Effect of feeding corn distillers dried grains with solubles naturally contaminated with deoxynivalenol on growth performance, meat quality, intestinal permeability, and utilization of energy and nutrients in broiler chickens

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ABSTRACT The objective of this experiment was to investigate the effect of feeding corn distillers dried grains with solubles (**DDGS**) naturally contaminated with deoxynivalenol (**DON**) on growth performance, meat quality, intestinal permeability, and utilization of energy and nutrients in broiler chickens. Two trials (growth and metabolism trials) were conducted. In the growth trial, a total of four hundred 7-day-old Ross 308 broiler chicks were allotted to 1 of 5 dietary treatments with 8 replicates in a completely randomized design. The diets were formulated to contain 5 inclusion levels of 0, 5, 10, 15, or 20% DON-contaminated DDGS in diets and were fed to birds for 21 d. Results indicated that increasing inclusion levels of DON-contaminated DDGS decreased (linear, $P < 0.01$) BW gain and feed efficiency of broiler chickens. The relative organ weights of the liver and breast were decreased (linear and quadratic, $P < 0.05$) by increasing inclusion levels of DON-contaminated DDGS in diets. The transepithelial electrical resistance values as a measure of intestinal permeability were decreased (linear, $P < 0.05$) by increasing inclusion levels of DON-contaminated DDGS in diets. In the metabolism trial, a total of twenty four 22-day-old Ross 308 broiler chickens were allotted to 1 of 3 dietary treatments consisting of 0, 10, or 20% inclusion of DON-contaminated DDGS in diets. Each treatment had 8 replicates. Increasing inclusion levels of DON-contaminated DDGS in diets decreased (linear and quadratic, $P < 0.05$) ME_n (AME_n and TME_n) and apparent total tract retention of nitrogen and acid-hydrolyzed ether extract in diets. In conclusion, feeding diets containing more than 10% DON-contaminated DDGS to broiler chickens has negative effects on growth performance, intestinal permeability, and utilization of energy and nutrients in diets. Therefore, it is suggested that if DDGS is contaminated with DON, inclusion level of DDGS should be limited, possibly at less than 5.0% in broiler diets.

Key words: broiler chicken, corn distillers dried grains with solubles, deoxynivalenol, growth performance, intestinal permeability

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INTRODUCTION

Corn distillers dried grains with solubles (**DDGS**) is a byproduct of corn grain fermentation in the ethanol industry. Because of the high nutritional value such as protein, fat, and phosphorus (**P**) in DDGS, the use of DDGS for animal feeds has attracted the great attention of the animal industry. However, the relatively high amounts of fiber lead to a limited use of DDGS in diets for swine and poultry. Several experiments have been conducted to determine the optimal inclusion level of DDGS in broiler diets. Lumpkins et al. (2004) reported that inclusion of less than 15% DDGS in diets for grower and finisher broiler chickens had no negative effects. Wang et al. (2007) studied the effects of increasing inclusion levels from 0 to 25% DDGS in broiler diets, and observed no adverse outcomes. Similarly, Wang et al. (2008) also reported that inclusion of 20% DDGS in diets did not affect growth performance of broiler chickens. Therefore, it has been suggested that up to 20% DDGS can be safely used for broiler diets (Wang et al., 2008; Kim et al., 2018).

The DDGS is produced mainly from corn fermentation and the corn is frequently contaminated with mycotoxins (Joint FAO/WHO Expert Committee on Food

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Additives, 2001). Thus, the incidence of mycotoxin contamination in DDGS has been widely reported. Previous experiments have reported that DDGS is frequently contaminated by various mycotoxins such as aflatoxin, zearalenone, ochratoxin, and deoxynivalenol (**DON**; Wu and Munkvold, 2008). Rodrigues and Naehrer (2012) analyzed mycotoxin contaminations in various feed ingredients and reported that DON contamination was the highest among 4 mycotoxins in DDGS.

In previous experiments studying the effects of DON-contaminated DDGS in animals, Patience et al. (2014) investigated the effect of feeding diets containing DON-contaminated DDGS (16 mg/kg DON) to pigs and reported that inclusion of 25% DON-contaminated DDGS in diets decreased BW gain (**BWG**) and feed intake (**FI**). However, Wickramasuriya et al. (2020) reported that increasing inclusion levels of DON-contaminated DDGS (5 mg/kg DON) from 0 to 20% in diets did not affect productive performance and egg quality of laying hens. Thus, the effect of feeding DON-contaminated DDGS to animals is likely dependent on inclusion levels of DDGS in diets and contamination levels of DON in DDGS. However, few experiments have been performed to evaluate the effects of feeding diets containing DON-contaminated DDGS to broiler chickens.

The objective of the current experiment, therefore, was to investigate the effect of feeding diets containing DON-contaminated DDGS on growth performance, meat quality, intestinal permeability, and utilization of energy and nutrients in broiler chickens.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved the Institutional Animal Care and Use Committee at Chung-Ang University (IACUC No. 201600108).

Mycotoxin Analysis

The mycotoxin contamination levels in DDGS used in this experiment were analyzed (Table 1). The contamination levels of aflatoxin, ochratoxin, and zearalenone in the DDGS were negligible; however, the contamination level of DON in the DDGS was 2.16 mg/kg, which showed that the main mycotoxin contaminant in DDGS was DON. It has been reported that average DON-contamination level of DDGS produced in United States was 0.62 mg/kg (Zhang et al., 2009), which indicated that DDGS used in the current experiment was contaminated with high level of DON. Other energy and nutrient compositions in the DON-contaminated DDGS are presented in Table 2.

Table 1. Mycotoxin contamination of corn distillers dried grains with solubles (DDGS) used in this experiment (as-fed basis).

Mycotoxins	Contamination levels, mg/kg
Deoxynivalenol	2.160
Zearalenone	0.201
Alfatoxin	ND[1]
Ochratoxin	ND[1]

[1]ND, not detected.

Table 2. Analyzed energy and nutrient compositions of corn distillers dried grains with solubles (DDGS) used in this experiment (as-fed basis).

Items	Contents
GE[1], kcal/kg	4,406
DM, %	88.26
Crude ash, %	5.43
CP, %	25.30
Ether extract, %	8.50
Amino acid, %	
Essential amino acid	
Arginine	1.09
Histidine	0.65
Isoleucine	1.44
Leucine	2.80
Lysine	0.81
Methionine	0.55
Threonine	1.00
Tryptophan	0.16
Phenylalanine	1.18
Valine	1.10
Nonessential amino acid	
Alanine	1.83
Aspartic acid	1.65
Cysteine	0.78
Glutamic acid	4.33
Glycine	1.05
Proline	2.04
Serine	1.31
Tyrosine	0.77

[1]GE, gross energy.

Experiment 1: Growth Trial

Animals, Diets, and Experimental Design. A total of six hundred 1-d-old Ross 308 broiler chicks were obtained from a local commercial hatchery (Dongsan hatchery, Cheonan, Republic of Korea) and housed in 40 battery cages (76 × 78 × 45 cm, width × length × height) placed in an environmentally controlled room. An equal number of birds were allotted to each battery cage and birds were provided with a commercial starter diet and fresh water for 7 d. At 7 d of age, all birds were weighed and the birds with extremely high and low BW were discarded to select birds with the similar initial BW. A total of four hundred 7-d-old broiler chickens (initial BW = 185.2 ± 1.15 g) were selected and allotted to 1 of 5 dietary treatments with 8 replicates, each replicate consisting of 10 birds, in a completely randomized design. Two galvanized iron troughs (76 cm long × 12 cm wide × 8 cm deep) hanging each outside of the battery cage were set for a feeder and a drinker. Grower diets were fed to birds for 21 d. Experimental diets were formulated to contain DON-contaminated DDGS at inclusion levels of 0, 5, 10, 15, or 20% (Table 3). The diets were in a mash form. All diets were formulated to meet or exceed the Ross 308 broiler nutrition specifications (Aviagen, 2014) for energy and nutrients in

Table 3. Composition and nutrient content of the experimental diets (as-fed basis).

	Inclusion levels of DON-contaminated DDGS[1], %				
Items	0	5	10	15	20
Ingredient, %					
Corn	45.72	44.14	42.54	41.15	39.48
Wheat	6.20	5.88	5.56	5.00	4.79
Soybean meal, 46% CP	37.69	35.05	32.38	29.75	27.08
DDGS[1]	0.00	5.00	10.00	15.00	20.00
Soybean oil	5.76	5.25	4.75	4.25	3.74
Monodicalcium phosphate	1.54	1.46	1.37	1.29	1.20
Limestone	1.26	1.31	1.38	1.44	1.49
Lysine sulfate	0.26	0.34	0.42	0.50	0.58
Threonine	0.12	0.12	0.12	0.12	0.12
DL-Methionine	0.35	0.34	0.33	0.32	0.31
L-Tryptophan	0.00	0.01	0.05	0.08	0.11
NaCl	0.30	0.30	0.30	0.30	0.30
Choline	0.10	0.10	0.10	0.10	0.10
Coccidiostats	0.10	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.30	0.30	0.30	0.30	0.30
Vitamin premix[2]	0.15	0.15	0.15	0.15	0.15
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00
Calculated energy and nutrient content[4]					
AME_n[5], kcal/kg	3,100	3,100	3,100	3,100	3,100
CP, %	21.75	21.75	21.75	21.75	21.75
Total lysine, %	1.32	1.32	1.32	1.32	1.32
Total methionine + cysteine, %	1.00	1.00	1.00	1.00	1.00
Total threonine, %	0.94	0.94	0.93	0.93	0.93
Total tryptophan, %	0.27	0.27	0.27	0.27	0.27
Digestible lysine, %	1.24	1.25	1.27	1.29	1.30
Digestible methionine + cysteine, %	0.91	0.91	0.91	0.91	0.91
Digestible threonine, %	0.83	0.82	0.81	0.80	0.79
Digestible tryptophan, %	0.24	0.23	0.23	0.23	0.22
Total calcium, %	0.87	0.87	0.87	0.87	0.87
Available phosphorus, %	0.44	0.44	0.44	0.44	0.44

[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.

[2]Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 13,000 IU; vitamin D_3, 5,000 IU; vitamin E (from DL-α-tocopheryl acetate), 80 IU; vitamin K_3, 4 mg; vitamin B_1, 4 mg; vitamin B_2, 10 mg; vitamin B_6, 6 mg; vitamin B_{12}, 20 μg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 200 μg; niacin, 60 mg.

[3]Provided per kilogram of the complete diet: Zn (as ZnO), 100 mg; Mn (as $MnSO_2 \cdot H_2O$), 120 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 60 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 16 mg; Co (as $CoCO_3$), 1,000 μg; I (as $Ca(IO_3)_2 \cdot H_2O$), 1.25 mg; Se (as Na_2SeO_3), 300 μg.

[4]Calculated values from the Ross 308 broiler nutrition specifications (Aviagen, 2014).

[5]Assumed AME_n value for DDGS (3,282 kcal/kg) was calculated from the prediction equation of corn co-products in broiler chickens (Rochell et al., 2011).

growing broiler chickens. During the experiment, birds were provided with feed and water ad libitum and were exposed to a lighting schedule of 23-h light:1-h dark. The room temperature was maintained at 30°C during the first week of the experiment and then gradually decreased to 24°C at the end of the experiment as recommended by Ross 308 broiler nutrition specifications (Aviagen, 2014). The BWG and FI were recorded at the conclusion of the experiment. Mortality was recorded daily. The feed efficiency (**FE**) was calculated by dividing BWG with FI. The BWG and FI were adjusted with the number and living days of dead birds (Kim et al., 2017).

Sample Collection and Chemical Analysis. The sample collections were performed with only 3 treatment groups of 0, 10, and 20% inclusion of DON-contaminated DDGS in diets because the contaminated levels of DON in DDGS were relative low (i.e., 2.16 mg/kg). At the conclusion of experiment (28 d of age), 2 birds with a BW close to the mean BW per replicated cage were sacrificed by CO_2 asphyxiation and dissected immediately. One bird was used for analyzing the relative organ weight, meat quality, and plasma measurements, whereas the other bird was used for analyzing the intestinal permeability at the jejunal mucosa using an Ussing Chamber.

Blood samples were immediately obtained from each bird via cardiac puncture into a 10-mL sodium heparin tube (Becton Dickinson and Co, Franklin Lakes, NJ). The blood samples were immediately centrifuged at 3,000 × *g* and at 4°C for 20 min to separate the plasma and stored at −20°C before analysis. The concentrations of aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), creatinine, and total protein in the plasma were analyzed using a HITACHI automatic analyzer 7020 (Hitachi Ltd., Tokyo, Japan). The liver, small intestine, kidney, spleen, bursa of Fabricius, and breast meat were excised and weighed to calculate the relative organ weight, which was expressed as a percentage of BW.

The right and left breast meat samples were collected for analyzing meat quality. The right breast meat was used for analyzing pH at 1 and 24 h postmortem using a pH meter (Hanna Instruments, Nusfalau, Romania) and meat color at 24 h postmortem using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) to measure CIE color scale for lightness (L*), redness (a*), and yellowness (b*). The left breast meat was used to determine water holding capacity (**WHC**) at 48 h postmortem. Briefly, 1.5 g of the breast meat sample was weighed and wrapped using a Whatman filter paper (Whatman Grade 3, Whatman International Ltd., Maidstone, England). The packed breast meat samples were placed into a 50-mL conical tube (SPL Life Sciences Co., Ltd, Gyeonggi-do, Republic of Korea) and centrifuged at 4°C and at the speed of 3,000 × *g* for 15 min. After centrifugation, the breast meat samples were weighed again. The WHC was determined as: WHC (%) = [(breast meat weight before centrifugation − breast meat weight after centrifugation)/breast meat weight before centrifugation] × 100. The right portion of breast meat was also used for analyzing thiobarbituric acid reactive substance (**TBARS**) value at 7 d of the 4°C storage as described by Lee et al. (2017) with minor modifications. Briefly, approximately 5 g of the breast meat samples was weighed and placed in a 50-mL conical tube. Afterwards, 15 μL butylated hydroxytoluene and 15 mL deionized distilled water were added into the 50-mL conical tube. The samples were homogenized on ice using a homogenizer (Daihan Scientific Co., Ltd, Gangwon-do, Republic of Korea). Approximately 1 mL of the supernatants was collected in a 15-mL test tube (Sarstedt AG & Co., Nümbrecht, Germany) and 2 mL thiobarbituric acid/trichloroacetic acid mixture was added, and the mixture was vortexed. The mixture was incubated in a water bath for 15 min (Daihan Scientific Co., Ltd, Gangwon-do, Republic of Korea) at 90°C. After incubation, the mixture was cooled on ice for a few minutes and then centrifuged at 4°C and at the speed of 3,000 × *g* for 10 min. The supernatant was collected and used to analyze the TBARS value. Absorbance at 531 nm was detected using a microplate reader (Spectramax 190, Molecular Devices Corp., Sunnyvale, CA).

The intestinal permeability was determined at the jejunal mucosa based on transepithelial electrical resistance (**TER**) value measured in a two-channel Ussing chamber system (U2500, Warner Instruments, Hamden, CT). The detailed procedure in a two-channel Ussing chamber system was described previously (Goo et al., 2019).

Experiment 2: Metabolism Trial

Experimental Design and Sample Collection. A total of twenty four 22-d-old commercial Ross 308 broiler chickens were allotted to 1 of 3 dietary treatments with 8 replicates per treatment and 1 bird per replicate. Birds were placed in metabolic cages (35.2 × 45.0 × 55.3 cm, width × length × height). Room temperature was set at 21°C and the light was provided for 24 h throughout the experiment. Three dietary treatments (i.e., 0, 10, or 20% DON-contaminated DDGS in diets) were used. The diets were identical to those used in the growth trial. All birds were provided with feed and water ad libitum before the start of the metabolism trial. The experimental procedure in the metabolism trail was followed by the method of Bourdillon et al. (1990) with a minor modification. In short, at the start of the experiment (22 d of age), average FI was determined using the basal diet for 2 d. Afterward, all birds were subjected to a 72-h adaptation period. For the first 55 h, birds were fed the designated treatment diets at 80% average FI (i.e., 160 g) to minimize ingredient selections in feeders, and birds were then fasted for 17 h to empty the gastrointestinal tract before the start of the collection period. The collection period lasted for 96 h. Birds were fed 160 g of each treatment diet at 9:00 AM for the first 79 h (i.e., 4 times) and fasted for the next 17 h. Excreta were collected continuously during the whole collection period. Finally, all birds were fasted again for an additional 24 h, and excreta were collected to measure the endogenous losses of energy and nitrogen (**N**) during the final fasting period.

Chemical Analysis. The excreta were collected daily and immediately stored at −20°C. The excreta samples were dried in a force-air drying oven at 60°C for 48 h and finely ground for further analysis. The experimental diets and excreta samples were analyzed for acid-hydrolyzed ether extraction (**AEE**; Method 996.01; AOAC, 2007), and N (Method 990.03; AOAC, 2007). The samples for diets and excreta were analyzed for gross energy (**GE**) using bomb calorimetry (Model 6400; Parr Instruments Co., Moline, IL). Calcium (**Ca**) and P concentrations in the diets and excreta were analyzed using inductively coupled plasma spectrometer (Optima 5300 DV, Perkin Elmer Inc., Shelton, CT) as demonstrated by AOAC (2007; Method 935.13) with minor modifications (Kim et al., 2016).

Calculations. The values for N-corrected ME (AME_n and TME_n) of the experimental diets containing 0, 10, or 20% DON-contaminated DDGS were calculated as followed (Wolynetz and Sibbald, 1984; Lee et al., 2018):

$$AME_n(kcal/kg) = [GEi - \{GEo + (Ni - No) \times 8.22\}]/FI,$$

$$TME_n(kcal/kg) = [GEi - \{GEo + (Ni - No) \times 8.22\} + \{GEe + (Ni - No) \times 8.22\}]/FI$$

where GEi indicates the GE intake; GEo indicates the GE output; Ni − No indicates the gram N balance; GEe indicates endogenous loss of energy; 8.22 equals the N retained value (Hill and Anderson, 1958). The values for endogenous loss of energy and N balance during 24-h fasting period were multiplied by 4 to calculate GEe and N balance for 96-h collection period.

The values for apparent total tract retention (**ATTR**) of N, AEE, Ca, and P were calculated as followed (Prola et al., 2013; Lee et al., 2018):

$$\text{ATTR of X} = [(\text{total X ingested} - \text{total X excreted})/\text{total X ingested}] \times 100$$

where X represents N, AEE, Ca, and P.

Statistical Analysis

For both experiment 1 and 2, all data were analyzed in a completely randomized design using PROC MIXED procedure of SAS (SAS Institute., Cary, NC). Each replicate was considered an experimental unit for all analyses. All data were checked for normal distribution and outliers with the UNIVARIATE procedure of SAS (Steel et al., 1997). The LSMEANS procedure was used to calculate the treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. The orthogonal polynomial contrast tests were used to determine linear and quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets (Seo et al., 2018). The statistical significance was set at $P < 0.05$.

RESULTS

Experiment 1: Growth Trial

Growth Performance. Increasing inclusion levels of DON-contaminated DDGS in diets decreased (linear, $P < 0.01$) BW, BWG, and FE of broiler chickens (Table 4). The BW and BWG of birds fed diets containing 0 or 5% DON-contaminated DDGS were greater ($P < 0.05$) than those of birds fed other diets. The FE of birds fed diet containing 5% DON-contaminated DDGS was greater ($P < 0.05$) than FE of birds fed diets containing 10, 15, or 20% DON-contaminated DDGS. The birds fed diets containing 20% DON-contaminated DDGS had the least ($P < 0.05$) BW, BWG, and FE. However, FI was not influenced by increasing inclusion levels of DON-contaminated DDGS in diets.

Relative Organ Weight. The relative liver and breast weights were decreased (linear and quadratic, $P < 0.05$) by increasing inclusion levels of DON-contaminated DDGS in diets (Table 5). There was a quadratic relationship ($P < 0.05$) for the relative kidney weight among 3 dietary treatments. The birds fed diets containing 20% DON-contaminated DDGS had less ($P < 0.05$) relative liver and breast weights than those fed diets containing 0 or 10% DON-contaminated DDGS. However, increasing inclusion levels of DON-contaminated DDGS in diets had no effects on the relative weights of the small intestine, spleen, and bursa of Fabricius.

Table 4. Effect of increasing inclusion levels of DON-contaminated DDGS in diets on growth performance of broiler chickens.[1, 2]

	Inclusion levels of DON-contaminated DDGS[1], %						*P*-value[4]		
Items[3]	0	5	10	15	20	SEM	T	L	Q
BW, g	1,505[a]	1,524[a]	1,448[b]	1,434[b]	1,348[c]	20	<0.01	<0.01	0.06
BWG, g	1,320[a]	1,339[a]	1,262[b]	1,249[b]	1,163[c]	20	<0.01	<0.01	0.06
FI, g	2,027	1,988	2,032	2,023	2,028	32	0.83	0.71	0.76
FE, g/kg	651[ab]	676[a]	621[b]	618[b]	574[c]	14	<0.01	<0.01	0.09

[a-c]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]BWG, BW gain; FI, feed intake; FE, feed efficiency (BWG:FI).
[4]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.

Table 5. Effect of increasing inclusion levels of DON-contaminated DDGS in diets on the relative organ weights of broiler chickens.[1, 2]

	Inclusion levels of DON-contaminated DDGS[1], %				*P*-value[4]		
Items[3]	0	10	20	SEM	T	L	Q
Liver, %	3.11[a]	3.26[a]	2.82[b]	0.08	<0.01	0.02	<0.01
Breast, %	19.81[a]	20.98[a]	18.10[b]	0.01	<0.01	0.03	<0.01
Small intestine, %	5.31	5.61	5.33	0.20	0.52	0.96	0.26
Kidney, %	0.55	0.60	0.51	0.27	0.09	0.29	<0.05
Spleen, %	0.11	0.12	0.10	0.01	0.27	0.56	0.13
BF[5], %	0.22	0.22	0.20	0.05	0.51	0.30	0.63

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]The relative organ weight was expressed as a percentage of BW.
[4]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.
[5]BF, bursa of Fabricius.

Table 6. Effect of increasing inclusion levels of DON-contaminated DDGS in diets on plasma measurements of broiler chickens.[1, 2]

	Inclusion levels of DON-contaminated DDGS[1], %				*P*-value[4]		
Items	0	10	20	SEM	T	L	Q
ALT[3], U/L	3.00	2.83	2.87	0.18	0.78	0.61	0.64
AST[3], U/L	262.07	261.06	252.62	6.89	0.57	0.34	0.65
Creatinine, mg/dL	0.31[a]	0.27[b]	0.30[a]	0.01	0.01	0.48	<0.01
Total protein, d/dL	3.74	3.72	3.77	0.10	0.93	0.84	0.75

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]ALT, alanine aminotransferase; AST, aspartate aminotransferase.
[4]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.

Table 7. Effect of increasing inclusion levels of DON-contaminated DDGS in diets on meat quality of broiler chickens.[1, 2]

		Inclusion levels of DON-contaminated DDGS[1], %				*P*-value[5]		
Items		0	10	20	SEM	T	L	Q
pH	1 h	6.39	6.42	6.47	0.04	0.42	0.20	0.85
	24 h	5.94	5.92	5.98	0.05	0.39	0.54	0.22
Meat color[3]	L*	51.10[a]	50.74[a]	48.01[b]	0.56	<0.01	<0.01	0.10
	a*	3.99	3.77	4.54	0.31	0.20	0.66	0.09
	b*	8.52	8.46	9.20	0.43	0.42	0.28	0.46
WHC[4], %		79.30	79.82	80.25	0.86	0.74	0.44	0.97
TBARS[4]		0.34	0.33	0.33	0.01	0.81	0.82	0.56

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]L*, lightness; a*, redness; b*, yellowness (breast meat color).
[4]WHC, water holding capacity; TBARS, thiobarbituric acid reactive substance (malondialdehyde equivalents per g of meat sample).
[5]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.

Plasma Measurements. Increasing inclusion levels of DON-contaminated DDGS in diets indicated a quadratic relationship ($P < 0.05$) for plasma creatinine concentrations with birds fed diets containing 10% DON-contaminated DDGS exhibiting the least concentrations (Table 6). However, plasma concentrations of ALT, AST, and total protein were not affected by increasing inclusion levels of DON-contaminated DDGS in diets.

Meat Quality. Increasing inclusion levels of DON-contaminated DDGS in diets decreased (linear, $P < 0.01$) breast meat lightness (L*; Table 7). The birds fed diets containing 20% DON-contaminated DDGS had less ($P < 0.05$) meat lightness (L*) than those fed diets containing 0 or 10% DON-contaminated DDGS. However, other breast meat quality including pH (1 h and 24 h), redness (a*), yellowness (b*), WHC, and TBARS values was not affected by increasing inclusion levels of DON-contaminated DDGS in diets.

Intestinal Permeability. The intestinal permeability at the jejunal mucosa was measured using an Ussing chamber (Table 8). The TER values as a measure of intestinal permeability were decreased (linear, $P < 0.05$) with increasing inclusion levels of DON-contaminated DDGS in diets. A quadratic relationship ($P < 0.05$) was observed for the Isc values. The TER values for birds fed diets containing 10 or 20% DON-contaminated DDGS were less ($P < 0.05$) than those fed diets containing no DON-contaminated DDGS.

Table 8. Effect of increasing inclusion levels of DON-contaminated DDGS in diets on intestinal permeability of broiler chickens.[1,2]

	Inclusion levels of DON-contaminated DDGS[1], %				*P*-value[4]		
Items[3]	0	10	20	SEM	T	L	Q
PD, mV	95	97	77	20	0.73	0.52	0.65
Isc, μa/cm^2	0.41	1.15	0.51	0.25	0.09	0.79	0.03
TER, Ω/cm^2	250[a]	129[b]	144[b]	35	<0.05	<0.05	0.11

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]PD, transepithelial voltage; Isc, short circuit current; TER, transepitherlial electrical resistance.
[4]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.

Table 9. Metabolizable energy and apparent total tract retention of nutrients in diets containing DON-contaminated DDGS in broiler chickens.[1, 2]

	Inclusion levels of DON-contaminated DDGS[1], %				*P*-value[4]		
Items	0	10	20	SEM	T	L	Q
AME_n, kcal/kg	3,022[a]	2,796[b]	2,808[b]	18	<0.01	<0.01	<0.01
TME_n, kcal/kg	3,119[a]	2,928[b]	2,903[b]	18	<0.01	<0.01	<0.01
N[3], %	64.35[a]	60.86[b]	61.32[b]	0.59	<0.01	0.01	<0.01
AEE[3], %	77.03[a]	69.43[b]	67.57[b]	0.99	<0.01	<0.01	0.02
Ca[3], %	50.76	49.67	54.00	2.61	0.45	0.38	0.38
P[3], %	46.99	46.35	46.12	1.11	0.84	0.58	0.87

[a,b]Means within a variable with no common superscript differ significantly ($P < 0.05$).
[1]DON, deoxynivalenol; DDGS, corn distillers dried grains with solubles.
[2]Data are least squares means of 8 observations per treatment.
[3]N, nitrogen; AEE, acid-hydrolyzed ether extract; Ca, calcium; P, phosphorus.
[4]T, overall effects of treatments; L, linear effects of increasing inclusion levels of DON-contaminated DDGS in diets; Q, quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets.

Experiment 2: Metabolism Trial

Increasing inclusion levels of DON-contaminated DDGS in diets decreased (linear and quadratic, $P < 0.01$) the values for AME_n and TME_n (Table 9). The birds fed diets containing 10 or 20% DON-contaminated DDGS had less ($P < 0.05$) values for AME_n and TME_n than those fed diets containing no DON-contaminated DDGS. Increasing inclusion levels of DON-contaminated DDGS in diets decreased (linear and quadratic, $P < 0.05$) ATTR of N and AEE. The birds fed diets containing 10 or 20% DON-contaminated DDGS had less ($P < 0.05$) ATTR of N and AEE than birds fed diets containing no DON-contaminated DDGS. However, the ATTR of Ca and P was not influenced by increasing inclusion levels of DON-contaminated DDGS in diets.

DISCUSSION

Previous experiments have evaluated the safe inclusion levels of DDGS in broiler diets and reported that inclusion of up to 20% inclusion of DDGS in diets has been reported to show no negative effects on broiler performance (Wang et al., 2008; Kim et al., 2018). However, variable results have also been reported. Lumpkin et al. (2004) reported that feeding diets containing 18% DDGS decreased BWG and FE of broiler chickens. In addition, Campasino et al. (2015) reported that broiler chickens fed diets containing 15% DDGS had less BW than those fed diets containing 0 or 5% DDGS. Loar et al. (2010) observed that feeding diets containing 22.5% DDGS decreased BWG of 28-day-old broiler chickens. Therefore, the safe inclusion levels of DDGS in broiler diets appear to be inconsistent and dependent on various factors such as animals, environment, and nutritional compositions of DDGS (Świątkiewicz and Koreleski, 2008; Salim et al., 2010; Liu, 2011).

In the current experiment, feeding diets containing more than 10% DON-contaminated DDGS decreased BW, BWG, and FE of broiler chickens, which suggests that DON contaminations of DDGS may be an important factor affecting the safe inclusion level of DDGS in broiler diets. Furthermore, several researchers have reported that broiler chickens are able to tolerate up to 15 mg/kg DON in diets (Kubena et al., 1997; Awad et al., 2004; Awad et al., 2006). In the present experiment, however, the calculated concentration of DON in diets containing 10% DON-contaminated DDGS was approximately 0.216 mg/kg DON, which was far less than the safe level of DON (i.e., 15 mg/kg) in broiler diets; however, broiler chickens exhibited decreased growth performance. The reason why broiler chickens are more sensitive to DON in DDGS is unclear; however, it may be related to the fact that DON in DDGS may be converted to various transforms (e.g., masked DON) during high heat processing, and these transforms of DON are likely more toxic to animals (Khaneghah et al., 2019). In addition, high amounts of some dietary components such as fiber in DDGS may negatively affect intestinal structure and function (Jørgensen et al., 1996; Jansman, 2016), which possibly facilitates DON absorption in the body. In the current experiment, we also observed increased intestinal permeability (i.e., decreased TER) by feeding diets containing more than 10% DON-contaminated DDGS to birds. This increased intestinal permeability may also be a consequence of impaired intestinal cell functions due to increased DON absorption in the intestinal cells.

It has been reported that one of negative effects of DON contamination in diets is decreased FI of animals and the extent of reduction in FI depends on the contamination levels (Wellington et al., 2020). However, several previous experiments have reported that up to 18 mg/kg DON contamination in diets had no effects on FI of broiler chickens (Kubena et al., 1997; Awad et al., 2004, 2006; Xu et al., 2011), which agreed with our observation. Therefore, it is suggested that the negative effect of DON contamination in broiler diets may not be directly caused by decreased FI of broiler chickens.

The relative liver weight was measured as a possible indicator of hepatic metabolic disorders associated with feeding diets containing DON-contaminated DDGS to broiler chickens. The results revealed that only 20% inclusion of DON-contaminated DDGS in diets exhibited negative effects on the relative liver weight of broiler chickens. Loar et al. (2010) demonstrated that increasing inclusion levels of DDGS in diets decreased the

relative liver weight of broiler chickens although the DDGS was not contaminated with mycotoxins. Loar et al. (2010) also reported that more than 22.5% inclusion of normal DDGS in diets decreased the relative liver weight of broiler chickens. Therefore, the decrease in the relative liver weight may be attributed to increasing inclusion levels of DDGS with little relation to DON-contamination. However, the reduction in the relative breast weight of birds fed diets containing 20% DON-contaminated DDGS was likely caused by the large reduction in the final BW. Unlike the relative weights of the liver and breast, however, the relative weights of other organs including the small intestine, spleen, and bursa of Fabricius were not affected by increasing inclusion levels of DON-contaminated DDGS in diets despite the reduction in the BW.

Plasma concentrations of ALT and AST have been used as an indicator of the liver damage or diseases (Ma et al., 2014). Plasma concentrations of total protein can be a measure of long-term dietary protein utilization in the body (Matthews and Southern, 2000; Ghareeb et al., 2012). Plasma concentrations of total protein have been reported to be decreased when birds were fed diets contaminated with DON and aflatoxins, which suggests that total plasma protein can also be a useful indicator of the effects of dietary mycotoxins in chickens (Manning et al., 1990; Ghareeb et al., 2012). However, we observed no effects on plasma concentrations of ALT, AST, and total protein in broiler chickens. Similar results were observed by Wickramasuriya et al. (2020) who reported that there were no linear and quadratic effects of increasing inclusion levels of DON-contaminated DDGS in diets from 0 to 20% on plasma concentrations of ALT, AST, and total protein of laying hens. Therefore, our observation may indicate that inclusion of up to 20% DDGS in diets, regardless of DON-contaminations, may have little detrimental effects on liver function and protein utilization in the body, although feeding diets containing 20% DON-contaminated DDGS decreased the relative liver weight.

The pH value for the meat is often considered a potential indicator of meat quality because the rapid reduction in the postmortem pH of meat represents increased protein denaturation, which induce a detrimental effect on meat quality (Briskey and Wismer-Pedersen, 1961). In this experiment, however, increasing inclusion levels of DON-contaminated DDGS in diets had no effects on the pH of breast meat. It was also reported that increasing inclusion levels of normal DDGS in diets had no effects on the pH of breast meat (Corzo et al., 2009). In general, pH of 5.8 to 6.2 of broiler breast meat is considered normal at 24-h postmortem (Alvarado et al., 2007), and the pH values measured in this experiment were in the normal range at 24-h postmortem. These results indicate that inclusion level or DON-contamination of DDGS in diets may have no negative effects on the pH of broiler breast meat. The breast meat L* value is considered an indicator of the paleness of breast meat (Alvarado et al., 2007). According to Van Laack et al. (2000), normal breast meat showed less than 55 L* value, whereas more than 60 L* values were considered the pale breast meat. In this experiment, increasing inclusion levels of DON-contaminated DDGS in diets decreased L* values of breast meat. Similar results were obtained by Foltyn et al. (2013) who reported that increasing inclusion levels of DDGS in diets decreased breast meat lightness. However, the breast L* values measured in this experiment were in a normal range (i.e., less than 55; Schilling et al., 2010), which indicates that inclusion of up to 20% DON-contaminated DDGS in broiler diets may have little negative effects on breast meat color. Increasing inclusion levels of DON-contaminated DDGS in diets had no effects on WHC and TBARS of breast meat. This observation was consistent with the results of previous experiments (Corzo et al., 2009; Schilling et al., 2010). Therefore, it is suggested that increasing inclusion levels of DON-contaminated DDGS up to 20% in diets may have no detrimental effects on broiler meat quality.

Intestinal permeability is a well-known measure of the intestinal barrier function, which is composed of epithelial layers in the mucosa, assisting in the defense mechanisms against the invasion of harmful microorganisms and toxins from the lumen of the gastrointestinal tract (Moeser et al., 2007). The TER values have often been measured as an indicator of intestinal permeability (Goo et al., 2019). Less values for TER represent higher intestinal permeability, indicating impaired intestinal barrier functions that can allow more antigenic agents (i.e., toxins, bacteria, and feed-originated hazards) to penetrate across the mucosal epithelium (Moeser et al., 2007). In the present experiment, inclusion of 10 or 20% DON-contaminated DDGS in broiler diets decreased TER values of the jejunal mucosa. This increased intestinal permeability may be the reason for decreased growth performance of broiler chickens in this experiment. It is appreciated that increased intestinal permeability is highly associated with intestinal and systemic inflammation, which adversely affects animal health (Moeser et al., 2007; Goo et al., 2019). It is unclear why DON impairs intestinal barrier function; however, it may be related to decreased cellular metabolism in mucosa. The DON has been reported to decrease nutrient absorption, especially glucose uptake in intestinal cells (Hunder et al., 1991). This decreased nutrition absorption may lead to a deficiency of energy and nutrients in intestinal cells (e.g., cellular starvation) and contribute to a decrease in cellular functions such as intestinal barriers (Awad et al., 2004). This is probably why we observed that increasing inclusion levels of DON-contaminated DDGS in broiler diets increased intestinal permeability (i.e., decreased TER values) of the jejunal mucosa. However, Awad et al. (2004) reported that less than 5 mg/kg DON contamination in broiler diets had no negative effects on intestinal barrier functions; however, in the current experiment, the concentrations of DON in diets (0.216 and 0.432 mg/kg DON for diets containing 10 and 20% DON-contaminated DDGS, respectively) were far less than 5 mg/kg

and we found a linear decrease in intestinal barrier functions. The reason for this observation is difficult to be explained; however, it may be associated with the possible interaction of DON contamination and its characteristics of DDGS as a feed ingredient. Moreover, it is likely that DON contaminated in DDGS may be more toxic to intestinal cells than a purified form of DON or DON contaminated in other ingredients because the heat processing of DDGS at the high temperature may transform DON to other toxic chemical formation (i.e., masked DON; Khaneghah et al., 2019). In addition, higher fiber concentrations of DDGS may induce an alterations in intestinal structure and function (Jørgensen et al., 1996; Jansman, 2016). This change is likely to cause increased absorption of DON in intestinal cells and subsequently the body, which may accelerate intestinal and systemic inflammations in animals.

Increasing inclusion levels of DON-contaminated DDGS in diets decreased ME_n values (i.e., AME_n and TME_n) of diets although ME_n concentrations in all diets were formulated to be equalized. Adeola and Zhai (2012) reported that increasing inclusion levels of DDGS in diets decreased ME values. The reason for this decrease has been related to higher fiber concentrations of DDGS and the corresponding negative effects on nutrient digestion and absorption. However, it is likely that DON-contamination of DDGS exhibits more negative effects than normal DDGS does. Previous experiments reported that more than 30% inclusion of normal DDGS decreased ME values for diets (Adeola and Zhai, 2012); however, we found that only 10% inclusion of DON-contaminated DDGS resulted in decreased ME values. The reason for this observation appears to be associated with the fact that DON impairs villous structure and function in the small intestine, which plays an important role in nutrient digestion and absorption (Awad et al., 2006). This is also likely related to decreased ATTR of N and AEE as inclusion levels of DON-contaminated DDGS in diets were increased. This decrease in available energy and nutrients is also the reason for decreased growth performance of broiler chickens fed diets containing increasing inclusion levels of DON-contaminated DDGS.

It should be noted, however, that the present study has a limitation to clearly separate the effects of DDGS itself or its DON-contamination because normal DDGS with no DON-contamination was not used as the control in treatment diets. Further researches are warranted to verify our findings of DON-contamination in DDGS for broiler chickens as compared to normal DDGS originated possibly from the same source of DON-contaminated DDGS.

CONCLUSIONS

Increasing inclusion levels of DON-contaminated DDGS in diets decrease growth performance and intestinal barrier function of broiler chickens with little negative effects on meat quality. In addition, energy and nutrient utilizations in diets are decreased by increasing inclusion levels of DON-contaminated DDGS in diets. The DON contamination is a significant factor affecting the safe inclusion level of DDGS in broiler diets. It is suggested that if DDGS is contaminated with DON, inclusion level of DDGS should be limited, possibly at less than 5.0% in broiler diets.

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DISCLOSURES

The authors declare no conflict of interest for the data presented in this experiment.

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