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Effects of DDGS nutrient composition (reduced-oil) on digestible and metabolizable energy value and prediction in growing pigs

January 2013

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A special thanks to our funding partners on this research: Minnesota Corn Research & Promotion Council and POET Nutrition, LLC. Thanks, too, to D. Hedges (University of Minnesota, St. Paul) and J. Cook (USDA-ARS, Ames) for their help with sample collection and laboratory analysis.

Contents

Abstract: Two experiments were conducted to determine the DE and ME content of corn distillers dried grains with solubles (DDGS) containing variable crude fat concentrations and to develop prediction equations for estimating DE and ME. Relative to this objective, EE ranged from 4.88% to 10.88% (DM basis) in Exp. 1 while in Exp. 2 the range was from 8.56% to 13.23% (DM basis). The DE and ME values for the corn-DDGS averaged 3,692 and 3,463 kcal/kg DM, respectively, for Exp. 1, and 3,635 and 3,425 kcal/kg DM, respectively, for Exp. 2. These small differences in DE and ME content between experiments may be due to the differences in pig BW and differences in DDGS particle size which averaged 338 µm in Exp. 1 compared to 791 µm in Exp. 2. In Exp. 1, no ingredient physical or chemical parameter measured (bulk density-BD, particle size-PS, GE, CP, starch-ST, TDF, NDF, ADF, hemicellulose-HC, EE, or ash) was significant at $P \leq 0.15$ to predict DE or ME content in corn DDGS. For Exp. 2, the best fit DE equation was DE, kcal/kg DM = 1,601- (54.48 × % TDF) + (0.69 × % GE) + (731.5 × BD), with $R^2 = 0.91$ and SE = 41.25. The best fit ME equation was: ME, kcal/kg DM = 4,558 + (52.26 × % EE) - (50.08 × % TDF), with $R^2 = 0.85$ and SE = 48.74. However, crude fat has the largest impact on the gross energy (GE) of DDGS, with PS having a secondary effect. Digestibility of various nutritional components within DDGS can be quite variable. These results suggest that although EE may be a good indicator of the GE in DDGS, it is not a primary indicator of DE or ME, and dietary fiber, namely ADF or TDF, is more important in determining the DE or ME value of DDGS to growing pigs.

Key words: energy, energy prediction, DDGS, growing-finishing pigs, lipid content

Introduction

Corn dried distillers grains with solubles (**DDGS**) has typically contained 10 to 11% ether extract (**EE**) with a ME content similar to corn (Stein and Shurson, 2009). However, the majority of U.S. ethanol plants have recently implemented oil extraction technology that has led to the production of DDGS with a wider range of EE (5 to 12%) than previously produced. Because oil contains approximately 2.25 times more energy than carbohydrates, removal of corn oil likely reduces the ME content in DDGS which can affect its economic value and dietary inclusion rates.

Four studies have been published (Stein et al., 2006; Pedersen et al. 2007; Stein et al., 2009; Anderson et al. 2012) which determined the DE and ME content of 30 sources of corn DDGS varying in EE from 9.6 to 14.3% (DM basis). Studies by Pedersen et al. (2007) and Anderson et al. (2012) also included DE and ME prediction equations based on chemical analysis to estimate DE and ME content. In contrast, only three studies have been published that estimated the effect of reduced-oil DDGS on ME content (Dahlen et al., 2011; Jacela et al., 2011; Anderson et al., 2012). In the studies by Jacela et al. (2011) and Anderson et al. (2012), oil was removed by hexane extraction, whereas Dahlen et al. (2011) evaluated a product with no solubles added to the final product. The processes used to produce reduced-oil DDGS in these studies are different than the centrifugation technologies used by ethanol plants today. Consequently, results from these studies may not be applicable for estimating the impact of oil extraction on ME content in reduced-oil DDGS because of the wide disparity in DE and ME estimates based on EE of the sources evaluated. Therefore, the objectives of this study were to obtain various sources of DDGS varying in EE content from which to determine DE and ME, and to develop prediction equations based upon ingredient composition.

Procedures

Animal Management

The Institutional Animal Care and Use Committee at Iowa State University (Ames) approved all experimental protocols. Gilts used in this research were offspring from PIC Camborough 22 sows × L337 boars (Pig Improvement Company, Hendersonville, TN). Three groups of 24 gilts (n = 72; BW = 105.6 ± 9.1 kg) were utilized in Exp. 1, and 6 groups of 24 gilts (n = 144; BW = 83.7 ± 8.3 kg) were utilized in Exp. 2. Gilts were housed individually in metabolism crates (Exp. 1: 1.2 × 2.4 m; Exp. 2: 0.7 × 1.5 m) that allowed for separate but total collection of feces and urine. Crates were equipped with a stainless steel feeder and a nipple waterer, to which the pigs had ad libitum access. Gilts were randomly assigned to either the basal or DDGS-containing diet resulting in 12 replications for pigs fed the control diet and 15 replications for pigs fed each DDGS source in Exp. 1, or 12 replications for pigs fed the control diet or each DDGS source in Exp. 2.

Diets

Gilts were fed a standard corn–soybean meal diet prior to experimentation and were weighed at the beginning and end of the metabolism trials. For each trial, the same basal diet was fed which contained 96.7% corn and supplemental vitamins and minerals, with corn being the sole energy-containing ingredient (**Table 1**). In Exp. 1, 4 DDGS samples varying in ether extract (EE) content from 4.88 to 10.88% (DM basis) were evaluated while in Exp. 2, 11 DDGS samples with EE content varying from 8.56 to 13.23% (DM basis) were evaluated. Particle size of DDGS sources varied from 294 to 379 g/cc in Exp. 1 and from 568 to 1,078 g/cc in Exp. 2. In both experiments, pigs were either fed 100% of the control diet or test diets which contained 70% of the basal diet and 30% of a specific DDGS sample. All diets were fed in a meal form. Test ingredients were not ground to a constant particle size, but rather were added to the diets at their original particle size as would be fed commercially. Feed was offered at approximately 3% of BW during the 9-d adaption and 4-d collection periods. Only pigs with constant and complete feed consumption during the adaptation period were used for the 4-d collection period.

Sample Collection

During the time-based 4-d total fecal and urine collection period, stainless steel wire screens were placed under each metabolism crate for total fecal collection and stainless steel buckets containing 30 mL of 6 N HCl were placed under each crate for the total urine collection. Feces and urine were collected once daily and stored at 0°C until the end of the collection period. Feces were pooled over the 4-d period, dried in a 70°C forced-air oven, weighed, and ground through a 1-mm screen and a subsample was taken for analysis. Likewise, urine samples were pooled over the 4 d period, thawed at the end of the collection period, weighed and a subsample was collected for analysis.

Chemical Analysis and Calculations

All corn-DDGS samples were ground through a 1-mm screen before chemical analysis. Samples were analyzed at various laboratories as described in **Table 2**, with the analyzed composition of the basal diet summarized in **Table 3**, and the composition of the DDGS samples summarized in **Tables 4 and 5** for Exp. 1 and 2, respectively. To determine DE and ME, GE of the feedstuffs, feces, and urine samples were determined using an isoperibol bomb calorimeter (Parr Instrument Company, Moline, IL) with benzoic acid used as a standard. For urine, 1 mL of filtered subsample urine was added to 0.5 g of dried cellulose and subsequently dried at 50°C for 24 h. Urine addition and subsequent drying was repeated 3 times, for a total of 3 mL of filtered urine, over a 72-h period before urinary GE determination. Gross energy in cellulose was also determined and urinary GE was calculated by subtracting the GE in cellulose from the GE in the samples containing both urine and cellulose.

Gross energy intake was calculated as the product of GE content of the treatment diet and the actual feed intake over the 4-d collection period. The DE and ME of each test ingredient was calculated by subtracting the DE or ME contributed by the basal diet from the DE or ME of the diet containing that particular test ingredient and then dividing the result by the inclusion rate of the test ingredient in the diet. Because corn was the only energy-containing ingredient in the basal diet, the energy concentration of corn was calculated by dividing the DE or ME of the basal diet by 0.9670. All energy values are reported on a DM basis.

Similar to the calculations for energy, apparent total tract digestibility (**ATTD**) of ADF, C, DM, GE, EE, NDF, N, P, and S were calculated by subtracting the respective component contributed by the basal diet from the similar component of the diet containing that particular test ingredient, and then dividing the result by the inclusion rate of the test ingredient in the diet. Digestibility coefficients were then determined by dividing grams of component digested by the grams of component consumed and are reported on a percentage basis.

Statistical Analysis

Using the individual pig as the experimental unit, data were subjected to ANOVA with group and treatment in the model (SAS Inst. Inc., Cary, NC), with treatment means reported as least squares means. The experiment was conducted as a completely randomized design with DE and ME of the basal diet used as a covariate to determine DE and ME values, respectively, among all groups of pigs. Stepwise regression was used to determine the effect of the feedstuff nutrient composition on apparent GE, DE, ME, and DE:ME ME:GE, and ME:GE; variables with P-values $\leq$ 0.15 being retained in the model. The R^2 and the SE of the estimate were used to define the best fit equation. Similar to the analysis of energy, the digestibility of each component in the basal diet was used as a covariate to determine the digestibility of each component in the test diet.

Results and Discussion

General and Compositional Evaluation

One of the main objectives of this study was to obtain DDGS samples with a range of EE from which to relate ingredient composition to *in vivo* DE and ME values. Relative to this objective, EE ranged from 4.88% to 10.88% (Table 4) in Exp. 1 while in Exp. 2 the range was from 8.56% to 13.23% (Table 5). The difference in concentration of TDF and NDF among DDGS sources was 2.25% and 3.40 percentage units, respectively in Exp. 1, but was greater in Exp. 2, where they differed by 6.46 and 15.18 percentage units, respectively. This is noteworthy because Anderson et al. (2012) and Pedersen et al. (2007) showed that a measure of fiber and EE value are often included in DE and ME prediction equations for corn DDGS. Ash and CP are also primary variables in DE and ME prediction equations (Noblet and Perez, 1993; Pedersen et al., 2007; Anderson et al., 2012). In the current study, the range in CP and ash were from 28.97 to 31.19% and 5.37 to 6.14%, respectively in Exp. 1, and from 27.69 to 32.93% and 4.32 to 5.31%, respectively, in Exp. 2. Although the range in composition was not as great as reported by Anderson et al. (2012), it is equal to or greater than the ranges in corn-DDGS composition reported by others (Spiehs et al., 2002; Stein et al., 2006, 2009; Pedersen et al., 2007). Other nutrient composition data (fatty acids, minerals, starch, etc.) provided (Table 4 and 5) are not discussed but are included because these data are lacking in the literature (NRC, 2012) and may be important relative to other research topics, such as the impact of feeding DDGS varying in EE content on carcass pork fat quality (Xu et al., 2010a, b; McClelland et al., 2012).

In the current experiment, DE and ME values for the corn-DDGS averaged 3,692 and 3,463 kcal/kg DM, respectively, for Exp. 1 (**Table 6**), and 3,635 and 3,425 kcal/kg DM, respectively, for Exp. 2 (**Table 7**). These small differences in DE and ME content between experiments may be due to the differences in pig BW which has been reported to affect energy digestibility (Noblet and Shi, 1993; Le Goff et al., 2002), but may also be due to differences in DDGS particle size which averaged 338 µm in Exp. 1 compared to 791 µm in Exp. 2, and is also known to have an impact on energy digestibility (Nuzback et al., 1984; Yanez et al., 2011; Liu et al., 2012). The DE and ME content of DDGS sources evaluated in the current study compare favorably to values reported by others (Stein et al., 2006; Pedersen et al., 2007; Stein et al., 2009; Anderson et al., 2012) and the NRC (2012) for 'normal' DDGS samples. Jacela et al. (2011) and Anderson et al. (2012) evaluated a hexane-extracted DDGS with dramatically lower (4.56 and 3.15%, respectively) EE content than the EE content of DDGS samples utilized in the current experiments. A reduced-oil DDG co-product (8.8% EE) was also evaluated by Dahlen et al. (2011), but it was produced by not adding solubles to the corn DDG. Among the reduced-oil DDGS sources evaluated in the current study, corn oil was partially removed by centrifugation. Large differences in composition in corn-DDGS result from differences in dry-grind ethanol plant design, oil extraction equipment, and efficiencies of oil extraction, which makes comparing DE and ME values between different experiments challenging. Thus, the development and use of prediction equations based upon nutrient composition to estimate energy content in feeds such as barley (Fairbairn et al., 1999), meat and bone meal (Adedokun and Adeola, 2005; Olukosi and Adeola, 2009), corn-DDGS (Pedersen et al., 2007; Anderson et al., 2012), and wheat-DDGS

(Cozannet et al., 2010), like those developed for complete diets (Just et al., 1984; Noblet and Perez, 1993), is a noteworthy task.

DE and ME Prediction Estimates

In Exp. 1, no ingredient physical or chemical parameter measured (bulk density-**BD**, particle size-**PS**, GE, CP, starch-**ST**, TDF, NDF, ADF, hemicellulose-**HC**, EE, or ash) was significant at $P \leq 0.15$ to predict DE or ME content in corn DDGS. Likewise, neither DE nor ME as a percentage of GE could be predicted by any measured parameter (BD, PS, GE, CP, ST, TDF, NDF, ADF, HC, EE, and ash). The best fit equation for ME as a percentage of DE was predicted as: 107.61 – (0.64 × % CP) + (0.96 × % ash) [R^2 = 0.99, SE = 0.67, P = 0.07]. The inability of these physical and chemical parameters to predict DE, ME, or ME as a percentage of GE was surprising because we expected that the differences in several parameters among the DDGS samples evaluated would have provided enough variation from which regression equations could have been generated. Although Pedersen et al. (2007) did not evaluate reduced oil-DDGS sources *per se*, the range in EE content in their experiment (4.66% on a DM basis) was less than the corn-DDGS samples utilized in the Exp. 1 (6.00% on a DM basis) of the current study, but the ranges in NDF (6.55%) and ash (1.47%) content were greater than in the current experiment (3.40% and 0.77%, respectively). Interestingly, GE differences among DDGS samples between the current Exp. 1 and that of Pedersen et al. (2007) were similar (333 and 317 kcal/kg DM, respectively).

For Exp. 2, stepwise regression and chemical analysis were useful in generating a series of prediction equations for DE (**Table 8**). The initial regression included TDF as the most important component to predict DE, with the addition or deletion of additional parameters to the regression model via stepwise regression resulting in the best fit equation, Eq. 3: DE, kcal/kg DM = 1,601- (54.48 × % TDF) + (0.69 × % GE) + (731.5 × BD), with R^2 = 0.91 and SE = 41.25. Because TDF analysis can be costly, time consuming, and less automated, we elected to exclude TDF from the parameters offered in the model. As a result, ADF became the most important predictor in the model, Eq. 4, followed by BD resulting in the best fit question, Eq. 5: DE, kcal/kg DM = 3,343- (73.15 × % ADF) + (2,276 × BD), with R^2 = 0.76 and SE = 61.81. By not offering TDF in the model and comparing the best fit models (Eq. 5 versus Eq. 3), the SE of the estimate increased and the coefficient of determination (R^2) decreased, indicating loss in prediction confidence. It was surprising that BD was a significant factor in these models because the range in BD was only 0.100 g/cc (Table 5) among DDGS samples. However, Kingsly (2010) showed that increasing the amount of condensed solubles added to the grains fraction before producing DDGS linearly increases several chemical (e.g. EE) and physical properties of DDGS including particle size and bulk density. Since the ethanol plants vary in the rate of condensed distillers solubles use to make DDGS, and partial oil extraction occurs in the thin stillage before dehydrating to produce condensed distillers solubles, it is plausible that bulk density is a meaningful parameter for prediction DE content of DDGS. Bulk density can be excluded from Eq. 3 and Eq. 5, however in both cases the SE of the estimate increased and the coefficient of determination decreased (Eq. 2 and 4, respectively). Equation 2 appears to predict DE relatively well (R^2 = 0.86 and SE = 45.75), but Eq. 4 does not (R^2 = 0.48 and SE = 85.73). Our data are similar to others where fiber is a central component in regression equations to predict DE, whether it be for a complete diet (Noblet and Perez, 1993), wheat-DDGS (Cozannet et al., 2010), or corn-DDGS (Pedersen et al., 2007; Anderson et al., 2012). Furthermore, removing TDF from the equation and replacing it with NDF (Anderson et al., 2012) or ADF (current study) reasonably predicts DE, but this is not unexpected because there is not a large difference between TDF and NDF in corn-based co-products (Tables 4 and 5, Anderson et al., 2012). Corn-DDGS does, however, contain an appreciable amount of β-glucans derived from yeast, and it has been estimated that 20% of the weight of corn-DDGS is dried yeast (Han and Liu, 2010; Liu, 2011). Unlike NDF, measurement of TDF includes β-glucans (NRC, 2012). Therefore, it would be expected that TDF is a better parameter to use in the DE and ME prediction models than NDF because TDF provides a more complete estimate of fiber in corn-DDGS. This issue may become more important as more corn oil is removed from DDGS, thereby concentrating all other compounds, including β-glucans.

A series of prediction equations was also generated for ME (**Table 9**). The initial regression included ADF as the most important component to predict ME, with the addition or deletion of additional parameters to the model via stepwise regression resulting in the best fit equation, Eq. 4: ME, kcal/kg DM = 4,558 + (52.26 × % EE) - (50.08 × % TDF), with R^2 = 0.85 and SE = 48.74. It is worthwhile to note that similar to DE, a fiber component, in this case ADF, was an initial parameter in the regression model suggesting that fiber is a primary factor affecting the ME content of corn DDGS. This is not surprising given that DE and ME content are highly correlated. We were surprised that BD came into the model, albeit it was not maintained in the final model. The fact that TDF was in the final model suggests that analysis of feedstuffs for TDF, even in corn co-products, should be actively pursued, and is supported by our previous work (Anderson et al., 2012) and by the indication that fermented co-products may contain appreciable amounts of β-glucans (Han and Liu, 2010; Liu, 2011). It is not known whether TDF would have come into models generated by others (Nobet and Perez, 1993; Fairbairn et al., 1999; Petersen et al., 2007; Cozannet et al., 2010), as TDF was not measured in their feedstuffs. In contrast for prediction equations for complete feeds (Noblet and Perez, 1993) or corn-DDGS (Petersen et al., 2007), ash did not come into our prediction equations for either DE or ME. It also did not come into the DE or ME equations presented by Anderson et al. (2012), but was a regression coefficient parameter for ME when TDF was not offered into the list of analytes (Anderson et al., 2012).

As expected, predicting DE as a percent of GE followed a similar pattern as the prediction of DE, where TDF was a primary component in the regression model (Eq. 1 and 2, **Table 10**). Likewise, if TDF was not offered as an analyte, another fiber measure, in this case NDF, came into the model, with BD being in the final model (Eq. 3 and 4, respectively). Not allowing TDF as an analyte in regression resulted in NDF being retained in the regression model, but the resultant equations had greater standard errors and reduced coefficients of determinations making us less confident in their ability to predict DE as a percent of GE. As stated previously, this may be due to corn-DDGS having appreciable amounts of β-glucans which is analyzed by the TDF assay, but not by the NDF assay.

Metabolizable energy as a percent of DE was negatively affected by ADF content, Eq. 1, with subsequent equations excluding ADF and including a negative effect of CP, but positive effects of ash and BD (**Table 11**). Although Fairbairn et al. (1999) and Petersen et al. (2007) did not measure ME as a percent of DE, previous work (Noblet and Perez, 1993; Anderson et al., 2012) supports the notion that CP has a negative effect on ME as a percent of DE. The positive coefficient of ash was not expected, given that ash typically has a negative effect on DE or ME (Noblet and Perez, 1993; Adedokun and Adeola, 2005; Pedersen et al., 2007; Olukosi and Adeola, 2009; Conzannet et al., 2010; Anderson et al., 2012). We have no explanation for this effect; however, it is interesting to note that Fairbairn et al. (1999) also reported a positive coefficient for ash in estimating DE.

It may be of interest to predict GE from the composition of an ingredient, then applying a ME as a percent of GE to estimate ME. Because the analysis of DDGS from Exp. 1 and 2 are independent of animal experimentation, their compositional data can be combined and utilized in stepwise regression to predict GE. The results of this are presented in **Table 12**, suggesting that EE has the largest impact on the GE of DDGS (Eq. 1), with PS having a secondary (Eq. 2) effect. The fact that EE is in the model while CP, starch, and a fiber component are not in the model is not surprising, given the fact that lipids contain approximately 2.25 times the energy of carbohydrates, and various carbohydrates and CP are of somewhat similar GE content. We cannot explain the negative coefficient associated with PS on GE, given that all samples were ground to a common size before GE analysis. Even though equating ME as a percent of GE is not a common practice in the nutritional literature (Noblet et al., 1993; Pedersen et al., 2007; Anderson et al., 2012), it could then be used with an estimated GE to predict ME. Metabolizable energy as a percent of GE could not be predicted from data obtained from pigs fed in Exp. 1, but data from pigs fed in Exp. 2 indicates that either TDF (ME, % of GE = 102.3 – (1.01 × %TDF); R^2 = 0.84, SE = 0.95, P = 0.01) or NDF (ME, % of GE = 84.7 – (0.45 × %NDF); R^2 = 0.63, SE = 1.43, P = 0.01) can be used as prediction equations.

Although some might consider that EE by itself could have been a primary factor in predicting DE and ME, given the energy concentration of lipids compared to carbohydrates, it was not maintained in DE prediction equations (Table 8) and a secondary effect in ME prediction equations (Table 9). Likewise, directly relating the EE content of DDGS to ME using simple linear regression were found to have a poor fit and were not found to be significant (Exp. 1: DE, kcal/kg DM = 3,461 + (31.83 × %EE), R^2 = 0.22, *P* = 0.54; ME, kcal/kg DM = 3,130 + (46.23 × %EE), R^2 = 0.32, *P* = 0.43; Exp. 2: DE, kcal/kg DM = 3,414 + (20.72 × %EE), R^2 = 0.05, *P* = 0.49; ME kcal/kg DM = 3,103 + (30.28 × %EE), R^2 = 0.11, *P* = 0.31). This is supported by Pedersen et al. (2007) and Anderson et al. (2012) where it was reported that a fiber measure (TDF, NDF, ADF, HC, etc.) typically cane into the prediction models prior to EE. One may speculate that because fiber is of a much higher percentage in the ingredient, 3.6 fold higher over the 15 DDGS samples in the current experiment, and because fiber has a large impact on energy metabolism (Fernandez and Jorgensen, 1986; Chabeauti et al., 1991), including effects on lipid digestion (Degen et al., 2007), that this would be expected.

Digestibility Coefficients

Apparent total tract digestibility of ADF, C, DM, GE, EE, NDF, N, P, and S for pigs fed the basal diet and each DDGS are also shown in Tables 6, and 7, respectively. Average ATTD of ADF, C, DM, GE, EE, NDF, N, P, and S for pigs fed the DDGS samples (ADF, 61.21 vs. 71.78; C, 73.69 vs. 71.61; DM, 72.48 vs. 70.89; GE, 75.06 vs. 72.36; EE, 72.36 vs. 59.03; NDF, 49.36 vs. 56.59; N, 81.11 vs. 81.00; P, 61.40 vs. 58.52, and S, 88.63 vs. 83.33; Exp. 1 and 2, respectively) varied somewhat between the two experiments. In comparison, Pedersen et al. (2007) reported an average ATTD of GE, N, and P of 82.9, 82.7, and 50.8%, respectively, for 10 corn-DDGS samples, while Stein et al. (2009) reported an average ATTD of ADF,DM, GE, EE, NDF, N, and P for 4 corn-DDGS samples of 70.2, 75.1, 75.1, 72.5, 69.6, 84.9, and 56.0%, respectively. Urriola et al. (2010) reported an average ATTD of ADF and NDF of 60.7 and 59.3%, respectively. Widyaratne and Zijlstra (2007) an ATTD of GE and P in a single DDGS sample of 78.7 and 55.5%, respectively, while Almeida and Stein (2010) reported an ATTD of P in a single DDGS sample of 68.6%. Consequently, digestibility of various components within DDGS can be quite variable.

In several cases, DE, ME, DE as a percentage of GE, ME as a percentage of DE, and ME as a percentage of GE could be predicted by utilizing various total tract digestibility coefficients, as has been done by others (Noblet and Perez, 1993). However, this analysis is beyond the scope and aim of our experimentation, and it necessitates conducting animal experimentation which is a goal of being able to predict energy (DE, ME, or various relationships) from the composition of an ingredient.

Given the time and expense of animal experimentation, development and use of prediction equations based upon nutrient composition to estimate energy content in feeds is a worthwhile endeavor. Although EE may be a good indicator of the GE in the corn DDGS samples evaluated in this study, it is not a primary indicator of DE or ME. The data also indicate that dietary fiber, namely ADF or TDF, is more important in determining the DE or ME value of DDGS to growing pigs.

Table 1. Ingredient composition of basal diet, as-fed basis[1]

Ingredient	Concentration, %
Corn	96.70
Monoammonium phosphate	0.75
Limestone	1.30
Sodium chloride	0.35
Titanium dioxide	0.50
Vitamin mix[2]	0.20
Trace mineral mix[3]	0.20

[1]Basal diet formulated to contain 0.50% Ca and 0.45% P.

[2]Provided the following per kilogram of diet: vitamin A, 7,716 IU; vitamin D_3, 1,929 IU; vitamin E, 39 IU; vitamin B_{12}, 0.04 mg; riboflavin, 12 mg; niacin, 58 mg; and pantothenic acid, 31 mg.

[3]Provided the following per kilogram of diet: Cu (oxide), 35 mg; Fe (sulfate), 350 mg; I (CaI), 4 mg; Mn (oxide), 120 mg; Zn (oxide), 300 mg; and Se (Na_2SeO_3), 0.3 mg.

Table 2. Methods of analysis

Analyte	Method of analysis
Bulk density[1]	USDA (1953)
GE[1]	Isoperibol bomb calorimeter (model 1281, Parr Instrument Co., Moline, IL)
Particle size[1]	Baker and Herrman (2002)
ADF[2]	AOAC (2005) official method 973.18 (A–D)
Ash[2]	AOAC (2005) official method 942.05
CP[2]	AOAC (2005) official method 990.03
DM[2]	AOAC (2005) official method 934.01
Ether extractt[2]	AOAC (2005) official method 920.39 (A), petroleum ether
Fatty acids[2]	AOAC (2005) official method 969.33; 963.22
Free fatty acids[2]	AOAC (2005) official method 940.28
Lysine[2]	AOAC (2005) official method 982.30 E (a)
Minerals[2]	AOAC (2005) official method 985.01 (A–D)
NDF[2]	Holst (1973)
Peroxide value[2]	AOAC (2005) official method 940.28
Thiobarbituric acid[2]	AOCS (2011) official method Cd 19-90
Total starch[2]	AACC International (1976); approved method 76–13.01; modified: Sigma Starch Assay Kit (Kit STA-20, St. Louis, MO)
Total dietary fiber[3]	AOAC (2005) official method 991.43
Aflatoxin B1, B2, G1, G2[4]	AOAC (2005) official method 994.08
Deoxynivalenol[4]	Trucksess et al. (1998)
Fumonisin B1, B2, B3[4]	AOAC (2005) official method 995.15
Ochratoxin A[4]	AOAC (2005) official method 2000.3
T-2 Toxin[4]	Croteau et al. (1994)
Zearalenone[4]	MacDonald et al.(2005)

[1]Analyzed by USDA-ARS, Ames, IA.

[2]Analyzed by University of Missouri, Columbia, MO.

[3]Analyzed by Eurofins, DesMoines, IA.

[4]Analyzed by Trilogy Analytical Laboratory, Washington, MO.

Table 3. Composition of corn basal diet utilized in Exp. 1 and 2, DM basis

Item	Basal
Bulk density, g/cc	-
Particle size, µm	-
Dry matter, %	85.88
GE, kcal/kg	4,025
CP, %	8.28
Lysine, %	0.28
Total starch, %	55.29
Total dietary fiber, %	7.10
NDF, %	10.65
ADF, %	2.90
Hemicellulose, %[1]	7.75
Ash, %	4.44
Chloride, %	0.29
Phosphorus, %	0.42
Potassium, %	0.40
Sodium, %	0.15
Sulfur, %	0.16
Crude fat, %	2.84
Fatty acid[2]	
Myristic, 14:0	ND
Palmitic, 16:0	15.06
Palmitioleic, 16:1	0.13
Stearic, 18:0	1.89
Oleic, 18:1	27.28
Linoleic, 18:2	53.04
Linolenic, 18:3	1.45
Arachidonic, 20:4	ND
Eicosapentaenoic, 20:5	ND
Docosapentaenoic, 22:5	ND
Docosahexaenoic, 22:6	ND
Free fatty acids, %	1.79
Thiobarbituric acid, A(532)	11.91
Peroxide value, mEq/kg	58.22
Mycotoxins	
Aflatoxin B1, ppb	ND
Aflatoxin B2, ppb	ND
Aflatoxin G1, ppb	ND
Aflatoxin G2, ppb	ND
Deoxynivalenol, ppm	0.23
Fumonisin B1, ppm	ND
Fumonisin B2, ppm	ND
Fumonisin B3, ppm	ND
Ochratoxin A, ppb	ND
T-2 Toxin, ppb	ND
Zearalenone, ppb	ND

[1]Calculated as NDF – ADF.

[2]Fatty acid composition is expressed as a percentage of total fat.

ND = not detected or below detection limit.

Table 4. Composition of corn distillers dried grains with soluble used in Exp. 1, DM basis

	Corn distillers dried grains with solubles source			
Item	2	3	4	5
Bulk density, g/cc	0.597	0.660	0.608	0.556
Particle size, µm	379	362	294	316
Dry matter, %	88.87	88.77	89.98	89.93
GE, kcal/kg	4,780	4,841	4,943	5,113
CP, %	31.19	30.56	30.80	28.97
Lysine, %	1.14	1.09	1.06	1.06
Total starch, %	3.26	3.26	2.53	3.26
Total dietary fiber, %	35.56	36.05	36.01	33.80
NDF, %	30.49	31.58	33.89	31.64
ADF, %	9.42	10.05	10.59	9.01
Hemicellulose, %[1]	21.07	21.53	23.30	22.63
Ash, %	5.82	6.14	5.67	5.37
Chloride, %	0.19	0.18	0.17	0.17
Phosphorus, %	0.91	0.91	0.87	0.90
Potassium, %	1.31	1.22	1.18	1.31
Sodium, %	0.23	0.35	0.41	0.16
Sulfur, %	1.31	1.27	1.39	1.16
Crude fat, %	4.88	5.61	7.45	10.88
Fatty acid[2]				
Myristic, 14:0	ND	ND	ND	0.06
Palmitic, 16:0	14.46	14.34	13.96	13.73
Palmitioleic, 16:1	0.15	0.14	ND	0.14
Stearic, 18:0	2.33	2.29	2.26	2.27
Oleic, 18:1	26.51	26.51	27.16	27.30
Linoleic, 18:2	53.55	53.33	53.71	53.36
Linolenic, 18:3	1.65	1.63	1.57	1.52
Arachidonic, 20:4	ND	ND	ND	ND
Eicosapentaenoic, 20:5	ND	ND	ND	ND
Docosapentaenoic, 22:5	ND	ND	ND	ND
Docosahexaenoic, 22:6	0.20	0.25	0.25	0.17
Free fatty acids, %	0.64	0.57	0.87	1.09
Thiobarbituric acid, A(532)	17.07	19.39	6.69	6.03
Peroxide value, mEq/kg	6.75	14.17	10.41	6.55
Mycotoxins				
Aflatoxin B1, ppb	ND	ND	ND	ND
Aflatoxin B2, ppb	ND	ND	ND	ND
Aflatoxin G1, ppb	ND	ND	ND	ND
Aflatoxin G2, ppb	ND	ND	ND	ND
Deoxynivalenol, ppm	1.46	1.46	1.44	1.33
Fumonisin B1, ppm	1.80	1.13	1.22	1.22
Fumonisin B2, ppm	0.34	0.11	0.33	0.33
Fumonisin B3, ppm	0.11	ND	ND	ND
Ochratoxin A, ppb	ND	ND	ND	ND
T-2 Toxin, ppb	ND	ND	ND	ND
Zearalenone, ppb	57.61	ND	ND	ND

[1]Calculated as NDF – ADF.

[2]Fatty acid composition is expressed as a percentage of total fat.
ND = not detected or below detection limit.

Table 5. Composition of corn distillers dried grains with solubles used in Exp. 2, DM basis

	Corn distillers dried grains with solubles source										
Item	2	3	4	5	6	7	8	9	10	11	12
Bulk density, g/cc	0.597	0.566	0.574	0.612	0.521	0.573	0.553	0.541	0.549	0.573	0.621
Particle size, µm	863	622	1054	1078	689	766	710	645	757	945	568
Dry matter, %	88.40	88.47	87.47	85.60	89.18	87.56	86.43	84.79	86.53	85.54	86.98
GE, kcal/kg	5,077	5,075	5,066	4,897	5,043	4,963	4,938	5,167	4,963	4,948	5,130
CP, %	27.69	29.67	29.67	32.93	30.97	30.15	30.31	30.61	29.77	32.71	32.10
Lysine, %	1.06	1.03	1.06	1.20	1.13	1.13	1.11	1.20	1.05	1.19	1.06
Total starch, %	1.76	3.89	1.61	0.84	0.89	3.38	2.20	1.26	2.84	0.97	1.09
TDF, %	37.78	33.91	35.33	32.48	35.66	30.84	33.90	32.43	31.32	33.90	33.46
NDF, %	43.97	36.49	38.62	35.70	38.89	33.30	38.23	34.00	28.79	35.85	38.92
ADF, %	14.02	12.14	13.92	13.40	12.90	10.47	12.45	9.87	10.33	13.71	13.29
Hemicellulose, %[1]	29.95	24.35	24.70	22.30	25.99	22.83	25.78	24.13	18.46	22.14	25.63
Ash, %	4.42	4.32	4.58	5.12	4.91	4.87	5.03	5.30	5.04	5.31	4.89
Chloride, %	0.15	0.11	0.15	0.16	0.17	0.16	0.16	0.17	0.13	0.16	0.16
Phosphorus, %	0.75	0.71	0.80	0.88	0.74	0.80	0.89	0.89	0.83	0.91	0.77
Potassium, %	1.09	1.03	1.09	1.34	1.23	1.18	1.21	1.30	1.09	1.15	1.15
Sodium, %	0.18	0.09	0.17	0.04	0.18	0.11	0.22	0.22	0.25	0.26	0.18
Sulfur, %	0.59	0.46	0.78	1.03	0.78	0.72	0.66	0.57	0.87	1.15	0.93
Crude fat, %	11.20	11.13	10.79	8.56	10.82	9.62	10.05	13.23	9.65	9.96	11.83
Fatty acid[2]											
Myristic, 14:0	0.07	0.07	0.07	0.08	0.06	0.08	0.08	0.06	0.07	0.07	0.06
Palmitic, 16:0	13.97	15.38	14.65	14.56	14.38	14.27	14.08	14.07	14.04	14.10	13.58
Palmitioleic, 16:1	0.14	0.12	0.14	0.15	0.16	0.14	0.14	0.13	0.15	0.13	0.13
Stearic, 18:0	2.09	2.01	2.62	2.06	2.08	2.08	2.03	2.05	2.09	2.12	2.05
Oleic, 18:1	25.94	24.96	27.03	25.16	24.81	25.78	25.53	26.69	25.80	26.22	25.65
Linoleic, 18:2	54.44	54.01	51.92	54.23	54.98	54.20	54.76	53.51	54.51	53.93	54.92
Linolenic, 18:3	1.60	1.72	1.35	1.76	1.66	1.64	1.64	1.57	1.58	1.59	1.76
Arachidonic, 20:4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eicosapentaenoic, 20:5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Docosapentaenoic, 22:5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Docosahexaenoic, 22:6	0.16	0.16	0.27	0.26	0.17	0.18	0.16	0.16	0.16	0.16	0.15
Free fatty acids, %	2.01	1.41	1.53	1.36	1.46	1.69	1.48	2.38	1.39	1.47	1.87
Thiobarbituric acid, A(532)	7.14	8.55	9.00	12.76	5.67	6.36	6.90	11.78	5.33	6.90	7.60
Peroxide value, mEq/kg	8.23	0.24	2.61	17.50	19.03	0.58	0.45	2.39	1.42	2.78	3.47
Mycotoxins											

Aflatoxin B1, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aflatoxin B2, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aflatoxin G1, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aflatoxin G2, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Deoxynivalenol, ppm	0.68	0.34	0.34	2.10	0.89	1.60	1.97	1.06	1.62	1.64	0.34
Fumonisin B1, ppm	0.90	0.34	0.34	0.35	0.34	0.91	0.93	ND	1.73	0.47	0.11
Fumonisin B2, ppm	0.11	ND	ND	ND	ND	0.11	0.12	ND	0.35	ND	ND
Fumonisin B3, ppm	ND	ND	ND	ND	ND	ND	ND	ND	0.12	ND	ND
Ochratoxin A, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T-2 Toxin, ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Zearalenone, ppb	74.55	ND	ND	113.55	ND	98.68	ND	65.81	61.48	100.07	ND

[1]Calculated as NDF – ADF.

[2]Fatty acid composition is expressed as a percentage of total fat.

ND = not detected or below detection limit.

Table 6. Apparent total tract digestibility and energy concentration of corn DDGS, Exp. 1

		DDGS Source				Statistics	
Item	Basal	2	3	4	5	SD	P
Observations	12	15	14	14	14	-	-
Total tract digestibility[1]							
ADF	59.12	58.59	70.19	55.15	60.92	11.20	0.01
C	89.66	73.62	78.05	69.01	74.07	8.03	0.05
DM	89.85	72.44	77.29	67.71	72.48	8.29	0.04
GE	88.63	74.65	79.11	70.77	75.70	7.66	0.05
EE	33.49	65.68	69.80	72.71	81.24	9.47	0.01
NDF	56.15	49.79	57.36	44.45	45.82	13.21	0.07
N	81.57	82.58	83.44	77.96	80.47	5.52	0.06
P	39.53	61.37	66.48	59.12	58.64	15.45	0.58
S	79.98	89.05	89.87	86.98	88.61	3.40	0.18
Energy concentration[2]							
GE	4,025	4,780	4,841	4,944	5,113	-	-
DE	3,574	3,568	3,829	3,500	3,870	375	0.03
ME	3,501	3,286	3,604	3,266	3,696	381	0.01
DE:GE	88.78	74.72	79.19	70.90	76.08	7.71	0.06
ME:DE	97.95	93.38	94.04	93.40	94.33	2.35	0.68
ME:GE	86.96	68.82	74.56	66.18	72.82	7.84	0.04

[1]Digestibility of the basal diet used as a covariate for subsequent digestibility values.

[2]Digestible and metabolizable energy value of the basal diet used as a covariate for subsequent DE and ME values of each reduced oil-DDGS sample. Data presented on a DM basis. Final BW and ADFI averaged 105.6 kg and 2,693 g/d, respectively.

Table 7. Apparent total tract digestibility and energy concentration of corn DDGS, Exp. 2

Item[1]	Basal	DDGS Source 2	3	4	5	6	7	8	9	10	11	12	Statistics SD	P
Observations	11	11	11	9	12	11	12	12	12	12	12	12		
Total tract digestibility[1]														
ADF	55.50	69.87	69.87	74.57	72.17	66.77	74.94	76.74	68.24	72.75	73.53	70.14	6.73	0.02
C	89.71	67.93	71.04	70.18	72.25	68.27	74.34	73.16	73.01	74.70	70.50	72.37	7.31	0.39
DM	89.69	66.80	70.52	69.64	73.30	67.41	74.18	71.89	71.57	73.78	70.35	70.38	7.89	0.41
GE	88.50	68.32	71.20	70.85	74.78	69.12	74.69	73.26	73.71	75.20	71.31	73.47	7.10	0.29
EE	38.82	54.82	57.20	54.70	67.13	59.42	53.34	52.69	68.53	58.13	57.59	65.80	12.11	0.01
NDF	55.89	56.26	57.39	58.18	51.81	54.15	60.56	61.54	57.05	55.17	57.16	53.26	11.66	0.69
N	80.95	76.89	79.73	81.79	77.02	82.12	82.78	80.61	80.99	84.80	81.83	82.48	5.63	0.03
P	47.09	52.37	66.31	64.65	54.54	53.06	61.37	52.33	57.08	56.28	62.23	63.54	13.93	0.18
S	79.18	79.63	77.19	84.55	83.55	82.42	84.54	83.24	81.05	87.44	87.06	85.99	5.16	0.01
Energy concentration[2]														
GE	4,025	5,077	5,075	5,066	4,897	5,043	4,963	4,938	5,167	4,963	4,948	5,130	-	-
DE	3,55	3,47	3,61	3,58	3,66	3,48	3,70	3,61	3,80	3,73	3,52	3,768	350	0.31

	1	4	9	4	3	4	6	7	7	1	7			
ME	3,477	3,302	3,400	3,360	3,362	3,277	3,513	3,423	3,603	3,550	3,327	3,553	346	0.30
DE:GE	88.24	68.44	71.32	70.74	74.81	69.08	74.67	73.24	73.69	75.18	71.29	73.45	6.99	0.27
ME:DE	97.91	94.92	93.70	93.78	93.64	94.02	94.74	94.52	94.75	95.08	94.10	94.21	1.70	0.46
ME:GE	86.47	65.00	66.95	66.37	68.69	64.98	70.79	69.32	69.72	71.55	67.24	69.28	6.95	0.33

[1]Digestibility of the basal diet used as a covariate for subsequent digestibility values.

[2]Digestible and metabolizable energy value of the basal diet used as a covariate for subsequent DE and ME values of each reduced oil-DDGS sample. Data presented on a DM basis. Final BW and ADFI averaged 83.7 kg and 2,399 g/d, respectively.

Table 8. Stepwise regression equations for DE in corn DDGS, Exp. 2

		Regression coefficient parameter[1]				Statistical parameter[2]	
Item	Intercept	TDF	GE	BD	ADF	SE	R^2
Eq. 1	5,126	-44.22	-	-	-	-	-
SE[3]	385.3	11.41	-	-	-	-	-
P-value[3]	0.01	0.01	-	-	-	72.38	0.63
Eq. 2	2,084	-53.65	0.67	-	-	-	-
SE[3]	834.7	7.62	0.18	-	-	-	-
P-value[3]	0.04	0.01	0.01	-	-	45.75	0.86
Eq. 3	1,601	-54.48	0.69	731.5	NA	-	-
SE[3]	805.2	6.89	0.16	433.87	NA	-	-
P-value[3]	0.09	0.01	0.01	0.14	NA	41.25	0.91
Eq. 4	4,265	NA	-	-	-50.77	-	-
SE[3]	222.5	NA	-	-	17.81	-	-
P-value[3]	0.01	NA	-	-	0.02	85.73	0.48
Eq. 5	3,343	NA	-	2,276	-73.15	-	-
SE[3]	371.3	NA	-	745.8	14.79	-	-
P-value[3]	0.01	NA	-	0.02	0.01	61.81	0.76

[1]Equations based on analyzed nutrient content expresses on a DM basis. Units are kcal/kg of DM for GE and DE, percent for ADF and TDF, and g/cc for bulk density (BD). NA = not applicable.

[2]SE = standard error of the regression estimated defined as the root of the mean square error, R^2 = coefficient of determination.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

Table 9. Stepwise regression equations for ME in corn DDGS, Exp. 2

		Regression coefficient parameter[1]				Statistical parameter[2]	
Item	Intercept	ADF	BD	EE	TDF	SE	R^2
Eq. 1	4,132	-57.05	-	-	-	-	-
SE[3]	196.3	15.71	-	-	-	-	-
P-value[3]	0.01	0.01	-	-	-	75.63	0.59
Eq. 2	3,291	-75.47	1874	-	-	-	-
SE[3]	351.3	13.98	705.6	-	-	-	-
P-value[3]	0.01	0.01	0.03	-	-	58.48	0.78
Eq. 3	2,939	-73.30	2,004	23.65	-	-	-
SE[3]	369.6	12.48	630.6	13.32	-	-	-

P-value[3]	0.01	0.01	0.02	0.12	-	51.91	0.85
Eq. 4	4,558	-	-	52.26	-50.08	-	-
SE[3]	266.6	-	-	12.72	7.99	-	-
P-value[3]	0.01	-	-	0.01	0.01	48.74	0.85

[1]Equations based on analyzed nutrient content expresses on a DM basis. Units are kcal/kg of DM for ME, percent for ADF, EE, and TDF, and g/cc for bulk density (BD).

[2]SE = standard error of the regression estimated defined as the root of the mean square error, R^2 = coefficient of determination.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

Table 10. Stepwise regression equations for DE as a percent of GE in corn DDGS, Exp. 2

		Regression coefficient parameter[1]			Statistical parameter[2]	
Item	Intercept	TDF	BD	NDF	SE	R^2
Eq. 1	108.89	-1.08	-	-	-	-
SE[3]	4.64	0.14	-	-	-	-
P-value[3]	0.01	0.01	-	-	0.87	0.87
Eq. 2	100.74	-1.10	14.97	NA	-	-
SE[3]	6.06	0.12	8.14	NA	-	-
P-value[3]	0.01	0.01	0.10	NA	0.76	0.91
Eq. 3	89.29	NA	-	-0.46	-	-
SE[3]	4.63	NA	-	0.13	-	-
P-value[3]	0.01	NA	-	0.01	1.55	0.60
Eq. 4	73.11	NA	33.67	-0.55	-	-
SE[3]	7.57	NA	13.74	0.11	-	-
P-value[3]	0.01	NA	0.04	0.01	1.24	0.77

[1]Equations based on analyzed nutrient content expresses on a DM basis. Units are kcal/kg of DM for ME, percent for ADF, EE, and TDF, and g/cc for bulk density (BD). NA = not applicable.

[2]SE = standard error of the regression estimated defined as the root of the mean square error, R^2 = coefficient of determination.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

Table 11. Stepwise regression equations for ME as a percent of DE in corn DDGS, Exp. 2

		Regression coefficient parameter[1]				Statistical parameter[2]	
Item	Intercept	ADF	CP	Ash	BD	SE	R^2
Eq. 1	96.65	-0.19	-	-	-	-	-
SE[3]	1.16	0.09	-	-	-	-	-
P-value[3]	0.01	0.07	-	-	-	0.45	0.31
Eq. 2	100.73	-0.17	-0.14	-	-	-	-
SE[3]	2.68	0.09	0.09	-	-	-	-
P-value[3]	0.01	0.09	0.14	-	-	0.41	0.49
Eq. 3	98.80	-	-0.39	1.52	-	-	-
SE[3]	1.85	-	0.08	0.38	-	-	-
P-value[3]	0.01	-	0.01	0.01	-	0.29	0.74
Eq. 4	96.30	-	-0.46	1.84	5.47	-	-
SE[3]	2.23	-	0.09	0.40	3.25	-	-
P-value[3]	0.01	-	0.01	0.01	0.14	0.26	0.82

[1]Equations based on analyzed nutrient content expresses on a DM basis. Units are kcal/kg of DM for ME, percent for ADF, EE, and TDF, and g/cc for bulk density (BD). NA = not applicable.

[2]SE = standard error of the regression estimated defined as the root of the mean square error, R^2 = coefficient of determination.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

Table 12. Stepwise regression equations for GE in corn DDGS, (Exp. 1 and Exp. 2 samples combined)

	Regression coefficient parameter[1]			Statistical parameter[2]	
Item	Intercept	EE	PS	SE	R^2
Eq. 1	4,553	45.63	-	-	-
SE[3]	49.2	4.94	-	-	-
P-value[3]	0.01	0.01	-	41.84	0.87
Eq, 2	4,583	50.61	-0.12	-	-
SE[3]	38.6	4.07	0.04	-	-
P-value[3]	0.01	0.01	0.01	31.87	0.93

[1]Equations based on analyzed nutrient content expresses on a DM basis. Units are kcal/kg of DM for GE, percent for EE, and µm for particle size (PS).

[2]SE = standard error of the regression estimated defined as the root of the mean square error, R^2 = coefficient of determination.

[3]SE and *P*-values of the corresponding regression coefficient parameter.

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