DEVELOPMENT AND VALIDATION OF PREDICTION EQUATIONS FOR THE METABOLIZABLE ENERGY CONTENT OF DISTILLERS DRIED GRAINS WITH SOLUBLES FROM DIFFERENT SOURCES FOR PIGS

BY

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DISSERTATION

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ABSTRACT: Three experiments were conducted to determine the apparent DE and ME of samples for distillers dried grains with solubles (DDGS) from 17 different sources, either unground (665.8 ± 284.4 μm) or ground to a common particle size (337.5 ± 39.0 μm). The experiments were conducted simultaneously using an incomplete block design to determine the apparent DE and ME of samples of DDGS as follows: Exp. 1 used 18 dietary treatments, a corn-based control diet (common to all experiments) and 17 diets composed of each of 17 DDGS samples unground. Exp. 2 used 17 dietary treatments, using 15 DDGS samples ground and two unground (from Exp. 1); Exp. 3 used 5 dietary treatments using one source with 5 different particle sizes (1,557, 1,180, 890, 560, and 351 μm). All results are expressed on a DM basis unless otherwise noted. Results for Exp. 1 showed that the mean values for DE and ME of unground DDGS samples were 3,842 ± 116.3, and 3,596 ± 108.4 kcal/kg, respectively. For Exp. 2, mean values for DE and ME of ground DDGS samples were 3,954 ± 117.7, and 3,719 ± 122.5 kcal/kg, respectively. In addition, data from Exp. 1 and 2 were combined to evaluate the effect of DDGS source and particle size, and the two-way interaction. There were no important interactions, suggesting that the effect of particle size reduction was constant across DDGS samples. There was an effect ($P < 0.01$) of DDGS source on DE and ME, in addition to an effect of particle size, with the ground DDGS samples having 134 and 144 kcal/kg greater ($P < 0.01$) DE and ME, respectively, compared to the unground DDGS samples. In Exp. 3, reducing particle size in a single sample of DDGS resulted in no difference in DE, however, grinding the sample to the lowest particle size (351 μm) resulted in a 234 kcal/kg increase ($P < 0.05$) in ME, compared to particle sizes of 560, 890, 1,180, and 1,557 μm. The data generated in these experiments was used, along with the chemical composition (CP, crude fat, crude fiber, ADF, NDF, ash, and starch) of each DDGS sample (analyzed by 2 laboratories), and GE and particle

size to develop regression equations to predict the ME of DDGS based on chemical composition and particle size. Regression equations to predict the ME of DDGS were developed using the PROC REG procedure of SAS. A series of equations were developed with those producing the greatest $\bar{R}^2$ values being selected. For Laboratories 1 and 2, $\bar{R}^2$ values were maximized using a 4-variable equation, however, different chemical components were included in the equation for each of the laboratories (crude fiber, ADF, NDF, and GE for the equation based on Laboratory 1; CP, crude fat, NDF, and starch for Laboratory 2; with $\bar{R}^2$ values 0.79 and 0.75, respectively). For validation purposes a separate experiment was conducted to determine the apparent ME of DDGS samples from 4 sources to check the accuracy of the selected equations. The root mean square error of prediction (RMSEP) and mean percent bias were used as criteria to evaluate the accuracy of the equations. The major finding was that the most accurate prediction of ME of DGGS was achieved when the same analytical laboratory was used both for the chemical analysis of the original samples used to develop the prediction equations and also for the analysis of the samples being evaluated (i.e., the samples for which ME was being predicted). This research, also, highlighted the need to develop standard procedures for the development and validation of equations to predict the energy concentration of DDGS and other ingredients, which is essential if users of equations are to have accurate predictions of energy value of feedstuffs.

I would like to dedicate the work in this dissertation to my wife Gloria and daughter Loree Adhelle, and to my family; without their love and support this would not have been possible.

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CHAPTER 1

INTRODUCTION

Historically, the majority of pigs in the Midwest of the U. S. have been fed diets based on corn and soybean meal with a limited amount of alternative ingredients. However, with the expansion of biofuels and the increased demand for corn for ethanol production, there was a marked increase in corn prices which elevated feed costs affecting profitability of swine producers (Cromwell et al., 2011).

With the increased production of ethanol, distillers dried grains with solubles (**DDGS**), a co-product from ethanol production, became available as an alternative ingredient in diets for pigs (Lumpkins et al., 2004). From 2008 to date, in the U. S., there has been a consistent increase in the utilization of DDGS in swine diets, from 0.7 to an estimated of 1.09 million metric tons for 2014 (Wisner, 2013). The official AAFCO definition of DDGS is "The product obtained after removal of ethyl alcohol by distillation from the yeast fermentation of a grain or grain mixture by condensing and drying at least three quarters of the solids of the resultant whole stillage by methods employed in the grain distilling industry". Since DDGS is a coproduct of a process to produce ethanol, there has been little incentive to standardize the nutrient content and quality of DDGS in the U. S. ethanol industry (Shurson et al., 2004).

With an increase in DDGS utilization, there was also an increase in research conducted to characterize its chemical composition and nutritional value, as this information is needed by nutritionists for accurate and efficient diet formulation for pigs. Therefore, the second chapter of this dissertation provides a review of the literature on published research on the utilization of DDGS in swine diets, in addition to summarizing the available literature on the chemical composition, and DE and ME estimates for DDGS. Furthermore, available data is presented on

prediction equations for DE and ME of DDGS based on chemical composition of DDGS; however, when these equations are used, they give different estimates for the same sample, which creates confusion as to what energy estimates to use.

Consequently, the research presented herein was conducted with the overall objective of developing prediction equations to accurately estimate the ME of DDGS. Chapter 3 presents data from 3 experiments on the measurement of ME of DDGS from different sources: unground, ground to a common particle size, and on one sample of DDGS at 5 different particle sizes. These data are used in the following chapter for development of prediction equations. Chapter 4 contains data on the development of multiple linear regression equations to predict the ME of DDGS based on chemical composition of the samples of DDGS used in Chapter 3 measured by two different laboratories. To complete the regression model-building process, certain equations were selected for validation purposes. Chapter 5 contains data on the measurement of ME of DDGS samples different to those used in Chapter 3. These samples were used for estimation of ME using the selected equations and a comparison of the measured and estimated values was conducted.

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CHAPTER 2

LITERATURE REVIEW

Distillers Dried Grains with Solubles Utilization in Swine

With the explosive growth in the production of ethanol from corn in the U.S. (Shurson and Noll, 2005), an increasing amount of the major co-product, distillers dried drains with solubles (**DDGS**), has become available for use in swine diets. The first step when processing cereal grains to produce ethanol is grinding to reduce particle size and then mixing with water and enzymes to produce a slurry. The starch in the slurry is then liquefied, saccharified, and fermented to produce ethanol, which is removed by distillation. The remaining non–fermentables are dewatered and dried to produce DDGS (Singh et al., 2002). For each 100 kg of corn fermented in a dry-grind ethanol plant, approximately 36 liters of ethanol, 32 kg of DDGS and 32 kg of carbon dioxide are produced (Shurson and Noll, 2005). It has been estimated that DDGS usage in swine has increased from 0.7 million metric tons in 2005 to more than double in 2009 with 1.8 million metric tons being fed to pigs (Wisner, 2010). Research with distillers co-products fed to swine has been conducted over the last 60 years, with an increasing amount more recently as a result of the increasing production and utilization of these co-products in the swine industry.

A literature review of research investigating the use of DDGS in diets of pigs has been carried out by Stein and Shurson (2009). In weanling pigs, 10 studies have been published with corn DDGS included at up to 30% of the diet with the following results:

- No changes in ADG were reported in any of the studies.
- ADFI was reduced in 2 studies and remained unchanged in 8.
- Gain:feed was increased in 5 studies, but not changed in the other 5 studies

Similarly, a summary of 25 studies where corn DDGS was fed at up to 40% of the diet to growing-finishing pigs was reported with the following results:

- ADG was reduced in 6 studies, 1 experiment reported an increase in ADG, with the other 18 studies reporting that growth rate remained unchanged relative to control diets with no DDGS.
- ADFI was reported in 23 studies, of which 2 indicated an increase, with 6 and 15 studies showing a reduction or no change, respectively, compared to diets without DDGS.
- Gain:feed was improved in 4 studies, 5 studies indicated a reduction, while 16 studies reported no change compared to a control diet without DDGS.

In general, this literature review showed that when DDGS is included in diets for weanling and growing-finishing pigs, comparable results to diets without DDGS can be obtained.

However, to fully exploit the utilization of DDGS, reliable values for the nutrient composition and digestibility need to be established to allow accurate diet formulation; several studies have been conducted to address this issue. Chemical composition (Spiehs et al., 2002), energy values and digestibility (Stein et al., 2006; Pedersen et al., 2007), and CP and AA digestibility (Stein et al., 2006; Fastinger and Mahan, 2006; Pahm et al., 2008) have been reported for corn DDGS. Moreover, the physical characteristics of DDGS, such as particle size and bulk density have been evaluated (Liu, 2008; Ileleji and Rosentrater, 2008). These results are discussed in the subsequent sections of this literature review.

Variation in the Chemical Composition of Distillers Dried Grains with Solubles.

Grains can be converted into ethanol by either wet milling or dry grind processing (Singh et al., 2001). In wet milling, the corn kernel is fractionated into different components, resulting in several co-products. In contrast, during dry-grind processing of grains, such as corn, the kernel is not fractionated and only one co-product, DDGS is generated (Rausch et al., 2005).

The starch in the corn kernel is then fermented to produce alcohol and carbon dioxide (Singh et al., 2002; Spiehs et al., 2002). Thus, removal of starch through fermentation, although not complete, increases the concentration of the other nutrients in the remaining co-product, by approximately threefold compared to the original grain (Spiehs et al., 2002). However, there is variation in the ethanol production process that results in considerable variation in the composition DDGS (Belyea et al., 2010).

The causes of this variation have been documented and the factors that contribute to create this variation have been categorized into the following: the raw material used, in this case corn, and processing factors that lead to the production of ethanol from corn (Olentine, 1986). The characteristics inherent to the corn that contribute to the variability in the chemical composition of DDGS are the corn variety and quality of the grain, which is a result of the conditions of the soil, the weather, and production methods, among others factors.

Moreover, variability is created when the corn is subject to the ethanol production processes, which can vary from plant to plant, and even from batch to batch within the same plant (Belyea et al., 2010). Other important factors that affect the variability in the chemical composition in DDGS are the ethanol production process conditions (Kingsly et al., 2010; Ileleji and Rosentrater, 2008). For instance, the degree of fermentation, the mixture ratio of the grains fraction and condensed distiller's solubles, and the differences in drying time and temperatures, among others factors can contribute to variation (Belyea et al., 1989; Spiehs et al. 2002; Carpenter, 1970; Olentine, 1986; US Grains Council, 2007). A significant amount of this variation can be attributed to the technology used in the ethanol plant, as the chemical composition can vary when comparing old vs. new generation (ethanol plants built after the mid 1990's; Spiehs et al., 2002).

Research by Rosentrater (2006) and Bhadra et al. (2007) indicated that DDGS is widely variable in its physical properties within and between ethanol plants (Table 2.1). This heterogeneity in physical properties could lead to differences in chemical composition among samples, which results from particle segregation caused by particle size differences and to a lesser extent by variations in density (Clementson et al., 2009). Thus, the end product will be variable in physical, chemical, and nutritional composition, and digestibility of nutrients (Cromwell et al., 1993; Belyea et al., 1989, 2004; Stein et al., 2006; Pedersen et al., 2007).

Besides the variation in the chemical composition of DDGS, more variation can be introduced from the analytical results reported by the laboratory. There is evidence that within laboratory variation can produce variable results. According to Rhodes (1977) variability can be expected to occur in any or all of the following steps within a given laboratory:

1. Sampling and sample preparation
2. Reagents used in the analysis
3. Calibration materials or devices
4. Environmental factors
5. Laboratory technicians
6. Instruments used

Furthermore, the use of different reference methodologies by the same laboratory for the same chemical component can yield different results, in addition to between-laboratory variability even when the same methodology is used. The results from a report from the AFIA (2007) showed that within- and between-laboratory variation exists for analysis of chemical composition of DDGS. However, only data for moisture, CP, crude fat and crude fiber were reported (Table 2.2).

Understanding the existing variation is critical for an efficient use of DDGS, in addition to having access to reliable values for the nutrient concentration in DDGS to ensure that accurate values are used for precise diet formulation. There are a number of published reports for the chemical composition of DDGS, a summary of which is presented in Table 2.3. It can be seen from these reports, that the chemical composition of DDGS is variable. For example, the starch content reported in 4 studies (Stein et al., 2006; Pedersen et al., 2007; Anderson et al., 2012; Kerr and Shurson, 2013) had a range of 2.3 to 8.2% with a range in CV of 14.0 to 59.8%, which reflects that the degree of fermentation in the ethanol production process is not consistent. Other chemical components, such as NDF content (Table 2.3) also showed wide variation, with a range of 27.6 to 51%, with a range of CV within experiment of 4.8 to 14.3, while CP, crude fat, crude fiber ADF, and ash had a maximum within experiment CV of 6.4, 23.3, 8.7, 28.4 and 14.7%, respectively. The major conclusion from this summary is that DDGS produced in the US is variable in its chemical composition. Thus, formulation of diets based on average nutrient composition, can result in diets that could either have insufficient nutrients such that animal performance can be compromised, or, on the other hand, excess nutrients which is unnecessary, expensive, and can have a negative environmental impact (Fabiosa, 2008; Belyea et al., 2004).

Variation in Energy and Dry Matter Digestibility of Distillers Dried Grains with Solubles

It has been established that the GE of DDGS is greater than that of corn, which is shown in the data summary presented in Table 2.4 with the average GE for DDGS (from several reports) being 19.2% greater than that of corn (NRC, 2012). When DDGS is produced there is a substantial increase in the crude fat and CP concentration compared to corn, which increases the GE of DDGS, despite the fact that the fiber concentration is also increased substantially.

However, compared to corn, the high fiber level in DDGS can negatively impact nutrient digestibility (Laplace et al., 1989; Stein and Shurson, 2009).

Previous research has shown that the DM digestibility of DDGS in pigs is less compared (Table 2.4) to that of corn. Stein et al. (2006), reported the DM digestibility of 10 sources of DDGS, was on average of 68.3% compared to 87.6% for the corn sample measured in the same experiment (Table 2.4). Similarly, studies from Feoli (2008), and Stein et al. (2009) reported the average DM digestibility of 2 and 4 sources, respectively, of DDGS to have a DM digestibility of 78.9 and 75.1%, respectively, whereas the corn samples within each experiment had a DM digestibility of 87.4 and 93.0%, respectively.

Likewise, the digestibility of energy of DDGS for pigs has been shown to be less compared to that of corn, with several studies reporting the digestibility of DDGS having a range from 65.6 to 78.7%, whereas for corn, it has been reported to have a range from 85.1 to 92.3%, with an average difference of 14.7 percentage units less for DDGS (Table 2.4). Differences in the digestibility of DM and energy can be attributed to differences in the dietary fiber content of DDGS relative to corn, with DDGS having approximately 3 times more dietary fiber than corn (Stein and Shurson, 2009).

Dietary fiber has a lower digestibility compared to other nutrients, with estimates of digestibility of dietary fiber generally ranging from 40 to 60%, whereas the digestibility of protein, fat, sugars and starch is above 80% (Noblet and Le Goff, 2001). The proposed mechanisms for the reduced nutrient digestibility of high fiber ingredients relative to corn, such as DDGS, are increased endogenous nutrient losses and rate of passage of digesta (Lenis et al., 1996; Lupton and Turner, 2000; Grieshop et al., 2001; Souffrant, 2001; Schulze et al., 1995). In addition, the efficiency of utilization of dietary fiber by pigs is lower compared to that of other

nutrients. When pigs are fed high fiber containing diets, the efficiency of energy utilization is decreased by between 9 to 22% due to a reduced absorption of glucose and nitrogen from the small intestine (Giusi-Perier et al., 1989; Noblet et al., 1994). This is because, in the pig, dietary fiber is resistant to digestion by mammalian enzymes in the small intestine. However, starch digestion is a more efficient process, and for most cereal grains, its digestion in the small intestine is greater than 95% (Bach Knudsen, 2001). Fiber reaching the hindgut of the pig, becomes available for partial or complete bacterial fermentation in the hindgut (Bindelle et al., 2008), with the end products being short chain fatty acids, which make a relatively small and variable contribution (between 5 and 28%) to the energy balance of the animal (Dierick et al., 1989; Farrell and Johnson, 1972; Rérat et al., 1987; Imoto and Namioka, 1978; Yen et al., 1991; Kass et al., 1980), due in part to energy losses due to gas production, and the heat of the fermentation associated with the process (Bindelle et al. 2008).

Energy Estimates for Distillers Dried Grains with Solubles in Swine.

The published estimates in the literature for the energy concentration of corn DDGS show substantial variation (Table 2.4). The wide variability in the chemical composition of DDGS (Table 2.3) explains part of the variation in the energy values reported. Moreover, different methodologies have been used to determine the energy concentration in DDGS, which may explain part of the differences in the energy estimates (Table 2.4).

Studies evaluating the DE and ME of DDGS have used different techniques to measure the energy concentration. Some studies have used metabolism studies with the total collection of feces and urine (Stein et al., 2005; Pedersen et al., 2007; Anderson, 2009; Stein et al., 2009; Kerr and Shurson, 2013), however, due to the duration, costs, labor and potential animal welfare concerns when using this type of experiment, other methodologies have also been used

(Anderson, 2009). For example, indigestible markers have been used in some studies, which eliminates the need for total collection of feces, although this only will provide estimates for the DE and not the ME (Stein et al., 2006; Feoli, 2008). Other methodologies that have been used include the use of growth assay where the energy values are determined via regression analysis (Hastad et al., 2004). It has been documented that different measurement techniques can give different energy values (Mroz et al., 1996; Hastad et al., 2004; Agudelo et al., 2010). In addition to these methodologies, prediction equations based on the chemical composition have been used to estimate the energy concentration of different DDGS sources (Spiehs et al., 2002).

In general, the DE and ME values commonly used for DDGS in diet formulation are derived from tables such as NRC (1998; 2012), which are based on a summary of studies that have measured DE and ME. The DE and ME of DDGS reported by NRC (1998) was 3,440 and 3,032 kcal/kg DM, respectively which is 86.8 and 78.9%, respectively, of the value reported for corn by NRC (1998). However, due to the increased production and use of DDGS in the last decade, more research has been conducted aimed at determining its energy value, which in most cases has shown that the energy concentration is generally greater than that of corn. A summary of the DE and ME of DDGS and corn is presented in Table 2.4. Spiehs et al. (2002) reported the calculated DE and ME (using regression equations published by Noblet and Perez, 1993) of 10 sources of DDGS obtained between 1997 to 1999 (to reflect DDGS from "new generation" ethanol plants in addition to 4 samples from an older Midwestern ethanol plant). The range in the calculated DE and ME (kcal/kg DM) values for the new generation sources was from 3,879 to 4,084 and 3,639 to 3,838, respectively, with average values of 3,990 and 3,749, respectively. The average values for the older Midwestern plant were 3,879 and 3,661 kcal/kg DM for DE and ME, respectively. Relative to corn (NRC, 1998) the new generation DE and ME values were

0.7% greater and 2.4% lower, respectively, while the values from the older plant for DE and ME were 2.1 and 4.6% lower, respectively.

Subsequently, research by Hastad et al. (2004) determined the energy concentration of 2 sources of DDGS by measuring DE and ME using the marker method and by using a growth assay. The average ME estimated using the growth assay was 9% less compared to that estimated using the index method (3,567 vs. 3,921 kcal/kg, respectively). Similarly, research by Allee et al. (2005) reported the ME (kcal/kg) of a sample of DDGS of 3,940 kcal/kg DM, which is 2.5% greater relative to the NRC (1998) value for corn. However, Stein et al. (2005) determined the average DE and ME of 4 sources of DDGS to be 3,639 and 3,378 kcal/kg DM, respectively. This ME value was 12.1% less compared to the ME of corn reported by NRC (1998) corn and approximately 16% less than the values reported by Hastad et al. (2004) and Allee et al. (2005).

In other research, Stein et al. (2006) determined that the average DE (kcal/kg DM) of 10 sources of corn DDGS was 3,556 (range from 3,382 to 3,811 kcal/kg DM), which was less than the DE for the corn sample evaluated (3,845 kcal/kg DM), which was similar to that reported by NRC (1998; 3,843 kcal/kg DM). Moreover, Pedersen et al. (2007) determined that the average DE of 10 sources of DDGS was 4,140 with a range from 3,947 to 4,593 kcal/kg DM and the average ME was 3,897 with a range from 3,674 to 4,336 kcal/kg DM. These values were 4.5 and 1.4% greater, respectively, than the DE and ME of corn reported in NRC (1998).

In more recent research by Anderson et al. (2009), the DE and ME (kcal/kg DM) concentrations of 7 samples of DDGS were measured. One sample of DDGS was subject to an additional oil extraction step after fermentation to produce a final crude fat content of 3.2% compared to 11.4% for the other 6 samples. The average DE and ME for these 6 DDGS samples

was 4,029 and 3,790. Relative to corn (NRC, 1998) the DE was 1.7% greater and the ME was 1.4% less. In contrast, the average DE and ME for the oil extracted sample was 4.0 and 3.7% less compared to the other 6 samples, and 2.3 and 5.0% less compared to corn (NRC, 1998).

In other studies, Stein et al (2009) reported the DE and ME of 4 sources of DDGS that used similar production technologies and of corn grown within a narrow geographical area (250 km). The DE and ME of the DDGS averaged 4,072 and 3,750 kcal/kg, respectively, which was less than the DE and ME in the corn sample evaluated (4,181 and 4,103 kcal/kg, respectively), but 2.8% greater and 2.4% less, respectively, than the DE and ME values of corn reported by NRC (1998).

More recently, a revised edition of NRC (2012) has reported the DE and ME for DDGS; however, in this report 3 different values were reported depending on the oil content of the DDGS used. For example, the ME for DDGS with greater than 10% oil was reported to be 3,845 kcal/kg, whereas for DDGS containing between 6 and 9% oil, and less than 4% oil, the values reported were 3,801, and 3,475 kcal/kg, respectively. Compared to the estimates for ME of corn presented in NRC 10th and 11th editions (1998 and 2012), which are almost identical (3,843 and 3,844), the DDGS ME for the three different oil content categories have on average 100.0, 98.9 and 90.4% of the ME in corn.

In summary, the estimates of DE and ME of DDGS are highly variable. In addition the methodologies to obtain the estimates for energy concentration are not always practical and are limited primarily to research situations (Anderson, 2009). Practical approaches to address this issue have relied on developing equations to predict the ME of DDGS based on the chemical composition of the samples and this area will be reviewed in the following section.

Prediction of Energy Concentration from Chemical Composition.

Currently, attempts to formulate to energy levels in swine diets are generally within a tolerance level of ± 1.5% (Fairbarn et al., 1999). This can be difficult to achieve in practice when using high levels of DDGS because, as discussed previously, the reported ME values in the literature show substantial variation among studies (Table 2.4). As already discussed, the composition of DDGS can vary substantially (Table 2.3) with resulting variation in its energy concentration (Table 2.4).

In commercial practice, variation in the nutrient content of a feedstuff can be accounted for by using prediction equations based on its chemical composition. However, there has been limited research to develop prediction equations specifically for DDGS. In the past few years, prediction equations developed by Noblet and Perez (1993) have been used, however, these equations were developed using 114 different diets and were not specific for individual ingredients. However, Pedersen et al. (2007) developed equations to predict the DE and ME contents specifically for DDGS based on samples from 10 ethanol plants (one sample from each plant), using diets with an inclusion level of DDGS of 50%. In other research, Anderson (2009) and Anderson et al. (2012) developed prediction equations for corn co-products in which 7 sources of DDGS were included, and more recently, Kerr and Shurson (2013) developed prediction equations from two experiments using 4 and 11 different sources, respectively, with varying oil content. However, the criteria used for selection of these equations were mostly based on R^2 values and the measure of error accompanying these equations is variable. For example, Noblet and Perez (1993) developed equations to predict ME and reported R^2 of 0.85 to 0.93 with the root mean square error (**RMSE**) also called residual standard deviation (**RSD**) ranging from 64 to 92 kcal/kg, whereas Pedersen et al. (2007) only reported R^2 ranging from 0.94

to 0.99, and Anderson (2009) and Anderson et al. (2012) reported R^2 values ranging from 0.91 to 0.95, and 0.43 to 0.72 with RMSE ranging from 306 to 424 and 323 to 464 kcal/kg, respectively. Likewise, Kerr and Shurson (2013) reported R^2 values from 0.48 to 0.91 with RMSE from 41 to 86 kcal/kg for equations to predict the ME of DDGS. As can be seen from these reports, studies have shown considerable variation in R^2 and RMSE statistics, which makes it difficult to determine which is the "best" equation to use. In addition, none of these studies conducted a validation of the selected equations, which is a critical step in the process of developing prediction equations to ensure validity and accuracy of prediction.

Particle Size Reduction and its Effect on Digestibility.

The common grinding method used to reduce the particle size of grains is to pass them through either a hammer mill or a roller mill (Lenser, 1985). The reduction of particle size involves a two-step process: disruption of the outer seed coat and exposure of the endosperm of the grain (Amerah et al., 2007). Further reduction of the particle size, allows more surface area to be exposed to digestive enzymes in the upper gastrointestinal tract. This allows an enhanced digestion of nutrients and improved utilization of the grain by the animal (Waldroup, 1997; Walker, 1999).

Particle size reduction of grains has been used to increase nutrient digestibility for many years. However, in recent years, the interest in feed particle size has increased, as the feed industry continues to search for ways to increase nutrient utilization and improving production efficiency. Nonetheless, the effects of reduced particle size on the nutritional value are not the same for all cereal grains (Healy et al., 1994). Research from Fraps (1932) and Aubel (1945; 1955) showed an improved nutrient digestibility and feed efficiency of ground sorghum compared to whole sorghum grain. In addition, Ohh et al., (1983) and Owsley et al. (1981) in

nursery and growing-finishing pigs, respectively, reported increased DM, starch and energy digestibility as the particle size of sorghum was decreased. Similar results have been shown for barley (Goodband and Hines, 1988).

In contrast, research by Hale et al. (1979) and Hale and Thompson (1986) reported a greater feed efficiency and growth performance, respectively, for pigs fed coarse wheat compared to finely ground wheat. However, the effects of particle size reduction of corn, in general, are consistent and show an improvement in growth rate and feed efficiency in pigs. A summary of published reports on the effects of particle size reduction of corn on growth performance is presented in Table 2.5. These improvements are the result of improvements in the digestibility of DM and GE; previous research has generally shown a linear improvement in the digestibility of DM and energy as the particle size of corn is reduced from coarse (900 to 1,200 µm) to fine (300 to 400 µm). A summary of previous research conducted using corn is presented in Figures 1 and 2.

However, research on the effects of particle size of by-products of cereal grains including DDGS has been limited. Even though much research has been conducted to characterize factors in DDGS such as compositional (Belyea et al., 2004), nutritional (Spiehs et al., 2002; Pedersen et al., 2007), and physical properties (Ileleji and Rosentrater, 2008), limited research has been conducted to determine the relationship of particle size to that of nutrient digestibility. Rausch et al., (2005) measured the particle size of both corn and corn DDGS and reported that these were poorly correlated ($r < 0.35$). Furthermore, Liu et al. (2008) reported that the particle size of DDGS from 11 sources varied greatly both within and among samples. In addition, it was shown that particle size distribution of DDGS had weak correlations with composition of whole DDGS, whereas sieved fractions of DDGS with different particle sizes were well correlated with some

chemical components. For example, protein content was negatively correlated (-0.63) with sieved fractions of DDGS, and oil and total carbohydrate content were positively correlated (0.58 and 0.39, respectively). In other words, protein content was greater in finer particle size fractions, whereas oil and total carbohydrate content were lower.

In recent years, research evaluating the effect of particle size reduction of DDGS has been conducted, although with a limited number of samples. Yañez et al. (2010) evaluated the effects of grinding DDGS on energy digestibility using DDGS produced from co-fermentation of corn and wheat in a 1:1 ratio. The particle size of the DDGS was reduced using a hammer mill from 517 to 383 µm, which resulted in improvements ($P < 0.05$) in the digestibility of energy from 69.6 to 70.7% for the ground DDGS and in DE (as-fed basis) from 3,280 kcal/kg to 3,338 kcal/kg. In addition, Liu et al. (2012) evaluated the effect particle size reduction of one source of DDGS on the digestibility of DM, and GE in addition to measuring the DE and ME; the sample had an initial particle size of 818 µm and was ground to 595 and 308 µm. Dry matter digestibility for the diets containing the 308 µm DDGS was 84.3% compared with the 595 and 818 µm, which was 83.9 and 82.8%. The digestibility of energy for the 308 µm DDGS was 82.7%, compared to 81.9 and 80.8 for the 595 and 818 µm DDGS samples, respectively. Moreover, the DE and ME of the samples with different particle sizes were measured, with values for the 308 µm DDGS sample being 4,006 and 3,862 kcal/kg, respectively, whereas the 595 and 818 µm DDGS samples had values of 3,932 and 3,745 kcal/kg, and 3,738 and 3,583 kcal/kg, for DE and ME, respectively. As can be seen from this research, digestibility of DM and energy can be improved by reduction of the particle size of DDGS, however, these studies were conducted with a limited number of samples, and as documented, DDGS can vary in both

the chemical composition and physical properties. Further research is needed to clearly understand the impact of grinding on nutrient digestibility in pigs.

In conclusion, DDGS can be used as an ingredient in diets for pigs without compromising growth performance. However, the energy values published for DDGS are variable, and this is a reflection of the variation in the chemical composition; consequently, it is important to understand this variation so that it can be accounted for in feeding programs using DDGS. In practical terms, variability in nutrient content can be accounted for by using prediction equations based on the chemical composition. However, there has been limited research to develop and validate prediction equations specifically for DDGS.

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TABLES AND FIGURES

Table 2.1. Range in reported values for the physical properties of distillers dried grains with solubles

	Reference	
Item	Rosentrater, 2006[1]	Bhadra et al., 2007[2]
Moisture, %	13.21 - 21.16	3.54 - 8.21
Water activity	0.527 - 0.634	0.42 - 0.53
Thermal conductivity, W/m ^{o}C	0.06 - 0.08	0.05 - 0.07
Thermal diffusivity, mm^2/s	0.13 - 0.15	0.10 - 0.17
Bulk density, kg/m^3	389.28 - 501.46	467.70 - 509.38
Angle of repose, ^{o}	26.51 - 34.23	25.7 - 47.04
Hunter		
L*	39.99 - 49.82	36.56 - 50.17
a *	8.00 - 9.81	5.20 - 10.79
b *	18.22 - 23.5	12.53 - 23.36

[1]Data from 6 dry grind ethanol plants.

[2]Data collected from 2 batches from 3 ethanol plants.

Table 2.2. Summary of analytical methods for analysis of distillers dried grains with solubles (AFIA, 2007)

Reference method	Description	CV (%)	
		Intralaboratory[1]	Interlaboratory[2]
Moisture			
AOAC 934.01	Loss on Drying (Vacuum)	2.34	7.93
AOAC 935.29	Loss on Drying (103^{o}C/5Hrs)	1.47	5.23
NFTA 2.2.2.5	Loss on Drying (105 ^{o}C /3Hrs)	1.82	4.62
AOAC 930.15	Loss on Drying (135 ^{o}C /2Hrs)	1.50	8.09
AOAC 2001.12	Moisture (Karl Fischer)	0.89	NA
CP			
AOAC 990.03	CP (Combustion)	0.67	1.58
AOAC 2001.11	CP (Kjedahl)	0.60	1.23
Crude fat			
AOAC 2003.05	Crude Fat (Ethyl Ether)	3.04	8.34
AOAC 954.02	Fat (Acid Hydrolysis)	4.37	8.07
AOAC 945.16	Crude Fat (Pet Ether)	2.71	2.95
AOAC 2003.06	Crude Fat (Hexane)	2.11	5.45
Crude fiber			
AOAC 978.10	Crude Fiber	4.09	17.84
AOCS Ba 6a-05	Crude Fiber (Ankom)	7.07	8.10

[1]Intralaboratory results are based on averages of 30 test samples analyzed in triplicate for each method.

[2]Interlaboratory results are based on average of 5 test samples analyzed in duplicate for each method at 23 laboratories.

Table 2.3. Summary of the chemical composition of distillers dried grains with solubles previously reported in the literature[1]

Reference	No. of samples	DM, %	CP, %	Starch, %	Crude fat, %	ADF, %	NDF, %	Ash, %	Crude fiber, %
NRC (1998)	-[2]	93.0	29.8	-	9.0	17.5	37.2	-	-
Spiehs et al., 2002[3]	118	88.9 (1.7)	30.2 (6.4)	-	10.9 (7.8)	16.2 (28.4)	42.1 (14.3)	5.8 (14.7)	8.8 (8.7)
Spiehs et al., 2002[4]	4	88.3 (0.9)	28.1 (2.4)	-	8.2 (12.6)	16.7 (-)	35.4 (1.8)	6.3 (17.5)	7.1 (4.2)
Stein et al., 2006	10	88.9 (1.3)	30.9 (4.1)	7.3 (14.0)	-	12.2 (13.1)	45.2 (4.8)	-	-
Pedersen et al., 2007	10	87.6 (1.4)	32.2 (6.4)	8.2 (39.9)	11.7 (13.6)	11.6 (11.5)	27.6 (7.1)	4.4 (10.6)	-
Anderson et al., 2012[5]	1	87.4	34.7	3.0	3.2	15.8	51.0	5.2	8.7
Anderson et al., 2012	6	89.1 (2.7)	31.3 (6.3)	4.3 (59.8)	11.4 (6.5)	12.1 (19.6)	40.4 (14.8)	4.5 (10.7)	7.8 (5.6)
NRC, 2012, > 10% oil	12-81	89.31	30.6	7.54	11.7	13.2	36.4	4.6	7.9
NRC, 2012, > 6 and < 9% oil	4-13	89.4	30.3	10.8	10.0	13.5	34.1	4.5	10.0
NRC, 2012, < 4% oil	1-2	89.3	31.2	11.2	4.0	19.0	37.8	5.2	6.9
Kerr and Shurson, 2013	14	87.6 (1.9)	30.5 (4.5)	2.2 (48.3)	9.7 (23.3)	11.7 (15.3)	38.9 (11.3)	5.1 (9.8)	-

[1]Values expressed on 100% DM basis. CV (%) presented in parenthesis when available.

[2]Data was not presented.

[3]New generation ethanol plants (plants built after 1997).

[4]Old generation ethanol plant (plant built before 197).

[5]DDGS sample was subject to oil extraction.

Table 2.4. Summary of published estimates for GE, DE, and ME (kcal/kg DM) and digestibility (%) of DM and energy of corn distillers dried grains with solubles and corn

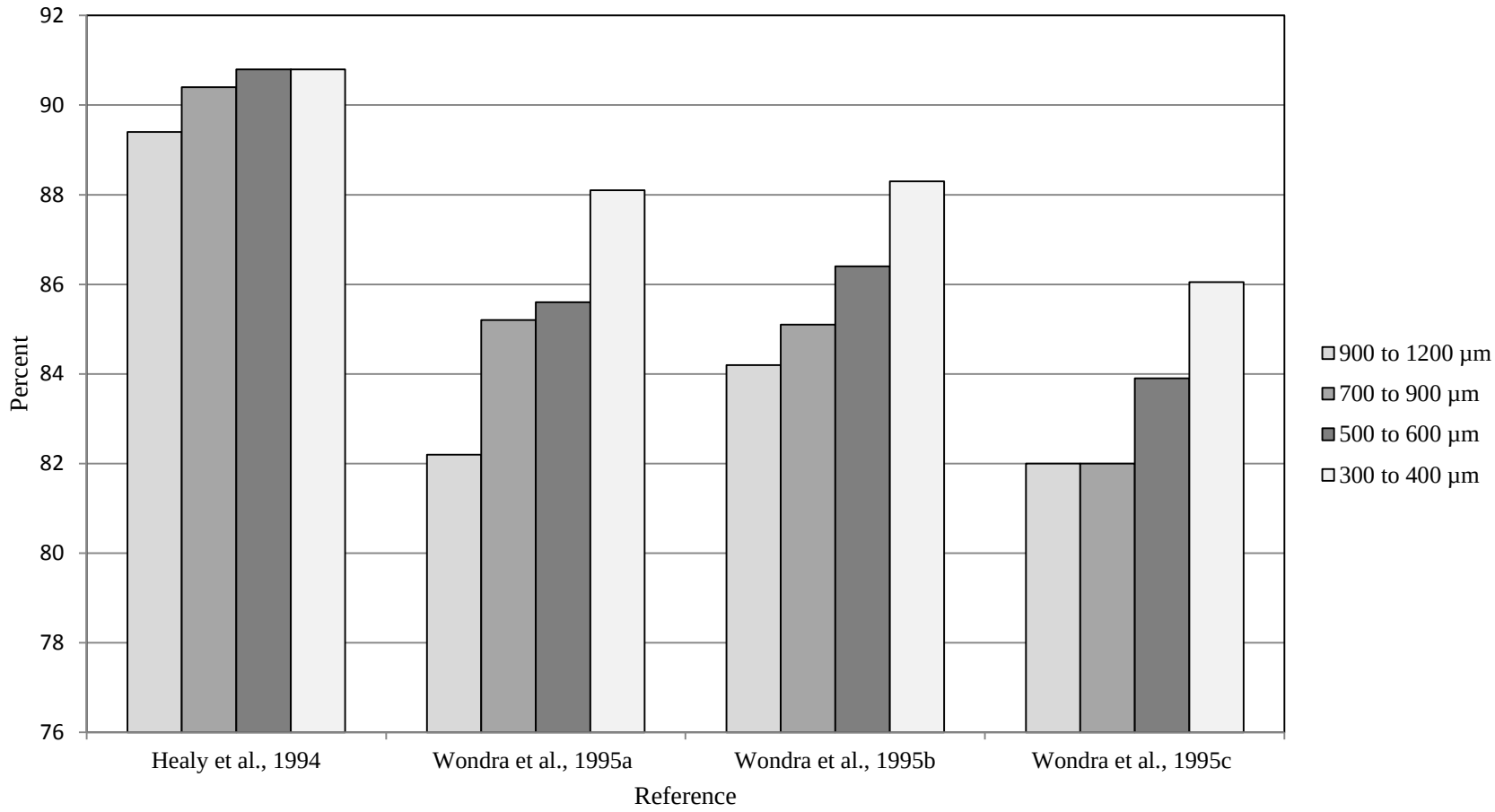
		DDGS					Corn					DDGS relative to NRC (2012) corn (%)	
					Digestibility					Digestibility			
Reference	Samples	GE	DE	ME	DM	Energy	GE	DE	ME	DM	Energy	DE	ME
NRC, 1998	-	-	3,440	3,032	-	-	-	3961	3,843	-	-	88.0	78.9
Spiehs et al., 2002[1]	4	-	3,879	3,661	-	-	-	-	-	-	-	99.3	95.2
Spiehs et al., 2002[2]	118	-	3,990	3,749	-	-	-	-	-	-	-	102.1	97.5
Hastad et al., 2004[3]	2	-	-	3,567	-	-	-	-	-	-	-	-	92.8
Hastad et al., 2004[8]	2	-	4,090	3,921	-	-	-	-	-	-	-	104.7	102.0
Allee, 2005[8]	1	-	-	3,940	-	-	-	-	3,864	-	-		102.5
Stein et al., 2005[8]	4	-	3,639	3,378	71.0	75.0	-	-	-	-	-	93.1	87.9
Fastinger and Mahan, 2006	5	-	-	-	-	68.0	-	-	-	-	-	-	-
Stein et al., 2006[4]	10	5,426	3,556	-	68.3	65.6	4,558	3,845	-	87.6	85.1	91.0	-
Feoli, 2008[4]	2	5,193	3,680	-	78.9	77.9	4,483	3,818	-	87.4	85.4	94.2	-
Widyaratne and Zijlstra, 2007	1	-	3,900	-	-	71.5	-	-	-	-	-	99.8	-
Pedersen et al., 2007[8]	10	5,434	4,140	3,897	-	76.8	4,496	4,088	3,989	-	90.4	105.9	101.4
Anderson et al., 2012[5,8]	1	5,076	3,868	3,650	-	-	-	-	-	-	-	99.0	95.0
Anderson et al., 2012[8]	6	5,420	4,029	3,790	-	-	-	-	-	-	-	103.1	98.6
Stein et al., 2009[8]	4	5,593	4,072	3,750	75.1	75.1	-	4,181	4,103	93.0	92.3	104.2	97.6
Dahlen et al., 2011[8]	1	-	3,351	2,964	-	-	-	-	-	-	-	85.7	77.1
Dahlen et al., 2011[6,8]	1	-	3,232	2,959	-	-						82.7	77.0
Jacela et al., 2011[7,8]	1	5,098	3,100	2,858	-	-	-	-	-	-	-	79.3	74.3
Liu et al., 2012[8]	3	5,423	3,892	3,730	-	-	5,022	3,682	3,577	-	-	99.6	97.0
NRC, 2012, > 10% oil	16	5,429	4,053	3,845	-	-	4,454	3,908	3,844	-	-	103.7	100.0
NRC, 2012, > 6 and < 9% oil	3	5,271	4,009	3,801	-	-	-	-	-	-	-	102.6	98.9
NRC, 2012, < 4% oil	2	5,712	3,687	3,476	-	-	-	-	-	-	-	94.3	90.4
Kerr and Shurson, 2013[8]	15	4,972	3,664	3,444	71.7	73.8	-	-	-	-	-	94.5	90.1

[1]DE and ME were calculated from chemical composition of an old generation (plant built before 1997) ethanol plant using the equations of Noblet and Perez (1993).
[2]DE and ME were calculated from chemical composition of new generation (plants built after 1997) ethanol plants using the equations of Noblet and Perez (1993).
[3]ME was determined using a growth assay.
[4]ME was determined using the index method.
[5]DDGS sample was subject to oil extraction prior to energy determination.
[6]Low-solubles DDGS.
[7]DDGS sample was subject to oil extraction prior to energy determination; DE measured via a digestibility experiment; ME was calculated using an equation from Noblet and Perez (1993) using the DE measured value.
[8]Energy concentration measured using standard experiments in which the apparent DE and ME are measured by difference.

Table 2.5. Published estimates of the effect of particle size reduction of corn on the growth performance of pigs

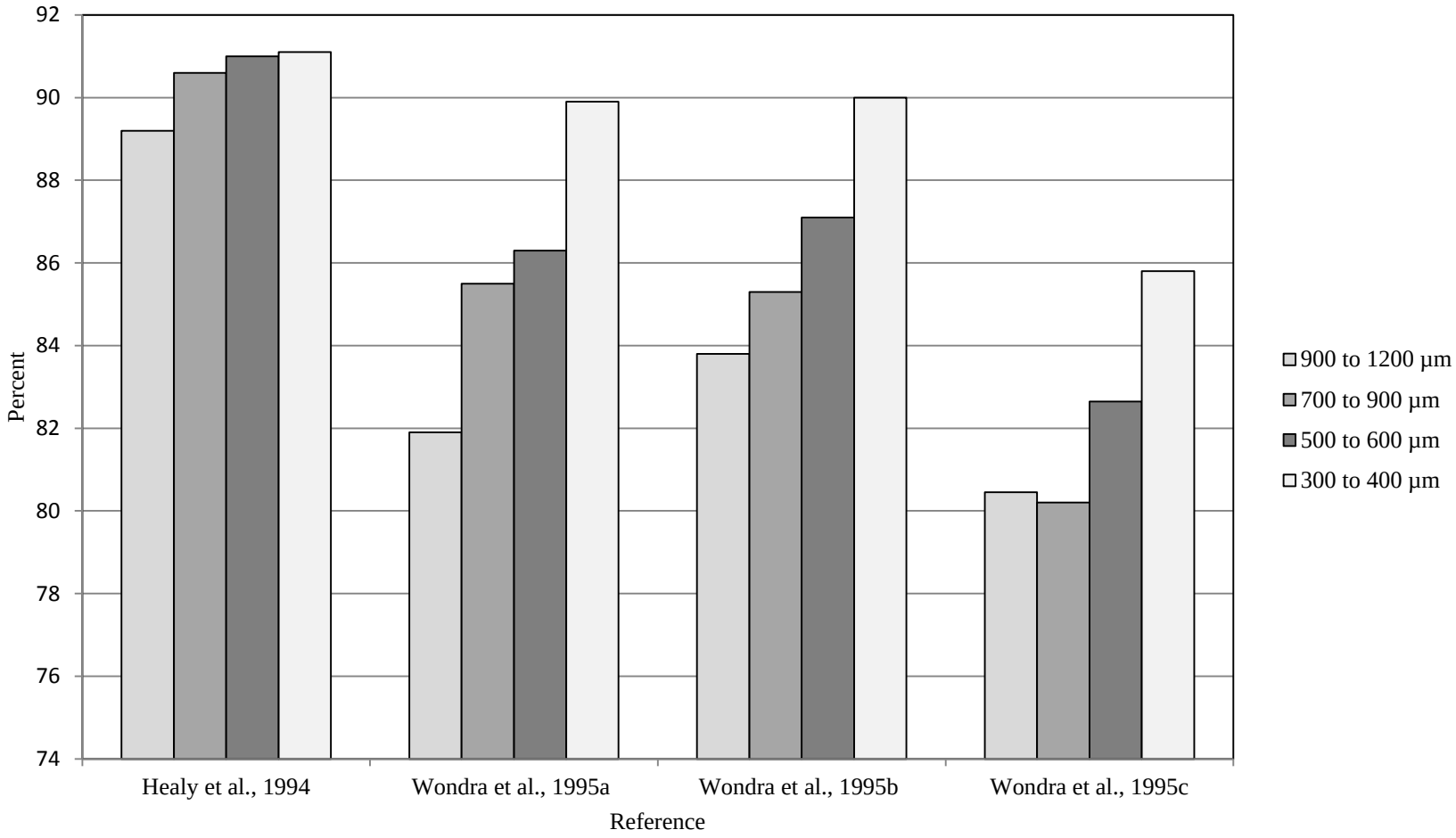
Reference	Feedstuff	Initial and final BW (kg)	No. Pigs	Item	Particle Size		
					Coarse (> 1,000 µm)	Medium (700 to 900 µm)	Fine (<600 µm)
Mahan et al., 1966	Corn	19-55	36	ADG (kg)	0.710	0.790	0.740
				G:F (kg:kg)	0.337	0.329	0.341
Hedde et al., 1985	Corn	35-97	160	ADG (kg)	0.680	-	0.730
				G:F (kg:kg)	0.266	-	0.288
Giesemann et al., 1990	Corn	32-91	192	ADG (kg)	0.686	-	0.719
				G:F (kg:kg)	0.257		0.279
Wondra et al., 1995b	Corn	55-115	160	ADG (kg)	0.980	0.980	0.990
				G:F (kg:kg)	0.298	0.305	0.321

Figure 2.1. Published estimates of the effect of particle size reduction of corn on the digestibility of DM[1 2]



[1]The studies of Healy et al., 1994, and Wondra et al., 1995a, b, c were carried out using nursery pigs, second parity sows, first parity sows, and finishing pigs, respectively.
[2]There was a linear effect of particle size reduction on digestibility ($P < 0.05$).

Figure 2.2. Published estimates of the effect of particle size reduction of corn on the digestibility of energy[1 2]



[1]The studies of Healy et al., 1994, and Wondra et al., 1995a, b, c were carried out using nursery pigs, second parity sows, first parity sows, and finishing pigs, respectively.
[2]There was a linear effect of particle size reduction on digestibility ($P < 0.05$).

CHAPTER 3

DIGESTIBLE AND METABOLIZABLE ENERGY OF DISTILLERS DRIED GRAINS WITH SOLUBLES SOURCES FROM A VARIETY OF ETHANOL PLANTS FED TO GROWING PIGS

ABSTRACT: Three experiments were conducted to determine the apparent DE and ME of samples of distillers dried grains with solubles (DDGS) from different ethanol plants. The three experiments were carried out using the same 36 barrows (17.2 ± 0.9 kg initial BW) in an incomplete block design with a total of 36 dietary treatments. A common corn-based control diet (89.5% corn + mineral and vitamin supplements and casein) was used and the experimental diets used in all 3 experiments were formulated by replacing 50.4% of the corn in the corn-based control diet with the same quantity of each sample of DDGS. Exp. 1 had 17 experimental diets, each containing one of the 17 DDGS samples unground; Exp. 2 had a total of 17 experimental diets, 15 diets each containing one of 15 DDGS samples ground and two unground (from Exp. 1); Exp. 3 had a total of 5 experimental diets, 3 diets containing samples of source DDGS-09 ground to 1,180, 890, and 560 microns and 2 diets also containing source DDGS-09, from Exp. 1 and 2 (unground and ground with particle size of 1,557 and 351 microns, respectively. A total of 9 experimental periods consisting of 4 d of adaptation to experimental diets, followed by 3 d of collection for feces and urine were used. GE of corn, DDGS, feces, and urine were determined by bomb calorimetry. Values are expressed on a DM basis, unless otherwise noted. For Exp. 1, mean values for DE and ME of the unground DDGS samples were 3,842 ± 116.3 and 3,596 ± 108.4 kcal/kg, respectively. For Exp. 2, mean values for DE and ME of the ground DDGS samples was 3,954 ± 117.7 and 3,719 ± 122.5 kcal/kg, respectively. Data from Exp. 1

and 2 were combined to evaluate the effect of DDGS source and particle size, and the two-way interaction. For DE and ME there were no interactions, indicating that the effect of particle size reduction was constant across DDGS samples. There was an effect of DDGS sample on the DE and ME, in addition to an effect of particle size, with the ground DDGS samples having 3.5% and 4.0% (134 and 144 kcal/kg, respectively) greater ($P < 0.01$) DE and ME, respectively, compared with the unground DDGS samples. In Exp. 3, reducing the particle size of the same sample of DDGS had no effect ($P > 0.05$) in DE, however, grinding the sample to the lowest particle size (351 μm) resulted in a greater ME, compared to the other 4 particle sizes. These experiments highlighted the large variation in ME of DDGS sources available in the US in 2008, and that in general, reducing the particle size of DDGS is an effective way to increase the apparent DE and ME of DDGS for growing pigs.

INTRODUCTION

In the U.S., expansion of the production of ethanol from corn has resulted in an increasing amount of the major co-product, distillers dried grains with solubles (DDGS), becoming available for use in swine diets. However, to use DDGS efficiently as an ingredient, estimates for the nutrient and energy concentration need to be available. This becomes especially important as nutritionists need this information for accurate diet formulation to develop feeding programs that maximize growth performance, in addition to being cost-effective. Estimates for the ME of DDGS can be obtained from the published literature (Table 2.4); however, published estimates of ME of DDGS are highly variable. These differences in the energy concentration of DDGS are a reflection of the variation in chemical composition, as can be seen from the reports in the literature (Table 2.3). Several factors have been identified and documented as the cause for the differences in the nutrient content of DDGS, and include the

initial composition of the corn, and the processing conditions to produce ethanol (Olentine, 1986; Belyea et al., 2010). These differences in composition of DDGS are not surprising given that corn also exhibits substantial batch to batch variation in ME (Kim, 1999).

Relative to NRC (2012) estimates for the DE and ME of corn (3,908 and 3,844 kcal/kg, respectively), DE and ME estimates for DDGS ranged from 79.3 to 109.8%, and 74.3 to 102.5%, respectively (Table 2.4). This wide range in the energy concentration of DDGS is a product of some of the factors mentioned above, in addition to differences in methodology for estimation of energy concentration (Table 2.4). These large differences contribute to confusion as to what energy value to use in diet formulation. The most accurate method to obtain information on the energy concentration of DDGS is to measure it through standard experiments in which the apparent DE and ME are determined by difference (Adeola, 2001), however, these are expensive, time consuming, and special facilities and equipment are required, which in general are only available at universities and research institutions. In practice, nutritionists have had access to prediction equations for the energy concentration of complete diets (Noblet and Perez, 1993) and feedstuffs as an alternative to direct measurement of energy concentration; however there has been limited research conducted to develop equations specifically for DDGS (Pedersen et al., 2007; Kerr and Shurson, 2013). Therefore, the objective of this research was to measure the DE and ME of DDGS samples from a wide variety of sources that encompasses the DDGS available to the industry for further development of prediction equations for the ME of DDGS based on chemical composition.

MATERIALS AND METHODS

General Procedures

Three experiments to determine the DE and ME of DDGS by difference as proposed by Adeola, (2001) were conducted using common materials and methodology. A total of 17 DDGS samples from different sources (ethanol plants) were chosen to represent the variation in chemical composition, particle size, and energy concentration that is commonly observed in commercial sources of DDGS from the major ethanol plants in the Midwest of the US (IA, IL, MO, MN, and SD). A corn sample was used to create a diet to be used as a control, having a total of 18 samples in the experiment. Choice of DDGS sources was based on the database of DDGS composition of the University of Minnesota website (http://www.ddgs.umn.edu). At least, 140 kg of material was obtained from each source in June and July 2008 and the experiments to measure the DE and ME of the samples of DDGS were carried out in August to October 2008 at the research facilities of the Pioneer Livestock Nutrition Center (**PLNC**; Polk City, IA).

The three experiments were carried out using the same group of pigs. Experiment 1 was carried out to determine the apparent DE and ME of the 17 samples as-received (unground; mean particle size was 665.8 ± 284.4 μm with a range from 265 to 1,557 μm). Experiment 2 was carried out to determine the apparent DE and ME using the 17 DDGS samples from Exp.1, with 15 of these samples ground (2 samples had relatively low initial PS and were not ground) using a hammer mill to a common particle size in an attempt to ensure that all samples had a similar particle size (Ground; mean particle size was 337.5 ± 39.0 μm with a range from 265 to 403 μm). Experiment 3 was carried out to determine the apparent DE and ME of a single DDGS sample, which had the largest particle size, and was evaluated unground (Exp. 1; 1,557 μm), or ground to particle sizes of 1,189, 890, 560, and 351 μm.

The geometric mean particle size of each DDGS sample for all experiments was measured using a Rotap sieve shaker (model RX-29, W.S. Tyler Co, Cleveland, OH; Table 3.1) fitted with 14 US Sieve sizes – 4, 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270 and a pan. Three rubber balls to help material pass were used in sieves 40, 50, 70, 100, 140, 200 and 270 and a synthetic amorphous precipitated silica was used as a flow agent (Sipernat ®) at a rate of 1 g, with a 100 g sample of DDGS, each sieve plus the rubber balls, and the pan were weighed prior to shaking which was carried out for 10 min. At the end of the 10 min, each of the sieves and the pan were weighted and the weight recorded was used to calculate mean and SD of particle size.

Experimental Design and Treatments

There were a total of 36 observations for the control diet, and 8 observations for the diets containing each one of the DDGS samples. Exp. 1 had 17 experimental diets, each containing one of the 17 DDGS samples unground; Exp. 2 had a total of 17 experimental diets, 15 diets each containing one of 15 DDGS samples ground and two unground (from Exp. 1); Exp. 3 had a total of 5 experimental diets, 3 diets containing samples of source DDGS-09 ground to 1,180, 890, and 560 microns and two diets, from Exp. 1 and 2 (unground and ground with particle size of 1,557 and 351 microns, respectively. Overall, there were 35 diets containing DDGS and 1 corn-based diet as the common control for a total of 36 diets. A requirement of the PLNC research facility was to obtain 36 observations of the corn-based control diet and 8 observations of each of the DDGS diets to reach the objectives of this research. A total of 9 experimental periods each consisting of a 4-d adaptation period followed by a 3-d collection period were carried out. Because of this, 4 pigs (experimental unit) were assigned the corn-based control diet in each of 9 experimental periods, which left 32 pigs and 35 diets with DDGS. Therefore, since

all treatments did not fit in each block, all experiments were carried out simultaneously using an incomplete block design (block = period, each with 36 metabolism crates) and included a corn-based diet as a common control. Pigs were randomly allotted to treatment at the start of each of the 9 adaptation and collection periods with the restriction that pigs received each of the dietary treatments only once during the experimental period.

Animals and Housing

The facility consisted of two identical environmentally controlled rooms with 18 metabolism crates per room for a total of 36 crates. Crates (Thorp Equipment Co., Thorp, WI) had dimensions of 0.71m × 1.63m × 0.91m and were fitted with adjustable rear and top panels, a feeder (0.61m wide × 0.23m deep), and a nipple-type water drinker. Temperature in the rooms was maintained between 18° and 21° C and the rooms were ventilated at the rate of approximately 12 air changes per h. The lighting was on between 0730 and 1600 h each d.

The same group of pigs was used throughout the experiment. A uniform set of commercial barrows (36 for the experiment and 6 extras) of the same genetic background and with an average initial weight of 17.2 ± 0.9 kg were obtained from a herd with a high health status and transported to the PLNC for use in the experiment.

Diet Preparation and Feeding

All diets were formulated to supply sufficient nutrients to support normal pig growth. For the corn sample diet, corn (89.5% of the diet; ground to a particle size of 500 μm) was supplemented with casein (sodium caseinate), an indigestible marker, and minerals and vitamins (Table 3.2). Casein was used as the AA source because of its high digestibility and favorable AA balance. A commercially available vitamin/trace mineral premix was used. The test diets were produced by substituting 50.4% of the corn with one of the DDGS sources (Table 3.2). The

dietary treatments were fed during both the adaptation and collection periods. Chromic oxide was included in the diet used for all meals during the adaptation and collection periods to enhance uniformity of fecal appearance due to differences in color of the different sample of DDGS; the first and last meals of the collection period included ferric oxide as the start and stop visual marker, respectively.

The general condition of each pig was observed daily and any health related issues were recorded and addressed based on the recommendations of the attending veterinarian. During both the adaptation and the collection periods, pigs were fed twice daily at 0730 and 1400 h ± 15 min. Each crate was checked immediately after feeding and any feed on the front screens or floor was added back to the feeder in order to minimize feed wastage. Water was available to the pigs on a restricted basis from 0730 to 0900 and 1400 to 1530 daily to minimize water wastage and variation in urine output.

The target daily feed intake was 3 times the maintenance ME requirement. The quantity of feed offered was calculated from the BW at the start of each adaptation period using the following assumptions:

Maintenance ME requirement = 106 kcal/kg$^{0.75}$ (NRC 1998)

Corn ME:GE ratio = 0.88

Corn ME = DDGS ME

ME of casein = 3,535 kcal/kg, 9% moisture basis (NRC 1998)

Based on these assumptions, the amount of feed offered daily was calculated using the following equation:

Daily feed offered, kg = [(BW, $kg^{0.75}$ × 106 kcal/kg) × 3] / [((Test material in diet (%) × GE of test material)) × (DM of test material (%)) × 0.88] + [casein in diet (%) × 3,535 kcal/kg]

Fecal Collection and Sample Preparation Procedures

The objective was to quantitatively and separately collect all feces and urine resulting from the digestion and metabolism of the test diet consumed during the 3-d collection period. Fecal collection began upon visual appearance of the ferric oxide (approximately 12 to 24 h after feeding the marker) and continued until the ferric oxide appeared again (feces containing the ferric oxide were not collected). Feces were collected and weighed twice daily after each collection and were immediately placed in a forced air oven at 62º C for 7 d.

Urine Collection and Sample Preparation Procedures

Urine collection was carried out on a fixed-time basis starting at 0930 on the first d of collection and ended at 1030 of the last d of collection for each of the collection periods. Urine collection vessels containing 10 ml of 6N HCl were placed under each metabolism pen. The pH of the urine in the collection vessels was checked daily and maintained between 1.5 and 2.5 by the addition of 6N HCl as needed. To achieve uniformity of collected urine, at the end of each collection period, urine was diluted to achieve a total weight of 5 kg by adding water, and the pH was adjusted to between 2 to 3 by addition of 6N HCl. The diluted urine sample was thoroughly mixed and a 250 ml sub-sample was collected into a plastic bottle, frozen (-20º C), and submitted to the laboratory for analysis.

Sample Analysis

Samples of the corn and DDGS samples were ground using a Knifetech Model 1095 sample mill (Rose Scientific Ltd, Edmonton, Canada), after which DM was determined in

duplicate for each sample by drying in an oven at 135ºC for 2 h (procedure 930.15; AOAC, 2005). The dried feces samples from each pig were weighed, composited, and placed into a re-sealable bag, which was labeled with the pig number and test sample identification. The entire composite fecal sample was ground through a Wiley mill (Thomas Scientific, Philadelphia, PA) through a 6 mm screen. Thereafter, a 30 g sub-sample was taken and ground in a Knifetech Model 1095 sample mill (Rose Scientific Ltd, Edmonton, Canada).

Gross energy of the corn and DDGS samples (Table 3.3), feces, and urine samples was determined in the laboratories of Pioneer Hi-Bred International, Inc., (Urbandale, IA) using bomb calorimetry (Isoperibol Bomb Calorimeter, Model Number 1281; Parr Instrument Co., Moline, IL) with the analysis being carried out in duplicate. Urine samples were placed onto Solka-Floc® (International Fiber Corporation, North Tonawanda, NY) and dried to produce a mixture with 16 to 18% urine by weight, which was subjected to bomb calorimetry determination, in addition to determination of the GE of the Solka-Floc® (Fent, 2001).

Assumptions and Calculations

The control diet was composed of 89.5% corn, 7.9% casein, minerals, vitamins, and an indigestible marker. In experiments where the apparent DE and ME are to be measured, and the test feedstuff (e.g., DDGS) cannot be fed alone, diets are formulated with other feedstuffs (e.g., corn) in addition to the test feedstuff, also supplying the component of interest (i.e., energy; Adeola, 2001). In addition, these type of experiments have average adaptation periods of 3 to 7 d, and collection periods of 4 to 6 d in duration. However, due to the number of dietary treatments and desired number of observations, the present experiments had a total of 9 adaptation and collection periods, which lasted a total 56 d. Due to the long duration of the experiment, and the fact that the same group of pigs was used, casein was added to the control

diet, so that the pigs would have sufficient quantities of AA so that their growth was not compromised. As the DE and ME of casein were not measured during these experiments, data for the energy values of casein to be used in calculations were obtained from published literature (Fent, 2001). The DE and ME used for casein in the research by Fent (2001) was 4,723 and 4,560 kcal/kg, which was obtained using pigs with an average initial BW of 30.6 kg, and with casein being included at 6.14% of the diet, compared to 7.9% of casein which was used in the current experiments. To obtain the DE and ME of the DDGS samples, the following calculations were used (all concentrations used in the calculations were expressed on a DM basis):

1) The DE and ME of the corn were calculated by subtracting the DE and ME contributed by the casein to the corn-casein diet.

2) By subtracting the DE and ME contributed by the corn and casein to the corn-casein-DDGS diets, the DE and ME contributed by each sample of DDGS was calculated by difference using the following equations:

DDGS DE = [exp. diet DE - (corn DE × (0.391/0. 974) - (casein DE (0.079/0.974)] /(0.504/ 0. 974)

DDGS ME = [exp. diet ME - (corn ME × (0.391/0. 974) - (casein ME (0.08/0.974)] /(0.504/0. 974)

For DM and energy digestibility, and energy metabolizability of the experimental diets, the following equations were used:

DM digestibility, % = [(DM intake (g) - DM in feces (g))/DM intake (g)] × 100

Energy digestibility, % = [(GE intake (kcal) - GE in feces (kcal))/GE intake (kcal)] × 100

Energy metabolizability, % = [(GE intake (kcal) - GE in feces (kcal) - GE in urine (kcal))/GE intake (kcal)] × 100.

Statistical Analyses

Prior to analysis, the PROC UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to verify normality and homogeneity of variances of each variable. For all experiments, the pig was the experimental unit and the data were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1996) with the model including the fixed effects of DDGS source and the value for the corn sample for each specific variable as a covariate, and the random effects of period, room nested within period, and pig nested within room. In addition, the data for Exp. 1 and 2 were combined to evaluate the effect of DDGS source, particle size (unground vs. ground) and DDGS source by particle size interaction, with the model including the fixed effects of DDGS source, particle size, and 2-way interaction (the corn sample for each specific variable was used as a covariate), and the random effects of period, room nested within period, and pig nested within room. The LSMEANS procedure was used to estimate mean values, and the PDIFF option was used to separate means. An alpha level of 0.05 was used assess differences among treatment means.

RESULTS AND DISCUSSION

All values presented and discussed are expressed on a DM basis, unless otherwise noted. Chemical composition for the corn and corn-based diet, DDGS samples, experimental diets with DDGS samples unground and ground are shown in Table 3.4, 3.5, 3.6, 3.7, and 3.8 respectively.

Effect DDGS Samples (unground) on the DE and ME – Experiment 1

For Exp. 1, the mean GE of the 17 unground DDGS samples was 5,317 ± 66.1 kcal/kg with the lowest value being for DDGS sample 1 (5,191 kcal/kg) and the highest value being for

DDGS sample 3 (5,452 kcal/kg; Table 3.3). On average, these GE values fall within the range in GE values reported for DDGS (Table 2.4).

Least-squares means for the effects of experimental diets on DE and ME for Exp. 1 are presented in Table 3.6. Gross energy intake was not different for pigs fed the experimental diets with DDGS; however, there were differences ($P < 0.01$) in the total energy concentration of feces (range 4,818 to 5,140 kcal/kg) and daily fecal energy output (range = 882 to 1,079 kcal/d) with source 8 having the greatest ($P < 0.01$) output for both measures, and sources 14 and 11 having the lowest total energy concentration in feces and daily fecal energy output, respectively. The energy concentration of urine and daily urinary energy output were not influenced by the experimental diets containing the DDGS sources. The DE and ME of experimental diets (range = 3,797 to 4,009, and 3,625 to 3,836 kcal/kg, respectively) were different ($P < 0.01$) among DDGS sources with the diet containing DDGS source 11 and 13 having the greatest DE and ME (4,009 and 4,003 kcal/kg, and 3,725 and 3,737 kcal/kg respectively), and DDGS source 1 having the lowest DE and ME (3,797 and 3,625 kcal/kg, respectively; Table 3.6). The DDGS sources with the greatest ($P < 0.01$) DM digestibility were sources 11 and 13, and source 8 had the lowest, with an average difference of 3.4 percentage units, whereas sources 11 and 8 had the greatest and lowest energy digestibility, respectively, with a difference of 4.1 percentage units. Energy metabolizability was also different between experimental diets containing the different DDGS sources ($P < 0.01$) with sources 11 and 13 having the greatest metabolizability and sources 1 and 8 having the lowest metabolizability, with a range of 3.6 percentage units between the highest and lowest. In addition, the DE and ME among DDGS sources (range = 3,585 to 3,994 and 3,330 to 3,737 kcal/kg, respectively) were different ($P < 0.01$) with sources 11 and 13

being similar and having both the greatest DE and ME, respectively, while source 1 had both the lowest DE and ME (Table 3.6).

In general, the DE and ME values for DDGS reported in this research are within the range of values reported for DDGS in the literature (Table 2.4); however, the DE and ME values are lower than those reported by NRC (2012) for DDGS with greater that 10% oil, which was the case for the samples used in these experiments (Table 3.5). However, the values reported in NRC (2012) were based on a limited number of samples and, therefore, may not be representative.

The apparent DE and ME values for corn determined in this experiment were 3,883 and 3,818 kcal/kg, respectively, values that are similar to those reported by NRC (1998; 2012).

The objective for the selection of the DDGS sources to use in this project was to provide material that represented the variation in nutrient composition likely to be found between commercial sources in the US. These results illustrate that the 17 DDGS sources used in the experiment represented a considerable range in energy concentration and clearly demonstrates that the selection process was successful and that these were appropriate sources to use to develop prediction equations that would apply to DDGS material available to the swine industry in the Midwest. The development of prediction equations for ME of DDGS is presented and discussed in the subsequent chapters.

Effect of DDGS Source and Particle Size on DE and ME – Experiment 2

For Exp. 2, the average GE of the 15 Ground DDGS samples (plus 2 samples that had initial low particle size) was 5,306 ± 67.1 kcal/kg with the lowest value being for DDGS sample 1 (5,164 kcal/kg) and the highest value being for DDGS sample 2 (5,401 kcal/kg; Table 3.3). Least-squares means for the effect of experimental diets, and ground DDGS samples on energy

concentration for Exp. 2 is presented in Table 3.9. Gross energy intake was not different for pigs fed any of the experimental diets; however, there were differences ($P < 0.01$) in the total energy concentration of feces (range 4,814 to 5,026 kcal/kg) with sources 7 and 14 having the greatest and lowest ($P < 0.01$) energy concentration in feces, respectively. Pigs fed experimental diet with source 1 had the greatest ($P < 0.01$) daily fecal energy output (1,042 kcal/d), whereas those fed the diet with source 13 had the lowest ($P < 0.01$) (843 kcal/d). In contrast, DDGS source had no effect on any of the urinary energy measures. The DE and ME of experimental diets (range = 3,842 to 4,071, and 3,669 to 3,921 kcal/kg, respectively) were different ($P < 0.01$) among the experimental diets with that containing DDGS source 2 having the greatest DE and ME (4,071 and 3,921 kcal/kg, respectively), and the diet with DDGS source 1 having the lowest DE and ME (3,842 and 3,669 kcal/kg, respectively; Table 3.9). For the digestibility of DM, energy and metabolizability, the experimental diets with source 1 had the lowest ($P < 0.01$; 79.9, 79.4 and 75.8%, respectively) and source 13 had the greatest ($P < 0.01$; 82.9, 82.9 and 79.5%, respectively). Source 2 had the greatest ($P < 0.01$) DE and ME (4,115 and 3,900 kcal/kg, respectively), and source 1 had the lowest ($P < 0.01$) DE and ME (3,673 and 3,414, respectively).

Data from Exp. 1 and 2, were combined to evaluate the effect of DDGS source, particle size and the 2-way interaction, with the least-squares means presented in Table 3.10. There was only one interaction ($P < 0.05$) between DDGS source and particle size, which was for GE of feces, however, for the remaining variables measured, there were no treatment interactions, therefore, only the main effects will be presented and discussed.

The GE intake was not different among the experimental diets containing the 17 DDGS sources (Table 3.10). However, the energy output in feces differed ($P < 0.01$) among the

experimental diets (range = 884 to 1,040 kcal/d). In contrast, the GE in urine and urinary energy output remained unaffected by dietary treatment (Table 3.10). As a consequence, the digestibility of DM and energy of the experimental diets containing the different sources of DDGS differed ($P < 0.05$) with a range of 79.5 to 82.6% and 78.9 to 82.3%, respectively (Table 3.10). In addition, the DE and ME of the experimental diets containing the DDGS sources were different ($P < 0.05$) with a range of 3,821 to 4,032, and 3,644 to 3,870 kcal/kg, respectively (Table 3.10). Moreover, the DE and ME of the DDGS sources was different ($P < 0.05$) with a range of 3,632 to 4,039 kcal/kg and 3,365 to 3,802 kcal/kg, respectively (Table 3.10).

Particle size reduction of DDGS had an effect on most of the variables measured. For example, GE intake was 50 kcal/d greater ($P < 0.05$) for the diets containing the ground compared to the unground DDGS sources. In addition, there was a 6.7% reduction ($P < 0.01$) in the fecal energy output for those pigs fed the experimental diets with the ground, compared to the unground DDGS sources. However, there was no effect of particle size on the energy concentration of urine (2,162 vs. 2,146 kcal/kg for the unground and ground sources, respectively) or on urinary energy losses (162 vs. 156 kcal/d, respectively). The DE and ME of the experimental diets with the ground DDGS sources was 69 and 75 kcal/kg greater ($P < 0.01$) compared to diets with the unground DDGS sources (Table 3.10). Similarly, experimental diets with the ground DDGS sources had a greater ($P < 0.01$) digestibility of DM (1.1 percentage units) and energy (1.5 percentage units), and greater metabolizability of energy (1.6 percentage units) compared to the experimental diets containing the unground DDGS sources (Table 3.9). Consequently, the DE and ME of the ground DDGS sources was on average 134 and 144 kcal/kg, respectively, greater ($P < 0.01$) compared to the unground DDGS sources.

Research evaluating the effect of particle size reduction has been conducted with corn with finishing pigs and sows. For example, in an experiment by Wondra et al. (1995b) with finishing pigs the digestibility of DM and energy was 6.1 and 8.3% greater ($P < 0.05$) for diets with corn ground to 400 compared to 1,000 µm, and 7.2 and 9.8% in diets were the corn particle size in lactation diets was reduced from 1,200 to 400 µm . However, more recently Yañez et al. (2010) evaluated the effects of grinding DDGS on energy digestibility using DDGS produced from co-fermentation of corn and wheat in a 1:1 ratio. The particle size of the DDGS was reduced from 517 to 383 µm, which resulted in improvements ($P < 0.05$) of 1.1 percentage units in the digestibility of energy, and 50 kcal/kg (as-fed) in DE. In addition, Liu et al. (2012) evaluated the effect particle size reduction of DDGS on digestibility of DM, and GE in addition to measuring the DE and ME of one source of DDGS. The original mean particle size was 818 µm and samples were ground to particle sizes of 595 and 308 µm. Dry matter and energy digestibility for the diets containing the DDGS ground to 308 µm was 1.5 and 1.8 percentage units greater ($P < 0.05$) compared to the DDGS at 818 µm with the DDGS ground to 595 µm being intermediate. In addition, the DE of DDGS was similar between the fine and medium ground DDGS, but was on 268 kcal/kg greater ($P < 0.05$) compared to the coarse DDGS, whereas the ME for the fine DDGS was 279 kcal/kg greater ($P <0.05$) compared to the coarse, with the medium being intermediate.

Effect of Particle Size Reduction within a Single Source of Distillers Dried Grains with Solubles on DE and ME – Experiment 3

For Exp. 3, the GE of sample DDGS ground to 1,180, 890 and 560 was, 5,363, 5,349, and 5,376, respectively (Table 3.11). Gross energy intake was similar among the different particle sizes. However, the concentration of energy in feces was less ($P < 0.05$) for the lowest particle

size compared to the other particle sizes, and the fecal energy output was less ($P < 0.05$) for the 351 and 1,557 µm particle sizes compared to the 1,180 and 560 µm, with the 890µm particle size being intermediate. Energy concentration in urine and urinary energy output were similar among particle sizes. There was no effect of particle size reduction on DE of the experimental diets; however, ME was greater for the diet containing the DDGS sample ground to 351µm compared to the other particle sizes. There was no effect of particle size reduction on the digestibility of DM and energy; however, for energy metabolizability, the diet containing DDGS sample ground to 351 µm was different *(*$P < 0.05$) from those ground to 1,180, 890, and 560 µm, but similar to the Unground (1,557 µm), with the Unground being similar to the other diets containing the intermediate particle sizes. For DE, there was no effect of particle size; however, there was a quadratic response ($P < 0.05$) to reducing particle size for ME which was greater ($P < 0.05$) for the 351 µm particle size than for the other particle sizes (Table 3.11).

These results are different from those reported by Wondra et al. (1995a,b) with corn in which the reduction of particle size from 1,000 to 400 µm in finishing pig diets and 1,200 to 400 µm in lactating sow diets resulted in a linear increase in the digestibility of DM and energy. It has been reported by Rausch et al. (2005) that the particle size distribution of ground corn was not correlated ($r < 0.35$) to the particle size distribution of DDGS. In addition, visual examination of the sample used in this experiment indicated that it was physically different from the other sources evaluated, with the distinct characteristic of containing a high proportion of spherical particles referred to by some as syrup balls (Ileleji et al., 2007), and this could explain the high particle size measured on this sample. Due to the limited published data on the effect of particle size reduction of DDGS on nutrient digestibility, more research needs to be conducted to determine the relationship between particle size and digestibility.

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TABLES

Table 3.1. Geometric mean particle size[1] of the samples of distillers dried grains with solubles

	Geometric mean particle size, µm		
Sample number	Experiment 1	Experiment 2	Experiment 3
DDGS-01	1,017	403	-
DDGS-02	740	393	-
DDGS-03	497	337	-
DDGS-04	637	354	-
DDGS-05	578	360	-
DDGS-06	534	300	-
DDGS-07	753	361	-
DDGS-08	566	307	-
DDGS-09[2]	1,557	351	1,180, 890, 560
DDGS-10	731	387	-
DDGS-11	318	318	-
DDGS-12	669	353	-
DDGS-13	620	321	-
DDGS-14	265	265	-
DDGS-15	597	347	-
DDGS-16	586	296	-
DDGS-17	653	285	-
All sources:			
Mean	665.8	337.5	907.6
SD[3]	284.4	39.0	481.4

[1]The geometric mean particle size for each sample was determined using a Rotap sieve shaker (model RX-29, W.S. Tyler Co, Cleveland, OH).

[2]For Exp. 3, Source DDGS-9 ground to three intermediate particle sizes to those used in Exp. 1 and 2 for a total of 5 particle sizes.

[3]Between-sample SD for the geometric particle size of DDGS source.

Table 3.2. Ingredient composition of the experimental diets (as-fed)

Ingredient, (%)	Corn	Corn-DDGS
Corn	89.50	39.10
DDGS	-	50.40
Sodium caseinate[1] + indigestible marker[2]	8.0	8.0
Limestone	1.0	1.0
Dicalcium phosphate[3]	0.65	0.65
Salt	0.40	0.40
Vitamin/trace mineral suplemment[4]	0.45	0.45

[1]Casein (sodium caseinate; 88.7% CP, 9% moisture, NRC, 1998).

[2]Indigestible marker = chromic oxide was used to enhance uniformity of fecal color and was mixed with the casein to achieve a concentration of 1.25% (0.568 kg of chromic oxide were added per 45.35 kg of casein). When the casein/chromic oxide mixture was included in the experimental diet at 8.0%, the final concentration of chromic oxide was 0.10%. Ferric oxide was used as a start/stop marker and was mixed with the casein to achieve a concentration of 3.75% (1.7 kg of ferric oxide were added per 45.35 kg of casein). When the casein/ferric oxide mixture was included in the experimental diet at 8.0%, the final concentration of ferric oxide was 0.30%.

[3]Dicalcium phosphate – 18.5% phosphorus; 22% calcium.

[4]The vitamin/trace mineral supplement contained a minimum of the following per kilogram of complete diet: vitamin A (Retinyl Acetate), 2,974 IU; vitamin D3 (Cholecalciferol), 892 IU; vitamin E, 20 IU; vitamin K (Menadione Dimethylpyrimidinol Bisulfate), 2 mg; Riboflavin, 2 mg; Niacin, 16 mg; Panthothenic acid (d-Calcium Panthothenate), 10 mg; Choline, 20 mg; vitamin B12 (Cyanocobalamin), 0.01 mg.

Table 3.3. Gross energy for the distillers dried grains with solubles and corn sample used in all experiments

	GE, kcal/kg[1]		
Sample number	Experiment 1[2]	Experiment 2[3]	Experiment 3
DDGS-1	5,191	5,164	-
DDGS-2	5,406	5,401	-
DDGS-3	5,452	5,390	-
DDGS-4	5,336	5,334	-
DDGS-5	5,327	5,302	-
DDGS-6	5,274	5,274	-
DDGS-7	5,349	5,353	-
DDGS-8	5,340	5,349	-
DDGS-9	5,351	5,392	5,363[4], 5,349[5], 5376[6]
DDGS-10	5,238	5236	-
DDGS-11	5,266	5,266	-
DDGS-12	5,318	5288	-
DDGS-13	5,280	5296	-
DDGS-14	5,249	5,249	-
DDGS-15	5,368	5388	-
DDGS-16	5,372	5278	-
DDGS-17	5,269	5237	-
All sources:			-
Mean[7]	5,317	5,306	5,366
SD[8]	66.1	67.1	18.0
Corn[9]	4,455	-	-

[1]The GE was measured using bomb calorimetry (Isoperibol Bomb Calorimeter, Model Number 1281; Parr Instrument Co., Moline, IL).
[2]Samples unground.
[3]Samples ground.
[4]GE (kcal/kg DM) for DDGS-9 ground to 1,180 µm.
[5]GE (kcal/kg DM) for DDGS-9 ground to 890 µm.
[6]GE (kcal/kg DM) for DDGS-9 ground to 560 µm.
[7]Mean for the GE of DDGS-09 used in Exp. 3 includes all five particle sizes.
[8]SD for the GE of DDGS-9 used in Exp. 3 includes all five particle sizes.
[9]Data for the corn sample was used for all three experiments.

Table 3.4. Analyzed composition of the corn sample and the control diet for Laboratories 1 and 2 (DM basis)

	Corn	
Item	Sample	Diet
Laboratory 1:		
DM, %	86.8	87.87
Ash, %	1.32	3.51
CP, %	9.56	14.2
Crude fat, %	3.52	3.36
Crude fiber, %	2.35	0.76
ADF, %	3.65	2.3
NDF, %	10.00	9.57
Total starch, %	73.77	63.48
Laboratory 2		
DM, %:	88.17	88.61
Ash, %	1.27	3.86
CP, %	8.95	14.24
Crude fat, %	3.94	3.80
Crude fiber, %	1.88	1.92
ADF, %	2.19	2.26
NDF, %	10.42	10.69
Total starch, %	72.09	65.40

Table 3.5. Analyzed composition of the 17 sources of distillers dried grains with solubles for Laboratories 1, 2, and for Laboratories 1 and 2 combined (average of the 2 laboratories; DM basis)

	DDGS source																
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Laboratory 1:																	
DM, %	85.19	87.91	87.71	90.80	89.61	91.50	90.09	93.19	87.68	89.06	91.75	91.30	92.59	93.14	88.28	90.94	90.88
Ash, %	5.10	4.37	3.57	4.20	4.53	4.01	4.35	3.73	4.01	4.81	4.54	4.46	4.50	5.42	4.43	4.68	5.59
CP, %	29.60	29.60	29.70	31.00	31.70	32.20	29.50	31.70	31.10	27.80	28.90	29.80	32.00	29.40	31.30	30.60	30.80
Crude fat, %	9.52	11.40	10.30	10.90	10.30	8.72	10.80	10.60	9.10	9.99	10.90	11.50	11.20	10.70	11.50	10.70	10.50
Crude fiber, %	8.32	7.96	8.36	6.61	7.79	8.39	7.98	6.32	8.38	8.00	8.62	5.87	8.09	7.08	8.87	7.76	8.07
ADF, %	17.90	13.60	15.60	13.10	14.20	14.40	10.50	13.00	15.60	12.20	10.20	12.40	10.50	9.82	11.40	14.20	13.20
NDF, %	29.40	30.10	33.50	28.50	31.70	26.40	27.80	31.40	26.90	25.90	27.20	26.70	25.80	25.30	31.60	33.30	29.80
Starch, %	4.57	4.33	2.45	5.80	3.56	4.81	5.29	6.03	9.01	8.94	7.27	7.37	5.11	6.33	4.81	3.19	4.38
Laboratory 2																	
DM, %:	90.27	89.53	91.18	91.13	91.35	91.70	90.51	93.64	88.83	90.94	92.56	93.18	91.05	93.85	91.71	92.78	92.35
Ash, %	5.15	4.64	3.64	4.37	4.31	4.02	4.21	3.50	4.04	4.56	4.15	4.37	4.76	5.36	4.33	4.33	5.09
CP, %	29.78	29.28	33.34	30.84	31.29	31.88	28.81	29.60	30.79	27.30	29.09	29.39	31.26	29.88	30.52	29.63	30.02
Crude fat, %	9.46	14.63	12.67	13.00	12.90	11.79	13.79	12.12	11.94	13.20	12.60	13.59	13.43	11.44	14.11	13.33	13.32
Crude fiber, %	7.88	6.89	7.71	6.86	7.32	7.47	7.00	7.67	6.97	6.64	6.62	7.00	6.86	7.61	7.34	8.13	7.04
ADF, %	13.42	11.75	13.43	10.91	12.45	16.70	10.21	12.68	14.77	10.04	9.18	12.21	10.54	9.12	12.68	12.61	10.90
NDF, %	42.74	41.55	43.51	41.86	42.61	38.36	40.02	43.34	40.72	39.80	36.35	39.82	35.55	32.41	42.24	42.38	40.63
Starch, %	2.63	3.35	1.63	5.40	2.76	3.77	3.20	3.52	9.08	6.14	5.60	3.62	3.90	4.99	3.33	1.79	2.99
Laboratory 1 and 2 combined[1]:																	
DM, %	87.73	88.72	89.45	90.97	90.48	91.60	90.30	93.42	88.26	90.00	92.16	92.24	91.82	93.50	90.00	91.86	91.62
Ash, %	5.13	4.51	3.61	4.29	4.42	4.02	4.28	3.62	4.03	4.69	4.35	4.42	4.63	5.39	4.38	4.51	5.34
CP, %	29.69	29.44	31.52	30.92	31.50	32.04	29.16	30.65	30.95	27.55	29.00	29.60	31.63	29.64	30.91	30.12	30.41
Crude fat, %	9.49	13.02	11.49	11.95	11.60	10.26	12.30	11.36	10.52	11.60	11.75	12.55	12.32	11.07	12.81	12.02	11.91
Crude fiber, %	8.10	7.43	8.04	6.74	7.56	7.93	7.49	7.00	7.68	7.32	7.62	6.44	7.48	7.35	8.11	7.95	7.56
ADF, %	15.66	12.68	14.52	12.01	13.33	15.55	10.36	12.84	15.19	11.12	9.69	12.31	10.52	9.47	12.04	13.41	12.05
NDF, %	36.07	35.83	38.51	35.18	37.16	32.38	33.91	37.37	33.81	32.85	31.78	33.26	30.68	28.86	36.92	37.84	35.22
Starch, %	3.60	3.84	2.04	5.60	3.16	4.29	4.25	4.78	9.05	7.54	6.44	5.50	4.51	5.66	4.07	2.49	3.69
GE, kcal/kg[2]	5,191	5,406	5,452	5,336	5,327	5,274	5,349	5,340	5,351	5,238	5,266	5,318	5,280	5,249	5,368	5,372	5,269

[1]Average of the results of the 2 laboratories.
[2]GE analyses conducted at Pioneer Hi-Bred International Inc., (Urbandale, IA).

Table 3.6. Least-squares means for the effect of experimental diets containing the unground distillers dried grains with solubles samples on DE and ME (DM basis); Exp. 1

		DDGS source								
Item	Corn[1]	1	2	3	4	5	6	7	8	9
GE:										
Intake, kcal/d	4,874	4,871	4,904	4,894	4,991	4,876	4,894	4,935	4,911	4,879
Output in dry feces,										
Total, kcal/kg	5,004	5,010^{fg}	5,089^{bc}	5,112^{ab}	5,062^{cde}	5,036^{def}	5,015^{fg}	5,127^{a}	5,140^{a}	5,081^{bc}
Daily, kcal/d	564	1,046^{ab}	985^{bcd}	1,044^{ab}	1,013^{abc}	1,019^{ab}	928de	985^{bcd}	1,079^{a}	939^{cde}
Output in dry urine,										
Total, kcal/kg	2,033	2,154	2,147	2,078	2,243	2,062	2,276	2,207	2,183	2,271
Daily, kcal/d	90	172	162	157	157	138	181	165	152	191
Energy concentration										
Experimental diet										
DE, kcal/kg	3,951	3,797^{f}	3,969^{ab}	3,905^{bcde}	3,949^{abc}	3,873^{e}	3,962^{ab}	3,944^{abcd}	3,838^{ef}	3,984^{a}
ME kcal/kg	3,878	3,625^{f}	3,800^{abcd}	3,733^{bcde}	3,787^{abcd}	3,725^{cde}	3,784^{abcd}	3,782^{abcd}	3,682^{ef}	3,787^{abcd}
Dry matter digestibility, %	89.6	79.0^{f}	80.5^{bcde}	78.9^{f}	80.7^{bcd}	79.2^{ef}	81.4^{ab}	80.7^{bcd}	78.8^{f}	81.3^{ab}
Energy digestibility, %	88.4	78.3^{gh}	80.0^{cdef}	78.3^{gh}	80.1^{cde}	78.7^{fgh}	81.0^{abcd}	79.9^{def}	77.9^{h}	80.7^{abcd}
Energy metabolizability, %	85.6	74.7^{f}	76.6^{abcde}	74.8^{ef}	76.9^{abcd}	75.7^{cdef}	77.3^{abc}	76.7^{abcde}	74.7^{f}	76.7^{abcd}
Sample										
DE, kcal/kg	3,883	3,585^{f}	3,917^{ab}	3,793^{bcde}	3,878^{abc}	3,732^{e}	3,904^{ab}	3,868^{abcd}	3,664^{ef}	3,946^{a}
ME kcal/kg	3,818	3,330^{f}	3,668^{abcd}	3,537^{bcde}	3,643^{abcd}	3,522^{cde}	3,636^{abcd}	3,633^{abcd}	3,440^{ef}	3,642^{abcd}

[1]Means from the corn sample was used as a covariate.

^{a,b,c,d,e,f,g,h}Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 3.6 (cont.). Least-squares means for the effect of experimental diets containing the unground distillers dried grains with solubles samples on DE and ME (DM basis); Exp. 1

	DDGS source									
Item	10	11	12	13	14	15	16	17	SEM	*P*-value
GE:										
Intake, kcal/d	4,893	4,949	4,910	4,945	4,872	4,869	4,917	4,948	63.7	0.60
Output in dried feces,										
Total, kcal/kg	4,998^{gh}	4,916^{i}	5,042^{def}	5,067^{cde}	4,818^{j}	5,034^{efg}	5,072^{cd}	4,965^{h}	14.8	<0.01
Daily, kcal/d	907^{e}	882^{e}	1,010^{abc}	910de	898^{e}	942^{cde}	1,036^{ab}	1,015^{abc}	37.3	<0.01
Output in dried urine,										
Total, kcal/kg	2,152	2,171	2,278	2,065	2,172	2,237	2,178	1,986	81.2	0.36
Daily, kcal/d	142	180	176	171	177	169	164	137	14.3	0.17
Energy concentration										
Experimental diet										
DE, kcal/kg	3,959^{abc}	4,009^{a}	3,889^{cde}	4,003^{a}	3,974^{ab}	3,979^{a}	3,906^{bcde}	3,874^{de}	26.6	<0.01
ME kcal/kg	3,816^{ab}	3,830^{a}	3,717^{de}	3,836^{a}	3,789^{abcd}	3,810^{abc}	3,735^{bcde}	3,735^{bcde}	32.5	<0.01
Dry matter digestibility, %	81.7^{ab}	82.1^{a}	79.9^{cdef}	82.3^{a}	81.2^{abc}	80.6^{bcd}	79.5^{def}	79.5^{def}	0.52	<0.01
Energy digestibility, %	81.2^{abcd}	82.0^{a}	79.3^{efg}	81.7^{ab}	81.4^{abc}	80.3^{bcde}	79.0^{efgh}	79.2^{efgh}	0.54	<0.01
Energy metabolizability, %	78.2^{a}	78.3^{a}	75.8^{cdef}	78.3^{a}	77.6^{ab}	76.9^{abcd}	75.5^{def}	76.3^{bcdef}	0.66	<0.01
Sample										
DE, kcal/kg	3,898^{abc}	3,994^{a}	3,763^{cde}	3,982^{a}	3,927^{ab}	3,937^{a}	3,796^{bcde}	3,735^{de}	51.5	<0.01
ME, kcal/kg	3,698^{ab}	3,725^{a}	3,507^{de}	3,737^{a}	3,646^{abcd}	3,687^{abc}	3,542^{bcde}	3,541^{bcde}	62.7	<0.01

^{a,b,c,d,e,f,g,h}Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 3.7. Analyzed composition of the experimental diets containing the unground distillers dried grains with solubles sources for Laboratories 1 and 2 (DM basis); Exp. 1

	DDGS source																
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Laboratory 1:																	
DM, %	86.20	87.73	88.19	89.15	88.89	89.60	88.93	90.24	86.89	87.45	88.82	88.64	89.00	88.78	87.20	88.22	88.88
Ash, %	4.93	4.71	4.37	4.31	4.56	4.42	4.63	4.18	4.67	4.92	4.78	4.74	5.67	5.42	4.75	5.41	5.57
CP, %	25.00	24.90	25.00	24.80	24.30	24.70	23.70	23.80	25.90	24.50	22.80	24.70	25.30	24.70	23.40	25.20	25.50
Crude fat, %	5.31	8.65	7.40	7.77	7.63	7.04	7.59	7.56	7.46	7.69	7.29	14.60	8.25	7.22	7.34	7.45	7.24
Crude fiber, %	4.10	4.24	4.28	3.78	4.21	3.61	3.82	5.46	3.00	4.35	4.09	4.39	3.80	4.94	3.41	5.18	5.16
ADF, %	9.08	6.84	7.59	6.65	6.79	7.28	6.05	7.80	9.75	7.29	6.64	8.00	5.35	5.48	6.12	8.70	7.08
NDF, %	21.20	20.20	19.40	17.00	18.60	14.90	16.10	18.10	15.60	16.60	16.40	19.70	18.40	19.40	20.00	24.40	20.80
Total starch, %	28.60	27.70	27.67	29.46	30.05	31.98	29.83	29.49	34.76	30.82	31.50	32.18	32.55	33.94	29.76	30.19	31.28
Laboratory 2																	
DM, %:	86.61	88.67	88.13	89.78	89.72	89.77	89.28	90.48	88.37	88.70	90.15	90.31	91.01	90.39	89.11	89.96	90.36
Ash, %	5.76	5.43	5.22	5.40	5.34	5.17	5.02	4.80	5.10	5.45	5.19	5.24	5.37	5.85	5.51	5.09	5.31
CP, %	23.30	23.89	26.02	24.82	24.72	25.37	23.96	24.26	24.87	23.06	24.06	23.96	25.04	24.43	24.53	24.39	24.14
Crude fat, %	5.24	8.52	7.23	7.83	8.23	6.35	7.48	7.38	7.03	7.11	7.49	7.91	7.99	7.11	7.81	7.16	7.32
Crude fiber, %	5.22	4.41	4.88	4.38	4.41	4.41	4.32	4.71	4.16	4.24	4.16	4.39	4.23	4.69	4.42	4.65	4.24
ADF, %	8.20	6.90	7.20	6.94	7.95	7.80	6.12	7.34	8.66	6.24	5.62	6.83	6.46	5.58	6.93	7.41	6.78
NDF, %	26.58	27.37	28.53	25.68	29.28	23.71	25.17	26.09	23.96	34.44	25.42	31.46	30.66	36.91	26.00	26.65	33.43
Total starch, %	30.74	31.59	29.99	31.96	32.83	31.58	32.65	30.10	32.86	31.04	30.74	30.21	29.54	29.77	31.40	30.01	28.88

Table 3.8. Analyzed composition of the experimental diets containing the ground distillers dried grains with solubles sources for Laboratories 1 and 2 (DM basis); Exp. 2

	DDGS source																
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Laboratory 1:																	
DM, %	88.09	88.70	88.43	89.48	89.43	89.68	89.33	90.42	87.84	88.51	88.82	88.83	89.00	88.78	87.63	88.66	88.52
Ash, %	4.95	4.72	4.48	5.00	4.86	4.74	4.41	4.11	5.00	5.25	4.78	4.72	5.24	5.42	4.60	5.55	5.44
CP, %	24.40	24.50	26.60	25.60	24.60	24.90	22.10	22.60	24.40	23.90	22.80	23.30	25.10	24.70	23.20	25.00	25.30
Crude fat, %	5.67	8.54	7.77	7.72	7.38	6.39	7.99	7.42	7.21	7.23	7.29	7.96	8.31	7.22	8.16	6.84	6.98
Crude fiber, %	4.27	4.96	4.50	3.83	4.78	2.44	4.28	4.32	3.99	4.34	4.09	3.74	4.58	4.94	4.24	4.17	4.96
ADF, %	6.79	6.06	6.60	7.11	7.90	8.20	4.70	6.44	8.15	7.08	6.64	5.92	7.27	5.48	6.80	7.94	7.09
NDF, %	19.20	18.70	19.30	16.20	17.20	15.90	14.70	18.90	16.30	16.20	16.40	16.20	16.70	19.40	19.40	22.20	22.40
Total starch, %	31.72	32.92	31.52	29.11	27.59	34.85	30.95	30.33	32.71	38.01	31.50	35.17	29.36	33.94	33.14	30.92	33.51
Laboratory 2																	
DM, %:	89.19	90.24	90.22	90.51	90.52	91.03	90.60	91.28	90.27	90.66	91.03	91.09	91.78	91.23	90.63	91.19	90.87
Ash, %	5.65	5.49	5.09	5.52	5.44	5.20	5.22	4.82	5.35	5.29	5.19	5.48	5.47	5.85	5.36	5.44	5.07
CP, %	23.44	23.16	25.85	25.26	24.78	25.52	23.71	24.22	24.34	23.19	24.06	23.90	24.73	24.43	23.92	24.26	24.46
Crude fat, %	5.61	8.43	8.24	7.62	7.83	6.69	7.35	7.06	5.79	7.18	7.49	7.72	7.62	7.11	7.73	7.22	7.34
Crude fiber, %	4.89	4.07	4.46	4.15	4.28	4.14	3.91	4.60	3.81	4.20	4.16	4.24	4.06	4.69	4.46	4.57	4.21
ADF, %	7.18	6.97	7.40	6.11	6.71	7.24	5.63	7.35	8.22	6.13	5.62	6.60	6.20	5.58	6.47	7.38	6.56
NDF, %	29.31	24.42	25.70	23.31	23.20	38.54	23.54	25.72	30.29	26.89	25.42	43.14	35.07	36.91	26.24	26.10	24.82
Total starch, %	32.85	32.66	28.75	32.14	31.05	31.01	32.51	31.85	32.75	32.23	30.74	30.52	30.66	29.77	30.60	29.54	29.80

Table 3.9. Least-squares means for the effect of experimental diets containing the ground distillers dried grains with solubles samples on DE and ME (DM basis); Exp. 2.

		DDGS source								
Item	Corn[1]	1	2	3	4	5	6	7	8	9
GE:										
Intake, kcal/d	4,874	4,976	4,919	4,810	4,912	4,848	4,850	4,925	4,876	4,870
Output in dried feces,										
Total, kcal/kg	5,004	4,952^{cdef}	4,969^{cd}	5,013^{a}	4,972^{c}	4,989^{abc}	4,934^{def}	5,026^{a}	5,012^{ab}	4,973^{bc}
Daily, kcal/d	564	1,042^{a}	876^{ef}	966^{abcd}	897^{cdef}	976^{abc}	868^{ef}	879^{def}	970^{abc}	897^{cdef}
Output in dried urine,										
Total, kcal/kg	2,033	2,339	1,983	2,128	2,189	2,164	2,139	2,072	2,120	2,212
Daily, kcal/d	90	182	143	154	166	155	132	162	140	160
Energy concentration										
Experimental diet										
DE, kcal/kg	3,951	3,842^{g}	4,071^{a}	3,959^{def}	4,024^{abcd}	3,935^{ef}	4,042^{abc}	4,052^{ab}	3,965^{def}	4,043^{abc}
ME kcal/kg	3,878	3,669^{g}	3,921^{a}	3,794^{cdef}	3,859^{abcd}	3,776^{def}	3,900^{ab}	3,901^{ab}	3,818^{bcdef}	3,875^{abc}
Dry matter digestibility, %	89.6	79.9^{h}	82.1^{abc}	80.1^{gh}	81.8^{abcde}	80.5^{efgh}	82.4^{ab}	82.4^{ab}	80.6^{defgh}	81.6^{abcdef}
Energy digestibility, %	88.4	79.4^{g}	82.1^{abc}	79.9^{g}	81.6^{abcd}	80.1^{efg}	82.3^{ab}	82.2^{ab}	80.4^{defg}	81.5^{abcde}
Energy metabolizability, %	85.6	75.8^{g}	79.1^{abc}	76.6^{fg}	78.3^{abcde}	76.9^{efg}	79.4^{a}	79.3^{ab}	77.4^{defg}	78.1^{abcdef}
Sample										
DE, kcal/kg	3,883	3,673^{g}	4,115^{a}	3,899^{def}	4,024^{abcd}	3,852^{ef}	4,058^{abc}	4,079^{ab}	3,909^{def}	4,060^{abc}
ME kcal/kg	3,818	3,414^{g}	3,900^{a}	3,655^{cdef}	3,784^{abc}	3,622^{def}	3,857^{ab}	3,860^{ab}	3,700^{bcdef}	3,778^{abc}

[1]Means from the corn sample was used as a covariate.

[a,b,c,d,e,f,g,h]Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 3.9 (cont). Least-squares means for the effect of experimental diets containing the ground distillers dried grains with solubles samples on DE and ME (DM basis); Exp. 2

	DDGS source									
Item	10	11	12	13	14	15	16	17	SEM	*P*-value
GE:										
Intake, kcal/d	4,921	4,838	4,913	4,937	4,845	4,873	4,899	4,853	72.6	0.39
Output in dried feces,										
Total, kcal/kg	4,933^{def}	4,912^{fg}	4,926^{efg}	4,927^{ef}	4,814^{h}	4,958^{cde}	4,974^{abc}	4,886^{g}	17.4	<0.01
Daily, kcal/d	890^{cdef}	868^{ef}	956^{abcde}	843^{f}	905^{bcdef}	911^{bcdef}	990^{ab}	937^{bcde}	44.1	<0.01
Output in dried urine,										
Total, kcal/kg	2,044	2,158	2,126	2,090	2,091	2,176	2,099	2,218	81.9	0.44
Daily, kcal/d	137	171	163	164	169	163	147	163	16.1	0.64
Energy concentration										
Experimental diet										
DE, kcal/kg	3,988^{bcdef}	4,002^{abcde}	3,964^{def}	4,066^{a}	3,974^{cdef}	4,020^{abcd}	3,922^{f}	3,931^{f}	27.6	<0.01
ME kcal/kg	3,847^{abcde}	3,826^{bcdef}	3,809^{bcdef}	3,898^{ab}	3,793^{def}	3,854^{abcde}	3,773^{ef}	3,760^{f}	34.0	<0.01
Dry matter digestibility, %	82.0^{abcd}	81.9^{abcde}	80.8^{cdefgh}	82.9^{a}	81.1^{bcdefgh}	81.4^{bcdefg}	80.4^{fgh}	80.6^{defgh}	0.54	<0.01
Energy digestibility, %	81.8^{abcd}	81.8^{abcd}	80.7^{cdefg}	82.9^{a}	81.4^{bcdef}	81.3^{bcdef}	80.1^{fg}	80.6^{defg}	0.56	<0.01
Energy metabolizability, %	78.9^{abcd}	78.2^{abcdef}	77.4^{cdefg}	79.5^{a}	77.7^{bcdef}	78.0^{abcdef}	77.1^{efg}	77.1^{efg}	0.68	<0.01
Sample										
DE, kcal/kg	3,953^{bcdef}	3,980^{abcde}	3,908^{def}	4,105^{a}	3,927^{cdef}	4,015^{abcd}	3,826^{f}	3,843^{f}	53.3	<0.01
ME kcal/kg	3,757^{abcdef}	3,714^{bcdef}	3,686^{bcdef}	3,854^{ab}	3,654^{cdef}	3,771^{abcde}	3,613^{ef}	3,597^{f}	65.2	<0.01

^{a,b,c,d,e,f,g,h}Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 3.10. Least-squares means for the effect of distillers dried grains with solubles source and particle size on DE and ME (DM basis); Exp. 2

	DDGS source										
Item	1	2	3	4	5	6	7	8	9	10	12
GE:											
Intake, kcal/d	4,957	4,964	4,872	4,950	4,893	4,917	4,949	4,905	4,876	4,938	4,924
Output in dried feces,											
Total, kcal/kg											
Unground	5,013^{ghi}	5,087^{bc}	5,114^{ab}	5,065^{cde}	5,041^{defg}	5,018^{ghi}	5,123^{ab}	5,138^{a}	5,076^{cd}	4,999^{hij}	5,034^{efgh}
Ground	4,958^{kl}	4,964^{kl}	5,018^{ghi}	4,970^{jk}	4,985^{ijk}	4,931^{lm}	5,027^{fgh}	5,004^{hij}	4,972^{jk}	4,930^{lm}	4,921^{m}
Daily, kcal/d	1,040^{a}	930^{efg}	1,007^{abc}	951^{cdef}	993^{abcd}	909^{fg}	929^{efg}	1,018^{ab}	912^{fg}	906^{fg}	978^{bcde}
Output in dried urine,											
Total, kcal/kg	2,252	2,070	2,139	2,201	2,148	2,192	2,121	2,165	2,224	2,087	2,166
Daily, kcal/d	183	156	159	160	153	161	159	145	168	136	161
Energy concentration											
Experimental diet											
DE, kcal/kg	3,821^{e}	4,022^{ab}	3,937^{cd}	3,988^{ab}	3,908^{d}	3,997^{ab}	4,001^{ab}	3,903^{d}	4,013^{ab}	3,975^{bc}	3,924^{cd}
ME kcal/kg	3,644^{d}	3,860^{a}	3,768^{bc}	3,825^{ab}	3,753^{c}	3,832^{a}	3,842^{a}	3,755^{c}	3,836^{a}	3,836^{a}	3,762^{c}
Dry matter digestibility, %	79.5^{d}	81.3^{b}	79.6^{d}	81.3^{b}	79.9^{cd}	81.8^{ab}	81.6^{ab}	79.7^{d}	81.4^{b}	81.9^{ab}	80.2^{cd}
Energy digestibility, %	78.9^{f}	81.1^{b}	79.2^{ef}	80.9^{bc}	79.5^{ef}	81.5^{ab}	81.1^{b}	79.1^{ef}	81.1^{b}	81.5^{ab}	79.9^{cde}
Energy metabolizability, %	75.2^{h}	77.8^{abcde}	75.8^{gh}	77.6^{bcde}	76.4^{fgh}	78.2^{abc}	77.9^{abcd}	76.1^{fgh}	77.6^{bcde}	78.7^{ab}	76.6^{efg}
Sample											
DE, kcal/kg	3,632^{e}	4,021^{ab}	3,855^{cd}	3,955^{ab}	3,800^{d}	3,972^{ab}	3,978^{ab}	3,790^{d}	4,001^{ab}	3,929^{bc}	3,831^{cd}
ME kcal/kg	3,365^{d}	3,786^{a}	3,603^{bc}	3,717^{a}	3,576^{c}	3,727^{a}	3,746^{a}	3,582^{c}	3,716^{a}	3,736^{a}	3,594^{c}

^{a,b,c,d,e,f,g,h}Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 3.10 (cont). Least-squares means for the effect of distillers dried grains with solubles source and particle size on DE and ME (DM basis); Exp. 2

	DDGS source					Particle Size			*P*-values		
Item	13	15	16	17	SEM	Unground	Ground	SEM	Source	PS	Source × PS
GE:											
Intake, kcal/d	4,947	4,923	4,948	4,940	62.8	4,902^{b}	4,952^{a}	57.2	0.35	<0.01	0.25
Output in dried feces,											
Total, kcal/kg											
Unground	5,063^{cdef}	5,041^{defg}	5,075^{cd}	4,970^{jk}	15.7	-	-	-	-	-	0.04
Ground	4,929^{lm}	4,953^{klm}	4,983^{ijk}	4,883^{n}	-	-	-	-	-	-	-
Daily, kcal/d	884^{g}	940^{defg}	1,018^{ab}	987^{abcd}	33.3	993^{a}	927^{b}	27.4	<0.01	<0.01	0.72
Output in dried urine,											
Total, kcal/kg	2,061	2,201	2,165	2,117	60.5	2,162	2,146	35.4	0.35	0.55	0.87
Daily, kcal/d	161	170	163	149	11.7	162	156	7.5	0.27	0.18	0.86
Energy concentration											
Experimental diet											
DE, kcal/kg	4,032^{a}	3,996^{ab}	3,912^{d}	3,900^{d}	20.4	3,921^{b}	3,990^{a}	11.6	<0.01	<0.01	0.76
ME kcal/kg	3,870^{a}	3,822^{ab}	3,750^{c}	3,747^{c}	25.1	3,756^{b}	3,831^{a}	15.3	<0.01	<0.01	0.84
Dry matter digestibility, %	82.6^{a}	80.9^{bc}	80.0^{cd}	80.0^{cd}	0.40	80.2^{b}	81.3^{a}	0.22	<0.01	<0.01	0.95
Energy digestibility, %	82.3^{a}	80.7^{bcd}	79.5^{ef}	79.9^{def}	0.41	79.7^{b}	81.2^{a}	0.23	<0.01	<0.01	0.91
Energy metabolizability, %	78.9^{a}	77.2^{cdef}	76.2^{fgh}	76.7^{defg}	0.50	76.3^{b}	77.9^{a}	0.31	<0.01	<0.01	0.94
Sample											
DE, kcal/kg	4,039^{a}	3,970^{ab}	3,807^{d}	3,784^{d}	39.5	3,824^{b}	3,958^{a}	22.4	<0.01	<0.01	0.76
ME kcal/kg	3,802^{a}	3,710^{ab}	3,568^{c}	3,567^{c}	48.0	3,581^{b}	3,725^{a}	29.0	<0.01	<0.01	0.88

PS = particle size.

Table 3.11. Least-squares means for the effect of particle size reduction of a sample of distillers dried grains with solubles on DE and ME (DM basis); Exp. 3

	Particle size (µm)						*P*-value		
Item	351	560	890	1180	1557	SEM	Treatment	Linear	Quadratic
GE:									
Intake, kcal/d	4,957	5,083	4,894	5,054	4,760	94.2	0.12	0.17	0.15
Output in dried feces,									
Total, kcal/kg	4,991[b]	5,053[a]	5,094[a]	5,079[a]	5,084[a]	16.1	<0.01	<0.01	<0.01
Daily, kcal/d	897[b]	1,028[a]	949[ab]	1,021 [a]	908[b]	45.1	0.02	0.90	0.01
Output in dried urine,									
Total, kcal/kg	2,201	2,310	2,327	2,124	2,256	66.9	0.14	0.69	0.14
Daily, kcal/d	155	206	181	190	185	18.3	0.28	0.40	0.20
Energy concentration									
Experimental diet									
DE, kcal/kg	4,040	3,959	3,912	3,956	3,973	31.1	0.09	0.17	0.02
ME kcal/kg	3,875[a]	3,765[b]	3,708[b]	3,760[b]	3,782[b]	35.3	0.02	0.06	0.01
Dry matter digestibility, %	81.7	80.5	80.0	80.6	81.1	0.63	0.40	0.61	0.07
Energy digestibility, %	81.5	80.0	79.3	80.1	80.5	0.63	0.17	0.33	0.03
Energy metabolizability, %	78.2[a]	76.1[b]	75.2[b]	76.1[b]	76.6[ab]	0.71	0.04	0.12	0.01
Sample									
DE, kcal/kg	4,054	3,899	3,807	3,893	3,925	60.1	0.09	0.17	0.02
ME kcal/kg	3,812[a]	3,600[b]	3,490[b]	3,590[b]	3,633[b]	68.3	0.02	0.06	0.01

[a] Means from the corn sample was used as a covariate.

CHAPTER 4

EQUATIONS TO PREDICT THE METABOLIZABLE ENERGY OF DISTILLERS DRIED GRAINS WITH SOLUBLES FROM DIFFERENT SOURCES FED TO PIGS

ABSTRACT: Regression equations to predict the ME of DDGS based on chemical composition and particle size were developed. Samples of DDGS from 17 sources were chosen to represent the variation in nutrient and energy concentration available to the industry in the US in 2008. The samples were evaluated as-received from the ethanol plants. An experiment was conducted to determine the apparent DE and ME of the DDGS samples (Chapter 3); in addition, the chemical composition (CP, crude fat, crude fiber, ADF, NDF, ash, and starch) of each DDGS sample was analyzed by 2 laboratories. The geometric particle size was also determined. Correlation analyses were conducted to evaluate the relationships between the chemical composition and particle size of the samples and energy concentration. Regression equations to predict the ME of DDGS, based on chemical composition analyzed by each laboratory and particle size, were developed using the PROC REG procedure of SAS. Equations that gave the greatest $\bar{R}^2$ values were selected. There was considerable variation in the chemical composition of the 17 DDGS sources, as well as large differences between the results of the chemical analyses for the 2 laboratories for a number of the chemical components. In general, the magnitude of correlations between DE and ME and chemical composition of the samples were relatively weak indicating that individual chemical components were poor predictors of the energy concentration of the DGGS samples. In addition, both the chemical components that showed the strongest correlation with ME in DDGS and the most accurate equation to predict ME differed between the 2 laboratories used to analyze the samples. For Laboratories 1 and 2,

$\bar{R}^2$ values were maximized using a 4-variable equation, however, different chemical components were included in the equation for each of the laboratories (crude fiber, ADF, NDF, and GE for the equation based on Laboratory 1; CP, crude fat, NDF, and Starch for Laboratory 2; with $\bar{R}^2$ values 0.79 and 0.75, respectively). This experiment highlighted the large variation in nutrient composition of DDGS sources available in the US in 2008 and, also, that the most accurate equation to predict the ME of DDGS differed between the 2 laboratories used for the chemical analysis.

INTRODUCTION

The energy concentration of feed ingredients for swine is a major determinant of nutritive value and its evaluation is, therefore, of great importance for diet formulation. Commonly, values of the energy concentration of individual feedstuffs are obtained from tables such as those from NRC (2012). However, the chemical composition of individual feed ingredients, including biofuel co-products, can be variable and, therefore, the energy concentration can also vary considerably. The most accurate estimate of energy concentration is obtained by direct measurement of the apparent DE and ME, but this approach is, obviously, time consuming, and special facilities and equipment are needed, and in the best circumstances, only a very limited number of samples can be evaluated (Leukule et al., 1990). In practice, variability in energy concentration of a feedstuff, for example distillers dried grains with solubles (**DDGS**), can be accounted for by using prediction equations based on the chemical composition of individual samples. Nonetheless, there has been limited research to develop prediction equations specifically for DDGS. Equations that are currently available to predict the ME of DDGS or corn co-products (including DDGS) based on chemical composition (Pedersen et al., 2007; Anderson, 2009; Anderson et al., 2012; Kerr and Shurson, 2013) give widely different values for the same sample, resulting in confusion over the most appropriate equation to use. In addition, over- or under-estimation of the energy value of DDGS can be very costly for swine producers, especially in times of high ingredient prices. Therefore, the research presented in this chapter aimed to develop equations to predict the ME of DDGS from a wide variety of sources that could be used with the range of materials available in 2008 from Midwestern ethanol plants.

MATERIALS AND METHODS

DDGS Sources and Sampling Procedures

Samples of DDGS were obtained from a total of 17 different sources (ethanol plants) located in the Midwest of the US (IA, IL, MO, MN, and SD). A sample of approximately 140 kg of material was obtained from each source in June and July 2008 and samples were shipped to the Pioneer Livestock Nutrition Center (Polk City, IA) where the experiments were conducted. The samples were placed in plastic containers (PROBOX® Millford, OH) for storage prior to being used for experimental diet manufacture.

A 2-kg sample of the material (as-received) was collected from each of the plastic containers using a grain probe with samples being collected at different depths and locations across the container to ensure that a representative sample of the material was obtained. The sample was thoroughly mixed and subsamples were taken for analysis.

Measurements

Chemical analyses of the samples were conducted at 2 independent laboratories (Midwest Laboratories, Omaha, NE and Experimental Station Chemical Laboratories, Univ. of Missouri, Columbia, MO) that are widely used by the feed industry in the US. Components analyzed by the 2 laboratories were ash, CP, crude fat, crude fiber, ADF, NDF, and total starch. Details of the analytical methodology used by the 2 laboratories are presented in Table 4.1 and, as can be seen, the laboratories used different methodology for some of the components.

Estimates of the GE (Table 3.3), DE, and ME (Table 3.6) of the samples came from the experiments reported in Chapter 3. In addition, the geometric mean particle size of each source of DDGS was measured as described in Chapter 3 (Table 3.1). The analyses of particle size of the DDGS samples and the energy concentration of experimental diets, feces, and urine reported

in Chapter 3 were carried out by a third laboratory (Pioneer Hi-Bred International, Inc., Urbandale, IA) and not by the laboratories that carried out the chemical analysis of the DDGS sources.

Statistical Analysis

Correlation analysis between measured GE, DE, and ME and the individual chemical components (ash, CP, crude fat, crude fiber, ADF, NDF, total starch) for the analysis carried out by each laboratory and for the combined laboratory analysis (average of the values of the 2 laboratories), and particle size was carried out using the PROC CORR procedure of SAS (SAS Inst. Inc., Cary, NC.). In addition, linear regression equations to predict the ME of DDGS based on chemical components (ash, CP, crude fat, crude fiber, ADF, NDF, total starch, and GE) and particle size were developed using the PROC REG procedures of SAS (Littell et al., 1996). The coefficient of determination, denoted as R^2 and adjusted coefficient of determination, denoted as $\bar{R}^2$ values were calculated as measures of how well the least-squares equation ($\hat{y} = bo + b_1x$) performs as a predictor of the dependent variable. These were calculated as follows:

$$R^2 = 1 - \left(\frac{SSE}{SSTO}\right)$$

$$\bar{R}^2 = 1 - \left(\frac{n-1}{n-p}\right) - \left(\frac{SSE}{SSTO}\right)$$

where SSE is the error sums of squares and SSTO the total sums of squares; *n* is the number of observations and *p* is the number of parameters in the equation.

However, the choice of equations was made using the $\bar{R}^2$ statistic as a criterion, with the equation giving the highest $\bar{R}^2$ being chosen within each approach described above. As noted in the equations above, the R^2 statistic does not take into account the number of parameters in the

regression model and, consequently, the R^2 value can never decrease as the number variables in the equation increases. This assumes that every independent variable in the model helps to explain the variation in the dependent variable, although some may not significantly contribute to the model. On the other hand, the $\bar{R}^2$ statistic takes into account the number of parameters in the regression model using the degrees of freedom (Kutner et al., 2004). Thus, the $\bar{R}^2$ statistic helps to explain the variation in the dependent variable by only those variables that truly affect the dependent variable. Because of this, the $\bar{R}^2$ statistic can decrease as the number of parameters in the model increases. This occurs when the increase in the R^2 by including additional variables in the model becomes so small that it is not sufficient to offset the loss of an additional degree of freedom. Thus, it penalizes the addition of independent variables that do not explain addition variation in the dependent variable.

In addition, the Mallow's C (p) statistic (Mallows, 1973) was used as additional criteria for the evaluation of the regression equations, which is defined as follows:

$$C_p = \frac{SSE_p}{s^2} - (n - 2p)$$

where SSE_p is the error sums of square of the model containing *p* explanatory variables including the intercept (i.e., the number of parameters in the subset model); s^2 is the mean square error for the model containing all explanatory variables.

When using the Mallow's C(p) statistic, we seek to identify subsets of independent variables for, which the C(p) is small and is close to *p* (i.e., the number of parameters in the equation). Equations with small C(p) values have a small total mean squared error, and when the C(p) is near p, the bias of the regression model is small (Kutner et al., 2004).

All three statistics above are used to assess the goodness of fit of the estimated regression model; however neither R^2, $\bar{R}^2$, or Mallow's C (p) statistics indicate the error associated with the

prediction. Therefore, the root mean square error (**RMSE**), also called the residual standard deviation (**RSD**) or standard error, provided an indication of the error term associated with the model selected, and it is defined as follows:

$$\text{RMSE} = \sqrt{MSE} = \sqrt{\frac{SSE}{n-2}}$$

where MSE is mean square error, SSE is error sums of square.

RESULTS AND DISCUSSION

Chemical Composition of the DDGS Sources

The results for the chemical analyses of the 17 DDGS samples carried out by Laboratory 1, Laboratory 2, and the combined analysis (the average of the results for the 2 laboratories) are presented in Table 4.2 and the descriptive statistics for these measures are presented in Table 4.3. Overall mean values for DM, ash, and CP content of the 17 samples were relatively similar with differences between Laboratory 1 and 2 of -1.6, 2.0 and 0.8%, respectively (Table 4.3). However, for the other chemical components (i.e., crude fiber, crude fat, ADF, NDF, and total starch) there were relatively large differences between the mean values for the 2 laboratories. For example, the average value for NDF for Laboratory 1 was 28.2% less compared to the average of NDF values measured by Laboratory 2, and the value for starch for Laboratory 1 was 38% greater compared to Laboratory 2. Differences between the average values from Laboratory 1 compared to Laboratory 2 for crude fiber, crude fat, and ADF were 7.6, -17.8 and 8.9%, respectively.

In addition, the within-laboratory variation in composition between the 17 sources was also different for the 2 laboratories for a number of the chemical components. This is illustrated

by both the SD and CV and the range in values between sources observed for the 2 laboratories for a number of the components (Tables 4.3). For example, the SD of crude fat content measured by Laboratory 2 was 33% greater than that for Laboratory 1 (Table 4.3) and the within-laboratory range in crude fat levels for the 17 DDGS samples from Laboratory 1 was from 8.7% to 11.5% and for Laboratory 2 was from 9.5 to 14.6% (Table 4.3).

The chemical composition for the corn and corn-based diet is shown in Table 3.4; in general, there was agreement (less than 1 percentage units difference) between the 2 laboratories in the analyzed values; however, for the corn sample, the DM, ADF, and total starch showed a greater difference between the laboratories (1.4, 1.5 and 1.7 percentage units, respectively). For the corn-based diet, crude fiber, NDF and total starch showed a difference between the laboratories of 1.2, 1.1, and 1.9 percentage units, respectively. These differences could, in part, be due to the differences between the 2 laboratories (Table 4.1) for the chemical components in which the differences exceeded 1 percentage units.

Correlations between DDGS GE, DE and ME, and Chemical Composition

Pearson correlation coefficients between the energy concentration, chemical composition, and particle size of the DDGS samples for the analyses carried out by Laboratory 1, Laboratory 2, and the average values for the 2 laboratories are presented in Table 4.4. In general, for both laboratories and for the combined laboratory data, the correlations between energy concentration and chemical components were weak to moderate in magnitude, and correlations between individual chemical components and particle size were also relatively weak. In addition, the statistically significant correlations differed ($P < 0.05$) depending on the laboratory used to analyze the chemical composition (Table 4.4).

The GE of the DDGS sources was not correlated with DE and ME (Table 4.4), which is similar to the findings of Cozannet el al. (2010) who showed that the correlation between GE and DE of wheat DDGS was weak (0.11). However, the correlation between DE and ME was very strong (0.97), which is in agreement with Löwgren et al. (1992) who reported that DE and ME of barley and triticale had a correlation coefficient of 0.99.

However, for both laboratories and the combined laboratory data, most nutrients showed relatively weak correlations with the GE of DDGS (Table 4.4). The exceptions for Laboratory 1 were for ash and NDF with correlations of -0.64 and 0.65, respectively. For Laboratory 2 and the combined laboratories (Table 4.4), ash was negatively correlated (-0.59 and -0.63, respectively), and crude fat and NDF were positively correlated (0.56 and 0.59, and 0.50 and 0.60, for Laboratory 2 and the combined laboratories, respectively) with GE. There is limited published information on correlations between energy concentration and chemical composition of corn DDGS, however, for wheat DDGS, Cozanet et al. (2010) reported significant correlations between GE and crude fiber and starch (0.67 and -0.69, respectively). Research by Noblet and Perez (1993), which was conducted with 114 types of compound feeds 7 of which included corn DDGS as a major ingredient, showed correlations between GE and crude fat (0.92) and starch (-0.37). Although in the present experiment, the correlation between starch and GE was not significant ($I > 0.05$), the correlations were negative (-0.42, -0.24, and -0.34 for Laboratories 1, 2 and the combined laboratories, respectively). Cozanet et al. (2010) and Noblet and Perez (1993) both reported significant negative correlations between GE and starch content of wheat DDGS and compound feeds, respectively.

There were negative correlations between the DE of DDGS and chemical components such as crude fiber (-0.57; Laboratory 2), ADF (-0.60 and -0.47 for Laboratory 1 and the

combined laboratories, respectively) and NDF (-0.49, -0.60, and -0.58 for Laboratory 1, 2, and the combined laboratories, respectively) with only one positive correlation, which was with starch for Laboratory 2 (0.53; Table 4.4). Five studies have reported correlation analyses between DE and chemical composition (Table 4.5); with 3 of the studies using compound feeds (Spanghero and Volpelli, 1999; Morgan et al., 1987; Noblet and Perez, 1993) and 2 used individual feedstuffs such as barley, triticale (Löwgren et al., 1992) and wheat DDGS (Cozanet et al., 2010). For these studies, the strongest relationships (all negative) between DE and chemical components were with NDF (3 out of the 5 studies), ADF (one experiment), and crude fiber and ash (one experiment).

The ME of the DDGS samples was negatively correlated with ADF (-0.67 and -0.56; Laboratory 1 and the combined laboratories, respectively), crude fiber (-0.66; Laboratory 2) and NDF (-0.55 and -0.54; Laboratory 2 and the combined laboratories, respectively) and positively correlated with crude fat (0.54; Laboratory 2; Table 4.4). Reports in the literature have shown substantial variation for the correlations between ME and chemical composition. For example, crude fat was positively correlated in the research reported by Just et al. (1984) in compound feeds and individual feedstuffs; in contrast, Löwgren et al. (1982) showed negative correlations between ME and crude fat (-0.81). In agreement with the present experiment, crude fiber showed negative correlations with ME in 3 studies (Morgan et al., 1987; Löwgren et al., 1992; Leukule et al., 1990; Table 4.5) and NDF in 1 experiment (Morgan et al., 1987; Table 4.5).

There were only a few significant correlations between individual chemical components in the present experiment; for example, for Laboratory 1, ADF was negatively correlated with crude fat (-0.60) and NDF was negatively correlated with starch (-0.72). For Laboratory 2, ADF was positively correlated with CP (0.54), and starch and crude fiber were negatively correlated (-

0.57). In addition, particle size was positively correlated with ADF for Laboratory 1 (0.55) and with starch for Laboratory 2 (0.47).

Equations to Predict the ME of DDGS from Chemical Composition

A total of 9 analyzed components of the DDGS sources (ash, CP, crude fiber, crude fat, ADF, NDF, starch, GE, and particle size) could potentially have been included in a regression equation to predict the ME of DDGS. In addition, the 9 variables were available for the separate and combined analysis for the 2 laboratories. Consequently, a large number of regression equations to predict ME were generated, ranging from single to 9-variable equations, and for the separate and combined laboratory analyses. Different approaches were taken to produce prediction equations. The information most commonly available commercially includes proximate components (ash, CP, crude fiber and crude fat), with equations using these components only presented in Table 4.6 (equations using one to four variables for both laboratories individually or in combination). In addition, in some situations, some or all of the additional components analyzed in this experiment (i.e., ADF, NDF, starch, GE, and particle size) may be available, and equations based either on these components in combination with proximate analysis components or alone are presented in Table 4.6 (equations using five to nine variables) and 4.7 (equations using one to five variables). Finally, selected equations using all possible combinations of variables (equations using one to nine variables) are presented in Table 4.7. A number of statistics were computed for all equations including the R^2, $\bar{R}^2$, the C(p) value, and the root mean square error (RMSE). The $\bar{R}^2$ statistic was used to select the most appropriate equation, with that giving the highest $\bar{R}^2$ being the equation of choice. In addition, the R^2, C(p) value and RMSE were computed and these are also presented for reference.

Analytical Laboratory Effects on Equations to Predict the ME of DDGS

There was variation between laboratories both in terms of the components of proximate analysis that had the strongest relationship (highest $\bar{R}^2$ value) with ME of DDGS and, also, for the actual parameters in the regression equations to estimate ME of DDGS (Tables 4.6, 4.7, and 4.8) which can be explained by the differences in the reference methods used between the two laboratories. For example, the equations with the highest $\bar{R}^2$ from Laboratory 1 that used proximate components and the additional components included NDF, starch, GE and particle size (Equation number 8; Table 4.6), whereas for Laboratory 2, it was based on proximate components plus NDF and starch (Equation number 15; Table 4.6).

There are numerous other examples of differences between the 2 laboratories used in this experiment for the regression relationships between chemical components of DDGS and ME. This has major implications for the use of equations to predict the ME of DDGS, and perhaps other ingredients, based on chemical components because it implies that such equations are specific to the laboratory that carries out the chemical analysis of the original samples that were used to develop the equations. In other words, these observations indicate that prediction equations need to be developed for individual laboratories and the results of the chemical analysis carried out by any laboratory should not be used to estimate ME based on an equation developed for another laboratory.

An obvious reason for differences between laboratories in the relationship between chemical components and ME of DDGS relates to the different methodology used for the chemical analysis (Table 4.1). Because of the major practical implications of this result it is important that further research is carried out to validate this finding and to establish the reason

(s) for this variation between laboratories, ideally involving a greater number of laboratories than used in the present experiment.

Equations to Predict the ME of DDGS from Proximate Analysis

Selected equations based on proximate analysis components only (ash, CP, crude fat and crude fiber) are presented in Table 4.6 (equations 1 to 4, 10 to 13, and 19 to 22 for Laboratories 1, 2, and combined, respectively). Individual components of proximate analysis were relatively poor predictors of ME, with the best predictor being crude fiber for both Laboratory 1 and 2 ($\bar{R}^2$ = 0.04 and 0.39, respectively; equations 1 and 10, respectively), and crude fat for the combined analysis for the two laboratories ($\bar{R}^2$= 0.15; Equation number 19). Including all 4 proximate analysis components in the prediction equation resulted in relatively low and even negative $\bar{R}^2$ values, indicating that the proximate analysis components alone or in combination are relatively poor predictors of the ME of DDGS.

Including the other components in the equation along with proximate analysis components increased $\bar{R}^2$ values (Table 4.6), with the magnitude of the increase decreasing as the number of variables in the equation increased. Thus, for Laboratory 1 the best 5-variable equation [proximate analysis plus ADF (Equation number 5)] had an $\bar{R}^2$ value of 0.54 compared to -0.01 (Equation number 4) for the 4-variable equation (Table 4.6). Adding additional variables to create 6-, 7- ,and 8-variable equations increased $\bar{R}^2$ to 0.80 (Equation number 8); adding an extra variable to the 8-variable equation lowered the $\bar{R}^2$ indicating that adding one more variable does not improve the equation (Table 4.6).

Interestingly for Laboratory 2, the 6-variable equation had the highest $\bar{R}^2$ value and included proximate analysis and NDF and starch (Equations number 15; Table 4.6), but for the combined laboratories, included proximate analysis plus NDF, starch and particle size ($\bar{R}^2$ =

0.79). Equations that included only the other chemical components besides proximate analysis components are presented in Table 4.7. In general, these produced relatively low $\bar{R}^2$ values with the highest $\bar{R}^2$ being 0.60 for equation number 30, which included ADF, NDF and GE.

Producing equations with from 1 to 9 variables by offering all possible variables in the prediction equations (without forcing any other variables into the model; Table 4.8) produced relatively higher $\bar{R}^2$ values compared to using only proximate components, proximate components plus other variables, and other variables alone. Including just one variable produced relatively higher $\bar{R}^2$ values, and these variables were ADF for Laboratory 1 and combined, and crude fiber for laboratory 2 ($\bar{R}^2$ values of 0.41 and 0.27, and 0.39, respectively). Adding more variables increased the $\bar{R}^2$ values up to a point, and then the $\bar{R}^2$ values started to decrease. For Laboratory 1, the $\bar{R}^2$ value was maximized using a 4-variable equation (there was an increase of 0.01 when including up to 7 and 8 variables). This equation (Eq. no. 52) included crude fiber, ADF, NDF and GE). Similarly, for Laboratory 2, the 4-variable equation (Eq. no. 77) produced the highest $\bar{R}^2$ value (0.75), however, the components in the equation were different, with CP, crude fat, NDF and starch being included. For the combined laboratories the equations with the highest $\bar{R}^2$ (0.80) value included 7 variables (Eq. no. 111), which were CP, crude fat, crude fiber, NDF, starch, GE and particle size.

A number of studies have developed prediction equations specifically for the ME of DDGS based on chemical composition and these are summarized in Table 4.9. Pedersen et al. (2007) reported 5 equations that included ash, CP, crude fat, starch, ADF, NDF and GE with R^2 values from 0.94 to 0.99, whereas the 3 equations reported by Anderson (2009) for the ME of corn co-products included GE, total dietary fiber (TDF), ash, CP, crude fat, and NDF that had R^2 values from 0.91 to 0.95 and RMSE from 306 to 424 kcal/kg. Another report by Anderson et al.

(2012) presented 7 equations for the ME of corn co-products, which included hemicellulose, GE, TDF, NDF, and ash that had R^2 values from 0.43 to 0.72 ($\bar{R}^2$ values were only presented for 2 equations) and RMSE from 323 to 464 kcal/kg. Kerr and Shurson (2013) reported 4 equations, which included ADF, bulk density, crude fat, and TDF that had R^2 values from 0.59 to 0.85 and RMSE from 48.7 to 75.6 kcal/kg.

Some of the published equations included other components that were not included in the equations developed in the current experiment. For example Anderson (2009) included TDF (which includes soluble and insoluble fiber) in two of the equations reported, whereas Anderson et al. (2012) included hemicellulose in addition to TDF, and Kerr and Shurson (2013) included bulk density and TDF. In general, published production equations have not included physical properties of DDGS apart from Kerr and Shurson (2013) who included bulk density, whereas some of the equations presented in the current experiment included particle size as a component in the prediction of ME.

The results of the current experiment illustrate the large variation in nutrient and energy composition of DDGS sources available in the US in 2008, which highlights the need for equations to predict ME. An important finding is that results of chemical composition analysis of DDGS vary markedly between laboratories and, also, the best equation to predict the ME of DDGS also differed between laboratories. These findings need to be validated, but have major implications for the choice of equations for use under practical conditions.

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TABLES

Table 4.1. Reference methods for chemical analysis of distillers dried grains with solubles sources used by the 2 laboratories

Component	Laboratory 1	Laboratory 2
DM	AOAC (2000) official method modified 935.29	AOAC (2006) official method 934.01
Ash	AOAC (2006) official method 942.05	AOAC (2006) official method 942.05
CP	AOAC (2005) official method 990.03	AOAC (2005) official method 990.03
Crude fat	AOAC (2005) official method 945.16	AOAC (2006) official method 920.39 (A)
Crude fiber	Ankom Filter bag procedures	AOAC (2006) official method 978.10
ADF	Ankom Filter bag procedures	AOAC (2006) official method 973.18 (A-D)
NDF	Ankom Filter bag procedures	JAOAC 56 (1973) 1352-1356
Starch	AACC (2000) 76-11	AACC (1975) approved method 76-13.01 modified: Sigma Starch Assay Kit (Kit STA-20 St. Louis, MO)

Table 4.2. Analyzed composition of the 17 sources of distillers dried grains with solubles for Laboratories 1, 2, and for Laboratories 1 and 2 combined (average of the 2 laboratories; DM basis)

	DDGS source																
Item	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Laboratory 1:																	
DM, %	85.19	87.91	87.71	90.80	89.61	91.50	90.09	93.19	87.68	89.06	91.75	91.30	92.59	93.14	88.28	90.94	90.88
Ash, %	5.10	4.37	3.57	4.20	4.53	4.01	4.35	3.73	4.01	4.81	4.54	4.46	4.50	5.42	4.43	4.68	5.59
CP, %	29.60	29.60	29.70	31.00	31.70	32.20	29.50	31.70	31.10	27.80	28.90	29.80	32.00	29.40	31.30	30.60	30.80
Crude fat, %	9.52	11.40	10.30	10.90	10.30	8.72	10.80	10.60	9.10	9.99	10.90	11.50	11.20	10.70	11.50	10.70	10.50
Crude fiber, %	8.32	7.96	8.36	6.61	7.79	8.39	7.98	6.32	8.38	8.00	8.62	5.87	8.09	7.08	8.87	7.76	8.07
ADF, %	17.90	13.60	15.60	13.10	14.20	14.40	10.50	13.00	15.60	12.20	10.20	12.40	10.50	9.82	11.40	14.20	13.20
NDF, %	29.40	30.10	33.50	28.50	31.70	26.40	27.80	31.40	26.90	25.90	27.20	26.70	25.80	25.30	31.60	33.30	29.80
Starch, %	4.57	4.33	2.45	5.80	3.56	4.81	5.29	6.03	9.01	8.94	7.27	7.37	5.11	6.33	4.81	3.19	4.38
Laboratory 2																	
DM, %:	90.27	89.53	91.18	91.13	91.35	91.70	90.51	93.64	88.83	90.94	92.56	93.18	91.05	93.85	91.71	92.78	92.35
Ash, %	5.15	4.64	3.64	4.37	4.31	4.02	4.21	3.50	4.04	4.56	4.15	4.37	4.76	5.36	4.33	4.33	5.09
CP, %	29.78	29.28	33.34	30.84	31.29	31.88	28.81	29.60	30.79	27.30	29.09	29.39	31.26	29.88	30.52	29.63	30.02
Crude fat, %	9.46	14.63	12.67	13.00	12.90	11.79	13.79	12.12	11.94	13.20	12.60	13.59	13.43	11.44	14.11	13.33	13.32
Crude fiber, %	7.88	6.89	7.71	6.86	7.32	7.47	7.00	7.67	6.97	6.64	6.62	7.00	6.86	7.61	7.34	8.13	7.04
ADF, %	13.42	11.75	13.43	10.91	12.45	16.70	10.21	12.68	14.77	10.04	9.18	12.21	10.54	9.12	12.68	12.61	10.90
NDF, %	42.74	41.55	43.51	41.86	42.61	38.36	40.02	43.34	40.72	39.80	36.35	39.82	35.55	32.41	42.24	42.38	40.63
Starch, %	2.63	3.35	1.63	5.40	2.76	3.77	3.20	3.52	9.08	6.14	5.60	3.62	3.90	4.99	3.33	1.79	2.99
Laboratory 1 and 2 combined[1]:																	
DM, %	87.73	88.72	89.45	90.97	90.48	91.60	90.30	93.42	88.26	90.00	92.16	92.24	91.82	93.50	90.00	91.86	91.62
Ash, %	5.13	4.51	3.61	4.29	4.42	4.02	4.28	3.62	4.03	4.69	4.35	4.42	4.63	5.39	4.38	4.51	5.34
CP, %	29.69	29.44	31.52	30.92	31.50	32.04	29.16	30.65	30.95	27.55	29.00	29.60	31.63	29.64	30.91	30.12	30.41
Crude fat, %	9.49	13.02	11.49	11.95	11.60	10.26	12.30	11.36	10.52	11.60	11.75	12.55	12.32	11.07	12.81	12.02	11.91
Crude fiber, %	8.10	7.43	8.04	6.74	7.56	7.93	7.49	7.00	7.68	7.32	7.62	6.44	7.48	7.35	8.11	7.95	7.56
ADF, %	15.66	12.68	14.52	12.01	13.33	15.55	10.36	12.84	15.19	11.12	9.69	12.31	10.52	9.47	12.04	13.41	12.05
NDF, %	36.07	35.83	38.51	35.18	37.16	32.38	33.91	37.37	33.81	32.85	31.78	33.26	30.68	28.86	36.92	37.84	35.22
Starch, %	3.60	3.84	2.04	5.60	3.16	4.29	4.25	4.78	9.05	7.54	6.44	5.50	4.51	5.66	4.07	2.49	3.69
GE, kcal/kg[2]	5,191	5,406	5,452	5,336	5,327	5,274	5,349	5,340	5,351	5,238	5,266	5,318	5,280	5,249	5,368	5,372	5,269

[1]Average of the results of the 2 laboratories.
[2]GE analyses conducted at Pioneer Hi-Bred International Inc., (Urbandale, IA).

Table 4.3 Descriptive statistics for analyzed composition of distillers dried grains with solubles sources for the analysis of Laboratories 1 and 2 and for Laboratories 1 and 2 combined (average of the 2 laboratories[1]; DM basis)

Component	Laboratory	Mean	SD	CV, %	Minimum	Maximum
DM	1	90.10	2.21	2.45	85.19	93.19
	2	91.56	1.39	1.51	88.83	93.85
	combined	90.83	1.68	1.85	87.73	93.50
Ash	1	4.49	0.54	11.94	3.57	5.59
	2	4.40	0.50	11.29	3.50	5.36
	combined	4.45	0.50	11.33	3.61	5.39
CP	1	30.39	1.22	4.03	27.80	32.20
	2	30.16	1.37	4.53	27.30	33.34
	combined	30.28	1.16	3.84	27.55	32.04
Crude fat	1	10.51	0.80	7.61	8.72	11.50
	2	12.78	1.20	9.40	9.46	14.63
	combined	11.65	0.92	7.90	9.49	13.02
Crude fiber	1	7.79	0.84	10.73	5.87	8.87
	2	7.24	0.45	6.18	6.62	8.13
	combined	7.52	0.47	6.20	6.44	8.11
ADF	1	13.05	2.19	16.79	9.82	17.90
	2	11.98	1.99	16.60	9.12	16.70
	combined	12.51	1.94	15.53	9.47	15.66
NDF	1	28.90	2.68	9.29	25.30	33.50
	2	40.23	3.04	7.56	32.41	43.51
	combined	34.57	2.70	7.82	28.86	38.51
Starch	1	5.49	1.85	33.76	2.45	9.01
	2	3.98	1.82	45.60	1.63	9.08
	combined	4.74	1.78	37.50	2.04	9.05
Energy concentration, (kcal/kg):						
GE[2]		5,316	67.6	1.27	5,191	5,452
DE		3,842	116.3	3.03	3,585	3,994
ME		3,596	108.4	3.02	3,330	3,737

[1]Combined analysis = average of results from the two commercial laboratories.
[2]GE analyses conducted at Pioneer Hi-Bred International Inc., (Urbandale, IA).

Table 4.4. Pearson correlation coefficients between GE, DE and ME, and particle size and chemical composition (DM basis) of the distillers dried grains with solubles sources (unground) analyzed by Laboratories 1, 2, and for Laboratories 1 and 2 combined (average of the 2 laboratories)[1]

Item	GE	DE	ME	Ash	CP	Crude Fat	Crude Fiber	ADF	NDF	Starch
Laboratory 1										
DE	0.14									
ME	0.13	**0.97**								
Ash	**-0.64**	-0.11	-0.05							
CP	0.20	-0.07	-0.09	-0.32						
Crude fat	0.30	0.17	0.24	0.11	-0.14					
Crude fiber	0.07	0.33	0.32	0.05	-0.04	-0.33				
ADF	0.09	**-0.60**	**-0.67**	-0.20	0.16	**-0.60**	0.15			
NDF	**0.65**	**-0.49**	-0.46	-0.27	0.21	0.16	0.11	0.44		
Starch	-0.42	0.36	0.33	0.05	-0.33	-0.13	-0.20	-0.30	**-0.72**	
Particle size	0.02	-0.10	-0.17	-0.14	0.08	-0.44	0.17	**0.55**	-0.09	0.35
Laboratory 2										
Ash	**-0.59**	-0.01	0.02							
CP	0.41	-0.02	-0.09	-0.30						
Crude fat	**0.56**	0.42	**0.54**	-0.17	-0.16					
Crude fiber	0.14	**-0.57**	**-0.66**	-0.06	0.34	-0.46				
ADF	0.23	-0.27	-0.37	-0.44	**0.54**	-0.30	0.45			
NDF	**0.50**	**-0.60**	**-0.55**	-0.46	0.15	0.09	0.31	**0.47**		
Starch	-0.24	**0.53**	**0.47**	-0.01	-0.24	-0.13	**-0.57**	-0.09	-0.36	
Particle size	0.02	-0.10	-0.17	-0.04	-0.05	-0.19	-0.12	0.43	0.35	**0.47**
Laboratory 1 and 2 combined										
Ash	**-0.63**	-0.06	-0.02							
CP	0.35	-0.05	-0.10	-0.38						
Crude fat	**0.49**	0.35	0.46	-0.05	-0.15					
Crude fiber	0.14	0.02	-0.03	0.03	0.24	-0.34				
ADF	0.17	**-0.47**	**-0.56**	-0.35	**0.47**	**-0.56**	0.40			
NDF	**0.60**	**-0.58**	**-0.54**	-0.41	0.25	0.10	0.27	**0.54**		
Starch	-0.34	0.46	0.42	0.00	-0.37	-0.17	-0.38	-0.22	**-0.57**	
Particle size	0.02	-0.10	-0.17	-0.09	0.01	-0.32	0.10	**0.53**	0.15	0.42

[1]Statistically significant correlations ($P < 0.05$) were correlations of ≥ 0.47 and are presented in bold.

Table 4.5. Summary of published literature of correlation analyses between GE, DE and ME, and chemical composition in compound feeds and feedstuffs

Reference	Material	Number of Samples	Item	GE	DE	ME
Just et al., 1984	Compound feed	321	DE	-	-	-
			ME	-	-	-
			Ash	-	-	-0.25
			CP	-	-	-0.44
			Crude fat	-	-	0.84
			Crude fiber	-	-	-0.02
			ADF	-	-	-
			NDF	-	-	-0.07
			Starch	-	-	-
Just et al., 1984	Feedstuffs	331	DE	-	-	-
			ME	-	-	-
			Ash	-	-	-0.30
			CP	-	-	-0.19
			Crude fat	-	-	0.73
			Crude fiber	-	-	0.07
			ADF	-	-	-
			NDF	-	-	0.06
			Starch	-	-	-
Morgan et al., 1987	Compound feed	36	DE	0.28	-	-
			ME	0.26	0.99	-
			Ash	-0.04	-0.64	-0.67
			CP	0.15	0.09	0.04
			Crude fat	-	-	-
			Crude fiber	-0.06	-0.89	0.90
			ADF	-0.05	-0.89	-0.90
			NDF	0.07	-0.91	-0.91
			Starch	-0.15	0.79	0.82
Leukule et al., 1990	Tropical feedstuffs	18	DE	-	-	-
			ME	-	-	-
			Ash	-	-	-
			CP	-	-	-0.11
			Crude fat	-	-	-
			Crude fiber	-	-	-0.82
			ADF	-	-	-
			NDF	-	-	-
			Starch	-	-	-
Löwgren et al., 1992	Barley	8	DE	-	-	-
	Triticale	3	ME	-	0.99	-
			Ash	-	-0.93	-0.93
			CP	-	-0.36	-0.47
			Crude fat	-	-0.75	-0.81

Table 4.5 (cont). Summary of published literature of correlation analyses between GE, DE and ME, and chemical composition in compound feeds and feedstuffs

			Crude fiber	-	-0.93	-0.95
			ADF	-	-	-
			NDF	-	-	-
			Starch	-	0.73	0.80
Noblet and Perez, 1993	Compound feed	114	DE	0.30	-	-
			ME	-	-	-
			Ash	-0.01	-0.65	-
			CP	0.16	0.14	-
			Crude fat	0.92	0.12	-
			Crude fiber	0.22	-0.71	-
			ADF	0.20	-0.72	-
			NDF	0.20	-0.80	-
			Starch	-0.37	0.49	-
Spanghero and Volpelli, 1999	Compound feed	40	DE	-	-	-
			ME	-	-	-
			Ash	-	-	-
			CP	-	0.66	-
			Crude fat	-	0.76	-
			Crude fiber	-	-0.79	-
			ADF	-	-0.75	-
			NDF	-	-0.91	-
			Starch	-	-	-
Cozzanet el al., 2010	Wheat DDGS	10	DE	0.11	-	-
			ME	-	-	-
			Ash	-0.36	-0.57	-
			CP	-	-	-
			Crude fat	0.58	-0.38	-
			Crude fiber	0.67	-0.41	-
			ADF	0.54	-0.73	-
			NDF	0.44	-0.17	-
			Starch	-0.69	0.01	-

Table 4.6. Selected equations to predict the ME of distillers dried grains with solubles (unground) based on proximate analysis and other components for Laboratories 1 and 2 and for Laboratories 1 and 2 combined (average of the 2 laboratories)

			Proximate analysis				Other chemical components								
Equation No.	No. Variables	Intercept	Ash	CP	Crude Fat	Crude Fiber	ADF	NDF	Starch	GE	Part. Size	R^2	$\overline{R}^2$	C(p)	RMSE
Laboratory 1															
1	1	3275.60	-	-	-	41.13	-	-	-	-	-	0.10	0.04	1.4	106.2
2	2	2604.93	-	-	51.84	57.30	-	-	-	-	-	0.23	0.12	1.3	101.6
3	3	2677.09	-24.28	-	54.18	58.87	-	-	-	-	-	0.25	0.07	3.0	104.5
4	4	2865.66	-27.83	-5.21	53.18	58.39	-	-	-	-	-	0.25	-0.01	5.0	108.5
5	5	4181.45	-49.32	-2.76	-15.17	54.45	-41.76	-	-	-	-	0.69	0.54	16.9	73.4
6	6	-2105.33	11.91	7.10	50.22	64.57	.	-40.39	-	1.05	-	0.78	0.65	12.1	64.0
7	7	-1111.29	8.99	4.00	4.77	55.60	-22.62	-28.49	-	0.97	-	0.85	0.73	9.6	56.4
8	8	-4510.07	46.75	18.77	21.88	72.74	.	-32.14	27.65	1.40	-0.17	0.90	0.80	8.1	48.8
9	9	-4876.64	50.18	20.16	26.15	74.75	3.57	-33.49	29.76	1.44	-0.19	0.90	0.77	10.0	52.1
Laboratory 2															
10	1	4748.04	-	-	-	-159.20	-	-	-	-	-	0.43	0.39	1.4	84.5
11	2	4164.35	-	-	26.76	-125.81	-	-	-	-	-	0.50	0.43	1.7	82.0
12	3	3898.72	-	11.79	26.72	-138.17	-	-	-	-	-	0.52	0.41	3.2	83.4
13	4	3705.22	19.85	14.09	28.77	-136.69	-	-	-	-	-	0.53	0.37	5.0	86.2
14	5	4359.69	-34.55	10.09	38.84	-79.57	-	-20.79	-	-	-	0.76	0.64	5.7	64.6
15	6	3238.50	-12.66	13.87	56.56	-12.11	-	-18.18	22.91	-	-	0.82	0.71	5.0	58.6
16	7	2983.84	-1.25	15.09	55.97	-9.49	-	-14.08	30.14	-	-0.06	0.83	0.69	6.7	60.4
17	8	1895.86	7.86	9.96	41.45	-33.57	-	-15.56	26.60	0.31	-0.06	0.83	0.66	8.4	63.0
18	9	1111.99	29.91	0.50	31.99	-65.64	11.28	-16.41	25.48	0.54	-0.10	0.84	0.64	10.0	65.4
Combined laboratories															
19	1	2970.76	-	-	53.69	-	-	-	-	-	-	0.21	0.15	-0.7	99.7
20	2	2656.31	-	-	59.39	32.99	-	-	-	-	-	0.23	0.11	1.1	102.0
21	3	2812.60	-	-5.71	58.83	36.08	-	-	-	-	-	0.23	0.05	3.0	105.6
22	4	2864.62	-5.37	-6.69	58.58	36.66	-	-	-	-	-	0.23	-0.03	5.0	109.9
23	5	3519.78	-77.78	-2.67	79.30	104.50	-	-34.89	-	-	-	0.77	0.67	9.1	62.2
24	6	2103.26	-43.01	12.22	93.49	128.49	-	-25.20	27.54	-	-	0.86	0.78	5.6	50.8
25	7	1697.45	-31.18	16.45	90.62	135.98	-	-19.47	39.14	-	-0.08	0.88	0.79	6.4	49.8
26	8	515.11	-15.02	14.21	79.11	126.96	-	-21.15	39.24	0.27	-0.08	0.89	0.77	8.1	51.6
27	9	444.57	-12.12	12.93	83.59	127.47	4.42	-21.99	40.66	0.27	-0.10	0.89	0.74	10.0	54.9

Table 4.7. Selected equations to predict the ME of distillers dried grains with solubles (unground) based on ADF, NDF, starch, GE and particle size for Laboratories 1 and 2 and for Laboratories 1 and 2 combined (average of the 2 laboratories)

			Chemical components								
Eq. No.	No. Variables	Intercept	ADF	NDF	Starch	GE	Particle Size	R^2	$\overline{R}^2$	C(p)	RMSE
Laboratory 1											
28	1	4027.42	-33.05	-	-	-	-	0.45	0.41	5.7	83.4
29	2	-1617.62	-	-38.10	-	1.19	-	0.53	0.46	4.8	79.4
30	3	-448.89	-21.52	-26.64	-	0.96	-	0.67	0.60	2.1	68.9
31	4	-432.04	-21.40	-28.19	-2.78	0.97	-	0.67	0.56	4.0	71.6
32	5	-403.61	-21.76	-28.08	-3.05	0.96	0.01	0.67	0.52	6.0	74.7
Laboratory 2											
33	1	4391.88	-	-19.78	-	-	-	0.31	0.26	10.0	93.2
34	2	145.63	-	-29.52	-	0.87	-	0.53	0.46	4.7	79.6
35	3	-332.41	-	-25.47	21.04	0.92	-	0.64	0.55	3.1	72.6
36	4	-237.06	-	-20.08	30.12	0.86	-0.09	0.66	0.54	4.4	73.2
37	5	-276.22	-6.84	-19.23	28.44	0.88	-0.06	0.67	0.52	6.0	75.2
Combined laboratories											
38	1	3989.76	-31.45	-	-	-	-	0.32	0.27	10.9	92.5
39	2	-1176.63	-	-39.14	-	1.15	-	0.62	0.57	2.3	71.4
40	3	-742.99	-13.38	-32.52	-	1.06	-	0.66	0.58	2.9	70.3
41	4	-901.84	-14.80	-27.42	11.83	1.05	-	0.68	0.59	4.1	70.4
42	5	-957.76	-12.77	-26.82	14.59	1.05	-0.03	0.69	0.54	6.0	73.3

Table 4.8. Selected equations to predict the ME of distillers dried grains with solubles (unground) based on all chemical components and particle size for Laboratories 1 and 2 and combined

Equation No.	No. Variables	Intercept	Chemical components									R^2	$\overline{R}^2$	C(p)	RMSE
			Ash	CP	Crude Fat	Crude Fiber	ADF	NDF	Starch	GE	Part. Size				
Laboratory 1															
43	1	4027.42	-	-	-	-	-33.05	-	-	-	-	0.45	0.41	25.5	83.4
46	2	3637.01	-	-	-	55.45	-36.25	-	-	-	-	0.62	0.57	15.1	71.0
49	3	3863.38	-47.66	-	-	58.10	-38.78	-	-	-	-	0.68	0.60	13.4	68.3
52	4	-649.39	-	-	-	54.65	-24.45	-26.85	-	0.92	-	0.84	0.79	3.8	49.3
55	5	-972.68	-	-	-	56.02	-20.16	-29.98	-	0.99	-0.04	0.85	0.78	5.4	50.6
58	6	-1388.45	-	-	-	60.88	-16.42	-26.78	11.50	1.03	-0.08	0.86	0.78	6.6	50.9
61	7	-5440.07	65.36	18.43	-	66.54	-	-32.73	29.56	1.61	-0.19	0.89	0.80	6.9	48.6
64	8	-4510.07	46.75	18.77	21.88	72.74	-	-32.14	27.65	1.40	-0.17	0.90	0.80	8.0	48.8
67	9	-4876.64	50.18	20.16	26.15	74.75	3.57	-33.49	29.76	1.44	-0.19	0.90	0.77	10.0	52.1
Laboratory 2															
68	1	4748.04	-	-	-	-159.20	-	-	-	-	-	0.43	0.39	12.0	84.5
71	2	3785.52	-	-	53.41	-	-	-21.68	-	-	-	0.66	0.61	4.2	68.1
74	3	3456.67	-	-	56.79	-	-	-16.85	22.95	-	-	0.78	0.73	0.6	56.1
77	4	2973.95	-	15.00	60.09	-	-	-17.41	25.62	-	-	0.81	0.75	1.2	53.9
80	5	2885.14	-	14.93	57.92	-	-	-14.11	31.70	-	-0.06	0.82	0.74	2.7	54.8
83	6	2389.96	-	11.95	53.56	-	-	-15.44	30.98	0.13	-0.06	0.83	0.72	4.6	57.1
86	7	1890.88	-	-	34.72	-55.98	8.04	-18.72	21.82	0.43	-0.07	0.83	0.71	6.3	58.9
89	8	1081.11	30.34	-	31.46	-66.73	11.52	-16.49	25.33	0.56	-0.10	0.84	0.68	8.0	61.2
92	9	1111.99	29.91	0.50	31.99	-65.64	11.28	-16.41	25.48	0.54	-0.10	0.84	0.64	10.0	65.4
Combined laboratories															
93	1	3989.76	-	-	-	-	-31.45	-	-	-	-	0.32	0.27	29.6	92.5
96	2	-1176.63	-	-	-	-	-	-39.14	-	1.15	-	0.62	0.57	12.7	71.4
99	3	-25.81	-	-	33.82	-	-	-35.55	-	0.84	-	0.68	0.60	11.1	68.3
102	4	2145.93	-	-	90.85	126.40	-	-20.06	28.55	-	-	0.80	0.73	5.5	56.1
105	5	2625.87	-56.79	-	90.00	129.94	-	-26.42	23.43	-	-	0.85	0.79	4.2	50.3
108	6	1178.04	-	22.99	91.87	135.69	-	-15.10	45.72	-	-0.09	0.87	0.79	5.1	49.5
111	7	-30.15	-	15.55	75.79	123.96	-	-20.31	41.34	0.36	-0.09	0.89	0.80	6.2	49.0
115	8	13.08	-	13.63	82.17	125.31	5.51	-21.56	42.60	0.34	-0.11	0.89	0.77	8.1	51.6
117	9	444.57	-12.12	12.93	83.59	127.47	4.42	-21.99	40.66	0.27	-0.10	0.89	0.74	10.0	54.9

Table 4.9 Summary of published prediction equations for ME of compound feeds and individual feedstuffs for pigs

Reference	Material	Number of samples	Chemical components in equation
Morgan et al., 1975	Compound feed	19	CP, crude fat, NFE[1]
Jorgensen, 1980	Compound feed	104	CP, crude fat, crude fiber, NFE[1]
Eeckhout and Moermans, 1981	Compound feed	24	Hemicellulose, ADF
Kirchgessner and Roth, 1981	Compound feed	48	Starch plus sugar, ADF, residual OM
Just et al., 1984	Compound feed	321	CP, crude fiber, NFE[1] substances, OM
Löwgren et al., 1992	Compound feed	11	Ash, crude fiber
Noblet and Perez, 1993	Compound feed	114	Ash, CP, crude fat, NDF, Hemicellulose, Cellulose, ADL[2], WICW[3], GE
	Individual feedstuff		
Wiseman and Cole, 1979	Cereals	24	Crude fat, crude fiber, soluble carbohydrate
Jorgensen, 1980	Diverse feedstuffs	154	CP, crude fat, crude fiber, soluble carbohydrate, hemicellulose
Just et al., 1984	Diverse feedstuffs	331	CP, crude fiber, NFE[1] substances, NDF, GE, OM
Leukule et al., 1990	Diverse feedstuffs	70	CP, crude fat, soluble carbohydrate
Fairbarn et al., 1999	Barley	5	ADF, ADL[2], GE, ash
Kang et al., 2004	Soybean meal	14	Ash, ADF, NFE[1], soluble carbohydrate
Pedersen et al., 2007	DDGS	10	Ash, CP, crude fat, ADF, NDF, GE
Anderson, 2009	Corn Co-products	20	CP, NDF, crude fat, ash, GE, TDF[4]
Anderson, 2012	Corn Co-products	20	CP, NDF, crude fat, ash, GE, TDF[4]
Kerr and Shurson, 2013	DDGS	11	ADF, bulk density, crude fat, TDF[4]

[1]NFE = nitrogen free extract; [2]ADL = acid detergent lignin; [3]WICW = water insoluble cell walls; [4]TDF = total dietary fiber.

CHAPTER 5

EVALUATION OF THE ACCURACY OF EQUATIONS TO ESTIMATE THE METABOLIZABLE ENERGY OF DISTILLERS DRIED GRAINS WITH SOLUBLES FED TO PIGS

ABSTRACT: An experiment was conducted to determine the apparent ME of DDGS samples from 4 ethanol plants to validate equations to predict the ME of distillers dried grains with solubles (DDGS) developed in Chapter 4. The 4 DDGS sources were chosen to cover the range of ME available in 2008 in the Midwest. The experiment was carried out using 20 gilts (50.7 ± 2.5 kg initial BW) in a randomized complete block design with a total of 5 dietary treatments; BW was used as the blocking factor. A corn-based diet (97.32% corn + mineral and vitamin supplements) was used as the control with the experimental diets formulated by mixing each DDGS sample in a 1:1 ratio with corn. The experimental period consisted of 7 d of adaptation to experimental diets, followed by 5 d of collection of feces and urine. The GE of diets, corn, DDGS, feces, and urine were determined by bomb calorimetry. The average ME of DDGS samples was 3,361 kcal/kg with a range from 3,105 to 3,527 kcal/kg DM, respectively. The chemical composition of the 4 DDGS sources was analyzed by 4 laboratories and these data were used in equations developed in Chapter 4 to predict the ME of the 4 DDGS sources evaluated in this experiment. Comparisons between measured and predicted ME for the sources of DDGS were used to assess the accuracy of the equations for predicting ME using the root mean square error of prediction (RMSEP) and mean percent bias statistics. The most accurate prediction of ME of DGGS was achieved when the same analytical laboratory was used both for the chemical analysis of the original samples used to develop the prediction equations and also

for the analysis of the samples being evaluated (i.e., the samples for which ME was predicted). Results also highlighted the need to develop standard procedures for the development and validation of equations to predict the ME in DDGS and other ingredients, which is essential if users of equations are to have accurate predictions of energy value of feedstuffs.

INTRODUCTION

Use of regression analysis for development of equations to predict the energy concentration of feeds and ingredients for pigs is a practical alternative compared to direct measurement. This is especially true as more ingredients, such as co-products from corn processing, are used in diet formulation. In many cases, the composition of these co-products, such as distillers dried grains with solubles (**DDGS**), is variable (Spiehs et al., 2002; Stein et al., 2006; Pedersen et al., 2007; Anderson, 2009; Anderson et al., 2012, Kerr and Shurson, 2013) and the method used for estimating energy concentration consist of direct measurements *in vivo*, an approach which is expensive, laborious, and time consuming (Noblet and Perez, 1993; Cromwell et al., 1999, 2000; Kerr et al., 2009; Anderson et al., 2012). In addition, the different published estimates of energy values are often difficult to compare because of the differences between laboratories in methodology, analytical procedures (Palmquist and Jenkins, 2003), in addition to differences in nutrient concentrations of the test materials. Consequently, the use of prediction equations to estimate the energy of ingredients such as DDGS, based on chemical composition is receiving more attention and more research is being carried out in this area (Pedersen et al., 2007; Anderson, 2009; Cozannet et al., 2010; Anderson et al., 2012; Kerr and Shurson, 2013).

The general methodology for development of prediction equations for diets and ingredients consists of characterization of the individual chemical components (i.e., the

independent variables) combined with direct measurement of the dependent variable in question (e.g., energy concentration; Morgan et al., 1987; Löwgren et al., 1992; Spanghero and Volpelli, 1999; Noblet and Perez, 1993; Pedersen et al., 2007; Anderson, 2009; Cozanet et al., 2010; Anderson et al., 2012; Kerr and Shurson, 2013). Subsequently, multiple regression models are developed to identify an equation or a set of equations that best predict the dependent variable as a linear function of the independent variables.

Before prediction equations are used, they should be validated using different samples of the ingredient than those used in the development of the equations (Snee, 1977). However, in the published literature relating to the development of prediction equations for nutrient content of ingredients or diets, validation of equations is seldom referenced and/or limited data are presented on how the models were validated (Eeckhout and Moermans, 1981; Morgan et al., 1987; Noblet and Perez, 1993; Fairbairn et al., 1999; Pedersen et al., 2007; Anderson, 2009; Anderson et al., 2012; Cozannet et al., 2010; Kerr and Shurson, 2013). In most cases, equations are selected by assessing the coefficient of determination (R^2), which is a measure of the goodness of fit of the model; nevertheless, this approach does not assess the predictive ability or accuracy of the models developed.

Ideally, the final step in the regression equation development process should be the validation of the selected prediction equations (Snee, 1977; Kutner et al., 2004). Validation of a regression model is conducted by: 1) collection of new data to check the accuracy of the regression model; 2) comparison of results with theoretical expectations, earlier empirical results from the published literature, or simulation results; 3) use of a separate sample or samples not used in development in the model sample to check the model and its accuracy. Generally

speaking, these steps are seldom conducted; however, before prediction equations are widely adopted, the accuracy of the selected equations should be investigated.

Therefore, the overall objective of this research was to validate the accuracy of equations to predict the ME of DDGS that were developed in a previous experiment (Chapter 4).

MATERIALS AND METHODS

The experimental protocol was approved by the University of Illinois Institutional Animal Care and Use Committee.

Experimental Design and Treatments

The animal component of the experiment was conducted at the Swine Research Center at the University of Illinois using a randomized complete block design with BW as the blocking factor (there were 8 blocks with 5 pigs/block) with the following 5 dietary treatments: a corn-based diet (97.32% corn) and 4 experimental diets formulated by mixing samples from each of 4 sources of DDGS in a 1:1 ratio with corn.

Ingredients and Diets

The samples from the 4 sources (ethanol plants) of DDGS used in this experiment were selected to have a range of ME estimated from a database that contained historical chemical composition data from a number of samples from each source collected from September 2009 to July 2011 (The Maschhoffs, LLC, Carlyle, IL). The ME of the DDGS samples was estimated using a proprietary equation (A. Gaines, personal communication). The DDGS sources in the database were ranked by predicted ME , and 4 sources that spanned the range in ME were selected. The predicted ME of the 4 sources were 3,429, 3,522, 3,592, and 3,695 kcal/kg DM. A sample (approximately 200 kg) of DDGS for use in the experiment was obtained from each of the 4 ethanol plants and transferred to the University of Illinois. In addition, a single batch of corn was obtained for use in the experiment.

The chemical composition of the 4 DDGS samples (i.e., CP, crude fiber, crude fat, ash, NDF, ADF, and starch) was analyzed at 4 different laboratories and the corn sample was analyzed for chemical composition at one of the laboratories (Laboratory 1). A summary of the analytical methods used by each of the laboratories is presented in Table 5.1. The DDGS and corn samples were analyzed in duplicate for GE at the University of Illinois using bomb calorimetry (Isoperibol Bomb Calorimeter, Model Number 1281; Parr Instrument Co., Moline, IL). Results for these analyses are presented in Table 5.2. The particle size of each DDGS sample was measured using a Rotap sieve shaker (model RX-29, W.S. Tyler Co, Cleveland, OH) fitted with 13 US Sieves and a pan and the geometric mean and SD are presented in Table 5.3.

Diet Preparation and Feeding

The experimental diets were manufactured using the same batches of corn and other ingredients. The corn-based control diet consisted of corn (97.32%) plus minerals and vitamins. The experimental diets that contained the samples of DDGS were produced by replacing 50% of the corn in the control diet with the same amount of one of the DDGS samples (i.e., the diets contained 48.66% corn, 48.66% DDGS, and minerals and vitamins; Table 5.4). Diets were formulated to meet or exceed NRC requirements for minerals and vitamins for growing pigs (NRC, 1998). The formulation and analyzed composition of the experimental diets are presented in Table 5.4.

Animal, Housing, and Allotment to Treatment

The experiment was carried out with 20 gilts (initial average BW, 50.7 ± 2.5 kg) that were the progeny of G Performer sires and Fertilis 25 dams (Genetiporc, Alexandria, MN). Before the start of the experiment, pigs were reared under standard conditions in a grower

facility, housed in groups of 4 pigs, and had ad libitum access to standard diets, formulated to meet or exceed the nutrient requirements recommended by NRC (1998), and to water.

The experiment was conducted in an environmentally controlled metabolism room in which the ambient temperature was maintained between 22 and 26^{o}C throughout the experiment period using thermostatically-controlled space heaters and fan ventilation; lights were on continuously. The metabolism crates were constructed of stainless-steel and were equipped with screens and trays that allowed for the total, but separate, collection of feces and urine. Crate dimensions were 1.52 × 0.84 m, providing a floor space of 1.28 m^2/pig and each crate was equipped with a single-space feeder and a nipple-type water drinker.

The experiment involved 2 experimental periods each of 12 d, consisting of an adaptation (7 d) and a collection (5 d) period, with the same pigs being used in each period. For the first adaptation and collection period, 4 blocks of 5 pigs of similar BW were formed, and pigs were randomly allotted from within block to metabolism crates located in the same area of the room; metabolism crates were randomly allotted to dietary treatment within block. For the second adaptation and collection period, pigs were re-allotted to dietary treatment within each block as described above, with the restriction that they were allotted to a different diet than the one they were fed during the first period.

Sample Collections and Analysis

Each 12-d experimental period consisted of a 7-d adaptation period, used to acclimate the pigs to the metabolism crates and the dietary treatments, followed by a 5-d collection period during which total collection of feces and urine was carried out. The daily amount of feed provided per pig was 2.5 times the energy requirement for maintenance proposed by NRC (1998; i.e., 106 kcal ME per kg BW$^{0.75}$). The daily feed allowance was divided into 2 equal meals,

which were given at 0800 and 1600 h ± 15 min; water was available to the pigs at all times. Chromic oxide (0.3%) was added to the feed given in the morning meal of d 8 (start of collection period) and ferric oxide (0.3%) was added to the feed given in the morning meal of d 13 (last meal of the collection period). Fecal collection was initiated as soon as the chromic oxide appeared in the feces after d 8 and ended on the first appearance of the ferric oxide in the feces after d 13 as described by Adeola (2001). Urine collection started 2 h ± 15 min after the pigs were fed in the morning of d 8 and ended 2 h ± 15 min after the pigs were fed in the morning of d 13.

During the collection period, fecal material was collected twice daily, approximately 15 min after feeding, weighed, and placed in a plastic storage bag, which was placed in a freezer (at -18^{o}C). Urine was collected into buckets (containing 50 mL of 6 N sulfuric acid) that were placed under the metabolism cages. Twice daily, after feeding in the morning and afternoon, the urine was weighed, and a sub-sample of 20% of the total weight was placed into plastic containers, which were stored in a freezer at -18^{o}C.

At the end of the collection period, fecal samples from each pig were dried in a forced-air oven at 60^{o}C for 72 h, ground through a 2-mm screen, and thoroughly mixed before a sub-sample of 100 g was taken for analysis. Urine samples from each pig were thawed, strained through cheesecloth to remove particulate matter, and mixed thoroughly before a sub-sample of 200 mL was taken for analysis.

All analyses were conducted in duplicate and were repeated if duplicate values differed by more than ± 2.5%. Samples of feces were analyzed for DM at 135 ^{o}C for 2 h (procedure 930.15; AOAC, 2000). The GE of diets, corn, DDGS samples, feces, and urine plus cellulose was determined via bomb calorimetry (Isoperibol Bomb Calorimeter, Model Number 1281; Parr

Instrument Co., Moline, IL). Urine (approximately 2 mL) was dried at 55°C for 24 h onto 1 g of dried cellulose as described by Fent (2001) before GE determination was carried out. Cellulose was dried at 135 °C for 2 h (procedure 930.15; AOAC, 2000) prior to being used in the GE determination.

Calculations and Statistical Analysis

All values used in calculations were adjusted to 100% DM. Gross energy intake was calculated by multiplying the GE of the diet by the total feed intake over the collection period. Apparent DE of the experimental diets was calculated by subtracting the total GE of feces from the total GE intake and dividing by total feed intake. The apparent ME of experimental diets was calculated by subtracting the total GE of feces and urine from the total GE intake and dividing by total feed intake. The apparent DE and ME of corn were calculated by dividing the DE and ME of the corn-based control diet by the amount of corn in the diet (97.32%) and multiplying by 100. Finally, the apparent DE and ME of each of the DDGS samples were calculated by subtracting the DE and ME contributed by the corn to the corn-DDGS experimental diets as described by Adeola (2001).

Prior to data analysis, the PROC UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to verify normality and homogeneity of variances of the variables. Observations for feed intake and urine output from three pigs exceeded their treatment mean by more than 3 SD and were considered outliers and were, therefore, removed from the data set before analysis. For comparison of the DDGS sources, data were analyzed using the PROC MIXED procedure of SAS (Littell et al., 1996) with the pig being the experimental unit and the model including the fixed effect of DDGS source, and the random effects of period and block. The LSMEANS procedure was used to calculate mean values, and the PDIFF option was used to separate

treatment means. An alpha value of 0.05 was used to assess differences among treatment means. A contrast statement was used for comparisons of the means for the corn-based Control treatment with those of each of the 4 DDGS sources.

The data collected in this experiment, which included chemical composition data (measured by 4 different laboratories) and ME of the DDGS sources, were used as a validation data set (independent from that collected in Chapter 3) with the objective of evaluating the accuracy of selected equations developed in Chapter 4 (Table 4.8) at predicting the ME of the 4 DDGS samples evaluated in this chapter. The accuracy of selected equations was evaluated using 2 statistics, namely, the root mean square error of prediction (RMSEP) and mean percent bias. The RMSEP is defined as the square root of the average of the square differences between predicted and measured values of the validation data and was calculated as follows:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}}$$

where y_i is the measured value obtained in the validation data set; $\hat{y}_i$ is the predicted value of the validation data set.

This measure is a direct estimate of the average prediction error andis expressed in the original measurement units for the measure of interest (Esbensen et al., 2010). The RMSEP measures the average error of the prediction model provided that the data used is completely independent of the original data set used to develop the prediction models (Faber, 1999). In addition, a feature of the RMSEP is that it can be used to compare different models, regardless of how the models were developed, the number of independent variables in the model, or the numbers of components used (Esbensen et al., 2010).

In addition, the mean percent bias was determined as follows:

$$\text{Bias}, \% = \frac{\sum_{i=1}^{n}\left(\hat{Y}_i - Y_i\right) \times 100}{\sum_{i=1}^{n}(Y_i)}$$

where Y_i is the measured value obtained in the validation data set; $\hat{Y}_i$ is the predicted value of the validation data set.

Bias can be used to assess if there is a systematic difference between the average predicted values and the measured values. The larger the bias the less accurate the equation will be at predicting the ME of a sample of DDGS. When there is no difference between the predicted and measured values, the mean percent bias will then be zero.

RESULTS AND DISCUSSION

All values presented and discussed are expressed on a DM basis, unless otherwise noted.

Ingredient Composition

The nutrient composition and GE of the corn and DDGS samples used in this experiment are presented in Table 5.2. The GE of the 4 DDGS samples averaged 5,203 kcal/kg with a range from 5,038 to 5,321 kcal/kg, and the GE of the corn sample was 4,288 kcal/kg (Table 5.2). The average GE of the DDGS samples used in this experiment is less than those evaluated in Chapter 3 (which averaged 5,317 kcal/kg) as well as compared to values reported in the literature. For example, Stein et al. (2006), Pedersen et al. (2007), and Anderson et al. (2009) reported GE values of 5,426, 5,434, and 5,454 kcal/kg, respectively, for a range of DDGS samples (Table 2.4). However, recent research conducted by Kerr and Shurson (2013) reported GE of samples of DDGS (varying in crude fat content from 4.9 to 10.9% and 8.6 to 13.2%) in 2 experiments of 4,919 and 5,025 kcal/kg, respectively, which are lower than those measured in this experiment. In addition, NRC (2012) reported values for the GE of DDGS of 5,429, 5,271, and 5,712 kcal/kg, for samples containing greater than 10% oil, between 6 and 9% oil, and less than 4% oil,

respectively. The GE value for DDGS with less than 4% oil from NRC (2012) was based on a small number of samples and this may explain this relatively high value compared to those for samples with greater oil content (NRC, 2012). In comparison, the average oil content (average of analyzed values from the 4 different laboratories) of the 4 DDGS samples used in this experiment was 9.6% with a range from 6.4 to 12.1% (Table 5.2).

Differences in the GE of DDGS samples will reflect differences in the chemical composition of the samples. For example, in comparison with the 10 DDGS samples used by Pedersen et al. (2007), the DDGS samples used in this experiment had, on average (average of analyzed values from the 4 different laboratories), similar ash content [4.45 vs. 4.37 % ash for the samples used in the current experiment and that of Pedersen et al. (2007), respectively] but less crude fat, CP, and starch (9.55 vs. 11.67%, 29.6 vs. 32.2 %; 5.2 vs. 8.2 % , respectively) and greater ADF and NDF (13.9 vs. 11.6 %; 34.0 vs. 27.6 %, respectively).

The GE of the corn sample in the current experiment (4,288 kcal/kg; Table 5.2) was lower than the values reported by Stein et al. (2006), Pedersen et al. (2007) and NRC (2012) for yellow dent corn of 4,436, 4,496 and, 4,454 kcal/kg, respectively.

Nevertheless, the 4 DDGS samples used in this experiment had variation in chemical composition and GE, which was the target of sample selection. Thus, these samples were appropriate to address the major objective of this experiment, which was to test the prediction equations developed in Chapter 4.

There was variation in particle size between the DDGS sources (Table 5.3), with DDGS source 1 having a mean particle size of 1,064 µm, compared to 678, 620, and 758 µm for DDGS sources, 2,3 and 4, respectively. Variation in particle size was also evident in the DDGS samples used to develop the prediction equations in Chapter 3 where the mean particle size of the DDGS

samples was 665.8 ± 284.4 μm with a range from 318 to 1,557 μm, which is greater than the range in particle size for the 4 DDGS samples used in the current experiment (Table 5.3). In contrast to the situation with corn, there has been limited research carried out to establish the impact of particle size on energy and nutrient digestibility of DDGS. Yañez et al. (2010) used one source of DDGS produced from co-fermentation of corn and wheat in a 1:1 ratio and showed that reducing particle size from 517 to 383 μm increased GE digestibility by 1.1 percentage units. In addition, Liu et al. (2012) compared corn DDGS with particle sizes of 308, 595, and 818 μm and showed that the lowest compared to the highest particle size had greater digestibility of DM (1.5 percentage units) and energy (1.8 percentage units) with the DDGS sample with a particle size of 595 μm being intermediate for these measures.

Therefore, the differences in particle size between the samples used in the current experiment could have contributed to the differences in the estimates of DE and ME discussed below. For this reason, particle size was included as one of the independent variables in one of the prediction equations evaluated later in this chapter.

Apparent DE and ME of DDGS

The ingredient and analyzed composition of the control and DDGS treatment diets used in the experiment are presented in Table 5.4. Least-squares means for the effect of experimental diets and DDGS sources on energy concentration are presented in Table 5.5. Daily feed DM intake was not different between dietary treatments (Table 5.5). However, there were differences ($P < 0.05$) among dietary treatments in GE intake, with pigs fed diets with DDGS source 4 having greater GE intake than those fed the diets containing the corn sample and DDGS sources 2 and 3, with those fed diets containing DDGS sample 1 being intermediate (Table 5.5). These differences reflect the combined effects of numerical (not statistically significant) differences in

feed DM intake and, mainly, differences in GE of the diets (Table 5.5). The numerical differences in feed DM intake were the result of a small amount of feed refusals from a limited number of the pigs on the experiment.

There was an effect (P <0.05) of dietary treatment on the total energy concentration in feces, which was highest for pigs fed diets containing DDGS sources 2 and lowest for pigs fed source 3 and the corn-based diet (Table 5.5). However, the daily output of energy in the feces was similar for pigs fed diets containing the 4 DDGS sources, but was greater(P < 0.05) for these sources than for pigs fed the corn-based diet. The energy concentration in urine was not different for the 5 dietary treatments, however, the daily energy output in urine was 65.4% less (P < 0.0.5) in pigs fed the corn-based diet compared to those fed the diets with the 4 DDGS sources (Table 5.5).

The DE of experimental diets, calculated from the energy intake and excretion in feces, differed (P < 0.05) among dietary treatments with the diet containing DDGS source 1 having the highest DE, followed by the diets with DDGS source 4, the corn-based diet, and DDGS source 2, with the diet containing DDGS source 3 having the lowest DE (Table 5.5). Similarly, the ME of the experimental diets, calculated from the energy intake and excretion in feces and urine, differed (P < 0.05) among treatments, with the diet containing DDGS source 4 being highest, followed by the corn-based diet and the diet containing DDGS source 1, and then the diet containing DDGS source 2, with the diet containing DDGS source 3 being lowest (Table 5.5).

The DE and ME of the corn sample, calculated based on the DE and ME of the corn-based diet and the inclusion rate of corn in the diet, were 3,829 and 3,701 kcal/kg, respectively (Table 5.5). DDGS sources 1 and 4 had DE values that were not different from the values for the corn sample, however, DDGS sources 2 and 3 had lower (P < 0.05) DE (6.7 and 11.0 %,

respectively) than the corn (Table 5.5). The corn sample and DDGS source 4 had the greatest ($P < 0.05$) ME, followed by DDGS sources 1 and 2, with DDGS source 3 having the lowest ($P < 0.05$) ME . Furthermore, the apparent total tract digestibility (**ATTD**) of energy and DM, and energy metabolizability was 7.2, 7.4 and 9.5 percentage units ($P < 0.01$) greater, respectively, for the corn-based diet compared to the average of the 4 diets containing the DDGS samples (Table 5.5). Similarly, the corn sample had 19.4, 19.7, and 23.7 percentage units greater ($P < 0.01$) ATTD of energy and DM, and energy metabolizability, respectively, compared to the average of the 4 DDGS samples (Table 5.5).

The average value for the DE of the 4 DDGS samples (3,648 kcal/kg) was greater than those reported by Stein et al. (2006 and 2009), but less than those reported by Pedersen et al. (2007), and Anderson (2009). In addition, the average value for the ME of the 4 DDGS samples (3,361 kcal/kg) was less than values reported in the literature (Pedersen et al., 2007; Anderson et al., 2009; Stein et al., 2009). When compared to the corn sample, the DDGS samples had, on average, a lower ($P < 0.05$) DE (4.7%) and ME (9.2%). However, the range in ME values between the 4 DDGS samples (i.e., 3,105 to 3,527 kcal/kg; Table 5.5) was considerable illustrating that these samples were suitable to evaluate equations to predict the ME of DDGS, which was the primary objective of this experiment and is discussed in the next section.

Evaluation of Equations to Predict the ME of DDGS

The prediction equations evaluated in this experiment were chosen from the equations that were developed in Chapter 4, which are presented in Tables 4.6, 4.7, and 4.8. From all the equations developed in Chapter 4, 6 candidate equations were selected as the best equations to use to predict the ME of DDGS based on the criteria described in Chapter 4. These were equations number 8, 30, and 52, which were developed based on the chemical composition data

from Laboratory 1, and equations 15, 35, and 77, which were developed using the chemical composition data determined by Laboratory 2 (Table 5.6).

The predictive ability of the selected equations was evaluated by comparing the measured and predicted ME of the 4 samples of DDGS evaluated in this experiment using the RMSEP and mean percent bias statistics. Equations with the lowest RMSEP and least mean percent bias are assumed to give the most accurate prediction of the ME of DDGS. A positive mean percent bias results from the equation over-predicting the ME value of the DDGS sample, whereas, a negative mean percent bias results from the equation under-predicting the ME value of the sample.

The measured ME values for the 4 DDGS samples were from the experiment conducted to determine DE and ME (Table 5.5). In addition, ME values for the 4 DDGS samples were predicted using each of the 6 selected equations (Table 5.6) and the chemical composition of these samples determined by the same 2 laboratories (Laboratories 1 and 2) that were used in the development of the prediction equations in Chapter 4 and 2 other laboratories (Laboratories 3 and 4; Table 5.2). The RMSEP and mean percent bias statistics for the comparisons of predicted and measured ME values are presented in Table 5.7.

There was considerable range in the RMSEP and mean percent bias across the 6 prediction equations and the 4 laboratories (Table 5.7). For example, RMSEP ranged from 134 (Equation 8; Laboratory 3) to 476 (Equation 35; Laboratory 1) kcal/kg and mean percent bias from 0.5 (Equation 52; Laboratory 1) to 13.4% (Equation 35; Laboratory 1; Table 5.7)

The RMSEP and mean percent bias were lowest when the prediction of ME was based on the chemical composition data from the same laboratory as was originally used to develop the equation. For example, using chemical composition data from Laboratory 1 in Equation 8 (which was originally developed using chemical composition data from Laboratory 1) to predict

the ME of the 4 DDGS samples resulted in RMSEP and mean percent bias of 149 kcal/kg and -0.9%, respectively. In contrast, when the chemical composition data from Laboratory 2 were used in Equation 8 the values for RMSEP and mean percent bias were 436 kcal/kg and -12.6%, respectively. These results suggest that the most accurate prediction of the ME of DDGS samples will be achieved when both the prediction equation and the chemical analysis of the sample being evaluated are carried out by the same laboratory.

Predictions based on the chemical analysis carried out by Laboratory 3 also resulted in relatively low values for RMSEP and mean percent bias when the prediction equations were based on the equations developed using the data from Laboratory 1 (i.e., Equations 8, 30, and 52; Table 5.7). For example, for Equation 30, RMSEP and mean percent bias values based on Laboratory 3 chemical analysis were 183 kcal/kg and 2.6%, which are similar to values based on Laboratory 1 analysis of 176 kcal/kg and 2.5%, respectively (Table 5.7). However, this was not the case when the chemical analysis from Laboratory 3 was used in equations developed from Laboratory 2. For example, for Equation 35, RMSEP and mean percent bias values when chemical analysis from Laboratory 3 was used were 469 kcal/kg and 13.3 %, respectively, compared to values of 160 kcal/kg and 2.6 %, respectively, when the chemical composition data from Laboratory 2 was used in this equation (Table 5.7). It is difficult to explain these results, particularly as the methods used by the laboratories for the chemical analysis differed for a number of the chemical components (Table 5.1). Nevertheless, these results clearly demonstrate that the accuracy of equations to predict the ME of DDGS is dependent both on the analytical laboratory used to analyze the samples of DDGS used in the development of the prediction equations and, also, on the laboratory used to analyze the chemical composition of the samples that are being evaluated.

This research, also, has implications for the future development of equations to predict the ME of feedstuffs. One factor that can result in an inaccurate prediction of energy is if the samples being evaluated fall outside of the range of chemical composition and/or energy compared to the samples that were used to develop the equations. As the composition of co-products is likely to change over time, for example with developments in milling and fat extraction technologies, ideally new prediction equations should be developed that are accurate for the range of chemical composition and/or energy of the co-product in question.

In addition, there has been no standardization of methodology for the development of equations to predict ME of feedstuffs such as has been suggested for other areas where prediction equations are widely used. For example, Schinckel and Rusk (2012) proposed guidelines to standardize the methodology involved in the development of equations to predict carcass lean content. Of particular concern in this regard, in relation to the development of prediction equations for use in feedstuffs based on chemical composition, is the variation in methodology between laboratories in the analytical methods used. This is clearly illustrated in the present research where the accuracy of prediction of ME depended on both the laboratory used initially in the development of the equations as well as the one used for the analysis of the material being evaluated. It would be interesting to evaluate the predictive accuracy of equations developed using a similar approach as used in this research using a number of laboratories that employ the same analytical techniques for all components of chemical composition.

Recommendations for the development of prediction equations to maximize accuracy of prediction might include the use of approved methodologies to measure the energy of DDGS and other ingredients, determination of chemical composition by multiple laboratories to encompass the accepted reference methodologies, publication of prediction equations only after validation,

and acceptance of certain minimum standards of prediction accuracy before equations can be recommended for use. One improvement in this area would be to adopt a recommendation proposed by Schinckel and Rusk (2012) for use in the development of equations to predict carcass lean content, which is to develop guidelines by subject matter content experts for development of more accurate prediction equations, to include standards of accuracy and methodology to produce the equations.

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TABLES

Table 5.1. Reference methods used for chemical analysis of distillers dried grains with solubles sources at the 4 laboratories

Component	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4
DM	AOAC (2000) official method modified 935.29	AOAC (2006) official method 934.01	NFTA 2.2.2.5	NFTA 2.2.2.5
Ash	AOAC (2006) official method 942.05	AOAC (2006) official method 942.05	AOAC (2000) official method 923.03	AOAC (2006) official method 942.05
CP	AOAC (2005) official method 990.03	AOAC (2005) official method 990.03	AOAC (2002) official method 990.03	AOAC (2002) official method 990.03
Crude fat	AOAC (2005) official method 945.16	AOAC (2006) official method 920.39 (A)	AOAC (2005) official method 945.16	AOCS Ba 3-38 Mod.
Crude fiber	Ankom Filter bag procedures	AOAC (2006) official method 978.10	AOAC (2005) official method 962.09	AOAC (2005) official method 962.09
ADF	Ankom Filter bag procedures	AOAC (2006) official method 973.18 (A-D)	AOAC 973.18	Ankom Filter bag procedures
NDF	Ankom Filter bag procedures	JAOAC 56 (1973) 1352-1356	AOAC 2002.04	Ankom Filter bag procedures
Starch	AACC (2000) 76-11	AACC (1975) approved method 76-13.01 modified: Sigma Starch Assay Kit (Kit STA-20 St. Louis, MO)	Modified AOAC 996.11 – Mary Beth Hall, USDA-ARS	EEC (1972) Ewers polarimetric method

Table 5.2. Analyzed nutrient composition and GE of the corn and distillers dried grains with solubles sources conducted by the 4 laboratories (DM basis)

		DDGS Source			
Component	Corn	1	2	3	4
Laboratory 1					
DM, %	87.31	90.68	90.99	89.42	89.25
Ash, %	1.74	4.68	4.64	4.59	3.91
CP,%	8.50	26.47	30.22	29.97	29.69
Crude fat, %	5.36	12.13	8.54	7.79	11.32
Crude fiber, %	2.57	6.37	6.65	6.87	8.75
ADF, %	3.84	16.21	11.98	15.54	13.78
NDF, %	11.68	30.22	29.34	27.51	31.93
Starch, %	66.59	6.10	6.43	5.67	3.98
Laboratory 2					
DM, %:	-	88.06	87.98	87.04	85.94
Ash, %	-	4.88	5.01	5.26	4.71
CP, %	-	31.90	27.78	30.93	30.28
Crude fat, %	-	8.62	11.17	6.71	10.89
Crude fiber, %	-	7.31	7.40	8.01	8.02
ADF, %	-	10.86	15.87	13.11	12.76
NDF, %	-	38.93	46.73	38.78	45.81
Starch, %	-	4.79	3.58	4.01	2.33
Laboratory 3					
DM, %	-	90.40	90.00	88.75	88.43
Ash, %	-	4.86	4.80	5.00	4.61
CP, %	-	27.32	31.56	30.42	29.97
Crude fat, %	-	11.46	8.72	7.03	11.29
Crude fiber, %	-	8.31	7.22	7.97	7.92
ADF, %	-	15.39	10.78	13.55	12.60
NDF, %	-	32.19	29.78	30.08	30.42
Starch, %	-	7.28	7.47	6.30	4.90
Laboratory 4					
DM, %	-	90.63	90.59	89.07	88.62
Ash, %	-	5.75	4.99	6.76	4.93
CP, %	-	26.79	30.46	30.93	29.32
Crude fat, %	-	11.24	8.08	6.44	11.11
Crude fiber, %	-	6.84	6.40	6.96	7.22
ADF, %	-	19.53	12.25	15.72	14.67
NDF, %	-	33.65	31.79	31.10	35.43
Starch, %	-	7.06	4.64	5.50	3.39
GE, kcal/kg[1]	4,288	5,215	5,236	5,038	5,321

[1]GE analyses conducted at UIUC using a bomb calorimeter (Isoperibol Bomb Calorimeter, Model Number 1281; Parr Instrument Co., Moline, IL).

Table 5.3. Geometric mean particle size of the distillers dried grains with solubles sources (as received)

DDGS source	Mean particle size, μm[1]	SD[2]
1	1,064	1.74
2	678	1.78
3	620	1.73
4	758	1.75
All sources:		
Mean	780	1.75
SD[3]	197.61	0.02

[1]The geometric mean particle size for each sample was determined using a Rotap 13-sieve shaker (model RX-29, W.S. Tyler Co, Cleveland, OH).
[2]Within-sample SD of geometric mean particle size.
[3]Between-sample SD for the geometric mean particle size and SD of the 4 samples of DDGS.

Table 5.4. Ingredient and analyzed composition (DM basis) of the corn-based control and experimental diets containing the distillers dried grains with solubles sources

		DDGS source			
	Control	1	2	3	4
Ingredient, (%):					
Corn	97.32	48.66	48.66	48.66	48.66
DDGS	-	48.66	48.66	48.66	48.66
Limestone	0.60	0.60	0.60	0.60	0.60
Dicalcium phosphate[1]	1.51	1.51	1.51	1.51	1.51
Salt	0.45	0.45	0.45	0.45	0.45
Trace minerals[2]	0.09	0.09	0.09	0.09	0.09
Vitamins[3]	0.03	0.03	0.03	0.03	0.03
Composition, %:[4]					
Dry matter, %	87.36	88.25	88.06	87.32	86.69
Ash, %	3.34	4.51	4.69	4.70	4.70
CP, %	6.75	15.50	16.10	17.10	15.60
Crude fat, %	2.97	6.94	5.61	4.84	6.22
Crude fiber, %	0.61	2.08	2.81	2.66	2.38
ADF, %	2.75	6.93	6.35	6.67	5.37
NDF, %	7.52	16.2	15.20	17.4	18.4
Starch, %	63.05	34.00	36.99	33.92	31.59
GE, kcal/kg[5]	3,767	4,192	4,104	4,041	4,182

[1]Dicalcium phosphate (18.5% phosphorus; 22% calcium).

[2]The mineral supplement provided the following per kilogram of complete diet: Copper, 6.75 mg; Iodine, .13 mg; Iron, 68 mg; Manganese, 16 mg; Selenium, .13 mg; Zinc, 68 mg.

[3]The vitamin supplement provided the following per kilogram of complete diet: vitamin A (Retinyl Acetate), 5,287 IU; vitamin D_3 (Cholecalciferol), 826 IU; vitamin E (dl or d – Alpha tocopheryl Acetate), 26.4 IU; vitamin K (Menadione Dimethylpyrimidinol Bisulfate) 1.5 mg; vitamin B_{12} (Cyanocobalamin), 0.02 mg; Niacin (Niacinamide or Nicotinic Acid), 25.1 mg; Panthothenic acid (d-Calcium Panthothenate), 21.5 mg; Riboflavin, 5.9 mg.

[4]Chemical composition analyzed by a commercial laboratory (Laboratory 1).

[5]GE analysis conducted at UIUC using bomb calorimetry (Isoperibol Bomb Calorimeter, Model Number 1281; Parr Instrument Co., Moline, IL).

Table 5.5. Least-squares means for the effect of experimental diets containing the corn and distillers dried grains with solubles sources on DE and ME, and digestibility of DM and energy (DM basis)

	Treatment						
		DDGS source				Treatment	
Item	Corn	1	2	3	4	SEM	*P* value
Number of pigs[1]	7	8	8	8	8		
Feed Intake, kg/d	1.38	1.33	1.29	1.33	1.37	0.035	0.21
GE:							
Intake, kcal/d	5,912^{b}	6,198^{ab}	5,924^{b}	6,032^{b}	6,430^{a}	160.3	0.05
Output in feces:							
Total, kcal/kg	5,012^{c}	5,061^{bc}	5,265^{a}	4,969^{c}	5,214^{ab}	73.6	<0.01
Daily, kcal/d	764^{b}	1,125^{a}	1,158^{a}	1,236^{a}	1,244^{a}	59.0	<0.01
Output in urine:							
Total, kcal/kg	1,971	2,385	2,371	2,265	2,050	225.2	0.57
Daily, kcal/d	183^{b}	287^{a}	276^{a}	292^{a}	264^{a}	28.6	0.05
Energy concentration							
Experimental diet:							
DE, kcal/kg	3,726^{bc}	3,833^{a}	3,701^{dc}	3,617^{d}	3,801^{ab}	33.5	<0.01
ME kcal/kg	3,596^{ab}	3,607^{ab}	3,488^{bc}	3,397^{c}	3,608^{a}	42.2	<0.01
Dry matter digestibility, %	88.9^{a}	83.3^{b}	83.0^{bc}	81.2^{c}	82.6^{bc}	0.633	<0.01
Energy digestibility, %	87.1^{a}	81.9^{b}	80.5^{bc}	79.5^{c}	80.7^{bc}	0.725	<0.01
Energy metabolizability, %	84.0^{a}	77.0^{b}	75.9^{b}	74.6^{b}	76.6^{b}	0.914	<0.01
Test ingredient:							
DE, kcal/kg	3,829^{a}	3,838^{a}	3,572^{b}	3,406^{b}	3,774^{a}	64.5	<0.01
ME kcal/kg	3,701^{a}	3,525^{ab}	3,287^{bc}	3,105^{c}	3,527^{a}	82.2	<0.01
Dry matter digestibility, %	91.4^{a}	75.2^{b}	74.6^{b}	71.1^{c}	73.8^{bc}	1.19	<0.01
Energy digestibility, %	89.5^{a}	74.4^{b}	71.5^{bc}	69.5^{c}	72.0^{bc}	1.39	<0.01
Energy metabolizability, %	86.4^{a}	68.0^{b}	65.5^{b}	63.1^{b}	67.0^{b}	1.77	<0.01

[1]Observations for feed intake for 1 pig fed the control diet, and urine collection data for 2 pigs fed the diets that included DDGS samples 1 and 2, respectively, exceeded their treatment mean by more than 3 SD, were considered outliers, and were, therefore, removed from the data set before analysis.

[a,b,c]Within a row, means with a different superscript letter differ ($P < 0.05$).

Table 5.6. Selected equations to predict the ME of distillers dried grains with solubles based on proximate analysis plus other chemical components and particle size, other chemical components only, and all possible chemical components and particle size for Laboratories 1 and 2 and combined (from Chapter 4)

			Chemical components									Statistics			
Eq. No.	No. Variables	Intercept	Ash	CP	Crude Fat	Crude Fiber	ADF	NDF	Starch	GE	PS[1]	R^2	$\overline{R}^2$	C(p)	RMSE
Proximate analysis plus other chemical components and particle size															
Laboratory 1															
8	8	-4,510.07	46.75	18.77	21.88	72.74	-	-32.14	27.65	1.40	-0.17	0.90	0.80	8.1	48.8
Laboratory 2															
15	6	3238.50	-12.66	13.87	56.56	-12.11	-	-18.18	22.91	-	-	0.82	0.71	5.0	58.6
Other chemical components only															
Laboratory 1															
30	3	-448.89	-	-	-	-	-21.52	-26.64	-	0.96	-	0.67	0.60	2.1	68.9
Laboratory 2															
35	3	-332.41	-	-	-	-	-	-25.47	21.04	0.92	-	0.64	0.55	3.1	72.6
All possible chemical components and particle size															
Laboratory 1															
52	4	-649.39	-	-	-	54.65	-24.45	-26.85	-	0.92	-	0.84	0.79	3.8	49.3
Laboratory 2															
77	4	2973.95	-	15.00	60.09	-	-	-17.41	25.62	-	-	0.81	0.75	1.2	53.9

[1]Particle Size.

Table 5.7. Predicted ME values (kcal/kg), root mean square error of prediction (kcal/kg), and mean percent bias (%) between measured and predicted ME values of distillers dried grains with solubles using the selected prediction equations and chemical data from four different laboratories

		Laboratory 1[1]			Laboratory 2[1]			Laboratory 3[1]			Laboratory 4[1]		
Eq. No.	No. Variables	Predicted	RMSEP	Bias	Predicted	RMSEP	Bias	Predicted	RMSEP	Bias	Predicted	RMSEP	Bias
Proximate analysis plus other chemical components and particle size													
Laboratory 1[2]													
8	8	3,330	149	-0.9	2,937	436	-12.6	3,403	134	1.2	3,237	179	-3.7
Laboratory 2[2]													
15	6	3,648	316	8.5	3,341	138	-0.6	3,632	291	8.1	3,531	202	5.1
Other chemical components only													
Laboratory 1[2]													
30	3	3,444	176	2.5	3,129	263	-6.9	3,448	183	2.6	3,332	189	-0.9
Laboratory 2[2]													
35	3	3,813	476	13.4	3,447	160	2.6	3,811	469	13.3	3,722	388	10.7
All possible chemical components and particle size													
Laboratory 1[2]													
52	4	3,378	159	0.5	3,093	300	-8.0	3,424	161	1.9	3,246	220	-3.4
Laboratory 2[2]													
77	4	3,632	298	8.1	3,342	137	-0.6	3,633	290	8.1	3,526	197	4.9

[1]Laboratory that was used to carry out the chemical analysis of the 4 DDGS samples that were used in this chapter to evaluate the prediction equations.

[2]Laboratory that was used to carry out the chemical analysis of the DDGS samples that were used in Chapter 4 to develop the prediction equations.