University of Arkansas, Fayetteville

ScholarWorks@UARK

Theses and Dissertations

7-2020

Distillers Dried Grains with Solubles: Determination of Metabolizable Energy Values Using Regression-Based Assays and their Correlation with Chemical Composition

Kenia Mitre
University of Arkansas, Fayetteville

Follow this and additional works at: https://scholarworks.uark.edu/etd

 Part of the Animal Experimentation and Research Commons, Animal Studies Commons, and the Poultry or Avian Science Commons

Citation

Mitre, K. (2020). Distillers Dried Grains with Solubles: Determination of Metabolizable Energy Values Using Regression-Based Assays and their Correlation with Chemical Composition. *Theses and Dissertations* Retrieved from https://scholarworks.uark.edu/etd/3792

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact ccmiddle@uark.edu.

Distillers Dried Grains with Solubles: Determination of Metabolizable Energy Values Using Regression-Based Assays and their Correlation with Chemical Composition

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Poultry Science

by

Kenia Mitre
University of Arkansas
Bachelor of Science in Animal Science, 2018

July 2020
University of Arkansas

This thesis is approved for recommendation to the Graduate Council.

_____
Samuel J. Rochell, Ph.D.
Thesis Director

_____
Ken Coffey, Ph.D.
Committee Member

_____
Edward Gbur, Ph.D.
Committee Member

ABSTRACT

The inherent variability and lack of standardization of in vivo assays to determine the metabolizable energy (**ME**) of DDGS has led to inconsistent values and limited its potential to use in broiler diets. Therefore, two experiments were conducted to characterize the content of nitrogen-corrected ME (**ME_n**) in 11 DDGS sources obtained from various ethanol plants using a regression-based broiler assay and to attempt to develop equations to predict ME_n based on its chemical composition. In experiment 1, the objectives were to determine the influence of inclusion level and feed intake on the resulting ME and ME_n values of a single DDGS source when fed to broilers at multiple dietary inclusion levels. The ME and ME_n values of DDGS determined by difference decreased as its dietary concentration increased. Pair-feeding the 30% DDGS diet to the 60% DDGS diet intake reduced the ME and ME_n values of DDGS, indicating there was an effect of feed intake on the ME value of DDGS. Additionally, the ME_n of DDGS was determined by two regression-based methods. First, the DDGS associated caloric intake was regressed against the amount of DDGS intake to generate linear regression equations with slopes corresponding to the ME_n value of DDGS. Secondly, the dietary ME_n was regressed against the DDGS inclusion level, and extrapolation of the regression line to 100% DDGS was used to estimate its ME value. Both regression methods yielded similar ME and ME_n values of DDGS. Experiment 2 determined the ME_n of 11 DDGS samples obtained from different biorefinery locations operated by a single ethanol producer and related these values with chemical composition and physical properties of the DDGS samples. Analyses of DDGS included gross energy, CP, Lys to CP ratio (**Lys:CP**), ether extract, DM, starch, total dietary fiber (**TDF**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), color scores, and particle size. On a DM basis, ME_n of the 11 DDGS sources ranged from 2,284 to 3,088 kcal/kg with a CV of 7%.

Hemicellulose was the only component found to be correlated with the ME_n of DDGS. As a result, the lack of correlations between DDGS composition and its ME_n precluded development of prediction equations. Overall, these results indicate that the ME_n of DDGS estimated in ME_n assays is influenced by its inclusion level in the test diet and partly due to effects on feed intake While the narrow variability in the chemical composition of the DDGS sources did not allow for the development of prediction equations, these results provide good insight into the energy utilization and uniformity of these sources for poultry feed formulations.

©2020 by Kenia Mitre
All Rights reserved

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my advisor Dr. Samuel Rochell for his constant support, encouragement, and patience through my master's program. Thank you for making me step out of my comfort zone and for pushing me farther than I thought I could go. I also would like to thank my committee members, Dr. Ken Coffey and Dr. Edward Gbur, for their helpful feedback and suggestions that contributed to this thesis. I am also very grateful to Dr. Susan Watkins for introducing me to poultry and inspiring me to pursue a master's degree.

I feel honored for the opportunity to work besides fantastic and hard-working colleagues like Brooke Bodle, Jean Rémi, Skyler west, and Trevor Lee. I am thankful for all their invaluable help, advice, and, more importantly, for their friendship. I also would like to thank our entire lab team as well as the staff of the Poultry Research Farm for helping me when needed.

My deepest gratitude is for my family, especially to my mom, Lizi, and my grandma, María. Thank you for being my daily motivation to keep moving forward even in difficult times. This journey would not have been possible without their support, prayers, care, and love even from a distance. To my best friends Zulany and Rafael, thank you so much for always listening and giving me words of encouragement throughout these years. To the rest of my friends in Fayetteville, thank you for all your help, but especially for being my family away from home.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1: INTRODUCTION

Corn has been used as the main source of energy in poultry diets for centuries. However, with increases in corn demand for alcohol and fuel production, there is a need to better characterize readily available and economical alternative feed ingredients to efficiently grow poultry and livestock. The use of corn for ethanol production has resulted in the availability of ethanol dry milling co-products such as distillers dried grain with solubles (DDGS). Corn contains around 70% starch, and after the fermentation of starch to produce ethanol, the remaining 30% which consists of oil, fiber and protein, is concentrated in the resulting DDGS (Singh et al., 2001; Belyea et al., 2010a). Since its widespread availability in the early 2000s, DDGS has gained attention as an efficient alternative feed ingredient from a nutritional and economic standpoint. Accordingly, nutritionists have included DDGS in their feed formulations based on its contribution of available phosphorous, amino acids and metabolizable energy. Previous experiments have shown that a percentage of corn and soybean meal, which are the main components of conventional poultry diets, can be replaced by adding DDGS (Świątkiewicz and Koreleski, 2008; Wamsley et al., 2013). However, even though DDGS has a desirable nutritional profile, the variability in its nutritional content, specifically in its ME content, has been the major influencing factor leading to its low inclusion levels (typically less than 10%) in broiler diets.

Pedersen et al. (2014) found that nutrients such as fat, fiber, protein, and minerals are the most concentrated in DDGS. Similarly, studies have shown that the energy content in DDGS can be directly influenced by its fat, fiber and crude protein content (Batal and Dale, 2006). In recent years, most ethanol plants have opted for a higher extraction of oil, leading to lower and more variable contents of fat among DDGS samples (Kerr et al., 2013). To better understand

relationships between the chemical composition of DDGS and its ME content, several ME prediction equations have been developed and validated in broilers (Batal and Dale, 2006; Rochell et al., 2011; Meloche et al., 2013, 2014). Indeed, development and validation of robust prediction equations would reduce the need to conduct individual ME assays for each DDGS source, which is infeasible for poultry integrators producing high volumes of feed each day.

Even though prediction equations have been established to better predict the ME of DDGS based its nutritional profile, the variability associated with these equations and their predictive capacity has limited their use. This variability may be partly associated with the inherent variability of ME assays. While several laboratories worldwide have made efforts to standardize ileal amino acid digestibility assays (Ravindran et al., 2017), there remains considerable variation and debate regarding the best approaches for ME determination (Mateos et al., 2019; Wu et al., 2020). Therefore, there are several factors within the ME in vivo assays that could be modified to better determine the energy content of feed ingredients such as DDGS and potentially improve the accuracy of energy prediction equations.

To improve *in vivo* assays for ME determination in DDGS, aspects such as inclusion level and feed intake should be considered. For instance, by adding multiple inclusion levels of the test ingredient, the variability of the ME estimate could be potentially reduced. However, the bird's feed intake might be affected when feeding a single ingredient at high inclusion levels due to unbalanced diets, dietary energy content, palatability, and the fiber content of the experimental diets (Walugembe et al., 2014). An approach that allows the addition of multiple inclusion levels is the regression method, which was first proposed by Potter et al. (1960). A benefit of this approach is that it eliminates the need to determine endogenous energy losses (Ravindran et al., 2017). This approach has been used to determine the ME of ingredients such as barley, corn,

soybean meal, and DDGS (Villamide et al., 1997; Lopez and Leeson, 2008; Adeola and Ileleji, 2009).

Despite the numerous studies conducted, there is uncertainty about the inclusion of high levels of DDGS in poultry diets due to nutrient variability and bird response. The amount of starch removed from corn for ethanol production will vary from plant to plant, and this will directly influence the resulting ME of the DDGS (Knott et al., 2017). Currently, there is a lack of experiments conducted to observe the effect of inclusion levels on ME estimate using DDGS sources coming from different plants of a single ethanol producer. Furthermore, improvements in ME bioassays could lead to less variable ME estimates, which will result in more accurate correlations to DDGS chemical composition. Indeed, accurate correlations could result in more reliable predictors for the development of prediction equations. Therefore, the objective of this thesis are as follows:

1. Identify the most appropriate DDGS inclusion levels for a regression-based ME assay and determine the effect of feed intake associated with its inclusion level on ME_n of DDGS.

2. Use the optimized regression assay to determine the ME_n content of 11 sources of DDGS produced from various ethanol plants and use compositional analyses to develop ME_n prediction equations.

CHAPTER 2: LITERATURE REVIEW

DISTILLERS DRIED GRAINS WITH SOLUBLES

The rapid increase in ethanol production from cereal grains in the last decade led to the widespread availability of co-products such as DDGS. The ethanol industry utilizes cereals such as wheat, corn, or a mix of both grains as feedstocks in their production plants (Nuez Ortín and Yu, 2009). The United States is the biggest producer of corn in the world, so corn is currently the primary feedstock for domestic biofuel production. According to USDA (2019), 37% of the corn used in the USA is used in ethanol production, and corn DDGS was the most valuable co-product of this industry. Corn can be converted to ethanol by both wet and dry milling processes. The dry milling process has been more commonly used due to lower costs and the ability to produce DDGS as a co-product (Rausch and Belyea, 2006). Similarly, due to the high production of DDGS, it has been commercialized as a valuable feed ingredient for livestock and poultry. Initially, the majority of the DDGS produced was fed mainly to ruminants (around 75%), and the remaining 20% was fed to swine and poultry due to its high fiber content (Leytem et al., 2008). Indeed, the limited ability of birds to digest complex carbohydrates within DDGS restricts it to low inclusion levels in poultry feeds. Similarly, the inconsistency in its nutrient content has made nutritionist cautious about including DDGS in their feeding programs. However, innovations in processing techniques in modern ethanol plants have improved the nutritional profile of DDGS as a more uniform source of digestible amino acids, phosphorous and metabolizable energy (Spiehs et al., 2002; Lumpkins et al., 2004).

Nutritional variability of DDGS

Nutrient variability can be a limiting factor when using an ingredient in diet formulation. Underestimating the nutritional content of an ingredient can directly affect growth performance

while overestimating nutritional values can lead to economic losses and negative impacts on the environment. During ethanol production, there are several factors that can directly influence the nutrient composition of the resulting DDGS. The fermentation process has shown to affect the physical properties (i.e. particle size, color) and chemical composition such as protein, fat and fiber content (Belyea et al., 2010b). Two of the biggest components of fiber, or non-starch polysaccharides (**NSP**), in corn are arabinoxylan and cellulose. Also, the NSP content of DDGS could be affected by the fermentation and drying process (Świątkiewicz et al., 2013). As such, it has been reported that the addition of fiber degrading enzymes during the fermentation process improve nutrient availability and the ME of ingredients with high insoluble polysaccharide content (Pedersen et al., 2014). A study reported that 23% of the variability in ethanol concentration was driven by the type of corn hybrids; however, when corn sources were from producers within the Minnesota-Dakota geographical area, lower variability in nutrient composition of DDGS was reported (Spiehs et al., 2002).

In addition to corn quality, nutritional variability of DDGS can arise from factors directly related to the fermentation process such as temperature, additives, and the type of yeast used (Rausch and Belyea, 2006). Another factor that has been shown to cause variability in DDGS is the drying process, as heating can directly impact its protein quality and digestibility (Young, 2008). Efforts have been made to modify the dry-grind ethanol techniques to allow for a better nutritional profile of the resulting DDGS. Kim et al. (2010) suggested that the removal of the fiber content of corn or DDGS before or after the fermentation process by using enzymatic milling (E-mill) or sieving and air classification (Elusieve) process, results in DDGS with higher TME_n, protein, and digestible amino acid content than conventional DDGS. In addition, the

chemical composition and processing techniques among plants are factors that have shown to contribute to the variability of DDGS (Belyea et al., 2004; Batal and Dale, 2006).

With new technologies, ethanol plants have been modifying their processing techniques, which has been one of the significant causes of nutrient variability in the resulting DDGS. According to Shurson et al. (2004), old ethanol plants (~ 40 years old) utilized various enzymes in their fermentation process, which influenced the fermentation and heating time, whereas second-generation plants (~ 20 years old) improved the DDGS quality by reducing heating time. In addition, modern ethanol plants are increasing oil extraction from DDGS which has downgraded its amino acid profile. A study conducted by Spiehs et al. (2002) reported higher values of gross energy, phosphorous and total amino acid levels such as Met, Thr and Lys in modern DDGS ethanol plants from Minnesota when compared to older ethanol plants over a three year period. In addition, Belyea et al. (2010) reported greater nutrient variability between batches at a single plant than between plants.

Phosphorous

The consumption of starch during ethanol production results in the concentration of the remaining components of corn, including phosphorus and amino acids (Belyea et al., 2010b). Due to the production of microbial phytase during the fermentation process in ethanol production, the available P content on DDGS has been reported to be higher than in corn (Lumpkins and Batal, 2005). According to the NRC (1994), DDGS contains 72% total P, and 54% of that is in the non-phytate P form. Modifications in the fermentation process, such as adding microbial phytase, citric acid, or increasing heating temperature, have been shown to improve P bioavailability on DDGS (Martinez Amezcua et al., 2004; Martinez-Amezcua et al., 2006). Phosphorous is not only an expensive nutrient; it can be detrimental to the environment

when it is excessively excreted by the birds. Therefore, efforts have been made to accurately determine the available P content on DDGS to minimize cost and contamination. Wang et al. (2007) developed a DDGS nutrient matrix based of previously published data and found higher available P (0.84%) on a new DDGS source than the calculated values (0.77%) from the nutrient matrix. This result agrees with the value of 0.89 % available P found by Spiehs et al. (2002) when analyzing 118 DDGS samples over a period of 3 years. Even though increasing temperature during fermentation can improve P availability, it has been reported that the digestibility of amino acids, such as lysine, is negatively affected by heat (Warnick and Anderson, 1968). Kingsly et al. (2010) conducted an experiment where multiple levels of condensed distillers solubles were added and they reported increases in the P content of DDGS as the amount of solubles increased. Therefore, they suggested that a possible way to control the P content of DDGS is by the amount of solubles that is added during drying.

Amino Acids

The amino acids in corn are also concentrated in DDGS following starch removal for ethanol production. However, the increase in temperatures during ethanol production can induce Maillard reaction where lysine reacts with reducing sugars, making it indigestible to the animal (Martinez-Amezcua et al., 2006). When exposed to different heat processing conditions such as oven drying or autoclaving, the lysine content and digestibility of DDGS was decreased from 0.9% to 0.6% and from 68 to 45%, respectively (Martinez Amezcua and Parsons, 2007). Accordingly, studies have shown that the least digestible amino acids in DDGS are lysine, cysteine and threonine (Batal and Dale, 2006; Szczurek, 2010). Similarly, Parsons et al. (2006) determined the amino acid profile of 20 DDGS sources and found the results to be in agreement with those reported for early generation DDGS by NRC (1994); however, the lowest digestibility

and higher variability was observed for lysine (72%). Similarly, in an experiment conducted by Lumpkins and Batal (2005) it was determined that the true lysine digestibility of DDGS was approximately 75%. Several studies have found color and amino acid digestibility of DDGS to be significantly correlated, with lighter colors often corresponding to better amino acid profiles (Batal and Dale, 2006; Fastinger et al., 2006; Adedokun et al., 2009). Foltyn et al. (2014) found lysine digestibility to range from 45.8% to 76.8%, with the lowest value corresponding to the darkest samples and the highest value corresponding the bright yellow samples. In addition, color has been highly correlated with ME of DDGS, where darker colors correspond to lower ME values (Jie et al., 2013b)

METABOLIZABLE ENERGY OF DDGS

Several studies have been conducted to determine the ME of DDGS (Table 1). Initially, the TME assay using precision fed roosters was the most commonly used method for DDGS evaluation. Batal and Dale (2006) determined the TME_n of 17 DDGS samples from 6 different ethanol plants ranged from 2,490 to 3,190 kcal/kg (86% DM basis). In addition, Parsons et al. (2006) determined the TME_n values of 20 DDGS samples by using the precision fed rooster assay and reported values from 2,607 to 3,054 kcal/kg. Similarly, another experiment reported the TME_n content of five DDGS samples to range from 2,484 and 3,014 kcal/kg (Fastinger et al., 2006). The TME_n values reported by Parsons et al. (2006), Batal and Dale (2006), Fastinger et al. (2006) agree with the ones reported in NRC (1994). Later, Rochell et al. (2011) evaluated 6 DDGS sources from modern ethanol plants by using the single ingredient replacement assay in broilers and determined their average nitrogen corrected apparent metabolizable energy (AME_n) value to be 2,678 kcal/kg.

One of the main driving factors that will influence the ME content of DDGS is the amount of solubles that is added. A study conducted by Kingsly et al. (2010) determined that the ratio of condensed distiller solubles to wet distillers grains will directly influence the chemical composition of DDGS. Their results showed that reducing the amount of condensed distiller solubles added, crude protein, acid detergent fiber, neutral detergent fiber, and amino acid content increased, but fat, sugars, ash and glycerol content decreased (Kingsly et al., 2010). Similarly, Noll et al. (2007) reported increases in ash and fat content (8 to 10.5%) and particle size, as well as an increase in TME_n from 2,712 kcal/kg for distillers dried grain to 3,743 kcal/kg when 100% of solubles were added back. Oil extraction in DDGS for biodiesel production or for human feed industry has been a modern practice that has been implemented in most U.S. ethanol plants and has resulted in a higher variability in the fat content of DDGS (Jacela et al., 2011; Shurson, 2017). For instance, DDGS that have experienced oil extraction can have fat contents that range from 4 to 12%, with conventional DDGS having fat content ranging from 9 to 14% (Shurson, 2017). Meloche et al. (2013) determined on broilers the AME_n content of 15 DDGS sources varying in ether extract content to range from 1,869 to 2,824 kcal/kg of DM.

DDGS and broiler performance

Several studies have been conducted to determine appropriate levels of DDGS that can be used without causing negative effects on the overall performance of the birds. A study conducted by Wang et al. (2007) determined that DDGS could be successfully included up to 10% of the diets when formulated on a digestible amino acid basis, but meat quality and growth performance were compromised when included at 30% of the diet. Similarly, Zhang et al. (2013) found improvements in the overall growth performance of broilers when DDGS was fed at a 10% inclusion level in combination with vitamin E when compared to higher inclusion levels;

however, decreases in feed intake were observed when fed at 20% inclusion. On the other hand, increases in feed intake and feed conversion ratio (FCR) have been observed when birds were fed diets containing 15% DDGS inclusion level (Youssef et al., 2008; Campasino et al., 2015). According to Waldroup et al. (1981), DDGS could be added at up to 25% to diets without causing any negative effect to performance if dietary energy levels are kept constant; however, negative effects on growth performance can result when the energy level is allowed to decrease at 15% DDGS inclusion level or more. Lumpkins et al. (2004) reported that an 18% inclusion level during the starter phase caused negatives effects on chick performance and attributed this to the low lysine and the high fiber content when DDGS is included at high levels. Nutritionist often opt for formulating diets with low inclusion levels due to the inconsistent results regarding the most appropriate levels of DDGS to include in the diets. Therefore, the results from the experiments presented above highlight the importance of having reliable ME estimates for DDGS to optimize its inclusion level in practical diets.

ME DETERMINATION

ME assay methodology for complete diets

Due to the importance of balancing dietary ME when incorporating DDGS in broiler feeds, it is critical to obtain accurate estimates of its ME contribution. Metabolizable energy, the most commonly used energy system in broilers is calculated based the simple determination of the energy intake from feed minus the energy lost in the excreta. A more commonly used term is apparent metabolizable energy (AME), and this is the energy that the animal is utilizing without accounting for endogenous energy losses. On the other hand, TME typically uses precision fed roosters, which allows for direct and exact distribution of the ingredient to calculate the ME while accounting for the endogenous losses accurately.

Several different techniques are currently being used in bioassays to determine the metabolizable energy of feed ingredients for broilers, with little standardization among laboratories. Most ME bioassays include feeding the birds *ad libitum* followed by a total collection period where feed energy intake and excreta energy output are determined. When the total collection approach cannot be conducted, the index method is used as an alternative. The index method requires the use of an undigestible marker such as titanium dioxide (TiO_2), typically included at 0.3 to 0.5% of the diet, which can be used to calculate the energy that was ingested and excreted based on the analyzed concentrations of the marker in the feed and excreta. The total collection method reduced the errors associated with undigestible marker recovery. However, the index method only requires a small sample and eliminates errors related to contamination with feathers and feed, excreta adherence to wire floors and excreta falling out of the tray (Short et al., 1996; Smeets et al., 2015). In both cases, if the excreta samples or trays are not weighted accurately or the undigestible maker is not completely recovered the resulting ME values will be affected.

To be able to compare ME values across factors such as bird age, growth rate, and breed, a nitrogen retention correction factor is applied (Sibbald, 1989; Farrell et al., 1991). The correction value commonly used is 8.22 kcal GE/g N retained which represents the energy value of uric acid (Hill and Anderson, 1958). According to Shrimpton et al. (1977) variability on ME estimates on high protein ingredients can be reduced by correcting for nitrogen retention. Miski and Quazi (1981) reported that endogenous urinary and metabolic fecal energy and nitrogen loses in broilers are directly influenced by the basal metabolic energy requirements of the birds and their age. Similarly, Dale and Fuller (1984) determined that nitrogen correction can affect high protein feed ingredients, resulting in significantly lower values. Furthermore, it is important

to provide ME values and ME nitrogen corrected (ME_n) values specially when conducting *in vivo* assays on broilers, which have a high rate of protein deposition and consume high protein feed ingredients.

ME assay methodology for individual feed ingredients

While there are several factors to consider to accurately determine the ME of complete diets, there can be additional complications when trying to determine the ME of individual feed ingredients. The ME value of feed ingredients can be calculated by the direct or substitution method. The direct method consists of feeding the bird the single ingredient only. Due to palatability issues of feeding single ingredients, this is often accomplished via tube-feeding in adult roosters termed ”precision fed roosters” (Sibbald, 1975). This assay has been widely used to determine the TME of grains, proteins and by-products (Batal and Dale, 2006; Fastinger et al., 2006). The advantage of using this method is that fewer birds are required, and it does not require formulation and mixing of complete diets. Another advantage is that adult roosters have a developed digestive system compared to the immature digestive system of broilers incapable of fully digesting and absorbing nutrients (Jin et al., 1998). However, one of the main concerns when determining the ME of feed ingredient by this method is that a large proportion of the excreta energy can be from endogenous losses when roosters are not fed *ad libitum*. In addition, broilers in commercial operations are fed complete diets and studies have shown that interactions between nutrients and ingredients play a role in the dietary energy that is available to the bird (Mateos et al., 2019).

Substitution method

In contrast to the direct method, the substitution methods consists of feeding the test ingredient as part of a complete diet and is the most widely used method to determine the ME of feed ingredients (Wu et al., 2020). There are different ways to incorporate the test ingredient into the diet when using the substitution method. One approach is the basal substitution initially proposed by (Sibbald and Slinger, 1963) in which a proportion of the basal diet is substituted by the test ingredient. The ME of the basal diet and the test diets is determined, and the ME value of the test ingredient can be calculated by difference. The test diets for this approach can be formulated with the same level of minerals and vitamins, and the energy to crude protein ratios are maintained to prevent deficiencies of these nutrients. The other approach is the single ingredient method where a well characterized ingredient such as dextrose is used as reference ingredient in a reference diet. Test diets are formed by replacing dextrose only with the test ingredient at one or multiple inclusion levels. Hill and Anderson (1958) conducted several experiments and determined the ME value of dextrose to be 3,640 kcal/kg. Several studies have used this approach to determine the metabolizable energy of feed ingredients (Pesti et al., 1986; Rochell et al., 2011; Meloche et al., 2013).

Effect of Inclusion level

In addition to the dietary component that is replaced by the test ingredient in the substitution method, the inclusion level of the test ingredient has also been shown to influence the resulting ME (Sibbald and Slinger, 1962). When conducting ME assays, the addition of dietary inclusions of the test ingredient reduces variability around the ME estimate because a higher proportion of the total dietary energy is derived from the test ingredient. Nonetheless, dietary nutritional balance, palatability and feed intake can be affected by high inclusion level of

ingredients such as DDGS. Guillaumb and Summers (1970), demonstrated that the apparent ME value (i.e., not corrected for endogenous losses) of a diet will be reduced at low feed intake due to the fact that a higher proportion of the excreted energy is of endogenous origin. Previously, several ME assays have been conducted using high inclusion levels up to 60% for barley and 75% for corn (Villamide et al., 1997; Lopez and Leeson, 2008). Lopez and Leeson (2008) observed increases in the AME_n values of soybean meal as inclusion levels increased from 10 to 30%. When conducting ME assays the most appropriate levels should be selected to reduce variability and be kept within a range that can be applicable to industry conditions.

Regression-based ME assays

When multiple inclusion levels of the test ingredients are fed to birds, the ME of the test ingredient is determined by the regression method. Within the regression method, there are two approaches that have been commonly used for ME determination. One of them is where the diet ME value is regressed against the test ingredient inclusion levels. Therefore, the ME value of feed ingredients is determined by extrapolating to 100% inclusion level of the test ingredient (Porter et al., 1960). This approach often includes the basal diet as the starting point of the regression line to better explain the existence of a relationship between inclusion levels and how that affects the ME of the complete diets. When extrapolating to 100% inclusion level the R^2 values, which indicate the variability (%) of the dependent variable that is explained by the model, are often low. Because of the nature of the data, there is not always a strong linear relationship between the independent and dependent variables.

Another regression model was proposed by Adeola and Ileleji (2009), where the ME or ME_n intake of the test ingredient (independent variable) is regressed against the test ingredient intake in kilocalories (dependent variable). The slope of the equation represents the ME or ME_n

of the test ingredient. The R^2 values obtained by this method are often high mainly due to the inherent increases in gross energy associated with increases in feed intake. Several researchers have previously used this approach for ME determination of feed ingredients such as corn DDGS, glycerin, soybean meal, canola meal, cottonseed meal, peanut meal, corn, and barley (Villamide et al., 1997; Dozier et al., 2008; Lopez and Leeson, 2008; Zhang and Adeola, 2017). In addition, the regression method has been previously used in amino acid digestibility determination on DDGS (Foltyn et al., 2014).

PREDICTION OF DDGS ME

The use of nutrient and energy matrix values for DDGS derived from static sources such as reference books (e.g., NRC publications) and research publications can lead to the underestimation or overestimation of the true nutritional potential of the actual DDGS available to the nutritionist. Therefore, to better determine the metabolizable energy of DDGS, prediction equations based on chemical composition have been developed in attempt to provide more lot-specific ME values without having to determine the ME value for every DDGS source that is used in formulations. One of the first groups to develop ME prediction equation for DDGS were Batal and Dale (2006). They used proximate analysis of 17 DDGS and determined that fat, fiber, protein, and ash were the most significant predictors for TME_n. In addition, Rochell et al. (2011) evaluated different corn co-products, including DDGS, to determine their AME_n in broilers. These DDGS samples had 10% of ether extract or higher, except for one sample that contained 3.15%. The most significant predictors for AME_n were crude fat, ash, and hemicellulose. However, when hemicellulose was removed from the equation the resulting model included neutral detergent fiber, crude protein, and gross energy as the main predictors. Later, Meloche et al. (2014) utilized an external set of 15 DDGS sources to validate the prediction equations

previously reported. The resulting root mean square errors were significantly high which in not ideal for best fit prediction equations. Another experiment conducted by Anderson et al. (2012) utilized corn co-products commonly used to feed swine and reported that the main predictors for ME were GE and TDF ($R^2 = 0.72$). However, when TDF was removed from the selection pool, a new fit model that contained GE, NDF and ash ($R^2 = 0.68$) was generated. It is clear from this previous work that the various measures of fiber have a substantial impact on the ME of DDGS.

While several experiments have been conducted to accurately determine the ME of DDGS, the lack of standardization of *in vivo* assays has resulted in a wide range of ME values. Determining the optimal DDGS inclusion in diets is extremely difficult when there are numerous ME values to base diet formulation on. Even though the literature shows a variety of methods used to determine the ME of DDGS, those values have been predominantly determined using one inclusion level of DDGS or a group of low inclusion levels. Therefore, our first experiment will evaluate various inclusion levels of a single DDGS source in a regression-based ME assay. Factors such as feed intake, as influenced by dietary DDGS inclusion level, and regression method will be studied to determine their effect on the resulting ME estimates for DDGS. Similarly, it was noted in previous experiments the ME of DDGS is influenced by geographical areas and plant-specific processes. Therefore, in the second experiment 11 DDGS sources from different plants from a single ethanol producer will be evaluated to develop prediction equations based on chemical composition. The ME_n content of these sources will be determined by using an optimized assay from experiment 1.

Table 2.1 Summary of metabolizable energy (ME) values of distillers dried grains with solubles (DDGS) determined by different methods in poultry[1,2]

					kcal/kg, DM basis			
Reference	Method	Bird type	Estimate	n	Minimum	Maximum	Average	CV (%)
Lumpkins et al. (2004)	Direct	Rooster	TME_n	1	-	-	2,905	-
Fastinger et al. (2006)	Direct	Rooster	TME_n	5	-	-	2,871	-
Batal and Dale (2006)	Direct	Rooster	TME_n	17	2,490	3,190	2,820	6
Rochell et al (2011)	Substitution	Broiler	AME_n	6	2,146	3,098	2,678	11
Jie et al. (2013)	Direct	Rooster	TME_n	30	1,739	3,234	2,761	13
	Difference	Rooster	AME_n		1,416	2,912	2,438	15
Meloche et al. (2013)	Substitution	Broiler	AME_n	15	1,869	2,824	2,309	12
Meloche et al. (2014)	Substitution	Broiler	AME_n	15	1,975	3,634	2,765	13

[1]Values are reported as average if more than one sample evaluated
[2]n corresponds to the number of samples analyzed

REFERENCES

Adedokun, S. A., P. Utterback, C. M. Parsons, O. Adeola, M. S. Lilburn, and T. J. Applegate. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350–358.

Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. Poult. Sci. 88:579–585.

Anderson, P. V., B. J. Kerr, T. E. Weber, C. J. Ziemer, and G. C. Shurson. 2012. Determination and prediction of digestible and metabolizable energy from chemical analysis of corn coproducts fed to finishing pigs. J. Anim. Sci. 90:1242–1254.

Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. J. Appl. Poult. Res. 15:89-93.

Belyea, R. L., K. D. Rausch, T. E. Clevenger, V. Singh, D. B. Johnston, and M. E. Tumbleson. 2010a. Sources of variation in composition of DDGS. Anim. Feed Sci. Technol. 159:122–130.

Belyea, R. L., K. D. Rausch, and M. E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresour. Technol. 94:293-298.

Campasino, A., M. Williams, R. Latham, C. A. Bailey, B. Brown, and J. T. Lee. 2015. Effects of increasing dried distillers' grains with solubles and non-starch polysaccharide degrading enzyme inclusion on growth performance and energy digestibility in broilers. J. Appl. Poult. Res. 24:135–144.

Dale, N., and H. L. Fuller. 1984. Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations. Poult. Sci. 63:1008-1012.

Dozier, W. A., B. J. Kerr, A. Corzo, M. T. Kidd, T. E. Weber, and K. Bregendal. 2008. Apparent metabolizable energy of glycerin for broiler chickens. Poult. Sci. 87:317–322.

Farrell, D. J., E. Thomson, J. J. Du Preez, and J. P. Hayes. 1991. The estimation of endogenous excreta and the measurement of metabolisable energy in poultry feedstuffs using four feeding systems, four assay methods and four diets. Br. Poult. Sci. 32:483–499.

Fastinger, N. D., J. D. Latshaw, and D. C. Mahan. 2006. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poult. Sci. 85:1212–1216.

Foltyn, M., M. Lichovníková, V. Rada, and A. Musilová. 2014. Apparent ileal amino acids digestibility of diets with graded levels of corn DDGS and determination of DDGS amino acids digestibility by difference and regression methods in broilers. Czech J. Anim. Sci. 59:164-169.

Guillaume, J., and J. D. Summers. 1970. Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolizable energy. Can. J. Anim. Sci. 50:363–369.

Hill, F. W., and D. L. Anderson. 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. J. Nutr. 64:587–603.

Jacela, J. Y., J. M. DeRouchey, S. S. Dritz, M. D. Tokach, R. D. Goodband, J. L. Nelssen, R. C. Sulabo, R. C. Thaler, L. Brandts, D. E. Little, and K. J. Prusa. 2011. Amino acid digestibility and energy content of deoiled (solvent-extracted) corn distillers dried grains with solubles for swine and effects on growth performance and carcass characteristics1,2. J. Anim. Sci. 89:1817–1829 .

Jie, Y. Z., J. Y. Zhang, L. H. Zhao, Q. G. Ma, and C. Ji. 2013. The correlationship between the metabolizable energy content, chemical composition and color score in different sources of corn DDGS. J. Anim. Sci. Biotechnol. 4:38.

Jin, S.-H., A. Corless, and J. L. Sell. 1998. Digestive system development in post-hatch poultry. Worlds. Poult. Sci. J. 54:335–345.

Kerr, B. J., W. A. Dozier, and G. C. Shurson. 2013. Effects of reduced-oil corn distillers dried grains with solubles composition on digestible and metabolizable energy value and prediction in growing pigs. J. Anim. Sci. 91:3231–3243.

Kim, E. J., C. M. Parsons, R. Srinivasan, and V. Singh. 2010. Nutritional composition, nitrogen-corrected true metabolizable energy, and amino acid digestibilities of new corn distillers dried grains with solubles produced by new fractionation processes. Poult. Sci. 89:44–51.

Kingsly, A. R. P., K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Stroshine, and S. Radcliff. 2010. The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) - Plant scale experiments. Bioresour. Technol. 101:193–199.

Knott, J., J. Shurson, and J. Goihl. 2004. Effects of the nutrient variability of distiller's solubles and grains within ethanol plants and the amount of distiller's solubles blended with distiller's grains on fat, protein and phosphorus content of DDGS. Protein and phosphorous content of DDGS.

Leytem, A. B., P. Kwanyuen, and P. Thacker. 2008. Nutrient excretion, phosphorus characterization, and phosphorus solubility in excreta from broiler chicks fed diets containing graded levels of wheat distillers grains with solubles. Poult. Sci. 87:2505–2511.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Lumpkins, B. S., and A. B. Batal. 2005. The bioavailability of lysine and phosphorus in distillers dried grains with solubles. Poult. Sci. 84:581–586.

Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poult. Sci. 83:1891–1896.

Martinez-Amezcua, C., C. M. Parsons, and D. H. Baker. 2006. Effect of microbial phytase and citric acid on phosphorus bioavailability, apparent metabolizable energy, and amino acid digestibility in distillers dried grains with solubles in chicks. Poult. Sci. 85:470–475.

Martinez Amezcua, C., and C. M. Parsons. 2007. Effect of increased heat processing and particle

size on phosphorus bioavailability in corn distillers dried grains with solubles. Poult. Sci. 86:331–337.

Martinez Amezcua, C., C. M. Parsons, and S. L. Noll. 2004. Content and relative bioavailability of phosphorus in distillers dried grains with solubles in chicks. Poult. Sci. 83:971–976.

Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2019. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. J. Appl. Poult. Res. 28:506–525.

Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poult. Sci. 93:1428-1439.

Meloche, K. J., B. J. Kerr, G. C. Shurson, and W. A. Dozier III. 2013. Apparent metabolizable energy and prediction equations for reduced-oil corn distillers dried grains with solubles in broiler chicks from 10 to 18 days of age. Poult. Sci. 92:3176–3183.

Miski, A. M., and S. Quazi. 1981. Influence of age and sex of growing broiler chicks and body weight of roosters on their endogenous and metabolic energy losses.Poult. Sci. 60:781-785.

NRC. 1994. Nutrient Requirements of Poultry. Nineth Rev. National Academic Press, Washington, DC.

Noll, S., J. Brannon, and C. Parsons. 2007. Nutritional value of corn distiller dried grains with solubles (DDGs): Influence of solubles addition. Poult.Sci.86:68.

Nuez Ortín, W. G., and P. Yu. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. J. Sci. Food Agric. 89:1754–1761.

Parsons, C. M., C. Martinez, V. Singh, S. Radhakrishman, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. Proc.Multi-State Poult. Nutr. Feeding conf., Indianapolis, IN.

Pedersen, M. B., S. Dalsgaard, K. E. Bach Knudsen, S. Yu, and H. N. Laerke. 2014. Compositional profile and variation of distillers dried grains with solubles from various origins with focus on non-starch polysaccharides. Anim. Feed Sci. Technol. 197:130-141.

Pesti, G. M., L. O. Faust, H. L. Fuller, N. M. Dale, and F. H. Benoff. 1986. Nutritive value of poultry by-product meal. 1. Metabolizable energy values as influenced by method of determination and level of substitution. Poult. Sci.65:2258-2267.

Rausch, K. D., and R. L. Belyea. 2006. The future of coproducts from corn processing. Appl. Biochem. Biotechnol. 128:47–86.

Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations. Anim. Feed Sci. Technol. 225:62–72.

Rochell, S. J., B. J. Kerr, and W. A. Dozier. 2011. Energy determination of corn co-products fed to broiler chicks from 15 to 24 days of age, and use of composition analysis to predict

nitrogen-corrected apparent metabolizable energy. Poult. Sci. 90:1999-2007.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Shrimpton, D. H., S. Leeson, and D. Lewis. 1977. Metabolisable energy studies with turkeys: Nitrogen correction factor in metabolisable energy determinations. Br. Poult. Sci. 18:373–379.

Shurson, G. C. 2017. The role of biofuels coproducts in feeding the world sustainably. Annu. Rev. Anim. Biosci. 5:229–254.

Shurson, G., M. Spiehs, and M. Whitney. 2004. The use of maize distiller's dried grains with solubles in pig diets. Pig news and information 25:75.

Sibbald, I. R. 1975. The effect of level of feed intake on metabolizable energy values measured with adult roosters. Poult. Sci. 54:1990–1997.

Sibbald, I. R. 1989. Metabolizable energy evaluation of poultry diets. Recent developments in poultry nutrition. Studies in the agricultural and food sciences. Pages 12–26.

Sibbald, I. R., S. J. Slinger, and G.C Ashton. 1961. Factors affecting the metabolizable energy content of poultry feeds. Poult. Sci.40:945-951.

Sibbald, I. R., and S. J. Slinger. 1963. Factors affecting the metabolizable energy content of poultry feeds. Poult. Sci. 42:707–710.

Singh, V., R. A. Moreau, K. B. Hicks, R. L. Belyea, C. H. Staff, R. A. Moreau, K. B. Hicks, and R. L. Belyea. 2001. Removal of fiber from distillers dried grains with solubles (DDGS) to increase value. Trans. ASAE 45:389–392.

Smeets, N., F. Nuyens, L. Van Campenhout, E. Delezie, J. Pannecoucque, and T. Niewold. 2015. Relationship between wheat characteristics and nutrient digestibility in broilers: Comparison between total collection and marker (titanium dioxide) technique. Poult. Sci. 94:1584–1591.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Świątkiewicz, S., and J. Koreleski. 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. Worlds. Poult. Sci. J. 64:257–265.

Szczurek, W. 2010. Standardized ileal digestibility of amino acids insome cereals, rapeseed products and maize DDGSfor broiler chickens at the age of 14 days. J. Anim. Feed Sci. 19:72–80.

Villamide, M. J., J. M. Fuente, P. Perez De Ayala, and A. Flores. 1997. Energy evaluation of eight barley cultivars for poultry: Effect of dietary enzyme addition. Poult. Sci. 76:834–840.

Waldroup, P. W., J. A. Owen, B. E. Ramsey, and D. L. Whelchel. 1981. The use of high levels of distillers dried grains plus solubles in broiler diets. Poult. Sci. 60:1479-1484.

Walugembe, M., M. F. Rothschild, and M. E. Persia. 2014. Effects of high fiber ingredients on the performance, metabolizable energy and fiber digestibility of broiler and layer chicks. Anim. Feed Sci. Technol. 188:46–52.

Wamsley, K. G. S., I. E. Loar, K. Karges, and J. S. Moritz. 2013. The use of practical diets and regression analyses to determine the utilization of lysine and phosphorus in corn distillers dried grains and solubles using Cobb 500 male broilers. J. Appl. Poult. Res. 22:279–297.

Wang, Z., S. Cerrate, F. Yan, P. Waldroup, Z. Wang, S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Utilization of distillers dried grains with solubles (DDGS) in broiler diets using a standardized nutrient matrix. Int. J. Poult. Sci. 6:470–477.

Warnick, R. E., and J. O. Anderson. 1968. Limiting essential amino acids in soybean meal for growing chickens and the effects of heat upon availability of the essential amino acids. Poult. Sci. 47:281–287.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. Poult. Sci. 99:385-406.

Youssef, I. M. I., C. Westfahl, A. Sunder, F. Liebert, and J. Kamphues. 2008. Evaluation of dried distillers' grains with solubles (DDGS) as a protein source for broilers. Arch. Anim. Nutr. 62:404–414.

Zhang, F., and O. Adeola. 2017. Energy values of canola meal, cottonseed meal, bakery meal, and peanut flour meal for broiler chickens determined using the regression method. Poult. Sci. 96:397–404.

Zhang, Y., A. Shan, W. Jiang, C. Bi, and Z. Li. 2013. The effect of vitamin E on growth performance and meat quality in broilers given diets containing distillers' dried grain with solubles (DDGS). Br. Poult. Sci. 54:138–143.

CHAPTER 3: EFFECT OF DIETARY INCLUSION LEVEL ON THE METABOLIZABLE ENERGY CONTENT OF DISTILLERS DRIED GRAINS WITH SOLUBLES DETERMINED IN BROILER CHICKS AT 21 D OF AGE

ABSTRACT

Assays to determine ME_n are inherently variable and can be influenced by a number of factors such as inclusion level of the test ingredient. Understanding these effects is particularly important for ingredients that can vary widely in ME_n content such as distillers dried grains with solubles (DDGS). An experiment was conducted to evaluate the influence of dietary DDGS concentration on the estimation of its ME and ME_n values in broilers, and to determine if any changes in these values are associated with changes in broiler FI as dietary concentrations of DDGS increase. Five treatments consisted of a reference basal diet and diets in which 15, 30, 45, or 60% DDGS was added at the expense of all energy providing ingredients in the basal diet for ME_n determination by the difference method and regression method. A sixth treatment consisted of the 30% DDGS diet pair-fed to equalize FI with birds fed the 60% DDGS *ad libitum*. A seventh treatment included an additional reference diet in which dextrose was included at 15% for ME_n determination of DDGS using the single ingredient replacement approach. Male broilers (448 total) were randomly allocated to 56 battery cages with 8 replicate cages per treatment (8 birds/cage) and were provided a common starter diet from 0 to 14 d post-hatch. Experimental diets contained 0.5% TiO_2 and were fed from 14 to 21 d. Diets and excreta were collected from 19 to 21 d and analyzed for nitrogen, gross energy and TiO_2 for determination of ME and ME_n using the index method. Polynomial contrasts were used to assess linear and quadratic effects of DDGS inclusion level. As expected, linear decreases in BWG and FI were observed ($P < 0.001$) as dietary DDGS concentration increased at the expense of the nutritionally complete basal diet.

There were decreases ($P < 0.001$) in ME and ME_n values of the complete diets and of DDGS as DDGS inclusion increased. Pair-feeding the 30% DDGS diet to the 60% DDGS diet reduced ($P < 0.05$) ME and ME_n values of DDGS when compared with those determined in birds fed the 30% DDGS diet *ad libitum*. The ME and ME_n values determined for DDGS when fed at 15% of the diet were higher ($P < 0.001$) when using the complete basal replacement method than when using the single ingredient replacement method. The first regression method resulted in ME and MEn values that were very similar and were within their 95% confidence interval. These results indicate that ME_n values determined for DDGS decreased as its inclusion within the test diet increased, with these reductions in ME_n partly due to decreased feed intake.

Key words: Metabolizable energy, broiler, DDGS, inclusion level, pair-feeding.

INTRODUCTION

Distillers dried grains with solubles (DDGS) continues to be an important feed ingredient for poultry, and ethanol plants continually improve processing techniques to more efficiently convert starch to ethanol and extract corn oil to market to biodiesel producers or as an animal feed ingredient (Saunders and Rosentrater, 2009). Thus, the resulting variability in the energy content of DDGS can be problematic when trying to accurately formulate poultry diets at least cost. In response to this issue, several ME prediction equations based on the chemical composition of DDGS have been developed and validated, though the predictive capacity of these equations remains limited (Meloche et al., 2014). This lack of predictive capacity may be partly associated with the inherent variability of in vivo ME determination (Wu et al., 2020), and therefore, it is possible that optimizing in vivo assays used to determine DDGS ME could potentially benefit subsequent ME prediction equations based on relationships between DDGS chemical composition and in vivo ME estimates.

Some factors that can impact the estimated ME of a test ingredient are the type of basal or reference diet used and how the test ingredient is incorporated into it, inclusion level of the test ingredient, and feed intake of the test diets (Sibbald, 1975; Wiseman and Salvador, 1991; Rodríguez et al., 2001). There are two substitution methods that are commonly used on ME assays when complete diets are fed to the birds. One approach is the complete basal replacement where the test ingredient is added at the expense of all the energy providing ingredients in the test diet. The second approach is the single ingredient replacement where the test ingredient is added at the expense of a single, well-defined ingredient within the basal diet such as dextrose (Anderson et al., 1958) or corn (Pesti et al., 1986). Dextrose has been used in several experiments to determine and compare different method of ME determination for feedstuff (Hill et al., 1960; Rochell et al., 2011; Meloche et al., 2013).

According to Sibbald and Slinger (1962), ME estimates are also impacted by inclusion level. Higher dietary inclusions of the test ingredient reduce variability around the ME estimate because a higher proportion of the total dietary energy is derived from the test ingredient. However, high dietary inclusions of most individual ingredients, including DDGS, can negatively influence nutritional balance, palatability, and ultimately, feed intake of the test diets. Guillaumb and Summers (1970), demonstrated that the apparent ME value (i.e., not corrected for endogenous losses) of a diet will be reduced at low feed intake due to the fact that a higher proportion of the excreted energy is of endogenous origin. Similarly, according to Sibbald (1975) at high intake levels there is a smaller effect of the excreta endogenous losses. One approach to partially circumvent variation associated with test ingredient inclusion level is to use a regression approach, as initially proposed by Potter et al. (1960), whereby multiple levels of the test ingredient are fed. An advantage of this approach is that it eliminates the need to correct for basal

endogenous energy losses, as these losses do not influence the slope of the regression line across the multiple inclusion levels of the test ingredient (Rodehutscord et al., 2004). Adeola and Zhai (2012) conducted an experiment using a regression approach to estimate the ME of DDGS when included at 0, 30 and 60% of the test diet and reported its ME_n value to be 2,688 kcal/kg (DM basis). However, there is still debate regarding the most appropriate method for calculating regression-based ME estimates (Wu et al., 2020).

The objectives of the current experiment were to determine the influence of the aforementioned factors of inclusion level and feed intake on the resulting ME and MEn values of a single DDGS source when fed to broilers at 15, 30, 45, or 60% of the diet from 14 to 21 d post-hatch. To isolate the effect of feed intake, an additional group was pair-fed the 30% DDGS diet such that feed intake was equalized with that of birds fed the 60% DDGS diet *ad libitum*. Finally, two methods of calculating regression-based ME and MEn estimates across different dietary inclusion levels were compared.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Arkansas approved all procedures in this protocol #19121

Bird Husbandry

A total of 448 male chicks from a Cobb 500 female line obtained from a commercial hatchery (Cobb Vantress Inc., Fayetteville, AR) were placed in 56 battery cages at 8 chicks per cage and reared to 21 d post-hatch. Battery cages (0.61m x 0.61m), had raised wired floors, and were equipped with trough feeders and nipple waters. Battery cages were housed within a climate-controlled room with LED lighting. The lighting program consisted of 23L:1D from day

0 to 7 and 16L:8D from day 8 to 21. Room temperatures were set to 32˚C the day of placement and decreased to 21˚C by the end of the trial.

Dietary Treatments

Birds were fed a common corn soybean meal-based diet *ad libitum* (AL) from day 0 to 14 d post-hatch. Experimental treatments were fed from 14 to 21 d and included corn soybean meal-based diets with 0, 15, 30, 45, or 60% DDGS, as well as an additional reference diet which contained 15% dextrose. Dextrose and DDGS were added at the expense of all energy-providing ingredients in the basal diet, with vitamins and mineral supplements held constant. A seventh treatment group consisted of pair feeding the diet containing 30% DDGS (30 PF) to equalize feed intake of birds to those fed the 60% DDGS (60 AL) diet. Treatments were randomly allocated to the cages. Daily FI was determined for birds fed the 60 AL diet, and that exact amount was fed to birds in the 30 PF group the following day. All experimental diets contained titanium dioxide at 0.5% inclusion to serve as the indigestible marker for determination of ME by the index method. All dietary treatments were provided in mash form.

The DDGS source used in this experiment was obtained from an ethanol plant in the Midwest and was found to contain 30.56% crude protein (CP), 28.23% neutral detergent fiber (NDF), 8.49% acid detergent fiber (ADF), 34.83% total detergent fiber (TDF) and 11.86% starch (as-is) as analyzed by the University of Missouri Agriculture Experiment Station Chemical laboratories (Columbia, MO). For DM determination, samples and diets were dried at 105° C in a drying oven (Isotemp oven, Fisher Scientific, Pittsburg, PA) for 12 hours [AOAC Official method 934.01 (for dry matter)]. In addition, the DDGS sample contained 9.36 % moisture and particle size of 490 μm (University of Arkansas laboratory) which was determined by using a series of 14 selected USA standard sieves (6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200 and

270). The sieving procedure was conducted according to a standard method (ASAE Standards, 2003).

Sample Collection and Analyses

Feed intake (FI) and excreta output were quantitatively measured from 19 to 21 d post-hatch after a 5 d adaption period to the experimental diets (14 to 19 d). Excreta were collected on stainless steel trays, and samples of homogenized excreta were collected from each cage and pan avoiding contamination with feed and feathers. Excreta samples were placed in cups, frozen, freeze-dried, and ground using an electric coffee grinder. Feed samples were ground using a grinder with a 1-mm screen (Perten LM 3100, Perten Instrument, Hägersten, Sweden). Feed and excreta samples were analyzed for gross energy and nitrogen content at the University of Arkansas Central Analytical Laboratory. Gross energy was determined using a Parr 6200 calorimeter (Parr Instruments, Moline, IL). Dry matter was determined on diets and lyophilized excreta samples were determined by drying at 105^{o} C (Isotemp oven, Fisher Scientific, Pittsburgh, PA) for 12 hours [AOAC Official methods 934.01 (for dry matter)]. Nitrogen content on excreta and feed samples were determined by using the combustion method [(AOAC Official Methods 990.03 (for nitrogen)]. The undigestible marker TiO2 was determined using the method proposed by Short et al.(1996).

Metabolizable Energy Determination of Experimental Diets and DDGS

The following calculations were used to determine the ME and ME_n (kcal/kg) of the experimental diets using the index method.

$$\text{Nitrogen-corrected dietary } ME_n \text{ (kcal/kg)} = GE_{diet} - [GE_{excreta} \times (TiO_{2,\ diet} / TiO_{2,\ excreta}) + (8.22 * \text{retained nitrogen}_{(g)})]$$

Where GE_{diet} and $GE_{excreta}$ represent the analyzed gross energy value (kcal/kg) and TiO_{2diets} and $TiO_{2excreta}$ corresponds to the analyzed TiO_2 values (%). To determine the ME_n value, a nitrogen correction factor of 8.22 kcal/g was used (Hill and Anderson, 1958).

The following equation was used to determine the ME content of the reference basal diet by the substitution method when dextrose served as the reference ingredient.

$$\text{Basal } ME_{n,\,(kcal/kg)} = [ME_{n,diet} - (DI\ \% \times 3{,}640)] / BI_{\,(\%)}$$

Where DI represents the dextrose inclusion level (%) and 3,640 represents the energy contribution from dextrose in kilocalories (Anderson et al., 1958). The product of that multiplication is subtracted from the $ME_{n,diet}$ which represents the energy (kcal/kg) from the basal reference diet containing 0% DDGS. BI represents the basal inclusion (%) of the dextrose reference diet.

The ME and ME_n of the DDGS (kcal/kg) was calculated by the difference method using the following equation.

$$\text{ME and } ME_n \text{ of DDGS (kcal/kg)} = [ME_{n,diet} - (ME_{n,basal} \times BI)] / TI$$

Where $ME_{n,diet}$ represent the analyzed ME_n values (kcal/kg) of the test diet and $ME_{n,basal}$ represents the analyzed value of the corn soybean meal basal diet or the calculated basal portion of the reference diet containing dextrose. The concentration (%) of the test ingredient is represented by TI and BI represent and basal portion in the experimental diet.

The intake of DDGS was calculated based on feed intake and DDGS inclusion. The caloric intake related to DDGS (kcal/kg) was calculated from the ME (kcal/kg) multiplied by the DDGS intake (kg) for each inclusion level provided in the feed.

Statistical Analyses and Regression-Based ME Estimates

There were 8 replicate cages per treatment with 8 birds per cage, and cage location was used as a blocking factor. Growth performance, nitrogen retention, and ME and ME_n data were analyzed as a randomized complete block design using a one-way ANOVA (PROC MIXED, SAS 9.4, Cary NC). Effects of DDGS inclusion level on these measurements were evaluated using linear and quadratic polynomial contrasts. A single degree of freedom contrast was used to compare the effect of basal replacement approach (complete basal versus dextrose) on the ME and ME_n values of DDGS when fed at 15%. Additionally, the effect of feed intake was evaluated with a single degree of freedom contrast between the birds fed the 30 AL and the 30 PF diet to equal intake of those fed the 60 AL.

Regression-based ME and ME_n estimates of DDGS were determined using two approaches. In the first approach, the DDGS associated caloric intake (kcal) was regressed against the amount of DDGS intake (kg) and linear regression equations were generated with slopes corresponding to the ME or ME_n value (kcal/kg) for DDGS (Adeola and Ileleji, 2009). In the second approach, ME and ME_n values of the experimental diets were regressed on DDGS inclusion (%), and ME and ME_n estimates were based on extrapolation of dietary ME or ME_n at a dietary DDGS inclusion of 100% (Mateos and Sell, 1980; Gonzalez-Esquerra and Leeson, 2000). Confidence intervals were determined and used to compare the slopes and intercepts for each set of inclusion levels of DDGS. Significance was considered at $P \leq 0.05$ for statistical analyses.

RESULTS AND DISCUSSION

Effect of basal component replacement on estimation of DDGS energy utilization

Factors such as diet palatability, feed intake and methodology are factors that can influence the in vivo ME estimates (Wu et al., 2020). The AME and AME_n of the DDGS at when fed at 15% dietary inclusion and determined by dextrose replacement was 1,440 and 1171 kcal/kg lower ($P < 0.001$) than when determined by CSBM basal substitution. When calculating the AME_n value of the CBSM basal portion using the diet containing dextrose by subtracting an assumed energy contribution of 3,640 kcal/kg for dextrose (Hill and Anderson, 1958; Rochell et al., 2011; Meloche et al., 2013), a higher value was obtained (3,113 kcal/kg) than when determining the energy value of this diet directly (2,905 kcal/kg). Therefore, the higher AME value estimated for the basal portion resulted in a lower estimation of the AME contribution from DDGS to the total test diet AME when using this method. Previous data from our lab indicate that the actual AME value of dextrose varies from the value of 3,640 published by Hill and Anderson (1958), depending on dextrose inclusion and other ingredients used. Although using dextrose replacement appeared to underestimate the AME of DDGS in the current experiment, previous researchers (Rochell et al., 2011; Meloche et al., 2013) have reported estimates of AME for DDGS using this method that are in better agreement with other published DDGS AME values. Nonetheless, it is clear that the proportion of basal diet replaced can influence the AME estimate of feed ingredients even when the proportion of replacement is constant.

Effect of dietary DDGS inclusion level on its estimated energy utilization

In addition to the basal diet components that are replaced, the inclusive level of the test ingredient can also influence its estimated ME value, in part related to effect on feed intake. In the current experiment, there were quadratic decreases in FI ($P = 0.014$) and BWG ($P < 0.001$)

and a quadratic increase in FCR (P = 0.001) as the amount of DDGS increased from 0 to 60% in the experimental diets (Table 3.2). This was not unexpected, as DDGS was added to the basal diet at the expense of all energy-providing ingredients without balancing for nutrient or energy content, similar to the approach taken by other researchers (Wiseman and Salvador, 1991). This facilitates calculation of the energy value of the test ingredient by difference through the assumption that energy value of the basal fraction will not change if all constituent ingredients are held in proportion. However, this also results in nutrient imbalances that can affect the feed intake and nutrient metabolism of the birds. Indeed, DDGS is known to be deficient in Lys, and despite the fact that we marginally increased the digestible Lys content of the basal diet above the bird's estimated requirement to account for the increasing amount of DDGS in the test diet, the digestible Lys content of the 60% DDGS diet was 0.90%, below the estimated requirement of 1.12 % for this age bird (Cobb-Vantress, 2018). On the other hand, nutrients which are inherently higher in DDGS, such as dietary fiber which can influence both feed intake and digesta transit time (Mateos et al., 2012), increased with dietary DDGS inclusion in the test diets. Such imbalances are unavoidable at when high inclusion levels of almost any single feed ingredient are fed and must be taken into consideration when interpreting results when nutrient digestibility or energy utilization is determined by difference as was the objective in the current experiment.

Increasing DDGS inclusion from 0 to 60% decreased the AME and AME_n of the complete diets quadratically (P <0.001) from 3,107 to 2,432 and 2,905 to 2,271 kcal/kg, respectively (Table 3.3), which indicates the energy content of the DDGS was in fact lower than that of the basal diet. The proportion of dietary nitrogen retained by the birds also decreased quadratically (P < 0.001) as DDGS inclusion increased from 0 to 60%, which may have been due to the

aforementioned imbalance in dietary amino acid content as DDGS inclusion increased. Furthermore, Parsons et al. (1983) showed that as the fiber content in the diet increases, the endogenous losses from the digestive tract increases, possibly contributing to the reduction in both energy utilization and nitrogen retention as DDGS inclusion increased. On the other hand, insoluble fiber increases rate of passage which could also affect the nitrogen and nutrient absorption by the birds. Both factors could lead to excessive energy excretion or poor utilization of nutrients due to the fiber content of DDGS.

There was a linear ($P < 0.001$) decrease in the AME and AME_n content of the DDGS when determined by difference as its dietary inclusion increased from 15 to 60% (Table 3.4). Previous research has shown that when using higher inclusion levels of the test ingredient, the resulting ME estimates are closer to their true value for the feedstuff (Sibbald and Slinger, 1962; Pesti et al., 1986). This is partly due to the fact that the variability associated with the ME estimates is directly and inversely proportional to its inclusion level. The standard deviations of the AME DDGS estimates were generally lower at higher DDGS inclusion levels, indicating less variability. Indeed, the potential negative interactions arising from feeding a high level of a single ingredient due to its imbalanced nutrient profile, as previously discussed must also be considered.

The 30 PF treatment was included in the current experiment to determine to what extent that the difference in ME and ME_n values for DDGS when fed at 30 and 60% could be accounted for by a reduction in FI per se. However, although a reduction in FI the 30PF group compared with the 30AL was achieved, birds in the 60AL group still had higher FI ($P = 0.041$) than those in the 30PF group because we did not adequately account for daily increases in feed intake. Nonetheless, reducing the intake of 30% DDGS diet by X% to an intake closer to that of birds

fed the 60% DDGS diet reduced the estimated AME and AME_n of DDGS by 113 (3.9%; P = 0.037) and 99 kcal/kg (3.6%, P = 0.018), respectively, when compared with the 30AL group. This indicates that FI does in fact influence the resulting ME estimate of the test ingredient, possibly due to the fact that when feed intake is low there will be a proportionally higher energy contribution from the endogenous losses in the collected excreta. However, Hill and Anderson (1958) stated that a 30% reduction of feed intake did not have any effects on the resulting ME of complete diets which could be explained by the effect of reverse peristalsis which is known to allow birds to better utilize nutrients when FI is reduced (Sacranie et al., 2006). Overall, the effects of dietary test ingredient inclusion level on energy utilization are multifaceted, and regression-based that incorporate the benefit of both high and low inclusion.

Comparison of 2 regression-based calculations of ME and ME_n values for DDGS

Potter et al. (1960) were one of the first groups to use regression approaches for ME determination assays. Their model consisted of regressing the ME of the complete diets against the inclusion level of the test ingredients used and extrapolating the test ingredient inclusion level to 100% (Potter et al., 1960). More recently, Adeola and Ileleji (2009) proposed a modified model where the ME intake associated with the ingredient is regressed against the test ingredient FI. While this approach has been used on several experiments, there still debate in the accuracy of this model (Wu et al., 2020). Table 3.6 contains the intercepts and slopes and their corresponding 95% confidence intervals of regression equations of dietary AME and AME_n on DDGS inclusion level. Additionally, estimates of ME_n for DDGS determined using these equations and extrapolating to 100% DDGS are included. Using, this approach, ME and ME_n values ranged from 2,050 to 2,669 kcal/kg and from 1,905 to 2,423 kcal/kg respectively. Table 3.5 contains the intercepts, slopes and corresponding 95% confidence intervals of linear

regression equations between DDGS associated ME or ME_n intake and DDGS intake when DDGS was fed at various inclusion levels. By using this modified regression method, the slope corresponds to the ME and ME_n values of DDGS which ranged from 2,113 to 2,787 kcal/kg and 1,971 to 2,533 kcal/kg (DM basis) respectively. The ME and ME_n values obtained by the model proposed by (Potter et al., 1960) are within the 95% confidence interval of the values found by using the regression model proposed by Adeola and Ileleji (2009). The higher R^2 associated with the model proposed by Adeola and Ileleji(2009) could be due to the fact that GE intake increases as FI increases, therefore indicating a high correlation which is not the case when inclusion levels are added as the independent variable as explained by (Wu et al., 2020).

In conclusion, the current study confirms previous research that dietary inclusion level of the test ingredient has an important impact on estimates of its energy utilization and reveals that this relationship is particularly important for DDGS *per se*. This supports the notion that regression-based ME assays, particularly when conducted using growing broilers fed *ad libitum*, may be superior to those based on a single level test ingredient inclusion. Additionally, although not equal in goodness of fit, both regression methods applied to this dataset resulted in similar ME and MEn values when using multiple inclusion levels. The methodology (i.e., single ingredient vs. basal replacement), used to calculate the AME of DDGS, significantly influenced the resulting values. Therefore, this should be thoroughly analyzed before formulating diets for the bioassay. Future research is needed to determine the interaction of dextrose with other ingredients in the diets that could influence the resulting ME of feed ingredients.

Table 3.1 Ingredient and nutrient (as-fed basis) composition of experimental diets

Item	Dextrose basal	CSBM basal	DDGS Inclusion (%)			
			15	30	45	60
Corn	49.12	58.17	49.12	40.71	31.02	21.97
Soybean meal	28.61	33.88	28.61	23.34	18.07	12.79
Soybean oil	3.21	3.80	3.21	2.61	2.02	1.43
L-lysine H.Cl	0.20	0.24	0.20	0.16	0.13	0.09
DL-methionine	0.22	0.26	0.22	0.18	0.14	0.10
L-threonine	0.04	0.05	0.04	0.04	0.03	0.02
DDGS	0.00	0.00	15.00	30.00	45.00	60.00
Dextrose	15.00	0.00	0.00	0.00	0.00	0.00
Limestone	0.90	0.90	0.90	0.90	0.90	0.90
Dicalcium phosphate	1.51	1.51	1.51	1.51	1.51	1.51
Titanium dioxide	0.50	0.50	0.50	0.50	0.5	0.50
Vitamin premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix[1]	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Santoquin	0.02	0.02	0.02	0.02	0.02	0.02
Selenium premix 0.06%	0.02	0.02	0.02	0.02	0.02	0.02
Calculated nutrient composition						
AME_n, kcal/kg	3,194	3,136	3,015	2,895	2,775	2,654
CP, %	17.52	20.75	21.63	22.50	23.38	24.25
Digestible Lys, %	1.07	1.27	1.15	1.03	0.91	0.79
Digestible TSAA, %	0.72	0.85	0.85	0.85	0.85	0.85
Digestible Thr, %	0.65	0.76	0.75	0.74	0.73	0.72
Ca, %	0.78	0.80	0.79	0.78	0.78	0.77
Available P, %	0.39	0.41	0.46	0.50	0.55	0.59
Analyzed composition						
CP, %	19.90	21.60	23.30	23.80	24.90	25.60
Gross energy, kcal/kg	3,930	3,984	4,026	3,970	4,046	4,108

[1]Supplied the following per kg of diet: vitamin A, 6,173 IU; vitamin D3, 4,409 ICU; vitamin E, 44 IU; vitamin B12, 0.01 mg; menadione, 1.20 mg; riboflavin, 5.29 mg; d-pantothenic acid, 7.94 mg; thiamine, 1.23 mg; niacin, 30.86 mg; pyridoxine, 2.20 mg; folic acid, 0.71 mg; biotin, 0.07 mg; manganese, 24 mg; zinc, 14.4 mg; selenium, 0.04 mg; copper, 0.68 mg; iodine, 0.47 mg

[2]DDGS = distillers dried grains with solubles

Table 3.2 Growth performance of male broilers fed experimental diets from day 14 to 21[1,2]

Item	21 d BW (g)	BWG (g)	FI (g)	FCR(g:g)
Dextrose basal	985[a]	489[a]	760[a]	1.56[cd]
CSBM basal	970[ab]	481[a]	674[b]	1.44[e]
15% DDGS	972[ab]	468[a]	677[b]	1.48[de]
30% DDGS	922[b]	418[b]	657[bc]	1.58[c]
45% DDGS	859[c]	361[c]	618[cd]	1.70[b]
60% DDGS	808[c]	308[d]	585[de]	1.87[a]
30% PF to 60% AL	820[c]	321[d]	556[e]	1.73[b]
SEM	13.9	8.6	9.6	0.02
P-values				
CSBM basal vs dextrose basal	0.425	0.514	<0.001	0.001
DDGS linear	<0.001	<0.001	<0.001	<0.001
DDGS quadratic	0.028	0.009	0.014	0.001
30% AL vs 30% PF	<0.001	<0.001	<0.001	<0.001
30% PF vs 60% AL	0.547	0.302	0.041	<0.001

[a-f]Means within a column with different superscript are significantly different ($P \leq 0.05$).
[1]Values are means of 8 replicate cages with 8 birds per cage.
[2]Abbreviations: AL= ad libitum; DDGS = distillers dried grains with solubles; PF = pair fed

Table 3.3 Nitrogen retention, AME, and AME_n values of experimental diets containing DDGS at different inclusion rates fed to male broilers from day 14 to 21[1,2]

Item	Nitrogen retention (%)	AME (kcal/kg)	AME_n (kcal/kg)
CSBM Basal	66.18[c]	3,107[b]	2,905[b]
15% DDGS	64.64[c]	3,060[b]	2,846[bc]
30% DDGS	70.66[ab]	2,974[bc]	2,759[cd]
45% DDGS	52.80[d]	2,716[d]	2,527[e]
60% DDGS	42.76[e]	2,432[e]	2,271[f]
30% PF to 60% AL	67.44[bc]	2,861[c]	2,660[d]
15% Dextrose replacement	73.21[a]	3,405[a]	3,193[a]
SEM	0.789	31	29
P-values			
ANOVA	<0.001	<0.001	<0.001
Basal vs 15% dextrose replacement	<0.001	<0.001	<0.001
DDGS Linear	<0.001	<0.001	<0.001
DDGS Quadratic	<0.001	<0.001	<0.001
30% AL vs 30% PF	0.006	0.037	0.018
30% PF vs 60% AL	<0.001	<0.001	<0.001

[a-f] Means within a column with different superscript are significantly different ($P \leq 0.05$).

[1]Values are means of 8 replicate cages with 8 birds per cage.

[2] Abbreviations: AL = ad libitum; AME = apparent metabolizable energy; DDGS = distillers dried grains with solubles; PF = pair fed

Table 3.4 Analyzed AME and AME_n values of DDGS determined using the difference method when fed at inclusion rates of 15%, 30%, 45%, 60%[1,2]

Item	AME (kcal/kg)	AME_n (kcal/kg)
15% DDGS	2,904 ± 342[a]	2,617 ± 337[a]
30% DDGS	2,780 ± 416[ab]	2,527 ± 373[ab]
45% DDGS	2,354 ± 219[bc]	2,173 ± 203[bc]
60% DDGS	2,098 ± 176[c]	1,956 ± 160[c]
30% PF to 60% AL	2,401 ± 316[bc]	2,195 ± 296[abc]
15% Dextrose replacement	1,464 ± 342[d]	1,446 ± 337[d]
SEM	111	105
P-values		
ANOVA	<0.001	<0.001
Single ingredient vs 15% dextrose replacement[3]	<0.001	<0.001
DDGS linear	<0.001	<0.001
DDGS quadratic	0.554	0.548
30% AL vs 30% PF	0.020	0.030
30% PF vs 60% AL	0.059	0.114

[a-d] Means within a column with different superscript are significantly different ($P \leq 0.05$)

[1]Values are means of 8 replicate cages with 8 birds per cage

[2]Abbreviations: AL = ad libitum; AME = apparent metabolizable energy; AME_n = nitrogen corrected apparent metabolizable energy; DDGS = distillers dried grains with solubles; PF = pair fed

[3]Comparison of ME and ME_n values of DDGS determined by the basal substitution and single ingredient replacement approach

Table 3.5 Intercepts, slopes and corresponding 95% confidence intervals of linear regression equations between DDGS associated ME or MEn intake (dependent variable) and DDGS intake (independent variable) when DDGS was fed at various inclusion levels (regression method 1)[1,2]

Item	Intercept (kcal)	Slope (kcal/kg)	r^2	*P*- value
ME[3]				
0, 15, 30%	7.89 (-71.20 to 86.99)	2,787 (2,523 to 3051)	0.96	<0.001
0, 15, 30, 45%	64.82 (-26.65 to 156.30)	2,400 (2,178 to 2,623)	0.94	<0.001
0, 15, 30, 45, 60%	126.08 (26.62 to 225.54)	2,113 (1,923 to 2,304)	0.93	<0.001
0, 30, 60%	90.57 (-51.64 to 232.78)	2,135 (1,875 to 2,394)	0.93	<0.001
ME_n				
0, 15, 30%	5.56 (-66.29 to -77.42)	2,533 (2,293 to 2,773)	0.96	<0.001
0, 15, 30, 45%	53.28 (-27.70 to 134.26)	2,210 (2,014 to 2,407)	0.95	<0.001
0, 15, 30, 45, 60%	104.30 (17.31 to 191.29)	1,971 (1,804 to 2,137)	0.94	<0.001
0, 30, 60%	75.57 (-47.60 to 198.74)	1,988 (1,763 to 2,212)	0.94	<0.001

[1]Slope corresponds to ME and ME_n values of DDGS

[2]Values in parenthesis are 95% confidence intervals of the intercept and slope

[3]ME = Metabolizable energy; ME_n = Nitrogen corrected metabolizable energy

Table 3.6 Intercepts, slopes and corresponding 95% confidence intervals of linear regression equations between dietary AME or AMEn and DDGS inclusion (%) and DDGS ME and MEn estimates based on extrapolation to 100% DDGS inclusion (regression method 2)

Item	Intercept (kcal)	Slope (kcal/kg)	r^2	*P* – value	ME or ME_n (kcal/kg)
ME[2]					
0, 15, 30%	3,113 (3,056 to 3,171)	-4.44 (-7.38 to -1.49)	0.30	0.005	2,669
0, 15, 30, 45%	3,153 (3,090 to 3,216)	-8.39 (-10.62 to -6.16)	0.66	<0.001	2,314
0, 15, 30, 45, 60%	3,196 (3,130 to 3,263)	-11.29 (-13.10 to -9.47)	0.81	<0.001	2,067
0, 30, 60%	3,175 (3,081 to 3,270)	-11.25 (-13.69 to -8.80)	0.81	<0.001	2,050
ME_n					
0, 15, 30%	2,910 (2,858 to 2,962)	-4.87 (-7.56 to -2.18)	0.39	0.001	2,423
0, 15, 30, 45%	2,943 (2,887 to 2,998)	-8.15 (-10.13 to -6.16)	0.70	<0.001	2,128
0, 15, 30, 45, 60%	2,979 (2,920 to 3,038)	-10.58 (-12.18 to -8.98)	0.82	<0.001	1,921
0, 30, 60%	2,962 (2,980 to 3,045)	-10.57 (-12.70 to -8.44)	0.83	<0.001	1,905

[1]Values in parenthesis are 95% confidence intervals of the intercept and slope

[2]ME = Metabolizable energy; ME_n = Nitrogen corrected metabolizable energy

Table 3.7 Comparison of ME and ME_n of DDGS obtained by two regression methods

Item	Regression method 1 (Kcal/kg)	Regression method 2 (Kcal/kg)
ME		
0, 15, 30	2,787	2,669
0, 15, 30, 45	2,400	2,314
0, 15, 30, 45, 60	2,113	2,067
0, 30, 60	2,135	2,050
ME_n		
0,15,30	2,533	2,423
0, 15, 30, 45	2,210	2,128
0, 15, 30, 45, 60	1,971	1,921
0, 30, 60	1,988	1,905

ME = Metabolizable energy; ME_n = Nitrogen corrected metabolizable energy

REFERENCES

Adedokun, S. A., P. Utterback, C. M. Parsons, O. Adeola, M. S. Lilburn, and T. J. Applegate. 2009. Comparison of amino acid digestibility of feed ingredients in broilers, laying hens and caecectomised roosters. Br. Poult. Sci. 50:350–358.

Adeola, O., and K. E. Ileleji. 2009. Comparison of two diet types in the determination of metabolizable energy content of corn distillers dried grains with solubles for broiler chickens by the regression method. Poult. Sci. 88:579–585.

Adeola, O., and H. Zhai. 2012. Metabolizable energy value of dried corn distillers grains and corn distillers grains with solubles for 6-week-old broiler chickens. Poult. Sci. 91:712–718.

Anderson, P. V., B. J. Kerr, T. E. Weber, C. J. Ziemer, and G. C. Shurson. 2012. Determination and prediction of digestible and metabolizable energy from chemical analysis of corn coproducts fed to finishing pigs. J. Anim. Sci. 90:1242–1254.

Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. J. Appl. Poult. Res. 15:89-93.

Belyea, R. L., K. D. Rausch, T. E. Clevenger, V. Singh, D. B. Johnston, and M. E. Tumbleson. 2010a. Sources of variation in composition of DDGS. Anim. Feed Sci. Technol. 159:122–130.

Belyea, R. L., K. D. Rausch, and M. E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresour. Technol. 94:293-298.

Campasino, A., M. Williams, R. Latham, C. A. Bailey, B. Brown, and J. T. Lee. 2015. Effects of increasing dried distillers' grains with solubles and non-starch polysaccharide degrading enzyme inclusion on growth performance and energy digestibility in broilers. J. Appl. Poult. Res. 24:135–144.

Carré, B. 2000. Effets de la taille des particules alimentaires sur les processus digestifs chez les oiseaux d'élevage. Prod. Anim. 2 (12), 131-136.

Cozannet, P., Y. Primot, C. Gady, J. P. Métayer, M. Lessire, F. Skiba, and J. Noblet. 2010. Energy value of wheat distillers grains with solubles for growing pigs and adult sows. J. Anim. Sci. 88:2382–2392.

Dale, N., and H. L. Fuller. 1984. Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations.Poult. Sci. 63:1008-1012.

Donadelli, R. A., D. A. Stone, C. G. Aldrich, and R. S. Beyer. 2019. Effect of fiber source and particle size on chick performance and nutrient utilization. Poult. Sci. 98:5820–5830.

Dozier, W. A., B. J. Kerr, A. Corzo, M. T. Kidd, T. E. Weber, and K. Bregendal. 2008. Apparent metabolizable energy of glycerin for broiler chickens. Poult. Sci. 87:317–322.

Farrell, D. J., E. Thomson, J. J. Du Preez, and J. P. Hayes. 1991. The estimation of endogenous

excreta and the measurement of metabolisable energy in poultry feedstuffs using four feeding systems, four assay methods and four diets. Br. Poult. Sci. 32:483–499.

Fastinger, N. D., J. D. Latshaw, and D. C. Mahan. 2006. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poult. Sci. 85:1212–1216.

Fastinger, N. D., and D. C. Mahan. 2006. Determination of the ileal amino acid and energy digestibilities of corn distillers dried grains with solubles using grower-finisher pigs1,2,3. J. Anim. Sci. 84:1722–1728.

Foltyn, M., M. Lichovníková, V. Rada, and A. Musilová. 2014. Apparent ileal amino acids digestibility of diets with graded levels of corn DDGS and determination of DDGS amino acids digestibility by difference and regression methods in broilers.

Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J. Agric. Food Chem. 55:10737–10743.

Ghazalah, A. A., M. O. Abd-Elsamee, and E. S. Moustafa. 2011. Use of distillers dried grains with solubles (DDGS) as replacement for soybean meal in laying hen diets. Int. J. Poult. Sci. 10:505–513.

Gonzalez-Esquerra, R., and S. Leeson. 2000. Studies on the metabolizable energy content of ground full-fat flaxseed fed in mash, pellet, and crumbled diets assayed with birds of different ages. Poult. Sci. 79:1603–1607.

Guillaume, J., and J. D. Summers. 1970. Maintenance energy requirement of the rooster and influence of plane of nutrition on metabolizable energy. Can. J. Anim. Sci. 50:363–369

Hill, F. W., and D. L. Anderson. 1958. Comparison of Metabolizable Energy and Productive Energy Determinations with Growing Chicks. J. Nutr. 64:587–603.

Hill, F. W., D. L. Anderson, R. Renner, and L. B. Carew. 1960. Studies of the metabolizable energy of grain and grain products for chickens. Poult. Sci. 39:573–579.

Jacela, J. Y., J. M. DeRouchey, S. S. Dritz, M. D. Tokach, R. D. Goodband, J. L. Nelssen, R. C. Sulabo, R. C. Thaler, L. Brandts, D. E. Little, and K. J. Prusa. 2011. Amino acid digestibility and energy content of deoiled (solvent-extracted) corn distillers dried grains with solubles for swine and effects on growth performance and carcass characteristics1,2. J. Anim. Sci. 89:1817–1829.

Jie, Y. Z., J. Y. Zhang, L. H. Zhao, Q. G. Ma, and C. Ji. 2013. The correlationship between the metabolizable energy content, chemical composition and color score in different sources of corn DDGS. J. Anim. Sci. Biotechnol. 4:38.

Jin, S.-H., A. Corless, and J. L. Sell. 1998. Digestive system development in post-hatch poultry. Worlds. Poult. Sci. J. 54:335–345.

Kerr, B. J., W. A. Dozier, and G. C. Shurson. 2013. Effects of reduced-oil corn distillers dried grains with solubles composition on digestible and metabolizable energy value and

prediction in growing pigs. J. Anim. Sci. 91:3231–3243.

Kim, E. J., C. M. Parsons, R. Srinivasan, and V. Singh. 2010. Nutritional composition, nitrogen-corrected true metabolizable energy, and amino acid digestibilities of new corn distillers dried grains with solubles produced by new fractionation processes. Poult. Sci. 89:44–51.

Kingsly, A. R. P., K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Stroshine, and S. Radcliff. 2010. The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) - Plant scale experiments. Bioresour. Technol. 101:193–199.

Knott, J., J. Shurson, and J. Goihl. 2004.Effects of the Nutrient Variability of Distiller's Solubles and Grains within Ethanol Plants and the Amount of Distiller's Solubles Blended with Distiller's Grains on Fat, Protein and Phosphorus Content of DDGS.Protein and phosphorous content of DDGS.

Leytem, A. B., P. Kwanyuen, and P. Thacker. 2008. Nutrient excretion, phosphorus characterization, and phosphorus solubility in excreta from broiler chicks fed diets containing graded levels of wheat distillers grains with solubles. Poult. Sci. 87:2505–2511.

Liu, K. 2008. Particle size distribution of distillers dried grains with solubles (DDGS) and relationships to compositional and color properties. Bioresour. Technol. 99:8421–8428.

Liu, K. 2011. Chemical Composition of Distillers Grains, a Review. J. Agric. Food Chem 59:1508–1526.

Lopez, G., and S. Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poult. Sci. 87:298–306.

Lumpkins, B. S., and A. B. Batal. 2005. The bioavailability of lysine and phosphorus in distillers dried grains with solubles. Poult. Sci. 84:581–586.

Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poult. Sci. 83:1891–1896.

Martinez-Amezcua, C., C. M. Parsons, and D. H. Baker. 2006. Effect of microbial phytase and citric acid on phosphorus bioavailability, apparent metabolizable energy, and amino acid digestibility in distillers dried grains with solubles in chicks. Poult. Sci. 85:470–475.

Martinez Amezcua, C., and C. M. Parsons. 2007. Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. Poult. Sci. 86:331–337.

Martinez Amezcua, C., C. M. Parsons, and S. L. Noll. 2004. Content and relative bioavailability of phosphorus in distillers dried grains with solubles in chicks. Poult. Sci. 83:971–976.

Mateos, G. G., L. Cámara, G. Fondevila, and R. P. Lázaro. 2019. Critical Review of the Procedures Used for Estimation of the Energy Content of Diets and Ingredients in Poultry. J. Appl. Poult. Res. 28:506–525.

Mateos, G. G., E. Jiménez-Moreno, M. P. Serrano, and R. P. Lázaro. 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics. J. Appl.

Poult. Res. 21:156–174.

Mateos, G. G., and J. L. Sell. 1980. True and apparent metabolizable energy value of fat for laying hens: influence of level of use. Poult. Sci.59:369-373.

Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poult. Sci. 93: 1428-1439.

Meloche, K. J., B. J. Kerr, G. C. Shurson, and W. A. Dozier III. 2013. Apparent metabolizable energy and prediction equations for reduced-oil corn distillers dried grains with solubles in broiler chicks from 10 to 18 days of age. Poult. Sci. 92:3176-3183.

Miski, A. M., and S. Quazi. 1981. Influence of age and sex of growing broiler chicks and body weight of roosters on their endogenous and metabolic energy losses.Poult. Sci. 60: 781-785.

Nielsen Almeida, F., J. Kyaw Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. J. Anim. Sci. Biotechnol. 4:44.

Noll, S., J. Brannon, and C. Parsons. 2007. Nutritional value of corn distiller dried grains with solubles (DDGS): Influence of solubles addition.Poult. Sci. 86:68

Nuez Ortín, W. G., and P. Yu. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. J. Sci. Food Agric. 89:1754–1761

Parsons, C. M., C. Martinez, V. Singh, S. Radhakrishman, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. Multi-State Poultry Nutrition and feeding conference.

Parsons, C. M., L. M. Potter, and R. D. Brown. 1983. Effects of dietary carbohydrate and of intestinal microflora on excretion of endogenous amino acids by poultry.Poult. Sci. 62: 483-489.

Pedersen, M. B., S. Dalsgaard, K. E. Bach Knudsen, S. Yu, and H. N. Laerke. 2014. Compositional profile and variation of Distillers Dried Grains with Solubles from various origins with focus on non-starch polysaccharides. Anim. Feed Sci. Technol. 197:130-141.

Pesti, G. M., L. O. Faust, H. L. Fuller, N. M. Dale, and F. H. Benoff. 1986. Nutritive value of poultry by-product meal. 1. Metabolizable energy values as influenced by method of determination and level of substitution. Poult. Sci. 65:2258-2267.

Potter, L. M., L. D. Matterson, A. W. Arnold, W. J. Pudelkiewicz, and E. P. Singsen. 1960. Studies in Evaluating Energy Content of Feeds for the Chick. Poult. Sci. 39:1166–1178.

Rausch, K. D., and R. L. Belyea. 2006. The Future of Coproducts From Corn Processing. Applied biochemestry and technology. 128: 47-86.

Ravindran, V., O. Adeola, M. Rodehutscord, H. Kluth, J. D. van der Klis, E. van Eerden, and A. Helmbrecht. 2017. Determination of ileal digestibility of amino acids in raw materials for broiler chickens – Results of collaborative studies and assay recommendations. Anim. Feed Sci. Technol. 225:62–72.

Rochell, S. J., B. J. Kerr, and W. A. Dozier. 2011. Energy determination of corn co-products fed to broiler chicks from 15 to 24 days of age, and use of composition analysis to predict nitrogen-corrected apparent metabolizable energy. Poult. Sci.91:1999-2007.

Rodehutscord, M., M. Kapocius, R. Timmler, and A. Dieckmann. 2004. Linear regression approach to study amino acid digestibility in broiler chickens. Br. Poult. Sci. 45:85–92.

Rodríguez, M. L., C. Alzueta, A. Rebolé, L. T. Ortiz, C. Centeno, and J. Treviño. 2001. British Poultry Science Effect of inclusion level of linseed on the nutrient utilisation of diets for growing broiler chickens Effect of inclusion level of linseed on the nutrient utilisation of diets for growing broiler chickens. Br. Poult. Sci. 42:368–375.

Sacranie, A., P. Iji, Mikkelsen, L., and Choct, M. 2006. Occurrence of reverse peristalsis in broiler and layer chickens. Poult. Sci.64:197.

SAS Institute. 2009. SAS User's Guide. Statistics. Version 9.2 Edition. SAS Inst. Inc., Cary, NC.

Saunders, J. A., and K. A. Rosentrater. 2009. Survey of US fuel ethanol plants. Bioresour. Technol. 100:3277–3284.

Short, F. J., P. Gorton, J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59:215–221.

Shrimpton, D. H., S. Leeson, and D. Lewis. 1977. Metabolisable Energy Studies With Turkeys: Nitrogen Correction Factor In Metabolisable Energy Determinations. Br. Poult. Sci. 18:373–379.

Shurson, G. C. 2017. The Role of Biofuels Coproducts in Feeding the World Sustainably. Annu. Rev. Anim. Biosci. 5:229–254.

Shurson, G., M. Spiehs, and M. Whitney. 2004. The use of maize distiller's dried grains with solubles in pig diets.Pig news and information 25:75.

Sibbald, I. R. 1975. The effect of level of feed intake on metabolizable energy values measured with adult roosters. Poult. Sci. 54:1990–1997.

Sibbald, I. R. Metabolizable energy evaluation of poultry diets.Recent developments in poultry Nutrition (2013): 12.

Sibbald, I. R., and S. J. Slinger. 1962. Factors Affecting the Metabolizable Energy Content of Poultry Feeds. Poult. Sci.39: 544-556.

Sibbald, I. R., and S. J. Slinger. 1963. Factors Affecting the Metabolizable Energy Content of Poultry Feeds. Poult. Sci. 42:707–710.

Singh, V., R. A. Moreau, K. B. Hicks, R. L. Belyea, C. H. Staff, R. A. Moreau, K. B. Hicks, and R. L. Belyea. 2001. Removal of fiber from distillers dried grains with solubles (DDGS) to increase valuE. Trans. ASAE 45:389–392.

Smeets, N., F. Nuyens, L. Van Campenhout, E. Delezie, J. Pannecoucque, and T. Niewold. 2015. Relationship between wheat characteristics and nutrient digestibility in broilers: Comparison between total collection and marker (titanium dioxide) technique. Poult. Sci.

94:1584–1591.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84:853–860.

Świątkiewicz, S., and J. Koreleski. 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. Worlds. Poult. Sci. J. 64:257–265.

Szczurek, W. 2010. Standardized ileal digestibility of amino acids insome cereals, rapeseed products and maize DDGSfor broiler chickens at the age of 14 days. J. Anim. Feed Sci. 19:72–80.

Villamide, M. J., J. M. Fuente, P. Perez De Ayala, and A. Flores. 1997. Energy Evaluation of Eight Barley Cultivars for Poultry: Effect of Dietary Enzyme Addition. Poult. Sci. 76:834–840.

Waldroup, P. W., J. A. Owen, B. E. Ramsey, and D. L. Whelchel. 1981. The Use of High Levels of Distillers Dried Grains Plus Solubles in Broiler Diets. Poult. Sci. 60: 1479-1484.

Walugembe, M., M. F. Rothschild, and M. E. Persia. 2014. Effects of high fiber ingredients on the performance, metabolizable energy and fiber digestibility of broiler and layer chicks. Anim. Feed Sci. Technol. 188:46–52.

Wamsley, K. G. S., I. E. Loar, K. Karges, and J. S. Moritz. 2013. The use of practical diets and regression analyses to determine the utilization of lysine and phosphorus in corn distillers dried grains and solubles using Cobb 500 male broilers. J. Appl. Poult. Res. 22:279–297.

Wang, Z., S. Cerrate, F. Yan, P. Waldroup, Z. Wang, S. Cerrate, C. Coto, F. Yan, and P. W. Waldroup. 2007. Utilization of distillers dried grains with solubles (DDGS) in broiler diets using a standardized nutrient matrix. Int. J. Poult. Sci. 6:470–477.

Warnick, R. E., and J. O. Anderson. 1968. Limiting essential amino acids in soybean meal for growing chickens and the effects of heat upon availability of the essential amino acids. Poult. Sci. 47:281–287.

Wiseman, J., and F. Salvador. 1991. The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fats fed to broilers. Poult. Sci.70:573-582.

Wu, S. B., M. Choct, and G. Pesti. 2020. Historical flaws in bioassays used to generate metabolizable energy values for poultry feed formulation: a critical review. Poult. Sci.99:385-406.

Youssef, I. M. I., C. Westfahl, A. Sunder, F. Liebert, and J. Kamphues. 2008. Evaluation of dried distillers' grains with solubles (DDGS) as a protein source for broilers. Arch. Anim. Nutr. 62:404–414.

Zhang, F., and O. Adeola. 2017. Energy values of canola meal, cottonseed meal, bakery meal, and peanut flour meal for broiler chickens determined using the regression method. Poult. Sci. 96:397–404.

Zhang, Y., A. Shan, W. Jiang, C. Bi, and Z. Li. 2013. The effect of vitamin E on growth performance and meat quality in broilers given diets containing distillers' dried grain with solubles (DDGS). Br. Poult. Sci. 54:138–143.

CHAPTER 4: APPARENT METABOLIZABLE ENERGY CONTENT OF 11 SOURCES OF DISTILLERS DRIED GRAINS WITH SOLUBLES DETERMINED IN BROILER CHICKENS AT 3 WEEKS OF AGE

ABSTRACT

Distillers dried grains with solubles (DDGS) is a co-product of the corn ethanol industry that is commonly used as a poultry feed ingredient in the United States. However, nutritional variability of DDGS, particularly in its metabolizable energy (ME) content, can be a limiting factor for its use. An experiment consisting of 2 identical trials was conducted to determine the ME and nitrogen-corrected ME (ME_n) of 11 DDGS samples obtained from different biorefinery locations operated by a single ethanol producer. The DDGS samples ranged in crude protein from 29.61 to 33.88%, ether extract from 5.82 to 9.66%, acid detergent fiber from 7.71 to 11.30%, and neutral detergent fiber from 24.08 to 31.35% (DM basis). In each trial, 576 off-sex male Cobb 500 breeder chicks were allocated to 72 battery cages. A total of 23 treatments included a basal diet with 0% DDGS and 22 test diets in which energy providing ingredients in the basal diet were replaced with 15 or 30% DDGS from each of the 11 sources. There were 12 replicates cages for the basal diet (6 per trial) and 6 replicates for the test diets (3 per trial) with 8 chicks per cage. Birds were provided a common starter diet from 0 to 14 d and experimental diets from 14 to 21 d, with a 48 h total excreta collection conducted from 19 to 21 d. Feed and excreta samples were analyzed for DM, nitrogen, and gross energy content to determine the ME and ME_n of complete diets using the total collection method. For each source, dietary ME or ME_n was regressed on DDGS inclusion (%) using simple linear regression, and ME and ME_n estimates were based on extrapolation of dietary ME or ME_n at a dietary DDGS inclusion of 100%. Linear regression slopes for ME and ME_n were significantly different than zero for 9 of

the 11 sources, with R^2 values ranging from 0.02 to 0.79 and 0.02 to 0.82, respectively. The ME values of the DDGS ranged from 2,477 to 3,263 and averaged 2,913 kcal/kg, while the ME_n values ranged from 2,284 to 3,088 and averaged 2,765 kcal/kg (DM basis). Pearson correlation coefficients resulted in hemicellulose as the only significant predictor of DDGS AME. Low CV from chemical composition suggested a consistent DDGS product among biorefinery locations using similar ethanol production practices.

Key words: Broilers, metabolizable energy, DDGS, energy prediction

INTRODUCTION

Distillers dried grains with solubles (DDGS) is the main co-product of the dry-grind ethanol industry, and it contains up to three-fold the amount of certain nutrients that are found in corn due to its starch removal during the fermentation process (Ghazalah et al., 2011). Even though DDGS has been used in poultry diets as a source of available phosphorous, digestible amino acids, and metabolizable energy (ME) for several decades, its inclusion in poultry diets generally remains low due in part to its nutritional variability (Cozannet et al., 2010; Liu, 2011). Its metabolizable energy content is particularly variable since it is directly affected by agronomic factors related to the hybrid of corn used for fermentation and the processing techniques that will vary between plants (Liu, 2008b; Kingsly et al., 2010).

The modernization of ethanol plants and modifications in processing techniques have led to changes in the nutritional content of DDGS over time. Furthermore, previous experiments have reported differences in the nutritional content of DDGS between bioethanol plants where corn is used as the primary cereal grain (Spiehs et al., 2002; Batal and Dale, 2006; Liu, 2008b, 2011; Kingsly et al., 2010). Factors such as temperature, yeast source, particle size, fiber

removal, and amount of solubles added will vary significantly between plant locations and batches (Singh et al., 2001; Noll et al., 2007; Belyea et al., 2010a). Some ethanol companies have aimed to standardize DDGS processing techniques across locations to improve the consistency in its nutrient and energy content. Stein et al. (2006) conducted an experiment in swine to evaluate 10 DDGS sources from a single ethanol producer and found differences in their digestible energy content, reflecting the need to understand the relationship between nutrient composition and energy utilization even for sources produced under similar conditions across various locations.

The relationship between chemical composition and ME content has been previously established for corn milling co-products, including DDGS (Batal and Dale, 2006; Rochell et al., 2011; Meloche et al., 2013). Similarly, several experiment have determined the influence of physical properties such as particle size and color to be highly correlated with the nutritional content of DDGS (Noll et al., 2007; Liu, 2008b; Jie et al., 2013a). In addition, the effect of heat damage on amino acids content have been correlated with the sample color (Batal and Dale, 2006; Fastinger et al., 2006). Indeed, relating *in vivo* ME utilization of DDGS in broilers to its characteristics that are routinely analyzed in feed mills and nutritional laboratories would be very helpful for nutritionists purchasing and formulating with different sources of DDGS.

The practical application of existing equations to estimate the ME of DDGS based its chemical composition has been limited by their high variability and poor predictive capacity (Batal and Dale, 2006; Meloche et al., 2014). The AME_n of the DDGS and corn co-products used in previous experiments for the development of prediction equations in broilers has been determined using a single inclusion level (15%) of the test ingredient within the test diets (Rochell et al., 2011; Meloche et al., 2013), but regression-based assays based on multiple

inclusion levels of DDGS inclusion might provide more robust ME estimates (Adeola and Zhai, 2012). Previous work from our lab determined DDGS inclusion level within the test diet influences its ME estimation, and that 15 and 30% inclusion levels may provide a compromise between lower variation in ME estimates without causing issues associated with nutrient imbalance observed at higher inclusion levels. Therefore, the objective of this experiment was to determine the ME and nitrogen-corrected ME (ME_n) of 11 DDGS samples obtained from different biorefinery locations operated by a single ethanol producer and to relate these values with chemical and physical properties of DDGS.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Arkansas approved all procedures (protocol #19121) in this experiment.

Bird Husbandry

A total of 1,152 off sex males chicks from a Cobb 500 female line obtained from a commercial hatchery (Cobb Vantress Inc., Fayetteville, AR) were used in 2 identical trials. In each experiment, 576 chicks were placed in 72 battery cages at 8 chicks per cage and reared to 21 d post-hatch. Battery cages (0.61 m x 0.61 m) had raised wired floors and were equipped with trough feeders and nipple waters. Battery cages were housed within climate-controlled rooms with LED lighting. The lighting program consisted of 23L:1D from day 0 to 7 and 16L:8D from day 8 to 21. Room temperatures were set to 32˚C at day of placement and were decreased to 21˚C by the end of the trial.

Dietary treatments and in vivo ME assay

Birds received a common corn-soybean meal-based diet *ad libitum* from 0 to 14 d post-hatch. Distillers dried grains with solubles sources were obtained from 11 different ethanol plants throughout the United States from a single commercial producer using similar ethanol and DDGS production techniques. Experimental treatments were fed from day 14 to 21 and consisted of a total of 23 treatments that included a basal diet with no DDGS, and 22 test diets in which energy providing ingredients in the basal diet were replaced with 15 or 30% DDGS from each of the 11 sources. There were 12 replicate cages for the basal diet (6 per trial), and 6 replicates for the test diets (3 per trial) with 8 chicks per cage.

Feed intake and body weights were recorded at days 14 and 21 to calculate body weight gain and FCR. Feed disappearance and total excreta output were recorded during a 48-h collection period from 19 to 21 d to determine energy and nitrogen intake and excretion. Excreta were collected on stainless steel trays below each cage, and samples of homogenized excreta were collected from each cage and pan, avoiding contamination with feed and feathers. Excreta samples were placed in cups, frozen, and freeze-dried before analysis.

Analysis of DDGS, feed, and excreta samples

All DDGS sources used in this experiment were analyzed for gross energy (GE), crude protein (CP), ether extract (EE), total dietary fiber (TDF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and starch by a commercial laboratory (University of Missouri Agriculture Experiment Station Chemical laboratories, Columbia, MO; Table 4.2). Hemicellulose calculation content was estimated by subtracting acid detergent fiber (ADF) from neutral detergent fiber (NDF). Particle size was determined on a 13 half-height sieve shaker (W.S Tyler, Model RX-

29,RO-TAP, Mentor, OH) according to a standard method (ASAE Standards, 2003), with data reported as the geometric mean (μm). The degree of lightness (L*), redness (a*), and yellowness (b*) of DDGS samples were determined using a Chroma meter colorimeter (model CR – 400, Konica Minolta). Reported color scores were the mean of three measurements, with the samples placed on top of a white surface, and readings were taken on the left, center, and right area of each sample. The color of each sample was expressed in terms of CIE values for lightness (L*; higher = lighter), redness (a*; higher = redder), and yellowness (b*; higher = more yellow).

Lyophilized excreta samples were ground using an electric coffee grinder, while feed samples were ground using a grinder with a 1-mm screen (Perten LM 3100, Perten Instrument, Hägersten, Sweden). Feed and excreta samples were analyzed for gross energy and nitrogen content at the University of Arkansas Central Analytical Laboratory. Gross energy was determined using a Parr 6200 calorimeter (Parr Instruments, Moline, IL). Nitrogen content on excreta and feed samples were determined by using the combustion method [AOAC Official Methods 990.03]. Dry matter of DDGS, feed, and excreta samples was determined by drying at 105^{o}C (Isotemp oven, Fisher Scientific, Pittsburgh, PA) for 12 hours [AOAC Official methods 934.01].

Calculations and regression-based ME estimates

The AME and AME_n values (kcal/kg) for each dietary treatment were determined using to the following equations:

$$AME\ (kcal/kg) = [GE_{intake} - GE_{excreted}]/\ FI$$

$$AME_n\ (kcal/kg) = GE_{intake} - [GE_{excreted} \times (8.22 * retained\ nitrogen_{(g)})]/FI$$

where GE_{intake} and $GE_{excreted}$ are the gross energy values (kcal/kg) in the feed intake and the excreta, and FI is the feed intake (FI). To determine the AME_n value, a nitrogen correction factor of 8.22 kcal/g was used (Hill and Anderson, 1958). The AME and AME_n of the DDGS (kcal/kg) at each inclusion level was calculated by the difference method using the following equation.

AME and AME_n of DDGS: $AME_{n,DDGS}$ (kcal/kg) = [$AME_{n,diet}$- ($AME_{n,basal}$ × BI)] / TI

Where $AME_{n,diet}$ and $AME_{n,basal}$ represent the analyzed AME_n values (kcal/kg) of the test and basal diets, and BI and TI represent the concentration (%) of the test ingredient and basal portion in the experimental diet.

Regression-based ME and ME_n estimates were determined by regressing the ME and ME_n values of the experimental diets on DDGS inclusion (%), and ME and ME_n of DDGS were based on extrapolation of dietary ME or ME_n at a dietary DDGS inclusion of 100% (Mateos and Sell, 1980; Gonzalez-Esquerra and Leeson, 2000). One of the advantages of the regression method is that it accounts for the endogenous losses; therefore, the energy is denoted as ME rather than AME. Confidence intervals (95%) on slopes and intercepts were determined, and significance was considered at $P \leq 0.05$ for statistical analyses.

Statistical Analyses

Trial by treatment interactions was evaluated and found not to be significant ($P > 0.05$); therefore, data from both trials were pooled, resulting in 12 replicate cages for the basal treatment and 6 replicates cages per each treatment containing DDGS. More replicates cages were used for the basal diet to determine its AME value more precisely to further serve as reference for the determination of AME and ME of DDGS by difference and regression method. Pearson correlation coefficients were determined among ME, ME_n, nutrient composition, particle

size, and color using the CORR procedure of SAS 9.4 (2012). All possible subset selection in multiple regression was used to determine the chemical component that were significant predictors of ME_n for 11 DDGS sources using the selection function in PROC REG procedure of SAS 9.4 (2012). The all possible subset approach fits all the possible models containing one, two, or more predictors in the models. From those, the models with the highest adjusted coefficient of determination (R^2 adj) and lowest Mallows' statistic (Cp) and Akaike information criterion (AIC) values are selected for further analysis.

RESULTS AND DISCUSSION

Due to the dynamic nature of DDGS composition, the use of predictive equations to estimate ME content from chemical components within DDGS could improve the accuracy of final feed formulations. The objective of the current experiment was to determine the ME and ME_n of 11 DDGS sources obtained from different plants using similar processing techniques and to establish correlations between chemical composition and physical properties that would lead to ME and ME_n prediction equations for similarly-produced DDGS. The proximate composition of samples produced from 20 different plants was evaluated, and 11 source locations were chosen to provide the greatest variation in these analyses (Table 2). On a dry matter basis, GE of the selected DDGS sources ranged from 4,247 to 4,499 kcal/kg (CV = 1.48%), CP content ranged from 29.61 to 33.88% (CV = 3.78%), starch content ranged from 5.13 to 15.65% (CV = 21.40%), NDF content ranged from 24.08 to 31.35% (CV = 7.56%), and EE content ranged from 5.82 to 9.66% (CV = 12.87%). In comparison, higher variability (CV = 2.33%) in GE was reported by Rochell et al. (2011) on 6 sources of DDGS. Similarly, the fat contents of the DDGS sources used in the experiments conducted by Batal and Dale (2006) and Meloche et al. (2013) were much more variable (CV = 26.5 and 28.9% respectively) than for the ones obtained in the

current experiment. Belyea et al. (2010) reported that fermentation batches were greater sources of variation than plant location when analyzing DDGS sources from 9 dry grind ethanol plants. The relatively lower variation among analyses of the current DDGS may be due to improved standardization of processing techniques and segregation of final products by the ethanol producers from which these samples were sourced.

Though the objective of the experiment was to determine the ME and ME_n of the DDGS sources by regression, assessing the energy utilization of the complete test diets as well for DDGS source individually by difference in Tables 4.3 and 4.4, respectively, provides insight into the subsequent regression-based estimates. The AME_n value of the basal diet was 3,156 kcal/kg DM (2,904 kcal/kg, as-is), which is in close agreement with the calculated value of 3,156 kcal/kg (Table 1). It can be noted at 15 and 30% DDGS inclusion the AME and AME_n values of the complete diets decreased from 3,354 and 3,184 kcal/kg to 3,175 and 3,018 kcal/kg respectively. A similar trend was observed by Adeola and Zhai (2012) where 30 and 60% DDGS inclusion levels decreased the dietary AME and AME_n value from 3,244 to 2,851 kcal/kg and from 3,137 to 2,752 kcal/kg respectively. The AME_n values of the complete diets containing 15% DDGS were higher than that of the basal diet, except for diets containing DDGS sources 1, 3, 9, and 10, which indicates that the energy content of the DDGS at 15% was higher than that of the basal diet. Likewise, the AME and AME_n values of the DDGS values decreased from 3,608 to 2,963 kcal/kg and from 3,401 to 2,815 kcal/kg at 15 and 30% DDGS inclusion, respectively. The decrease in the ME and ME_n of the DDGS could be explained by the nutritional imbalance of diets and increases in endogenous losses associated with high fiber ingredients leading to ineffective utilization of nutrients and energy by the birds.

Intercepts, slopes, and their corresponding 95% confidence intervals of regression equations of dietary AME and AME_n on DDGS inclusion level are presented in Table 4.5. Estimates of ME_n for DDGS were determined using these equations and extrapolating to 100% dietary DDGS inclusion. Except source 5 and 11, regression slopes were significantly different than zero and negative to indicate decrease in dietary ME_n as the inclusion level increased. The lack of significant regression slopes for sources 5 and 11 could be explained by the fact that the differences in ME between the basal diet and the DDGS at 15% was not large enough to allow a decrease as the DDGS inclusion increased. Using this approach, ME and ME_n values ranged from 2,477 to 3,263 kcal/kg and from 2,284 to 3,088 kcal/kg and averaged 2,913 and 2,765 kcal/kg respectively. Adeola and Zhai (2012) determined the ME_n of DDGS to be 2,688 kcal/kg by using a modified regression method where the DDGS caloric intake (kcal) was regressed against the DDGS intake (kg). Of the 11 DDGS sources tested, 8 had ME_n values within one SD of the mean of 2,765 kcal/kg DM, 1 source was nearly 2 SD below the average, and the 2 sources that did not have significant regression equations were over 1 SD above the mean. Similarly, the ME_n of the DDGS sources had a CV of 7% which is lower than the ones from the AME_n of 15 (CV = 12%) and 30 (CV = 15%) DDGS sources analyzed by Meloche et al. (2013) and Jie et al. (2013a) respectively. Meloche et al. (2013) and Jie et al. (2013a) determined the AME_n of DDGS by the single ingredient replacement method and by the difference method respectively; therefore, the low variability reported in this experiment could be attributed not only to similarities in processing techniques between plants, but also to the use of the regression method for ME determination.

Pearson correlation coefficients between chemical components of DDGS and their ME_n are presented in Table 6. Hemicellulose was the only component found to be correlated with

DDGS ME_n (r = 0.61, *P* = 0.03), which agrees with the results obtained by Rochell et al. (2011). However, these authors found hemicellulose to be negatively correlated with AME_n, whereas a positive relationship between hemicellulose and ME_n was observed in the current study. Determination of hemicellulose is costly and not practical since it requires the determination of NDF and ADF. Hence, Rochell et al. (2011) removed hemicellulose form the initial list of predictors and the resulting models contained NDF, GE, CP and starch. Also, prediction equations generated by Meloche et al. (2013) included TDF and CP as significant predictors of DDGS ME This suggests that further investigation into relationship between specific fiber components and DDGS ME is needed. The lack of correlations between chemical components and the ME_n in our experiment, could perhaps be explained by the narrow range in chemical composition when compared to previous experiments where the variability was much higher (Batal and Dale, 2006; Rochell et al., 2011; Meloche et al., 2013).

Indicators of heat damage such as DDGS color, and more recently Lys:CP ratios, have been related to nutrient and energy utilization in DDGS (Fastinger and Mahan, 2006; Jie et al., 2013a; Nielsen Almeida et al., 2013). In the current experiment, L* scores of the DDGS sources ranged from 44.75 to 55.45 (CV =), a* scores ranged from 11.22 to 15.91, and b* scores ranged from 39.13 to 49.15, with strong correlations (r = 0.87, $P < 0.01$) between L* values and b* scores. These results agree with the color range and correlations reported by Jie et al. (2013) for 30 DDGS sources. Low ME, and TME_n values have been previously reported for DDGS sources with high a* and low L* values (Fastinger et al., 2006; Jie et al., 2013b). Lysine is one of the most affected amino acids by heat damage due to the effect of Maillard reactions which directly affects its bioavailability (Stein et al., 2006). Fontaine et al. (2007) reported that lysine to CP ratio (Lys:CP) is the most significant indicator of lysine damage and that values of 3.2% is

indicative of properly processed DDGS, while those with values of 2.3% are considered severely damaged by heat. The Lys:CP ratio for DDGS in our experiment ranged from 2.44 to 3.06 and averaged 2.69%, and no correlations among DDGS color, Lys:CP, and its ME_n of DDGS were found. Therefore, the lys:CP ratio of the DDGS sources in the current experiment suggests that the sources were affected by heat damage but within an acceptable range.

The particle size of DDGS sources ranged from 364 to 760 µm (CV = 22.38%). Particle size was negatively correlated with a*and b* scores and CP content, which agrees with the results obtained by Liu (2008a) who found more starch content and less crude protein on DDGS samples with larger particle sizes. Studies have shown that slowing rate of passage can be achieved through the use of coarse particle size, which improves nutrient digestibility and energy utilization by allowing digestive enzymes to be in close contact with nutrients for a longer time (Carré, 2000; Liu, 2008a). Though there is limited information on the effect of particle size on energy utilization from DDGS, previous studies on corn have shown increased TME_n and ileal energy digestibility when feeding coarse and medium particle size corn compared with smaller particle sizes (Xu et al., 2015; Donadelli et al., 2019). However, the results from this experiment did not support a relationship between particle size and DDGS ME_n within the ranges tested.

Overall, the 11 DDGS sources selected from different ethanol plants using similar DDGS production practices evaluated in this experiment had a generally consistent chemical composition. The average ME and ME_n values of the DDGS samples were 2,913 and 2,765 kcal/kg DM when determined by regression. These results provide a good insight into the potential for consistency in DDGS from different plants is produced with similar processes and segregated based on analyses of final products. This study also reaffirms that to establish robust

prediction equations, it may be advantageous to utilize ingredients with a wide range of values in both chemical composition and nutrient utilization.

Table 4.1 Analyzed composition of corn distillers dried grains with solubles sourced from 11 different ethanol plants using similar production processes[1]

Item, %	DDGS -1	DDGS - 2	DDGS -3	DDGS -4	DDGS -5	DDGS -6	DDGS - 7	DDGS -8	DDGS -9	DDGS -10	DDGS -11
Moisture	8.38	9.10	9.51	9.86	10.31	9.30	8.47	10.42	10.99	9.54	9.39
Gross energy (kcal/kg)	4,467	4,499	4,421	4,465	4,489	4,442	4,449	4,389	4,247	4,413	4,448
CP	33.88	32.13	30.28	29.61	32.62	32.17	31.06	31.68	31.09	31.88	33.37
Starch	11.92	14.21	15.65	12.81	12.75	12.38	12.58	12.04	9.80	12.23	5.13
Total dietary fiber	30.42	32.61	35.59	34.95	31.90	36.21	36.00	34.52	38.07	35.03	27.16
Acid detergent fiber	9.36	8.89	10.20	10.09	8.56	11.01	10.13	9.62	11.30	9.18	7.71
Neutral detergent fiber	24.08	27.77	31.03	27.47	29.40	31.35	31.23	29.02	29.37	28.87	25.87
Hemicellulose[2]	14.72	18.87	20.83	17.38	20.84	20.34	21.10	19.39	18.06	19.69	18.15
Ether extract	7.29	9.21	7.19	8.55	9.66	7.57	8.83	7.74	7.74	7.73	5.82
Lys:CP ratio	2.78	3.06	2.63	2.83	2.69	2.73	2.79	2.44	2.55	2.56	2.50
Particle size, µm	418	416	467	760	426	364	390	453	535	422	425
Color measurements											
L*	52.11	55.45	44.75	45.08	45.61	53.33	52.05	51.92	51.30	52.74	52.85
a*	15.91	14.55	14.68	11.22	15.23	15.37	15.86	14.14	14.14	13.54	12.89
b*	48.06	49.15	39.13	35.53	41.16	45.95	46.79	44.65	40.59	44.54	45.82

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

Table 4.2. Ingredient and nutrient composition of basal and experimental diets

	CSBM	DDGS inclusion (%)	
Item	Basal	15	30
Corn	58.99	49.81	40.62
Soybean meal	33.07	27.92	22.78
Soybean oil	3.94	3.33	2.71
L-lysine HCl	0.13	0.11	0.09
DL-methionine	0.21	0.18	0.15
L-threonine	0.02	0.02	0.02
DDGS[2]	0.00	15.00	30.00
Limestone	0.90	0.90	0.90
Dicalcium phosphate	1.51	1.51	1.51
Titanium dioxide	0.50	0.50	0.50
Vitamin & mineral premix[1]	0.25	0.25	0.25
Sodium chloride	0.36	0.35	0.35
Choline chloride	0.10	0.10	0.10
Santoquin	0.02	0.02	0.02
Calculated nutrient composition, % unless noted otherwise[3]			
AME_n, kcal/kg	3,156	-	
CP	20.27	-	
Digestible Lysine	1.16	-	
Digestible TSAA	0.80	-	
Digestible Threonine	0.73	-	
Ca	0.80	-	
Nonphytate P	0.41	-	

[1]Supplied the following per kg of diet: vitamin A, 6,173 IU; vitamin D3, 4,409 ICU; vitamin E, 44 IU; vitamin B12, 0.01 mg; menadione, 1.20 mg; riboflavin, 5.29 mg; d-pantothenic acid, 7.94 mg; thiamine, 1.23 mg; niacin, 30.86 mg; pyridoxine, 2.20 mg; folic acid, 0.71 mg; biotin, 0.07 mg; manganese, 24 mg; zinc, 14.4 mg; selenium, 0.04 mg; copper, 0.68 mg; iodine, 0.47 mg

[2]DDGS = distillers dried grains with solubles

[3]Calculated composition of experimental diets containing 15 or 30% DDGS varied with DDGS source and concentration.

Table 4.3 Apparent metabolizable energy and nitrogen corrected metabolizable energy of complete diets containing 15% or 30% of 11 sources of DDGS sourced from different location and determined in broilers chicks[1,2]

	AME (kcal/kg)		AME_n (kcal/kg)	
Source	15%	30%	15%	30%
Basal	3,322		3,156	
1	3,245	3,065	3,057	2,886
2	3,356	3,209	3,177	3,038
3	3,292	3,187	3,122	3,028
4	3,345	3,181	3,164	3,019
5	3,424	3,284	3,248	3,109
6	3,405	3,202	3,225	3,035
7	3,350	3,154	3,175	2,990
8	3,377	3,188	3,190	3,028
9	3,309	3,128	3,133	2,967
10	3,332	3,192	3,155	3,034
11	3,462	3,231	3,283	3,068

[1]AME and AME_n was determined by a 48-h excreta collection following a 7-d adaptation period.
[2]Values are means of 12 replicates for and 6 replicates for the rest of the diets (DM basis)
[3]Abbreviations: AME = apparent metabolizable energy; AME_n = nitrogen corrected apparent metabolizable energy

Table 4.4 AME and AME_n of the 11 DDGS sources determined by difference when 15% and 30% inclusion level in broilers chicks[1,2]

	AME (kcal/kg)		AME_n (kcal/kg)	
Source	15%	30%	15%	30%
1	2,876	2,568	2,609	2,374
2	3,620	3,048	3,414	2,882
3	3,193	2,973	3,043	2,846
4	3,549	2,954	3,322	2,816
5	4,070	3,298	3,890	3,117
6	3,945	3,023	3,733	2,871
7	3,582	2,863	3,398	2,721
8	3,758	2,979	3,501	2,846
9	3,306	2,778	3,122	2,643
10	3,461	2,992	3,264	2,866
11	4,325	3,122	4,116	2,981

[1]Values are means of 6 replicates cages with 8 birds per cage and reporter on a DM basis
[2]Abbreviations: AME = apparent metabolizable energy; AME_n = nitrogen corrected apparent metabolizable energy

Table 4.5 Slopes and intercepts of regression equations between dietary concentration of DDGS and dietary AME or AME_n for 11 sources of DDGS

Source	Intercept (95% CI)	Slope (95% CI)	R^2	*P*-value	ME or ME_n (kcal/kg)[1]
ME					
1	3,340 (3,307 to 3,373)	-8.63 (-10.60 to - 6.66)	0.79	<0.001	2,477
2	3,348 (3,313 to 3,383)	-3.58 (-5.66 to -1.50)	0.37	0.002	2,990
3	3,338 (3,305 to 3,371)	-4.65 (-6.62 to -2.67)	0.52	<0.001	2,873
4	3,348 (3,309 to 3,387)	-4.51 (-6.84 to -2.17)	0.42	<0.001	2,897
5	3,353 (3,311 to 3,396)	-0.90 (-3.44 to 1.63)	0.02	0.467	3,263
6	3,357 (3,308 to 3,406)	-3.51 (-6.45 to -0.58)	0.22	0.021	3,006
7	3,352 (3,310 to 3,393)	-5.29 (-7.24 to -2.62)	0.47	<0.001	2,823
8	3,353 (3,305 to 3,402)	-4.08 (-6.96 to -1.20)	0.28	0.008	2,945
9	3,346 (3,310 to 3,383)	-6.33 (-8.52 to -4.13)	0.62	<0.001	2,713
10	3,345 (3,310 to 3,380)	-4.24 (-6.31 to -2.16)	0.45	<0.001	2,921
11	3,365 (3,310 to 3,419)	-2.27 (-5.53 to 0.98)	0.09	0.162	3,138
ME_n					
1	3,163 (3,132 to 3,194)	-8.79 (-10.63 to 6.96)	0.82	<0.001	2284
2	3,171 (3,138 to 3,203)	-3.45 (-5.39 to -1.50)	0.38	0.001	2826
3	3,162 (3,130 to 3,194)	-4.12 (-6.03 to -2.20)	0.48	<0.001	2750
4	3,170 (3,134 to 3,206)	-4.13 (-6.26 to -2.00)	0.42	<0.001	2757
5	3,177 (3,136 to 3,219)	-0.89 (-3.35 to 1.60)	0.02	0.464	3088
6	3,180 (3,134 to 3,226)	-3.26 (-5.99 to -0.53)	0.22	0.022	2854
7	3,175 (3,136 to 3,214)	-4.93 (-7.24 to -2.62)	0.47	<0.001	2682
8	3,174 (3,131 to 3,217)	-3.69 (-6.25 to -1.14)	0.29	0.007	2805
9	3,169 (3,135 to 3,206)	-5.89 (-7.93 to -3.85)	0.62	<0.001	2580
10	3,167 (3,135 to 3,199)	-3.72 (-5.63 to -1.81)	0.43	<0.001	2795
11	3,187 (3,136 to 3,239)	-1.91 (-4.99 to -1.67)	0.07	0.211	2996

[1]Abbreviation: ME = metabolizable energy; ME_n = nitrogen corrected metabolizable energy

[2]ME and ME_n of DDGS was estimated by extrapolating the regression line to 100% DDGS inclusion.

Table 4.6. Pearson correlation coefficients between ME and MEn content of DDGS and is chemical and physical properties based on analysis 11 DDGS sources[1]

Item	ME_n	GE	CP	EE	TDF	ADF	NDF	Starch	Hcell	Lys:cp	PS	L*	a*	b*
ME_n	1													
P-value														
GE	0.281	1												
P-value	0.40													
CP	-0.034	0.274	1											
P-value	0.922	0.41												
EE	0.167	0.293	-0.274	1										
P-value	0.62	0.38	0.42											
TDF	-0.203	-0.552	-0.727	0.321	1									
P-value	0.55	0.08	0.01	0.34										
ADF	-0.431	-0.578	-0.575	0.09	0.891	1								
P-value	0.19	0.06	0.06	0.79	<0.01									
NDF	0.341	-0.235	-0.559	0.311	0.742	0.557	1							
P-value	0.31	0.49	0.07	0.35	0.01	0.08								
Starch	-0.147	0.259	-0.463	0.599	0.511	0.363	0.440	1						
P-value	0.67	0.44	0.15	0.05	0.11	0.27	0.18							
Hcell	0.646	0.041	-0.35	0.322	0.393	0.111	0.887	0.326	1					
P-value	0.03	0.91	0.29	0.33	0.23	0.75	<0.01	0.33						
Lys:cp	-0.153	0.585	-0.065	0.591	-0.009	0.016	-0.092	0.494	-0.118	1				
P-value	0.65	0.058	0.85	0.055	0.98	0.96	0.79	0.12	0.73					
PS	-0.097	-0.162	-0.653	0.114	0.218	0.231	-0.175	0.048	-0.338	0.071	1			
P-value	0.78	0.63	0.03	0.74	0.52	0.49	0.61	0.89	0.31	0.84				
L*	-0.164	-0.064	0.528	-0.195	-0.147	-0.107	-0.191	-0.338	-0.170	0.096	-0.57	1		
P-value	0.63	0.85	0.10	0.57	0.67	0.75	0.57	0.31	0.62	0.78	0.07			
a*	-0.273	0.09	0.426	0.195	0.076	0.151	0.258	0.300	0.225	0.167	-0.78	0.269	1	
P-value	0.42	0.79	0.19	0.56	0.83	0.66	0.44	0.37	0.51	0.62	0.05	0.42		
b*	-0.157	0.273	0.718	-0.093	-0.368	-0.316	-0.227	-0.164	-0.096	0.22	-0.798	0.869	0.577	1
P-value	0.64	0.42	0.01	0.79	0.27	0.34	0.5	0.63	0.78	0.52	0.01	<0.01	0.06	

CP = (N x 6.25); TDF = total dietary fiber; NDF = neutral detergent fiber; ADF = acid detergent fiber; Hcell = hemicellulose; PS = particle size; ME = metabolizable energy; ME_n = nitrogen corrected metabolizable energy

REFERENCES

Adeola, O., and H. Zhai. 2012. Metabolizable energy value of dried corn distillers grains and corn distillers grains with solubles for 6-week-old broiler chickens. Poult. Sci. 91:712–718.

Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles.J. Appl. Poult. Res. 15:89-93.

Belyea, R. L., K. D. Rausch, T. E. Clevenger, V. Singh, D. B. Johnston, and M. E. Tumbleson. 2010. Sources of variation in composition of DDGS. Anim. Feed Sci. Technol. 159:122–130.

Carré, B. 2000. Effets de la taille des particules alimentaires sur les processus digestifs chez les oiseaux d'élevage. Prod. Anim. 2 (12), 131-136.

Cozannet, P., Y. Primot, C. Gady, J. P. Métayer, M. Lessire, F. Skiba, and J. Noblet. 2010. Energy value of wheat distillers grains with solubles for growing pigs and adult sows. J. Anim. Sci. 88:2382–2392.

Donadelli, R. A., D. A. Stone, C. G. Aldrich, and R. S. Beyer. 2019. Effect of fiber source and particle size on chick performance and nutrient utilization. Poult. Sci. 98:5820–5830.

Fastinger, N. D., J. D. Latshaw, and D. C. Mahan. 2006. Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. Poult. Sci. 85:1212–1216.

Fastinger, N. D., and D. C. Mahan. 2006. Determination of the ileal amino acid and energy digestibilities of corn distillers dried grains with solubles using grower-finisher pigs1,2,3. J. Anim. Sci. 84:1722–1728.

Fontaine, J., U. Zimmer, P. J. Moughan, and S. M. Rutherfurd. 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers dried grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. J. Agric. Food Chem. 55:10737–10743.

Ghazalah, A. A., M. O. Abd-Elsamee, and E. S. Moustafa. 2011. Use of distillers dried grains with solubles (DDGS) as replacement for soybean meal in laying hen diets. Int. J. Poult. Sci. 10:505–513.

Gonzalez-Esquerra, R., and S. Leeson. 2000. Studies on the metabolizable energy content of ground full-fat flaxseed fed in mash, pellet, and crumbled diets assayed with birds of different ages. Poult. Sci. 79:1603–1607.

Hill, F. W., and D. L. Anderson. 1958. Comparison of Metabolizable Energy and Productive Energy Determinations with Growing Chicks. J. Nutr. 64:587–603.

Jie, Y. Z., J. Y. Zhang, L. H. Zhao, Q. G. Ma, and C. Ji. 2013b. The correlationship between the metabolizable energy content, chemical composition and color score in different sources of corn DDGS. J. Anim. Sci. Biotechnol. 4:38.

Kingsly, A. R. P., K. E. Ileleji, C. L. Clementson, A. Garcia, D. E. Maier, R. L. Stroshine, and S. Radcliff. 2010. The effect of process variables during drying on the physical and chemical

characteristics of corn dried distillers grains with solubles (DDGS) - Plant scale experiments. Bioresour. Technol. 101:193–199.

Liu, K. 2008. Particle size distribution of distillers dried grains with solubles (DDGS) and relationships to compositional and color properties. Bioresour. Technol. 99:8421–8428.

Liu, K. 2011. Chemical Composition of Distillers Grains, a Review. J. Agric. Food Chem 59:1508–1526.

Mateos, G. G., and J. L. Sell. 1980. True and apparent metabolizable energy value of fat for laying hens: influence of level of use. Poult. Sci.59:369-373.

Meloche, K. J., B. J. Kerr, N. Billor, G. C. Shurson, and W. A. Dozier. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poult. Sci.93:1428-1439.

Meloche, K. J., B. J. Kerr, G. C. Shurson, and W. A. Dozier III. 2013. Apparent metabolizable energy and prediction equations for reduced-oil corn distillers dried grains with solubles in broiler chicks from 10 to 18 days of age. Poult Sci.92:3176-3183.

Almeida, F. N., J. Kyaw Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. J. Anim. Sci. Biotechnol. 4:44.

Noll, S., J. Brannon, and C. Parsons. 2007. Nutritional value of corn distiller dried grains with solubles (DDGs): Influence of solubles addition. Poult Sci. 86:68.

Rochell, S. J., B. J. Kerr, and W. A. Dozier. 2011. Energy determination of corn co-products fed to broiler chicks from 15 to 24 days of age, and use of composition analysis to predict nitrogen-corrected apparent metabolizable energy. Poult. Sci.90: 1999-2007.

Singh, V., R. A. Moreau, K. B. Hicks, R. L. Belyea, C. H. Staff, R. A. Moreau, K. B. Hicks, and R. L. Belyea. 2001. Removal of fiber from distillers dried grains with solubles (DDGS) to increase value. Trans. ASAE 45:389–392.

Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J. Anim. Sci. 80:2639–2645.

Stein, H. H., M. L. Gibson, C. Pedersen, and M. G. Boersma. 2006. Amino acid and energy digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. J. Anim. Sci. 84:853–860.

Xu, Y., C. R. Stark, P. R. Fernet, C.M. Williams, W.J. Pacheco, and J. Brake. 2015. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poult. Sci. 94: 53-60.

CONCLUSION

The variability in energy content is one of the main concerning factors for nutritionists when formulating diets containing DDGS. Accurate energy values are necessary to optimally utilize DDGS to reduce feed costs and improve bird growth performance. The overall results from these experiments showed that the inclusion level of the test ingredient used in the experimental diets for *in vivo* assays will influence the resulting ME_n values. Increases in the inclusion level of DDGS decreased its ME when determined by the difference method. Further, decreased feed intake was observed in response to increasing DDGS up to 60%, possibly due to the high fiber content in diets containing high inclusions of DDGS. In fact, a reduction in feed intake through pair feeding decreased the ME_n of DDGS which supports the fact that at low FI there may be a reduction in the ME estimate due to a relatively higher excretion of energy through endogenous losses. Using these same data, 2 regression approaches based on multiple DDGS inclusion levels were used, and in both cases, the resulting ME values closely agreed. It was determined that diets containing 15 and 30% DDGS inclusion level did not compromise the bird's growth performance and may allow for an accurate determination of ME when using the regression method.

When analyzing 11 DDGS sources in experiment 2, relatively low variability was observed in nutrient composition as well as in the final ME_n values determined by regression. The importance of fiber content of DDGS as a predictor for ME determination was reflected in this experiment where hemicellulose was the only chemical component correlated with the ME_n. The low variability found among the DDGS sources could be explained by the similarities in processing techniques in every plant from which the sources were obtained. While no predictive

equations were developed, these results reflect the potential for relative consistency and uniformity in DDGS sources when obtained from a single ethanol producer.

Future research should be conducted to evaluate the additivity of the ME values when fed as part of complete diets. In addition, when conducting ME assays close evaluation should be considered when using the single ingredient or basal replacement approach when formulating diets for in vivo ME assays since they both influence the resulting ME. Also, in experiment 2, fiber showed to be significantly correlated for DDGS ME determination, therefore should be taken into consideration when trying to develop prediction equations.

APPENDIX

To:	Samuel Rochell
Fr:	Craig Coon
Date:	June 3rd, 2019
Subject:	IACUC Approval
Expiration Date:	October 6th, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your personnel addition(s) of Kenia Mitre, Derrell Trevor Lee, Jean-Remi Teyssier, and Ethan Collins to protocol # **17029:** *Optimization of bioassays to determine the metabolizable energy content of feed ingredients for broiler chickens.*

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond October 6th, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp