Effects of feeding distillers dried grains with solubles to broilers from 0 to 28 days posthatch on broiler performance, feed manufacturing efficiency, and selected intestinal characteristics[1]

R. E. Loar II,* J. S. Moritz,† J. R. Donaldson,‡ and A. Corzo*[2]

*Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762;
†Department of Animal and Nutritional Science, West Virginia University, Morgantown 26506;
and ‡Department of Biological Sciences, Mississippi State University, Mississippi State, MS 39762

ABSTRACT This study evaluated the effect of 2 levels (0 vs. 8%) of distillers dried grains with solubles (DDGS) in a starter broiler diet (0 to 14 d; 45 replicates/treatment) after these same birds were subsequently fed a grower diet (14 to 28 d) with either 0, 7.5, 15, 22.5, or 30% DDGS (9 replicates/treatment). Ross × Ross 308 male broilers were used in this experiment, and evaluation criteria consisted of feed mill parameters, broiler growth, relative liver weight, ileal viscosity, and cecal content count of *Clostridium perfringens* and *Escherichia coli* analyzed by both selective media and real-time PCR. Increased inclusion of DDGS resulted in a nonlinear response for production rate ($P < 0.05$), conditioner energy usage ($P < 0.01$), and pellet mill energy usage ($P < 0.05$). Increasing DDGS resulted in a linear decrease in pellet quality ($P < 0.001$) and an increase ($P < 0.001$) in total fines. Inclusion of DDGS decreased ($P < 0.001$) energy usage at the pellet mill and decreased ($P < 0.05$) bulk density of the diets. The DDGS levels fed during the starter phase (0 vs. 8%) had no effect on the broilers at 14 or at 28 d of age. Increasing DDGS inclusion levels during the grower phase resulted in a linear decrease ($P < 0.001$) in BW gain and liver relative weight ($P < 0.001$). A DDGS starter × grower interaction ($P < 0.05$) was observed for feed consumption, in which birds that consumed no DDGS during the starter phase exhibited a decrease in feed consumption with the higher inclusion levels of DDGS during the grower phase, whereas birds that received 8% DDGS during the starter phase were unaffected by DDGS inclusion level in the grower phase. Feed conversion, mortality, ileal viscosity, and cecal *C. perfringens* and *E. coli* concentrations were unaffected by DDGS level in the grower diet. The feed intake response suggests a beneficial effect of exposing broiler chicks to DDGS if inclusion levels of 22.5% or higher are to be fed after 14 d of age. However, the data suggest that the young broiler can be negatively affected with inclusion levels of 15% DDGS or higher up to 28 d of age.

Key words: distillers dried grains with solubles, *Clostridium perfringens*, *Escherichia coli*, viscosity

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INTRODUCTION

The production of ethanol continues to increase dramatically. There is also an increased interest in the primary by-product associated with its production, distillers dried grains with solubles (**DDGS**). There have been major increases in the production of ethanol feed co-products, with DDGS representing the majority, and estimates are that the increases will continue (Renewable Fuels Association, 2008). Although it is not certain how long this trend will continue, it is only reasonable to take advantage of this economic and readily available feedstuff. With any new product, there are limitations in its use. An initial limitation is associated with feed throughput and pellet quality. Behnke (2007) suggested that once DDGS in the diet exceeds levels of 5 to 7%, pellet throughput, as well as pellet quality, may be negatively affected. However, little data exist to support these observations. Beyond the simple physical factors of pellet quality and throughput, it has been well documented that nutritional variability among DDGS sources can become a major issue when formulating diets of growing broilers (Cromwell et al., 1993; Batal and Dale, 2006; Fastinger et al., 2006).

Food safety is a major issue throughout all aspects of the food industry and is constantly on the mind of

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[2]Corresponding author: acorzo@poultry.msstate.edu

any major integrator. A major portion of food safety is concerned with the presence of pathogenic bacteria and the prevention of these bacteria in different products. Two pathogens of particular interest to the broiler industry are *Escherichia coli* and *Clostridium perfringens*. Although there is little or no research as to the effects that DDGS inclusion in the diet can have on the colonization of these bacteria in poultry, the same is not true of beef cattle. Jacob et al. (2008) reported a positive association between the use of DDGS in the diet of feedlot cattle and the prevalence of *E. coli* O157 in fecal material.

It is of utmost importance that we understand the potential limitation and challenges of using high inclusion levels of DDGS in broiler diets. This study was designed to address some of those concerns: 1) evaluate the effects of varying levels of DDGS on pelleting characteristics and feed mill efficiency, 2) observe the effects on performance caused by feeding increasing levels of DDGS (0, 7.5, 15, 22.5, and 30%) to growing broilers from 14 to 28d of age after being fed 0 or 8% DDGS during the starter phase, and 3) determine the effects that various levels of DDGS may have in the grower diet on intestinal viscosity and cecal populations of *E. coli* and *C. perfringens* of young broilers.

MATERIALS AND METHODS

Feed Milling

This portion of the study was conducted over a 4-d period at the West Virginia University pilot feed mill in Morgantown. Equipment used included the following: Weigh-Tronix stationary feed mill SFM-2000 (Avery Weigh-Tronix, Fairmont, MN): integrated hammer mill, scale, microingredient mixer; 15-horsepower horizontal shaft hammer mill (screen size: 1/8 in.); 907.2-kg-capacity single-screw vertical mixer in series with a pellet mill; CPM 2288A master model pellet mill (California Pellet Mill Co., Crawfordsville, IN) with a 40-horsepower main drive motor, 30.48-cm diameter, 0.476 × 4.496-cm die. There were 4 separate grower-phase diets that varied in DDGS inclusion level: 0, 15, 30, and 30 plus 2% sand. This study was designed as a Latin square and treatments were blocked by day of production and run order. Each treatment was replicated 4 times, with each batch being 453.6 kg and representing an experimental unit. The treatment utilizing the 2% sand addition was included to see if the sand could act similar to rock phosphate sources by providing a pellet die scouring effect. Initially, the 0 and 30% DDGS diets were made and the 15% DDGS diet was the result of blending. The 0 and 30% DDGS diets manufactured are shown in Table 1. These same diets would later be used to manufacture the diets that would be fed during the growout portion of the study. All diets were mixed for 15 min in a single-screw vertical mixer. Before pelleting, all diets were batched into their 453.6-kg aliquots in mash form. For the 30% DDGS plus sand treatment, sand was included at the expense of the total diet. The sand used had an average particle size of 450 μm. Once all feed was batched, each individual batch was transferred back into the mixer where it was then conveyed to the conditioner-pellet mill.

Mash was conditioned to a steady-state temperature of 82°C (180°F). Steam pressure at the gauge was 262 kPa (38 psi) through use of a globe valve. Feed temperature was monitored with a digital thermometer inserted directly into the stream of conditioned mash and was controlled by throttling steam into the conditioner using a ball valve. Rate of feed entering the conditioner was held constant across all treatments. Pellets were formed using a California Pellet Mill [California Pellet Mill Co., 1.295-m-length, 0.31-m-diameter short-term CPM conditioner (3 steam inlet ports), 429 rpm shaft speed; 21 picks; 10-s feed retention time] and were cooled on a horizontal belt cooler using forced ambient air. Relative electrical energy usage at both the conditioner and pellet mill were determined using Powerlogic power meters (Schneider Electric, LaVergne, TN) attached to the 3-phase leads of the pellet mill main drive and conditioner motor (Square D, Schneider Electric). Production rate, percentage of fines, and bulk density were also estimated. One representative bag from each manufacturing run was reserved for determination of pellet quality, as measured by pellet durability index and modified pellet durability index. Pellet quality was assessed on the day of manufacture via a tumbling box according to ASAE standard S269.4 (ASAE, 1997). Because of the use of a 3/16 × 1.5-in. die, pellets were sifted in a No. 6 American Society for Testing and Materials screen. The modified pellet durability index was determined in a similar manner, with the exception of adding five 13-mm hex nuts to the pretumbled sample to obtain added pellet agitation (ASAE, 1997).

Growout

The growout portion of the study encompassed the period between 0 to 28 d of age using Ross × Ross 308 males obtained from a commercial hatchery. One-day-old chicks were randomly placed in each of 90 floor pens (15 birds/pen; 1,350 birds total; 0.07 m^2/bird). The close-sided house had thermostatically controlled heating, cool cells, and cross ventilation. Each pen contained built-up litter, a hanging feeder (22.5-kg capacity), and nipple drinkers (3 nipples/pen). The lighting program was 23L:1D and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via in ovo administration at d 18) as well as Newcastle disease and infectious bronchitis (via coarse spray at hatch).

To ensure accurate formulation of the experimental diets, samples of DDGS, corn, soybean meal, and ProPlus (H. J. Baker & Bro. Inc., Little Rock, AR) were analyzed for total amino acids and CP composition (AOAC, 2006). Digestible amino acid values were calculated from published digestible coefficients (Ajino-

Table 1. Experimental starter- and grower-phase diet composition of diets varying in distillers dried grains with solubles (DDGS) inclusion levels (% as is)

	Starter diets (0 to 14 d)		Grower diets (14 to 28 d)	
Item	0% DDGS	8% DDGS	0% DDGS	30% DDGS
Ingredients				
Corn	58.0	53.0	63.7	38.7
Soybean meal (48% CP)	35.1	32.2	28.9	22.1
DDGS	—	8.0	—	30.0
ProPlus[1]	1.50	1.50	2.50	2.50
Poultry fat	2.99	2.57	1.90	3.88
Defluorinated phosphorus	1.52	1.36	1.37	0.73
Calcium carbonate	0.04	0.29	0.63	1.19
Premix[2]	0.25	0.25	0.25	0.25
NaCl	0.24	0.09	0.22	0.12
DL-Methionine	0.21	0.30	0.21	0.14
L-Lysine HCl	0.06	0.30	0.18	0.26
Coccidiostat[3]	0.05	0.05	0.05	0.05
L-Threonine	—	0.08	0.05	0.03
Calculated composition				
AME (kcal/kg)	3,100	3,100	3,125	3,125
Available P (%)	0.46	0.46	0.45	0.45
Ca (%)	0.92	0.92	0.90	0.90
CP (%)	23.2	23.2	21.4	24.0
Digestible TSAA (%)	0.90	0.90	0.82	0.82
Digestible Lysine (%)	1.25	1.25	1.14	1.14
Digestible Threonine (%)	0.84	0.88	0.74	0.74

[1]Animal protein blend, with a CP value of 60% (H. J. Baker & Bro. Inc., Little Rock, AR).

[2]The vitamin and mineral premix contained the following per kilogram of diet: retinyl acetate, 2,654 μg; cholecalciferol, 110 μg; DL-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; vitamin B_{12}, 0.01 mg; folic acid, 0.6 μg; choline, 379 mg; D-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin, 33 mg; thiamin, 1.0 mg; D-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; and selenium, 0.3 mg.

[3]Dietary inclusion of coccidiostat provides 60 g of salinomycin sodium per 907.2 kg of feed.

moto Heartland LLC, 2004) by using the analyzed total amino acid content of the ingredients. Crude protein was not assigned a minimum value during formulation, and essential digestible amino acids were maintained in all dietary treatments by setting minimum formulation ratios relative to digestible Lys as follows: TSAA, 75; Thr, 65; Val, 78; Ile, 68; Trp, 17; and Arg, 105 and following previously published recommendations (Lemme et al., 2004). All other essential nutrients were formulated to meet or exceed nutrient recommendations (NRC, 1994). Upon receiving the results for CP and amino acid analysis of the feed ingredients, the nutrient matrix was updated and the feed formulas were solved using linear programming (Table 1).

During the starter phase (0 to 14 d), 2 DDGS inclusion levels (0 vs. 8%) were fed to the 90 floor pens (2 treatments; 45 replicates/treatment). Subsequently, each of these 2 DDGS levels provided during the starter phase was fed 0, 7.5, 15, 22.5, or 30% DDGS during the grower phase (14 to 28 d), resulting in a 2 × 5 factorial study (2 DDGS in starter × 5 DDGS in grower), and was replicated 9 times for a total of 90 experimental units. The feed was provided to the birds from 0 to 14 d of age in crumbles and from 14 to 28 d as pellets (Table 1). Treatments were blocked completely, according to location within the house. Table 1 shows the 0 and 30% DDGS grower diets used in both the feed milling and growout portions of the study. The 7.5, 15, and 22.5% DDGS grower diets were the result of blending between the 0 and 30% grower diets. Feed and water were provided for ad libitum consumption.

All birds in each pen were weighed collectively at the beginning and end of each feed phase. Feed consumption and mortality were monitored throughout the study and feed conversion was corrected for the weight of mortality and represents the following: (g of feed consumed by all birds in a pen)/(g of BW per pen + weight of dead birds). All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Bacterial Quantification

The *E. coli* strain 25922 and the *C. perfringens* strain 13124 were acquired through the American Type Culture Collection (Manassas, VA). *Escherichia coli* was cultured on trypticase soy agar at 37°C under aerobic conditions and *C. perfringens* was cultured on reinforced clostridial medium at 37°C under anaerobic conditions. Anaerobic conditions were established using a Mitsubishi Anaeropak jar (Mitsubishi Gas Chemical America Inc., New York, NY) and anaerobic indicators were used to ensure that these conditions were maintained during the incubation periods.

At 28 d of age, 1 bird per pen was randomly selected and killed via cervical dislocation. Ceca were removed and weighed pre and postextraction of contents. Cecal contents were diluted 1:10 in sterile PBS (1 mL total

volume) and vortexed for 1 min at maximum setting to homogenize the sample. Dilutions of the samples were then plated on selective media. For detection of *E. coli* in the samples, dilutions of the cells were plated on MacConkey agar supplemented with MUG (Remel, Lenexa, KS) and incubated for 24 h at 37°C. For detection of *C. perfringens* in the samples, dilutions of the cells were plated on Perfringens agar base with TSC selective supplement (Oxoid, Basingstoke, Hampshire, UK) for 24 h at 37°C under anaerobic conditions. Anaerobic conditions were established and monitored as described previously. *Clostridium perfringens* colonies were counted based on the appearance of black and opaque colonies. *Escherichia coli* colonies were counted after examination with a long-wave UV lamp for confirmation.

Deoxyribonucleic acid was isolated from 24-h cultures of *E. coli* 25922 and *C. perfringens* 13124 for standardizations using the DNeasy tissue kit, following the manufacturer's included protocol, from Qiagen (Hilden, Germany). Deoxyribonucleic acid from the cecal samples collected and analyzed above by selective media was isolated using the QIAamp DNA stool mini kit (Qiagen) following the manufacturer's included protocol. Briefly, intestinal samples diluted 1:10 in sterile PBS were treated with 0.5% Tween 20, vortexed for 1 min, and incubated at room temperature for 10 min. After this initial lysis period, 200 μL of sample was used for DNA isolation following the manufacturer's protocol (Qiagen). Primer and MGB probe sets for *C. perfringens* and *E. coli* were designed against the 16S rDNA of each strain using the Applied Biosystems Custom Taqman Assay Design Tool (Applied Biosystems, Carlsbad, CA). Sequences for each set are listed in Table 2. Quantitative PCR was performed separately for *C. perfringens* and *E. coli* assay sets for all cecal samples and bacterial standards. Each reaction contained the following: 9 μL of genomic DNA, 1 μL of assay mix (specific for either *C. perfringens* or *E. coli*), and 10 μL of Taqman Universal PCR Master Mix (Applied Biosystems). Standard curves were generated for *C. perfringens* and *E. coli* using DNA from pure cultures diluted 1:2, starting with 100-ng concentrations. Reactions were performed with a StepOne system from Applied Biosystems under standard cycling conditions: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Quantitations of *C. perfringens* and *E. coli* present in cecal samples were calculated by applying the threshold cycle (**Ct**) values generated to the standard curve of the corresponding target using the software associated with the StepOne system. It should be noted that Ct values represent an inverse response; therefore, a higher Ct value is not indicative of a higher concentration but rather a lower one. The experimental unit for bacterial quantification by either selective media or qPCR corresponded to the bird that was randomly selected from each replicate pen.

Viscosity and Liver Measurements

At 28 d of age, 2 birds per pen were randomly selected. Each bird was weighed individually and killed via cervical dislocation before having the liver removed and individually weighed to determine the relative liver weight. At this time, each bird also had the contents of the ileum (Meckel's diverticulum to ileocecal junction) removed and the ileal contents of both birds were placed in a common container and homogenized via manual mixing. After thorough mixing, two 1.5-mL Eppendorf tubes were filled with the digestive contents and centrifuged at 14,500 × *g* for 2 min. After centrifugation was complete, 250 μL of the supernatant was transferred to the sample cup of a Brookfield digital viscometer (model LVDV-II+P CP, Brookfield Engineering Laboratories Inc., Middleboro, MA) for a total sample volume of 0.5 mL. Based on previous research by Bedford and Classen (1993), viscosity (in centipoise, cp = 1/100 dyne second per cm^2) was determined at a shear rate of 60 s^{-1} at 37°C (Table 3).

Statistical Analysis

The feed mill portion of the study was analyzed using the PROC GLM option of SAS software (SAS Institute, 2004) with a *P*-value ≤0.05 indicating significance. The data were tested for linear and quadratic contrasts using the 3 DDGS inclusion levels (0, 15, and 30%) and without the 30% DDGS + sand treatment. When overall significant differences ($P < 0.05$) existed among all 4 treatments, Fisher's least significant difference option of SAS was used to separate treatment means (SAS Institute, 2004). Treatments were blocked by day of production and run order, thus replicating each treatment 4 times, with each 453.6-kg batch rep-

Table 2. Primers used for the detection of *Escherichia coli* and *Clostridium perfringens* by real-time PCR

Primer or probe	Target gene	Sequence (5′→3′)
Primers		
APEC_R	*E. coli* 16S RNA	GTGGACTACCAGGGTATCTAATCCT
APEC_F		CCCCCTGGACGAAGACTGA
CPERF_R	*C. perfringens* 16S RNA	GTGGACTACCAGGGTATCTAATCCT
CPERF_F		GCGACTCTCTGGACTGTAACTG
Probes		
APEC_M FAM	*E. coli* 16S RNA	TCCCCACGCTTTCG
CPERF_M FAM	*C. perfringens* 16S RNA	CTCCCCACGCTTTCG

resenting an experimental unit. Data generated from the growout and subsample collection for analysis of *E. coli*, *C. perfringens*, and ileal viscosity were evaluated using a 2-way ANOVA (DDGS in starter phase vs. DDGS in grower phase) in a randomized complete block design with the pen representing an experimental unit and using the PROC GLM option of SAS software (SAS Institute, 2004) with a P-value ≤ 0.05 indicating significance. The data were also tested for linear and quadratic contrasts with incremental levels of DDGS during the grower phase (0, 7.5, 15, 22.5, and 30%).

RESULTS

To prevent confusion for the reader in the results, discussion, and tables, we would like to further define some aspects of the experimental methods. The data presented and discussed in Figure 1 and Tables 3 and 4 correspond to data obtained at 28 d of age. However, one of the aims of this study was to explore potential carryover effects of feeding 0 versus 8% DDGS levels from 0 to 14 d. Therefore, a main effect noted in the text and illustrations as pregrower will correspond to the effect that feeding 0 versus 8% DDGS during the starter phase (0 to 14 d) had on the performance of the broilers during the grower phase (14 to 28 d). As a reminder, grower-phase levels of DDGS consisted of 0, 7.5, 15, 22.5, or 30%.

Significant results were obtained for all milling variables measured (Table 5). Quadratic contrast analysis showed that increasing levels of DDGS resulted in a nonlinear response for production rate ($P < 0.05$), conditioner energy usage ($P < 0.01$), and pellet mill energy usage ($P < 0.05$). Inclusion levels of 30% DDGS resulted in a decrease in pellet quality ($P < 0.001$) and pellet mill energy usage ($P < 0.001$) and an increase ($P < 0.02$) in conditioner electrical energy usage and amount of total fines ($P < 0.001$). The control diet resulted in greater pellet mill relative energy ($P < 0.001$) usage and diet bulk density ($P < 0.05$) compared with the diets containing 15 and 30% DDGS. Analysis of the data suggests that the use of sand did not have any effect when added to a diet with 30% DDGS diet when compared with the diet that was 30% devoid of it (Table 5).

At the end of the starter phase (14 d), analysis of the data showed that BW (0% DDGS = 362 g; 8% = 363

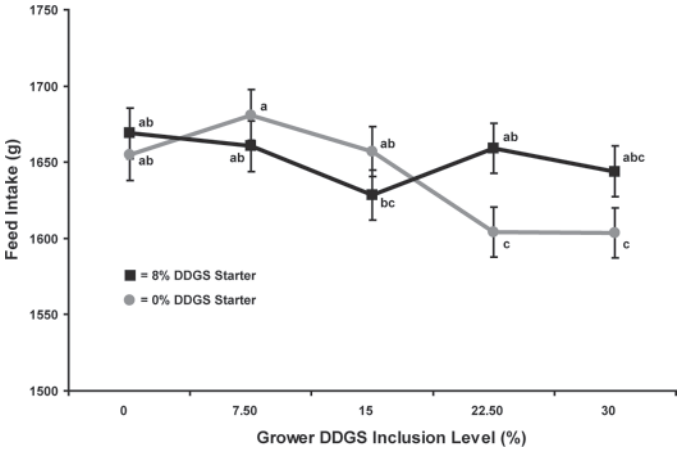

Figure 1. Interactive effects of feed consumption during the grower phase (from 14 to 28 d) in broilers fed various levels of distillers dried grains with solubles (DDGS) during the grower phase and with or without DDGS during the starter phase (from 0 to 14 d). Points not sharing a common superscript differ ($P < 0.05$).

Table 3. Live production, relative liver weight, and ileal viscosity at 28 d of age of broilers fed various levels of distillers dried grains with solubles (DDGS) from 0 to 28 d of age

Item	BWG[1] from 14 to 28 d (g)	Feed intake from 14 to 28 d (g)	Feed conversion from 14 to 28 d	Mortality from 14 to 28 d (%)	Relative liver weight[2] (%)	Viscosity (cp[3])
Grower DDGS (%)						
0	1,073^{ab}	1,661^{ab}	1.55	0.78	2.49^{a}	1.89
7.5	1,077^{a}	1,671^{a}	1.55	0.78	2.46^{ab}	1.99
15	1,053^{bc}	1,643^{abc}	1.56	1.81	2.41^{abc}	1.99
22.5	1,048^{c}	1,632^{bc}	1.56	0.00	2.37^{bc}	2.07
30	1,026^{d}	1,629^{c}	1.57	0.78	2.36^{c}	2.13
SEM	7.7	11.7	0.006	0.695	0.033	0.090
Pregrower						
No (0%)	1,053.4	1,644	1.55	0.50	2.41	2.02
Yes (8%)	1,058.5	1,652	1.56	1.19	2.43	2.02
SEM	4.9	7.4	0.004	0.437	0.021	0.056
ANOVA			*P*-value			
Pregrower DDGS	0.29	0.25	0.09	0.29	0.57	0.95
Grower DDGS	<0.001	0.03	0.20	0.56	0.02	0.44
Pregrower × grower	0.08	0.04	0.28	0.85	0.76	0.64
DDGS grower linear	<0.001	0.008	0.05	0.76	0.0004	0.07
DDGS grower quadratic	0.25	0.83	0.84	0.63	0.64	0.96

[a–d]Means within a column not sharing a common superscript differ ($P < 0.05$).

[1]Body weight gain.

[2]Expressed as a percentage of total BW.

[3]Centipoise, cp = 1/100 dyne second per square centimeter.

Table 4. *Clostridium perfringens* and *Escherichia coli* present at 28 d of age in broilers fed various levels of distillers dried grains with solubles (DDGS) from 14 to 28 d of age

Item	*E. coli* ($\log_{10}$ cfu/g of cecal contents)	*C. perfringens* ($\log_{10}$ cfu/g of cecal contents)	*E. coli* (Ct[1] values)	*C. perfringens* (Ct values)
Grower DDGS (%)				
0	6.33	4.46	23.09	24.29
7.5	5.61	4.28	23.78	24.47
15	5.99	4.04	22.31	23.66
22.5	6.25	4.44	24.14	23.81
30	5.93	4.19	23.54	23.60
SEM	0.23	0.21	0.59	0.37
Pregrower				
No (0%)	5.87	4.17	23.27	23.98
Yes (8%)	6.21	4.41	23.44	23.97
SEM	0.15	0.13	0.37	0.23
ANOVA			*P*-value	
Pregrower DDGS	0.11	0.23	0.72	0.97
Grower DDGS	0.20	0.57	0.24	0.35
Pregrower × grower	0.24	0.23	0.92	0.78
DDGS linear	0.76	0.59	0.49	0.07
DDGS quadratic	0.45	0.56	0.69	0.89

[1]Threshold cycle.

g; SEM = 4.0), feed consumption (0% DDGS = 455 g; 8% = 464 g; SEM = 5.0), or feed conversion (0% DDGS = 1.25; 8% = 1.27; SEM = 0.007) were not different ($P > 0.05$).

With one exception of feed consumption, the results indicate that the different DDGS levels fed during the starter phase did not affect how birds performed from 14 to 28 d of age. At 28 d of age, increasing dietary DDGS linearly decreased BW gain ($P < 0.001$) and liver relative weight ($P < 0.001$) (Table 3). There was also a linear trend ($P = 0.05$) suggesting an increase in feed conversion with incremental DDGS levels to the grower phase diet. Mortality and viscosity were unaffected by DDGS grower phase level. The only pregrower DDGS × grower DDGS interaction ($P < 0.05$) observed during the study was for feed consumption, in which birds that consumed no DDGS during the starter phase had a decrease in feed consumption at 22.5 and 30% DDGS levels during the grower phase when compared with birds that did consume DDGS in the starter phase (Figure 1). Bacterial levels of both *E. coli* and *C. perfringens*, measured through selective media or qPCR, were shown to be unaffected by dietary DDGS (Table 4). However, a marginal linear ($P = 0.07$) trend was observed for *C. perfringens* with increasing levels of DDGS when analyzed via qPCR, showing a Ct value that decreased with increasing inclusion of DDGS, therefore suggesting that the presence of cecal *C. perfringens* linearly

Table 5. Effects of various levels of distillers dried grains with solubles (DDGS) on feed mill efficiency and pellet quality of grower-phase broiler diets[1]

Item	Production rate (metric tons/h)	Conditioner relative energy usage (kWh/MT)	Pellet mill relative energy usage (kWh/MT)	PDI[2] (%)	MPDI[3] (%)	Bulk density (kg/m^3)	Total fines[4] (%)
DDGS (%)							
0	1.211	0.659^{bc}	6.531^{a}	74.4^{a}	56.3^{a}	631.8^{a}	30.8^{c}
15	1.266	0.646^{c}	5.127^{b}	66.8^{b}	43.5^{b}	622.8^{b}	41.7^{b}
30	1.143	0.749^{a}	4.775^{c}	62.1^{c}	34.1^{c}	618.3^{b}	54.2^{a}
30 + sand[5]	1.149	0.723^{ab}	5.019^{bc}	62.3^{c}	37.5^{c}	616.9^{b}	54.5^{a}
SEM	0.013	0.007	0.0798	0.54	1.50	2.60	1.52
P-value	0.07	0.02	0.001	0.001	0.001	0.02	0.001
Linear contrast[6]	0.039	0.003	0.001	0.001	0.002	0.001	0.002
Quadratic contrast	0.013	0.007	0.013	0.12	0.42	0.07	0.69

[a–c]Means within a column not sharing a common superscript differ ($P \leq 0.05$).

[1]Observed means correspond to a treatment mean obtained from 4 replicate batches.

[2]Pellet durability index.

[3]Modified pellet durability index.

[4]Percentage of total feed produced that was fines.

[5]Sand was included at the expense of all ingredients in the diet at a rate of 2%. The *P*-value corresponds to an orthogonal contrast between the treatments with 30% DDGS and 30% DDGS + sand.

[6]Linear and quadratic orthogonal contrasts were tested using the incremental dietary DDGS treatments (0, 15, and 30%) except the 30% DDGS + sand treatment.

increased with DDGS (higher Ct values correspond to lower DNA concentration). Conversely, this effect was not supported by the values from selective media bacterial growth.

DISCUSSION

Feed Milling

There is some agreement, mostly in the form of popular belief, of the effects that DDGS play in feed manufacturing, but to our knowledge, no supportive scientific evidence exists. On the other hand, it has been well documented that feeding high-quality pellets can result in improved gain and conversion in broilers (Jensen et al., 1962; Nir et al., 1994). Production rate was shown to be affected primarily by the 30% DDGS inclusion level in the diet (Table 5). This effect could be attributed to the decrease in the level of inorganic phosphate, which is known to have a scrubbing effect inside the die, although this effect also lacks scientific validation and warrants further research. Dietary DDGS inclusion resulted in decreased pellet quality, likely because this ingredient has a reduced starch component in comparison to ground corn, which could result in less starch gelatinization and decreased pellet binding. However, when looking at the composition of the grower diets (Table 1), higher DDGS inclusion levels resulted in higher amounts of added fat. Salmon (1985) added 3, 6, and 9% of added fat to pellets and reported a decrease with increased fat levels, and in this study, all fat was added at the mixer. If fat level is indeed a primary cause of decreased pellet quality, then one could recommend application of a portion of the fat using postpellet application when diets contain elevated levels of DDGS.

In this study, bulk density declined with DDGS addition to the diet. This decline is most likely due to the lower bulk density that DDGS has compared with regular corn. As DDGS increased in the diet, the amount of fines also increased, in agreement with previous reports by Min et al. (2009); this trend is typically a proportional response of pellet quality and is in complete agreement with the current study (Table 5).

Inclusion of 30% DDGS resulted in an increased energy usage at the conditioner, whereas the use of no DDGS resulted in an increase in energy usage at the pellet mill. Arguably, this increase in energy usage at the conditioner is of less interest than the decrease seen at the pellet mill, considering that it is the pellet mill that traditionally has much larger motors and consequently higher energy inputs. It is possible that in both cases, the changes observed are the result of added fat in the diet. It is widely believed that a diet with a higher oil inclusion will have increased pellet mill throughput compared with a diet with less oil, mostly due to the lubricating action of fat at the mash die interface (Thomas et al., 1998). As for the increased energy used at the conditioner, it can be hypothesized that higher supplemental fat amounts create a thicker, more viscous mash that may require more energy to be augered through the conditioner.

Another recent popular belief is that sand aids in overall mill performance when manufacturing pelleted diets that are low in rock phosphates, such as those diets with high levels of DDGS. It is believed that incorporating sand in a diet could create a die-scouring effect during pelleting, by reducing friction through scrubbing the die, thus easing overall diet throughput and decreasing energy usage. However, as observed in the current results, the 30% + sand diet did not affect any of the parameters measured when compared with its counterpart devoid of sand.

Growout and Liver Weight

Absence of a pregrower main effect for the performance of broilers at 28 d of age (Table 3) was expected based on previous research conducted by Lumpkins et al. (2004), in which DDGS were fed at a rate of 15% of the starter diet. These results agree with Parsons et al. (1983), who reported that up to 20% of the soybean meal in a chick diet could be replaced by DDGS without any detrimental effect on growth rate. The birds were affected by the different levels of DDGS fed during the grower phase. A linear trend was observed toward decreasing BW gain. Inclusion of DDGS past 7.5% of the diet resulted in lower BW gain values. However, it is important to note that the birds receiving 15% DDGS in the grower phase did not exhibit a significantly lower BW gain compared with the control birds. This is in close agreement with the suggestion made by Lumpkins et al. (2004) that DDGS from modern ethanol plants can be safely used at levels from 12 to 15% in the grower period. Wang et al. (2008) also reported that once DDGS in the diet exceeded 20%, there was a significant decline in BW gain in the grower period. In each of the 2 studies mentioned previously, the level of DDGS in the diet changed at different increments from the current study and thus it is hard to discern the line of maximum inclusion for DDGS in the grower phase. Wang et al. (2008) reported an increase in feed conversion ratio (**FCR**) as DDGS in the diet increased from 20 to 30% of the diet in the grower phase, whereas Lumpkins et al. (2004) reported similar results to our study with no difference in FCR, with DDGS comprising up to 18% of the diet. Although not as prominent as the results of the previous research mentioned, the current study showed a linear trend for increased FCR when DDGS inclusion was increased in the grower-phase diet. This current trend could be due to different factors. Two such factors are changes in gut viscosity as well as a possible toxicity as a result of the high DDGS inclusion, and both are factors that were evaluated in the current study.

Feed intake exhibited a linear trend toward decreased consumption as DDGS increased in the diet, but an interaction observed between the pregrower and grower

DDGS inclusion levels demonstrated how birds that consumed no DDGS during the starter phase exhibited a decrease in feed consumption at 22.5 and 30% during the grower phase, whereas birds that received 8% DDGS during the starter phase were unaffected by DDGS grower level (Figure 1). This feed consumption pattern exhibited by the birds that received no DDGS during the starter phase suggests an inability to adapt to the presence of DDGS in the grower diet and thus the relatively high levels of 22.5 and 30% may have caused that decrease in consumption. There was also a linear trend seen for relative liver weight (Table 3), whereas DDGS increased there was a decrease in relative liver weight. The liver was chosen to be weighed in this study as an evaluator for any metabolic challenge that could perhaps be associated with feeding high levels of DDGS. This linear decrease in the relative weight of the liver associated with feeding increasing dietary levels of DDGS may have been caused by a marginal toxicity resulting in atrophy of the liver, but until biochemical and histological analyses are conducted, it is difficult to ascertain.

Microflora and Viscosity

Past research performed by Jacob et al. (2008) has shown a positive association between dietary DDGS and fecal prevalence of *E. coli* in cattle. No changes in *E. coli* presence were observed in this study in cecal contents of these birds. A likely explanation for this difference may lie in the difference between the 2 species' gastrointestinal tracts. The digestive system of cattle widely differs from the monogastric system of the broiler, and therefore too many factors could have resulted in the bacterial increase reported in cattle but not seen in broilers. Although there is little research into effects that different feedstuffs have on *E. coli* levels of the broiler digestive system, Rubio et al. (1998) reported that *E. coli* counts were unaffected by the inclusion of sweet lupin seed meal in the diet, compared with a commercial type, wheat and soybean meal-based diet.

Because of potential economic impact to broiler operations throughout the world, the presence of *C. perfringens* and *E. coli* was evaluated in the present study to determine if varying concentrations of DDGS present in feed would affect the colonization of these microbes in broilers. Our results indicate that no significant differences were observed. There may be some indication ($P = 0.07$) that *C. perfringens* could possibly be increasing in cecal concentration as the percentage of DDGS increased per our qPCR results. In contrast, this *C. perfringens* was unsupported by the selective media counts, thus warranting further investigation. The variation between the qPCR and viable plate counts could be attributed to the presence of spore DNA or dead cells, or both, present in our samples analyzed by qPCR. Slight decreases were also observed in the presence of *E. coli* in the cecal samples, although these changes were also not significant. To our knowledge, research evaluating the use of DDGS in the diet and its effect on cecal concentration of *C. perfringens* in broilers is not readily available. Annett et al. (2002) reported that *C. perfringens* is more prevalent in broilers fed a wheat- or barley-based diet, as compared with those fed a corn-based diet. Changes in the levels of *C. perfringens* have also been associated with changes in protein source and level as shown by Drew et al. (2004). These authors established that the level of dietary CP did have an effect on the *C. perfringens* levels at 28 d of age in broilers. However, Drew et al. (2004) promoted *C. perfringens* colonization by inoculating this microorganism in the feed and not including an antibiotic or coccidiostat in the experimental diets. Furthermore, diets fed by Drew et al. (2004) were not pelleted. Although there was a measurable difference in the CP levels of the experimental diets used in the current study, these feeds were steam-pelleted and were not inoculated with *C. perfringens*. Therefore, this potential decrease in viable *C. perfringens* present with increasing dietary DDGS levels observed herein is the result of an effect that simulates commercial conditions closer and thus should be further evaluated for its practical commercial implication.

It has long been accepted that viscous grains are known to increase the viscosity of the digestive contents in broilers (Bedford, 1996; Jozefiak et al., 2007; Jia et al., 2009) when compared with corn-based diets. The marginal ($P = 0.07$) increase in viscosity with DDGS seems to suggest that a trend may be emerging and could possibly become significant when birds are fed DDGS for longer periods. Lee et al. (2003) observed a relationship in which increasing intestinal viscosity corresponded with growth depression in broilers fed guar meal germ and hull fractions in the diet. This response should be further evaluated, particularly in older broilers with a more functional and developed small intestine.

In conclusion, DDGS seem to be safe at inclusion levels of 8% from 0 to 14 d of age. During the grower phase, it seems clear that high levels of DDGS in the diet caused pellet quality and bulk density to decrease while reducing energy usage at the pellet mill. The marginal trends observed for increased intestinal viscosity and cecal *C. perfringens* viability with increasing DDGS in the diet warrant further investigation. When feeding the grower diets to broilers from 14 to 28 d of age, it appears that the limit of inclusion may be between 7.5 and 15% of the diet. It may be advantageous to the bird to have DDGS in the starter diet to condition the digestive system to this by-product before being exposed to even higher levels in the grower phase.

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