[b]	1.59[a]	1.53[b]	1.54[b]	0.01	0.009
0–41 d	1.52[b]	1.60[a]	1.51[b]	1.53[b]	0.009	<0.001

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

reported or calculated ME values for ingredients are used in the formulation to estimate the ME value of a complete feed that will meet the nutrient requirements of the bird (Wu et al., 2020). Not to mention, using consistent ME values for feed ingredients is important when variations in nutrient composition exist depending on antinutritional factors, the region or environment the feedstuff is grown and sourced (Scott et al., 1998). Overall, the efficiency and utilization of a feed ingredient depend on the energy content of the diet (Kleyn, 2013); thus, deficient or overestimated energy content can affect growth and efficiency (Meloche et al., 2014).

Carcass Traits. Carcass traits were not significantly affected by corn and grain sorghum treatments ($P > 0.05$, Table 8). No differences observed in LBW, HCW, BrW, CY, or BrY are consistent with the findings of Garcia et al. (2013), Gualtieri and Rapaccini (1990), and Torres et al. (2013), who fed broilers varying levels of low-tannin or tannin-free grain sorghum.

RNA Sequencing

The mRNA-seq revealed 46 DEG (Table 9), 23 upregulated and 23 downregulated in the red/bronze-fed birds when compared with birds in the corn treatment. The GSEA using gene ontology showed an upregulation of genes that were associated with signaling receptor regulator/activator/binding activities, and receptor ligand activity in birds fed the red/bronze treatment when compared with corn-fed broilers (Figure 1). Similarly, GSEA using KEGG also showed an upregulation in neuroactive ligand receptor interaction in birds fed the red/bronze treatment. The Reactome pathway associated with metal-sequestering metallothioneins was over-represented among genes that were downregulated in broilers fed the red/bronze treatment (R-GGA-5661231 Padj = 8.346×10^{-5}).

The RNA sequencing was conducted to give preliminary insight on the effect of grain sorghum on the gene expression in the jejunum of broilers. Red/bronze grain

Table 7. Effect of dietary treatment on weekly broiler BW gain, feed consumption, and adjusted feed conversion ratio

	Dietary treatment				SEM	
Variable and phase	Corn	Red/bronze	White/tan	US No. 2	(n = 8)	*P*-value
BW gain, g						
Starter						
0–7 d	166[a]	162[a]	164[a]	149[b]	3.00	0.002
7–14 d	384[a]	363[bc]	377[ab]	360[c]	5.66	0.021
Grower						
14–21 d	622[a]	590[b]	635[a]	619[a]	7.10	0.001
21–28 d	879[a]	824[b]	840[ab]	828[b]	13.17	0.033
Finisher						
28–35 d	986	942	928	931	23.13	NS
35–41 d	433	575	521	499	67.03	NS
Feed intake, g						
Starter						
0–7 d	185[a]	186[a]	181[a]	163[b]	4.60	0.011
7–14 d	443	437	435	421	8.47	NS
Grower						
14–21 d	873[a]	880[a]	847[b]	844[b]	8.29	0.014
21–28 d	1,259[a]	1,248[ab]	1,212[bc]	1,202[c]	14.93	0.021
Finisher						
28–35 d	1,589	1,632	1,538	1,572	24.71	NS
35–41 d	1,130[b]	1,197[a]	1,103[b]	1,123[b]	21.44	0.034
Adjusted feed conversion ratio, g						
Starter						
0–7 d	1.11	1.14	1.10	1.09	0.04	NS
7–14 d	1.14	1.20	1.15	1.17	0.03	NS
Grower						
14–21 d	1.42[b]	1.50[a]	1.36[b]	1.38[b]	0.02	<0.001
21–28 d	1.48[b]	1.54[a]	1.47[b]	1.48[b]	0.01	0.040
Finisher						
28–35 d	1.66	1.75	1.69	1.72	0.03	NS
35–41 d	1.94	1.90	1.84	1.81	0.04	NS

[a–c]Means within the same column lacking a common superscript are significantly different at $P \leq 0.05$.

Table 8. Effect of dietary treatment on broiler live BW (LBW), hot carcass weight (HCW), breast weight (BrW), carcass yield (CY), and breast yield (BrY) at 42 d of age

	Dietary treatment				SEM	
Variable	Corn	Red/bronze	White/tan	US No. 2	(n = 8)	*P*-value
LBW, g	3,584	3,458	3,467	3,506	34.56	0.073
HCW,[1] g	2,796	2,659	2,671	2,707	39.20	0.064
BrW,[2] g	1,100	1,043	1,029	1,068	24.53	0.206
CY,[3] %	78.04	76.86	77.05	77.20	0.85	0.778
BrY,[3] %	39.37	39.23	38.46	39.37	0.68	0.769

[1]Carcass with skin on and bone in.

[2]Breast with skin on and bone in.

[3]Yields represent hot carcass parts relative to LBW and HCW.

Table 9. Differentially expressed genes in the jejunum of broilers fed the red/bronze treatment compared with that of broilers fed the corn treatment[1]

Ensembl_ID	Gene symbol[2]	Log_2FC	LogCPM	FDR
ENSGALG00000017184	*MMP7*	6.575	3.335	3.42E-05
ENSGALG00000028886	*LOC428593*	3.916	2.190	3.04E-03
ENSGALG00000043257	*LYGL*	3.752	6.989	9.94E-04
ENSGALG00000048869	*PTCHD3*	2.404	6.558	2.30E-02
ENSGALG00000016283	*BEND6*	2.346	1.302	3.45E-02
ENSGALG00000005257	*VCAM1*	2.173	2.073	3.94E-02
ENSGALG00000010645	*ENPP6*	2.134	6.589	1.18E-03
ENSGALG00000053764	*CYYR1*	2.008	0.120	1.75E-03
ENSGALG00000028047	*RHOV*	1.912	0.620	1.18E-03
ENSGALG00000052009	*LOC107049467*	1.815	4.325	3.64E-02
ENSGALG00000052425	*BATF3*	1.801	3.340	1.10E-02
ENSGALG00000012117	*STEAP3*	1.759	0.568	4.17E-02
ENSGALG00000016128	*B3GALT5*	1.518	4.117	1.75E-03
ENSGALG00000013268	*DNAH5L*	1.471	0.298	2.21E-02
ENSGALG00000028376	*FGF19*	1.387	6.456	3.92E-02
ENSGALG00000017085	*SLC7A1*	1.367	3.194	3.54E-02
ENSGALG00000026862	*CLDN1*	1.306	2.434	1.70E-02
ENSGALG00000007077	*CPT1A*	1.291	4.855	3.45E-02
ENSGALG00000012601	*GOLM1*	1.239	5.280	1.18E-03
ENSGALG00000000919	*PIGR*	1.235	10.270	4.81E-02
ENSGALG00000008701	*XDH*	1.115	5.725	4.03E-02
ENSGALG00000006322	*CLCA1*	1.054	11.673	1.92E-02
ENSGALG00000001975	*TMEM120A*	1.031	7.030	4.03E-02
ENSGALG00000003261	*PTRF*	−1.031	1.285	4.60E-02
ENSGALG00000015004	*RIOK3*	−1.107	9.468	4.60E-02
ENSGALG00000003053	*PRDX6*	−1.189	7.275	2.30E-02
ENSGALG00000029746	*NT5DC3*	−1.192	3.104	3.94E-02
ENSGALG00000039447	*KRT23*	−1.275	6.168	4.03E-02
ENSGALG00000027159	*CNKSR3*	−1.300	5.022	3.94E-02
ENSGALG00000003073	*RHOBTB1*	−1.304	6.627	3.94E-02
ENSGALG00000047910	NA	−1.358	3.149	5.36E-03
ENSGALG00000013568	*NR4A3*	−1.369	3.170	3.14E-02
ENSGALG00000016165	*TFF3*	−1.636	1.512	4.23E-02
ENSGALG00000032614	*ART1L3*	−1.766	2.289	3.04E-03
ENSGALG00000040044	NA	−1.811	1.292	3.45E-02
ENSGALG00000039705	*GFPT2*	−1.927	0.972	3.14E-02
ENSGALG00000016485	*RHOB*	−2.024	6.258	5.36E-03
ENSGALG00000008231	*PACSIN3*	−2.097	1.128	8.90E-03
ENSGALG00000012119	*MARCO*	−2.281	2.335	5.40E-03
ENSGALG00000047199	*RP11–400G3.5*	−2.367	5.259	2.64E-02
ENSGALG00000048390	NA	−2.446	0.335	9.58E-03
ENSGALG00000003803	*DAB2*	−2.751	1.657	2.65E-02
ENSGALG00000028451	*MT4*	−2.880	9.423	3.33E-02
ENSGALG00000050737	*SLC27A2*	−2.911	2.412	2.46E-02
ENSGALG00000048295	NA	−3.608	3.424	1.18E-02
ENSGALG00000014616	*MT3*	−4.914	5.584	1.70E-03

[1]Log_2FC = log fold change; FDR = false discovery rate. Genes were considered differentially expressed if the FDR was <0.05 and $|log_2FC|$ was >1 using the likelihood ratio test. LogCPM = log counts per million.

[2]NA = not applicable.

sorghum was specifically selected, and gene expression was compared with the corn treatment. Among the tannin-free grain sorghum varieties in this study, red/bronze is the most highly pigmented and known to have a greater content of polyphenolic compounds (Khoddami et al., 2015). Such compounds have been reported to have the ability to

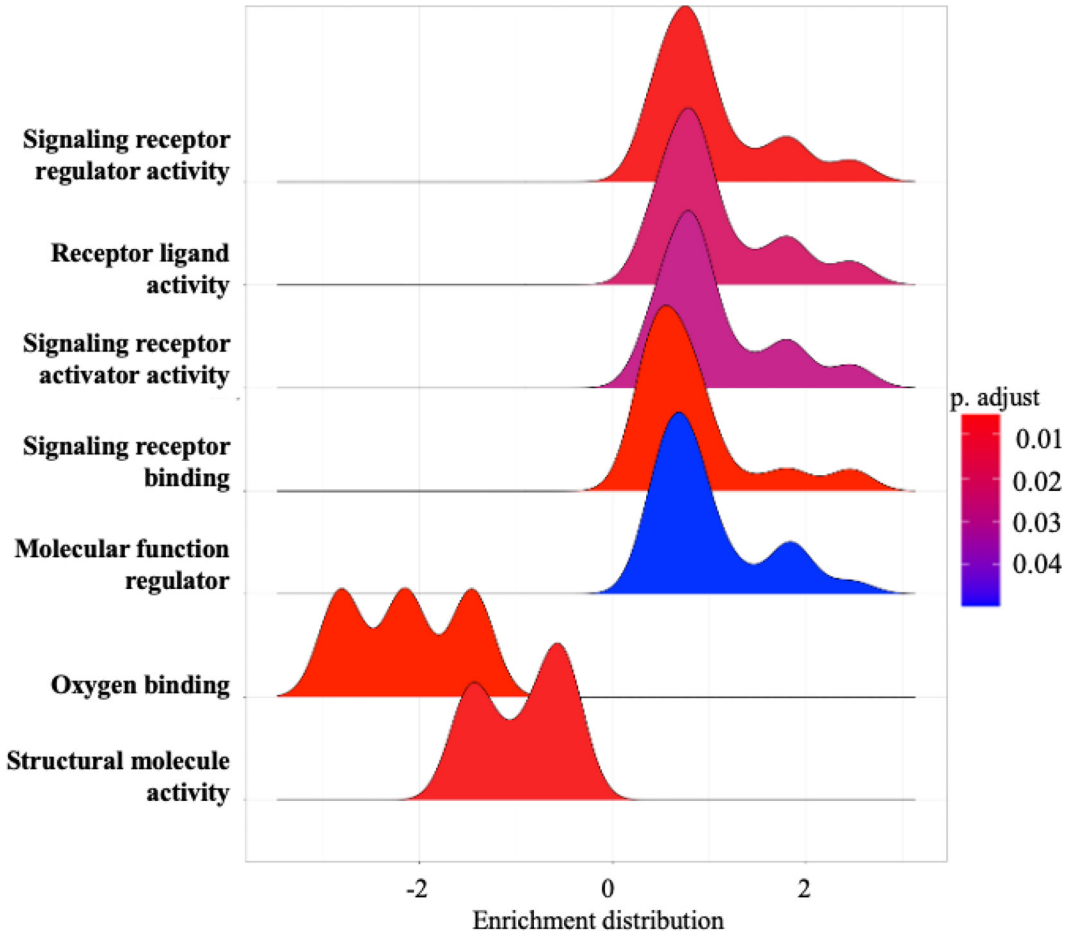

Figure 1. Density of gene ontology (GO) enrichment of expressed genes in the jejunum of broilers fed the red/bronze treatment compared with the corn treatment. p. adjust = *P*-value used in multiple hypotheses testing for the enrichment distribution of expressed genes. The figure shows the distribution of genes expressed (x-axis) that are upregulated (to the right of zero) and downregulated (to the left of zero). Those expressed genes shown in the density plot peaks were analyzed using GO to identify the gene associated functions as shown (y-axis) for upregulated and downregulated genes.

protect the host from pathogens by modulating cell signaling pathways and have inhibitory effects on *Escherichia coli* and *Salmonella enteritidis* (Al-Zoreky, 2009).

Differential expression analysis and GSEA suggest changes in metal homeostasis pathways when broilers were fed with red/bronze grain sorghum. Genes *MMP7* and *LYGL* were significantly upregulated and are associated with functions for zinc-ion binding and lysozyme activity, respectively, whereas *MT3* was significantly downregulated and is associated with metal ion binding functions. In other words, results suggest that birds fed red/bronze sorghum had an increased activity for zinc-ion binding but a decreased storage capacity. Metallothionein, in particular, is a major storage protein for Zn (Troche et al., 2015). Nutritionally, Zn is an important trace mineral but also has a functional role as a cofactor and has been known to reduce oxidative stress and inflammatory cytokines (Prasad et al., 2011; Bortoluzzi et al., 2019).

In addition, the upregulation of neuroactive ligand receptor interaction may suggest that DEG could affect the pathway for appetite regulating peptides, such as ghrelin. In broilers, ghrelin has been reported as a signaling peptide for energy utilization and maintaining energy homeostasis (Classen, 2017; Song et al., 2019). In a previous study by Song et al. (2019), increased mRNA levels of ghrelin in the duodenum, liver, and abdominal fat were reported in broilers fed a low-energy diet compared with a high-energy diet. These findings suggest that this low-energy-level diet may be a result of the observed upregulation of genes associated with appetite regulating peptides (ghrelin); thus, increased levels of genes involved in the pathway for ghrelin in the red/bronze treatment could stimulate greater feed intake. Although dietary factors can have a major influence on feed intake and it is consistently reported that decreased energy content can increase feed intake, other factors can influence feed intake including access to feed and water, health of the birds, and environmental stress (Ferket and Gernat, 2006). Overall, variability was observed within the replicate mucosa samples for the red/bronze and corn treatment groups upon expression analy-

sis, which may be a result of lack of quality and quantity of the mucosa sample for RNA extraction and sequencing and have affected the number of possible DEG.

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 (data not shown). Intestinal morphology was analyzed specifically in the jejunum of this study because this is a major site for nutrient absorption (Liu et al., 2016) and any changes on 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 indicate an increased demand for cell proliferation to maintain optimal gut function. Tannin-free grain sorghum did not affect jejunum morphology in this present study, and these results are consistent with previous findings by Torres et al. (2013) showing no effect on villus height and crypt depth in low-tannin grain sorghum diets.

APPLICATIONS

Based on the overall results in the present study, birds fed the corn treatment had a greater BWG and FI compared with those fed the grain sorghum treatments, but the replacement of corn by tannin-free grain sorghum did not adversely affect carcass traits. Also, the replacement of corn with white/tan and No. US 2 did not affect AdjFCR. Intestinal morphology was unaffected in broilers, and gene expression provides future direction for analyzing more specific genes of interest that may influence the intestinal morphology of sorghum-fed birds. Results may also allow nutritionists in the commercial poultry industry to consider using an inclusion of tannin-free grain sorghum as an alternative to corn. The inclusion of tannin-free grain sorghum in commercial poultry diets will be dependent on cost, availability, and overall interest of nutritionists and poultry integrators of its use in diets.

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LITERATURE CITED

Abdel-Moneim, A.-M. E., A. M. Shehata, S. O. Alzahrani, M. E. Shafi, N. M. Mesalam, A. E. Taha, A. A. Swelum, M. Arif, M. Fayyaz, and M. E. Abd El-Hack. 2020. The role of polyphenols in poultry nutrition. J. Anim. Physiol. Anim. Nutr. (Berl.) 104:1851–1866. https://doi.org/10.1111/jpn.13455.

AgriStats. 2022. AgriStats Monthly Live Production, July. Agri Stats Inc.

Al-Zoreky, N. S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. Int. J. Food Microbiol. 134:244–248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002.

Alshelmani, M. I., E. A. Abdalla, U. Kaka, and M. A. Basit. 2021. Nontraditional feedstuffs as an alternative in poultry feed. Pages 19–20 in Advances in Poultry Nutrition Research. IntechOpen.

Andrews, S. 2010. FASTQC. A quality control tool for high throughput sequence data. Accessed Mar. 2022. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Ashley, D., D. Marasini, C. Brownmiller, J. A. Lee, F. A. Carbonero, and S. O. Lee. 2019. Impact of grain sorghum polyphenols on microbiota of normal weight and overweight/obese subjects during in vitro fecal fermentation. Nutrients 11:217. https://doi.org/10.3390/nu11020217.

Biloni, A., C. F. Quintana, A. Menconi, G. Kallapura, J. Latorre, C. Pixley, S. Layton, M. Dalmagro, X. Hernandez-Velasco, A. Wolfenden, B. M. Hargis, and G. Tellez. 2013. Evaluation of effects of early bird associated with FloraMax-B11 on Salmonella Enteritidis, intestinal morphology, and performance of broiler chickens. Poult. Sci. 92:2337–2346. https://doi.org/10.3382/ps.2013-03279.

Bortoluzzi, C., B. Lumpkins, G. F. Mathis, M. França, W. D. King, D. E. Graugnard, K. A. Dawson, and T. J. Applegate. 2019. Zinc source modulates intestinal inflammation and intestinal integrity of broiler chickens challenged with coccidia and *Clostridium perfringens*. Poult. Sci. 98:2211–2219. https://doi.org/10.3382/ps/pey587.

Bradbury, E. J., S. J. Wilkinson, G. M. Cronin, P. C. Thomson, M. R. Bedford, and A. J. Cowieson. 2014. Nutritional geometry of calcium and phosphorus nutrition in broiler chicks. Growth performance, skeletal health and intake arrays. Animal 8:1071–1079. https://doi.org/10.1017/S1751731114001037.

Buck, S. F. 1960. A method of estimation of missing values in multivariate data suitable for use with an electronic computer. J. R. Stat. Soc. B 22:302–306. https://doi.org/10.1111/j.2517-6161.1960.tb00375.x.

Chen, J., G. Tellez, J. D. Richards, and J. Escobar. 2015. Identification of potential biomarkers for gut barrier failure in broiler chickens. Front. Vet. Sci. 2:1–10. https://doi.org/10.3389/fvets.2015.00014.

Classen, H. L. 2017. Diet energy and feed intake in chickens. Anim. Feed Sci. Technol. 233:13–21. https://doi.org/10.1016/j.anifeedsci.2016.03.004.

Corzo, A., M. W. Schilling, R. E. Loar II, L. Mejia, L. C. G. S. Barbosa, and M. T. Kidd. 2010. Responses of Cobb×Cobb 500 broilers to dietary amino acid density regimens. J. Appl. Poult. Res. 19:227–236. https://doi.org/10.3382/japr.2010-00172.

Ewels, P., M. Magnusson, S. Lundin, and M. Käller. 2016. MultiQC: Summarize analysis results for multiple tools and samples in a single report. Bioinformatics 32:3047–3048. https://doi.org/10.1093/bioinformatics/btw354.

Fagundes, N. S., R. Pereira, C. Bortoluzzi, J. M. Rafael, G. S. Napty, J. G. M. Barbosa, M. C. M. Sciencia, and J. F. M. Menten. 2017. Replacing corn with sorghum in the diet alters intestinal microbiota without altering chicken performance. J. Anim. Physiol. Anim. Nutr. (Berl.) 101:e371–382. https://doi.org/10.1111/jpn.12614.

Ferket, P. R., and A. G. Gernat. 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci. 5:905–911. https://doi.org/10.3923/ijps.2006.905.911.

Fraps, G. S. 1943. Relation of the protein, fat, and energy of the ration to the composition of chickens. Poult. Sci. 22:421–424. https://doi.org/10.3382/ps.0220421.

Garcia, R. G., A. A. Mendes, I. C. L. Almeida Paz, C. M. Komiyama, F. R. Caldara, I. A. Nääs, and W. S. Mariano. 2013. Implications of the use of sorghum in broiler production. Braz. J. Poult. Sci. 15:257–262. https://doi.org/10.1590/S1516-635X2013000300013.

Gous, R. M., A. S. Faulkner, and H. K. Swatson. 2018. The effect of dietary energy: protein ratio, protein quality and food allocation on the efficiency of utilisation of protein by broiler chickens. Br. Poult. Sci. 59:100–109. https://doi.org/10.1080/00071668.2017.1390211.

Gualtieri, M., and S. Rapaccini. 1990. Sorghum grain in poultry feeding. Worlds Poult. Sci. J. 46:246–254. https://doi.org/10.1079/WPS19900024.

Harbertson, J. F., E. A. Picciotto, and D. O. Adams. 2003. Measurement of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. Am. J. Enol. Vitic. 54:301–306.

He, W., P. Li, and G. Wu. 2021. Amino acid nutrition and metabolism in chickens. Pages 109–131 in Amino Acids in Nutrition and Health. Springer. https://doi.org/10.1007/978-3-030-54462-1_7.

Hulan, H. W., and F. G. Proudfoot. 1982. Nutritive value of sorghum grain for broiler chickens. Can. J. Anim. Sci. 62:869–875. https://doi.org/10.4141/cjas82-105.

Jackson, S., J. D. Summers, and S. Leeson. 1982. Effect of dietary protein and energy on broiler carcass composition and efficiency of nutrient utilization. Poult. Sci. 61:2224–2231. https://doi.org/10.3382/ps.0612224.

Khoddami, A., H. H. Truong, S. Y. Liu, T. H. Roberts, and P. H. Selle. 2015. Concentrations of specific phenolic compounds in six red sorghums influence nutrient utilisation in broiler chickens. Anim. Feed Sci. Technol. 210:190–199. https://doi.org/10.1016/j.anifeedsci.2015.09.029.

Kidd, M. T., C. D. McDaniel, S. L. Branton, E. R. Miller, B. B. Boren, and B. I. Fancher. 2004. Increasing amino acid density improves live performance and carcass yields of commercial broilers. J. Appl. Poult. Res. 13:593–604. https://doi.org/10.1093/japr/13.4.593.

Kim, D., J. M. Paggi, C. Park, C. Bennett, and S. L. Salzberg. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 37:907–915. https://doi.org/10.1038/s41587-019-0201-4.

Kleyn, R. 2013. Chicken Nutrition: A Guide for Nutritionists and Poultry Professionals. Context.

Leeson, S., L. Caston, and J. D. Summers. 1996. Broiler response to diet energy. Poult. Sci. 75:529–535. https://doi.org/10.3382/ps.0750529.

Li, T., G. Xing, Y. Shao, L. Zhang, S. Li, L. Lu, Z. Liu, X. Liao, and X. Luo. 2020. Dietary calcium or phosphorus deficiency impairs the bone development by regulating related calcium or phosphorus metabolic utilization parameters of broilers. Poult. Sci. 99:3207–3214. https://doi.org/10.1016/j.psj.2020.01.028.

Liao, Y., G. K. Smyth, and W. Shi. 2014. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930. https://doi.org/10.1093/bioinformatics/btt656.

Liu, S. Y., H. H. Truong, A. Khoddami, A. F. Moss, P. C. Thomson, P. H. Roberts, and P. H. Selle. 2016. Comparative performance of broiler chickens offered ten equivalent diets based on three grain sorghum varieties as determined by response surface mixture design. Anim. Feed Sci. Technol. 218:70–83. https://doi.org/10.1016/j.anifeedsci.2016.05.008.

Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier III.. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poult. Sci. 93:1428–1439. https://doi.org/10.3382/ps.2013-03712.

Moritz, A. H., S. K. Krombeen, B. Presgraves, M. E. Blair, R. E. Buresh, R. M. Kaminski, W. C. Bridges, M. Arguelles-Ramos, and T. A. Wilmoth. 2022. Apparent metabolizable energy and performance of broilers fed selected grain sorghum varieties. Appl. Anim. Sci. 38:268–278. https://doi.org/10.15232/aas.2022-02271.

Noy, Y., and D. Sklan. 1998. Metabolic responses to early nutrition. J. Appl. Poult. Res. 7:437–451. https://doi.org/10.1093/japr/7.4.437.

Ollion, J., J. Cochennec, F. Loll, C. Escudé, and T. Boudier. 2013. TANGO: A generic tool for high-throughput 3D image analysis for studying nuclear organization. Bioinformatics 29:1840–1841. https://doi.org/10.1093/bioinformatics/btt276.

Oviedo-Rondón, E. O. 2019. Holistic view of intestinal health in poultry. Anim. Feed Sci. Technol. 250:1–8. https://doi.org/10.1016/j.anifeedsci.2019.01.009.

Prasad, A. S., B. Bao, F. W. Beck, and F. H. Sarkar. 2011. Zinc-suppressed inflammatory cytokines by induction of A20-mediated inhibition of nuclear factor-κB. Nutrition 27:816–823. https://doi.org/10.1016/j.nut.2010.08.010.

Raudvere, U., L. Kolberg, I. Kuzmin, T. Arak, P. Adler, H. Peterson, and J. Vilo. 2019. g:Profiler: a web server for functional enrichment analysis and conversions of gene lists. Nucleic Acids Res. 47(W1):W191–W198. https://doi.org/10.1093/nar/gkz369 Accessed 13March2022.

Robinson, M. D., D. J. McCarthy, and G. K. Smyth. 2010. EdgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140. https://doi.org/10.1093/bioinformatics/btp616.

Rokyta, D. R., A. R. Lemmon, M. J. Margres, and K. Aronow. 2012. The venom-gland transcriptome of the eastern diamondback rattlesnake (*Crotalus adamanteus*). BMC Genomics 13:1–23. https://doi.org/10.1186/1471-2164-13-312.

Scott, T. A., F. G. Silversides, H. L. Classen, M. L. Swift, M. R. Bedford, and J. W. Hall. 1998. A broiler chick bioassay for measuring the feeding value of wheat and barley in complete diets. Poult. Sci. 77:449–455. https://doi.org/10.1093/ps/77.3.449.

Shen, S., R. Huang, C. Li, W. Wu, H. Chen, J. Shi, S. Chen, and X. Ye. 2018. Phenolic compositions and antioxidant activities differ significantly among sorghum grains with different applications. Molecules 23:1203. https://doi.org/10.3390/molecules23051203.

Shields, L., Y. Gang, K. Jordan, S. Sapkota, L. Boatwright, X. Jiang, S. Kresovich, and R. Boyles. 2021. Genome-wide association studies of antimicrobial activity in global sorghum. Crop Sci. 61:1301–1316. https://doi.org/10.1002/csc2.20348.

Sibbald, I. R. 1980. Metabolizable energy in poultry nutrition. Bioscience 30:736–741. https://doi.org/10.2307/1308333.

Smith, E. R., and G. M. Pesti. 1998. Influence of broiler strain cross and dietary protein on the performance of broilers. Poult. Sci. 77:276–281. https://doi.org/10.1093/ps/77.2.276.

Song, X., H. Jiao, J. Zhao, X. Wang, and H. Lin. 2019. Ghrelin serves as a signal of energy utilization and is involved in maintaining energy homeostasis in broilers. Gen. Comp. Endocrinol. 272:76–82. https://doi.org/10.1016/j.ygcen.2018.11.017.

Torres, K. A. A., J. M. Pizauro Jr., C. P. Soares, T. G. A. Silva, W. C. L. Nogueira, D. M. B. Campos, R. L. Furlan, and M. Macari. 2013. Effects of corn replacement by sorghum in broiler diets on performance and intestinal mucosa integrity. Poult. Sci. 92:1564–1571. https://doi.org/10.3382/ps.2012-02422.

Troche, C., S. D. Eicher, and T. J. Applegate. 2015. The influence of dietary zinc source and coccidial vaccine exposure on intracellular zinc homeostasis and immune status in broiler chickens. Br. J. Nutr. 114:202–212. https://doi.org/10.1017/S0007114515001592.

Valable, A. S., A. Narcy, M. J. Duclos, C. Pomar, G. Page, Z. Nasir, M. Magnin, and M. P. Létourneau-Montminy. 2018. Effects of dietary calcium and phosphorus deficiency and subsequent recovery on broiler chicken growth performance and bone characteristics. Animal 12:1555–1563. https://doi.org/10.1017/S1751731117003093.

Venäläinen, E., J. Valaja, and T. Jalava. 2006. Effects of dietary metabolisable energy, calcium and phosphorus on bone mineralisation, leg weakness and performance of broiler chickens. Br. Poult. Sci. 47:301–310. https://doi.org/10.1080/00071660600741776.

Wu, G. 2013. Amino acids: Biochemistry and Nutrition. CRC Press.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: A critical review. Poult. Sci. 99:385–406. https://doi.org/10.3382/ps/pez511.

Yan, F., R. Angel, C. Ashwell, A. Mitchell, and M. Christman. 2005. Evaluation of the broiler's ability to adapt to an early moderate deficiency of phosphorus and calcium. Poult. Sci. 84:1232–1241. https://doi.org/10.1093/ps/84.8.1232.

Yu, G., L. G. Wang, Y. Han, and Q. Y. He. 2012. clusterProfiler: An R package for comparing biological themes among gene clusters. OMICS 16:284–287. https://doi.org/10.1089/omi.2011.0118.

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