The Development and Validation of Prediction Equations for the Apparent Metabolizable Energy of Distillers Dried Grains with Solubles in Broilers

by

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ABSTRACT

The objective of this research was to develop regression equations that accurately predict the AME_n content of distillers dried grains with solubles (DDGS) samples varying in ether extract content and to cross-validate these prediction equations using a set of independent DDGS samples. Experiment 1 determined the nutrient composition and AME_n content of 15 DDGS in order to develop prediction equations for AME_n in broilers. On a DM-basis, AME_n of the DDGS samples ranged from 1,869 to 2,824 kcal/kg. Analyses were conducted to determine gross energy, CP, ether extract, DM, starch, total dietary fiber, neutral detergent fiber, acid detergent fiber, and ash content of the DDGS samples. Stepwise selection resulted in the following best-fit equation for AME_n (DM-basis) based upon the adjusted coefficient of determination (R^2_{adj}), root mean square error (RMSE), and prediction error sum of squares (PRESS): AME_n, kcal/kg = -12,282 + (2.60 × gross energy) + (89.75 × CP) + (125.80 × starch) – (40.67 × total dietary fiber) ($P \leq 0.001$; $R^2 = 0.90$; $R^2_{adj} = 0.86$; RMSE = 99; PRESS = 199,819). Experiment 2 determined the AME_n content of 15 DDGS in order to validate 4 prediction equations for AME_n of corn DDGS in broilers. On a DM-basis, AME_n of the 15 DDGS samples ranged from 1,975 to 3,634 kcal/kg. Application of the 4 equations to the validation data resulted in RMSE values of 335, 381, 488, and 502 kcal/kg, respectively. The least absolute shrinkage and selection operator technique (LASSO) was applied to proximate analysis data for 30 corn co-products adapted from prior research and resulted

in the following best-fit equation: AME_n (kcal/kg) = 3,673 – (121.35 × crude fiber) + (51.29 × ether extract) – (121.08 × ash) ($P \leq 0.001$; $R^2 = 0.70$; $R^2_{adj} = 0.67$; RMSE = 457). These results indicated that validation is necessary to accurately determine the risk of error associated with the practical application of prediction equations to external data.

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I. INTRODUCTION

For the past decade, growing concerns over the United States' dependence on foreign oil and the environmental impact of fossil fuel consumption have provoked major changes in economic and farm policies. In response to these concerns, Congress enacted the Energy Policy Act of 2005, which instituted tax incentives and subsidies for the producers of alternative energy sources. This legislation established yearly production targets for renewable fuels with the ultimate goal of increasing annual biofuel production to 36 billion gallons by 2022. The rapid expansion of the ethanol and biodiesel industries to meet this goal has consequently created competition with animal agriculture for common energy feedstocks such as corn, oils, and fats. The cost of these ingredients has increased exponentially over the past decade, resulting in a challenging economic environment for poultry producers (Donohue and Cunningham, 2009).

The development of the ethanol industry has also resulted in a concomitant increase in the availability of distillers dried grains with solubles (**DDGS**), the primary co-product of ethanol production (Rausch and Belyea, 2006). In 2012, approximately 38 million short tons of DDGS were produced by the United States ethanol industry, with nearly 2 million short tons utilized in domestic poultry feed (Wisner, 2013). Poultry producers have capitalized on the increased availability of DDGS, which is a good source of energy, as well as CP, phosphorus, and sulfur amino acids. However, due to agronomic effects on the original corn, as well as processing differences between ethanol

plants, energy content may vary substantially between DDGS sources. Such variability may limit their inclusion in broiler diets (Batal and Dale, 2006). Furthermore, widespread implementation of novel oil extraction technologies has allowed the ethanol industry to generate additional revenue through the production and marketing of crude corn oil. It is expected that approximately 80% of United States ethanol producers will be employing oil extraction processes to some extent by 2013. Oil extraction substantially alters the chemical composition of DDGS, which not only increases the observed variation among DDGS sources but may also necessitate higher concentrations of supplemental fat in formulation (Saunders and Rosentrater, 2009).

In order to mitigate the adverse consequences of nutrient variability between DDGS sources, prediction equations have been developed to estimate ME based on their chemical composition (Batal and Dale, 2006; 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. Previous research has demonstrated that robust prediction equations can be generated for multiple corn fermentation co-products, including DDGS (Rochell et al., 2011). However, the precision of such equations is highly dependent on selection of test products with a wide range of nutrient composition. The practical value of any prediction equation should be determined through cross-validation with a sample set that is independent of the test products used to generate the equation.

Currently, cross-validated prediction equations for AME_n that specifically address the wide range of fat content now observed among modern DDGS sources have not been

reported in the literature. Therefore, the proposed research was conducted with the objective of developing robust and accurate equations for the prediction of AME_n content of modern DDGS in broilers. The first experiment determined the AME_n content of 15 samples of DDGS in order to develop a set of plausible prediction equations based on chemical composition. The second experiment determined the AME_n content of 15 additional samples of DDGS with which to validate the previously developed equations, as well as the equations of Rochell et al. (2011).

II. LITERATURE REVIEW

UTILIZATION OF DDGS IN BROILER DIETS

Distillers co-products from both the beverage and fuel ethanol industries have been successfully utilized in broiler diets for nearly a century. Early reports recognized the value of DDGS as a source of energy, protein, and minerals when included at low concentrations in poultry diets (Scott, 1970; Parsons et al., 1983). Distillers dried grains with solubles had been reported to contain a beneficial "unidentified growth factor," and were typically included in broiler diets at less than 5% (Jensen, 1981). However, several studies indicated that higher inclusion rates could be adopted if compensations were made for the lower energy and Lys content of DDGS (Waldroup et al., 1981; Parsons et al., 1983; Wang et al., 2007a,b; Shim et al., 2011). Waldroup et al. (1981) observed no negative effects on performance when DDGS from beverage alcohol production was included at 25% of the diet if adjustments were made to balance energy and Lys content. Additionally, Parsons et al. (1983) observed that DDGS could replace up to 30% of the soybean meal in a 24% CP starter diet without negatively affecting performance with proper supplementation of Lys.

With the expansion of the fuel ethanol industry in the late 20th century, distillers grains from beverage producers were reduced to less than 1% of the available supply (Shurson, 2002). Initial comparisons of early fuel alcohol DDGS with contemporaneous brewery DDGS indicated that both sources had similar nutritional profiles (Cromwell et

al., 1993). However, the continual innovation of biorefining technologies eventually resulted in the production of modern DDGS with altered nutritional profiles in comparison with their predecessors (Spiehs et al., 2002; Lumpkins et al., 2004). As these "next generation" distillers grains from fuel ethanol producers became more widely available, establishing practical dietary inclusion concentrations in broiler diets became a priority. Loar et al. (2010) determined that DDGS may be included at up to 8% of the diet from 0 to 14 d of age and up to 15% of the diet from 14 to 42 d of age without negatively affecting live performance. Likewise, Lumpkins et al. (2004) observed no detrimental effects on either live performance or carcass yield with inclusion rates of up to 6% DDGS in the starter phase, and up to 12 and 15% DDGS in the grower and finisher phases, respectively. Inclusion of DDGS in starter formulation is necessarily lower due to the limited ability of young chicks to digest complex carbohydrates, a major component of DDGS (Montagne et al., 2003). A maximum inclusion rate of 15% in the grower and finisher phases may be attainable through the use of high quality DDGS and formulation on a digestible amino acid basis (Wang et al., 2007a,b) Other authors have reported the use of up to 20% DDGS from 1 to 18 d (Min et al., 2009) and up to 24% DDGS from 1 to 42 d (Shim et al., 2011) without negatively affecting live performance. In each of these studies, diets were formulated on a digestible amino acid basis, in contrast to the use of total amino acid values in formulation in earlier studies (Parsons et al., 1993; Lumpkins et al., 2004). Furthermore, Shim et al. (2011) utilized set formulation minimums for Met, TSAA, Thr, Val, Ile, Trp, and Arg in order to maintain ideal digestible amino acid ratios relative to Lys. These adjustments allowed for higher inclusions of DDGS, without

negatively impacting bird performance, by precisely balancing their quality and composition.

Despite these conclusions, however, most commercial broiler integrators tend to formulate diets with much lower inclusion rates. A typical industry program might include DDGS at 5, 7, and 9% of the starter (1 to 14 d), grower (14 to 28 d), and finisher diets (29 to 42 d), respectively. Lower concentrations mitigate the effects of nutrient variability, the primary concern when formulating with DDGS (Waldroup et al., 2007). Variations in ME, Lys, phosphorus, and sodium content may negatively impact the precision of formulation, resulting in adverse effects on live performance. Therefore, an understanding of the average nutrient content and expected variability of DDGS is necessary to optimize their inclusion in broiler diets.

METABOLIZABLE ENERGY OF CONVENTIONAL DDGS

The nutrient profile of modern DDGS makes it appealing as an alternative ingredient for formulating broiler diets. Because feed accounts for approximately 70% of the live production cost for poultry, even small reductions in diet cost through the use of alternative ingredients can prove immensely valuable (Donohue and Cunningham, 2009). Inclusion of DDGS spares increasingly costly ingredients such as corn, soybean meal, and sources of phosphorus. In order to capitalize on the economic value of DDGS as a source of protein and energy, it is necessary to obtain accurate estimates of its nutrient composition. The nutrient composition of DDGS originating from beverage alcohol producers has been reported (NRC, 1994); however, these values may not adequately reflect the nutrient composition of modern DDGS. Furthermore, corn may vary substantially in nutrient composition, potentially resulting in nutrient variability of

DDGS. Commercial ethanol production relies on the conversion of starch to alcohol and carbon dioxide through yeast fermentation (Bothast and Schlicher, 2005). Removing starch from the grain concentrates the remaining nutrients, producing DDGS with an approximately 3-fold increase in protein, non-starch carbohydrates, fat, and minerals compared with corn (Liu, 2011).

Efficient utilization of DDGS as an alternative source of dietary energy has become progressively more important with increasing corn prices. Although the gross energy **(GE)** of DDGS is comparable with that of corn, its ME in poultry may be greatly reduced. Therefore, the extent to which DDGS may be economically used as a source of protein and phosphorus is partially dictated by the magnitude of the reduction in available energy associated with DDGS. A balance must be maintained between the inclusion of DDGS as an alternate source of nutrients and the amount of dietary energy available to efficiently utilize those nutrients. High inclusion rates may require the addition of expensive supplemental fat, the cost of which may exceed the savings associated with DDGS use. Therefore, an accurate assessment of the energy value of DDGS is of the utmost importance to ensure its successful incorporation into poultry diets.

Many estimates of the ME content of DDGS in poultry have been reported, often with considerable variability. Batal and Dale (2006) reported TME_n values of 17 DDGS samples from 6 different ethanol plants that ranged from 2,490 to 3,190 kcal/kg with an average of 2,820 kcal/kg on a DM basis. Likewise, Fastinger et al. (2006) evaluated 5 DDGS samples for which TME_n ranged from 2,484 to 3,047 kcal/kg with an average of 2,871 kcal/kg (DM-basis). Parsons et al. (2006) also observed similar TME_n values for a set of 20 DDGS samples, averaging 2,863 kcal/kg with a range of 2,607 to 3,054 kcal/kg

(DM-basis). The average TME_n values reported in these studies are in good agreement with the published reference value of 2,864 kcal/kg (NRC, 1994). However, Kim et al. (2009) determined a TME_n value of 3,554 kcal/kg for 1 sample of conventional DDGS. Nitrogen-corrected AME assays may also be applied to DDGS, though it has been observed that AME_n values are generally about 20% lower than TME_n values for the same sample (NRC, 1994). Rochell et al. (2011) reported AME_n content determined in broiler chicks for 6 DDGS samples. The average AME_n content was 2,678 kcal/kg with a range from 2,146 to 3,098 kcal/kg (DM-basis).

Each of the aforementioned studies indicates that the ME content of DDGS is highly variable. Such large variations are cause for concern, as even small deviations from the formulated energy content of a diet may adversely affect both feed cost and growth performance. Variation in dietary ME has been shown to affect feed conversion ratio in broilers (Leeson et al., 1996; Dozier et al., 2011). The extreme variation observed both between and within individual sources may hinder consistent and effective inclusion at high concentrations (Cromwell et al., 1993; Spiehs et al., 2002; Batal and Dale, 2006;). Furthermore, the nutritional content of DDGS may be affected by agronomic differences, processing conditions, and the utilization of novel oil extraction technologies.

FACTORS AFFECTING THE NUTRIENT CONTENT OF DDGS

Nutrient variability is a concern for all feed ingredients, as the inadvertent miscalculation of nutrient content may create substantial difficulties during diet formulation. Overestimating the nutrient content of a feedstuff may result in marginal or deficient diets, which have the potential to negatively impact live performance.

Alternatively, formulation with artificially low values results in needlessly increased feed costs through the inclusion of additional sources of any nutrient perceived as limiting in the diet. In the latter case, superfluous nutrients in the diet may be wasted, and may even pose an environmental issue. Variations in nutrient content may be attributed to a variety of causes, such as factors associated with crop production and processing.

Agronomic Factors

Agronomic and geographical effects on the composition of feedstock corn are often cited as a possible cause of variation in the resultant DDGS (Amezcua et al., 2004; Pedersen et al., 2007; Rochell et al., 2011). Indeed, the nutrient composition of corn may vary substantially between sources (Cowieson, 2005; Gehring et al., 2013). However, studies designed to investigate the influence of corn source on DDGS composition have not produced consistent results. In a study of a single dry-grind ethanol plant over a 5-yr period, Belyea et al. (2004) determined that no significant correlations existed between corn composition and the resultant DDGS over time. Furthermore, a subsequent study found significant differences in the mineral composition of DDGS from 9 dry-grind ethanol plants receiving corn sources with similar mineral composition (Belyea et al., 2005). Even when corn is specifically selected within a narrow geographical area to reduce variability, the resultant DDGS may have significant differences in nutrient composition (Stein et al., 2009). These results indicate that factors other than geographical and yearly variations in corn composition may be primarily responsible for the nutrient variability observed in DDGS. In contrast, Liu (2009) determined that significant correlations existed between the protein and non-starch carbohydrate **(NSC)** contents of 6 sources of corn and the subsequent concentrations of these nutrients in

DDGS produced by different dry-grind ethanol plants. In the same study, processing conditions were determined to affect DDGS composition as well.

Processing Conditions

Although dry-grind plants utilize the same basic method for ethanol production, minor variations in process variables may produce significant fluctuations in DDGS nutrient composition. Processing additives, drying conditions, and the ratio of solubles to dried grains may contribute to variations between ethanol plants (Spiehs et al., 2002; Belyea et al., 2008) as well as among batches from the same plant (Belyea et al., 1998; Belyea et al., 2004). Processing additives are utilized to maintain optimal conditions for the enzymatic breakdown of starch followed by yeast fermentation. Optimal pH may be maintained though the use of sulfuric acid and cross-contamination with undesirable microbes may be prevented by washing equipment with sodium hydroxide. However, the addition of these chemicals may produce unintended variations in the sulfur and sodium content of the subsequent DDGS, as reported by Batal and Dale (2003).

Drying conditions have been shown to account for most of the observed variability in nutrient composition. Exposure to high temperatures during drying is strongly associated with decreases in protein quality (Cromwell et al., 1993; Fastinger et al., 2006; Liu, 2011) and phosphorus bioavailability (Amezcua and Parsons, 2007). Furthermore, the amount of solubles added to the distillers grains during drying has been demonstrated to significantly alter the protein, fiber, fat, and mineral content of the resultant DDGS (Kingsley et al., 2010).

Corn Oil Extraction

Modifications of the conventional dry-grind method have been developed to allow for the extraction of specific non-fermentable corn fractions in DDGS. Recently, the demand for corn oil as a feedstock for biodiesel production has created an economic incentive for ethanol producers to implement post-fermentation oil extraction strategies (Liu, 2011). The removal of an additional 3 to 6% ether extract **(EE)** results in a concentrating effect on other nutrients in reduced-oil DDGS (Jacela et al., 2011). Data are limited on the ME value of reduced-oil DDGS for poultry; however, Rochell et al. (2011) reported a significantly lower AME_n content of 2,146 kcal/kg (DM basis) for 1 sample of reduced oil DDGS.

Given the substantial impact of these factors on the nutrient content of DDGS, it may be necessary to conduct a thorough chemical analysis of each source of DDGS to be used in formulation. As the ethanol industry continues to implement new processes, the nutrient content of the resultant DDGS changes, causing disparities between previously published values and the nutrient content of contemporary DDGS marketed to poultry producers. This is particularly true for ME values as ethanol producers continue to adopt novel oil-extraction strategies. However, *in vivo* assays for the determination of ME are both time-consuming and costly. Therefore, alternative methods for accurately and rapidly determining the ME content of DDGS may prove a valuable asset to poultry producers.

USE OF PREDICTION EQUATIONS FOR METABOLIZABLE ENERGY

Prediction equations to estimate the ME content of various feed ingredients based upon their chemical composition have been employed for decades (Sibbald et al., 1963; Moir and Connor, 1977; Adedokun and Adeola, 2005). In order to develop these

equations, ME values of a feedstuff are first determined through *in vivo* assays. Multiple linear regression analysis is then applied to generate equations that define a mathematical relationship between the ME value and 1 or more chemical components. Prediction equations eliminate the need to conduct further *in vivo* assays for every DDGS source to be used in diet formulations. Additionally, the use of prediction equations is more accommodating to progressive alterations to the nutritional profile of DDGS due to technological advances than quickly outdated published values. The energy value of DDGS is primarily dependent upon the concentration of the following nutritional components: protein, carbohydrates, and fats. Table 2.1 summarizes previously reported nutrient composition values for DDGS.

Nutrient Content of DDGS

Protein

The value of DDGS as a replacement for soybean meal is primarily dictated by its CP content and amino acid profile. The CP content of conventional DDGS has remained relatively consistent over time. An earlier study reported a range from 26.0 to 31.7% CP (CV = 5.3) among 9 DDGS sources, including 2 from beverage alcohol producers (Cromwell et al., 1993). A large-scale survey of 118 DDGS samples from 10 fuel ethanol plants determined a similar range from 28.7 to 31.6% CP (CV = 6.4) (Spiehs, et al., 2002). Additionally, analysis of 235 samples from a single ethanol plant over a 5-yr period resulted in a range from 30.8 to 33.3% CP (CV = 6.3) (Belyea et al., 2008). The similarity of these results indicates that the CP content of DDGS is relatively stable both among and within ethanol plants. Corn fractionation technologies do allow for the production of high-protein DDGS, which have been reported to contain CP contents as

high as 44.1% (Kim et al., 2008a) and 53.39% (Applegate et al., 2009). However, these modified DDGS are specifically marketed as a premium product, allowing for formulations to be adjusted appropriately.

The amino acid profile of DDGS is very similar to that of corn, and is therefore particularly limiting in Lys. Cromwell et al. (1993) reported Lys contents varying from 0.48 to 0.97% (CV = 18.71%). Similarly, Spiehs et al. (2002) reported a range of 0.72 to 1.02% Lys (CV =17.3%). Each of these studies determined Lys content to be the most variable among the essential amino acids. This is likely attributed to the susceptibility of Lys to heat damage during the processing and drying of DDGS. During the heating stages of DDGS production, the Maillard reaction may occur between reducing sugars present in the DDGS and the ε-amino group of Lys. This reaction results in DDGS with a darker color and reduces the bioavilability of Lys and other susceptible amino acids (Lumpkins and Batal, 2005; Batal and Dale, 2006; Fastinger et al., 2006). Although DDGS contain a moderate concentration of CP, the quality of the protein is far inferior to that of soybean meal. Therefore, its inclusion as a replacement source of protein is limited, and must be accompanied by the use of supplemental amino acids such as Lys.

Carbohydrates

As previously mentioned, CP content of conventional DDGS is relatively consistent, and therefore does not contribute greatly to energy variability. In contrast, the carbohydrate composition of DDGS is highly variable, and may contribute to substantial differences in ME content between sources. Carbohydrates compose the largest individual macronutrient fraction of DDGS with analyzed total carbohydrate values typically between 50 to 60% (DM-basis) (Kim et al., 2008b; Liu, 2009).

Corn is predominantly composed of starch, the majority of which is utilized by yeast during the fermentation process. As a result, DDGS contain low amounts of starch and relatively high amounts of NSC. The NSC fraction includes a variety of compounds, which are difficult to quantify due to vast differences in their nutritional, chemical, and physical characteristics. In DDGS, NSC are most commonly quantified on the basis of their solubility in neutral or acidic detergent solutions by the method of Van Soest et al. (1991). Determination of neutral detergent fiber **(NDF)** employs a neutral detergent solution to dissolve soluble compounds such as pectins and cell contents. The residual compounds are predominantly portions of the cell wall, including hemicellulose (**HC**), cellulose, and lignin. Further treatment with an acid detergent solution removes the HC fraction to produce acid detergent fiber **(ADF)**. Both NDF and ADF represent relatively indigestible fractions for poultry and are associated with reductions in ME content of feedstuffs (Annison and Choct, 1991; Bedford, 1996; Mateos et al., 2012). Stein and Shurson (2009) reported an average starch content of 7.3% among 46 samples of DDGS with a range of 3.8 to 11.4%. Concomitantly, NDF ranged from 20.1 to 32.9% with an average of 25.3% and ADF ranged from 7.2 to 17.3% with an average value of 9.9% for the same sample set.

In addition to NDF and ADF, NSC may be characterized on the basis of their solubility. The total dietary fiber **(TDF)** system allows for the additional recovery and quantification of soluble cell wall fractions that are lost in the determination of NDF (Van Soest et al., 1991). Total dietary fiber may be further divided into soluble and insoluble fiber components. The former includes pectins, arabinans, β-glucans, and resistant starches, whereas the latter includes structural components such as HC, cellulose, and

lignin and is roughly analogous to NDF content. Each of these fractions produces different physiological effects in monogastric animals. Insoluble fiber is largely indigestible, and therefore serves as a diluent of dietary energy (Annison and Choct, 1991). Soluble fiber produces viscous gels in the gastrointestinal tract that hinder the digestion and absorption of other nutrients, causing a reduction in ME that far surpasses the energy dilution effect of the indigestible fiber itself (Annison and Choct, 1991; Mateos et al., 2012; Perryman and Dozier, 2012).

Fats

In addition to protein and carbohydrates, fat also contributes to the energy value of DDGS. Cromwell et al. (1993) reported oil content ranging from 9.1 to 14.1% with an average of 10.7% for 9 DDGS samples from both beverage and ethanol sources. Similarly, Spiehs et al. (2002) determined oil content among 118 DDGS samples with an observed range of 10.2 to 11.4% and an average of 10.9%. Slightly higher oil concentrations of 12.0 and 11.7% were reported by Belyea et al. (2008) and Liu (2008), respectively. These results might suggest that oil content is relatively constant among conventional DDGS sources, particularly in comparison with other more variable nutrients such as carbohydrates. However, with the rapid adoption of lucrative post-fermentation oil extraction technologies, reduced-oil DDGS have become more common. These DDGS samples typically have EE contents lower than 9.0% with some samples as low as 2.0 to 4.0 % EE (Saunders and Rosentrater, 2009; Rochell et al., 2011; Anderson et al., 2012). Studies in swine have indicated that extracted oil is generally more digestible than intact oil (Adams and Jensen, 1984; Kil et al., 2010; Kim et al., 2013). Therefore, in addition to decreasing the overall oil content, oil extraction may also

preferentially remove more readily available oil, leaving the less digestible oil fractions in the resultant DDGS. As reduced-oil varieties of DDGS become more prevalent, it will become increasingly important to account for the additional effects of oil extraction on ME variability.

Prediction Equations

Previous research has demonstrated the development of prediction equations for the TME_n of corn DDGS (Batal and Dale, 2006) as well as wheat DDGS (Cozannet et al., 2010). Batal and Dale (2006) determined that crude fat, crude fiber, CP, and ash were the best predictors of TME_n ($R^2 = 0.45$) in mature roosters. However, this model was based on measures of proximate analysis. Cozannet et al. (2010) developed equations for wheat DDGS using NDF, ADF, acid detergent lignin, water insoluble cell walls, and starch in addition to proximate analysis. Of these chemical measures, only ADF was determined to be significantly predictive of AME_n content in broilers ($R^2 = 0.77$).

Additionally, Rochell et al. (2011) developed prediction equations for several corn fermentation co-products, including DDGS, using an AME_n assay. The 6 DDGS samples in this study had EE contents above 10.0%, with the exception of 1 sample with an EE content of 3.15%. The best predictors of AME_n in broilers were determined to be crude fat, ash, and HC ($R^2 = 0.89$). Removal of HC from the model resulted in the selection of NDF, GE, and CP as the primary predictors ($R^2 = 0.87$).

The importance of measures of fiber in the prediction of ME has been documented in poultry (Rochell et al., 2011) and in swine (Pedersen et al., 2007; Anderson et al., 2012). Pedersen et al. (2007) determined that ash, ADF, and GE were the primary predictors of ME for 10 samples of DDGS in swine ($R^2 = 0.94$). However, all of

the DDGS utilized in this study had EE contents greater than 9.5%. A similar study was conducted by Anderson et al. (2012) using 20 corn co-products, including 6 conventional DDGS samples with EE concentrations greater than 10%, and 1 reduced-oil sample with an EE content of 3.16%. For this study, the primary predictors for ME of corn co-products in swine were determined to be GE and TDF (R^2 = 0.72). The removal of TDF from the variable selection pool resulted in a model containing GE, NDF, and ash (R^2 = 0.68). These results in swine further emphasize the importance of fiber fractions such as TDF, NDF, and ADF in the prediction of ME.

It must be noted, however, that the persistent reliance on R^2 as a measure of model performance in the literature not only prevents proper comparisons from being made between proposed models, but may also cause misleading assumptions to be made concerning prediction accuracy. The R^2 value undergoes an inherent and unavoidable increase as predictors are added to a model. Thus, it is inappropriate to compare models with varying numbers of predictors on the basis of R^2. Instead, it is better to compare using adjusted R^2 values, which account for the number of predictors and the relative contribution of each to reducing the error of the overall model. Furthermore, R^2 value in multiple linear regression pertains only to the samples used to generate each model. For example, the equation reported by Batal and Dale (2006), with an R^2 of 0.45, would be expected to explain approximately 45% of the observed variation in TME_n within the sample set of 17 DDGS sources used in model development. Yet this statistic confers no information about the predictive performance of the model when applied to external data. The prediction capabilities of a model should be considered unproven until a validation has been conducted with an independent dataset, regardless of the R^2 value.

KNOWLEDGE GAPS IN THE LITERATURE

The precision of prediction equations depends greatly on the selection of an adequate number of test products with a sufficiently wide range of nutrient composition, and the inclusion of relevant chemical analyses in the regression analysis. Prior research has often omitted influential measures of fiber such as NDF, ADF, and TDF in the development of regression equations. The practical value of any prediction equation should be determined through cross-validation with a sample set that is independent of the test products used to generate the equation. Previously published prediction equations have been developed primarily using conventional DDGS. However, basic compositional analyses and AME_n content of DDGS with reduced oil content in broilers have not yet been published in the literature. Therefore, the development of prediction equations for AME_n that specifically address the wide range of nutrient content observed among modern DDGS is warranted. Furthermore, previously reported prediction equations for corn co-products have not undergone cross-validation with an independent sample set to validate their practical application. In order to address these knowledge gaps, the proposed research will determine AME_n content of 15 samples of DDGS in order to develop a set of robust and accurate prediction equations for AME_n based on chemical composition. The second experiment will determine AME_n content of 15 additional samples of DDGS for the purpose of cross-validating the equations developed in the first experiment, as well as the equations developed for corn co-products by Rochell et al. (2011)

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Table 2.1 Composition of corn distillers dried grains with solubles reported in the literature[1,2]

	Cromwell et al., 1993 (n = 9)				Spiehs et al., 2002 (n = 118)				Belyea et al., 2004 (n = 235)				Fiene et al., 2006 (n = 150)			
	Min	Max	Mean	CV	Min[3]	Max	Mean	CV	Min[4]	Max	Mean	CV[4]	Min	Max	Mean	CV
DM	87.	92.7	90.5	1.8	87.2	90.2	88.9	1.7					85.3	93.1	89.9	1.9
CP	26.	31.7	29.7	5.3	28.7	31.6	30.2	6.4	30.8	33.3	31.4	6.3	20.2	31.0	26.1	8.9
EE	9.1	14.1	10.7	6.3	10.2	11.4	10.9	7.8	10.9	12.6	12.0	5.6	3.0	13.8	9.9	28.3
Ash	3.7	8.1	5.3	27.7	5.2	6.7	5.8	14.7	4.3	5.0	4.6	5.7	2.1	7.0	4.34	19.8
Starch									4.7	5.9	5.3	9.7				
CF					8.3	9.7	8.8	8.7			10.2	3.7	4.7	23.1	6.3	24.5
TDF																
NDF	38.	33.1	43.9	10.0	36.7	49.1	42.1	14.3								
ADF	15.	11.4	20.8	21.1	13.8	18.5	16.2	28.4	15.4	19.3	16.8	9.3				
	Parsons et al., 2006 (n=20)				Batal and Dale, 2006 (n=17)				Stein et al., 2006 (n=10)				Rochell et al., 2010 (n=6)			
	Min	Max	Mean	CV	Min	Max	Mean	CV	Min	Max	Mean	CV	Min	Max	Mean	CV
DM	85.0	89.0	88.0	0.9			86.0		86.	90.8	88.9	1.3	86.6	93.2	89.7	2.8
CP					23.0	30.0	27.0	7.4	28.	32.7	30.9	4.1	26.5	34.7	30.3	9.2
EE	14.7	18.2	15.9	4.8	2.3	10.6	8.8	26.1					3.2	11.7	9.8	33.8
Ash	4.2	5.0	4.5	5.0	3.9	5.4	4.4	9.1					4.2	5.4	4.7	10.4
Starch									5.9	8.9	7.3	14.0	3.0	7.9	4.8	40.0
CF					5.1	8.1	6.6	12.1					7.0	8.7	7.7	8.2
TDF													30.3	38.1	35.0	8.4
NDF									41.	49.1	45.2	4.8	27.7	51.0	37.8	21.0
ADF									9.0	14.4	12.2	13.1	8.6	15.8	11.7	23.9

[1]Nutrient values expressed on % DM-basis; EE = ether extract; CF = crude fiber; TDF = total dietary fiber; NDF = neutral detergent fiber; ADF =acid detergent fiber.

[2]CV = coefficient of variation, (%).

[3]Minimum and maximum values for means of 10 sample locations.

[4]Minimum, maximum, and CV values for means of 5 sample groups.

III. APPARENT METABOLIZABLE ENERGY AND PREDICTION EQUATIONS FOR REDUCED-OIL CORN DISTILLERS DRIED GRAINS WITH SOLUBLES IN BROILER CHICKS FROM 10 TO 18 DAYS OF AGE

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) in order 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 m^2/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 in order 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 gross energy, 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 upon the adjusted coefficient of determination (R^2_{adj}), RMSE, 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) (R^2_{adj} = 0.86; RMSE = 98.76; PRESS = 199,819; $P \leq$ 0.001). These results indicated that the composition of DDGS with variable EE content may be used to predict AME_n in broiler chicks.

INTRODUCTION

Continued expansion of the ethanol industry has increased the amount of corn fermentation co-products available to livestock producers as an alternative feed ingredient. Specifically, DDGS have been increasingly utilized in poultry diets. However, recent advances in biorefining technologies have allowed the ethanol industry to remove an additional 2 to 6% 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.

In order to mitigate the adverse consequences of nutrient variability among DDGS sources, prediction equations have been developed to estimate metabolizable energy 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 co-products with a wide range of nutrient composition allows for the development of robust metabolizable energy prediction equations (Rochell et al., 2011). The equations reported by Rochell et al. (2011) indicate that fiber measures such as NDF or 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) has likewise shown that metabolizable energy may be predicted from TDF values, which were also not reported by Batal and Dale (2006) or Cozannet et al. (2010). Additionally, because Cozannet et al. (2010) utilized wheat DDGS, the equations developed therein are not applicable to corn DDGS. Furthermore, 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 3.1) + 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 body weight 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 in order to calculate energy and nitrogen intake and excretion. Multiple subsamples were collected from the

total amount of accumulated excreta 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 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 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 3.2 and 3.3) unless otherwise described. Neutral detergent fiber (Holst, 1973) was determined after pretreatment with a thermostable amylase. Values for 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.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon$$

where *y* is the response variable, AME_n; (x_1, x_2, …, x_k,) is the set of k regressor variables corresponding to each analyzed nutrient; the parameters β_j, j = 0, 1, …, k, are the partial regression coefficients representing the expected change in response *y* per unit change in x_i when all the remaining regressor variables, x_i (i≠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 in order 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 re-evaluated 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 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 RMSE of the regression coefficients, the adjusted coefficient of multiple determination ($\mathbf{R^2_{adj}}$), the Mallows' statistic ($\mathbf{C_p}$), the prediction error sum of squares (**PRESS**), and the prediction coefficient of determination ($\mathbf{R^2_{Pred.}}$), as defined below:

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

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

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

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

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 co-products 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 United States ethanol industry and made available to commercial poultry producers. The selected DDGS samples ranged in EE content from 3.15 to 13.23% (Table 3.2). Previous research in both poultry and swine has emphasized the importance of various fiber fractions in the development of prediction equations for the metabolizable energy of corn co-products such as DDGS (Rochell et al., 2011; Anderson et al., 2012; Pederson et al., 2007). 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 utilized 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 semi-purified diet (Adeola and Ileleji, 2009). Substitution of the test ingredient at a high level reduces variance in the calculated AME_n, but may negatively impact 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 when 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 metabolizable energy 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 3.4). 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 3.4; $P \leq 0.05$). 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 3.5). Rochell et al. (2011) reported an average AME_n value of 2,678 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 utilize DDGS as an energy source.

Pearson correlation coefficients between the chemical components of DDGS and their AME_n value are shown in Table 3.6. 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 co-products (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 co-products 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 metabolizable energy, it was not significantly correlated to AME_n (r = -0.20, *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 3.7). 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 R^2_{Adj} of 0.44. The subsequent selection of TDF (*P* = 0.02) on the basis of its partial correlation coefficient increased the model R^2_{Adj} to 0.63 (Equation 2). Successive addition of CP (*P* = 0.06; equation 3, R^2_{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 DM) – (40.67 × % TDF DM) + (89.75 × % CP DM) + (125.80 × % starch DM); R^2_{Adj} = 0.86, RMSE = 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 3.8). 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, $R^2_{Adj} = 0.50$) instead as the second predictor after GE. Crude protein ($P = 0.01$; Equation 3, $R^2_{Adj} = 0.71$) and starch ($P = 0.01$; Equation 4, $R^2_{Adj} = 0.71$) were again added as the third and fourth predictor variables for the revised selection equation. The model was then improved 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, kcal/kg of DM = -14,322 + (2.69 × GE kcal/kg DM) + (117.08 × % CP DM) + (149.41 × % starch DM) – (18.30 × % NDF DM); $R^2_{Adj} = 0.88$, RMSE = 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 (Pederson, 2007; $R^2 = 0.94$) and TDF (Anderson, 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 metabolizable energy 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 which 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}, RMSE, and C(p) values compared to 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 to a value 227,477 for the model in which NDF is included. A model with a higher PRESS value will explain the variation external to 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). While 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 in order 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 (Anderson et al., 2012; Pederson et al., 2007). 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., 2012). 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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Table 3.1 Ingredient and calculated nutrient composition of the basal diet

Item	Amount
Ingredient (%, "as-is")	
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
Non-phytate P	0.46
Na	0.21

[1]Vitamin 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 B_{12} (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.
[2]Mineral 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.
[3]BioCox 60 provided 60 g/907 kg of salinomycin (Alpharma, Fort Lee, NJ).
[4]Values 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, 2004).

Table 3.2 Analyzed composition of 15 corn distillers dried grains with solubles sources[1]

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

Item (%)	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

[1]All values on a DM basis. Values reported on a percentage basis unless noted otherwise.
[2]Hemicellulose was calculated as neutral detergent fiber minus acid detergent fiber.

Table 3.3 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)
Dry matter	AOAC official method 934.01
Starch	AACC approved method 76-13. Modified: Starch Assay Kit (product code STA-20, Sigma, St. Louis, MO)
Crude protein	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

[1]Unless otherwise noted, all methods of analysis were determined by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO).
[2]AOAC = Association of Official Analytical Chemists; AACC =American Association of Cereal Chemists.
[3]Determined by Auburn University Laboratory (Auburn, AL).

Table 3.4 Feed intake and BW gain of broiler chicks fed diets containing 15% distillers dried grains with solubles from 10 to 18 d of age[1]

Treatment	Feed Intake (g/bird)	BW Gain (g/bird)
Control[1]	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 ($P < 0.05$). Observed means for feed intake and BW gain are based on 9 replicate pen means per treatment.
[1]Control diet contained 15% dextrose.

Table 3.5 Determined gross energy (GE) and AME_n of corn distillers dried grains with solubles (DDGS) samples in broiler chicks[1,2]

DDGS			AME_n	
Sample	n	GE	kcal/kg	% of GE
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 ($P < 0.05$).

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

Table 3.6 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									
Gross energy	0.69	1.00								
P-value	0.01									
Crude protein	-0.20	-0.51	1.00							
P-value	0.48	0.05								
Starch	0.09	-0.23	-0.45	1.00						
P-value	0.75	0.41	0.09							
TDF	-0.56	-0.16	0.289	-0.39	1.00					
P-value	0.03	0.57	0.30	0.15						
NDF	-0.52	-0.35	0.59	-0.35	0.88	1.00				
P-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			
P-value	0.05	0.22	0.01	0.05	0.01	0.01				
Ether extract	0.35	0.74	-0.24	-0.46	0.16	-0.06	0.09	1.00		
P-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	
P-value	0.98	0.22	0.02	0.22	0.26	0.87	0.84	0.28		
Hemicellulose	-0.48	-0.33	0.52	-0.23	0.83	0.98	0.78	-0.14	-0.04	1.00
P-value	0.07	0.22	0.05	0.40	0.01	0.01	0.01	0.63	0.90	

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

Table 3.7 Stepwise selection of a regression model for AME_n based on the nutrient composition of 15 corn distillers dried grains with solubles samples

	Regression coefficients[1]					Statistical parameters[2]			
AME_n equation	Intercept	GE	TDF	CP	Starch	RMSE	R^2_{Adj}	C(p)	PRESS
Equation 1	-5,584	1.59	-	-	-	198	0.44	28.72	-
SE	2,294	0.46	-	-	-	-	-	-	-
Estimate *P*-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 *P*-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 *P*-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 *P*-value	0.01	0.01	0.01	0.01	0.01	-	-	-	

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

[2]R^2_{Adj} is the adjusted coefficient of determination; RMSE 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 3.8 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

	Regression coefficients[1]						Statistical parameters[2]			
AME_n equation	Intercept	GE	ADF	CP	Starch	NDF	RMSE	R^2_{Adj}	C(p)	PRESS
Equation 1	-5,584	1.59	-	-	-	-	198	0.44	28.72	-
SE	2,294	0.46	-	-	-	-	-	-	-	-
Estimate *P*-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 *P*-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 *P*-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 *P*-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 *P*-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 *P*-value	0.01	0.01	-	0.01	0.01	0.01	-	-	-	

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

[2]R^2_{Adj} is the adjusted coefficient of determination; RMSE 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.

IV. VALIDATION OF PREDICTION EQUATIONS FOR APPARENT METABOLIZABLE ENERGY OF CORN DISTILLERS DRIED GRAINS WITH SOLUBLES IN BROILER CHICKS

ABSTRACT

An experiment consisting of 3 nearly identical trials was conducted to determine the AME_n content of distillers dried grains with solubles (DDGS) in order to validate 4 previously published prediction equations for AME_n of corn DDGS in broilers. In addition, prior research data were utilized to generate a best-fit equation for AME_n based on proximate analysis. Fifteen samples of DDGS ranging in ether extract (EE) from 4.98 to 14.29% (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, male Ross × Ross 708 chicks were housed in grower battery cages and received a common starter diet until the experimental period. Each cage was randomly assigned to 1 of the dietary treatments (Trial 1 and Trial 2: Control + 6 test diets, 13 replicates per diet; Trial 3: Control + 3 test diets, 12 replicates per diet). Experimental diets were fed over a 6-d acclimation period, followed by a 48 h total excreta collection period. On a DM-basis, AME_n of the 15 DDGS samples ranged from 1,975 to 3,634 kcal/kg. Analyses were conducted to determine gross energy, CP, EE, DM, starch, total dietary fiber, neutral

detergent fiber, crude fiber (CF), acid detergent fiber, and ash content of the DDGS samples. All results were reported on a DM basis. Application of the 4 equations to the validation data resulted in root mean square error (RMSE) values of 335, 381, 488, and 502 kcal/kg, respectively. Least absolute shrinkage and selection operator (LASSO) technique was applied to proximate analysis data for 30 corn co-products adapted from prior research and resulted in the following best-fit equation: [AME_n (kcal/kg) = 3,673 – (121.35 × CF) + (51.29 × EE) – (121.08 × ash); $P < 0.01$; $R^2 = 0.70$; $R^2_{adj} = 0.67$; RMSE = 270 kcal/kg]. The RMSE values obtained through validation were not consistent with the expectation of predictive performance based on internal measures of fit for each equation. These results indicated that validation is necessary to quantify the expected error associated with practical application of each individual prediction equation to external data.

INTRODUCTION

Over the past decade, dietary energy costs have increased substantially due to the diversion of a large portion of the corn supply to meet the demands of the renewable fuel industry (Donohue and Cunningham, 2009). As the ethanol industry has expanded, the availability of DDGS, the primary co-product of ethanol production, has increased correspondingly. Distillers dried grains with solubles have been increasingly utilized in poultry diets as a cost-effective substitute for portions of traditional ingredients such as corn and soybean meal. However, both agronomic and processing differences can contribute to substantial variability in nutrient content among DDGS sources (Belyea et al., 2004; Stein et al., 2009; Kingsly et al., 2010; Liu, 2011). Recently, the implementation of novel biorefining technologies, which allow for the post-fermentation

extraction of oil from thin stillage during the processing of DDGS, has further exacerbated this inherent variability (Meloche et al., 2013). The accuracy of AME_n values used in diet formulation can substantially impact feed costs as well as the profitability of broiler production. If AME_n values are overestimated in diet formulation, marginal or deficient dietary energy content may adversely affect growth performance (Leeson et al., 1996; Dozier et al., 2011). In contrast, if AME_n content is underestimated, dietary formulation may require the addition of supplemental fat to meet dietary energy needs (Saunders and Rosentrater, 2009).

In vivo determination of AME_n content is not only time-consuming and costly, but the determined values also apply only to the specific samples evaluated in the assay. Robust prediction equations that estimate the ME content of DDGS based on nutrient composition may provide an inexpensive, rapid, and accurate alternative for the determination of ME (Pedersen et al., 2007; Rochell et al., 2011, Anderson et al., 2012; Kerr et al., 2013; Meloche et al., 2013). Previous studies have led to the development of linear regression equations for estimating the AME_n of corn DDGS based on nutrient composition (Rochell et al., 2011, $R^2 = 0.89$; Meloche et al., 2013, $R^2 = 0.90$). Additionally, equations for TME_n of DDGS based on proximate analysis have been developed for use in poultry (Batal and Dale, 2006, $R^2 = 0.44$), but no analogous equation has been reported for AME_n. Although each of these equations successfully fits the DDGS samples utilized in model development, there is no guarantee that the accuracy of predicting AME_n will be similar when these equations are applied to nutrient composition data from additional DDGS samples. Therefore, proper validation of these models is warranted.

To our knowledge, an independent validation of prediction equations for AME_n of DDGS has not been reported in poultry. Therefore, the objective of this study was to evaluate the AME_n content of 15 DDGS samples varying in EE content in order to develop an independent validation data set with which to evaluate the predictive performance of the equations of Rochell et al. (2011) and Meloche et al. (2013). Additionally, proximate analysis data adapted from the work of Rochell et al. (2011) and Meloche et al. (2013) was used to develop an alternate cross-validated equation for AME_n.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at Auburn University approved the use of live birds in this experimental protocol (PRN 2012-2128; 2013-2221).

Broiler Husbandry

Three similarly designed energy balance trials were conducted in broilers (Trial 1: 10 to 18 d of age; Trial 2: 13 to 21 d of age; Trial 3: 24 to 30 d of age). In total, 1,754 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. The experimental facility for all 3 trials was a solid-sided house with temperature control. Temperature was set at 33°C at placement and was decreased gradually with increasing bird age to 27°C at the conclusion of each trial. A 23L:1D lighting schedule was used for the duration of each trial. In each trial, chicks (Trial 1: 637 chicks, 91 cages, 7 per cage; Trial 2: 637 chicks, 91 cages, 7 per cage; Trial 3: 480 chicks, 48 cages, 10 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. After a 6-d acclimation period, a 48-h energy balance assay was conducted (Trial 1: 16 to 18 d of age; Trial 2: 19 to 21 d of age; Trial 3: 28 to 30 d of age).

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. In each trial, dietary treatments consisted of a control diet (85% basal diet + 15% dextrose) and test diets (Trial 1: Control + 6 test diets, 13 replicates; Trial 2: Control + 6 test diets, 13 replicates; Trial 3: Control + 3 test diets, 12 replicates) each containing 15% of an individual DDGS sample substituted at the expense of dextrose (85% basal diet + 15% DDGS; Table 1). Broilers were fed a common corn-soybean meal starter diet from placement until the start of experiment (Trial 1: 10 d of age; Trial 2: 13 d of age; Trial 3: 24 d of age). At this time, birds were randomly assigned to 1 of the dietary treatments. All dietary treatments were offered in mash form.

Measurements

Feed consumption was recorded to verify acceptance of the dietary treatments over the experimental feeding period. Feed disappearance and total excreta weights (wet-basis) were recorded during the 48 h collection period in order to calculate energy and nitrogen retention. Multiple subsamples were collected from the total amount of accumulated excreta on the pan beneath each cage. 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 GE using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IA). Nitrogen content of the experimental diets and excreta were determined for duplicate 0.25-g samples using a combustion analyzer (Elementar Americas Inc., Mt. Laurel, NJ) according to an established method (AOAC International, 2006; method 968.06).

Apparent ME_n for each dietary treatment was calculated using 8.73 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: 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 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 chemical composition (University of Missouri Agriculture Experiment Station Chemical Laboratories, Columbia; Eurofins Scientific, Inc., Des Moines, IA; Tables 2 and 3) unless otherwise described. Neutral detergent fiber (Holst, 1973) was determined after pretreatment with a thermostable amylase. Values for 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. All analyses are reported on a DM basis.

Statistical Analyses

Data were analyzed as a randomized complete block design with cage location as the blocking factor. Feed intake, BW gain, and AME_n were analyzed for each trial using PROC MIXED (SAS Institute, 2009) by the following mixed-effects model:

$$Y_{ij} = \mu_{..} + \rho_i + \tau_j + \varepsilon_{ij}$$

where $\mu..$ is the overall mean; the ρ_i are identically and independently normally distributed random block effects with mean 0 and variance σ^2_ρ; the τ_j are the fixed factor level effects corresponding to the *j*th treatment (DDGS) such that $\Sigma\tau_j = 0$; and the random errors ε_{ij} are identically and independently normally distributed with a mean 0 and a variance σ^2.

Prediction performance of the established prediction equations was evaluated on the basis of the difference between the predicted and observed AME_n values. The percent difference was calculated on the basis of absolute values to prevent the calculation of artificially low averages due the presence both positive and negative variation among samples. The R^2, R^2_{adj}, and C_p for the established prediction models, as defined below, provide context for this comparison. Additionally, the RMSE, PRESS, and the $R^2_{Pred.}$, were calculated as defined below:

$$\boldsymbol{R^2} = 1 - \left(\frac{SS_{Res}}{SS_T}\right)$$

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

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

$$\boldsymbol{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}[\hat{y}_i - y_i]^2}{n}}, i = 1, 2, \ldots, n$$

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

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

where SS_{Res} is the residual sum of squares, SS_T is the total sum of squares, *n* is the number of observations in the sample, *p* is the number of regressors included in the model, $\hat{\sigma}^2$ is the estimate of σ^2 (error variance), y_i is the observed value for the *i*th observation, $\hat{y}_i$ is the predicted value for the *i*th observed response, and $\hat{y}_{(i)}$ is the predicted value for 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). High values of R^2, R^2_{adj}, and R^2_{Pred} are indicative of better model fit. Conversely, low values of C_p, RMSE, and PRESS are considered optimal.

The least absolute shrinkage and selection operator (**LASSO**) technique was applied to the original data sets of Rochell et al. (2011) and Meloche et al. (2013) using PROC GLMSELECT (SAS Institute, 2009). The models generated through this more recently developed selection technique were used to evaluate the efficacy of the stepwise selection method used by Rochell et al. (2011) and Meloche et al. (2013). This method solves the following L_1-norm penalized minimization problem:

$$\sum_{i=1}^{n}\left(y_i - \sum_{j} x_{ij}\beta_j\right)^2 + \lambda\sum_{j=1}^{p}|\beta_j|, \qquad i = 1, 2, \ldots, n \ and \ j = 1, 2, \ldots, p$$

where n is the number of observations in the sample, y_i is the set of centered response values, x_{ij} is the set of standardized regressors, β_j is the slope coefficient associated with the *j*th regressor, λ is a tuning parameter constraining the sum of the absolute value of the coefficients, and p is the number of predictors in the equation (Tibishirani, 1996, 2011). The LASSO selection technique was also applied to corn co-product composition data adapted from Rochell et al. (2011) and Meloche et al. (2013) to generate a model for AME_n based on proximate analyses only. Statistical significance was considered at $P \leq 0.05$. All tests were determined to have statistical power $\beta \geq 0.80$ (Faul et al., 2009).

RESULTS AND DISCUSSION

In order to adequately validate the equations of Rochell et al. (2011) and Meloche et al. (2013), it was necessary to select DDGS samples with a sufficient amount of variation in the predictive nutrient components (Table 3). The selected DDGS samples ranged in EE content from 4.98 to 14.23% on a DM-basis. Each of the equations of interest contains at least 1 fiber measurement, such as HC, NDF, TDF. In the selected DDGS samples, HC ranged from 19.29 to 28.96%, NDF ranged from 27.84 to 43.78 %, and TDF ranged from 26.50 to 36.60%. Gross energy content of the DDGS samples ranged from 4,841 to 5,254 kcal/kg, CP ranged from 28.40 to 34.21%, starch ranged from 2.33 to 10.01%, and ash ranged from 4.58 to 5.63%.

Feed intake among birds receiving DDGS treatments was similar within each trial over the 8-d experimental feeding period (Table 4). However, birds receiving DDGS sources 9, 10, 13, 14, and 15 consumed less feed when compared with birds receiving the dextrose control diet within their respective trials ($P \leq 0.05$). Because this experiment

was not intended to investigate treatment effects on bird performance, feed intake and BW gain were recorded for the sole purpose of monitoring potential feed refusals. No adjustments were made in diet formulations for the nutrient content of the selected DDGS samples, resulting in variable nutrient density in the experimental diets. It has been reported that broilers may alter their feed intake in order to accommodate their nutrient requirements when fed diets with varying nutrient density (Pesti and Smith, 1984; Leeson et al., 1996; Plavnik et al., 1997; Brickett et al., 2007). Comparable differences in feed intake among diets containing DDGS were reported by Meloche et al. (2013) in a similarly designed AME_n study. In Trial 2, BW gain was lower for birds receiving DDGS source 9 compared with the dextrose control (Table 4; $P \leq 0.05$), but similar among all other treatments within both Trial 1 and Trial 2. Body weight was not recorded for Trial 3.

Apparent ME_n values for the 15 samples of DDGS ranged from 1,975 to 3,634 kcal/kg of DM with an average value of 2,764 kcal/kg of DM (Table 5). Rochell et al. (2011) reported a similar average AME_n value of 2,678 kcal/kg for 6 samples of DDGS. Alternatively, Meloche et al. (2013) reported a much lower average AME_n value of 2,309 kcal/kg for 15 samples of DDGS. Because GE content varied substantially among DDGS samples, AME_n as a percentage of GE was calculated. In the current study, average AME_n value as a percentage of GE was 60.9%, compared with only 46.2% reported by Meloche et al. (2013).

The equations of Rochell et al. (2011) and Meloche et al. (2013) were developed to predict AME_n content of corn co-products (including DDGS) and DDGS, respectively, on the basis of specific chemical components selected through stepwise multiple

regression (Table 6). However, because the current validation study was conducted using only DDGS samples, the results presented here are not necessarily applicable to the other co-products assessed by Rochell et al. (2011). In order to more clearly differentiate between equations from the same author, these equations will be referenced as Equation 1 to Equation 4 the remainder of the text (Table 6).

Numerous statistical techniques exist for assessing the accuracy of linear regression models within the data set used to develop the equation. These measures of internal fit include R^2, R^2_{adj}, and RMSE. Traditionally, R^2 has been utilized as the primary measure of model performance in the literature (Batal and Dale, 2006; Pedersen et al., 2007; Rochell et al., 2011; Anderson et al., 2012; Kerr et al., 2013; Meloche et al., 2013). The R^2 values for Equation 1 ($R^2 = 0.89$) and Equation 2 ($R^2 = 0.87$) seem to indicate poorer fit than those of Equation 3 ($R^2 = 0.90$) and Equation 4 ($R^2 = 0.89$). However, caution must be used when comparing models strictly on the basis of R^2 alone. As predictors are added to a model, the residual sum of squares must decrease, causing an unavoidable increase in model R^2 (Montgomery et al., 2012). For this reason, it is considered inappropriate to compare models with varying numbers of predictors on the basis of R^2. Rather, it is better to compare using R^2_{adj} values, which account for the number of predictors and the relative contribution of each to reducing the error of the overall model. The R^2_{adj} values indicated relatively similar fit among all 4 models (Table 6).

Furthermore, R^2 values in multiple linear regression pertain only to the samples used to generate each model. For example, Equation 1 has an R^2_{adj} of 0.86 and thus would be expected to explain approximately 86% of the observed variation in AME_n

within the sample set of 15 corn co-products used in model development. This corresponds to an expectation that approximately 14% of the variation in AME_n, on average, is not explained by the model. For a sample with average AME_n within the model building data set, 14% variation represents about 388 kcal/kg. The RMSE of the equation is equivalent to the standard deviation of residuals. For normally distributed residuals, approximately 68% of the observed values would be expected to fall within a distance equal to 1 times the RMSE, and approximately 95% of the observed values would be expected to fall within a distance equal to 2 times the RMSE (Pukelsheim, 1994). For Equation 1, a RMSE value of 191 indicates that approximately 95% of the observed AME_n values within the set of 15 co-products used to generate the equation would fall within 382 kcal/kg of the fitted value determined by the regression model, corresponding well with the R^2_{adj}. The same conclusions may be made on the basis of R^2_{adj} and RMSE for the other 3 equations. Yet, these statistics confer no information about the predictive performance of the model when applied to new DDGS samples. Although the models under consideration here fit the data well by traditional internal measures, they may not necessarily be successful when applied to new data.

Some indication of the predictive potential of a model may be ascertained by using the model building data itself to calculate the PRESS statistic. This measure of fit is generated through an iterative calculation that repeatedly fits the model with 1 observation omitted. The predicted value for each omitted observation is then used in calculation of the PRESS statistic. Substituting the PRESS statistic for the residual sum of squares in a calculation similar to that of traditional R^2 produces the R^2_{Pred}, which represents the amount of variation in AME_n that is expected to be explained by the model

when applied to new data. Equations 1 and 2 have R^2_{Pred} values of 0.74 and 0.78, respectively (Table 6). These values indicate that, on average, predictions based on a new data set should be within 26% (727 kcal/kg) and 22% (600 kcal/kg) of the actual value when Equations 1 and 2 are applied to new data. For Equations 3 and 4, R^2_{Pred} values of 0.80 and 0.71 are obtained, resulting in an expected average error of 20% (470 kcal/kg) and 29% (670 kcal/kg), respectively, when Equations 3 and 4 are applied to new data (Table 6).

However, in order to determine whether these internal measures of fit are reliable estimates of prediction accuracy, the models must be applied to a validation data set (Table 7). By calculating the difference between the observed and fitted values for the validation data set, it is possible to again calculate the RMSE for the prediction data, which will be referred to as the $RMSE_P$. The $RMSE_P$ was larger for all 4 equations compared with the RMSE observed for the original data. Equations 1 and 2 had the smallest increase in error, with $RMSE_p$ values of 335 and 380 kcal/kg, respectively. These values indicated that approximately 95% of the observations for a new data set would be expected to fall within 670 and 762 kcal/kg above or below the predicted value from these 2 equations, respectively. These values correspond to 24% and 28% of the average observed AME_n value for the prediction data set and are in good agreement with the expected deviation determined from R^2_{Pred}. Conversely, the $RMSE_p$ values for Equation 3 ($RMSE_P$ = 488 kcal/kg) and Equation 4 ($RMSE_P$ = 502) were substantially larger than the RMSE values for the original data. For a new data set, approximately 95% of the observations would be expected to fall within 976 (35.3% of average AME_n) and 1,004 kcal/kg (36.3% of average AME_n) above or below the predicted value from these 2

equations, respectively. The failure of Equations 3 and 4 to meet their expected prediction potential may be explained by the lower variation among samples in the original data set compared with the prediction data set. The approximate distribution of the residuals, determined using the RMSE above, is only accurate for predictions within the smallest convex set containing all of the original data points. This set is known as the regressor variable hull (**RVH**; Montgomery et al., 2012). If the observed values for the prediction data set fall outside the RVH, they are considered extrapolative.

Table 8 provides a summary of the nutrient composition of the samples used to develop Equations 1 and 2 (15 corn co-products), as well as Equations 3 and 4 (15 DDGS), in comparison with the samples used in the current study (15 DDGS). The development of Equations 1 and 2 utilized a variety of different corn co-products, including DDGS (6 samples), corn germ (2 samples), high protein DDG (2 samples), corn gluten feed, corn gluten meal, corn bran, corn germ meal, and dehulled, degermed corn (Rochell et al., 2011). The use of an array of different, yet related, corn co-products allowed for the development of an equation covering a wide range of nutrient values for the explanatory variables. Indeed, none of the validation samples used in the current study exceeded the RVH of Equations 1 and 2, despite considerable variation (Table 8). Conversely, the range of nutrients observed within the validation samples exceeded the RVH of Equations 3 and 4 for GE, starch, TDF, EE and ash. The farther an extrapolative data point is from the range of explanatory variables used in model development, the greater the potential for error in prediction. In the validation data, 12 out of 15 samples exceeded the range for starch values in Equations 3 and 4. (Table 8) Because starch has a large slope coefficient in these equations, minor changes in starch value can greatly

influence the predicted AME_n value. Therefore, the use of extrapolative data in the validation study may have contributed to the poor performance of these equations in comparison with Equations 1 and 2.

Poor predictive performance may also be attributable to other common issues in multiple linear regression, such as multicollinearity, inadequate sample size, or omitted variable bias (Montgomery et al., 2012). Multicollinearity is defined as near-linear dependence between predictor variables. When severe multicollinearity is present, the estimates of the slope coefficients become unstable and the associated standard errors become inflated. Additionally, strong dependence between variables makes it difficult to separate their effects during model selection, resulting in the omission of important variables. Related variables are considered redundant by traditional model selection processes such as stepwise, and therefore may be incorrectly excluded from the final prediction model. As a result, the erroneous predictions observed when using extrapolative data become even more pronounced (Meloun et al., 2002). The detrimental effects of multicollinearity in model selection can be partially alleviated by the application of more complex model selection techniques. Specifically, the LASSO technique constrains the sum of the absolute values of the slope estimates, alleviating the estimate inflation associated with related predictors (Tibishirani, 1996).

Utilizing LASSO to perform model selection on the original data for Equations 1 and 2 yielded the following best-fit equation: [Table 9; AME_n (kcal/kg) = 2,655 – (18.29 × NDF) + (44.14 × EE) + (0.21 × GE) – (10.91 × TDF) - (91.08 × ash); $R^2 = 0.92$; $R^2_{Adj} = 0.87$; RMSE = 182; PRESS = 662,319; $P \leq 0.0001$]. Application of this model to the validation data resulted in a $RMSE_P$ value of 321 kcal/kg, a slight improvement in

prediction accuracy compared with Equation 1 and 2. The production of a divergent model through LASSO indicates that multicollinearity within the original data may be slightly reducing the prediction efficacy of Equations 1 and 2. It must be noted that the *P*-values for the individual t-tests associated with NDF, GE, and TDF are non-significant ($P \geq 0.05$). It is possible for an important regressor to have a non-significant result for the individual *P*-value due to small sample size, measurement errors in the predictors, or multicollinearity with another predictor variable. However, considering that Equations 1 and 2 are not only more parsimonious, but also use predictors that are each individually significant, the small loss of accuracy associated with multicollinearity may be worthwhile. The application of LASSO selection to the original data for Equations 3 and 4 yielded an identical model to Equation 3, indicating that multicollinearity did not affect model selection through stepwise regression for those equations. Therefore, it is likely that the observed prediction error for Equations 3 and 4 is primarily due to the undesirable effects of extrapolation within the validation data.

In order to select a successful prediction model, a balance must be maintained between 2 conflicting objectives. A subset model should contain as few variables as possible to allow for practical application, yet must contain enough variables to produce accurate predictions. Specifically, when developing models intended to predict ME on the basis of nutrient composition, it is advantageous to select the least expensive, most accurate, and least time-consuming chemical components to assess. Analyses for proximate composition are assumed to be relatively simple and are widely utilized in the poultry industry to assess nutrient variability among ingredient sources. Therefore, the components of proximate analysis are well suited for use in practical prediction

equations. Batal and Dale (2006) reported that TME_n of DDGS could be predicted based on EE, crude fiber (**CF**), CP, and ash ($R^2 = 0.45$). The inclusion of fewer regressors reduces variance associated with the slope coefficients as well as the variance of the predicted response. However, the decrease in variance comes at the cost of introducing omitted variable bias into the estimates (Montgomery et al., 2012). Indeed, the authors acknowledged these equations are intended for use as a general guide due to the low R^2 value (Batal and Dale, 2006).

An analogous equation was developed for AME_n from proximate analysis data for 15 corn co-products (Rochell et al., 2011) and 15 DDGS samples (Meloche et al., 2013). Selection using LASSO resulted in the following best fit equation: [Table 10; AME_n (kcal/kg) = 3,673 – (121.35 × CF) + (51.29 × EE) – (121.08 × ash); $R^2 = 0.70$; $R^2_{Adj} = 0.67$; RMSE = 270; PRESS = 2,374,246; $P \leq 0.0001$.] Application of this model to the validation data resulted in a $RMSE_P$ value of 457 kcal/kg, a slight improvement in prediction accuracy compared with Equation 3 and 4. For a new data set, approximately 95% of the observations would be expected to fall within 914 kcal/kg (33.1% of average AME_n) above or below the predicted value from this equation. Although this equation does not perform as well in prediction as Equations 1 and 2, it utilizes more commonly available measures of chemical content, and thus may be more applicable in practice. Additionally, this equation requires fewer chemical components as regressors than the equation for TME_n reported by Batal and Dale (2006). Although it is not possible to directly compare the prediction accuracy of these 2 equations based on the available data, the lower R^2 value for the Batal and Dale (2006) equation indicates a limitation on predictive capacity.

In conclusion, this validation study indicated that greater caution should be taken when interpreting model selection data, particularly when R^2 is used as the primary measure of fit. Prior to practical application, a thorough and explicit explanation of the consequences of inherent model error in terms of the dependent variable is critical to ensure proper interpretation. Application of any model to extrapolative data may reduce the reliability of all measures of internal fit, and may result in residuals which exceed the maximum allowable error in diet formulation. Therefore, it is suggested that rigorous analysis and validation of prediction equations developed hereafter is warranted to establish the risk of error associated with practical application, and to better communicate these risks to the end user.

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Table 4.1 Ingredient and calculated nutrient composition of the basal diets

Item	Amount	
	Trials 1 and 2	Trial 3
Ingredient (%, "as-is")		
Corn	57.01	62.92
Soybean meal (48% CP)	36.96	32.88
Poultry oil	2.27	---
Dicalcium phosphate	2.06	1.72
Calcium carbonate	0.52	1.13
DL-methionine	0.27	0.28
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
Sodium chloride	0.23	0.52
L-Lys-HCl	0.09	---
Salinomycin[3]	0.05	0.05
L-Thr	0.04	---
Calculated nutrient composition (%)[4]		
AME_n (kcal/kg)	3,025	2,393
CP	21.78	20.18
Digestible Lys	1.13	0.97
Digestible Met	0.58	0.57
Digestible TSAA	0.85	0.81
Digestible Thr	0.74	0.64
Digestible Val	0.88	0.82
Digestible Ile	0.81	0.75
Digestible Arg	1.32	1.21
Digestible Trp	0.22	0.20
Ca	0.97	0.90
Non-phytate P	0.46	0.44
Na	0.21	0.23

[1]Vitamin 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 B_{12} (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.
[2]Mineral 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.
[3]BioCox 60 provided 60 g/907 kg of salinomycin (Alpharma, Fort Lee, NJ).
[4]Values 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, 2004).

Table 4.2 Methods of analysis used to determine feed composition of 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)
Dry matter	AOAC official method 934.01
Crude protein[3]	AOAC official method 990.3
Starch	AACC approved method 76-13. Modified: Starch Assay Kit (product code STA-20, Sigma, St. Louis, MO)
Total dietary fiber[4]	AOAC official method 985.20 (A-C)
Neutral detergent fiber	Holst (1973)
Acid detergent fiber	AOAC official method 973.18 (A-D)
Crude Fiber	AOAC official method 978.10
Ether extract	AOAC official method 920.39 (A) petroleum ether
Ash	AOAC official method 942.05

[1]Unless otherwise noted, all methods of analysis were determined by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO).
[2]AOAC = Association of Official Analytical Chemists; AACC = American Association of Cereal Chemists.
[3]Determined by Auburn University Laboratory (Auburn, AL).
[4]Determined by Eurofins Scientific, Inc. (Des Moines, IA).

Table 4.3 Analyzed composition of 15 corn distillers dried grains with solubles sources[1]

Item (%)	1	2	3	4	5	6	7	8
Gross energy (kcal/kg)	5,254	5,139	5,061	5,009	4,978	5,194	4,841	5,148
Moisture	11.34	11.12	10.66	10.20	9.48	8.70	12.25	11.32
CP	29.65	32.00	31.59	30.58	32.21	29.83	34.21	34.09
Starch	2.50	2.33	3.82	4.93	4.40	4.68	5.64	3.98
Total dietary fiber	31.47	31.62	31.12	32.41	32.81	32.10	29.30	30.50
Neutral detergent fiber	38.27	38.49	39.58	30.95	31.05	27.84	31.43	37.12
Acid detergent fiber	11.48	12.14	11.60	8.90	8.55	8.55	8.81	13.22
Hemicellulose[2]	26.79	26.35	27.98	22.05	22.50	19.29	22.62	23.90
Crude fiber	9.49	9.37	8.59	8.25	8.59	9.12	8.80	9.29
Ether extract	13.34	10.41	9.11	8.01	6.99	11.38	4.98	8.16
Ash	4.79	4.71	5.38	5.63	5.51	5.53	5.63	5.13

Item (%)	9	10	11	12	13	14	15
Gross energy (kcal/kg)	4,951	5,254	5,098	4,884	4,934	5,052	5,154
Moisture	11.45	12.11	10.86	10.58	10.33	10.60	9.25
CP	32.69	32.96	28.40	32.32	30.30	28.48	30.69
Starch	8.08	4.03	10.01	5.99	4.94	5.85	6.69
Total dietary fiber	29.15	26.50	28.05	31.70	31.40	36.60	33.58
Neutral detergent fiber	43.78	32.83	38.12	34.57	32.92	37.14	32.96
Acid detergent fiber	14.83	12.13	10.70	9.35	9.16	9.66	8.21
Hemicellulose[2]	28.96	20.70	27.42	25.22	23.77	27.49	24.75
Crude fiber	9.95	8.11	8.64	11.60	9.95	9.39	8.77
Ether extract	10.72	14.29	12.04	5.86	6.06	8.80	11.59
Ash	4.66	4.61	4.58	5.63	5.43	5.35	5.03

[1]All analyses performed in duplicate (DM-basis). Values reported as a percentage unless noted otherwise.
[2]Hemicellulose was calculated as neutral detergent fiber minus acid detergent fiber.

Table 4.4 Feed intake and BW gain of broiler chicks fed diets containing 15% distillers dried grains with solubles

Treatment	Trial[1]	Feed intake (g/bird)	BW gain (g/bird)
Control[2]	1	424	288
1	1	398	289
2	1	408	290
3	1	409	290
4	1	400	287
5	1	395	285
6	1	398	282
SEM		34.8	28.4
Control[2]	2	599[a]	413[ab]
7	2	592[a]	444[a]
8	2	561[ab]	409[b]
9	2	551[b]	414[ab]
10	2	552[b]	413[ab]
11	2	564[ab]	421[ab]
12	2	585[ab]	445[a]
SEM		39.0	32.7
Control[2]	3	832[x]	---
13	3	765[y]	---
14	3	759[y]	---
15	3	757[y]	---
SEM		43.2	

[1]Trial 1 was conducted from 10 to 18 d of age. Trial 2 was conducted from 13 to 21 d of age. Trial 3 was conducted from 26 to 30 d of age.
[2]Control diet contained 15% dextrose.
[a-b]Means not sharing a common superscript within a column differ significantly ($P < 0.05$). Observed means for 13 to 21 d feed intake and BW gain are based on 13 replicate pen means per treatment (7 birds per pen).
[x-y]Means not sharing a common superscript within a column differ significantly ($P < 0.05$). Observed means for 26 to 30 d feed intake are based on 12 replicate pen means per treatment (10 birds per pen).

Table 4.5 Determined gross energy (GE) and AME_n of corn distillers dried grains with solubles (DDGS) samples in broiler chicks[1]

DDGS		GE	AME_n	
sample	n[2]	kcal/kg	kcal/kg	% of GE
1	13	5,254	3,634[a]	69.16
2	13	5,139	2,553[c]	49.68
3	13	5,061	2,869[bc]	56.68
4	13	5,009	2,781[bc]	55.52
5	13	4,978	2,523[c]	50.69
6	13	5,194	2,535[c]	48.82
7	13	4,841	2,903[bc]	59.96
8	13	5,148	2,640[bc]	51.29
9	13	4,951	2,461[cd]	49.70
10	13	5,254	3,120[ab]	59.38
11	13	5,098	3,111[b]	61.02
12	13	4,884	2,581[c]	52.84
13	12	4,934	1,975[d]	40.03
14	12	5,052	2,644[bc]	52.33
15	12	5,154	3,137[ab]	60.86
SEM			106	1.80

[1] Gross energy and AME_n are expressed as kilocalories per kilogram of DM. Gross energy was determined in duplicate using an adiabatic oxygen bomb calorimeter (Parr Instruments, Moline, IA). Apparent metabolizable energy was determined by a 48-h excreta collection following a 6-d adaptation period.
[2] Observed means for AME_n are based on n pen means per treatment (Treatments 1 to 12: 7 birds per pen; Treatments 13 to 15: 10 birds per pen).
[a-d]Means not sharing a common superscript within a column differ significantly ($P < 0.05$).

Table 4.6 Prediction equations for AME_n of corn co-products developed by Rochell et al. (2011) and Meloche et al. (2013)

	AME_n equation[1,2]	Measures of internal fit[3]						
		P	R^2	R^2_{Adj}	RMSE	C(p)	PRESS	$R^2_{Pred.}$
Equation 1 Rochell et al., 2011	AME_n, kcal/kg = 3,517 + (46.02 × EE) – (82.7 × Ash) – (33.27 × HC)	≤ 0.01	0.89	0.86	191	-2.57	939,667	0.74
Equation 2 Rochell et al., 2011	AME_n, kcal/kg = (– 30.19 × NDF) + (0.81 × GE, kcal/kg) – (12.26 × CP)	≤ 0.01	0.87	0.84	196	–	776,353	0.78
Equation 3 Meloche et al., 2013	AME_n, kcal/kg = -12,282 + (2.60 × GE, kcal/kg) + (89.75 × CP) + (125.80 × starch) – (40.67 × TDF)	≤ 0.01	0.90	0.86	99	2.58	199,819	0.80
Equation 4 Meloche et al., 2013	AME_n, kcal/kg = -14,322 + (2.69 × GE, kcal/kg) + (117.8 × CP) + (149.41 × starch) – (18.30 × NDF)	≤ 0.01	0.92	0.88	90	1.35	227,477	0.71

[1]All regressors expressed on a percent DM-basis, unless otherwise noted.
[2]EE = ether extract; HC = hemicellulose; NDF = neutral detergent fiber; GE = gross energy (kcal/kg); TDF = total dietary fiber.
[3]*P* is the *P*-value for the overall F-test associated with each regression equation; R^2 is the multiple coefficient of determination; R^2_{Adj} is the adjusted multiple coefficient of determination; RMSE is the root of the mean square error; C(p) is the Mallows statistic; PRESS is the prediction error sum of squares; and $R^2_{Pred.}$ is the prediction multiple coefficient of determination.

Table 4.7 Validation of AME_n prediction equations on an external set of 15 corn distillers dried grains with solubles samples

DDGS[1]	AME_n (kcal/kg)	Equation 1[2]	Residual	Equation 2[2]	Residual	Equation 3[3]	Residual	Equation 4[3]	Residual
1	3,634	2,845	-789	2,737	-897	3,076	-558	2,957	-677
2	2,553	2,731	178	2,609	55	2,961	408	2,894	340
3	2,869	2,562	-307	2,517	-351	2,929	60	2,839	-30
4	2,781	2,688	-93	2,748	-33	2,789	8	2,903	122
5	2,523	2,636	113	2,700	177	2,772	249	2,930	406
6	2,535	2,943	408	3,001	465	3,183	648	3,331	796
7	2,903	2,530	-373	2,553	-349	2,896	-7	2,975	72
8	2,640	2,675	34	2,632	-9	3,426	786	3,435	795
9	2,461	2,662	202	2,288	-173	3,356	896	3,229	769
10	3,120	3,106	-14	2,861	-259	3,768	648	3,673	553
11	3,111	2,781	-330	2,630	-481	3,642	531	3,516	405
12	2,581	2,483	-97	2,516	-65	2,782	201	2,862	281
13	1,975	2,557	582	2,631	656	2,612	637	2,634	659
14	2,644	2,566	-78	2,622	-22	2,660	16	2,799	155
15	3,137	2,812	-325	2,804	-333	3,351	214	3,533	396
	RMSE[4]	191		196		99		90	
	$RMSE_P$[5]	335		380		488		502	

[1]DDGS = distillers dried grains with solubles.
[2]Rochell, et al., 2011.
[3]Meloche et al., 2013.
[4]RMSE = root mean square error of the equation as applied to the original data.
[5]$RMSE_P$ = root mean square error of the equation as applied to the validation data in the current study.

Table 4.8 Summary of nutrient composition data for corn co-products used to develop prediction equations for AME_n

Study[2]	Summary statistics[3]	Component[1]									
		AME_n (kcal/kg)	GE (kcal/kg)	CP	Starch	TDF	NDF	ADF	CF	EE	Ash
Rochell et al., 2011 (n = 15)	Minimum	1,746	4,397	8.3	0.5	2.6	4.3	0.5	0.6	0.2	0.5
	Maximum	3,495	5,811	66.3	88.0	47.8	61.1	25.4	10.7	18.5	6.8
	Average	2,780	5,163	30.3	15.8	29.4	32.8	10.3	6.5	7.8	4.2
	CV (%)	18.2	7.4	51.7	136.3	40.1	45.1	55.9	45.1	73.4	44.3
Meloche et al., 2013 (n = 15)	Minimum	1,869	4,678	26.5	0.8	28.9	27.0	7.7	8.7	3.2	4.3
	Maximum	2,824	5,167	34.7	3.9	37.8	51.0	15.8	12.1	13.2	5.3
	Average	2,309	4,998	30.3	2.3	33.4	35.7	11.9	10.2	9.5	4.9
	CV (%)	12.0	2.3	7.2	49.6	7.4	18.4	19.9	10.0	29.9	6.5
Current Study (n = 15)	Minimum	1,975	4,841	28.4	2.3	26.5	27.8	8.2	8.1	5.0	4.6
	Maximum	3,634	5,254	34.2	10.0	36.6	43.8	14.8	11.6	14.3	5.6
	Average	2,764	5,064	31.3	5.2	31.2	35.1	10.5	9.2	9.4	5.2
	CV (%)	14.0	2.6	5.8	38.6	7.7	12.0	19.0	9.0	29.8	7.9

[1]Nutrient values expressed on % DM-basis, unless otherwise noted; GE = gross energy; TDF = total dietary fiber; NDF = neutral detergent fiber; ADF =acid detergent fiber; EE = ether extract.
[2]Rochell et al. (2011) data included DDGS (n = 6), corn germ (n = 2), high protein DDGS (n = 2), corn gluten feed, corn gluten meal, corn bran, corn germ meal, and dehulled, degermed corn; Meloche et al. (2013) data included 15 samples of DDGS; Current study includes 15 samples of DDGS.
[3]CV = coefficient of variation, (%).

Table 4.9 Selection of a regression model for AME_n (kcal/kg) based on the nutrient composition of 15 corn co-products using the least absolute shrinkage and selection operator (LASSO) technique[1]

Regression coefficients[2]								Fit Criteria[3]				
	Intercept	NDF	EE	GE	TDF	Ash	Starch	CP	$P_{Overall}$	R^2	R^2_{Adj}	RMSE	C(p)
	2,780								1.00	0.00	0.00	490	65.5
	3,358	-17.47							< 0.01	0.58	0.55	317	22.0
	3,378	-18.91	3.51						0.23	0.63	0.57	298	20.0
	3,209	-19.91	4.92	0.04					0.28	0.67	0.58	282	19.0
	2,712	-17.45	14.24	0.15	-8.09				0.03	0.79	0.71	223	11.3
	2,668	-18.10	37.46	0.20	-10.28	-70.74			< 0.01	0.91	0.86	147	4.0
	502	-20.70	32.43	0.55	-0.75	-51.50	7.51		0.31	0.92	0.86	137	5.1
	449	-20.83	32.97	0.55	0.00	-50.76	8.02	0.54	0.88	0.92	0.84	137	7.1
	522	-20.08	36.49	0.51	---	-52.39	8.80	2.24	1.00	0.92	0.87	136	5.1
Selected model[4]									$P_{Overall}$	R^2	R^2_{Adj}	RMSE	R^2_{Pred}
AME_n =	2,656	-18.29	44.14	0.21	-10.91	-91.08	---	---	<0.01	0.92	0.87	182	0.82
SE	774	10.59	12.53	0.15	13.31	36.96	---	---					
P	< 0.01	0.12	<0.01	0.19	0.43	0.04	---	--					

[1]Nutrient composition data for corn co-products adapted from Rochell et al. (2011).
[2] NDF = neutral detergent fiber; EE = ether extract; GE = gross energy; TDF= total dietary fiber.
[3] *P* is the *P*-value for the individual t-test associated with each added predictor. $P_{overall}$ is the *P*-value for the overall F-test associated with the regression equation; R^2_{Adj} is the adjusted coefficient of determination; RMSE = root of the mean square error; C(p) is the Mallows statistic; and $R^2_{Pred.}$ is the prediction multiple coefficient of determination.
[4]Parameter estimates and statistical measures for the selected model were generated using PROC REG to model the set of predictors determined to optimize the fit criteria in LASSO.

Table 4.10 Selection of a regression model for AME_n (kcal/kg) based on the proximate composition of 30 corn co-products using the least absolute shrinkage and selection operator (LASSO) technique[1]

Regression coefficients[2]						Fit criteria[3]				
	Intercept	CF	EE	Ash	CP	$P_{Overall}$	R^2	R^2_{Adj}	RMSE	C(p)
	2,558					1.00	0.00	0.00	461	59.69
	3,232	-80.18				< 0.01	0.47	0.45	335	19.61
	3,273	-94.74	9.35			0.05	0.55	0.51	310	15.33
	3,507	-110.30	33.87	-70.79		< 0.01	0.68	0.64	262	6.28
	3,499	-124.65	55.17	-116.70	4.91	0.08	0.72	0.67	246	5.00
Selected model[4]						$P_{Overall}$	R^2	R^2_{Adj}	RMSE	R^2_{Pred}
AME_n =	3,673	-121.35	51.29	-121.08	---	< 0.01	0.70	0.67	270	0.62
SE	209	18.51	13.75	45.26	---					
P	< 0.01	< 0.01	< 0.01	0.01	---					

[1]Nutrient composition data for corn co-products adapted from Rochell et al. (2011) and Meloche et al. (2013).
[2] CF = crude fiber; EE = ether extract.
[3] *P* is the *P*-value for the individual t-test associated with each added predictor. $P_{overall}$ is the *P*-value for the overall F-test associated with the final regression equation; R^2_{Adj} is the adjusted coefficient of determination; RMSE = root of the mean square error; C(p) is the Mallows statistic; and $R^2_{Pred.}$ is the prediction multiple coefficient of determination.
[4]Parameter estimates and statistical measures for selected model generated using PROC REG to model the set of predictors determined to optimize the fit criteria in LASSO.

V. CONCLUSIONS

The rapid increase in corn and soybean meal prices over the past decade has resulted in a concomitant increase in the cost of live production. The inclusion of alternative feed ingredients, such as DDGS, may provide significant cost-savings to poultry producers. Approximately 80% of ethanol producers in the United States currently employ post-fermentation oil extraction technology. The impact of oil extraction on the energy content and nutrient variability of the resultant DDGS is a cause for concern among animal nutritionists. Inaccurate estimates of energy content may negatively impact bird performance, and may also substantially increase diet cost. In order to capitalize on lower cost feed ingredients, nutritionists require accurate assessments of nutrient content. The research presented herein evaluated the potential use of prediction equations that estimate the energy content of DDGS based on nutrient composition as an inexpensive, rapid, and accurate alternative to time-consuming and costly *in vivo* energy determination assays.

The first experiment evaluated the AME_n content of 15 DDGS samples in order to develop prediction equations based on chemical composition. Stepwise selection resulted in a model including GE, TDF, CP, and starch. Removal of TDF from the variable selection pool resulted in a model including GE, NDF, CP, and starch. These results demonstrated the influence of fiber fractions on the AME_n content of DDGS. The high R^2 values of these equations indicated that a strong relationship existed between chemical

composition and AME_n content for the 15 samples used in model development. However, these results did not provide adequate information to determine the predictive performance of these equations on an external dataset.

The second experiment was designed to evaluate the AME_n content of 15 additional DDGS samples for the purpose of validating the equations developed in the first experiment, as well as the equations of Rochell et al. (2011). Each of the equations had high R^2_{pred} values, and thus was expected to have high prediction performance. The equations of Rochell et al. (2011) had a prediction RMSE that corresponded well with the expectation from R^2_{pred}. However, the equations developed in Experiment 1 of the current study had a larger prediction RMSE values than expected from the R^2_{pred}. Large differences in expected and actual prediction performance may preclude the effective use of a model in practical application.

These prediction equations for AME_n of DDGS have the potential to provide general estimates of energy value; however, a thorough assessment of the associated error of prediction is necessary prior to practical application. Although common measures of fit such as R^2, R^2_{adj}, and R^2_{pred} can be used to ascertain a rough estimate of expected prediction performance, these values may be highly inaccurate if issues such as multicollinearity, extrapolation, or sampling error were present during model selection. Furthermore, a small change in the fit of a model may correspond with a large change in practical efficacy when expressed in kcal/kg of expected prediction error. Therefore, caution must be exercised during the development and analysis of prediction models in order to accurately assess the risks associated with practical application, and to effectively communicate any inherent limitations to the end user of the model.