# Contribution of exogenous dietary carbohydrases to the metabolizable energy value of corn distillers grains for broiler chickens

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**ABSTRACT** The objectives of this study were to determine the ileal digestible energy (IDE), ME, and  $ME_n$  contents of corn distillers grains (CDG) for broiler chickens and to quantify these energy utilization responses to carbohydrase supplementation by the regression method. The CDG sample used in the current experiment contained (by analysis) 936 g/kg of DM, 4,894 kcal/kg of gross energy, 315.1 g/kg of CP, 94.6 g/kg of crude fat, 94.8 g/kg of crude fiber, 495.6 g/kg of neutral detergent fiber, 179.1 g/kg of acid detergent fiber, 0.19 g/kg of Ca, and 4.8 g/kg of P. The studies were conducted at 2 locations (Purdue or Louisiana State University) and CDG were incorporated into a practical corn-soybean meal diet at 3 levels (0, 300, or 600 g/kg without or with added carbohydrase in a 2  $\times$  3  $\times$  2 factorial arrangement. The carbohydrase premix was added to supply 2,000 U of xylanase + 1,800 U of amylase/kg of feed. The diets were fed to 288 broiler chickens from d 15 to 22 posthatch with 6 birds per cage and 8 replicate cages per diet in a randomized complete block design at each of 2 locations. The broiler chicks were fed a standard broiler starter diet from d 1 to 15 posthatch. The IDE of diets decreased both linearly (P < 0.01) and guadratically (P < 0.05)as CDG increased from 0 to 600 g/kg regardless of carbohydrase supplementation. There was a linear (P< 0.01) decrease in ME of diet from 3,239 to 2,510 kcal/kg as CDG increased from 0 to 600 g/kg in the diets without added carbohydrase, whereas for birds fed the carbohydrase-supplemented diets, there were both linear (P < 0.01) and quadratic (P < 0.01) decreases from 3,398 to 2,613 kcal/kg as CDG increased from 0 to 600 g/kg. Dietary  $ME_n$  linearly decreased (P < 0.01) regardless of carbohydrase supplementation as CDG increased from 0 to 600 g/kg. Supplementation with carbohydrase improved (P < 0.01) IDE, ME, and  $ME_n$ . Regressions of CDG-associated IDE, ME, or ME<sub>n</sub> intake in kilocalories against kilograms of CDG intake without added carbohydrase generated the following: IDE = 44 + 2,340X,  $r^2 = 0.953$ ; ME = 10 +2,315X,  $r^2 = 0.993$ ; and  $ME_n = 10 + 2,132X$ ,  $r^2 =$ 0.991. Corresponding regressions when carbohydrase was added were as follows: IDE = -17 + 2,622X, r<sup>2</sup> = 0.985; ME = -25 + 2,448X, r<sup>2</sup> = 0.979; and ME<sub>n</sub> = -22 + 2,264X, r<sup>2</sup> = 0.978. These data indicate that the respective IDE, ME, and  $ME_n$  values (kcal/kg of DM) of the CDG sample evaluated were 2,340, 2,315, and 2,132 when carbohydrase was not added and 2,622, 2,448, and 2,264 when carbohydrase was added. Comparison using ANOVA procedures indicated that the slope when carbohydrase was added was greater (P <(0.05) than when carbohydrase was not added. This response implies that carbohydrase supplementation improved (P < 0.05) the IDE, ME, and ME<sub>n</sub> of CDG in practical corn-soybean meal-based diets used in this current study by 12, 5.7, and 6.2%, respectively.

Key words: broiler chick, corn distillers grains, enzyme, ileal digestible energy, metabolizable energy

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#### INTRODUCTION

The final stages of the dry-grind processing of corn for ethanol production leave solids called wet distillers grains and condensed distillers solubles. When dried, the distillers grains contain mostly proteins and fiber, whereas the condensed distillers solubles contain mostly residual sugars (Wu, 1994) and oil. During drying, the condensed distillers solubles are added back to the wet distillers grains; the amount added as well as the drying conditions contribute to the differences in the composition and nutritional value of corn distillers dried grains with solubles. Because corn distillers grains (**CDG**) do not have the solubles added, they usually contain less sugars and more fiber than CDG with sol-

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**Table 1.** Ingredient composition of starter diet fed from d 1 to15 posthatch on an as-fed basis

| Item                                  | Amount |
|---------------------------------------|--------|
| Ingredient, g/kg                      |        |
| Corn                                  | 542.2  |
| Soybean meal $(47.5\% \text{ CP})$    | 360.0  |
| Soybean oil                           | 50.0   |
| Dicalcium phosphate <sup>1</sup>      | 20.0   |
| Limestone (38% Ca)                    | 13.0   |
| NaCl                                  | 4.0    |
| Vitamin-mineral premix <sup>2</sup>   | 3.0    |
| DL-Met                                | 3.8    |
| L-Lys·HCl                             | 2.9    |
| L-Thr                                 | 1.1    |
| Total                                 | 1,000  |
| Calculated nutrient content           |        |
| Protein, g/kg                         | 226.0  |
| ME, kcal/kg                           | 3,208  |
| Ca, g/kg                              | 10.0   |
| P, g/kg                               | 7.5    |
| Ca:P                                  | 1.3    |
| Nonphytate P, g/kg                    | 4.9    |
| Total indispensable amino acids, g/kg |        |
| Arg                                   | 14.6   |
| His                                   | 5.9    |
| Ile                                   | 9.2    |
| Leu                                   | 18.9   |
| Lys                                   | 14.3   |
| Met                                   | 7.2    |
| TSAA                                  | 10.8   |
| Phe                                   | 10.5   |
| Phe + Tyr                             | 19.1   |
| Thr                                   | 9.4    |
| Trp                                   | 3.0    |
| Val                                   | 10.2   |

<sup>1</sup>20% Ca, 18.5% P.

<sup>2</sup>Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D<sub>3</sub>, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B<sub>12</sub>, 13.2  $\mu$ g; biotin, 55.2  $\mu$ g; thiamine mononitrate, 2.2 mg; folic acid, 990  $\mu$ g; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; and Se, 300  $\mu$ g.

ubles and thus, nutritionally usable energy is expected to be lower. Adeola and Ileleji (2009) recently reported that the ME and  $ME_n$  content of CDG with solubles in a practical corn-soybean meal-based diet for broiler chickens was determined by the regression method to be 2,904 and 2,787 kcal/kg of DM, respectively. We are not aware of data on the energy value of CDG for broiler chickens.

Supplementing livestock feeds with exogenous enzymes enables the use of feedstuffs that are otherwise unusable or allows inclusion of such feedstuffs at proportions in the feed that would be otherwise impractical. Exogenous enzymes act by complementing the endogenous supply of the enzyme or by providing the enzymes that the animal is not able to produce by itself. Carbohydrases such as xylanase and amylase target specific fiber and starch portions of plant feedstuffs and have been reported to improve energy utilization and performance in an age- and ingredient-related manner in broiler chickens and pigs (Olukosi et al., 2007a,b, 2008). Given that CDG contains mostly protein and fiber, it is important for diet formulation purposes to assess energy utilization response to carbohydrase supplementation. The experiment reported in this communication was designed to determine the energy value of CDG for broiler chickens and quantify energy utilization responses to carbohydrase supplementation.

#### MATERIALS AND METHODS

Experiments were conducted at 2 locations, Purdue University and the Louisiana State University Agricultural Center (LSUAC), and all animal procedures were approved by the Institutional Animal Care and Use Committees at each location. Starter and experimental diets were mixed and prepared from the same batch of each ingredient at Purdue University and used in the experiments at Purdue and LSUAC. Three hundred thirty-two male Ross 308 and 336 male Ross 708 broiler chicks were used at Purdue and LSUAC, respectively, and were fed a standard broiler starter diet (Table 1) from d 1 to 15 posthatch. The average d 1 and 15 posthatch BW and 14-d feed intakes were 36, 331, and 355 g for male Ross 308 birds, respectively; corresponding numbers for male Ross 708 birds were 41, 346, and 424 g, respectively.

## Diets

Analyzed gross energy and chemical composition of the CDG used in the study are presented in Table 2. The CDG were incorporated in a practical corn-soybean meal diet at 3 levels (0, 300, or 600 g/kg) without or with added carbohydrase in a  $3 \times 2$  factorial arrangement. The carbohydrase premix was prepared to provide 2,000 U of xylanase + 1,800 U of amylase/ kg of feed and was supplied by Danisco Animal Nutrition (Marlborough, UK). Ingredient composition of the 6 diets is shown in Table 3. Energy-yielding ingredients such as corn, soybean meal, soy oil, and cornstarch were replaced by CDG in such a way as to maintain the same ratio of corn, soybean meal, soy oil, and cornstarch across the experimental diets. These ratios were 1.353, 9.740, 8.969, 7.200, 6.630, and 0.921 for corn:soybean meal, corn:soy oil, corn:cornstarch, soybean meal:soy oil, soybean meal:cornstarch, and soy oil:cornstarch, respectively, for the diets in Table 3. This substitution method is important due to energy contribution of basal ingredients and test (CDG) ingredient to the experimental assay diets when using the regression method in energy utilization studies (Adeola and Ileleji, 2009).

## **Experimental Procedures**

In each location (Purdue and LSUAC), 288 of the initial 332 or 336 birds were sorted by BW and assigned to 8 cages per diet with 6 birds per cage in such a way that the average initial BW was similar across diets.

Cages were divided into 8 blocks of 6 diets and diets were randomly assigned to cages within each block. Birds were provided ad libitum access to water and dietary treatments from d 15 to 22 posthatch.

Excreta were collected twice daily on d 19, 20, and 21 posthatch. During collection, waxed paper was placed in trays under the cages and excreta on the waxed paper were collected. The collected excreta samples were pooled per cage over the 3 d, stored in a freezer, dried, and ground to pass through a 0.5-mm screen using a mill grinder (Retsch ZM 100, GmbH & Co. K.C., Haan, Germany). On d 22 posthatch, feeders and birds were weighed to determine weight gain and feed intake, birds were killed, and ileal digesta were collected from the Meckel's diverticulum to about 2 cm cranial to the ileocecal junction. Ileal contents from birds were flushed with distilled water into plastic containers, pooled by cage, and stored in a freezer  $(-20^{\circ}C)$  until freeze-dried and ground. Frozen excreta and ileal digesta samples were shipped to Purdue University from LSUAC for processing and analyses.

## Analyses

Ileal digesta, excreta, and diet samples were analyzed for gross energy to determine the ileal digestible energy (IDE), ME, and ME<sub>n</sub>. Gross energy in samples was determined in a bomb calorimeter (Parr 1261 bomb calorimeter, Parr Instrument Co., Moline, IL) using benzoic acid as a calibration standard. Samples were dried at 105°C in a drying oven (Precision Scientific Co., Chicago, IL) for 24 h for DM determination. Nitrogen was determined using the combustion method (Leco model) FP-2000 N analyzer, Leco Corp., St. Joseph, MI) using EDTA as a calibration standard. Chromium concentration in the diets and excreta samples was determined using the method of Fenton and Fenton (1979). Diets were analyzed for xylanase and amylase where 1 U of xylanase is defined as the quantity of the enzyme that liberates 1  $\mu$ mol of xylose equivalent per minute and 1 U of amylase is defined as the amount of the enzyme catalyzing the hydrolysis of 1 µmol of glucosidic linkage per minute. Xylanase activity in diets was measured using a kit (Megazyme International Ireland Ltd., Bray, Ireland) based on the method by McCleary (1991). Amylase activity in feed was measured using Phadebas (Megazyme International Ireland Ltd.) tablets based on the method published by Barnes and Blakeney (1974) and McCleary and Sheehan (1989). Dry matter, gross energy, and N in CDG were determined as described above. Proximate analyses [methods 990.03] (for N), 942.05 (for ash), 920.39 (for crude fat), 978.10 (for crude fiber), 934.01 (for moisture), 973.18 (A, B, C, D) (for neutral detergent fiber and acid detergent fiber), 985.01(A, B, D) (for Ca and P), and 982.30 E (a,b,c) (for amino acid); AOAC International, 2000] of the CDG were conducted at the University of Missouri Experiment Station Chemical Laboratories, Columbia.

#### Calculations and Statistical Analysis

Coefficients (C) of ME or IDE were calculated as follows:

$$C = 1 - [(Md/Mo) \times (Eo/Ed)],$$

where Md is the concentration of the marker Cr in the diet, Mo is the concentration of the marker Cr in the excreta or ileal digesta output, Eo is the concentration of energy in the excreta or ileal digesta output, and Ed is the concentration of energy in the diet. The product of C and the gross energy (kcal/kg) concentration of the diet give the ME (kcal/kg) or IDE (kcal/kg) of the diet. Because catabolic compounds in excreted N can contribute to energy loss, ME was corrected to zero N retention (ME<sub>n</sub>) using a factor of 8.22 kcal/g (Hill and Anderson, 1958). The energy-yielding ingredients contributed 955 g/kg of diet to the gross energy concentration. Ileal digestible energy and ME in CDG were determined by correcting substitution rate for the energy contributions of basal ingredients and CDG to the total dietary energy as described previously (Adeola and Ileleji, 2009). The coefficients (C) of ME, ME<sub>n</sub> or IDE for assay diets (diets in which a portion of the basal diet is substituted with the test ingredient CDG). basal diet, and test ingredient are Cad, Cbd, and Cti, respectively. The proportional contribution of energy by the basal diet and test ingredient to the assay diet

Table 2. Analyzed gross energy and chemical composition of the corn distillers grains used in the study on an as-fed basis<sup>1</sup>

| Item                            | Amount |
|---------------------------------|--------|
| DM, g/kg                        | 936.3  |
| Gross energy, kcal/kg           | 4,894  |
| CP (N $\times$ 6.25), g/kg      | 315.1  |
| Crude fat, g/kg                 | 94.6   |
| Crude fiber, g/kg               | 94.8   |
| Neutral detergent fiber, g/kg   | 495.6  |
| Acid detergent fiber, g/kg      | 179.1  |
| Ash, g/kg                       | 18.6   |
| Ca, g/kg                        | 0.19   |
| P, g/kg                         | 4.8    |
| Indispensable amino acids, g/kg |        |
| Arg                             | 12.9   |
| His                             | 8.8    |
| Ile                             | 12.9   |
| Leu                             | 43.6   |
| Lys                             | 9.3    |
| Met                             | 6.7    |
| Phe                             | 17.2   |
| Thr                             | 11.6   |
| Trp                             | 2.0    |
| Val                             | 17.2   |
| Dispensable amino acids, g/kg   |        |
| Ala                             | 25.0   |
| Asp                             | 20.1   |
| Cys                             | 6.0    |
| Glu                             | 52.2   |
| Gly                             | 11.7   |
| Pro                             | 25.7   |
| Ser                             | 13.2   |
| Tyr                             | 13.1   |

<sup>1</sup>Values presented are from 1 replicate analysis for amino acids and means of duplicate analyses for the other nutrients.

are Pbd and Pti, respectively; by definition Pbd + Pti = 1 or Pbd = 1 - Pti. The assumption of additivity in diet formulation implies that

$$Cad = (Cbd \times Pbd) + (Cti \times Pti).$$

Solving for Cti gives

$$Cti = [Cad - (Cbd \times Pbd)]/Pti.$$

Substituting 1 - Pti for Pbd gives

$$Cti = \{Cbd + [(Cad - Cbd)/Pti]\}.$$

The product of Cti at each level of test ingredient CDG substitution rate (300 or 600 g/kg), the gross energy (kcal/kg) concentration of CDG, and kilograms of

of dry CDG intake (0.3 or 0.6) is the CDG-associated ileal digestible energy, ME, or  $ME_n$  intake in kilocalories.

Growth performance and energy utilization response data were analyzed as a  $2 \times 3 \times 2$  factorial of location (Purdue or LSUAC), CDG (0, 300, or 600 g/kg), and carbohydrase (not added or added) in a randomized complete block design using the GLM procedures of SAS Institute (2006). The model included a location (1 df), replicate (7 df), CDG (2 df), carbohydrase (1 df), and interactions. The initial analysis indicated that there was no interaction among location and the other factors (location × CDG; location × carbohydrase; location × CDG × carbohydrase) in the model; therefore, those interactions were pooled into the error term.

The regression of CDG-associated IDE, ME, or  $ME_n$ intake in kilocalories against kilograms of CDG intake

Table 3. Ingredient composition of experimental diets fed from d 15 to 22 posthatch on an as-fed basis

|  |               |                    | Dietary corn dist | illers grain, g/kg |                   |         |
|--|---------------|--------------------|-------------------|--------------------|-------------------|---------|
|  | Car           | rbohydrase not add | led               |                    | Carbohydrase adde | d       |
| Item   | 0             | 300                | 600               | 0                  | 300               | 600     |
| Ingredient, g/kg                                 |               |                    |                   |                    |                   |         |
| Corn   | 487.0         | 333.4              | 179.8             | 477.0              | 323.4             | 169.8   |
| Soybean meal $(47.5\%)$                          | 360.0         | 246.5              | 132.9             | 360.0              | 246.5             | 132.9   |
| Soybean oil                                      | 50.0          | 34.2               | 18.5              | 50.0               | 34.2              | 18.5    |
| Dicalcium phosphate <sup>1</sup>                 | 20.0          | 20.0               | 20.0              | 20.0               | 20.0              | 20.0    |
| Limestone  | 13.0          | 13.0               | 13.0              | 13.0               | 13.0              | 13.0    |
| NaCl   | 4.0           | 4.0                | 4.0               | 4.0                | 4.0               | 4.0     |
| Vitamin-mineral premix <sup><math>2</math></sup> | 3.0           | 3.0                | 3.0               | 3.0                | 3.0               | 3.0     |
| DL-Met   | 2.8           | 2.8                | 2.8               | 2.8                | 2.8               | 2.8     |
| L-Lvs:HC]  | 0.7           | 0.7                | 0.7               | 0.7                | 0.7               | 0.7     |
| L-Thr  | 0.2           | 0.2                | 0.2               | 0.2                | 0.2               | 0.2     |
| Cornstarch <sup>3</sup>                          | 34.3          | 17.2               | 0.1               | 34.3               | 17.2              | 0.1     |
| Carbohydrase premix <sup>4</sup>                 | 0.0           | 0.0                | 0.0               | 10.0               | 10.0              | 10.0    |
| $Cr oxide premix^5$                              | 25.0          | 25.0               | 25.0              | 25.0               | 25.0              | 25.0    |
| Corn distillers grains                           | 20.0          | 300.0              | 600.0             | 20.0               | 300.0             | 600.0   |
| Total  | 1 000 0       | 1 000 0            | 1 000.0           | 1 000 0            | 1 000 0           | 1 000 0 |
| Calculated composition                           | 1,000.0       | 1,000.0            | 1,000.0           | 1,000.0            | 1,000.0           | 1,000.0 |
| ME_kcal/kg                                       | 2 992         | 3 111              | 2 000             | 3 991              | 3 100             | 2 007   |
| $CP (N \times 6.25) $ g/kg                       | 218 /         | 232.8              | 2,000             | 218 /              | 232.8             | 2,337   |
| $C_1 (1 \times 0.25), g/Rg$                      | 10.02         | 0.77               | 0.53              | 10.02              | 0.77              | 0.53    |
| D g/log  | 7 30          | 7.00               | 9.00<br>8.60      | 7 20               | 7.00              | 8.60    |
| Nonphytato P. g/kg                               | 1.30          | 5.68               | 6.48              | 1.23               | 5.68              | 6.48    |
| Total amino acida g/kg                           | 4.00          | 5.08               | 0.40              | 4.00               | 5.08              | 0.40    |
| Ang  | 11 20         | 19.99              | 19.97             | 14 99              | 19.99             | 19.97   |
| Hig<br>Lis                                       | 14.00<br>5 79 | 6.08               | 6.44              | 14.30<br>5.72      | 6.08              | 6.42    |
|  | 0.75          | 0.08               | 0.44              | 0.75               | 0.08              | 0.45    |
| lie<br>Lorr                                      | 9.04          | 9.22               | 9.40              | 9.04               | 9.22              | 9.40    |
| Leu  | 10.00         | 22.00              | 20.79             | 10.00              | 22.00             | 20.79   |
| Lys  | 12.47         | 11.05              | 9.03              | 12.47              | 11.05             | 9.03    |
| Met  | 6.09          | 6.70<br>10.66      | (.31              | 6.08               | 6.70<br>10.66     | (.31    |
| TSAA   | 9.55          | 10.66              | 11.77             | 9.55               | 10.66             | 11.77   |
| Phe  | 10.27         | 11.05              | 11.83             | 10.27              | 11.05             | 11.83   |
| Phe + Tyr  | 18.76         | 19.89              | 21.03             | 18.75              | 19.89             | 21.02   |
| Thr  | 8.34          | 8.96               | 9.57              | 8.34               | 8.95              | 9.57    |
| Trp  | 2.96          | 2.65               | 2.35              | 2.96               | 2.65              | 2.35    |
| Val  | 9.94          | 10.86              | 11.77             | 9.94               | 10.85             | 11.77   |

<sup>1</sup>20% Ca, 18.5% P.

<sup>2</sup>Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D<sub>3</sub>, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B<sub>12</sub>, 13.2  $\mu$ g; biotin, 55.2  $\mu$ g; thiamine mononitrate, 2.2 mg; folic acid, 990  $\mu$ g; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; and Se, 300  $\mu$ g.

<sup>3</sup>Adjusted cornstarch level to accommodate cornstarch in the chromic oxide premix.

 $^{4}$ Carbohydrase premix prepared to provide 2,000 U of xylanase + 1,800 U of amylase/kg of feed. The xylanase and amylase were supplied by Danisco Animal Nutrition (Marlborough, UK).

<sup>5</sup>Prepared as 1 g of chromic oxide added to 4 g of cornstarch.

for cage of birds was conducted and the solutions option was used to generate the intercept and slope using the GLM procedures of SAS Institute (2006). There were 16 blocks (8 blocks in each of 2 locations) and each block consisted of 3 cages of 0, 300, or 600 g of CDG/ kg per carbohydrase (not added or added). For each block, regression of CDG-associated IDE, ME, or ME<sub>n</sub> intake in kilocalories against kilograms of CDG intake generated 16 intercepts and 16 slopes per carbohydrase (not added or added). With a block of 3 cages serving as the experimental unit, the intercept and slope data were analyzed using the GLM procedures of SAS Institute (2006) in a randomized complete block design using the intercept or slope as the dependent variable and carbohydrase as the independent variable with 7 df for replicate, 1 df for location, 1 df for carbohydrase, 1 df for location  $\times$  carbohydrase interaction, and 21 df for the error term. Interaction was pooled into the error term because it was not significant, giving 22 df for the error term. Statistical significance was determined at an  $\alpha$  level of 0.05.

#### RESULTS

The results of carbohydrase analysis of diets indicated respective xylanase of 329, <100, or 329 U/kg in experimental diets containing CDG levels at 0, 300, or 600 g/kg without added carbohydrase and 1,816, 1,757, or 1,885 U/kg in experimental diets containing CDG levels at 0, 300, or 600 g/kg with added carbohydrase. Amylase in diets without added carbohydrase was less than 100 U/kg, but the amylase (U/kg) of diets with added carbohydrase was 1,547, 1,858, or 1,645 for diets containing CDG at 0, 300, or 600 g/kg, respectively. The enzyme formulation did not contain protease. The CDG used in the current study contained 4,894 kcal of gross energy/kg, 315.1 g of CP/kg, and 936 g of DM/kg (Table 2). Increasing the dietary substitution of CDG linearly and quadratically reduced (P < 0.01) final weight, weight gain, feed intake, and G:F of birds over the 7-d period regardless of dietary supplementation with carbohydrase (Table 4). Addition of carbohydrase improved (P < 0.05) the weight gain, tended to improve (P = 0.052) feed intake, but had no effect on G:F ratio of birds over the 7-d period. There were interactions (P < 0.01) between CDG level and carbohydrase supplementation in IDE, ME, and ME<sub>n</sub> of broilers fed the experimental diets containing CDG levels at 0, 300, or 600 g/kg without or with added carbohydrase fed from d 15 to 22 posthatch (Table 4). The IDE of diets decreased both linearly (P < 0.01) and quadratically (P< 0.05) as CDG increased from 0 to 600 g/kg regardless of carbohydrase supplementation. There was a linear (P < 0.01) decrease in ME of diets from 3,239 to 2,510 kcal/kg as CDG increased from 0 to 600 g/kg in the diets without added carbohydrase, whereas for birds fed the carbohydrase-supplemented diets, there were both linear (P < 0.01) and quadratic (P < 0.01) decreases from 3,398 to 2,613 kcal/kg as CDG increased from 0 to 600 g/kg. Likewise, dietary ME<sub>n</sub> linearly decreased (P < 0.01) from 3,071 to 2,346 kcal/kg without added carbohydrase and linearly (P < 0.01) and quadratically (P < 0.01) decreased from 3,224 to 2,449 kcal/kg with carbohydrase supplementation as CDG increased from 0 to 600 g/kg. Supplementation with carbohydrase improved (P < 0.01) IDE, ME, and ME<sub>n</sub> (Table 4).

Regressions and comparison of carbohydrase (not added or added) for intercepts and slopes of the regressions in the determination of IDE, ME, and ME<sub>n</sub> of CDG are presented in Table 5. The IDE regression equation for the diet without added carbohydrase was 44 + 2,340X,  $r^2 = 0.953$ , which implies an IDE value of 2.340 kcal/kg for the CDG. The IDE regression equation for the diet with added carbohydrase was -17 + $2,622X, r^2 = 0.985$ , which indicates an IDE value of 2,622 kcal/kg. Likewise, ME regression equation for the diet without added carbohydrase was Y = 10 + 2.315X,  $r^2 = 0.993$ , which indicates a ME value of 2,315 kcal/ kg for the CDG, and that for the diet with added carbohydrase was Y = -25 + 2,448X,  $r^2 = 0.979$ , which connotes a ME value of 2,448 kcal/kg. For ME<sub>n</sub>, regression equation for the diet without added carbohydrase was Y = 10 + 2,132X,  $r^2 = 0.991$ , which indicates a ME value of 2,132 kcal/kg for the CDG, and that for the diet with added carbohydrase was Y = -22 + 2,264X,  $r^2 = 0.978$ , which implies a ME value of 2,264 kcal/ kg. The slopes were greater (P < 0.05) when carbohydrase was added than without carbohydrase supplementation, which indicates that carbohydrase supplementation improved the IDE, ME, and ME<sub>n</sub> of CDG in practical corn-soybean meal-based diets used in this current study.

# DISCUSSION

The primary objectives of the current study were to determine the IDE, ME, and  $ME_n$  of CDG for broiler chickens and to quantify the contribution of added carbohydrase to the energy value of CDG using the regression method. By analysis, the CDG sample used in the current experiment contained 936 g/kg of DM, 4,894 kcal/kg of gross energy, 315.1 g/kg of CP, 94.6 g/kg of crude fat, 94.8 g/kg of crude fiber, 495.6 g/kg of neutral detergent fiber, 179.1 g/kg of acid detergent fiber, 0.19 g/kg of Ca, and 4.8 g/kg of P. The above-stated nutritional characteristics are expectedly different from those of CDG with solubles, whose typical composition is 4.8 kcal/g of gross energy, 265 g/kg of CP, 108 g/kg of crude fat, 61 g/kg of crude fiber, 250 g/kg of neutral detergent fiber, 99 g/kg of acid detergent fiber, 0.3 g/ kg of Ca, and 6.3 g/kg of P (Kim et al., 2008; Adeola and Ileleji, 2009; Stein and Shurson, 2009). Because CDG does not have the condensed solubles added back, the concentration of structural, less digestible carbohydrates tends to be higher in CDG than those in CDG with solubles and thus consistent with the composition of CDG used in the current study. Published data on nutrient composition of CDG are limited. Pahm et al. (2008) used CDG that contained 287 g/kg of CP, 373 g/kg of neutral detergent fiber, 182 g/kg of acid detergent fiber, 8.1 g/kg of Lys, and 5.6 g/kg of Met compared with the CDG used in the current study. For high-protein CDG, the following ranges of nutrient composition have been reported: 411 to 534 g/kg of CP, 29 to 37 g/kg of crude fat, 165 to 340 g/kg of neutral detergent fiber, 87 to 273 g/kg of acid detergent fiber, 9.5 to 13.2 g/kg of Lys, and 8.1 to 10.6 g/kg of Met (Widmer et al., 2007; Kim et al., 2008, 2009; Applegate et al., 2009).

Given that the study was designed to quantify the contribution of carbohydrase to the IDE, ME, and  $ME_n$  contents of CDG using the regression method, the use of a relatively high concentration of CDG in diets is essential for generating reliable energy values. The ME and  $ME_n$  of the diet that contained 0 g of CDG/kg with no added carbohydrase are similar to the 3,313 and 3,129 kcal/kg reported by Adeola and Ileleji (2009). The diets containing 600 g of CDG/kg would decrease growth performance and energy and nutrient utilization during the relatively short period of feeding the diets, but this was necessary to attain the objective of determining the IDE, ME, and  $ME_n$ values of CDG. Indeed, there was a linear reduction in the 7-d weight gain, feed intake, and feed efficiency of birds as dietary concentration of CDG increased from 0 to 600 g/kg, which in part resulted from the linear reduction in dietary energy available to the birds for growth. It is insightful that there was a 23% reduction in the average of IDE, ME, and ME<sub>n</sub>, which mirrored the 26% reduction in the average of the 7-d weight gain, feed intake, and feed efficiency of birds as dietary level of CDG increased from 0 to 600 g/kg. During the dry-grind processing of corn for ethanol production, most of the starch in the grain is converted to ethanol and only a small amount of starch is present in CDG. However, the fiber in corn is not converted to ethanol, and as a result, CDG with solubles contains approximately 35% insoluble and 6% soluble dietary fiber (Stein and Shurson, 2009). The digestibility of dietary fiber is low, which reduces the digestibility of DM; this in addition to the low oil content is the reason the digestibility of energy in CDG is reduced and thus the reduced dietary IDE, ME, and  $ME_n$  as dietary CDG increased.

The ME value of CDG evaluated in the current study is 2,315 kcal/kg of DM, which is lower than the 2,904 kcal/kg of DM recently reported for CDG with solubles using similar experimental procedures and facilities (Adeola and Ileleji, 2009). Although not in the same study, the 589 kcal of higher ME in CDG with solubles is consistent with the 50% lower neutral detergent fiber concentration and 45% lower acid detergent fiber concentration in CDG with solubles compared with CDG. After fermentation of the starch in corn into ethanol, the remaining nutrients (protein, fat, and fiber) are concentrated approximately 3 times. The concentration of fat and residual starch in the condensed solubles is

|                   |        |             | CDG,    | , g/kg |             |       |       |        |           |                       |        |        |        |                |
|-------------------|--------|-------------|---------|--------|-------------|-------|-------|--------|-----------|-----------------------|--------|--------|--------|----------------|
|                   | Carbol | hydrase not | t added | Carb   | ohydrase aa | lded  |       |        |           | P.                    | -value |        |        |                |
| Item              | 0      | 300         | 600     | 0      | 300         | 600   | SEM   | Enzyme | CDG level | Enzyme ×<br>CDG level | $L^3$  | $Q^3$  | $L^4$  | $\mathrm{Q}^4$ |
| Initial weight, g | 370    | 370         | 370     | 370    | 370         | 370   |       |        |           |                       |        |        |        |                |
| Final weight, g   | 730    | 691         | 597     | 763    | 708         | 602   | 15.98 | 0.162  | < 0.01    | 0.671                 | < 0.01 | 0.159  | < 0.01 | 0.195          |
| Weight gain, g    | 360    | 321         | 227     | 393    | 338         | 232   | 11.07 | 0.045  | < 0.01    | 0.439                 | < 0.01 | 0.044  | < 0.01 | 0.059          |
| Feed intake, g    | 511    | 490         | 441     | 547    | 512         | 453   | 14.44 | 0.052  | < 0.01    | 0.710                 | < 0.01 | 0.415  | < 0.01 | 0.498          |
| G:F, g:kg         | 700    | 658         | 519     | 721    | 665         | 512   | 10.65 | 0.423  | < 0.01    | 0.435                 | < 0.01 | < 0.01 | < 0.01 | < 0.01         |
| IDE, kcal/kg      | 3,317  | 3,052       | 2,554   | 3,432  | 3,006       | 2,779 | 37.4  | < 0.01 | < 0.01    | < 0.01                | < 0.01 | 0.013  | < 0.01 | 0.032          |
| ME, kcal/kg       | 3,239  | 2,895       | 2,510   | 3,398  | 2,862       | 2,613 | 30.9  | < 0.01 | < 0.01    | < 0.01                | < 0.01 | 0.588  | < 0.01 | < 0.01         |
| $ME_n$ , kcal/kg  | 3,071  | 2,730       | 2,346   | 3,224  | 2,705       | 2,449 | 28.4  | < 0.01 | < 0.01    | < 0.01                | < 0.01 | 0.540  | < 0.01 | < 0.01         |

Table 4. Growth performance, ileal digestible energy (IDE), ME, and ME<sub>n</sub> of broilers fed the diets containing corn distillers grains (CDG) levels at 0, 300, or 600 g/kg without or

by Danisco Animal Nutrition (Marlborough, UK) <sup>2</sup>Carbohydrase premix prepared to supply 2,000 U of xylanase + 1,800 U of amylase/kg of feed. The xylanase and amylase were supplied <sup>3</sup>Linear (L) and quadratic (Q) contrasts for the diets without enzyme.

<sup>4</sup>Linear (L) and quadratic (Q) contrasts for the diets with enzyme.

greater than in CDG; thus, the addition of condensed solubles to CDG dilutes the concentrations of CP, neutral detergent fiber, and acid detergent fiber but increases concentrations of residual starch and fat. These are expected to affect energy utilization differences between CDG and CDG with solubles.

For dehydrated CDG of the beverage industry origin, NRC (1994) reported  $ME_n$  of 2,097 kcal/kg of DM, which compares with the 2,132 kcal/kg of DM determined in the current study. We are not aware of published data on the ME of dry-grind processing of corn for ethanol-produced CDG for broilers. In studies that determined the ME of high-protein CDG for broilers, Kim et al. (2008) reported a TME<sub>n</sub> of 3,246 kcal/kg of DM and Applegate et al. (2009) reported AME<sub>n</sub> and AME of 2,685 and 2,626 kcal/kg of DM, respectively. Because energy utilization is affected by a variety of factors including protein quality of a feed, it is common to correct ME for N retention that occurs during the assay period, which in the current study resulted in an approximately 8% reduction in the ME content of the CDG. Reductions in the range of 4 to 10% have been reported in several studies with broilers and ducks (Hong et al., 2002; Adeola et al., 2007; Adeola and Ileleji, 2009). In all probability, protein amount and quality are contributory, in part, to the differences in N-corrected reductions in ME. Addition of CDG to the diet at 300 or 600 g/kg resulted in positive N retention in the birds. Because diets with added CDG contained N that supplied part of the bird's need for protein, there was reduced catabolism of body protein and hence a positive N retention.

One of the objectives of the current study was to quantify the contribution of an added carbohydrase to the IDE, ME, and ME<sub>n</sub> of CDG. To attain this objective, the study was designed to generate IDE, ME, or ME<sub>n</sub> for each block of 3 cages that used 0, 300, or 600 g of CDG substitution/kg for each level of carbohydrase (not added or added). Ileal digestible energy, ME, or  $ME_n$  values from each of 16 blocks (8 per location) were subsequently compared between diets without and with added carbohydrase. Carbohydrase supplementation improved the IDE, ME, and ME<sub>n</sub> of CDG in practical corn-soybean meal-based diets used in this current study by 12, 5.7, and 6.2%, respectively.

The IDE represents energy available to birds from a feed ingredient before microbial fermentation of energy substrates in the ceca and the relatively short colon. A greater carbohydrase-related improvement in IDE (12%) than ME (5.7%) in the current study is perhaps explained by the fact that less energy substrates are available for cecal and colonic microbial fermentation because smaller amounts of undigested structural dietary components are passing from the ileum into the ceca and colon. This would indeed improve the efficiency of dietary energy utilization because glucose, absorbed from the midgut, is a more efficient energy substrate than volatile fatty acids absorbed from the hindgut. Adeola and Bedford (2004) observed 6 and 3%improvements in IDE and ME for duck diets supplemented with xylanase. Other studies with broilers have also shown that a larger improvement in IDE is accompanied by a smaller improvement in ME (Cowieson and Adeola, 2005; Olukosi et al., 2007b).

Some structural carbohydrates, as may be in CDG, act as antinutrients by limiting the access of digestive enzymes to substrates and also by increasing digesta viscosity, thus impairing nutrient utilization. Carbohydrases may hydrolyze structural carbohydrates, thereby reducing the barrier to nutrient digestibility and thus enhancing the utilization of the feedstuff. There are several possible mechanisms to explain the carbohydrase-related improvement in utilization of dietary energy. Parkkonen et al. (1997) observed in vitro that xylanase increased the permeability of the aleurone layer through hydrolysis of structural carbohydrates, which may enhance contact of digestive or exogenous enzymes and their substrates. Supplementation of diets

 $r^2$ Item Intercept, kcal Slope, kcal/kg CDG IDE No added carbohydrase 2.3400.95344.3Added carbohydrase<sup>1</sup> -17.12,622 0.9859.93 SEM 51P-value < 0.01< 0.01CDG ME 9.9 2,3150.993 No added carbohydrase -25.4Added carbohydrase 2,4480.97933SEM 6.81 P-value 0.002< 0.01CDG ME<sub>n</sub> 2,132No added carbohydrase 10.30.991Added carbohydrase<sup>1</sup> -21.92,2640.9787.0535SEM P-value 0.0040.014

Table 5. Comparison of carbohydrase (not added or added) for intercepts and slopes of the regressions in the determination of ileal digestible energy (IDE), ME, and ME<sub>n</sub> of corn distillers grains (CDG) on a DM basis

<sup>1</sup>Carbohydrase premix prepared to supply 2,000 U of xylanase + 1,800 U of amylase/kg of feed. The xylanase and amylase were supplied by Danisco Animal Nutrition (Marlborough, UK).

with carbohydrases that hydrolyze viscosity-inducing water-soluble carbohydrates would be expected to ameliorate these effects and improve nutrient utilization. Indeed, this was demonstrated by Adeola and Bedford (2004), in which supplementation of a high-viscosity wheat-based diet with xylanase mitigated the growth performance reduction with an accompanying decrease in duodenal and ileal digesta viscosity and a subsequent increase in energy and nutrient utilization. Xylanase and amylase may improve IDE, ME, and ME<sub>n</sub> due to improved digestibility of starch (Gracia et al., 2003), sparing effects on endogenous amino acids and energy associated with a reduction in endogenous amylase production (Mahagna et al., 1995; Ritz et al., 1995; Gracia et al., 2003), or improved access to cellular contents associated with hydrolysis of structural carbohydrates (Bedford, 2002). Perhaps the higher energy resulting from carbohydrase supplementation is a consequence of a combination of the mechanisms discussed above.

The respective IDE, ME, and ME<sub>n</sub> values of the CDG sample evaluated were 2,340, 2,315, and 2,132 kcal/kg of DM, respectively. Xylanase and amylase supplementation improved the IDE, ME, and ME<sub>n</sub> of CDG in practical corn-soybean meal-based diets used in this study by 12, 5.7, and 6.2%, respectively. Given the like-lihood that the response of different feeds to xylanase and amylase supplementation may be different, more research is needed to quantify the energy utilization responses of a variety of feeds to carbohydrase supplementation.

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