

METABOLISM AND NUTRITION

Apparent metabolizable energy and prediction equations for reduced-oil corn distillers dried grains with solubles in broiler chicks from 10 to 18 days of age¹

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ABSTRACT An experiment consisting of 2 identically designed trials was conducted to determine the nutrient composition and AME_n content of distillers dried grains with solubles (DDGS) to develop prediction equations for AME_n in broilers. Fifteen samples of DDGS ranging in ether extract (EE) from 3.15 to 13.23% (DM basis) were collected from various dry-grind ethanol plants and were subsequently fed to broiler chicks to determine AME_n content. A corn-soybean meal control diet was formulated to contain 15% dextrose, and test diets were created by mixing the control diet with 15% DDGS at the expense of dextrose. In each trial, 672 male Ross × Ross 708 chicks were housed in grower battery cages with 7 birds per cage (0.06 m²/bird) and received a common starter diet until 10 d of age. Each cage was randomly assigned to 1 of 16 dietary treatments, with 6 replicate pens per treatment. Experimental diets were fed over a 6-d acclimation period from 10

to 16 d of age, followed by a 48-h total excreta collection period. Gross energy (GE) and CP of the experimental diets and excreta were determined to calculate AME_n for each DDGS sample. On a DM basis, AME_n of the 15 DDGS samples ranged from 1,869 to 2,824 kcal/kg. Analyses were conducted to determine the GE, CP, EE, DM, starch, total dietary fiber (TDF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash content of the DDGS samples. Stepwise regression resulted in the following best-fit equation for AME_n (DM basis) based on the adjusted coefficient of determination (R²_{adj}), SE, and prediction error sum of squares (PRESS): AME_n, kcal/kg = -12,282 + (2.60 × GE) + (89.75 × CP) + (125.80 × starch) - (40.67 × TDF; R²_{adj} = 0.86; SE = 98.76; PRESS = 199,819; P ≤ 0.001). These results indicated that the composition of DDGS with variable EE content may be used to predict AME_n in broiler chicks.

Key words: broiler, dried distillers grains with solubles, fiber, metabolizable energy, prediction equation

2013 Poultry Science 92:3176–3183
<http://dx.doi.org/10.3382/ps.2013-03290>

INTRODUCTION

Continued expansion of the ethanol industry has increased the amount of corn fermentation coproducts available to livestock producers as an alternative feed ingredient. Specifically, distillers dried grains with solubles (DDGS) have been increasingly used in poultry diets. However, recent advances in biorefining technologies have allowed the ethanol industry to remove an additional 2 to 6% ether extract (EE) from DDGS as a strategy to generate additional revenue through the production and marketing of crude corn oil. As a result,

the AME_n value of reduced oil DDGS may be decreased by as much as 300 to 600 kcal/kg. Differences in oil content exacerbate the inherent energy variability among DDGS sources, and therefore may limit their utility in broiler diets.

To mitigate the adverse consequences of nutrient variability among DDGS sources, prediction equations have been developed to estimate ME based on their chemical composition (TME_n of corn DDGS, Batal and Dale, 2006; AME_n of wheat DDGS, Cozannet et al., 2010). Prediction equations provide an estimated energy value that is more accommodating to the inherent variation among modern DDGS sources than published values, while also eliminating the need for costly and time-consuming in vivo assays for every DDGS source to be used in diet formulations. Evaluation of corn coproducts with a wide range of nutrient composition allows for the development of robust ME prediction equations (Rochell et al., 2011). The equations reported by Rochell et al. (2011) indicate that fiber measures such as

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Received May 6, 2013.

Accepted August 15, 2013.

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neutral detergent fiber (**NDF**) or hemicellulose (**HC**) are important predictors for AME_n content. However, neither of these measures was reported by Batal and Dale (2006). Recent data in swine (Anderson et al., 2012) have likewise shown that ME may be predicted from total dietary fiber (**TDF**) values, which were also not reported by Batal and Dale (2006) or Cozannet et al. (2010). Additionally, because Cozannet et al. (2010) used wheat DDGS, the equations developed therein are not applicable to corn DDGS. Furthermore, the EE content of most of the DDGS samples used to develop equations in the work of Batal and Dale (2006) and Rochell et al. (2011) was greater than 9.0%. Therefore, the application of these equations to reduced oil DDGS would require undesirable extrapolation. Prediction equations for AME_n that specifically address the wide range of EE content now observed in corn DDGS sources have not been reported. Therefore, the objectives of this study were to evaluate the AME_n content of 15 DDGS samples varying in EE content and to develop regression equations that accurately predict the AME_n content of reduced oil DDGS in broilers based upon chemical composition.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2012–2056).

Dietary Treatments

Fifteen DDGS samples were obtained from various dry-grind ethanol plants throughout the Midwestern United States. These samples were selected to represent a wide range of EE content. Sixteen dietary treatments consisted of a control diet [85% basal diet (Table 1) + 15% dextrose] and 15 test diets each containing 15% of an individual DDGS sample substituted at the expense of dextrose (85% basal diet + 15% DDGS). All dietary treatments were offered in mash form. At 10 d of age, birds were randomly assigned to 1 of the 16 dietary treatments. Each treatment was represented by 12 replications (6 replicates per trial).

Broiler Husbandry

Two identical energy balance trials were conducted in broilers from 10 to 18 d of age. One thousand three hundred forty-four male Ross × Ross 708 (Aviagen Inc., Huntsville, AL) chicks were obtained from a commercial hatchery and received vaccines for Marek's disease, Newcastle disease, and infectious bronchitis. In each trial, 672 chicks (7 per cage) were placed into grower battery cages (Petersime, Gettysburg, OH). Each cage (68 × 68 × 38 cm) was equipped with a trough feeder and a trough waterer. The experimental facility was a solid-sided house with temperature control. For both trials, temperature was set at 33°C at placement and

decreased gradually with increasing bird age to 27°C at the conclusion of the trial. A 23L:1D lighting schedule was used for the duration of the trial. Broilers were fed a common corn-soybean meal starter diet from placement to 10 d of age.

Measurements

Birds were placed on experimental diets at 10 d of age. After a 6-d acclimation period, a 48-h energy balance assay was conducted from 16 to 18 d of age. Feed consumption and BW gain were recorded to verify acceptance of the dietary treatments over the 8-d experimental feeding period. Feed disappearance and total excreta weights (wet basis) were recorded during the 48-h collection period to calculate energy and nitrogen intake and excretion. Multiple subsamples were collected from the total amount of accumulated excreta

Table 1. Ingredient and calculated nutrient composition of the basal diet

Item	Amount
Ingredient (% as-is basis)	
Corn	57.01
Soybean meal (48% CP)	36.96
Poultry oil	2.27
Dicalcium phosphate	2.06
Calcium carbonate	0.52
DL-Methionine	0.27
Vitamin premix ¹	0.25
Mineral premix ²	0.25
Sodium chloride	0.23
L-Lys-HCl	0.09
Salinomycin ³	0.05
L-Thr	0.04
Calculated nutrient composition (%) ⁴	
AME_n (kcal/kg)	3,025
CP	21.78
Digestible Lys	1.13
Digestible Met	0.58
Digestible TSAA	0.85
Digestible Thr	0.74
Digestible Val	0.88
Digestible Ile	0.81
Digestible Arg	1.32
Digestible Trp	0.22
Ca	0.97
Nonphytate P	0.46
Na	0.21

¹Vitamin premix provided the following per kilogram of diet: vitamin A (vitamin A acetate), 8,000 IU; vitamin D (cholecalciferol), 2,000 IU; vitamin E (DL- α -tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg; folic (folic acid), 0.5 mg; D-pantothenic acid (calcium pantothenate), 15 mg; riboflavin, 5.4 mg; niacin (niacinamide), 45 mg; thiamine (thiamine mononitrate), 1 mg; D-biotin, 0.05 mg; pyridoxine (pyridoxine hydrochloride), 2.2 mg; and choline (choline chloride), 500 mg.

²Mineral premix provided the following per kilogram of diet: Mn (manganous oxide), 65 mg; Zn (zinc oxide), 55 mg; Fe (iron sulfate monohydrate), 55 mg; Cu (copper sulfate pentahydrate), 6 mg; I (calcium iodate), 1 mg; Se (sodium selenite), 0.3 mg.

³BioCox 60 provided 60 g/907 kg of salinomycin (Alpharma, Fort Lee, NJ).

⁴Values reported as percentages unless noted otherwise. Digestible amino acid values were determined from digestible coefficients and calculated total amino acid content of the ingredients (Ajinomoto Heartland, 2004).

Table 2. Analyzed composition of 15 corn distillers dried grains with solubles sources¹

Item (%)	1	2	3	4	5	6	7	8
Gross energy (kcal/kg)	4,678	4,990	5,022	4,897	4,963	4,963	4,948	4,938
Moisture	11.23	13.13	9.13	11.71	10.48	9.45	10.04	12.58
CP	34.74	27.91	28.93	32.93	30.05	29.77	32.31	30.31
Starch	3.04	3.73	3.32	0.84	3.38	2.84	0.97	2.20
Total dietary fiber	37.20	30.50	28.50	32.50	30.80	31.30	33.90	33.90
Neutral detergent fiber	50.96	27.33	27.03	35.70	33.30	28.79	35.85	38.23
Acid detergent fiber	15.82	7.65	8.16	13.40	10.47	10.33	13.71	12.45
Hemicellulose ²	35.14	19.68	18.87	22.30	22.83	18.46	22.14	25.78
Ether extract	3.15	4.19	6.31	8.56	9.62	9.65	9.96	10.05
Ash	5.16	4.84	5.20	5.12	4.87	5.04	5.31	5.03
	9	10	11	12	13	14	15	
Gross energy (kcal/kg)	5,066	5,043	5,075	5,077	5,008	5,130	5,167	
Moisture	9.89	10.82	10.99	11.57	8.95	9.20	14.83	
CP	29.67	30.97	29.67	27.69	26.48	32.10	30.61	
Starch	1.61	0.89	3.89	1.76	3.30	1.09	1.26	
Total dietary fiber	35.30	35.70	33.90	37.80	32.69	33.50	32.40	
Neutral detergent fiber	38.62	38.89	36.49	43.97	27.72	38.92	34.00	
Acid detergent fiber	13.92	12.90	12.14	14.02	9.75	13.29	9.87	
Hemicellulose ²	24.70	25.99	24.35	29.95	17.97	25.63	24.13	
Ether extract	10.79	10.82	11.13	11.28	11.52	11.83	13.23	
Ash	4.58	4.91	4.32	4.42	4.48	4.89	5.30	

¹All values on a DM basis. Values reported on a percentage basis unless noted otherwise.

²Hemicellulose was calculated as neutral detergent fiber minus acid detergent fiber.

on the pan beneath each pen. Each excreta sample was then homogenized, and a 250-g representative sample was reserved in a plastic bag.

Representative samples of feed and excreta were frozen and subsequently dried at 55°C for 48 h in a forced-air oven. Dried samples were then ground through a mill equipped with a 1-mm screen to ensure a homogeneous mixture. Duplicate 0.8-g samples of feed and excreta were analyzed for gross energy (GE) using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IA). Nitrogen contents of the experimental diets and excreta were analyzed by a commercial laboratory (University of Missouri Agricultural Experiment Station Chemical Laboratories, Columbia; method 990.03; AOAC International, 2006).

Apparent ME_n for each dietary treatment was calculated using 8.73 kcal/g as the nitrogen correction factor (Titus, 1956), and subtracting the AME_n contribution from dextrose (3,640 kcal/kg; Hill and Anderson, 1958) from the control diet by using the following equations: total AME_n intake (kcal) = (GE intake (kcal) - GE excretion (kcal) - {8.73 (kcal/g) × [N intake from diet (g) - N excretion (g)]}); basal AME_n intake (kcal) = [AME_n of control diet (85% basal + 15% dextrose; kcal) - 3,640 kcal of ME/kg of dextrose]; DDGS AME_n (kcal/kg) = {[total AME_n intake (kcal) - basal AME_n intake (kcal)]/DDGS intake (kg)}.

All DDGS samples were analyzed by a commercial laboratory for proximate composition (University of Missouri Agriculture Experiment Station Chemical Laboratories, Columbia; Tables 2 and 3) unless otherwise described. Neutral detergent fiber (Holst, 1973) was determined after pretreatment with a thermostable amylase. Values for acid detergent fiber [ADF; method 973.18 (A-D); AOAC International, 2006] and NDF

were expressed without residual ash. Hemicellulose was determined as the difference between ADF and NDF.

Statistical Analyses

Data were analyzed as a randomized complete block design (SAS Institute, 2009) with cage location as the blocking factor. Each treatment was represented by 12 replications (6 replicates per trial).

Stepwise regression was used to determine the relationship between nutrient composition and AME_n described by the following model:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \dots + \beta_kx_k + \varepsilon,$$

where y is the response variable, AME_n; (x_1, x_2, \dots, x_k) is the set of k regressor variables corresponding to each analyzed nutrient; the parameters β_j , $j = 0, 1, \dots, k$ are the partial regression coefficients representing the expected change in response y per unit change in x_1 when all the remaining regressor variables, x_1 ($i \neq j$), are held constant; and ε is an independent and normally distributed random error component. The stepwise selection procedure begins by first including the regressor variable with the highest simple correlation to the dependent variable. As each regressor is entered into the model, the partial correlation coefficients of the remaining candidate regressors are calculated to adjust for effect of each selected variable on the dependent variable. The candidate regressor with the largest partial correlation coefficient then enters the model. At each step, the regressors in the model are reevaluated for significance and may be removed if they exceed the criteria for entry. The process is repeated until no further candidate regressors meet the criteria for entry or

Table 3. Methods of analysis used to determine feed composition on 15 corn distillers dried grains with solubles sources

Analysis ¹	Method of analysis ²
Gross energy ³	Isoperibol bomb calorimeter (model no. 6300, Parr Instrument Co., Moline, IL)
DM	AOAC official method 934.01
Starch	AACC approved method 76–13. Modified: Starch Assay Kit (product code STA-20, Sigma, St. Louis, MO)
CP	AOAC official method 990.3
Ether extract	AOAC official method 920.39 (A) petroleum ether
Total dietary fiber	AOAC official method 985.20 (A–C)
Neutral detergent fiber	Holst (1973)
Acid detergent fiber	AOAC official method 973.18 (A–D)
Ash	AOAC official method 942.05

¹Unless otherwise noted, all methods of analysis were determined by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO).

²AOAC = AOAC International (Gaithersburg, MD); AACC = American Association of Cereal Chemists (St. Paul, MN).

³Determined by Auburn University Laboratory (Auburn, AL).

elimination (Montgomery et al., 2012). In the current study, entry and elimination criteria were set at $P \leq 0.05$.

The resultant best fit equation was chosen based upon the SE of the regression coefficients, the adjusted coefficient of multiple determination (R^2_{Adj}), the Mallows' statistic (C_p), the prediction error sum of squares (**PRESS**), and the prediction coefficient of determination ($R^2_{Pred.}$), as defined below:

$$R^2_{Adj} = 1 - \left(\frac{SS_{Res}/(n-p)}{SS_T/(n-1)} \right);$$

$$C_p = \frac{SS_{Res}(p)}{\hat{\sigma}^2} - n + 2p;$$

$$PRESS = \sum_{i=1}^n [y_i - \hat{y}_{(i)}]^2, \quad i = 1, 2, \dots, n;$$

$$R^2_{Pred.} = \left[1 - \left(\frac{PRESS}{SS_T} \right) \right],$$

where n is the number of observations in the sample, p is the number of regressors included in the model, SS_{Res} is the residual sum of squares, SS_T is the total sum of squares, $\hat{\sigma}^2$ is the estimate of σ^2 , y_i is the predicted value for the i th observation, and $\hat{y}_{(i)}$ is the predicted value of the i th observed response based on a model fit to the remaining $(n-1)$ sample points when the i th observation is removed (Montgomery et al., 2012).

The adjusted coefficient of multiple determination was chosen as a selection criterion because its value only increases in response to the addition of variables that reduce the residual mean square of the model. Thus, the R^2_{adj} provides a more straightforward approach of comparing models with different numbers of regressors than the unadjusted coefficient of multiple determination, which increases inherently when variables are added to the model (Montgomery et al., 2012). Additionally, full and partial correlations between nutrient composition and AME_n were calculated to assist in interpreting the results of stepwise selection. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

Multiple linear regression analysis of the nutrient composition of feedstuffs, with the aim of developing prediction equations for energy, has been successfully applied to a variety of ethanol coproducts in the past (Batal and Dale, 2006; Cozannet et al., 2010; Rochell et al., 2011). To generate a prediction equation that is not only robust and accurate, but also of practical use in a commercial poultry production setting, the selection of representative samples with a wide range of nutrient content is required. Therefore, in the current research, DDGS samples were selected to represent the wide range of EE content observed in the modern DDGS now being produced in the US ethanol industry and made available to commercial poultry producers. The selected DDGS samples ranged in EE content from 3.15 to 13.23% (Table 2). Previous research in both poultry and swine has emphasized the importance of various fiber fractions in the development of prediction equations for the ME of corn coproducts such as DDGS (Pedersen et al., 2007; Rochell et al., 2011; Anderson et al., 2012). In the selected DDGS samples, TDF ranged from 28.90 to 37.80%, NDF from 27.03 to 50.96%, and ADF from 7.65 to 15.82%. Gross energy content of the DDGS samples ranged from 4,678 to 5,167 kcal/kg of DM, CP from 26.48 to 34.74%, starch from 0.84 to 3.89%, and ash from 4.32 to 5.31%. Extensive variation in nutrient composition is characteristic of DDGS, and reflects the variation in ethanol processing procedures as well as inherent variation in the original corn source (Cromwell et al., 1993; Spiehs et al., 2002).

Prior research has used TME_n assays as a method for determining energy content when evaluating DDGS samples in poultry (Lumpkins et al., 2004; Batal and Dale, 2006; Fastinger et al., 2006; Kim et al., 2010). However, AME_n assays permit ad libitum feeding of the experimental diet, which better simulates feeding practices in the broiler industry. To determine AME_n , DDGS may be substituted for a portion of either a practical diet or a semipurified diet (Adeola and Ileleji, 2009). Substitution of the test ingredient at a high level reduces variance in the calculated AME_n , but may negatively affect feed intake and DM digestibility of

the diet (Adeola and Zhai, 2012). In the current study, utilization of a practical corn-soybean meal basal diet with the inclusion rate of DDGS set at the recommended maximum of 15% for the grower phase (Lumpkins et al., 2004) allows the approximation of an industry diet without compromising the accuracy of the AME_n determination for the test ingredient. However, Adeola and Ileleji (2009) observed lower ME_n values for DDGS fed in a practical basal diet compared with those fed in a semipurified diet. Furthermore, AME_n values for DDGS are approximately 20% lower than the corresponding TME_n values (NRC, 1994). Therefore, any comparisons between published ME values must take the assay type and basal diet into account.

Feed intake between birds receiving DDGS treatments was similar over the 8-d experimental feeding period (Table 4). However, when compared with birds receiving the dextrose control diet, birds receiving DDGS sources 3, 4, 5, and 11 consumed less feed ($P \leq 0.05$). Consequently, BW gain was lower for birds receiving DDGS sources 4, 5, and 11 compared with those receiving the dextrose control (Table 4; $P \leq 0.05$). Sibbald (1975) demonstrated a hyperbolic relationship between apparent ME and test diet intake with apparent ME approaching the true ME value of the test diet at high levels of intake. However, the numerical differences in daily feed intake for this experiment were much less extreme than those observed by Sibbald (1975), and therefore it is not expected that a significant depression in AME_n content occurred for those treatments.

Apparent ME_n values for all 15 samples of DDGS ranged from 1,869 to 2,824 kcal/kg of DM with an average value of 2,309 kcal/kg of DM (Table 5). Rochell et al. (2011) reported an average AME_n value of 2,678

Table 5. Determined gross energy (GE) and AME_n of corn distillers dried grains with solubles (DDGS) samples in broiler chicks¹

Item	n	GE	AME _n	
			kcal/kg	% of GE
DDGS sample				
1	12	4,678	1,869 ^e	39.95
2	12	4,990	2,551 ^{abc}	51.12
3	12	5,022	2,487 ^{abcd}	49.52
4	12	4,897	2,103 ^{cde}	42.94
5	12	4,963	2,401 ^{abcd}	48.37
6	12	4,963	2,526 ^{abcd}	50.89
7	12	4,948	2,309 ^{abcde}	46.66
8	12	4,938	2,068 ^{de}	41.89
9	12	5,066	2,273 ^{bcde}	44.86
10	12	5,043	2,012 ^{bcde}	39.91
11	12	5,075	2,418 ^{abc}	47.64
12	12	5,077	2,074 ^{de}	40.85
13	12	5,008	2,032 ^{bcde}	40.59
14	12	5,130	2,824 ^a	55.04
15	12	5,167	2,687 ^{ab}	52.00
SEM			108	2.16

^{a-e}Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

¹Gross energy and AME_n are expressed as kilocalories per kilogram of DM. Apparent ME was determined by a 48-h excreta collection following a 6-d adaptation period.

kcal/kg for 6 samples of DDGS. However, this may be attributed to the analysis of DDGS with greater AME_n values than those used in the current study. Because the DDGS in the current study varied widely in GE content between samples, AME_n as a percentage of GE was calculated. The average AME_n value as a percentage of GE was 46.2%, indicating that broilers did not efficiently use DDGS as an energy source.

Pearson correlation coefficients between the chemical components of DDGS and their AME_n value are shown in Table 6. Gross energy was highly correlated with AME_n ($r = 0.69$, $P = 0.01$), and may be attributed to the inherent arithmetic limitation placed on potential AME_n value by the GE content of the DDGS sample. Total dietary fiber also displayed a strong correlation with AME_n ($r = -0.56$, $P = 0.03$), followed closely by ADF, and NDF ($r = -0.52$, -0.52 , respectively, $P = 0.05$). Similarly, strong correlations between fiber fractions and energy value have been observed in previous research for a variety of corn coproducts (Rochell et al., 2011) and wheat DDGS (Cozannet et al., 2010). In contrast to the observations of Rochell et al. (2011), HC did not correlate significantly with AME_n ($r = -0.48$, $P = 0.07$) in the reduced oil DDGS sources evaluated in the current study. This disparity may be attributable to the wide range of HC content (3.78 to 48.56%) observed in the array of corn coproducts analyzed by Rochell et al. (2011), in comparison with the relatively narrow HC content of the DDGS used in the current study (17.97 to 35.14%). Neither starch ($r = 0.09$, $P = 0.75$) nor ash ($r = 0.01$, $P = 0.98$) displayed a significant correlation to AME_n due to their low content in the DDGS samples evaluated. Although CP contributes to ME, it was not significantly correlated to AME_n ($r = -0.20$,

Table 4. Feed intake and BW gain of broiler chicks fed diets containing 15% distillers dried grains with solubles from 10 to 18 d of age¹

Treatment	Feed intake (g/bird)	BW gain (g/bird)
Control ²	421 ^a	276 ^{ab}
1	419 ^{ab}	295 ^a
2	399 ^{abc}	266 ^{abc}
3	384 ^{bcd}	262 ^{bc}
4	361 ^d	218 ^d
5	378 ^{cd}	238 ^{dc}
6	403 ^{abc}	285 ^{ab}
7	401 ^{abc}	283 ^{ab}
8	398 ^{abc}	278 ^b
9	410 ^{abc}	273 ^{ab}
10	398 ^{abc}	262 ^{bc}
11	380 ^{cd}	238 ^{dc}
12	411 ^{abc}	274 ^{ab}
13	397 ^{abc}	275 ^{ab}
14	390 ^{abcd}	266 ^{abc}
15	407 ^{abc}	281 ^{ab}
SEM	7.22	6.59

^{a-d}Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

¹Observed means for feed intake and BW gain are based on 9 replicate pen means per treatment.

²Control diet contained 15% dextrose.

Table 6. Pearson correlation coefficients between chemical composition and AME_n for 15 corn distillers dried grains with solubles samples

Item ¹	AME _n	GE	CP	Starch	TDF	NDF	ADF	EE	Ash	HC
AME _n	1.00									
GE	0.69	1.00								
<i>P</i> -value	0.01									
CP	-0.20	-0.51	1.00							
<i>P</i> -value	0.48	0.05								
Starch	0.09	-0.23	-0.45	1.00						
<i>P</i> -value	0.75	0.41	0.09							
TDF	-0.56	-0.16	0.289	-0.39	1.00					
<i>P</i> -value	0.03	0.57	0.30	0.15						
NDF	-0.52	-0.35	0.59	-0.35	0.88	1.00				
<i>P</i> -value	0.05	0.20	0.02	0.20	0.01					
ADF	-0.52	-0.34	0.64	-0.52	0.86	0.90	1.00			
<i>P</i> -value	0.05	0.22	0.01	0.05	0.01	0.01				
EE	0.35	0.74	-0.24	-0.46	0.16	-0.06	0.09	1.00		
<i>P</i> -value	0.21	0.01	0.40	0.09	0.58	0.82	0.76			
Ash	0.01	-0.33	0.61	-0.34	-0.31	-0.05	-0.06	-0.30	1.00	
<i>P</i> -value	0.98	0.22	0.02	0.22	0.26	0.87	0.84	0.28		
HC	-0.48	-0.33	0.52	-0.23	0.83	0.98	0.78	-0.14	-0.04	1.00
<i>P</i> -value	0.07	0.22	0.05	0.40	0.01	0.01	0.01	0.63	0.90	

¹GE = gross energy; TDF = total dietary fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; HC = hemicellulose.

P = 0.48), and was likely due to the homogeneous CP content of the analyzed DDGS samples. The weak correlation of EE (*r* = 0.35, *P* = 0.21) with AME_n is unexpected given the high energy contribution of fat, as well as the purposeful selection of DDGS samples with a wide range of EE content. Nevertheless, this finding is supported by Rochell et al. (2011), where a similarly poor correlation of EE with AME_n (*r* = 0.39, *P* = 0.15) was reported.

Stepwise multiple linear regression analysis was used to identify the combination of nutritional components that most effectively predicted AME_n for the 15 DDGS sources (Table 7). Because GE has the highest simple correlation with AME_n, the first model is a simple linear regression of AME_n on GE (equation 1) with an R²_{Adj} of 0.44. The subsequent selection of TDF (*P* = 0.02) on the basis of its partial correlation coefficient increased the model R²_{Adj} to 0.63 (equation 2). Successive addition of CP (*P* = 0.06; equation 3, R²_{Adj} =

0.72) and starch (*P* = 0.01) resulted in the final model: [AME_n, kcal/kg of DM = -12,282 + (2.60 × GE kcal/kg of DM) - (40.67 × % TDF DM) + (89.75 × % CP DM) + (125.80 × % starch DM); R²_{Adj} = 0.86, SE = 99].

However, analysis of feedstuffs for TDF is less automated than that of other nutrient components and is consequently more costly, time-consuming, and labor-intensive. Therefore, TDF was excluded from the pool of potential predictors made available for selection into the model (Table 8). Gross energy was the first variable to enter the revised selection model (equation 1). With TDF omitted from the variable selection pool, stepwise selection included ADF (*P* = 0.13; equation 2, R²_{Adj} = 0.50) instead as the second predictor after GE. Crude protein (*P* = 0.01; equation 3, R²_{Adj} = 0.71) and starch (*P* = 0.01; equation 4, R²_{Adj} = 0.71) were again added as the third and fourth predictor variables for the revised selection equation. The model was then improved

Table 7. Stepwise selection of a regression model for AME_n based on the nutrient composition of 15 corn distillers dried grains with solubles samples

AME _n equation	Regression coefficient ¹					Statistical parameter ²			
	Intercept	GE	TDF	CP	Starch	SE	R ² _{Adj}	C(p)	PRESS
Equation 1	-5,584	1.59	—	—	—	198	0.44	28.72	—
SE	2,294	0.46	—	—	—	—	—	—	—
Estimate <i>P</i> -value	0.03	0.01	—	—	—	—	—	—	—
Equation 2	-3,096	1.42	-49.17	—	—	161	0.63	15.06	—
SE	2,061	0.38	17.59	—	—	—	—	—	—
Estimate <i>P</i> -value	0.16	0.01	0.02	—	—	—	—	—	—
Equation 3	-6,187	1.82	-57.4	44.56	—	141	0.72	9.97	—
SE	2,312	0.38	15.91	20.79	—	—	—	—	—
Estimate <i>P</i> -value	0.02	0.01	0.01	0.06	—	—	—	—	—
Equation 4	-12,282	2.60	-40.67	89.75	125.80	99	0.86	2.58	199,819
SE	2,372	0.35	12.12	19.42	35.74	—	—	—	—
Estimate <i>P</i> -value	0.01	0.01	0.01	0.01	0.01	—	—	—	—

¹GE = gross energy; TDF = total dietary fiber.

²R²_{Adj} is the adjusted coefficient of determination; SE is the standard error of the regression equation defined as the root of the mean square error; C(p) is the Mallows statistic; and PRESS is the prediction error sum of squares.

Table 8. Stepwise selection of a regression model for AME_n based on the nutrient composition of 15 corn distillers dried grains with solubles samples with total dietary fiber removed from the model

AME _n equation	Regression coefficient ¹						Statistical parameter ²			
	Intercept	GE	ADF	CP	Starch	NDF	SE	R ² _{Adj}	C(p)	PRESS
Equation 1	-5,584	1.59	—	—	—	—	198	0.44	28.72	—
SE	2,294	0.46	—	—	—	—	—	—	—	—
Estimate <i>P</i> -value	0.03	0.01	—	—	—	—	—	—	—	—
Equation 2	-3,894	1.33	-36.38	—	—	—	187	0.50	23.61	—
SE	2,403	0.46	22.50	—	—	—	—	—	—	—
Estimate <i>P</i> -value	0.13	0.01	0.13	—	—	—	—	—	—	—
Equation 3	-8,213	1.82	-73.58	76.81	—	—	144	0.71	10.66	—
SE	2,327	0.39	21.16	25.17	—	—	—	—	—	—
Estimate <i>P</i> -value	0.01	0.01	0.01	0.01	—	—	—	—	—	—
Equation 4	-13,631	2.59	-49.89	110.44	124.25	—	107	0.84	3.84	—
SE	2,434	0.38	17.40	21.50	39.35	—	—	—	—	—
Estimate <i>P</i> -value	0.01	0.01	0.02	0.01	0.01	—	—	—	—	—
Equation 5	-14,317	2.69	-0.29	117.07	149.23	-18.22	95	0.87	3.35	—
SE	2,204	0.34	30.60	19.52	37.56	9.68	—	—	—	—
Estimate <i>P</i> -value	0.01	0.01	0.99	0.01	0.01	0.09	—	—	—	—
Equation 6	-14,322	2.69	—	117.08	149.41	-18.30	90	0.88	1.35	227,477
SE	2,012	0.31	—	18.51	30.66	4.67	—	—	—	—
Estimate <i>P</i> -value	0.01	0.01	—	0.01	0.01	0.01	—	—	—	—

¹GE = gross energy; ADF = acid detergent fiber; NDF = neutral detergent fiber.

²R²_{Adj} is the adjusted coefficient of determination; SE is the standard error of the regression equation defined as the root of the mean square error; C(p) is the Mallows statistic; and PRESS is the prediction error sum of squares.

further with the addition of NDF ($P = 0.09$; equation 5, $R^2_{Adj} = 0.87$). However, the presence of NDF in the revised equation reduces the significance of ADF as a predictor, forcing its removal from the equation to yield the final model: $[AME_n, \text{kcal/kg of DM} = -14,322 + (2.69 \times \text{GE kcal/kg of DM}) + (117.08 \times \% \text{ CP DM}) + (149.41 \times \% \text{ starch DM}) - (18.30 \times \% \text{ NDF DM})$; $R^2_{Adj} = 0.88$, $SE = 90$].

The strong influence of fiber fractions observed in the current study corresponds well with the work of Rochell et al. (2011), in which HC was selected as the primary predictor, followed by NDF when HC was omitted from the model. Similarly, Cozannet et al. (2010) reported that ADF effectively predicted AME_n in wheat DDGS. Comparable results were acquired for prediction equations in swine, where ADF (Pedersen et al., 2007; $R^2 = 0.94$) and TDF (Anderson et al., 2012; $R^2 = 0.77$) were selected as major predictors for ME. In contrast to these results, Batal and Dale (2006) reported that TME_n was best predicted by EE content. However, direct comparisons are difficult between the present study and that of Batal and Dale (2006) due to the use of different ME assays. Furthermore, the prediction equations reported by Batal and Dale (2006) were based solely on the proximate composition of the DDGS, and therefore did not account for the specific fiber fractions addressed in the current study. Because hemicellulose is calculated as the difference between NDF and ADF, it cannot be included in the variable pool for selection without first removing NDF and ADF. Selection based on HC produced models that were inferior in all selection criteria and are therefore not addressed here.

Although the inclusion of NDF produces a model with improved R^2_{Adj} , SE, and C(p) values compared with the prior model including TDF, it is important to note that these values reflect the efficacy of a regression

model in explaining the variability of the data used to produce the model. Upon examining the PRESS values for each model, it is evident that each has different expected prediction capabilities for other data. The model that includes TDF has a PRESS value of 199,819 compared with a value 227,477 for the model in which NDF is included. A model with a higher PRESS value will explain the variation outside the data range less effectively than a model with a lower PRESS value. For models containing TDF or NDF, the R^2_{Pred} values calculated from PRESS were 0.80 or 0.71, respectively. The R^2_{Adj} for the model including NDF indicates that it explains approximately 2% more of the variation in the current set of DDGS samples than the equation including TDF. However, the R^2_{Pred} values indicate that the equation including TDF is expected to explain approximately 9% more of the variation in a new set of DDGS samples. The importance of TDF in prediction of AME_n for DDGS may be due to the high levels of β -glucans present in DDGS due to residual yeast from the ethanol fermentation process (Liu, 2011). Whereas TDF accounts for the presence of β -glucans, NDF does not (NRC, 2012). Thus, TDF may be better suited for inclusion in prediction equations for DDGS, despite the associated analytical costs.

Although the DDGS sources used in this study were specifically selected to represent a wide range of EE content to evaluate the effect of oil extraction technologies on the energy value of DDGS, EE did not enter the AME_n prediction model. Restricting the model to contain EE alone resulted in a poor model for which the overall regression was not significant ($AME_n = 2,034 + 35.55 \times EE$; $R^2_{adj} = 0.05$, $P = 0.20$). Similar to the findings of this study, Rochell et al. (2011) reported that EE did not enter one of the best-fit models generated for AME_n. Additionally, prediction models for ME in

swine included EE only as a secondary predictor after the inclusion of fiber fractions (Pederson et al., 2007; Anderson et al., 2012). The limited effect of EE as a predictor of AME_n is likely due to the predominance of fiber fractions as a percentage of DDGS composition. This may be particularly true when the EE content has been further reduced by oil extraction technologies, because the removal of oil has a concentrating effect on other components of DDGS, such as fiber. The detrimental impact of fiber fractions on energy digestibility in poultry is well-documented (Annison and Choct, 1991; Bedford, 1996; Mateos et al., 2012), and is reflected in the negative regression coefficients associated with fiber fractions in the current study. Furthermore, studies in swine have shown that intact sources of corn oil are much less digestible than supplemental corn oil (Adams and Jensen, 1984; Kim et al., 2013). This effect may be exacerbated by the presence of high concentrations of dietary fiber (Bach Knudsen and Hansen, 1991; Dégen et al., 2007).

In conclusion, modern DDGS sources selected for variable EE content exhibited a wide range of AME_n values. Stepwise selection in multiple linear regression determined that GE, TDF, CP, and starch were the best predictors of AME_n in DDGS. Omission of TDF from the variable selection pool to develop a more practical model resulted in the inclusion of NDF in lieu of TDF. Ether extract did not effectively predict AME_n , and hence was not included in the model. Rigorous validation of these models with an independent set of DDGS samples is warranted to verify their practical value as prediction equations for AME_n in broiler diets.

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