

NUTRITIONAL EVALUATION OF NOVEL ETHANOL CO-PRODUCTS FOR POULTRY

BY

SHELBY PAIGE CORRAY

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2017

Urbana, Illinois

Adviser:

Professor Carl M. Parsons

ABSTRACT

The objective of the first study was to evaluate the nutritional value of Glutenol™, a new coproduct of the ethanol industry. Glutenol™ was produced by Quality Technology International in a modified wet-milling plant using a hybrid process, NextGenFrac™, which fractionates the corn kernel components prior to fermentation without the use of sulfur dioxide. Glutenol™ was analyzed to contain 52.3% CP, 1.7% Met + Cys, 1.32% Lys, 1.69% Thr, and 2.23% Val on a DM basis. Two precision-fed rooster assays with conventional and cecectomized roosters were conducted to determine TME_n and amino acid digestibility, respectively. The TME_n of Glutenol™ was determined to be 3256 kcal/kg DM. Standardized digestibility values for Lys, Met, Cys, Thr and Val were 80.1%, 90.4%, 74.1%, 81.1% and 84.9% respectively. In addition, a three-week broiler chick assay was conducted with increasing levels of dietary Glutenol™. Diet 1 was a standard corn/soybean meal diet with 0% Glutenol™. Diets 2, 3 and 4 had increasing levels of Glutenol™ at 4%, 8% and 12% respectively. As the level of Glutenol™ increased, the level of SBM in the diet decreased from 35.77% in Diet 1 to 29.42% in Diet 4 to keep the protein levels equal among treatments. The diets were fed to Ross 308 male broilers housed in battery cages from 3 to 22 days post hatch. All diets were formulated to be equal in ME and digestible amino acids. Weight gain, feed intake and gain/feed ratio were measured. No significant differences in growth performance were observed among dietary treatments, leading to the conclusion that Glutenol™ can be fed up to at least 12% in the diet of broiler chickens if diets are formulated to be equal in ME and digestible amino acids. In the second study, a series of 48-hour precision-fed rooster assays were conducted to determine TME_n using conventional roosters and/or amino acid digestibility using cecectomized roosters for distiller's dried grains with solubles (DDGS) produced from human food waste at high solids content (FWDDGS), DDGS

produced from two mutant corn hybrids containing either increased protein or increased levels of select dietary indispensable amino acids such as lysine, arginine, and tryptophan, and an increased-protein ethanol co-product that is produced by a process which separates a high concentration protein and yeast fraction from ethanol stillage (Still Pro™, Fluid Quip Process Technologies, Cedar Rapids, IA). The original raw materials for the FWDDGS consisted mainly of, mashed potatoes, sweet corn, and white bread. The results of the rooster assays indicated that the digestibility of the amino acids in the FWDDGS was similar to typical DDGS (e.g., 61% for lysine), whereas the TME_n for the FWDDGS was higher than for typical DDGS. The DDGS produced from the high protein mutant corn hybrid was shown to have a higher protein content of 34% compared with DDGS from its control corn hybrid, which contained 28% protein. Using Lys as an example, there was a large difference in Lys content between the two samples; the high protein mutant DDGS contained 1.60% Lys versus 1.05% for the control DDGS. The digestibility of amino acids was generally similar for the mutant DDGS and the control DDGS. Similar results were observed for Lys, Arg, and Trp for the DDGS produced from the second mutant corn hybrid. The Still Pro™ sample was analyzed to contain 53% protein on a dry matter basis with 2.22 % Lys, 1.05% Met, 0.90% Cys, and 2.06% Thr. The digestibility values for these amino acids were 84%, 92%, 87%, and 86% respectively. The TME_n of the Still Pro™ sample was determined to be 3372 kcal/kg on a dry matter basis. The results of the two studies indicate that the nutritional value of the four novel ethanol coproducts is generally superior to the nutritional value of conventional DDGS.

DEDICATION

This thesis is dedicated to my husband, Jace. Thank you for always supporting and encouraging me. It is also dedicated to my parents, David and Ginger Reed for believing in me and helping me achieve my goals.

“Anything worth doing is worth overdoing. Moderation is for cowards.”

- Shane Patton, USN

ACKNOWLEDGEMENTS

Without the patience, dedication, hard work, and guidance of my adviser, Dr. Carl Parsons, I could not have completed my research or this thesis. I am extremely appreciative of all the things he has done for me and the opportunities he has given me. I am honored to have studied Poultry Nutrition under one of the best nutritionists and professors in the industry. Thank you Dr. Parsons.

To Aly Gautier, Christina Hanna, and Angela Price, three women who I could not have made it through this journey without. You were the light on the days that seemed to be the roughest and my encouragement when I needed that extra push. You were my supporters, cheerleaders, and encouragers and I will forever be grateful for that. The memories, laughs, and advice will forever be cherished in my heart. Thank you for all you are and all that you continue to do for me. Many thanks to Dr. Sam Rochell for your encouragement, advice and for being that “calm factor” when we all needed it. Thank you to my fellow graduate students Patrick Von Schaumburg and John Munoz for your help and friendship.

To the Poultry Research Farm staff, thank you to everyone who helped shape me and my career and for the immense amount of patience. I learned the most through the difficulties I faced and I am truly grateful for the ups and the downs. It was hard work and I am glad I took the step, worked extra hard, pushed myself beyond what I thought were my limits, and never gave up. A special thank you to Brandon Zech for your helpfulness, hard work, and understanding while I was jumping back and forth between the farm and the office. I could not have done it without you. Thank you to Katie Grott and Annalise Anderson for your patience, laughter and friendship.

To my parents, for making every opportunity possible available to me, and for showing me what hard work and determination is all about. Thank you for all of the life advice you have given me and for your endless support, Mom and Dad. I also want to thank my Grandma, a very special woman in my life whose support and love I could not go without.

To my husband, Jace, my best friend, biggest fan, and number one supporter: you mean the world to me. Thank you for listening to my rants and always being my calm in a storm. For making me laugh every single day and reminding me what really matters. For saying “do the very best you can and everything will be okay”. I love you more than you will ever know.

TABLE OF CONTENTS

CHAPTER 1 LITERATURE REVIEW.....	1
INTRODUCTION.....	1
ETHANOL PRODUCTION.....	2
CONVENTIONAL PRODUCTION OF DDGS.....	3
THE USE OF ETHANOL CO-PRODUCTS IN POULTRY NUTRITION.....	4
NOVEL ETHANOL CO-PRODUCTS.....	11
THE USE OF DDGS IN BROILER NUTRITION.....	18
CONCLUSION AND OBJECTIVES.....	21
LITERATURE CITED.....	23
CHAPTER 2 NUTRITIONAL EVALUATION OF GLUTENOL: A CO-PRODUCT OF ETHANOL PRODUCTION.....	31
ABSTRACT.....	31
INTRODUCTION.....	32
MATERIALS AND METHODS.....	34
RESULTS AND DISCUSSION.....	36
LITERATURE CITED.....	40
TABLES.....	43
CHAPTER 3 NUTRITIONAL EVALUATION OF THREE TYPES OF NOVEL ETHANOL CO-PRODUCTS.....	48
ABSTRACT.....	48
INTRODUCTION.....	49
MATERIALS AND METHODS.....	50

RESULTS AND DISCUSSION.....	53
LITERATURE CITED.....	58
TABLES.....	60

CHAPTER 1
LITERATURE REVIEW
INTRODUCTION

Corn distillers dried grains with solubles (DDGS) is a co-product produced from the fermentation of corn for ethanol production. This ethanol co-product is added to many animal diets. In 2015, the poultry industry alone used approximately 8% of the DDGS produced (Renewable Fuels Association, 2015). The DDGS can be used as an alternative feed ingredient to replace some of the corn and soybean meal in a diet. This aids in bringing diet cost down as well as helping the ethanol industry by providing an outlet for the co-products of ethanol production. The increased demand for ethanol has led to an increase in co-products produced, mostly DDGS. Determining the suitability of ethanol co-products for use in the poultry industry is important. The nutrient profile and physical characteristics of DDGS can be variable. Animal scientists are interested in studying the composition of DDGS to use it in livestock diets, since it is a good protein source and can replace some of the more expensive feed ingredients such as soybean meal. At the end of 2010, over 13.1 million gallons of ethanol was produced and there were a total of 204 fuel ethanol manufacturing plants operating in the United States (Renewable Fuels Association, 2011). By the end of 2015, over 14.8 million gallons of ethanol were produced in the United States. Ethanol production continues to increase while demand only increases slightly (Gavilon, 2015). One-third of every bushel of grain that enters the ethanol process is returned to the livestock feed market, usually in the form of distillers dried grains with or without solubles. These co-products are fed to beef cattle, dairy cattle, swine, poultry, and fish all around the world. In the 2013/2014 production year, over 40 million metric tons (mmt) of DDGS for animal feed were produced from ethanol production

facilities, making the renewable fuels sector one of the largest feed processing segments in the U.S (Renewable Fuels Association). These DDGS production volumes are expected to remain constant for the next couple of years.

ETHANOL PRODUCTION

History

Ethanol was first used in 1826 to power an engine and again in 1876 to power a combustion engine. It was also used as lighting fuel in the 1850's until it was taxed as liquor to help pay for the civil war (Bevill, 2008). In 1908, after the tax was repealed, ethanol was used to fuel Henry Ford's Model T car. In the 1920's and 30's, gasoline blended with ethanol was used because of the high fuel demand during World War II (Bevill, 2008). The ethanol industry that we know today began in the early 1970's during the oil crisis. Growing interest in alternative fuels is occurring due to two main reasons, according to Berndes et al. (2001) and Farrell et al. (2006) The latter authors stated that the first is due to oil supply insecurity and its impending peak. The second reason is the vital need to lower carbon dioxide (CO₂) emissions from fossil fuel use in order to ease global climatic change. Due to the abundance and the ease of transforming it into alcohol, corn became the leading feedstock for ethanol production. Government subsidies for ethanol helped keep the fuel in production as prices fell and rose in the 1980's and 1990's. In 2005, with two goals in mind, decreasing oil dependency and using environmentally friendly fuels, the Renewable Fuels Standard became a law as part of the United States' energy policy. In 2007, the Energy Independence and Security Act was signed and required renewable fuel usage to increase to 26 billion gallons annually by 2022. In 2015, 14.81 billion gallons of ethanol was produced (Renewable Fuels Association 2015). This shows that the ethanol industry has continued to grow and will keep growing to reach the 2022

goal. Ethanol production has now become important not only for fuel but also for its co-products produced. Feeding agricultural animals is very important and if producers can reduce feed costs by adding ethanol co-products to their diet formulations, it will be a more efficient grow-out process.

CONVENTIONAL PRODUCTION OF DDGS

Dry Grind Process

Over 95 percent of ethanol production in the US comes from corn, the rest is made from wheat, barley, milo, cheese whey, and beverage residues. One bushel of corn (56 lbs.) produces approximately 16.5 lbs. of DDGS and 2.8 gallons of ethanol (Renewable Fuels Association 2015). According to 2015 data from Renewable Fuels Association, an estimated 40 million metric tons of DDGS was produced by ethanol plants in 2015, the majority being DDGS. The most common process used to produce these products is a dry-grind process. According to the U.S. Department of Agriculture, approximately 90% of the DDGS produced in the ethanol industry comes from a dry grind plant. The dry fractionation process begins with grinding and aspirating to separate the pericarp from the germ and the endosperm. The endosperm is then crushed into starch granules and flattened using a roller mill. Screening separates the fines (starch) from the germ flakes which contain oil. Next the contents are mixed with water and heated. Alpha amylases are added to aid in the breakdown of starch to maltooligosaccharides. The product is then cooled in saccharification tanks where glucoamylase is added. The temperature is lowered again in the tanks to 35°C, additional glucoamylase is added, and the product is fermented for 48-64 hours. After fermentation the product is distilled to 95% ethanol vapor, the ethanol product is produced and the rest of the liquid and solids are centrifuged and dried to produce DDGS (Abbas et al., 2009).

Wet Milling Process

In conventional wet-milling, corn is processed for the purification of starch and several co-products, including germ, gluten, fiber and steep liquor. Currently, the end products of wet milling are germ, corn gluten meal, starch slurry and corn gluten feed (Ramirez et al., 2008). Corn that is processed using the conventional wet milling technique is first cleaned and then put into steep tanks containing water and SO₂ for 24-36 hours at 52°C. The product is then ground to remove approximately 85% of the germ. The rest of the contents are put through a second grind where the remaining 15% of the germ is extracted. From the second grind the product goes through a third grind to extract the fiber. The last of the contents are sent to a centrifuge and the products obtained after this process are protein (Corn gluten meal) and starches. These starches will be used for food such as nutritive sweeteners or for fuel production (Renewable Fuels Association). Davis (2001) reported the average yield per bushel breakdown of corn when it is wet-milled; 31 lbs. starch, 12.5 lbs. gluten feed, 2.5 lbs. gluten meal and 1.6 lbs. corn oil. New techniques are being developed using a wet-fractionation procedure to produce DDGS as a co-product; these will be discussed later.

THE USE OF ETHANOL CO-PRODUCTS IN POULTRY NUTRITION

Introduction

DDGS has primarily been fed to ruminant animals due to its nutrient variability and high fiber content (Cromwell et al., 1993). It was a less common practice to feed DDGS to non-ruminants like poultry and swine. Yet, it has been shown by many researchers that feeding DDGS to poultry, whether it be laying hens or broilers, has shown positive results. The results of the addition of DDGS to poultry diets will be discussed in the upcoming paragraphs. In the past, the lack of feeding DDGS to non-ruminants may have been due to the competition with ruminant

producers for the relatively limited supply. However, the supply of DDGS has changed considerably compared to 40-50 years ago. New policies have encouraged the ethanol industry to increase production and therefore a greater quantity of DDGS is available to feed to ruminants and non-ruminants. According to the Renewable Fuels Association, in 1999 only 2.3 million metric tons of distillers grains were produced from ethanol biorefineries compared to the 40 million metric tons that were produced in 2015.

General Composition of Corn Compared to DDGS

The major feedstock used in the ethanol industry is corn. The composition of the corn kernel is important to know as it directly relates to the nutritional qualities of corn and ethanol co-products, particularly DDGS. The corn kernel consists of a hard endosperm on the outside and a soft floury endosperm towards the middle of the kernel, a germ or embryo, and the bran or pericarp which coats the kernel (Hopkins et al., 1974). A typical kernel of corn is approximately 75% starch, 9% protein, 4.0% oil, 1.5% ash, 1.7% carbohydrates/sugars and 8.9% fiber. The endosperm contains 88.4% starch, representing most of the starch in the entire kernel. The germ contains the most protein at 18.4%, and contains oil at 29.6%. The fiber is highest in the bran layer which contains 86.9% fiber. The composition of DDGS is much different than corn due to the processing methods it goes through in the ethanol plant. The protein content of DDGS on a dry matter basis has been reported by Spiehs et al. (2002) to be 30.2%. Belyea et al. (2004) reported a crude protein value of 31.3% and Y. Kim et al. (2008) averaged several samples and found the crude protein content to be 30.7%. More recently Pedersen et al. (2014) reported a value of 31.4% for crude protein of DDGS compared to the reported corn value of 8.3% protein. Because the protein content is more than three times higher than corn, DDGS becomes a good protein source for feed formulators and producers to add to their animal diets compared to corn.

Corn also contains lower values for crude fat and crude fiber with values at 3.9% and 2.5%, compared to DDGS which contains 10.6% crude fat and 8.5% crude fiber (Pedersen et al., 2014). Pedersen et al. (2014) also reported ADF and NDF values for corn and DDGS of 3.8%, 9.3%, and 11.3%, 37.3%, respectively. Total non-starch polysaccharides (NSP) in corn has a value of 7.9% while in DDGS total NSP is 32.5% (Pedersen et al. 2014). Crude fat in DDGS has been shown to range from 10.9% (Spiehs et al. 2002) to 13.0% (Johnston & Moreau 2017). Pedersen et al. (2014) reported 11.1% crude fat and Belyea et al. (2004) reported 11.9%. Overall, DDGS compositional values are approximately 3 times higher than values reported for corn.

Nutritional Composition and Value of DDGS

The co-products produced from ethanol production are important economic aspects of the ethanol and grain crop industries. Determining the nutritional value of DDGS is crucial so that agricultural animal producers can accurately use DDGS and other co-products of ethanol production in their livestock diets. The nutritional characteristics of DDGS depend on the composition of the feedstock (corn) used and on the processing conditions (Belyea et al., 2004). Not only are producers and diet formulators concerned with the nutritional value of feed ingredients in their diets but also with diet cost. Protein is the most expensive nutrient in animal diets. Commercial broiler diets generally contain between 18-22% crude protein, depending on the phase of growth. In the United States, protein in poultry diets is mostly coming from soybean meal (SBM) which is typically at a 25-35% inclusion rate in broiler diets. The DDGS can be a good protein source for broilers, but determining the optimal inclusion rate is needed. In order to do this, we need to gather information on the composition before DDGS can be included in the formulations at high levels. If DDGS is included in the diet, it could replace some of the soybean meal, which is more expensive. Feed costs are critical to producers; thus, exploring ways to be

more efficient and save on feed costs without decreasing growth performance of the broilers is important. Soybean meal prices fluctuate daily, this past year prices ranged from \$290 per ton to \$450 per ton (U.S. Grains Council 2016-2017). If DDGS is less expensive per ton than SBM, the producer could decrease SBM used in the diet and increase DDGS to reduce feed costs. The markets are always changing; price per ton of SBM and DDGS can vary and is not always consistent. The DDGS prices have historically been lower than SBM. In 2016 DDGS prices varied from \$140 per ton to \$180 per ton (DTN Progressive Farmer), which is considerably lower than SBM prices. If these market prices continue and SBM continues to cost more than DDGS, it will likely encourage producers to include this feed ingredient in their formulations. Of course, knowing the accurate nutritional composition of DDGS is very important. Therefore, evaluations of DDGS have been conducted for many years with somewhat varying results. As ethanol production increases and new plants are built, the process by which ethanol is made may change, which in turn could change the composition of the co-products produced. The protein fraction in DDGS derives from the endosperm, germ and bran. Around 75% of the protein in corn is in the endosperm tissue (Shukla and Cheryan, 2001). The rest of the protein is coming from the bran (11-12%) and germ (Leathers, 2003). Once the corn is taken to an ethanol processing plant and DDGS is produced, the protein content triples compared with corn. In the 1994 NRC reported the protein content of DDGS to be 27.2%. Ten years later, Belyea et al. (2004) reported the protein content of conventional DDGS to range from 28% to 34%. Batal et al. (2006) collected 17 DDGS samples from 6 different ethanol plants and found CP content to range from 23%-30% with an average of 27%, which is similar to the reported value in the NRC (1994).

Overall protein content is important, and so is amino acid content. It is very important to know the amino acid composition of a feed ingredient when formulating diets, especially because DDGS is heated and this tends to destroy some of the amino acids, which in turn will affect the nutritional value of the product. It is important to formulate diets based on available or digestible amino acids rather than on total amino acids (Parsons, 1996). Therefore, determining the digestible amino acid content of DDGS is imperative. The slope-ratio assay (bioavailability), balance assays (digestibility) and the standardized ileal amino acid digestibility assay (SIAAD) are the most common methods used to determine digestibility of amino acids in feed ingredients. A digestibility assay that is commonly used for poultry is the precision-fed cecectomized rooster assay (PFR; Parsons, 2002). In the past, studies have been evaluated to compare SIAAD and PFR with sometimes varying amino acid digestibility values for some feed ingredients (Garcia et al., 2007; Adedokun et al., 2009). During the precision fed rooster assay, roosters are tube fed 24-30 grams of a feed ingredient and the excreta are collected 48 hours after tube feeding and analyzed for amino acids. Endogenous corrections for amino acids are made using roosters that have been fasted for 48 hours. Kim et al. (2011) compared the amino acid digestibility of 6 DDGS samples by the PFR assay and the SIAAD assay. They found the value for methionine to be significantly different between the two assays. The PFR digestibility value for methionine was 88.9% while the SIAAD value was 96.9%. However, depending on the samples, most of the other standardized amino acid digestibility values were not significantly different between the two assays, such as, arginine, cystine, valine, etc. Lysine digestibility of the different DDGS samples was significantly different between the assays for 4 out of the 6 samples. For example, one sample had a value of 65.3 % digestibility for the PFR assay and a value of 75.1 for the SIAAD assay (Kim et al., 2011). Even though there were some individual differences found,

there were not consistent differences between the PFR assay and SIAAD assay. In 2012 Kim et al. compared the amino acid digestibility coefficients of DDGS samples among three bioassays, PFR, SIAAD and also the precision-fed chick assay (PFC). In the PFC, 21 day old broiler chicks are precision-fed 8 to 10 grams of a feed ingredient and placed on a battery with water access only for 4 hours. Once 4 hours has passed, the chicks are euthanized and ileal digesta is collected and pooled per replicate group. In this study, the PFR assay consistently yielded higher amino acid digestibility values for the DDGS samples compared to the PFC assay values. The SIAAD assay values were similar to the PFR values (Kim et al., 2012). Because values may differ among methodologies, nutritionists should be cautious about the digestibility coefficients they are using and be aware of how they were obtained (Adedokun et al., 2009).

The DDGS is deficient in lysine, tryptophan, and arginine for poultry. The latter is similar to corn, except DDGS is more concentrated and the amino acid levels are magnified about 3 times since the starch is removed during processing (Spiehs et al., 2002). Although relatively high concentrations of amino acids are required to support the rapid growth of broilers, a nutritionist's goal is to provide sufficient amounts of digestible amino acids, especially the limiting amino acids, without feeding excess protein that will increase nitrogen excretion. Lysine is usually the second most limiting amino acid in broiler chicken diets (NRC, 1994). The requirement for lysine was reported to be 1.10% of the diet during the first 3 weeks of growth and 1.00% from week 3 to week 6 (NRC, 1994). Baker and Han (1994) reported the average lysine requirement (based on the Illinois Ideal Chick Protein, 1999) to be 1.22% of the diet, which is slightly higher than the NRC (1994) suggests. Conventional DDGS contains 0.75% lysine and the percentage of total amino acid contained in the feedstuff that does not show up in the excreta (digestibility coefficient) for lysine is 65% (NRC, 1994).

There are several other amino acids that are vital to chick growth. These include, threonine, valine, arginine and the sulfur amino acids, methionine and cystine among others. Threonine is usually the third limiting dietary amino acid in broiler chickens; its requirement is often expressed as a ratio to dietary lysine and it is an amino acid that is required for optimal breast meat yield (Kidd, 2000; Kidd et al., 1997). Baker and Han (1994) reported the average threonine requirement for broiler chicks from 0 to 3 weeks of age to be 0.82% of the diet. Conventional DDGS contains 0.92% threonine with a digestibility coefficient of 72% (NRC, 1994). Methionine is historically the first-limiting amino acid in broiler chickens. The DDGS contains 0.60% methionine and 1.04 % methionine plus cysteine (NRC, 1994).

The removal of fiber is important for creating a product that is more beneficial to feed to poultry and other nonruminants. Distillers dried grains with solubles has primarily been fed to ruminant animals due to a high fiber content, but now there are new methods for producing lower fiber and higher protein DDGS products (Rausch and Belyea, 2006; Singh et al., 2005). When fiber is removed from DDGS, a higher protein and a higher fat product is produced and, this will make it a more viable feed ingredient for poultry (Singh et al., 2005; Srinivasan et al., 2005). Fiber is not only poorly digested by poultry, but adding it to the diet can also decrease digestibility of other nutrients in the diet (Janssen et al., 1985). The reduction of fiber content usually increases the protein concentration in the product and therefore produces a more digestible and valuable coproduct to be used in poultry diets.

The metabolizable energy (ME) content of DDGS for poultry is also very important. A significant amount of the ME in DDGS comes from its fat content. Parsons et al. (2006) summarized data collected in his lab from 90 conventional DDGS samples from 2004 to 2006 and found the average fat content of DDGS to be 14%. The fat in DDGS originates largely from

the germ fraction of the corn kernel but also some fat is coming from the bran and endosperm fractions (Rausch and Belyea 2005). Fat is an important component as it relates to available energy concentrations (Belyea et al., 2004). Fat content in DDGS is increased compared with the corn that is processed into DDGS. Corn has an average fat concentration of 4.21% while DDGS was found to have an average concentration of 12% and 10.9% crude fat (Belyea et al., 2004; Spiehs et al., 2002). True metabolizable energy (TME_n) in DDGS is directly related to the amount of crude fat in DDGS. Following protein, energy content is the next most important factor of DDGS. Broiler diets typically contain 3,050 kcal ME/kg to 3,200 kcal ME/kg. The mean TME_n of conventional DDGS was found to be 2,863 kcal/kg on an as-fed basis (Parsons et al., 2006; Dale and Batal, 2003). The TME_n of corn is typically 3,350 kcal/kg on an as-fed basis (NRC, 1994). The ME value for DDGS is lower than that of corn due to the higher fiber content of DDGS when compared with corn. Another important topic in poultry nutrition is mineral availability especially phosphorus availability. Phosphorus availability is higher in DDGS than in corn due to the yeast phytase during the fermentation phase of ethanol production. According to the NRC (1994), the concentration of total phosphorus (P) in corn is 0.28% and there is approximately 0.72% of total P in DDGS. However, the bioavailability of the P in DDGS is approximately 60-70% compared with only 20-30% in corn.

NOVEL ETHANOL CO-PRODUCTS

Modified Ethanol Production Introduction

There is great interest in modifying traditional processes by which ethanol is produced in order to make higher quality end products that can be better utilized by nonruminants. The majority of ethanol is processed by a dry grind method. As described before, the dry fractionation process begins with the corn being ground, then it is put into a slurry, cooked,

liquefied, fermented/saccharified (releasing carbon dioxide), and the product is distilled to create ethanol. The remaining water and non-fermentable solids are mixed and dried to produce DDGS (Davis 2001). The wet milling process includes grain cleaning, steeping, germ separation and recovery, fiber separation and recovery, gluten separation and recovery and starch separation (Ramirez et al., 2008). Ethanol process units are often managed as separate front-end and back – end processes, a practice derived from the industry’s early association with the distilled spirits industry (Monceaux and Keuhner, 2009). A discussion of front and back end processing will be presented in the following sections along with a discussion of the new methods that are being developed in an effort to increase the value of DDGS in livestock diets.

Front end Processing

The dry fractionation process is referred to as front end processing where milling, mashing, cooking, liquefaction, saccharification and fermentation occur. The products of the previously described dry grind method have been tested for their nutritional value. Singh et al. (2005), collected corn samples, processed them with the conventional dry grind method and analyzed the samples for crude protein, crude fat, ash and acid detergent fiber (ADF) on a dry matter basis (DMB). Crude protein of the DDGS produced by the conventional dry grind process was 28.5%, crude fat was 12.7%, ash was 3.61% and ADF was 10.8%. Kingsly et al., (2010) studied the effect of processing variables during drying on the physical and chemical characteristics of DDGS. During drying, wet distillers grains (WDG) and condensed distillers solubles (CDS) are blended. During the dry milling process, product variability of DDGS was mostly due to the levels of CDS added during drying (Kingsly et al., 2010). Kingsly et al. (2010) showed that crude protein, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were increased by reducing CDS levels while fat, ash, sugars and glycerol content decreased with

reducing levels of CDS. Crude protein of DDGS was found to range from 26.69% to 32.33% in the 4 samples of DDGS that were analyzed. Fat ranged from 7.76% to 10.80%, ash ranged from 2.07% to 4.00%, ADF ranged from 15.98% to 10.06% while NDF ranged from 33.18% to 44.49% (Kingsly et al. 2010). A process that is less widely used is wet milling or wet fractionation. It is another front end process in which DDGS is an end product. Since the germ, pericarp and endosperm fiber are high in fiber, a type of wet fractionation process called enzymatic milling (E-mill) removes the pericarp fiber and the germ components before fermentation to yield a product lower in fiber (Singh et al., 2005) and the endosperm is removed after fermentation (Li et al., 2014). The E-mill is a modified procedure that uses proteases and starch-degrading enzymes to separate nonfermentable fiber before fermentation (Kim et al., 2010). In the latter study, the authors analyzed E-mill samples and found the fiber concentration was greatly reduced to 6.79% NDF when compared with conventional DDG that was analyzed to have an average of 33% NDF (Srinivasan et al., 2005). The reduction in fiber is due to the removal of the high fiber fractions of the corn kernels before fermentation. By removing the fiber fractions, the protein content is concentrated, increasing it from 27-33% in conventional DDGS to 56.2% protein in E-mill DDG (Kim et al., 2010). The high protein value led to increased total amino acid concentrations in the E-mill DDG and also increased digestibility values for amino acids compared with conventional DDGS. For example, Kim et al. (2010) reported a lysine digestibility coefficient of 85.8% for E-mill DDG which was significantly different than the value for conventional DDGS (76.2%). There were also significant differences found in Met and Cys digestibility between E-mill DDG and conventional DDGS with the E-mill values being reported at 93.7% for Met and 87.8% for Cys and conventional DDGs values reported at 84.3% for Met and 75.1% for Cys (Kim et al 2010). Another study comparing E-mill to conventional DDGS

reported the protein concentration of E-mill DDG to be 51.9% and the NDF to be 1.8% compared with conventional DDGS samples, which were 34.3% protein and 17.1% NDF (Li et al. 2014). The researchers also reported the oil content of the E-mill DDGS was 0.7% which is significantly lower than the oil content of conventional DDGS (12.7%) (Li et al. 2014). Oil content directly relates to TME_n content of DDGS; therefore, E-mill DDG may have a lower TME_n than conventional DDGS and this could be a potential concern when feeding to non-ruminants. More will be discussed on the subject of oil content and energy values in the later sections.

Another method of front end processing is the dry degerm defiber method (3-D method). In the 3-D process, corn is tempered with hot water or steam for 5-10 minutes and ground in a degerminator to remove the germ and pericarp from the corn endosperm (Murthy et al. 2006). This process leads to a product with decreased fiber and fat concentrations and increased protein concentrations (Martinez-Amezcu et al. 2007). Martinez-Amezcu et al. (2007) evaluated the composition of DDGS produced from the 3-D process and reported the protein content to be 23.8% and the fat content at 8.7%. Martinez-Amezcu et al. (2007) also evaluated conventional dry grind DDGS samples to compare to the 3-D sample values. Compared to the values for conventional dry grind DDGS, the 3-D process yielded increased protein from 21.2% to 23.8% on a DMB and the crude fat content decreased from 13.9% to 8.7%. Total dietary fiber (TDF) was reduced from 36% in conventional DDGS to 28% in the 3-D samples (Martinez-Amezcu et al. 2007).

Two additional front end processes used to increase ethanol and modified DDGS production are the quick germ process (QG) described by Singh and Eckhoff (1996) and the quick germ-quick fiber process (QGQF) described by Singh et al. (1999). During the QG

process, the whole corn kernel is soaked in 60°C water for 12 hours. After soaking, the germ is recovered and the remaining corn fractions are ground and processed for ethanol production. The QGQF process recovers both the pericarp fiber and the germ prior to fermentation by soaking the ground corn in water with alpha-amylase for 12 hours to increase specific gravity which will allow the germ and fiber to float. The benefits of both processes are the recovery of high quality germ for corn oil production and fiber for corn oil production and the removal of germ and fiber to increase the protein and reduce the fiber content of the DDGS product (Singh et al. 1999). Singh et al., (2005) analyzed several DDGS samples from the different processes for protein content, crude fat, ash, and ADF. The protein content of the samples produced from the conventional process, QG process, and QGQF process were 28.5%, 35.91%, and 49.31%, respectively. These values show that the removal of the germ and fiber in the front end of processing increased the protein content of the final product.

The final front end process to discuss is a process called NextGenFrac™, developed by Quality Technology International to produce Glutenol™ as the end product. NextGenFrac™ is a type of modified wet milling using a hybrid process in which the corn kernel components are fractionated prior to fermentation without the use of sulfur dioxide. The result is a product with a crude protein content of approximately 52%.

Back end processing

In a dry grind processing plant, back-end processing consists of centrifugation, evaporation, and drying. Modifications can be made to this process to change the DDGS produced, mainly by the efficient removal of fiber or oil. Singh et al., (2002) obtained DDGS samples from three different ethanol plants and aspirated each sample to remove the pericarp fiber portion from the germ fraction. Aspiration is defined as the act or the result of removing,

carrying along or drawing by suction. The aspirated material was called aspirated DDGS and the remaining sample was called residual DDGS. They found that the removal of the aspirated DDGS from the original DDGS created a residual DDGS with a slightly increased oil and crude protein content. For example, one sample had an increase in oil content from 15.10% to 17.02% and another sample increased from 7.89% to 8.08%. Crude protein only slightly increased with the aspiration technique as the original DDGS was 28.01%, 29.92%, and 28.42% and the residual DDGS contained 28.76%, 31.36%, and 29.25%, respectively. The theory was that aspirating the material would allow the separation of some of the fiber so that it could be removed and the concentration of fat (mostly coming from the oil in the corn kernel) and protein would increase in the resulting DDGS. This study did not show big improvements in the value of DDGS, only small improvements. Srinivasan et al., (2004) used a combination of two separation methods, sieving and elutriation to separate fiber from DDGS. Elutriation is defined as the separation of particles by means of upward flowing stream of air. Sieving is a process used to separate particles based on difference in size. Together they called this process the Elusieve process. When air is passed through, it carries part of the fiber since the fiber has a lower density compared to non-fiber components in DDGS. After processing, the samples were analyzed for nutrient composition. A vibratory screen was used to sieve DDGS samples into size categories. The screens were 24T (869 μm), 34T (582 μm), 35M (447 μm), 60M (234 μm) and pan. The letter “T” referred to tensil bolt cloth and “M” referred to market-grade cloth. After sieving the original DDGS samples, the two smallest size categories 60M fraction and Pan size fraction showed increased levels of protein. Sample 1 increased from 33.6% protein in the original sample to 37.5% in the 60M fraction and 42.2% in the Pan size fraction and Sample 2 increased from 32.9% in the original to 37.5% 60M fraction and 40.1% in the Pan size fraction (Srinivasan

et al. 2005). The NDF decreased in Sample 1 and 2 from the original sample, showing the decrease in fiber concentration. In the original material, NDF was 32.5% for Sample 1 and it decreased to 29.3% and 19.0% for 60M fraction and Pan size fractions in the size categories, respectively. Sample 2 was similar with an NDF value of 33.6% in the original material decreasing to 29.7% and 27.9% in the 60M and Pan size categories (Srinivasan et al. 2005). Elutriation of the screened fractions resulted in increased protein and fat and decreased fiber in the heavier fractions. Srinivasan et al, (2006) suggested the elusieve process (combination of sieving and elutriation) would benefit dry grind corn processors. To confirm this, in 2006 these same authors completed an economic feasibility study to determine if the elusieve process would in fact benefit the corn processors. After performing an economic analysis including equipment costs, capital investment, operating costs, foodstuff prices, revenue and payback period, Srinivasan et al. (2006) concluded adding the Elusieve process to dry grind plants would benefit dry-grind corn processors, as it would increase the value of dry-grind coproducts.

Another avenue for an increased revenue for the corn processors is by the removal of some of the oil from DDGS. The oil is extracted from thin stillage after it is removed from the whole stillage using centrifugation. The now partially concentrated thin stillage is heated and the corn oil is extracted by a second centrifuge. The biofuel industry has been developing products for their use in fuel production, the oil in DDGS is a good product to use in the production of biodiesel. The oil can also be used in the human food industry. Removing oil from DDGS would subsequently increase fiber, protein and mineral concentrations and it may also improve flowability of the DDGS product in processing plants and during transportation (Ganesan et al., 2009). The nutrient composition of low-oil or deoiled DDGS has been studied for a few years. Most recently, Jacela et al. (2014) reported the crude protein of deoiled DDGS to be 35.58%

(DMB) which was greater than the crude protein content previously reported (26%-31%) for conventional DDGS. Since some of the oil is being removed, the fat content is expected to also decrease. Jacela et al. (2014) analyzed the fat content of deoiled DDGS to be 4.56% and Saunders and Rosentrater (2009) reported the fat content of deoiled DDGS to be 2.7%. These differences could be examples of the inefficiency of fat extraction procedures (Jacela et al., 2014). NDF had a value of 39.46% (DMB) in the deoiled DDGS, which is greater than conventional DDGS value of 31.61% (DMB) (Curry et al., 2014). Amino acid concentrations are also affected by the removal of oil. Jacela et al. (2014) and Curry et al. (2014) showed an increase in amino acid concentrations, except Trp, in deoiled DDGS compared with conventional DDGS. Dozier et al. (2015) examined amino acid digestibility of 3 DDGS samples varying in ether extract content fed to broilers from 23 to 31 days of age. The three samples were low oil DDGS (L-DDGS) which had a crude fat content of 6.06% (DMB), moderate oil DDGS (M-DDGS) which had a crude fat content of 8.81% (DMB) and high oil DDGS (H-DDGS) which had a crude fat content of 11.59% (DMB). The broilers fed the L-DDGS and the M-DDGS had lower digestibility coefficients for Ala, Arg, Asp, Lys, Glu, Met, Pro, Ser, Thr, Trp, and Tyr when compared to the broilers fed the H-DDGS. Jacela et al. (2014) concluded deoiled DDGS has increased crude protein and amino acid concentrations but less energy and slightly less Lys digestibility compared to conventional DDGS. Overall, more research is needed to determine the effects of extracting oil from DDGS and consequently feeding the reduced oil products to poultry.

THE USE OF DDGS IN BROILER NUTRITION

Variability in nutritional value

As discussed above, DDGS has nutritional value for poultry based on its inherent nutritional composition. However, one of the factors that has limited its use in broiler diets is that the nutritional composition and digestibility of DDGS is highly variable and depends on the corn and the techniques used during processing. The nutritional value of DDGS is comprised mainly by its metabolizable energy (ME) and digestible amino acid content. Gross energy (GE) is the total amount of energy that is present in the chemical bonds in the feedstuff. Digestible energy (DE) is GE minus what is lost in feces and ME is digestible energy minus what is lost in urine. Since we cannot easily separate urine and feces in poultry, metabolizable energy values are used most often when comparing feedstuffs. Several studies have shown that the GE and ME, of DDGS can vary greatly. Adeola and Zhai, (2012) determined the GE of their DDGS sample to be 4,762 kcal/kg on an as fed basis using bomb calorimetry. The GE of 6 DDGS samples was reported by Anderson et al. (2015) ranging from 5,314 to 5,500 kcal/kg DMB. Anderson et al. (2015) also examined the ME of DDGS by analyzing ileal digesta and excreta from broilers fed a corn-soybean meal reference diet with the addition of DDGS and determined the ME_n of DDGS to be 2,688 kcal/kg. Rochell et al. (2011) reported GE values of 6 DDGS samples ranging from 4,397 kcal/kg to 5,811 kcal/kg of DM. Rochell et al. (2011) chose to evaluate AME_n values instead of TME_n values. The TME_n values for corn and DDGS tend to be 3.5% to 20% higher than AME_n values, respectively (NRC, 1994). The AME_n values reported by Rochell et al. (2011) averaged from 2,146 to 3,098 kcal/kg of DM. These values were lower than previously reported values by Batal and Dale (2006) who reported TME_n values that ranged from 2,490 kcal/kg to 3,190 kcal/kg. The average reported by Batal and Dale (2006) (2,820 kcal/kg) is similar to the TME_n value listed in the NRC (1994) of 2,864 kcal/kg.

In addition to ME, the concentration and digestibility of amino acids can vary greatly among DDGS samples. Overprocessing and excessive heating can be a critical issue with DDGS. Heat damaged DDGS has decreased amino acid availability, mainly Lys due to Maillard reactions with carbohydrates (Spiehs et al., 2002). The color of DDGS often reflects whether or not the product has been overheated. Batal et al. (2006) tested 8 DDGS samples for color using a Minolta Chroma Meter CR-300. They discovered the samples that were lighter in color and more yellow had higher total and digestible amino acid levels. The light samples were found to have 0.79% total lysine and 0.60% digestible lysine compared to the 2 darker samples which had 0.64% and 0.39% total lysine and 0.37% and 0.18% digestible lysine levels, indicating respectively, that a significant amount of lysine was destroyed during processing. Cromwell et al. (1993) conducted an experiment with 9 DDGS samples and found 4 of them to be dark to very dark in color. There was a difference in lysine concentration, the darker samples were as low as 0.43% while the lighter samples were as high as 0.89% lysine. The other amino acids were also variable between the light and the dark DDGS samples but not to the same extent as lysine (Cromwell et al., 1993). Overall, Cromwell et al. (1993) found physical appearance of DDGS to be correlated with nutritional properties. The darker DDGS was lower in nutritional value than the lighter colored samples. For example, 4 out of the 9 sources had a color score of 4 or 5 meaning the appearance was dark to very dark. All 4 samples had decreased Lys concentrations. The DDGS samples with a 1 or 2 color score had Lys concentrations of .82% to .89%, the samples with a 3 for color score had a Lys concentration ranging from .68% to .77% while the samples with a color score of 4 or 5 had Lys concentrations ranging from .43% to .79%. Overall crude protein was also decreased as the color score increased. Almeida et al. (2013) reported amino acid digestibility values for DDGS samples that they autoclaved for 10, 20, or 30 minutes.

Digestible Lys decreased from 0.82% in the non-autoclaved DDGS to 0.65%, 0.73%, and 0.68% when autoclaved at 130°C for 10, 20, 30 minutes, respectively. This showed that heating affected lysine digestibility in a negative way. Although there are variabilities among DDGS, it has been shown that it is a valuable feed ingredient to include in broiler diets. It has been concluded that DDGS should not be included in starter diets at levels greater than 12% and grower diets could contain 12-15% DDGS (Lumpkins et al., 2004). The inclusion rate of 18% DDGS in the diet depressed chick weight gain during the starter (0-16 d) period (Lumpkins et al., 2004). During the grower/finisher phase, Lumpkins et al. (2004) did not detect a difference in weight gain among any of the dietary treatments of DDGS inclusion levels (0, 6, 12, 18%). In one experiment, Shim et al. (2011) found only one performance difference among diets with increased DDGS inclusion levels and this was body weight differences. They noted the birds fed 24% DDGS had a 2g per bird total body weight difference compared with the birds fed 0% DDGS and concluded broilers may perform well when fed high-quality DDGS and the diets are properly balanced on a digestible amino acid basis (Shim et al. 2011). Loar et al. (2012) reported varying levels of DDGS did have an effect on body weight gain and feed intake from 28 to 42 d and overall from 0 to 42 d. Body weight gain during these periods exhibited a linear decrease as DDGS increased in the ration once past the inclusion level of 7% (Loar et al., 2012).

CONCLUSIONS AND OBJECTIVES

DDGS is considered a more valuable ingredient than before (Lumpkins et al., 2004), but there are still improvements to be made. New processing techniques are being developed and evaluated. Researchers must assess the new and improved products from the current processing plants and determine if they are nutritionally valuable and can be included in animal diets, specifically poultry diets. It has been shown in several instances that changing the conventional

ethanol production process can improve the nutritional value of the co-products produced; however, these products still tend to be variable. The range in composition of DDGS can be due to original corn sources, processing methods, degree of starch fermentation, heat processing, proportion of solubles added back to the dried distiller's grains, and drying methods (Belyea et al., 2010; Martinez-Amezcu et al., 2007). By changing techniques in the front end and back end of processing, DDGS can be produced with decreased fiber and increased protein content. Although, DDGS is variable among sources it has been determine to be an acceptable feed ingredient for poultry. The products evaluated in this thesis are Glutenol™, DDGS from food waste, DDGS from two mutant corn hybrids and another product with increased protein called Still-Pro™. The general objectives were to evaluate each product and compare it with conventional DDGS. One of the products evaluated was Glutenol™, which is a co-product produced in a modified wet milling plant using a hybrid process called Hydromilling™. During Hydromilling™ the corn kernel components are fractionated prior to fermentation without the use of sulfur dioxide to create a higher protein DDGS product. Another product examined is DDGS produced from human food waste at high solids content. The raw materials used for this were mainly mashed potatoes, sweet corn, and white bread. We also examined DDGS produced from two mutant corn hybrids containing increased protein and/or increased select dietary indispensible amino acids (HPDDGS). The final product evaluated was an increased-protein ethanol co-product that is produced by a process that separates a high concentration protein and yeast fraction from ethanol stillage called Still-Pro™.

LITERATURE CITED

- Abbas, C., K. E. Beery, T. P. Binder, and T. Gottemoller. 2009. Dry fractionation of corn. Patent Application Publication. US. 1009/0029432 A1.
- 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 cecectomized roosters. *British Poultry Science*. 50:350-358.
- Almeida, F. N., J. K. Htoo, J. Thomson, and H. H. Stein. 2013. Amino acid digestibility of heat damaged distillers dried grains with solubles fed to pigs. *Journal of Animal Science and Biotechnology*. 4(1):44-54.
- Anderson, C. "Market Conditions Drop DDG Prices Dramatically." DTN Weekly Distillers Grains Update. N.p., 11 July 2016 Web. 2 Jan 2017.<<https://www.greatamericancrop.com/news-resources/article/2016/07/22/dtn-distillers-grain-weekly-update>>.
- Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *Journal of Applied Poultry Research*. 15:89-93.
- 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. *Bioresource Technology*. 94:293-298.
- 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. *Animal Feed Science and Technology*. 159:122-130.
- Berndes, G., C. Azar, T. Kaberger, and D. Abrahamson. 2001. The feasibility of large-scale lignocellulose-based bioenergy production. *Biomass and Bioenergy*. 20(5):371-83.

- Bevill, K. 2008. "Building the 'Minnesota Model'." *Ethanol Producer Magazine*, April, pp. 114-120. <https://www.ag.ndsu.edu/energy/biofuels/energy-briefs/history-of-ethanol-production-and-policy>.
- Combs, G. F., and E. H., Bossard. 1969. Further studies on available amino acid content of corn distillers dried grains with solubles. *Proc. 24th Distillers Feed Research Council*, pp. 53-58.
- Cromwell, G. L., K. L Kerkelman, and T.S. Stahly. 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *Journal of Animal Science*. 71:679-786.
- Curry, S. M., D. M. Navarro, F. N. Almeida, J. A. S. Almeida, and H. H. Stein. 2014. Amino acid digestibility in low-fat distillers dried grains with solubles fed to growing pigs. *Journal of Animal Science and Biotechnology*. 5:27-33.
- Dale, N., and A. Batal. 2003. Nutritional value of distillers dried grains with solubles for poultry. 19th Annual Carolina Nutrition Conf. Research Triangle Park (NC).
- Davis, K. 2001. Corn milling, processing and generation of co-products. 62nd Minnesota Nutrition Conference & Minnesota Corn Growers Association Technical Symposium. September 11-12, Bloomington, MN.
- Dozier, W. A., K. R. Perryman, and J. B. Hess. 2015. Apparent Ileal amino acid digestibility of reduced-oil distillers dried grains with solubles fed to broilers from 23 to 31 days of age. *Poultry Science*. 94:379-383.
- Liu, K., and K. A. Rosentrater. *Distillers Grains: Production, Properties, and Utilization*. Boca Raton: CRC/Taylor & Francis Group. 2012. Print.

- Farrell, A. E., R. J. Plevin, B. T. Turner, A. D. Jones, M. O'Hare, and D. M. Kammen. 2006. Ethanol can contribute to energy and environmental goals. *Science*. 311:506-508.
- Fernandez S. R., S. Aoyagi, Y. Han, C. M. Parsons, and D. H. Baker. 1994. Limiting order of amino acids in corn and soybean meal for growth of the chick. *Poultry Science*. 73:1887-1896.
- Ganesan V., K. A. Rosentrater, and K. Muthukumarappan. 2009. Physical and flow properties of regular and reduced fat distillers dried grains with solubles (DDGS). *Food Bioprocess Technol*. 2:156-166.
- Harms, R. H., R. S. Moreno, and B. L. Damron. 1969. Evaluations of distillers dried grains with solubles in diets of laying hens. *Poultry Science*. 48:1652-1655.
- Hopkins C. G., L. H. Smith, and E. M. East. 1974. The structure of the corn kernel and the composition of its different parts. University of Illinois, PhD thesis.
- Janssen, W. M. M. A., and B. Carré. 1985. Influence of fiber on digestibility of broiler feeds. Pages 78–93 in *Recent Advances in Animal nutrition*. D. J. A. Cole and W. Haresign, ed. Butterworths, London, UK.
- Jensen, L. S., L. Falen, and C. H. Chang. 1974. Effect of distillers dried grains with solubles on reproduction and liver fat accumulation in laying hens. *Poultry Science*. 53:586-592.
- Jensen, L. S., C. H. Chang, and S. P. Wilson. 1978. Interior egg quality: Improvement by distillers feeds and trace elements. *Poultry Science*. 57:648-654.
- Kidd, M. T. 2000. Nutritional considerations concerning threonine in broilers. *World's Poultry Science Journal*. 56:139-151.
- Kidd, M. T., B. J. Kerr, and N. B. Anthony. 1997. Dietary interactions between lysine and threonine in broilers. *Poultry Science*. 76:608-614.

- 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. *Poultry Science*. 89:44-51.
- Kim, E. J., P. L. Utterback, T. J. Applegate, and C. M. Parsons. 2011. Comparison of amino acid digestibility of feedstuffs determined with the precision-fed rooster assay and the standardized ileal amino acid digestibility assay. *Poultry Science*. 90:2511-2519.
- Kim, Y., N. S. Mosier, R. Hendrickson, T. Ezeji, H. Blaschek, B. Dien, M. Cotta, B. Dale, and M. R. Ladisch. 2008. Composition of corn dry-grind ethanol co-products: DDGS, wet cake, and thin stillage. *Bioresource Technology*. 99:5165-5176.
- 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 distiller's grains with solubles (DDGS) - Plant scale experiments. *Bioresource Technology*. 101:193-199.
- Leathers, T. D. 2003. Minireview: Bioconversion of maize residues to value-added coproducts using yeast like fungi. *FMES Yeast Research*. 3:133-140.
- Li S., W. Liu, K. D. Rausch, M. E. Tumbleson, and V. Singh. 2014. Comparison of protein concentrate, protein isolate and wet sieving processes for enriching DDGS protein. *Journal of American Oil Chemists' Society*. 91:867-874.
- Loar II, R. E., J. R. Donaldson, and A. Corzo. 2012. Effects of feeding distillers dried grains with solubles to broilers from 0 to 42 days posthatch on broiler performance, carcass characteristics, and selected intestinal characteristics. *Journal of Applied Poultry Research*. 21:48-62.

- Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. *Poultry Science*. 83:1891-1896.
- Martinez-Amezcu, C., C. M. Parsons, V. Singh, R. Srinivasan, and G. S. Murthy. 2007. Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. *Poultry Science*. 86:2624-2630.
- Martinez-Amezcu, C. 2005. Nutritional evaluation of corn distillers dried grains with solubles for poultry. University of Illinois-Urbana Champaign, IL. PhD Thesis.
- Monceaux, D. A., and D. Kuehner. "Dryhouse technologies and DDGS production." *The Alcohol Textbook*. 5th Edn., WM Ingledew, DR Kelsall, GD Austin and C. Kluhspies, Eds., Nottingham University Press: Nottingham (2009):303-322.
- Murthy, G. S., V. Singh, D. B. Johnston, K. D. Rausch, and M. E. Tumbleson. 2006. Evaluation and strategies to improve fermentation in characteristics of modified dry grind corn processes. *Cereal Chemistry*. 83:455-459.
- National Research Council. 1994. *Nutrient Requirements of Poultry*, Ninth Revised Edition. Washington, D.C.: National Academy Press.
- Pahm, A. A., C. S. Scherer, J. E. Pettigrew, D. H. Baker, C. M. Parsons, and H. H. Stein. 2009. Standardized amino acid digestibility in cecectomized roosters and lysine bioavailability in chicks fed distillers dried grains with soluble. *Poultry Science*. 88:571-578.
- Parsons, C. M. 1996. Digestible amino acids for poultry and swine. *Animal Feed Science Technology*. 59:147-153.

- Parsons, C.M. 2002. Digestibility and bioavailability of protein and amino acids. Pages 115-135 in Poultry Feedstuffs: Supply, Composition, and Nutritive Value. J. M. McNab and K. N. Boorman, ed. Centre for Agricultural Bioscience International (CABI), Oxon, UK.
- Parsons, C. M., C. Martinez, V. Singh, S. Radhakrishnan, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. Multi-State Poultry Nutrition and Feeding Conference.
- Parsons, C. M., D. H. Baker, and J. M. Harter. 1983. Distillers Dried Grains with Solubles as a Protein Source for the Chick. Poultry Science. 62: 2445-2451.
- 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. Animal Feed Science and Technology. 197:130-141.
- Ramirez E. C., D. B. Johnston, A. J. McAloon, W. Yee, and V. Singh. 2008. Engineering process and cost model for a conventional corn wet milling facility. Industrial Crops and Products. 27:91-97.
- Renewable Fuels Association. 2014. Fueling a Nation, Feeding the World. The Role of the U.S. Ethanol Industry in Food and Feed production. Available: http://ethanolrfa.org/wp-content/uploads/2015/09/9864ff506e6519057b_t5m6brouu.pdf. Accessed: September 29, 2016.
- Renewable Fuels Association. 2016. Leading the U.S. Ethanol Industry: Outlook. Available: <http://ethanolrfa.org/> Date Accessed: September 29, 2016.
- Runnels, T. D. 1966. The biological nutrient availability of corn distillers dried grains with solubles in broiler feeds. Proc. 21st Distillers Feed Research Council, pp. 11- 15.

- Rausch, K. D. and R. L. Belyea. 2006. The future of coproducts from corn processing. *Appl. Biochem. Biotechnol.* 128:47-86.
- Saunders J. A., and K. A. Rosentrater. 2009. Properties of solvent extracted low-oil corn distillers dried grains with solubles. *Biomass and Bioenergy.* 33:1486-1490.
- Scott, M. L. 1970. Twenty-five years of research on distillers feeds for broilers. *Proc. 25th Distillers Feed Research Council*, pp. 19-24.
- Shim, M. Y., G. M. Pesti, R. I. Bakalli, P. B. Tillman, and R. L. Payne. 2011. Evaluation of corn distillers dried grains with solubles as an alternative ingredient for broilers. *Poultry Science.* 90:369-376.
- Shukla, R., and M. Cheryan. 2001. Zein: the industrial protein from corn. *Industrial Crops and Production.* 13:171-192.
- Singh, V., D. B. Johnston, K. Naidu, K. D. Rausch, R. L. Belyea, and M. E. Tumbleson. 2005. Comparison of modified dry-grind corn processes for fermentation characteristics and DDGS composition. *Cereal Chemistry.* 82:187-190.
- Singh, V., R. A. Moreau, L. W. Doner, S. R. Eckhoff, and K. B. Hicks. 1999. Recovery of fiber in the corn dry-grind ethanol process: A feedstock for valuable coproducts. *Cereal Chemistry.* 76:868-872.
- Singh, V., and S. R. Eckhoff. 1996. Effect of soak time, soak temperature, and lactic acid on germ recovery parameters. *Cereal Chemistry.* 73:716-720.
- 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. *Journal of Animal Science.* 80:2639-2645.

Srinivasan, R., R. A. Moreau, K. D. Rausch, R. L. Belyea, M. E. Tumbleson, and V. Singh.

2005. Separation of fiber from distillers dried grains with solubles (DDGS) using sieving and elutriation. *Cereal Chemistry*. 82:528-533.

Srinivasan, R., V. Singh, R. L. Belyea, K. D., Rausch, R. A. Moreau, and M. E. Tumbleson.

Economics of fiber separation from distillers dried grains with solubles (DDGS) using sieving and elutriation. 2006. *Cereal Chemistry*. 83:324-330.

Urbanchuk, J. M. Contribution of the Ethanol industry to the economy of the United States in

2015. Prepared for the Renewable Fuels Association, Washington, DC, February 5th, 2016.

"2016 State of the U.S. Ethanol Industry." *Ethanol RFA*. N.p., n.d. Web. 12 Feb. 2017.

<<http://www.ethanolrfa.org/wp-content/uploads/2016/02/2016-State-of-the-Industry-address.pdf>>.

CHAPTER 2

NUTRITIONAL EVALUATION OF GLUTENOL™: A CO-PRODUCT OF ETHANOL PRODUCTION

ABSTRACT

The objective of this study was to evaluate the nutritional value of Glutenol™, a new coproduct of the ethanol industry. Glutenol™ was produced by Quality Technology International in a modified wet-milling plant using a hybrid process, NextGenFrac™, which fractionates the corn kernel components prior to fermentation without the use of sulfur dioxide. Glutenol™ was analyzed to contain 52.3% CP, 1.7% Met + Cys, 1.32% Lys, 1.69% Thr, and 2.23% Val on a DM basis. Two precision-fed rooster assays with conventional and cecectomized roosters were conducted to determine TME_n and amino acid standardized digestibility, respectively. The TME_n of Glutenol™ was determined to be 3256 kcal/kg DM. Standardized digestibility values for Lys, Met, Cys, Thr and Val were 80.1%, 90.4%, 80.1%, 74.1%, 81.1% and 84.9% respectively. In addition, a three-week broiler chick assay was conducted with increasing levels of dietary Glutenol™. Diet 1 was a standard corn/soybean meal diet with 0% Glutenol™. Diets 2, 3 and 4 had increasing levels of Glutenol™ at 4%, 8% and 12% respectively. As the level of Glutenol™ increased, the level of SBM in the diet decreased from 35.77% in Diet 1 to 29.42% in Diet 4 to keep the protein levels equal among treatments. The diets were fed to Ross 308 male broilers housed in battery cages from 3 to 22 days post hatch. All diets were formulated to be equal in ME and digestible amino acids (AA). Weight gain, feed intake and gain/feed ratio were measured. No differences in growth performance were observed among dietary treatments. In

conclusion, Glutenol™ can be fed up to at least 12% in the diet of broiler chickens if diets are formulated to be equal in ME and digestible AA.

INTRODUCTION

Ethanol co-products are fed to many animal species. The poultry industry utilized approximately 8% of the DDGS produced in 2015 (Renewable Fuels Association, 2015). There has been a substantial amount of growth in the quantity and type of ethanol co-products that are produced. In 2015, 14.7 billion gallons of ethanol for high-octane renewable fuel was produced along with 40 million metric tons of DDGS for animal feed (Renewable Fuels Association, 2015). As of January 2016, 90% of fuel ethanol is produced using a dry-grind process, while 10% of fuel ethanol is produced using a wet-milling process (Renewable Fuels Association, 2015). The wet-milling process is used to gain more value in the co-products. In wet-milling, the grain is steeped in water and diluted with SO₂ for 24-48 hours, which facilitates separation of the grain components. Quality Technology International produces Glutenol™ by using a hybrid process called NextGenFrac™ in a modified wet milling plant. During this process, corn is fractionated into the corn kernel components (germ, fiber, protein and starch) prior to fermentation without the use of SO₂. This helps to remove more of the fiber in the front end to produce an end product that is higher in protein. The main nutritional characteristics of Glutenol™ are its higher CP and AA concentrations compared to conventional DDGS. Conventional DDGS averages 27% protein according to many researchers (NRC, 1994; Batal et al. 2006; Belyea et al. 2004; Belyea et al. 2010). With the modified production process to produce Glutenol™, the amino acid profile of the product produced also changes. One of the main limitations with DDGS can be the lower amino acid concentrations and standardized digestibility values due to over-processing and overheating. Conventional DDGS is deficient in Lys, Trp, and Arg when fed to poultry (Spiehs et al. 2002). The

standardized digestibility of Lys has been shown to be the most affected of all amino acids by overheating DDGS due to Maillard reactions with carbohydrates (Spiehs et al., 2002). If DDGS is dark in color, it suggests that it has been overheated or heat-damaged and may have decreased amino acid availability. Several DDGS samples that ranged in color were evaluated by Batal and Dale, (2006); the lighter colored samples had a total Lys concentration of 0.79% and a 0.60% digestible Lys concentration while the darker colored samples averaged 0.64% total Lys and 0.31% digestible Lys. Another characteristic of DDGS that is important is the energy content. True metabolizable energy (TME_n) of DDGS is usually related to the amount of fat in DDGS. In earlier studies, conventional DDGS was found to have an average concentration of 12% and 10.9% crude fat (Belyea et al., 2004; Spiehs et al., 2002). The mean TME_n of similar conventional DDGS that contained approximately 10% fat was found to be 2,863 kcal/kg on an as-fed basis (Lumpkins et al., 2004; Parsons et al., 2006). Recently, most ethanol plants are removing some oil from the DDGS using centrifugation, resulting in the DDGS containing only 5-7% fat, which reduces the ME (metabolizable energy) content (Jacela et al., 2011; Dozier et al., 2015; Saunders and Rosentrater, 2009). Finding new ways to process corn for ethanol and simultaneously create higher ME and higher crude protein ethanol co-products would produce a more valuable feed ingredient for poultry. The objectives of this study were to determine the amino acid standardized digestibility and TME_n of Glutenol™, a new ethanol industry co-product, using a precision-fed rooster assay, and to determine the effect of diets containing increasing levels of Glutenol™ on broiler growth performance.

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use committee at the University of Illinois.

Test Ingredients

Glutenol™ (Quality Technology Ingredients, 1707 N. Randall Rd, Ste. 300 Elgin, IL), a high protein ethanol co-product produced in a modified wet milling plant using a hybrid process called NextGenFrac™, was evaluated. The NextGenFrac™ process fractionates the corn kernel components prior to fermentation without the use of sulfur dioxide to separate fiber. In addition, a sample of commercial solvent-extracted dehulled soybean meal was evaluated to provide a comparison of Glutenol™ to a commonly used high-protein ingredient in poultry diets.

TME_n and Amino Acid Standardized digestibility

Two precision-fed rooster assays were conducted to evaluate TME_n and amino acid standardized digestibility. Single comb white-leghorn roosters were fasted for 26 hours and subsequently tube-fed 30 grams of the test product, Glutenol™ or dehulled soybean meal. Conventional roosters were used to determine TME_n and cecectomized roosters were used to determine amino acid standardized digestibility. After tube feeding, each individual rooster was placed into an individual cage with a collection tray underneath. Excreta were collected quantitatively for 48 hours after tube feeding. The excreta were then freeze dried, weighed, ground and analyzed. Feed samples and excreta samples from conventional roosters were analyzed for gross energy (GE) using an adiabatic bomb calorimeter (Model 6300; Parr Instruments, Moline, IL), crude protein (CP) by combustion (Method 990.03; AOAC International, 2007) and TME_n was calculated as described by Parsons et al. (1992). Excreta from the cecectomized roosters were analyzed for amino acids at the Agricultural Experiment

Station Laboratory, University of Missouri-Columbia using method 982.30 E (a,b and c); AOAC International, 2007. Endogenous amino acid losses were determined using roosters that had been fasted for 48 hours and then standardized amino acid values were calculated.

Broiler chick experiment

Ross 308 male broiler chicks were housed in thermostatically controlled starter battery cages with raised wire floors in an environmentally controlled room. Chicks were weighed, wingbanded and allotted to pens at 3 days post hatch. There were 5 chicks per pen and each pen had an average starting weight of 44.5 grams per chick. Feed and water were provided for ad libitum consumption. At the end of the experiment, at 22 days of age, all chicks and feeders were weighed and recorded for analysis. Body weight gain, feed consumption, and feed efficiency were then calculated for each pen of chickens.

Dietary Treatments

All diets were formulated to be equal in TME_n and digestible amino acids based on the TME_n values of Glutenol™ and dehulled soybean meal determined in the precision-fed rooster assays. A TME_n value of 3,470 kcal/kg (NRC, 1994) was used for the corn and a TME_n value of 8,800 kcal/kg was used for the soybean oil. The digestible amino acid concentrations for the corn were calculated based on NRC (1994) table values. This experiment was a completely randomized design (CRD), with 4 diets per treatment, 10 replicates per treatment, and 5 chicks per replicate. Diet 1 was a corn-soybean meal control diet and Diets 2, 3 and 4 were the corn-soybean meal diet plus 4%, 8% or 12% Glutenol™, respectively. This was a 20-day assay, with the diets being fed from Day 3 until Day 22 of age. The composition of the four diets is shown in Table 2.1.

Statistical Analysis

Data from the broiler chicken assay were analyzed using the ANOVA procedure of SAS (SAS Institute Inc., Cary, NC) for completely randomized designs. Statistical significance of differences among individual treatments was assessed using the least significant difference test (Carmer and Walker, 1985). Significance was assessed at $P < 0.05$. The TME_n and amino acid standardized digestibility values for the Glutenol™ and soybean meal were also analyzed by ANOVA using SAS and the differences between means for the two ingredients were assessed using the least significant difference test.

RESULTS AND DISCUSSION

Experiment 1, Rooster Assay

The TME_n values for the Glutenol™ and dehulled soybean meal are presented in Table 2.2. The TME_n of Glutenol™ was determined to be 3256 kcal/kg of DM which is significantly greater than the determined TME_n value of the dehulled soybean meal. The TME_n value of 3256 kcal/kg of DM is only slightly less than the value of 3330 kcal/kg of DM listed in the NRC (1994) for DDGS containing 9% ether extract and the value reported by Batal and Dale (2006) of 3279 kcal/kg DM, also for DDGS containing a mean of 9% fat. When comparing the TME_n value of Glutenol™ with high-protein DDGS products evaluated in earlier studies, the ME of the Glutenol™ is generally higher than that of DDGS products containing increased protein levels. For example, Kim et al. (2008) reported that the TME_n of a high-protein DDGS containing 44% CP (DM basis) was 2957 kcal/kg. In addition, the mean TME_n of 8 samples of high-protein DDGS (mean=48% CP) was 3130 kcal/kg DM (Jung and Batal, 2009). Rochell et al. (2011) reported that the AME_n of a high protein DDGS containing 46.6% CP (DM basis) was 2879 kcal/kg DM when determined in broiler chickens. Also, in the latter study, the AME_n of a higher-

protein DDGs containing 62.2% CP (DM basis) was 3179 kcal/kg DM. Likewise, Kim et al. (2010) reported a high TME_n value of 3656 kcal/kg DM for an enzymatic-milled DDG containing 61% CP on a DM basis. The latter ingredient contained a very low level of NDF (6.8%) due to both germ and pericarp fiber being removed. The level of NDF in DDGS samples is usually approximately 35-37% on a DM basis (Kim et al., 2010). Thus, the ME of DDGS can be increased greatly by processing it to increase its CP and decreasing its fiber content.

The CP of Glutenol™, was determined to be 52.3% on a DM basis (Table 2.3) and is substantially increased from the CP value of 27% for conventional DDGS (NRC, 1994) and not different than the CP of dehulled soybean meal which had a value of 54.3%. The CP of conventional DDGS has been reported to range from 27%-34% (Batal and Dale, 2006; Belyea et al. 2004; Belyea et al. 2010) for DDGS containing 9% ether extract. As expected, due to the increased CP level, the total amino acid concentrations in Glutenol™ were increased and were similar to or in some instances, increased from those in soybean meal (Table 2.3). The total amino acid concentrations in Glutenol™ were also increased compared with those reported by the NRC (1994) for conventional DDGS. For example, the total Lys concentration for Glutenol™ was 1.32% compared with conventional DDGS at 0.75% (NRC, 1994). Total amino acid concentrations in Glutenol™ for Met, Thr, Val, Arg, and Ile were 0.88%, 1.69%, 2.23%, 1.65%, and 1.88%, respectively (Table 2.3). For amino acid standardized digestibility values, in many cases, Glutenol™ had amino acid standardized digestibility values that were not different from that of soybean meal. Because feed formulators would like to decrease diet cost if possible, replacing some of the more expensive soybean meal in the diet with Glutenol™ could possibly be beneficial and would not decrease dietary levels of most digestible amino acids. The amino acid standardized digestibility values for Glutenol™ are generally not different from those

reported in other studies for high-protein DDGS ingredients. Using cecectomized roosters, Godoy et al. (2009) reported most amino acid standardized digestibility values for a corn protein product containing 49.7% CP on a DM basis were not different than those determined herein for Glutenol™. One exception was that the standardized digestibility of Lys in the corn protein product was only 55.5% compared with a higher value of 74.1% in the current study. In two other previous studies, amino acid standardized digestibility values determined in cecectomized roosters for a high-protein DDGS product containing 44% CP (Kim et al. 2008) and 8 samples of a high-protein DDG (mean of 48% CP; Jung and Batal, 2009) were not different from the values obtained in the current study for Glutenol™. The cecectomized rooster amino acid standardized digestibility values for a very high-protein (61% CP) enzymatic-milled DDG (Kim et al, 2010) were generally slightly increased compared with the values for Glutenol™. The low fiber content of the enzymatic-milled DDG may have contributed to the higher amino acid standardized digestibility values. Listed in Table 2.3 are the digestible amino acid concentrations for Glutenol™ and dehulled soybean meal. Although the digestible Lys content is much higher in Glutenol™ when compared with conventional DDGS, the digestible Lys content of Glutenol™ is still lower than in the soybean meal (2.68%). In contrast, the digestible amino acid concentrations for Met, Cys, Pro, Ala, and Leu are higher in Glutenol™ compared with soybean meal. The higher digestible content of Met and Cys is particularly important for poultry nutrition because these amino acids are the first limiting in most poultry diets. Glutenol™ was determined to contain 0.80% digestible Met vs 0.69% in soybean meal and digestible Cys was 0.66% for Glutenol™ and 0.57% for soybean meal.

Experiment 2, Broiler Chick Assay

As mentioned earlier, the chick diets were formulated to be equal in TME_n and digestible amino acids based on the precision fed rooster assay results for Glutenol™ and soybean meal. In Diets 1 and 2, the CP level was 22% and in Diets 3 and 4, the CP level was 22.9% and 23.8%, respectively (Table 1.1). The protein level increased for Diets 3 and 4 to meet the digestible Arg requirement, which was 1.26%. When Glutenol™ was added at 8% and 12%, the digestible Arg requirement could not be met unless the dietary protein level was increased. Alternatively, L-Arg could have been added to the diets to maintain a consistent 22% CP level in all diets, but L-Arg is not currently supplemented in commercial broiler diets because it is too expensive. Therefore, in attempt to simulate commercial diet formulation, L-Arg was not added to the diets containing Glutenol™ in the current study. Body weight gain, feed intake and gain/feed ratio were not statistically different among any of the 4 treatments (Table 2.4). These results indicate that Glutenol™ can be included in broiler chicken diets from 3-22 days of age at levels of at least 12% with no significant effects on growth performance if the diets are formulated to be equal in TME_n and digestible amino acids. The results of the current study indicate that the new ethanol co-product, Glutenol™, has substantial nutritional value for poultry. Furthermore, based on current results for CP, digestible amino acids, and TME_n, Glutenol™ has greatly increased nutritional value compared with conventional DDGS.

LITERATURE CITED

- AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.
- Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *Journal of Applied Poultry Research*. 15:89-93.
- 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. *Bioresource Technology*. 94:293-298.
- 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. *Animal Feed Science and Technology*. 159:122-130.
- de Godoy, M. R. C., L. L. Bauer, C. M. Parsons, and G. C. Fahey Jr. 2009. Select corn coproducts from the ethanol industry and their potential as ingredients in pet foods. *Journal of Animal Science*. 87:189-199.
- Dozier, W. A., K. R. Perryman, and J. B. Hess. 2015. Apparent Ileal amino acid digestibility of reduced-oil distillers dried grains with solubles fed to broilers from 23 to 31 days of age. *Poultry Science*. 94:379-383.
- Jacela, J. Y., J. M. DeRouchey, S. S. Dritz, M. D. Tokach, R. D. Goodband, J. L. Nelssen, 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 characteristics. *Journal of Animal Science*. 89:1817-1829.

- Jung, B., and A. Batal. 2009. The nutrient digestibility of high-protein corn distillers dried grains and the effect of feeding various levels on the performance of laying hens. *Journal of Applied Poultry Research*. 18:741-751.
- Kim, E. J., C. Martinez-Amezcu, P. L. Utterback, and C. M. Parsons. 2008. Phosphorus bioavailability, true metabolizable energy, and amino acid digestibilities of high protein corn distillers dried grains and dehydrated corn germ. *Poultry Science*. 87:700-705.
- 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. *Poultry Science*. 89:44-51.
- Lumpkins, B. S., A. B. Batal, and N. M. Dale. 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. *Poultry Science*. 83:1891-1896.
- National Research Council. 1994. *Nutrient Requirements of Poultry*, Ninth Revised Edition. Washington, D.C.: National Academy Press.
- Parsons, C. M., C. Martinez, V. Singh, S. Radhakrishman, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. *Proceeding of Multi-State Poultry Nutrition and Feeding Conference*, Indianapolis, IN.
- Renewable Fuels Association. 2014. *Fueling a Nation, Feeding the World. The Role of the U.S. Ethanol Industry in Food and Feed production*. Available: http://ethanolrfa.org/wp-content/uploads/2015/09/9864ff506e6519057b_t5m6brouu.pdf. Accessed: September 29, 2016.
- Renewable Fuels Association. 2016. *Leading the U.S. Ethanol Industry: Outlook*. Available: <http://ethanolrfa.org/> Date Accessed: September 29, 2016.

- Rochell, S. J., B. J. Kerr, and W. A. Dozier III. 2011. Energy determination of corn co-products fed to broiler chicks from 15-24 days of age, and use of composition analysis to predict nitrogen-corrected apparent metabolizable energy. *Poultry Science*. 90:1999-2007.
- SAS Institute. 2010. SAS® User's Guide: Statistics. Version 9.2 Edition. SAS Institute, Inc., Cary, NC.
- Saunders J. A., and K. A. Rosentrater. 2009. Properties of solvent extracted low-oil corn distillers dried grains with solubles. *Biomass and Bioenergy*. 33:1486-1490.
- 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. *Journal of Animal Science* 80:2639-2645.

TABLES

Table 2.1 Diet composition for the broiler chick assay (%)

<u>Ingredient</u>	<u>Dietary treatment</u>			
	<u>Diet 1</u>	<u>Diet 2</u>	<u>Diet 3</u>	<u>Diet 4</u>
Corn	57.27	57.15	54.25	51.26
Glutenol™	0.00	4.00	8.00	12.00
Dehulled soybean meal	35.77	31.97	30.65	29.42
Soybean oil	2.59	2.44	2.72	3.02
Limestone	1.15	1.19	1.22	1.25
Dicalcium phosphate	1.75	1.71	1.66	1.62
Salt	0.46	0.44	0.43	0.41
Vitamin mix ¹	0.20	0.20	0.20	0.20
Mineral mix ²	0.15	0.15	0.15	0.15
DL-Met	0.28	0.27	0.25	0.22
L-Lys HCl	0.17	0.26	0.27	0.27
L-Thr	0.10	0.11	0.09	0.07
Choline chloride (60%)	0.07	0.07	0.07	0.07
Bacitracin-MD premix ³	0.04	0.04	0.40	0.04
<u>Calculated analysis</u>				
Crude protein	22.00	22.00	22.88	23.80
Ca	0.95	0.95	0.95	0.95
Non-phytate P	0.45	0.45	0.45	0.45
Na	0.20	0.20	0.20	0.20
Digestible Met + Cys	0.86	0.86	0.86	0.86
Digestible Met	0.59	0.59	0.58	0.57
Digestible Lys	1.20	1.20	1.20	1.20
Digestible Thr	0.80	0.80	0.80	0.80
Digestible Val	0.96	0.95	0.97	1.00

Table 2.1 Cont.

Digestible Arg	1.33	1.26	1.26	1.26
----------------	------	------	------	------

¹Provided per kilogram of diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 µg; DL- α -tocopheryl acetate, 11 IU; vitamin B12, 0.01 mg; riboflavin, 4.41 mg; D-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.

²Provided as milligrams per kilogram of diet: manganese, 75 from $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; iron, 75 from $\text{FeSO}_4 \cdot \text{H}_2\text{O}$; zinc, 75 mg from ZnO ; copper, 5 mg from $\text{CuO}_4 \cdot 5\text{H}_2\text{O}$; iodine, 75 from ethylene diamine dihydroiodide; selenium, 0.1 from Na_2SeO_3 .

³Contributed 13.75 mg/kg of bacitracin methylene disalicylate (5.5%).

Table 2.2 True metabolizable energy values for Glutenol™ and dehulled soybean meal

Sample	Gross Energy (kcal/kg DM)	Dry Matter (%)	TME _n (kcal/kg DM) ¹	Pooled SEM
Glutenol™	5523	84.0	3256 ^a	0.039
Dehulled soybean meal	4769	89.1	2796 ^b	

^{a-b} The TME_n of Glutenol™ was significantly different from soybean meal ($P < 0.05$).

¹ Values are means of 8 individually-caged conventional roosters.

Table 2.3 Total amino acids, amino acid standardized digestibility values, and digestible amino acid concentrations for Glutenol™ and dehulled soybean meal (DM basis) (%)

Amino Acid ¹	Glutenol™			Dehulled soybean meal			Pooled SEM ³
	Total	Digest. value	Digest. conc. ²	Total	Digest. value	Digest. conc. ²	
Met	0.88	90.4 ^a	0.79	0.76	91.2 ^a	0.69	0.65
Cys	0.82	80.1 ^a	0.66	0.73	77.8 ^a	0.57	1.69
Lys	1.32	74.1 ^b	0.98	3.43	87.5 ^a	3.00	0.99
Thr	1.69	81.1 ^b	1.37	2.10	85.9 ^a	1.80	0.89
Val	2.23	84.9 ^b	1.89	2.66	88.4 ^a	2.35	0.78
Arg	1.65	89.2 ^a	1.47	3.87	91.6 ^a	3.54	0.80
Ile	1.88	86.7 ^b	1.63	2.50	90.8 ^a	2.27	0.70
Asp	2.88	79.7 ^b	2.29	6.16	88.7 ^a	5.46	0.73
Ser	2.01	85.3 ^b	1.71	2.45	88.9 ^a	2.18	0.81
Glu	7.37	88.9 ^b	6.55	9.62	92.7 ^a	8.85	0.53
Pro	3.79	90.3 ^a	3.42	2.62	90.7 ^a	2.38	0.65
Ala	3.61	88.8 ^a	3.20	2.32	86.8 ^a	2.01	0.85
Leu	5.85	91.5 ^a	5.19	4.18	90.2 ^a	3.77	0.56
Tyr	1.93	90.5 ^a	1.75	1.99	90.6 ^a	1.80	0.51
Phe	2.38	89.9 ^a	2.14	2.78	91.3 ^a	2.54	0.54
His	1.25	84.9 ^a	1.12	1.45	87.2 ^a	1.26	0.57
Trp	0.32	91.6 ^b	0.29	0.83	96.1 ^a	0.80	0.60

^{a-b} Standardized digestibility values within the same row with different superscripts are significantly different (P<0.05).

¹ The CP of the Glutenol™ and dehulled soybean meal were 52.3% and 54.3%, respectively, on a DM basis.

² Digestible concentration= total × standardized digestibility values.

³ Pooled SEM for standardized digestibility values.

Table 2.4 Growth performance of broiler chicks fed increasing levels of Glutenol™ (G) from 3 to 22 d of age ¹

Treatment	Feed intake, g	Body weight gain, g	Gain:feed, g/kg
1. Control (No G)	1146	824	720
2. As 1 + 4% G	1106	807	732
3. As 1 + 8% G	1121	826	737
4. As 1 + 12% G	1099	822	749
Pooled SEM	20.7	14.4	13.6
P-Value (P<0.05)	0.41	0.78	0.50

¹Values are means of 10 pens containing 5 chicks per pen.

CHAPTER 3

NUTRITIONAL EVALUATION OF THREE TYPES OF NOVEL ETHANOL CO-PRODUCTS

ABSTRACT

A series of 48-hour precision-fed rooster assays were conducted to determine TME_n using conventional roosters and/or amino acid standardized digestibility using cecectomized roosters for distillers dried grains with solubles (DDGS) produced from human food waste at high solids content (FWDDGS), DDGS produced from two mutant corn hybrids containing either increased protein or increased levels of select dietary indispensable amino acids such as lysine, arginine, and tryptophan, and an increased-protein ethanol co-product that is produced by a process which separates a high concentration protein and yeast fraction from ethanol stillage (Still Pro™, Fluid Quip Process Technologies, Cedar Rapids, IA). The original raw materials for the FWDDGS consisted mainly of mashed potatoes, sweet corn and white bread. The results of the rooster assays indicated that the standardized digestibility of the amino acids in the FWDDGS was not different than typical DDGS (e.g., 61% for lysine; NRC, 1994), whereas the TME_n for the FWDDGS was higher than for typical DDGS. The DDGS produced from the high protein mutant corn hybrid had a higher protein content of 34% compared with the 28% protein found in DDGS from the control corn hybrid. Using Lys as an example, there was a large difference in Lys content between the two samples; the high protein mutant DDGS contained 1.60% Lys versus 1.05% for the control DDGS. The standardized digestibility of amino acids was generally not different for the mutant DDGS and the control DDGS. Similar results were observed for Lys, Arg, and Trp for the DDGS produced from the second mutant corn hybrid. The Still Pro™ sample was analyzed to contain 53% protein on a dry matter basis with 2.22 % Lys, 1.05% Met, 0.90% Cys and 2.06% Thr. The standardized digestibility values for these amino

acids were 84%, 92%, 87% and 86% respectively. The TME_n of the Still Pro™ sample was determined to be 3372 kcal/kg on a dry matter basis. In conclusion, the results of this study indicate that the nutritional value of the three novel ethanol coproducts is in general superior to the nutritional value of conventional DDGS.

INTRODUCTION

Ethanol is by far the most significant biofuel in the United States, accounting for 94% of all biofuel production in 2012 (USDA, 2013). The production of ethanol continues to evolve to find new ways to increase efficiency and produce end products that have increased value for nonruminant producers. To limit the “food versus fuel” competition, researchers have been searching for ways to reduce the amount of corn and sugar cane used to produce ethanol and find other reliable sources to produce a large amount of ethanol. One potential input source is human food waste. The latter is a complex biomass from various sources such as households and restaurants and supplies have been estimated to be more than 36 million tons per year (U.S. EPA, 2014). Haung et al. (2015) reported that ethanol could be efficiently produced from food waste at high solids content with vacuum recovery technology. The metabolizable energy and standardized digestibility of amino acids in the DDGS produced from the food waste has not been determined. In addition, there is much interest in producing ethanol co-products that contain increased CP and digestible amino acids because the use of DDGS in poultry diets is often limited due to its combination of decreased and highly variable amino acid standardized digestibility values, particularly for lysine.

One potential method of producing higher protein DDGS is to develop new corn hybrids that contain increased CP or at least increased levels of important dietary indispensable amino acids. Consequently, the DDGS produced from the ethanol processing of such corn hybrids

should contain increased levels of digestible amino acids. Indeed, Ramchandran et al. (2017) recently reported that DDGS produced from two mutant corn hybrids contained increased levels of protein and/or dietary important amino acids such as Lys and Trp than DDGS produced from control corn hybrids. The standardized digestibility of amino acids in the DDGS produced from the new mutant corn hybrids has not been reported. Another new increased CP ethanol coproduct has recently been developed by Fluid Quip Process Technologies, Cedar Rapids, IA. The ingredient is named Still Pro™ and is produced by a post fermentation process that separates a high concentration protein and yeast fraction from ethanol stillage, yielding a product that contains approximately 50% CP. No research with poultry has been conducted to evaluate the nutritional value of the Still Pro™ product. The objective of this study was to evaluate 3 types of novel ethanol co-products, namely, food waste DDGS (FWDDGS), DDGS produced from two new mutant corn hybrids and their control hybrids, and Still Pro™ for TME_n and/or amino acid standardized digestibility .

MATERIALS AND METHODS

The protocols for these studies were reviewed and approved by the Institutional Animal Care and Use committee at the University of Illinois.

Test Ingredients

One sample of Food Waste DDGS (FWDDGS), 8 DDGS samples produced from two mutant corn hybrids and two control corn hybrids using two different processing methods, and one sample of Still Pro™ were obtained. Each sample will be described in the next few paragraphs.

The FWDDGS was produced from materials commonly found in human food waste. The original raw materials for the FWDDGS consisted mainly of mashed potatoes, sweet corn, and

white bread as described by Huang et al. (2015). The composition of the food waste (DM basis) was 63.5% starch, 4.3% glucose, 13.9% protein, 4.1% oil, 5.2% neutral detergent fiber and 3.4% ash. Vacuum recovery technology was used to produce the FWDDGS evaluated herein (Huang et al., 2015). This technology is a vacuum fermentation system in which the produced ethanol is removed under vacuum during fermentation; thus, a low ethanol concentration can be maintained in the fermenter, which eliminates or minimizes yeast ethanol inhibition (Huang et al., 2015). The ethanol conversion efficiency for the vacuum fermentation was 93.6% which is higher than the value for conventional fermentation at 85.3% (Huang et al., 2015). The DDGS produced by this process was collected and TME_n and amino acid standardized digestibility were determined in roosters to estimate its nutritional value for poultry.

The DDGS produced from the two mutant corn hybrids, was obtained from the Department of Agricultural and Biological Engineering, University of Illinois. Mutations that increase Lys and Trp were combined by breeding them into two corn hybrid backgrounds that differ in grain protein concentration (Ramchandran et al., 2017). There were four hybrids evaluated in this study: FMo2C28, a high-protein mutant corn hybrid, and its control non-mutant corn hybrid FM, as well as FRHo2C28, the second mutant corn hybrid and its control non-mutant corn hybrid FRH. All hybrids were grown in each of three replicated field plots in 2012 at the Crop Sciences Research and Education Center located near the University of Illinois in Urbana, IL. Corn was harvested, sieved, cleaned, and ground using a hammer mill. Then each of the four corn hybrids were processed using two different laboratory scale processes. The first process was a conventional dry-grind procedure and the second process was a modified dry-grind procedure using granular starch hydrolyzing enzyme (GSHE) (Ramchandran et al., 2017). Thus, a total of 8 DDGS products were produced (four corn hybrids and two processing

methods). In the conventional dry-grind process, ground corn was mixed with water to obtain a 30% slurry. Sulfuric acid was added for the liquefaction process. Liquefaction, saccharification and fermentation included a mixture of alpha amylase, glucoamylase and protease enzymes, urea and yeast. Post fermentation, ethanol was evaporated from fermented mash and DDGS was produced from remaining material by drying in a convection oven and subsequently ground into a powder (Ramchandran et al., 2017). The modified dry-grind process (GSHE) was not different than the conventional process except that different alpha amylases and glucoamylase enzymes were used. Ethanol evaporation and DDGS preparation were the same as the conventional process (Ramchandran et al., 2017).

The Still Pro™ product (SP50) was produced through a process developed by Fluid-Quip Process Technologies, Cedar Rapids, IA. The SP50 is made in a conventional dry-grind ethanol plant. It is a post-fermentation process in which the whole stillage goes through stillage washing, protein concentration and protein drying. From the whole stillage tank, the product goes through a Fluid Quip filtration centrifuge to separate some of the protein, oil and solubles which are then put through a Fluid Quip disc/nozzle centrifuge. The clarified thin stillage is then sent to evaporators to produce oil and a high protein and high yeast fraction are sent through stillage decanters and then a dryer to produce a product containing approximately 50% protein.

TME_n and Amino Acid Standardized digestibility

Five precision-fed rooster assays were conducted to determine TME_n and amino acid standardized digestibility of the 10 test ingredients. Single Comb White-Leghorn roosters were fasted for 26 hours and subsequently tube-fed 30 grams of the test products. In each assay, conventional roosters were used to determine TME_n and cecectomized roosters were used to determine amino acid standardized digestibility. After tube-feeding, each individual rooster was

placed into an individual cage with a collection tray underneath. Excreta were then collected quantitatively for 48 hours. The excreta were then freeze dried, weighed, ground and analyzed. Feed samples and excreta samples from conventional roosters were analyzed for gross energy (GE) using an adiabatic bomb calorimeter (Model 6300; Parr Instruments, Moline, IL), crude protein (CP) by combustion (Method 990.03; AOAC International, 2007) and TME_n , which was calculated as described by Parsons et al., (1992). Excreta from the cecectomized roosters were analyzed for amino acids at the Agricultural Experiment Station Laboratory, University of Missouri-Columbia using method 982.30 E (a,b and c); (AOAC International, 2007). Endogenous amino acid losses were determined using roosters that had been fasted for 48 hours and then standardized amino acid standardized digestibility values were calculated. The first two rooster assays were conducted to determine TME_n and amino acid standardized digestibility in the FWDDGS, respectively (8 roosters per sample). The third rooster assay was conducted to determine standardized digestibility of amino acids in the 8 DDGS samples produced from the four corn hybrids processed by two different methods (3 roosters per sample). The last two rooster assays were conducted to determine TME_n and amino acid standardized digestibility of the SP50 ingredient (4 roosters per sample).

Statistical Analysis

The TME_n and amino acid standardized digestibility values were analyzed using the ANOVA procedure of SAS (1994). Differences among means were assessed by using the least significance difference test (Carmer and Walker, 1985).

RESULTS AND DISCUSSION

The CP, gross energy and TME_n values for FWDDGS are presented in Table 3.1. The TME_n of FWDDGS was determined to be 3890 kcal/kg of DM, which is greater than what has

usually been reported for conventional DDGS. Many samples of conventional DDGS were found to have a mean TME_n of 3279 kcal/kg on a DM basis (Parsons et al., 2006; Dale and Batal, 2006) and a value of 3330 kcal/kg on a DM basis according to the NRC (1994). The CP of FWDDGS was determined to be 27.3% on a DM basis. This CP value is slightly lower than what is reported in the NRC (1994) for conventional DDGS, 29.5% on a DM basis. The total amino acid concentrations in FWDDGS were generally higher than those previously reported for conventional DDGS (NRC, 1994) (Table 3.2). For example, Lys in conventional DDGS is listed as 0.81% on a DM basis (NRC, 1994), whereas the FWDDGS contained 1.45% total Lys. The differences in total amino acid content between the FWDDGS and the conventional DDGS (NRC, 2014) was greatest for Lys. Thus, part of the large difference for Lys could be associated with how the DDGS was dried. Excessive heating during drying can destroy Lys (Martinez and Parsons, 2007), suggesting that the drying conditions for the FWDDGS may have been less severe than those for conventional DDGS. Another possible contributing factor to the higher total Lys content of the FWDDGS is that the raw materials (mashed potatoes, sweet corn, and white bread) may have had a higher Lys content than corn which is the raw material for the DDGS included in the NRC (1994) table. The amino acid standardized digestibility values for the FWDDGS did not show any consistent pattern of differences when compared with NRC (1994) table values. When examining some of the most important amino acids in poultry diet formulation, standardized digestibility values for Lys and Thr were not different, standardized digestibility values for Met and Cys were decreased, and the standardized digestibility value for Val was increased compared with the NRC (1994) values for DDGS. When the total amino acid concentrations in the FWDDGS were multiplied by the standardized digestibility values, the resulting digestible amino acid concentrations were generally not different than or often higher

than the values calculated from the NRC (1994) DDGS table values, due to the higher total amino acid levels in FWDDGS. Overall, the results of the current study indicate that the protein or amino acid value of DDGS produced from food waste is equal to or superior to conventional DDGS that is produced from corn.

The DDGS produced from a higher protein mutant corn hybrid (FMo2C28) yielded a product with increased CP and much higher levels of total amino acids compared with its control hybrid (FM) (Table 3.3). For example, the total Lys content of the FMo2C28 mutant DDGS was 1.60% compared with 1.05% for the FM control DDGS for the conventional processing method. The modified processing method had little to no effect on total amino acid levels in the FM control and FMo2C28 DDGS products. When comparing amino acid standardized digestibility values for the DDGS from the two corn hybrids, there was no significant ($P < 0.05$) difference between the samples for many amino acids when the conventional processing method was used. However, the standardized digestibility values for several other amino acids were decreased ($P < 0.05$) for the FMo2C28 mutant DDGS compared with the FM control DDGS. For the modified processing method, there were no differences in amino acid standardized digestibility values between the two hybrids for most of the amino acids, with the notable exceptions that Lys standardized digestibility was substantially decreased for the FM control hybrid and the opposite was observed for Trp standardized digestibility. There was no consistent effect of processing method on amino acid standardized digestibility values for the FMo2C28 and FM DDGS. Due to the higher CP and total amino acid content, the digestible amino acid concentrations were generally much higher for the FMo2C28 mutant DDGS than for the FM control DDGS. The latter differences were observed for both the conventional and modified processing methods.

For the two DDGS samples produced from the other corn hybrid, the FRHo2C28 DDGS had similar CP values compared with its control hybrid, FRH. The levels of total amino acids for the FRHo2c28 mutant DDGS were sometimes less than the FRH control DDGS (Table 3.3). For example, the total Met content of the FRHo2C28 mutant DDGS was 0.49% compared with 0.60% for the FRH control DDGS for the conventional processing method and the levels of Leu and Phe were also much lower in the DDGS from the FRHo2C28. Fortunately, the latter two amino acids are normally present at levels in excess of the requirements in poultry diets and thus, are not usually important economically. However, the total amino acid levels for some other amino acids such as Lys, Arg and Trp were increased for the FRHo2C28 mutant DDGS compared with the FRH control DDGS. As observed earlier for the FMo2C28 DDGS products, the modified processing method had little to no effect on total amino acid levels for FRHo2C28 or FRH DDGS. When comparing the amino acid standardized digestibility values for the DDGS from the two corn hybrids, there were only a few significant ($P<0.05$) differences between FRH control DDGS and FRho2C28 mutant DDGS when the conventional processing method was used. For the modified processing method, there were only two significant ($P<0.05$) differences between FRH control DDGS and the FRHo2C28 mutant DDGS. Using the latter process, Lys standardized digestibility was significantly increased ($P<0.05$) in the mutant DDGS compared with the control DDGS, whereas the opposite effect was observed for Leu. Due to the similarities in CP content and amino acid standardized digestibility values between the FRH control DDGS and the FRHo2C28 mutant DDGS, differences in digestible amino acid concentrations between the two types of DDGS were mainly related to their differences in total amino acid values. For example, the DDGS from the FRHo2C28 mutant contained increased levels of digestible Lys, Arg, and Trp than the DDGS from the FRH control.

The final product tested was SP50. The CP, gross energy, and TME_n values for SP50 are presented in Table 3.4. The TME_n of SP50 was determined to be 3372 kcal/kg on a DM basis, which is slightly higher than the mean TME_n value of 3279 kcal/kg DM reported by Batal and Dale (2006) for conventional DDGS. The values in the latter study ranged from 2895 kcal/kg to 3709 kcal/kg on a DM basis among DDGS samples. The TME_n value of 3372 kcal/kg DM is not different than the value of 3330 kcal/kg DM according to the NRC (1994). The value determined for CP was 53% on a DM basis which is significantly greater than the CP value reported for conventional DDGS. The protein content of DDGS on a DM basis has been reported by Spieths et al. (2002) to be 30.2%. Belyea et al. (2004) reported a crude protein value of 31.3%, and Kim et al. (2008) averaged several samples and found the CP to be 30.7%, and the NRC (1994) reported a value of 29.5% CP on a DM basis. As expected, due to the high CP level of SP50, this product had increased total levels of all amino acids compared to those previously reported in the NRC (1994) (Table 3.5). For example, Lys in conventional DDGS is listed at 0.81% on a DM basis in the NRC (1994), whereas the SP50 contained 2.22% total Lys on a DM basis. The amino acid standardized digestibility values for SP50 were generally high (84-94%). These values are substantially higher than most amino acid standardized digestibility values reported by the NRC (1994). Notably, the amino acid standardized digestibility value for Lys in SP50 was 84% compared with 65% for conventional DDGS (NRC, 1994). Due to both the increased total amino acid levels and the increased amino acid standardized digestibility values, digestible amino acid concentrations were calculated for SP50 to be much higher than conventional DDGS (NRC, 1994). Overall, the results of the current study indicate that the nutritional value of SP50 is superior to conventional DDGS that is typically produced from corn.

LITERATURE CITED

- AOAC International. 2007. Official methods of analysis. 18th ed. Rev. 2. AOAC Int., Gaithersburg, MD.
- Batal, A. B., and N. M. Dale. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *Journal of Applied Poultry Research* 15:89-93.
- 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. *Bioresource Technology*. 94:293-298.
- Carmer, S. G., and W. M. Walker. 1985. Pairwise multiple comparisons of treatment means in agronomic research. *Journal of Agronomy Education*. 14:19-26.
- Haung, H., N. Qureshi, M. Chen, W. Liu, and V. Singh. 2015. Ethanol production from food waste at high solids content with vacuum recovery technology. *Journal of Agriculture and Food Chemistry* 63:2760-2766.
- Kim, Y., N. S. Mosier, R. Hendrickson, T. Ezeji, H. Blaschek, B. Dien, M. Cotta, B. Dale, and M. R. Ladisch. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. *Bioresource Technology*. 99:5165-5176.
- Martinez, A. M., and C. M. Parsons. 2007. Effect of increased heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles (DDGS). *Poultry Science*. 86:331-337.
- National Research Council. 1994. *Nutrient Requirements of Poultry*, Ninth Revised Edition. Washington, D.C.: National Academy Press.

- Parsons, C. M., C. Martinez, V. Singh, S. Radhakrishnan, and S. Noll. 2006. Nutritional value of conventional and modified DDGS for poultry. Multi-State Poultry Nutrition and Feeding Conference.
- Ramchandran, D., S. P. Moose, K. Low, J. Arp, C. M. Parsons, and V. Singh. 2017. Ethanol yields and elevated amino acids in distillers dried grains with solubles from maize grain with higher concentrations of essential amino acids. *Industrial Crops and Products*. 103:244-250.
- 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. *Journal of Animal Sciences*. 80:2639-2645.
- United States Department of Agriculture (USDA). Bioenergy Statistics; USDA; Washington, D.C., 2013; www.ers.usda.gov/data-products/us-bioenergy-statistics.asp#.VCK0tNdFOXd (accessed June 22, 2017).
- United States Environmental Protection Agency (U.S. EPA). Food Waste Basics; U.S. EPA: Washington, D.C., 2014; www.epa.gov/foodrecovery/ (accessed June 22, 2017).

TABLES

Table 3.1 Crude protein, gross energy, and true metabolizable energy values for food waste dried distillers grains with solubles (DM basis).

Crude protein (%)	Gross energy (kcal/kg)	TME _n (kcal/kg) ¹	SEM ²
27.3	5493	3890	48

¹ The TME_n value is a mean of 8 individually-caged conventional roosters.

² SEM for TME_n value.

Table 3.2 Total amino acids, amino acid standardized digestibility values, and digestible amino acid concentrations of food waste dried distillers grains with solubles (DM basis) (%)¹

Amino acid	Total	Digest. value	Digest. conc. ²	Pooled SEM ³
Met	0.49	75.47	0.37	1.87
Cys	0.56	70.09	0.39	3.02
Lys	1.45	61.24	0.89	2.85
Thr	1.18	70.08	0.81	2.71
Val	1.56	72.18	1.13	2.23
Arg	1.25	76.64	0.96	2.09
Ile	1.30	73.53	0.95	2.04
Asp	2.07	67.45	1.39	2.62
Ser	1.36	72.25	0.99	2.46
Glu	5.33	82.85	4.42	1.53
Pro	2.32	86.04	1.99	1.37
Ala	1.50	68.68	1.03	2.44
Leu	2.57	79.52	2.04	1.65
Tyr	1.10	77.65	0.85	1.90
Phe	1.40	78.98	1.10	1.71
His	0.76	77.71	0.59	1.77
Trp	0.31	83.79	0.26	2.58

¹ Standardized digestibility values are means of 8 individually-caged cecectomized roosters.

² Digestible concentrations = total × standardized digestibility value.

³ Pooled SEM for standardized digestibility values.

Table 3.3 Total amino acids, amino acid standardized digestibility values, and digestible amino acid concentrations for distillers dried grains with solubles produced from two different mutant corn hybrids and their controls and produced by either a conventional process or modified granular starch hydrolyzing enzyme (GSHE) process (as-fed basis) (%)

	Conventional process						Modified (GSHE) process						
	FM (Control)			FMo2C28 (Mutant)			FM (Control)			FMo2C28 (Mutant)			
Amino acid	Total	Digest value	Digest. conc. ¹	Total	Digest value	Digest conc.	Total	Digest value	Digest conc.	Total	Digest value	Digest conc.	Pooled SEM ²
Met	0.51	87.32 ^a	0.45	0.54	83.52 ^{dc}	0.45	0.47	80.08 ^e	0.38	0.49	80.31 ^e	0.39	0.95
Cys	0.57	84.44 ^a	0.48	0.72	80.40 ^a	0.58	0.54	81.24 ^a	0.44	0.70	81.27 ^a	0.57	2.92
Lys	1.05	72.70 ^{ab}	0.76	1.60	74.02 ^a	1.18	1.06	61.83 ^c	0.66	1.60	74.04 ^a	1.19	2.39
Thr	1.08	80.81 ^a	0.87	1.32	77.61 ^{ab}	1.02	1.08	72.45 ^b	0.78	1.36	78.55 ^{ab}	1.07	2.34
Val	1.42	82.72 ^a	1.17	1.84	80.15 ^a	1.47	1.36	73.38 ^b	1.00	1.82	80.52 ^a	1.47	1.83
Arg	1.22	90.79 ^{ab}	1.11	2.07	90.69 ^{ab}	1.88	1.18	85.51 ^c	1.01	2.06	90.77 ^{ab}	1.87	1.18
Ile	1.14	84.61 ^a	0.96	1.27	80.24 ^{bc}	1.02	1.11	76.63 ^c	0.85	1.29	80.93 ^{ab}	1.04	1.30
Asp	1.80	81.35 ^a	1.46	2.28	78.46 ^a	1.79	1.76	73.15 ^b	1.29	2.29	77.59 ^a	1.78	1.35
Ser	1.28	87.23 ^a	1.12	1.47	81.89 ^{bc}	1.20	1.25	79.30 ^c	0.99	1.49	83.49 ^{abc}	1.24	1.74
Glu	3.97	89.90 ^a	3.57	4.36	86.91 ^{bc}	3.79	3.85	85.01 ^c	3.27	4.28	86.01 ^c	3.68	0.88
Pro	2.23	92.14 ^a	2.05	2.33	89.08 ^a	2.08	2.11	88.23 ^a	1.86	2.27	88.58 ^a	2.01	1.31
Ala	1.97	88.38 ^a	1.74	2.14	84.39 ^{bc}	1.81	1.96	82.44 ^c	1.62	2.13	84.21 ^{bc}	1.79	1.05
Leu	3.24	91.22 ^a	2.96	2.97	85.97 ^b	2.55	3.04	86.86 ^b	2.64	2.80	86.21 ^b	2.41	0.79
Tyr	0.82	87.74 ^a	0.72	0.96	84.95 ^{abc}	0.82	0.83	79.84 ^c	0.66	0.90	82.28 ^{bc}	0.74	1.72
Phe	1.31	88.41 ^a	1.16	1.43	84.91 ^{bcd}	1.21	1.23	81.81 ^e	1.01	1.33	84.38 ^{cde}	1.12	1.01
His	0.83	80.00 ^a	0.66	1.12	79.23 ^a	0.89	0.80	75.76 ^a	0.61	1.16	81.20 ^a	0.94	2.10
Trp	0.20	95.07 ^a	0.19	0.34	71.79 ^e	0.24	0.18	88.66 ^{abc}	0.16	0.33	74.99 ^{de}	0.25	3.06
CP	27.75			34.00			28.00			33.81			

^{a-e} Standardized digestibility values within the same row with no common superscripts are significantly different (P<0.05).

¹ Digestible concentrations=total × standardized digestibility values.

² Pooled SEM calculated for standardized digestibility values.

Table 3.3 Cont.

Table 3.3 Cont.

Conventional process							Modified (GSHE) process						
FRH (Control)				FRHo2C28 (Mutant)			FRH (Control)				FRHo2C28 (Mutant)		
Amino acid	Total	Digest value	Digest. conc. ¹	Total	Digest value	Digest conc.	Total	Digest value	Digest conc.	Total	Digest value	Digest conc.	Pooled SEM ²
Met	0.60	86.86 ^{ab}	0.52	0.49	82.43 ^{de}	0.40	0.61	86.18 ^{abc}	0.53	0.50	84.38 ^{bcd}	0.42	0.95
Cys	0.57	79.78 ^a	0.45	0.60	79.76 ^a	0.48	0.55	83.65 ^a	0.46	0.61	83.03 ^a	0.51	2.92
Lys	1.02	67.58 ^{abc}	0.69	1.40	71.27 ^{ab}	1.00	1.04	65.67 ^{bc}	0.68	1.42	73.58 ^a	1.04	2.39
Thr	1.17	77.25 ^{ab}	0.90	1.23	77.97 ^{ab}	0.96	1.16	78.14 ^{ab}	0.91	1.23	78.82 ^{ab}	0.97	2.34
Val	1.51	80.20 ^a	1.21	1.64	79.91 ^a	1.31	1.51	80.65 ^a	1.22	1.65	81.45 ^a	1.34	1.83
Arg	1.20	87.79 ^{bc}	1.05	1.64	90.16 ^{ab}	1.48	1.20	88.92 ^{abc}	1.07	1.65	91.78 ^a	1.51	1.18
Ile	1.24	82.39 ^{ab}	1.02	1.16	81.08 ^{ab}	0.94	1.24	81.88 ^{ab}	1.02	1.18	81.86 ^{ab}	0.97	1.30
Asp	1.96	79.04 ^a	1.55	2.12	78.64 ^a	1.67	1.95	79.34 ^a	1.55	2.13	80.22 ^a	1.71	1.35
Ser	1.45	84.78 ^{ab}	1.23	1.33	82.40 ^{abc}	1.10	1.43	85.36 ^{ab}	1.22	1.33	83.74 ^{abc}	1.11	1.74
Glu	4.76	90.00 ^a	4.28	3.52	85.72 ^c	3.02	4.69	89.16 ^{ab}	4.18	3.57	86.86 ^{bc}	3.10	0.88
Pro	2.35	90.76 ^a	2.13	2.15	89.40 ^a	1.92	2.36	91.04 ^a	2.15	2.15	90.46 ^a	1.94	1.31
Ala	2.29	87.88 ^a	2.01	1.94	83.65 ^c	1.62	2.28	86.92 ^{ab}	1.98	1.94	84.33 ^{bc}	1.64	1.05
Leu	3.82	91.30 ^a	3.49	2.35	85.18 ^b	2.00	3.79	90.97 ^a	3.49	2.37	85.75 ^b	2.03	0.79
Tyr	1.01	88.11 ^a	0.89	0.81	81.94 ^c	0.66	0.99	87.35 ^{ab}	0.86	0.80	83.31 ^{abc}	0.67	1.72
Phe	1.51	87.51 ^{ab}	1.32	1.18	83.67 ^{de}	0.99	1.50	87.15 ^{abc}	1.31	1.19	84.91 ^{bcd}	1.01	1.01
His	0.80	78.16 ^a	0.63	0.86	77.05 ^a	0.66	0.82	78.32 ^a	0.64	0.90	79.82 ^a	0.72	2.10
Trp	0.20	91.18 ^{ab}	0.18	0.29	80.25 ^{cde}	0.23	0.20	91.92 ^{ab}	0.18	0.29	83.78 ^{bcd}	0.24	3.06
CP	30.44			30.50			30.87			30.18			

^{a-e} Standardized digestibility values within the same row with no common superscripts are significantly different (P<0.05).

¹ Digestible concentrations=total × standardized digestibility values.

² Pooled SEM calculated for standardized digestibility values.

Table 3.4 Crude protein, gross energy and true metabolizable energy values for Still Pro™ (DM basis)

Crude protein (%)	Gross energy (kcal/kg)	TME _n (kcal/kg) ¹	SEM ²
53.0	5335	3372	125

¹ The TME_n value is a mean of 4 individually-caged conventional roosters.

² SEM for TME_n value.

Table 3.5 Total amino acids, amino acid standardized digestibility values, and digestible amino acid concentrations for Still Pro™ (DM basis) (%) ¹

Amino acid	Total	Digest. value	Digest. conc. ²	Pooled SEM ³
Met	1.05	92.41	0.97	0.49
Cys	0.90	86.63	0.78	1.23
Lys	2.22	83.62	1.86	0.99
Thr	2.06	86.44	1.78	1.00
Val	3.08	86.90	2.67	0.79
Arg	2.49	94.02	2.34	0.56
Ile	2.24	90.02	2.00	0.64
Asp	3.62	87.15	3.15	0.85
Ser	2.25	89.40	2.00	0.86
Glu	7.61	93.03	7.08	0.55
Pro	3.46	92.52	3.20	0.64
Ala	3.51	90.67	3.18	0.59
Leu	5.80	93.79	5.44	0.42
Tyr	2.08	91.80	1.91	0.59
Phe	2.67	91.99	2.46	0.54
His	1.41	91.59	1.29	0.58
Trp	0.45	86.88	0.38	0.69

¹ Standardized digestibility values are means of 4 individually-caged cecectomized roosters.

² Digestible concentrations=total × standardized digestibility value.

³ Pooled SEM for standardized digestibility values.