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THE EVALUATION OF DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) AS AN ALTERNATIVE FEED INGREDIENT IN POULTRY DIETS

By

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In times of economic hardship, everyone must find ways to adapt, and the poultry industry is no exception. The majority of the cost in an integrated poultry operation is feeding the birds. Distillers dried grains with solubles (DDGS) has been around for decades as a by-product of the beverage industry and more recently as a co-product of the ethanol industry. Over the past decade, there has been a dramatic increase in the production of DDGS from U.S. ethanol biorefineries, making the co-product a very economical choice. DDGS have not held a common place in the poultry industry for long though, as issues with nutritional variability, storage, transportation, etc. all have led to the product being avoided by nutritionists much of the time. Even now, DDGS are fed at a relatively low inclusion level compared to other major feed ingredients such as corn, wheat and soybean meal.

With the often volatile price of corn grain, DDGS are receiving more attention as a feasible alternative in commercial poultry diets as research, such as that presented in this dissertation, continues to elucidate the nutritional, economical and dietary inclusion aspects of this once neglected ingredient. The primary purpose of this research has been

to determine the efficacy of DDGS as an ingredient in poultry diets, and also to further elaborate on the suitable inclusion rates in a ration. In the end it is clear that DDGS are an effective and suitable choice for inclusion into both broiler and layer diets. DDGS can be incorporated into layer diets at up to one-third of the ration with no deleterious effects on performance or egg quality. DDGS can be added to broiler diets at varying inclusion levels, depending on bird age, as the research points towards increased tolerance of the co-product as the bird ages, without harming bird health or performance. It is also shown that further processing of DDGS, primarily fiber separation, can have a positive effect on bird performance. In conclusion, DDGS inclusion in poultry rations is a sound choice provided attention is paid to the nutritional profile of this co-product.

DEDICATION

I would like to dedicate this research to those who were there and always supportive of what I did for no reason other than their love for me.

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INTRODUCTION

In today's economic volatility, everyone must find ways to cut costs and adapt to the changes brought about by our ever-changing economy. This is true for pretty much every person, business, organization, etc. and the poultry industry is no exception. As any poultry scientist knows, the majority of the cost in an operation is feeding your birds. This is where the co-product known as distillers dried grains with solubles (DDGS) can offer interesting attributes. For decades, DDGS was primarily available as a by-product of the beverage industry, but has gained more prominence as a co-product of the ethanol industry during the last decade. This has been a direct result of a dramatic increase in the production of DDGS from U.S. corn-derived ethanol biorefineries. DDGS have long been fed to the ruminant animal because their digestive system could so easily digest the product. However, in the commercial poultry industry, DDGS have not held a common place in the feed mills that long. Currently, DDGS is still fed at relatively low levels in most cases due to concerns of nutritional variability, among other things. With the increased attention given to DDGS over the last few years, these concerns are slowly disappearing as more ethanol producers are paying close attention to the quality of this co-product. However, issues still exist with feed manufacturing aspects of the product as it tends to create decreased physical quality of the industry standard pelleted diets.

As is well known, corn prices can be unstable and often volatile. This has led to DDGS having more use in commercial poultry diets as economics, more often than not,

are the primary factor that dictates the dietary inclusion of this ethanol co-product. The fact that there are a few companies researching different processing techniques and methods to provide a consistent and nutritional DDGS product is also helping to increase the inclusion rates. It is a likely assumption that DDGS are here to stay and are not just a craze that will disappear when grain prices drop, because ethanol can be an ingredient in gasoline, and as we have all seen it is listed as a component on many gas pumps. As long as there is ethanol production on a large scale there will be DDGS availability, and its value, both economically and nutritionally, cannot be ignored. Also, poultry integrators turning to DDGS will likely result in DDGS producers paying more attention to this co-product, and producing a more uniform and reliable DDGS product in a nutritional sense.

It is clear that DDGS is already an accepted ingredient in the poultry industry, but more research needs to be conducted to ensure industry confidence when it comes to including the co-product at higher rates. As with any dietary constituent, it is also necessary to determine a standard limit of inclusion. The following chapters will further explore the effects of varying inclusion levels and processing techniques of DDGS in commercial poultry diets. Results presented will not be limited to growth and performance, but will include effects on digestive organs, as well as the gastrointestinal (GI) tract environment. Some attention is also given to the effects that DDGS in the diet may have on feed manufacturing efficiency as well as the final product that a consumer would purchase.

CHAPTER II

REVIEW OF LITERATURE

The Process

Distillers dried grains with solubles (DDGS) are simply corn after being depleted of the starch in the endosperm. To produce ethanol from corn grain the producer grinds the corn, adds water and then "cooks" this mixture. Once the starch has been gelatinized, as a result of the heating, enzymes are added to cleave glucose from the long starch chains, and yeast is added for the fermentation process. The fermented mixture is distilled to yield the desired ethanol, and the leftover portion of the corn grain is dried. Once this is complete, the DDGS co-product now contains a higher concentration of crude protein, fiber, etc. and lower energy content, due to the loss of the starch, compared to the corn grain. There are new technologies however, that allow for the production of ethanol and the resultant DDGS co-product while totally removing the "cooking" step. The DDGS produced as a result of this process possess improved physical characteristics that are important to the poultry industry, such as increased pelletability and flowability (Gibson and Karges, 2006). When marketing the DDGS to poultry producers, the ethanol producers will dry the product, hence the name, as opposed to when the distiller's grains are marketed to ruminant producers and are often fed "wet". It is in this drying process that many ethanol producers neglect the DDGS product as many of the issues associated with nutritional variability in the product have been attributed to the producers' drying techniques.

Composition and Variability

DDGS has had a long history of variability and inconsistency where nutrient profiles are concerned. In 1993, Cromwell et al. evaluated the physical characteristics, chemical composition, and nutritional value of DDGS samples obtained from seven beverage alcohol and two fuel ethanol plants. The scientists stated that color scores, nutrient measures, and even odor varied widely among the samples. The research continued with the feeding of diets containing DDGS from the nine different locations in growth trials for both chicks and pigs. In both species weight gain, feed intake, and feed/gain were affected by the source of the DDGS. In the end, the researchers concluded that there is great variability in the physical, chemical and nutritional characteristics of DDGS across the different sources available to commercial livestock producers.

It is widely accepted that lysine, the second limiting amino acid (AA) in poultry production, is the AA of primary concern where DDGS are being used at any significant level in the diet. This is so because of lysine's importance as the second limiting AA and also because Cromwell et al. (1993) suggested that as a heat sensitive AA, lysine availability can be decreased as a result of Maillard reactions that occur as a result of the processing and/or drying conditions that DDGS endure. Pahm et al. (2009) compared the true digestible (measured using cecectomized roosters) and relative bioavailable (measured using a standard curve generated from 9 day chick weight gain) lysine levels in seven different DDGS samples. The researchers also obtained color scores for lightness (L), redness (a), and yellowness (b) for each sample. The mean standardized digestible lysine value was 61.4% while the mean relative bioavailability was found to be 69%. There were no differences in true digestible lysine and the concentration of bioavailable lysine for five of the seven samples tested. Greater L scores were associated

with an increased concentration of bioavailable lysine, and the researchers concluded that the L score of a DDGS sample may be used to estimate its' bioavailable lysine content. Furthermore, Pahm et al. (2009) reported that the concentration of true digestible lysine does not overestimate the bioavailable lysine levels in DDGS for poultry.

In research pertaining to the mineral composition of DDGS, Batal and Dale (2003) analyzed twelve samples of commercial DDGS for mineral composition. It is worth noting that in general the expected level of most minerals in DDGS is simply a 3-fold increase of the levels in corn grain. Batal and Dale (2003) report this because approximately two-thirds of the weight of corn is converted to either carbon dioxide or ethanol during the fermentation process. Thus, the unfermented portions, such as the minerals, should be increased roughly 3-fold. The most surprising result seen was for sodium content, where the values ranged from 0.09 to 0.44% and averaged roughly 0.23%. Calcium and sulfur also revealed ranges that differed greatly from the NRC (1994) values, while in general the other minerals assessed agreed with projected values based on a 3-fold increase. As is the expected recommendation, the researchers suggest that the mineral content of the DDGS needs to be determined on an individual supplier basis, prior to attempting to balance rations for poultry.

Batal and Dale (2006) obtained seventeen DDGS samples from 6 different ethanol plants in the Midwestern United States over a period of two years for analysis of true metabolizable energy (TME_n) as well as total and digestible AA. Color of each sample was also measured. The researchers stated that considerable differences were found among the samples concerning true AA digestibility. After comparing these results with those of the color scores, the researchers concluded that DDGS samples that were more yellow and lighter in color had undergone less heat damage and thus had higher

total and digestible AA levels. This also led the researchers to recommend confirmatory analysis on samples of DDGS from new suppliers, prior to feeding, due to the variations seen in both TME_n and AA digestibility.

In support of the work done by Batal and Dale (2006), Fastinger et al. (2006) discovered that a color score can be a useful tool in determining nutritional quality of DDGS. Once again, the samples tested were from a number of Midwestern United States ethanol plants and were evaluated for AA, color score and TME_n. These researchers concluded that once the color score reached a lightness between 28 and 34, that AA availability and true metabolizable energy levels may be negatively affected. They also noted that the reduction in AA availability was extremely evident for Lysine, which as stated, is the AA of primary concern in DDGS. Fastinger et al. (2006) went on to suggest that since the darkest colored DDGS sample was also the sample with the lowest digestibility, the dark color may indicate overheating during the drying process. As a result of the overheating, the researchers felt that Maillard reaction may have occurred to a greater extent and thus caused the AA availability and TME_n reductions.

If there was any doubt that the heating of the DDGS product was what was causing the reduced AA availability and digestibility, those doubts were put to rest by Amezcua and Parsons (2007). In a study conducted to test the effects of heat processing and particle size on the phosphorus availability of DDGS, the researchers found that the increased heating did in fact increase relative phosphorus bioavailability. However, they also noted that the increased heating led to reduced AA digestibility, particularly for lysine. Also, Belyea et al. (2004) investigated the relationship between the composition of corn grain and the composition of the resultant DDGS from that of corn grain after it was processed to yield ethanol. The authors obtained their corn and DDGS samples from

a dry grind ethanol plant over a five year period. Corn samples were analyzed for dry matter, protein, fat, crude fiber, and starch while DDGS samples were analyzed for dry matter, fiber, fat, starch, lignocellulose, and ash. Upon completion of their experiment, the authors reported that variation in the composition of the DDGS was not related to variation in the corn grain. The researchers suggested that variation in the processing of the corn to DDGS caused the variation in the product and thus the actual processing technique is what must be modified.

Feed milling and Physical Characteristics

As the use of DDGS in livestock diets becomes more common place, it becomes necessary to more thoroughly understand the physical characteristics of the product. By understanding the physical qualities of any feed ingredient, a producer will be more able to understand and deal with any issues that occur with storage, transport and milling. In 2006, Rosentrater performed extensive analysis on DDGS samples from six different dry grind ethanol plants in South Dakota. Several physical characteristics of the product were measured, including moisture content, color score, bulk density, angle of repose, etc. He was able to conclude that DDGS have physical properties similar to that of corn gluten feed, hominy feed, and other-corn based feeds.

While it is imperative to understand the physical properties of any feed ingredient that will be used at a significant level in a large scale feed production system, an understanding of these properties doesn't mean you can necessarily prevent problems associated with the physical characteristics. Behnke (2007) suggested that when DDGS exceeds 5-7% of a pelleted diet that both pellet throughput and pellet quality will suffer. The reasoning behind this is that since starch is generally involved with pellet binding,

via starch gelatinization, the very low levels of starch in DDGS leads to poor binding. Behnke (2007) goes on to suggest that since DDGS can be higher in oil content, compared to the grains they are replacing, this oil can coat the feed particles and with its' hydrophobic properties, disrupt particle binding. In agreement with the aforementioned suggestions, Min et al. (2009) reported an increase in the amount of fines found in a diet as DDGS concentration increased. A trend towards increasing fines usually mirrors a trend of decreasing pellet quality. Wang et al. (2008) also noted reduced bulk density and pellet quality with higher levels of DDGS inclusion and suggested this as a possible cause for reduced broiler performance.

Processing Techniques

In an attempt to develop a more nutritious and/or consistent DDGS product, different processing techniques have arose that yield a different kind of DDGS. One such process is the bio-refining process described by Gibson and Karges (2006). In this process, the corn grain is milled into different fractions before undergoing any other processing such as the fermentation associated with ethanol production. As a result, the different fractions can be directed to different production areas. The portion that has the highest corn starch concentration, thus primarily endosperm with much of the bran and germ removed, is what actually goes into the fermenting areas. The resultant product is what Gibson and Karges (2006) refer to as a "true DDG" and is reported to have high protein and AA levels along with low fat and phosphorus levels.

A modified dry milling process that yields what is known as a high protein distillers dried grain (HP-DDG) has received some attention in recent years. The process that produces this HP-DDG is essentially the same as the process described by Gibson

and Karges (2006), as the resultant product has a higher protein content. In 2008, Kim et al. evaluated the phosphorus bioavailability, TME_n, and AA digestibility of some HP-DDG. Tibia ash results from chick studies were used to determine phosphorus bioavailability, while cecectomized roosters were used to determine AA digestibility and TME_n. The HP-DDG yielded significantly lower values for TME_n when compared to those of the conventional DDGS while both treatments yielded similar AA digestibility results. Overall, the HP-DDG contained lower levels of phosphorus versus the conventional DDGS, and after the bioavailability coefficient was multiplied by the total phosphorus level, it was also found that the HP-DDG had a decreased level of bioavailable phosphorus compared to the conventional DDGS.

In another study on HP-DDG, Applegate et al. (2009) first determined the nutrient digestibility of HP-DDG which had a stated crude protein value of 54%. Analysis yielded an AME_n of 2,526 kcal/kg and standardized ileal digestibilities of 73.0, 84.9, and 73.0% for lysine, methionine, and threonine, respectively. A second study was then conducted where Applegate et al. (2009) utilized the AA and AME_n results from the first study. The researchers compared a standard industry diet regimen to that of either an approximate 25 or 50% replacement for the level of soybean meal (48% CP) inclusion in the diet. Replacement of up to 50% of the soybean meal inclusion with the HP-DDG had no negative effects on performance at 14 or 42 days of age or breast yield at 42 days. There was a decreased body weight gain and increased feed conversion ratio observed from 14 to 28 days of age. Also, birds that received the 50% replacement of soybean meal with the HP-DDG consumed more nitrogen and fiber versus the industry regimen, and as a result, excreted 31.8% more nitrogen and 21.9% more dry matter than the birds that were fed the industry regimen. Therefore, while HP-DDG may feasibly constitute a large

percentage of the diet without producing and deleterious effects on bird growth or yield, it will result in more manure and manure nitrogen.

In a less explored processing technique, Oryschak et al. (2010) tested the effects of extrusion on both wheat and corn DDGS. Two experiments were conducted with both extruded and non-extruded wheat and corn DDGS. Their results pertain to both wheat and corn DDGS and thus a distinction is not made between the results of each respective grain. The first experiment was done to determine apparent ileal digestibility coefficients of energy and nutrients of the test ingredients. The second study was a 42 day grow-out that assessed the performance of birds fed either 0, 5, or 10% DDGS. Experiment one results revealed that twin screw extrusion increased the apparent ileal digestibility of AA in DDGS by 10 – 34% when compared to non-extruded DDGS. Experiment two led the researchers to conclude that DDGS can be fed at up to 10% of the diet without any deleterious effects on growth performance or breast meat yield.

Another procedure entails modifying the DDGS co-product after the fermentation procedure is long past and the DDGS has been dried. The process involves a combination of air classification and sieving to remove a portion of the fiber component from the DDGS, and is known as the Elusieve procedure. In 2007, Martinez-Amezcua et al. reported that the Elusieve procedure increased the protein, AA, and fat content of DDGS, while decreasing the total dietary fiber from 34.5 to 19.7%. The process had no effect on the AA digestibility of DDGS. Kim et al. (2010) studied the effects that the Elusieve process had on the nutritional characteristics of DDGS. Compared with conventional DDGS, the researchers stated that the Elusieve process produced a DDGS product with an increased protein content and TME_n. As would be expected, the higher fiber fractions obtained from the Elusieve process exhibited lower protein, AA concentration and

digestibility, and TME_n when these values were compared to those for the lower fiber fractions of the Elusieve process. The authors concluded that the Elusieve procedure can be used to increase the nutritional value of conventional DDGS for poultry.

Commercial Layers

One particular sector of commercial poultry production that has seen a considerable amount of DDGS related research is the area of commercial laying hens. This is likely because laying hens in general require less energy in the diet, although balanced micronutrients are very important, and the diets are fed in mash form so all of the issues associated with DDGS and pellet production are not a concern. In 1965, Matterson et al. conducted two trials with Leghorn type birds fed DDGS at up to 20% of the ration. The results indicated no significant differences in either of the trials, between the DDGS and non-DDGS containing diets. The researchers also provided extra lysine in their second trial and found no performance improvements and thus concluded that the DDGS used had sufficient available lysine for laying hens. In their overall conclusion the researchers state that DDGS is an acceptable component of a laying ration, and that DDGS can be used to comprise up to one-third of the gross protein content. Harms and colleagues (1969) chose to evaluate DDGS at graded levels of methionine in the diet since methionine (TSAA) is the first limiting AA in poultry rations. The authors included DDGS at 10% of the ration and compared results to a standard corn and soybean meal based ration. They report that the addition of DDGS in the ration did not influence performance, regardless of the level of sulfur AA in the ration. The researchers were able to observe a response in egg production with increasing levels of methionine in the diet.

In conclusion the researchers state that DDGS can be successfully incorporated at a 10% rate, assuming the diet is formulated on the basis of AA content of the DDGS.

Jensen et al. (1978) chose to focus on the potential for DDGS to improve interior egg quality, as measured by Haugh units. Over a course of several experiments involving DDGS, brewers dried grains, and a multitude of trace elements, the researchers tested the effects of these various dietary additions on interior egg quality. These researchers reported that DDGS contain an "element" that will generally lead to improvements in interior egg quality. They further suggest that these "elements" may be trace elements. In more current research, Lumpkins and colleagues (2005) looked at the effects of DDGS inclusion in layer diets as a result of the increased availability of DDGS due to increased ethanol production. The researchers fed either a commercial or low-density layer ration that was formulated to contain either 0 or 15% DDGS for an 18 week period. The results show no differences between hens fed either 0 or 15% DDGS for the majority of the parameters evaluated; however, those hens receiving the 15% DDGS low-density ration had significantly decreased hen-day egg production through 35 weeks of age. Hens that were fed the 15% DDGS commercial density diet showed no statistical differences for the parameters measured when compared to the 0% DDGS commercial density ration, although there was numerically lower egg production to 32 weeks of age for the 15% DDGS fed hens.

The effect that large scale animal agriculture can have on the environment is often a concern of major producers and the general public. The commercial egg producing industry is no exception and that has led researchers such as Roberts et al. (2007a) to investigate the effect of different crude protein and fiber levels on ammonia emissions from laying hen manure. One of the researchers' fiber treatments was 10% DDGS

addition to the ration. The 10% DDGS ration lowered the 7 day cumulative ammonia emission by more than half that of the corn and soybean meal based control diet and lowered the daily ammonia emission rate as well. Roberts et al. (2007b) investigated the effects of dietary crude protein and fiber on nitrogen balance and production of commercial laying hens. Once again, 10% DDGS was one of their fiber treatments, and a corn and soybean meal based diet served as their control for the fiber portion of the study. The DDGS-containing diet resulted in no significant effects for any of the production parameters assessed. The 10% DDGS diet did result in a significant increase in nitrogen consumption, although excretion was unaffected compared to the control diet. In conclusion, Roberts et al. states that diets formulated to contain 10% DDGS had no effect on production or nitrogen excretion.

In further research pertaining to emissions and laying hen performance, Wu-Haan et al. (2010) evaluated the effects of feeding diets containing either 0, 10, or 20% DDGS to laying hens for a period of five weeks, on emission of hydrogen sulfide and ammonia, as well as production. Dietary treatment had no effect on the production parameters measured which included egg production. The diet containing 20% DDGS decreased daily hydrogen sulfide and ammonia emissions by 58 and 24% respectively, when compared to the control diet. It was concluded that 20% DDGS can be fed to laying hens to decrease emissions without any negative effects on the birds' production. Another group of researchers looked at the potential for DDGS to be included in a non-feed-withdrawal molt program for layers. Mejia et al. (2010) fed a 94% DDGS diet at either 36.3, 45.4, or 54.5 g/hen per day to comprise three treatments, while a 94% corn diet was fed at the same levels to comprise three additional treatments. Finally, a 47% corn and 47% soybean hulls diet was fed ad libitum to make the final treatment. The researchers

intended to feed all seven treatments for a 28 day molt period, but the DDGS treatments experienced low intake and thus rapid weight loss, and were consequently switched to a 16% crude protein corn and soybean mash diet on day 19. On day 28 all treatments received an identical corn and soybean meal layer ration for 39 weeks. It is reported that post-molt egg production was higher for hens that received the DDGS diets versus those that received the corn molt diets. The researchers report no other consistent differences in the parameters measured in the postmolt period. In the end they state that it is possible to utilize a DDGS based diet in a non-feed-withdrawal molt program and produce long term postmolt performance similar to that seen with the ad libitum feeding of a corn and soybean hulls diet.

Broilers

Of primary interest is the effect that DDGS inclusion has on the growth and performance of commercial broilers. Work with DDGS containing diets and their effects on commercial broilers has also been the focus of other researchers for several years. Parsons et al. (1983) wished to evaluate the efficacy of DDGS as a protein source in the young chick via a series of trials. The DDGS was being compared to soybean meal for assessment of the protein quality. The authors state that the DDGS was found to have a crude protein level of 28.6% and a 0.72% lysine level, which was also the first limiting AA in the DDGS. Chicks grew more efficiently with the soybean meal versus the DDGS diet, until the DDGS were supplemented with lysine, at which point growth and efficiency became similar to that of the birds fed the soybean meal. In conclusion, the researchers suggest that roughly 20% of the soybean meal in a corn and soybean meal

diet may be replaced by DDGS, and with lysine supplementation, up to 40% could be replaced without major detrimental effects to the chick.

Waldroup and colleagues (1981) determined the effects of high levels of DDGS in broiler diets by feeding the co-product at up to 25% of the ration for 42 days. If the energy level of the diet was held constant for all treatments when formulating, there were no significant differences observed at 42 days for body weight or feed conversion that were attributable to the level of DDGS in the diet. The authors also tested what would happen if they were to allow dietary energy to decline as DDGS increased in the diet, while energy:nutrient ratios were kept constant. In that case, once DDGS reached 15% of the diet, deleterious effects were observed for weight gain and feed efficiency.

More recently, Lumpkins et al. (2004) conducted two experiments to determine the efficacy of feeding DDGS from modern ethanol plants to commercial broilers. The first experiment was a 2 x 2 factorial where diets contained either 0 or 15% DDGS and either a high or low nutrient density. Data indicated that within each of the two density levels, the level of DDGS fed had no significant effects on chick performance. In the second trial conducted, diets were isocaloric and isonitrogenous and contained either 0, 6, 12, or 18% DDGS. Data showed no significant differences in performance or yield over the course of the 42 day study, with the exception of a decreased BW gain and feed efficiency for birds fed 18% DDGS during the starter phase. In the end, the authors suggest that the limitation of DDGS from commercial ethanol plants in the starter phase is around 6% while they estimate between 12 and 15% for the grower and finisher phases.

One research group recently went to extremes of DDGS inclusion in broiler rations. Wang et al. (2008) formulated broiler diets to contain 0, 10, 20, 30, 40, or 50%

DDGS and fed the diets for a period of 49 days. These researchers state that DDGS level had minimal effect on body weight at any age until inclusion levels exceeded 20% DDGS. They observed that feed intake was only affected by DDGS level during the starter phase from 0 to 14 days of age. Increasing DDGS level led to a significantly increased calorie conversion ratio while linearly decreasing dressing percentage. Significant reductions in breast and leg quarter yield were also noted with increasing DDGS levels in the diet, although birds fed higher levels of DDGS had higher wing yields. The authors conclude that if it were an economically sound decision, that DDGS could be fed at up to 30% of the diet. They suggested that the low energy density of the diet is most likely the primary factor in meeting the needs of a chick.

DDGS inclusion in the diet may have effects on the resultant poultry products such as the eggs, meat, etc. Corzo et al. (2009) evaluated the effects of feeding either 0 or 8% DDGS to broilers on meat quality. The researchers reported no differences among the DDGS treatments for the parameters of color, ultimate pH, cook loss, and shear values. For the consumer acceptance data, it was shown that the control (0% DDGS) breast meat was preferred slightly over the meat from birds that were fed 8% DDGS in both the flavor and overall acceptability categories. However, both treatments had scores that fall under the “like moderately” heading on the 9-point hedonic scale and in a difference-from-control test consumers could not differentiate between the meat from birds fed 8% DDGS and those fed no DDGS. DDGS-fed birds had thigh meat with more total polyunsaturated fatty acids and a greater percentage of linoleic acid which may indicate a greater degree of susceptibility to oxidation. Overall, the data points towards both 0 and 8% DDGS diets producing a high-quality broiler meat with minimal differences between the two treatments.

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CHAPTER III

EFFECTS OF FIBER REMOVAL FROM DISTILLERS DRIED GRAINS WITH SOLUBLES ON THE PERFORMANCE AND CARCASS CHARACTERISTICS OF MALE BROILERS

Abstract

This study was conducted to determine the effect of low fiber distillers dried grains with solubles (DDGS) on broiler performance. Recently, a technique known as the Elusieve (ELU) process, a combination of elutriation (air classification) and sieving, was developed to remove some of the fiber component of the DDGS to increase the overall nutritional value of the product. In the ELU process, DDGS are first sieved into four different sizes: large, medium, small and pan. Fiber is removed from the three biggest sizes by air classification, and the product obtained by mixing the remaining material is called “Big DDGS”. The pan material has a lower fiber concentration than DDGS and is called “Pan DDGS”. Four different dietary treatments with 12 replicates each were fed to Ross × Ross 308 male broilers from hatch until 42 d of age. A corn-soybean meal based diet without DDGS served as control (treatment 1); treatment 2 had conventional DDGS included at a concentration of 8% (UMD); treatment 3 consisted of 8% Big DDGS inclusion (PMD), and the fourth treatment consisted of 8% Pan DDGS (ED). Final body weight (BW) was observed to be superior by the birds fed the ED-based diets compared to those weights of birds fed the control diet and the PMD-based diet, while the UMD treatments had intermediate weights. However, contrast analysis shows a significant

difference in BW (P = 0.08) for birds fed ED-based diets when compared to birds fed UMD-based diets. Feed intake was shown to be higher in birds fed ED-based diets compared to those birds fed PMD-based diets. No other differences were found in bird performance or carcass traits. No differences in mortality were observed between the treatments. Results show partial improvement of DDGS via the use of the ELU process judging by a marginal improvement in 42 day BW.

Introduction

As the demand for alternative fuels continues to grow in the wake of major increases in fossil fuel prices, ethanol production has seen an increase. Ethanol production from bio-refineries in the U.S. has increased by over 10 million metric tons in the past seven years (Renewable fuels association, 2007), due in large part to government mandates that make it more profitable for corn producers to sell their corn crop for ethanol production rather than animal feeds. As a direct result of this increase in ethanol production, the feed industry has seen an increase in the availability of DDGS, the major by-product left after the starch has been converted to ethanol. This product is being included in some broiler diets, but the inclusion level of DDGS in commercial poultry diets has been fairly irregular up to this point. There have been field reports of problems with unloading the product from the standard rail cars, trucks and other transporting containers as a result of the product “setting up” over time (Koch, 2006). There is more space required for storage of DDGS, as it has a bulk density lower than that of conventional whole corn (Rosentrater, 2006). Furthermore, nutritional quality and consistency have been identified as issues with DDGS. It has been shown that during the thermal processing certain amino acids necessary for chick growth can be negatively

affected (Parsons et al., 1983). Batal and Dale (2006) reported that DDGS can exhibit large variability in TME_n and amino acid (AA) digestibility among samples from different suppliers. Batal and Dale (2006), along with Fastinger et al. (2006), report that the majority of the variability in the nutritional profiles of samples from different suppliers can be attributed to differences in processing and drying temperature.

With the increasing supply of DDGS, and the increasing costs of feeding broilers, it becomes necessary to address this list of concerns and look for ways to increase overall nutritional characteristics of DDGS. It has been long recognized that broilers do not have the capacity of digesting fibrous carbohydrates as efficiently as cattle or as swine. Thus, a reduced concentration of fiber in DDGS is expected to enable broilers to better digest this feed ingredient. Recently, a technique known as the "Elusieve" (ELU) process, has been reported to help increase the nutritional profile of DDGS (Parsons et al., 2006). In the ELU process, DDGS are first sieved into four different sizes: large, medium, small and pan (Srinivasan et al., 2005). Fiber is removed from the three biggest sizes by air classification and the product obtained by mixing the remaining material is called "Big DDGS". The pan material is a product by itself called "Pan DDGS", because it already has lower fiber, and higher protein, fat, and phosphorus concentrations than in conventional DDGS. The objectives of this study were: 1) compare the performance of broilers fed diets with DDGS at an 8% inclusion level, versus diets without DDGS; 2) measure the effects that DDGS products from ELU processing has on live performance and carcass characteristics of broilers when compared to a conventional corn and soybean meal diet, as well as a diet containing equal amounts of conventional DDGS.

Materials and Methods

General Procedures

This grow-out study encompassed the period between 0 to 42 days of age using Ross × Ross 308 males obtained from a commercial hatchery. Day-old chicks were randomly placed in each of 48 floor pens (12 birds/pen; 576 birds total) at a density of 0.09m^2/bird. The house had thermostatically controlled heating, curtains, cool cells and cross ventilation. Each pen had built-up litter, a hanging feeder (22.5kg capacity) and a water line (3 nipples/pen). The lighting program was 23 h light and 1 h dark and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via *in ovo* administration at day 18), Newcastle disease and infectious bronchitis (via coarse spray at hatch).

Treatments

There were 4 different dietary treatments with each treatment being replicated 12 times. Treatments were blocked completely, according to location within the house. A conventional corn-soybean meal based diet served as the control (treatment 1). Treatments 2, 3 and 4 were DDGS containing diets, with each diet being formulated to have a DDGS inclusion level of 8% during the starter and grower phases (Table 3.1). Treatment 2 represents original and unaltered DDGS (UMD), while treatment 3 represents partially modified DDGS (PMD), also known as "Big DDGS". Treatment 4 represented the diet that contained ELU Pan DDGS (ED), which contained the "Pan DDGS. All DDGS-containing diets were formulated using the same dietary formula, expecting that nutrient composition would vary sufficiently enough as to being able to

detect improvements in performance of diets fed the ELU-obtained DDGS when compared to a diet containing UMD.

Table 3.1 Experimental diet composition[1] (% as-is)

	0-21 d		21-42 d	
Ingredients	C/SBM	+ DDGS	C/SBM	+ DDGS
Corn	60.843	54.338	68.325	61.823
Soybean meal	33.048	30.701	26.021	23.674
Distillers dried grains with solubles (DDGS)	-	8.0	-	8.0
Poultry oil	2.629	3.517	2.258	3.145
Dicalcium phospate	1.776	1.659	1.665	1.548
Calcium carbonate	0.676	0.762	0.716	0.802
NaCl	0.439	0.404	0.443	0.408
Vitamin/Mineral premix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.221	0.207	0.165	0.151
L-Lysine	0.068	0.111	0.106	0.149
Sacox[3]	0.05	0.05	0.05	0.05
Calculated composition				
AME (kcal/kg)	3,100	3,100	3,150	3,150
Ca (%)	0.90	0.90	0.84	0.84
Available P (%)	0.45	0.45	0.42	0.42

[1]C/SBM refers to the control diet and +DDGS is representative of the nutritional composition of all three experimental diets.
[2] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.3 mg.
[3]Dietary inclusion of Sacox provides 60 g salinomycin sodium per 907.2 kg of feed.

The corn-soybean meal diet and the UMD-based diets were formulated to be isocaloric, isonitrogenous, and similar in calcium, phosphorus and all limiting amino

acids. Table 3.1 displays the different dietary treatments used. The "+DDGS" column is representative of the ingredient composition of all DDGS-containing treatments, but nutrient-wise only representative of the UMD diet. Proximate analysis was performed on the experimental diets and values are reflected in Table 3.2 (AOAC, 2006). Standardized digestible amino acid values were determined for the starter phase diets, via the use of precision-fed cecectomized roosters as described by Parsons (1986), and are displayed in Table 3.2. Total amino acid analysis and CP analyses were performed for the grower phase diets (AOAC, 2000). The PMD treatment was not analyzed for nutrient composition because this was an intermediate of treatments between the UMD- and the ED-based diets. All diets met or exceeded current recommendations for nutrients as set forth by the NRC (1994), and feed and water was provided on an ad libitum basis.

Measurements

All birds in each pen were weighed collectively at the beginning and at the end of the study. Pen weight was also obtained at 21 days of age when feed was changed from starter to grower. Feed consumption and mortality were monitored throughout the study and feed conversion was corrected for mortality, and represents grams of feed consumed by all birds in a pen divided by grams of body weight gain per pen. At 42 days of age, 6 birds per pen were randomly selected, tagged, individually weighed and cooped 12hr before processing. Birds were processed at a pilot processing plant

Table 3.2 Analysis of nutrient composition of experimental diets (%)[1]

	0-21 d			21-42 d		
Nutrient	C/SBM	UMD	ED	C/SBM	UMD	ED
	Proximate Analysis Values					
Ash	5.0	5.8	5.4	4.8	4.9	4.9
CP	-	-	-	20.3	19.0	19.1
Fat	3.4	5.4	5.3	5.1	6.1	6.5
Crude fiber	3.3	3.1	3.2	1.8	2.2	2.1
	Standardized Digestible AA values			Total AA Values		
TSAA	0.81	0.90	0.87	0.75	0.74	0.77
Lysine	1.13	1.11	1.10	1.14	1.06	1.07
Threonine	0.70	0.69	0.71	0.75	0.73	0.75
Valine	0.99	1.00	0.96	0.93	0.91	0.93
Isoleucine	0.88	0.88	0.82	0.80	0.77	0.82
Aspartic Acid	1.95	1.87	1.83	1.99	1.86	1.89
Serine	0.87	0.86	0.88	0.98	0.95	0.97
Glutamic acid	3.62	3.59	3.46	3.56	3.48	3.57
Proline	1.14	1.22	1.17	1.13	1.14	1.28
Alanine	1.00	1.08	1.04	1.04	1.07	1.11
Cystine	0.33	0.34	0.35	0.309	0.30	0.31
Methionine	0.48	0.56	0.52	0.46	0.44	0.45
Leucine	1.78	1.90	1.84	1.73	1.78	1.85
Tyrosine	0.70	0.70	0.68	0.56	0.59	0.61
Phenylalanine	1.00	1.01	0.96	1.01	0.99	1.02
Histidine	0.53	0.53	0.53	0.52	0.51	0.52
Arginine	1.32	1.29	1.26	1.29	1.20	1.23
Tryptophan	0.26	0.23	0.23	0.20	0.18	0.19

[1]C/SBM refers to the control diet, UMD refers to the diet containing unmodified dried distillers grains with solubles (DDGS), and ED refers to the diet containing the Pan DDGS.

Electrical stunning was performed by applying 11.5 volts (<0.05 mA, AC to DC current), for 3 sec for each broiler, and broiler carcasses were scalded, picked and eviscerated automatically using commercial prototype equipment. Carcass and abdominal fat weights were obtained as birds were manually removed from the line. Birds were then chilled for 4 h at which point all birds were manually deboned and weights were obtained for breast, wings, thighs and drums. Absolute and relative weights (% of live weight) were determined for wings, drums, thighs, carcass and boneless-skinless breast meat. Occurrence of deep pectoral myopathy in Pectoralis minor muscles was monitored for and recorded. All procedures were approved by the Mississippi State University's Institutional Animal Care and Use Committee.

Statistical Analysis

Data in this experiment were evaluated using analysis of variance in a randomized complete block design with one pen representing an experimental unit. Percentage data for mortality were transformed to arcsine √% for analysis. All data were analyzed by the GLM procedure of SAS (2004) and treatment effects ($P \leq 0.05$) were separated using Tukey's multiple comparisons test option of SAS (2004) using an α of 0.05.

Results and Discussion

Determination of ash, CP, fat and total fiber showed that UMD- and ED-based diets were different in composition when compared to the corn-soybean meal diet (Table 3.2). However, no definitive pattern (increase or decrease) of any of the previously mentioned nutrients was observed between the UMD- and the ED-based diets, suggesting that an 8% inclusion level of DDGS, whether or not these have been submitted to an ELU process, was not sufficient enough to result in detectable laboratory analysis differences

between these nutrients. Standardized digestible Lys, TSAA and Thr values that were determined via the use of cecectomized roosters (Table 3.2) were found to be in close agreement among all treatments and in close proximity to the calculated values for the starter phase. The grower phase values exhibited similar agreement across treatments, and with the calculated values. Furthermore, results for the standardized digestible AA values (starter phase) of the experimental diets show similar results between the UMD- and the ED- based diets. The results of the total amino acid analysis of the grower diets showed a slight increase in the amount of all amino acids between the UMD- and the ED-based diets, suggesting that perhaps total amino acid analysis methodology posses lower experimental variability, thus a higher sensitivity than that associated with digestibility assays.

On day 21, live performance data showed that there were no significant differences among treatments (Table 3.3). The BW results agree with those of previous research by Lumpkins et al. (2004) that showed the inclusion of conventional DDGS at up to 12% of the diet had no effect on weight gain up to 42 days of age. At 42 days of age the birds fed the ED diet exhibited higher BW compared to the control birds and the birds fed the PMD diet, whereas birds fed the UMD diet had intermediate values (Table 3.3). There was no difference found for BW between the birds fed ED- and those fed the UMD-based diet at 42 days, but this was marginal as there was a proximity to significance ($P = 0.08$) as observed by contrast analysis. Martinez et al. (2007) showed that the ELU process increased fat, protein and AA levels of DDGS, while decreasing the total dietary fiber content from 34.5 to 19.7%. These diet changes could explain the BW results mentioned previously. At 42 days of age feed consumption for the birds fed the ED diet was significantly higher than that of the birds fed the PMD diet (Table 3.3).

However, neither of these two treatments had a feed intake that differed significantly from that of the control or UMD treatments. There were no differences in mortality or feed conversion ratio among any of the treatments throughout the study (Table 3.3).

Processing data at 42 days of age showed no differences between treatments for any of the characteristics measured (Table 3.4). Previous research has suggested that DDGS levels in excess of 8% are acceptable if dietary energy levels are held constant, and have shown to have no negative effects on bird performance (Waldroup et al., 1981) or yields (Lumpkins et al., 2004), at up to 42 days, as in the present experiment. Therefore, based on results from the present experiment, carcass traits were unaffected by the inclusion of DDGS, when compared to those obtained by birds fed diets without DDGS.

Table 3.3 Live performance of male broilers fed various dietary treatments up to 21 and 42 days of age

Treatments[1]	0 to 21 days of age				0 to 42 days of age			
	BW (g)	Feed intake (g/bird)	FCR[2]	Mortality (%)	BW (g)	Feed intake (g/bird)	FCR[2]	Mortality (%)
C/SBM	736	1,248	1.560	4.2	2,793 [b]	4,947 [ab]	1.742	5.6
UMD	750	1,276	1.542	0.7	2,834 [ab]	4,979 [ab]	1.736	1.7
PMD	738	1,259	1.543	3.5	2,801 [b]	4,867 [b]	1.740	5.6
ED	765	1,294	1.537	2.1	2,907 [a]	5,059 [a]	1.720	4.9
SEM	12.6	18.3	0.015	1.19	28.6	48.3	0.007	1.69
P Value	0.37	0.33	0.54	0.18	0.03	0.05	0.11	0.14

[a-b]Means within a column not sharing a common superscript differ ($P \leq 0.05$).
[1]C/SBM refers to the control diet, UMD refers to the diet containing unmodified dried distillers grains with solubles (DDGS), PMD refers to the diet containing Big DDGS, and ED refers to the diet containing Pan DDGS.
[2]Values represent the feed to gain ratio after being corrected for mortality.

It is critical to provide sufficient amino acid levels of critical amino acids to growing broilers. There can be a wide range of variability in the nutrient profiles, physical characteristics and overall composition of DDGS from one source to the next as reported by Cromwell et al. (1993), and processing techniques such as excess heat applied during drying DDGS can have major effects on the amino acid content of feedstuffs (Parsons et al., 1992). Previous research has suggested options such as measuring color score of DDGS samples as a relatively quick way of determining those with poor amino acid digestibility (Fastinger et al., 2006). The ELU process could be a way to increase the nutritional value of DDGS by increasing the digestible amino acid concentrations, as well as that of other nutrients, and could possibly lead to an increase in nutritional uniformity in DDGS from different suppliers. Future research should further establish the amount of increase or decrease of nutrients for DDGS collected from PMD or ED selection.

Table 3.4 Carcass characteristics of male broilers fed various dietary treatments up to 42 days of age

Treatments[1]	Absolute weight (g)						Yield (% relative to live weight)					
	Carcass	Abdominal Fat	Breast	Wings	Thighs	Drumstick	Carcass	Abdominal Fat	Breast	Wings	Thighs	Drumstick
C/SBM	1,914	44	564	214	349	271	67.5	1.57	19.9	11.3	18.5	14.2
UMD	1,930	49	564	217	356	277	67.4	1.71	19.6	11.3	18.7	14.4
PMD	1,919	48	559	219	355	276	67.2	1.69	19.5	11.3	18.5	14.2
ED	1,943	50	564	221	361	280	67.3	1.71	19.6	11.4	18.6	14.5
SEM	19.5	1.7	7.4	2.2	4.7	3.0	0.15	0.055	0.19	0.06	0.15	0.09
P Value	0.76	0.14	0.96	0.21	0.34	0.36	0.56	0.20	0.33	0.84	0.87	0.12

[a-b]Means within a column not sharing a common superscript differ ($P \leq 0.05$).

[1]C/SBM refers to the control diet, UMD refers to the diet containing unmodified dried distillers grains with solubles (DDGS), PMD refers to the diet containing Big DDGS, and ED refers to the diet containing the Pan DDGS.

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CHAPTER IV

EFFECT OF DIETARY INCLUSION LEVEL OF DISTILLERS DRIED GRAINS WITH SOLUBLES ON LAYER PERFORMANCE, EGG CHARACTERISTICS, AND CONSUMER ACCEPTABILITY

Abstract

Recent availability and interest in distillers dried grains with solubles (DDGS) has resulted from the increased production of ethanol. A study was designed to evaluate second cycle Bovans white laying hens that were fed with varying levels of DDGS. Layer performance, egg characteristics and consumer acceptability served as evaluating criteria. Hens were fed a commercial diet formulated to contain 0, 8, 16, 24, or 32% DDGS for a period of 15 weeks. The varying levels of DDGS did not have adverse effects on any of the parameters measured. The 16% DDGS treatment resulted in significantly higher egg production than the 0, 8, or 24% treatments, while the 32% was intermediate and not significantly different from any other treatment. Inclusion of DDGS in the diet lead to a significantly darker (L*) and redder (a*) yolk, with a tendency to increase Haugh unit values ($P = 0.03$), indicating potentially increased interior egg quality. During taste panels, consumers slightly preferred the eggs that were derived from DDGS fed hens over eggs that were obtained from hens that were fed no DDGS in both the flavor acceptability ($P = 0.04$) and overall acceptability ($P = 0.02$) categories, but liked eggs from all treatments. Based on the results from the present study, DDGS could be

included at a rate of up to one- third of a commercial layer diet without any significant detrimental effects on the production or egg characteristics of second cycle hens.

Introduction

DDGS production has greatly increased over the past several years, mostly due to the increase in ethanol production. The DDGS production from ethanol biorefineries increased by over eight million metric tons from 2007 to 2008 (Renewable fuels association, 2008). These increases have mainly been due to government mandates, and while no one can be sure how long these mandates will remain in effect, it is only reasonable to examine ways of utilizing this abundant and often competitively priced co-product of ethanol production in commercial poultry diets. Also, as conventional feed ingredient prices continue to increase, the inclusion of products such as DDGS to replace portions of the more expensive ingredients will receive increased attention. This is by no means a simple matter of replacing one ingredient with another. The nutritional profile of a given DDGS sample can be highly variable. Researchers such as Batal and Dale (2006) have reported substantial variation in both total metabolizable energy (TME_n) and amino acid (AA) digestibility in DDGS samples from different ethanol plants. Also, some AA that are necessary for chick growth have been affected negatively in DDGS due to the thermal processing that corn undergoes during ethanol manufacturing (Parsons et al., 1983). Similarly, Batal and Dale (2006) and Fastinger et al. (2006) attribute the bulk of the variability in the nutritional profiles of DDGS samples from different suppliers largely, to differences in processing and drying temperature. There have also been problems reported with product storage since DDGS takes up more space than corn and other feed ingredients due to a lower bulk density (Rosentrater, 2006).

Once a producer is able to obtain accurate information regarding the nutritional profile and characteristics of a given DDGS source, they are more able to formulate a nutritionally sufficient diet. With the nutritional issues just mentioned, it becomes imperative to understand the limits of inclusion, especially in layer diets where information is limited. The objectives of this study were to: 1) evaluate the effects of increasing levels of DDGS on second-cycle layer performance when compared to that of layers on a conventional diet; 2) evaluate the effects of feeding incremental levels of DDGS on egg characteristics; and 3) evaluate consumer acceptability of the eggs, and ability to detect differences in the eggs, from hens that are fed varying levels of DDGS compared to those fed a conventional diet.

Materials and Methods

General Procedures

This study was conducted over a 15 week period using second cycle Bovans white laying hens that were 72 weeks old at the beginning of the study. Birds were housed in raised wire cages that were two staggered rows high and equipped with nipple drinkers and a manually filled feed trough. The facility was a curtain-sided (not blackout curtains) house equipped with forced-air furnaces for heat. Following the molt period, 300 hens were placed 2 per cage (0.625 ft^2/bird) with 5 cages comprising a replicate unit, for a total of thirty experimental units. Maximum environmental daylength time during the molt phase was 12 h and thus the amount of daylight the hens were experiencing in a curtain-sided house. Weather and daylength history for the area, during the dates the molt and study were conducted, was obtained from Weather Underground (www.wunderground.com) using the history option for the Golden Triangle Region of

Mississippi. Mississippi State University and the poultry research farm are wholly contained in this region. The lighting program began on the first day following the molt period when daylength was increased from 12 h to 13 h, with daylength increased by 30 min each of the following 2 weeks. Thereafter, daylength was increased weekly by 15 min until a total of 16 h light per d was reached and then held constant for the remainder of the study. The study was conducted from late March through early July and all procedures were approved by the Mississippi State University animal care and use committee.

Treatments

All hens were fed a common pre-lay diet prior to the study beginning and the actual test diets being fed. There were 5 dietary treatments that were replicated 6 times. A conventional corn-soybean meal based diet served as the control (treatment 1). Treatments 2, 3, 4 and 5 were DDGS containing diets, with each diet having a DDGS inclusion level of 8, 16, 24 and 32%, respectively. Treatments were blocked according to location within the house. All diets were fed in mash form and were formulated to be isocaloric, isonitrogenous, and similar in calcium, available phosphorus and limiting amino acids (Table 4.1). Diets were formulated to meet or exceed nutrient recommendations (NRC, 1994). The experimental diets containing 0 and 32% DDGS were first mixed, and then progressive amounts of each of these two were blended in order to generate the 8, 16 and 24% DDGS treatments. Feed and water was provided on an ad libitum basis.

Measurements

At the beginning and the end of the experimental period, birds in each replicate unit were collectively weighed. House temperature, egg production, and mortality were recorded daily. Eggs were collected daily and the incidence of cracked, soft shelled egg, and hard shelled egg were recorded. Feed was added on a daily basis to the feed troughs to ensure that no restriction occurred, and feed consumption was calculated once a week for each replicate unit. Shell quality was examined by specific gravity and breaking strength. Specific gravity measurements were obtained once a week on all suitable eggs from a single day by Archimedes's Principle as described by Peebles and McDaniel (2004). Near the end of the study (week 13), all eggs from a single d were collected and shell strength was evaluated using an Instron 5544 universal testing machine (Instron, Norwood, MA), with Merlin software, to apply pressure to the shell until cracking occurred, at which point force applied was recorded.

An average of 8 eggs for each of the 5 treatments (within each of the 6 replications) were selected on 2 separate occasions (weeks 5 and 11) and used for determination of instrumental color using a chroma meter (Chromameter Model CR-200, Minolta Camera Co., Ltd., Osaka, Japan Serial No C8202489) that was calibrated using a standard white calibration plate (Model No 20933026, Japan). One measurement was taken on each egg yolk approximately 1 min after cracking the egg into a small mixing bowl. The color for each sample was expressed in terms of CIE values for lightness (L*), redness (a*), and yellowness (b*) (CIE, 1978).

Also during weeks 5 and 11, all eggs from a different d than that of the color data eggs were collected and used to determine Haugh unit measurements via a Technical Services and Supplies QCD (display and power supply) and QCH (albumen height gauge

and calibration block) with Eggware software version 1.06.4 (Technical Services and Supplies Ltd. Chessingham Park, Dunnington, York, YO19 5SE, England). Data in this experiment were evaluated using analysis of variance in a randomized complete block design with each group of five pens representing an experimental unit. Data were tested for two-way interactions (DDGS level × Week). Data were analyzed by the GLM procedure of SAS (2007) and DDGS treatment effects ($P \leq 0.05$) were separated using Fisher's protected LSD test option of SAS (2007) at $\alpha \leq 0.05$.

Table 4.1 Experimental diet composition (% as-is)

Ingredients	0% DDGS	32% DDGS
Corn	61.42	44.64
Soybean meal (48%CP)	26.00	10.50
Distillers dried grains with solubles (DDGS)[2]	-	32.00
Calcium carbonate-coarse	5.00	5.00
Calcium carbonate-fine	4.80	5.15
Poultry fat	1.15	1.55
Dicalcium Phosphate	0.95	0.45
NaCl	0.40	0.30
L-Lysine HCl	-	0.27
DL-Methionine	0.15	-
Trace mineral Premix[3]	0.06	0.06
Choline Chloride 70%	0.05	0.05
Vitamin premix[4]	0.03	0.03
Biofix Select[5]	0.1	0.1
Calculated Composition		
AME (kcal/kg)	2,800	2,800
Available P (%)	0.38	0.38
Ca (%)	4.0	4.0
CP (%)	17.4	17.4
Total TSAA (%)	0.72	0.73
Total Lysine (%)	0.92	0.92

[1] 0% DDGS refers to treatment 1 (control), and 32% DDGS refers to treatment 5. All other treatments were the products of blending treatments 1 and 5.
[2]DDGS nutrient values were analyzed to be: Crude Protein = 27.8%, Fat = 9.35%, Calcium = 0.05%, Total Phosphorus = 0.84%, Sodium = 0.21%
[3]Contained per kg of diet: Vit A (retinyl palmitate) 7715 IU; Vit D_3 (cholecalciferol) 2755 ICU; Vit E (DL-α-tocopheryl acetate) 8.8 IU; Vit B_{12} .01 mg; Menadione (menadione sodium bisulfate complex) 0.18 mg; Riboflavin 4.41 mg; Pantothenic acid (D-calcium pantothenate) 5.51 mg; Niacin 19.8 mg; Folic acid 0.28 mg, Pyridxine (pyridoxine-HCL) 0.55 mg; Phytase 300 units.
[4]Contained per kg of diet: Manganese (manganese sulfate) 50 mg; Zinc (zinc sulfate) 50 ppm; Iron (ferrous sulfate) 25 ppm; Copper (copper sulfate) 2.5 mg; Iodine (calcium iodate) 1.0 mg; Selenium (sodium selenite) 0.15 mg.
[5]Biofix select was included in an attempt to deal with excess mycotoxin presence in case there was a high mycotoxin concentration in the DDGS used in this study. It was included in equal amounts in all diets so as to assure it would not be the cause of any production or egg characteristic differences.

Sensory Analysis

Two consumer based sensory panels (n = 100) were conducted using eggs from weeks 6 and 12 to evaluate the acceptability of eggs from laying hens that were fed diets with 0, 16, or 32% DDGS. These sensory panels consisted of a difference-from-control test and a consumer acceptability test (n = 50-55 panelists per replication for both tests). Each panel consisted of students, staff, and faculty at Mississippi State University. Participants were recruited by e-mails sent on the day of the test with information regarding panel details, and by asking people passing by the vicinity of the test (word of mouth) if they were interested in participating in the test.

Eggs from each treatment were cooked individually in frying pans (Farberware®, 15 cm diameter Non-Stick Cookware, Needham, MA) in the form of an omelet, and cut into 8 uniform pieces (2.5 × 3.0 cm). Eggs were cooked on a medium burner level for approximately 1 min on each side until slight browning occurred on each side of the omelet. Omelet pieces were then kept warm (60-70^{o}C) in an 8-quart chafer dish (Polarware Co.) until panelists evaluated the samples. Samples were only stored in chafer dishes for approximately 10-15 min to prevent drying out of the samples. Random three digit numbers were utilized to identify the samples. Sample order was randomized to account for sampling order bias. Consumers evaluated egg samples for the difference-from-control test first. The samples were then removed and samples for the consumer acceptance test were then provided to the panelist. Water and unsalted crackers were provided, and panelists were asked to expectorate and rinse their mouths between each sample.

For the difference-from-control test, each panelist was presented with an egg sample labeled as “control” (no DDGS), and 3 more coded egg samples, in which 1 of the

coded egg samples was the same as the control egg sample and the other 2 coded egg samples were from layers that were fed 16% and 32% DDGS of the diet, respectively. Panelists were asked to evaluate the control sample first and then determine how different (in terms of flavor), the other 3 coded samples were from the control sample by rating this difference on a scale from 0 to 4, where 0 = no difference, 1 = slight difference, 2 = moderate difference, 3 = large difference, and 4 = very large difference. For the consumer panel, each panelist was asked to evaluate 3 coded egg samples from hens fed diets without DDGS (control), and diets with 16% and 32% DDGS for texture, flavor, and overall acceptability using a 9 point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely (Meilgaard et al., 2007).

All sensory panel data was analyzed as a randomized complete block design (replications and panelists as blocks) with 2 replications was utilized to test the treatment effects ($P \leq 0.10$) of diet on the ability of panelists to perceive a difference from the control, the overall acceptability and the acceptability of texture and flavor (SAS, 2007). Fisher's protected LSD test was utilized to separate treatment means when significant differences ($\alpha \leq 0.05$) occurred.

Results and Discussion

Layer Performance

The results for many of the live production parameters are, by any standards, unique. The Bovans White management guide (2006) states that post molt hens are expected to peak at around 85% production. In the current study, the poorest performing groups achieved 85 % production. The hens also experienced higher, or worse, than

expected feed conversion and egg weight results (Bovans White management guide, 2006).

Total egg production increased rapidly from the time that experimental diets were fed until the third week of the study, at which point production began to level out for all treatments. When layers were fed 16% DDGS, birds had a significantly higher total egg production over the course of the study than all other treatments, with the exception of birds that were fed 32% DDGS (Table 4.2). These results are not typical when compared to previous reports where there were no significant differences in egg production when DDGS was included in the diet at levels ranging from 10 to 20% (Roberts et al., 2007, Matterson et al., 1965, Lumpkins et al., 2005). Conversely, Pineda et al. (2008) showed a linear decrease in egg production as DDGS concentration in the diet increased to levels in excess of 20%. The authors feel that the substantial differences seen in layer performance can most likely be attributed to bird age and strain. Previous research by Roberts et al. (2007), Lumpkins et al. (2005), and Pineda et al. (2008) was primarily done using the Hy-Line strain of bird with an age range of 23-49 weeks at the start of the experiment. In the current study, Bovans White hens at 72 weeks of age were used to conduct the trial. Matterson et al. (1965) did not specify hen age or strain, only that the birds used were leghorn type. The 16% DDGS treatment also produced a significantly higher hard shell egg (eggs that were not soft shelled, or cracked) production than treatments with 8 and 24% DDGS, while not differing from the control and 32% DDGS treatment.

Feed conversion values, expressed as feed/dozen (Table 4.2), were significantly lower for the 16 and 32% DDGS treatments than those for the 8 and 24% DDGS, while hens consuming the control diet were intermediate. In contrast to the results observed in this study, Lumpkins et al. (2005) showed no significant differences in kg of feed/dozen

eggs between layers fed commercial diets containing either 0 or 15% DDGS, while Matterson et al. (1965) showed similar results in birds fed up to 20% DDGS. Once again, these results were obtained in hens of a much younger age and different strain than the hens used in the current study.

In parallel with egg production and feed conversion, birds fed 16% DDGS produced greater total egg mass than birds fed 8 or 24% DDGS, but not different from those fed the control or the 32% DDGS diet (Table 4.2). Similarly to production and feed conversion data, previous research by Roberts et al. (2007) reported no significant differences in egg mass between commercial layers fed a control corn-soybean meal based diet and those fed a diet containing 10% DDGS.

Table 4.2 Live production characteristics of commercial laying hens fed various levels of DDGS

DDGS[1] (%)	Total Egg Production[2] (%)	Hard Shell Egg Production (%)	Feed/ Dozen	Egg Mass (kg)	Feed/Hen/Day (kg)	Egg Wt (g)	Mort. (%)
0	87[b]	85[ab]	1.63[ab]	4.2[ab]	0.114	71.4	1.8
8	85[b]	81[b]	1.72[a]	3.9[b]	0.113	70.4	2.1
16	93[a]	90[a]	1.55[b]	4.5[a]	0.115	71.4	0.4
24	85[b]	81[b]	1.72[a]	3.9[b]	0.114	69.2	0.0
32	89[ab]	87[ab]	1.58[b]	4.1[ab]	0.113	69.4	2.6
SEM	2.0	2.2	0.038	0.13	0.0015	0.82	1.2
Analysis of Variance (P)							
DDGS	0.04	0.03	0.01	0.03	0.84	0.22	0.46
Week	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
DDGS x Week	0.61	0.78	0.99	0.91	0.005	0.27	0.95

[1]Distillers dried grains with solubles
[2] Hen day production
[a-b] Means within a column not sharing a common superscript differ (P < 0.05).

Feeding DDGS did not affect mortality, feed intake (feed/hen/day), or egg weight (Table 4.2). However, a treatment by week interaction was observed for feed consumption (Table 4.3), and the bulk of the interaction could be attributed to the first week, in which birds fed the control diet had a lower feed intake than birds fed any other treatment. During this first week, hens were beginning production and there was more variation in the data than at any other week for most parameters measured. The first week was when the hens were most likely experiencing a high energy requirement as a result of just coming out of molt and also due to the cooler temperatures observed that week. As a result all the hens would be expected to eat more, but as previously stated, the control group consumed significantly less than any other treatment for that week. The authors feel that this may have occurred as a result of dietary differences, where tabular values used for ME_n of DDGS may have slightly overestimated their energetic content. Batal and Dale (2006) reported that the TME_n from different sources of DDGS could vary from 2,490 to 3,190 kcal/kg. Furthermore, when looking at the results for feed consumption, there was no observable effect in egg production or any of the other production data when an increase in feed consumption was recorded for a given week.

Table 4.3 Interactive effects of DDGS over time on feed consumption of commercial laying hens (feed/hen/day; g)[1]

DDGS [2] (%)	Week														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	118^{c}	125^{b}	121^{ab}	121^{a}	115	122^{a}	111	114^{ab}	116^{ab}	109	108	106	110	104	105^{b}
8	126^{ab}	125^{b}	119^{b}	118^{ab}	113	121^{ab}	111	115^{a}	115^{ab}	108	106	107	107	105	107^{ab}
16	127^{a}	126^{ab}	124^{a}	120^{ab}	116	122^{a}	111	115^{a}	117^{a}	110	109	107	109	107	109^{a}
24	126^{ab}	128^{a}	122^{ab}	119^{ab}	116	121^{ab}	109	114^{ab}	116^{ab}	108	108	106	109	106	107^{ab}
32	123^{b}	128^{a}	123^{ab}	114^{c}	113	118^{b}	108	109^{c}	113^{b}	110	109	105	109	105	107^{ab}
Mean	124	126	122	118	115	121	110	113	115	109	108	106	109	105	107
Temp[3]	12.8	16.7	18.3	12.2	20.0	17.8	20.6	17.8	22.8	22.8	26.1	25.0	23.9	26.1	25.0

[a-c] Means within a column not sharing a common superscript differ ($P < 0.05$).
[1] SEM = 2.0013
[2] Distillers dried grains with solubles
[3]Represents mean temperature in the area for given week in degrees Celsius.

As a result of this, we explored the environmental conditions during the time (www.wunderground.com) that the experiment was conducted and noticed that the increases in feed intake for a given week appeared to mimic cooler mean temperatures in the region. Therefore, being that the layer facility was a curtain-sided house with limited means of climate control to avoid cold stress, it is suggested that this effect may have resulted in the birds consuming more feed in response to cooler environmental temperatures. Furthermore, once regional temperatures ceased to drastically drop in the evenings (approximately one month into the study), the heaters were turned off and curtains were left down for the duration of the study for ventilation purposes.

Egg Characteristics

As DDGS concentration increased in the diet, the yolk increased in L* value, indicating a slightly darker yolk (Table 4.4). This result is in agreement with previous research of Roberts et al. (2007), Lumpkins et al. (2005), and Pineda et al. (2008) who reported that DDGS in the diet of layers had a darkening effect on the yolk of the egg. In addition, there was a slight increase in a* (redness) of the egg yolk as the DDGS concentration of the diet increased (Table 4.4). This trend for increased a* value has been previously documented by Pineda et al. (2008). No major differences were observed in the b* value among treatments which is in agreement with past research (Roberts et al., 2007, Lumpkins et al., 2005, Pineda et al., 2008) that showed that the b* value was not affected by DDGS concentration in the diet.

Haugh units, a measure of interior egg quality, were evaluated and shown to be reduced when hens were fed 8% DDGS when compared to those fed 24 and 32% DDGS,

while the control and 16% DDGS were intermediate (Table 4.4). There appears to be a tendency for Haugh units to be increased as DDGS concentration increases in the diet.

Table 4.4 Egg and eggshell characteristics of commercial laying hens fed various levels of DDGS

DDGS[1] (%)	L[2]	a[3]	b[4]	Haugh Units	Specific Gravity	Shell Strength (kgf)[5]
0	59.6[a]	-4.3[e]	30.4	74.8[ab]	1.0815	3.3
8	59.4[a]	-3.5[d]	30.9	73.8[b]	1.0821	3.4
16	58.1[b]	-2.8[c]	30.0	75.4[ab]	1.0823	3.3
24	58.7[ab]	-2.4[b]	31.3	77.7[a]	1.0835	3.4
32	58.3[b]	-1.7[a]	30.9	77.5[a]	1.0833	3.4
SEM	0.34	0.09	0.36	1.09	0.00056	0.08
Analysis of Variance (*P*)	0.003	<.0001	0.09	0.03	0.09	0.35

[a-e] Means within a column not sharing a common superscript differ (P < 0.05).
[1] Distillers dried grains with solubles.
[2]Lightness (yolk), scale from 0 to100 with 100 being white.
[3]Red/green (yolk), scale from -60 (green) to +60 (red).
[4]Yellow/blue (yolk), scale from -60 (blue) to +60 (yellow).
[5]kgf = kilogram-force.

Jensen et al. (1978) reported that feeding 10 % DDGS in the diet significantly increased Haugh units when compared to a conventional diet. Also, Lilburn and Jensen (1984), showed that 10% corn fermentation solubles, another fermentation by-product, in the diet of laying hens had the ability to significantly increase interior egg quality as measured by an increase in Haugh units. Conversely, in more recent research, interior egg quality was not affected as much as previously reported, by an increased concentration of DDGS in the diet (Lumpkins et al., 2005, Pineda et al., 2008). The authors attribute the Haugh unit increase observed in this study to the same factor that Jensen et al. (1978) attributed their increase in Haugh units to, trace elements. With the starch portion of the grain being removed during the fermentation process, resultant DDGS have a higher concentration of trace elements than corn. This idea is further supported by the work of Jensen and colleagues when they showed that the addition of extra trace elements further increased Haugh unit values. There were no differences in specific gravity among the treatments (Table 4.4), in agreement with previously reported effects when feeding DDGS to commercial laying hens (Pineda et al., 2008).

Consumer Acceptability

Results for the consumer sensory and acceptability panels are displayed in Table 4.5 and are presented as texture, flavor, overall acceptability, and difference from control. No significant differences were reported by consumers with respect to acceptability of texture. However, when looking at flavor and overall acceptability it was noted that DDGS containing treatments (16 and 32% DDGS) led to slightly higher scores, hence were slightly more desirable to consumers than those eggs derived from the control treatment. However, all scores were in the range of 6.4 to 6.9, which indicated that all

eggs were liked between slightly and moderately by consumers. When panelists were asked to differentiate the DDGS treatments from the control, consumers were not able to differentiate the 16 and 32 % DDGS treatments from the control (Table 4.5). All three treatments were rated as slightly different from the control, including the control itself. Therefore, it is not likely that consumers could tell a difference between eggs cooked as omelets that were produced from layers fed concentrations between 0 and 32 % DDGS. In addition, the results of this sensory difference test, with a slight increase in acceptability of eggs that were produced from layers that were fed DDGS, may not be of practical significance for the industry.

Table 4.5 Consumer acceptability of eggs

DDGS[1] (%)	Texture[2]	Flavor[2]	Overall Acceptability[2]	Difference from Control[3]
0	6.5	6.4[b]	6.4[b]	1.0
16	6.9	6.8[a]	6.8[a]	1.1
32	6.8	6.8[a]	6.9[a]	1.3
SEM	0.13	0.13	0.13	0.09
Analysis of Variance (*P*)	0.12	0.04	0.02	0.09

[a-b] Means within a column not sharing a common superscript differ ($P < 0.05$).
[1] Distillers dried grains with solubles
[2] Hedonic scale was based on 9-point scale: 1 = dislike extremely, 5 = neither like nor dislike, 9 = likes extremely.
[3] Difference from control evaluation: 0 = no difference, 1 = slight difference, 2 = moderate difference, 3 = large difference, 4 = very large difference. Panelists were asked how different the control sample was in terms of flavor to the other samples.

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CHAPTER V

EFFECTS OF VARYING LEVELS OF DISTILLERS DRIED GRAINS WITH SOLUBLES ON BROILER CHICKS

Abstract

There is no distinct line, regarding dietary concentration, when it comes to feeding distillers dried grains with solubles (DDGS) to young broilers. In two studies the effects of varying levels of DDGS in the diet, on young broilers, was observed from 0 to 14d of age. Diets ranged from 0 to 32% DDGS concentration, with 8% increments. Increasing DDGS led to decreased body weight gain (BWG), while feed intake and mortality were relatively unaffected. However, there was an increase seen in feed conversion (FCR) in conjunction with the increase in DDGS in the diet.

Introduction

When corn is fermented to be used in the production of ethanol, the bulk of the starch component of the grain is what is actually fermented off to make the biofuel, while the rest becomes a by-product commonly known as DDGS. There has been a dramatic increase in the amount of the primary by-product of corn fermentation, DDGS, produced as a result of ethanol production over the last several years (Renewable Fuels Association, 2008). DDGS can be a feasibly economical choice for inclusion in broiler diets, especially through periods of economic hardship like the ones we have found ourselves in recently. However, there are problems that have been noted with the product ranging from the areas of transport to milling to nutrition. In the two studies presented

here we are focusing on the nutritional aspect of the product. More specifically, the researchers wished to evaluate the effects that different levels of DDGS would have on broiler chicks during the first two weeks posthatch. Other research has pointed out that the low energy density of DDGS diets may be the limiting factor in meeting the energy needs of young broilers (Wang et al. 2008).

Research evaluating inclusion levels of DDGS in the diet of young broilers are sparse and some have recommended up to 25% DDGS may be included in the diet with no negative effects on body weight gain (BWG), feed conversion (FCR) or feed intake (Min et al. 2009). The objective of the work presented herein was to further investigate the maximum limits of DDGS inclusion for broiler chicks during a starter phase that ranged from 0 to 14d of age. Diets from the first study were formulated using calculated total amino acids (AA) and calculated apparent metabolizable energy (AME) values for the ingredients utilized. Based on the results from the first study the researchers felt it was necessary to conduct a follow up study where the DDGS would be submitted for analysis of true digestible AA and true metabolizable energy (TME_n) prior to diet formulation.

Materials and Methods

The first study encompassed the period between 0 to 14 d of age using Ross × Ross 308 males and females obtained from a commercial hatchery. Day-old chicks were randomly placed in a sex-separate manner, across 80 floor pens (15 birds/pen; 1200 birds total) at a density of $0.07 m^2$/bird. The second study utilized the same strain and age period, but only males were employed for a total of 35 floor pens (15 birds/pen; 525 birds total). In each case the house used was close-sided, with thermostatically controlled

heating, cool cells and cross ventilation. Each pen had built-up litter, a hanging feeder (22.5kg capacity) and a water line (3 nipples/pen). The lighting program was 23 h light and 1 h dark and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via *in ovo* administration at d 18), Newcastle disease and infectious bronchitis (via coarse spray at hatch).

There were 5 different DDGS dietary levels evaluated and both sexes for a total of 10 treatments with 8 replications each during study one. Study two was based on the same 5 DDGS inclusion levels with 7 replications and using only males. Treatments were blocked according to location within the house. A conventional corn-soybean meal based diet served as the control (treatment 1). Subsequent treatments represented 8, 16, 24 and 32% DDGS in the diet. All diets were formulated to be isocaloric and similar in calcium, phosphorus and all limiting AA and were fed in crumble form. Diets were formulated to meet or exceed nutrient recommendations (National Research Council 1994). Table 5.1 displays the diets used in each study, and in each case only the control and 32% DDGS diets are shown, as the 8, 16 and 24% diets were the result of blending the control and the 32% diet. Feed and water were provided ad libitum. Based on the results from study one, researchers collected two samples of the control and 32% DDGS diets, as well as two additional samples of the DDGS used, and had them sent to the veterinary diagnostic laboratory at Iowa State University to test for the following mycotoxins: aflatoxin G2, aflatoxin G1, aflatoxin B2, zearalenol, ochratoxin A, vomitoxin and T-2 toxin.

Table 5.1 Experimental diet composition (% as-is)

	Study I		Study II	
Ingredient	0%DDGS	32%DDGS	0%DDGS	32%DDGS
Corn	58.036	39.043	54.935	34.666
Soybean meal	35.099	20.648	36.659	25.304
DDGS	-	32.00	-	32.00
Pro-Plus[1]	1.5	1.5	1.5	1.5
Poultry oil	2.989	4.27	3.861	3.892
Deflourinated	1.517	1.067	1.522	1.67
Calcium carbonate	0.042	0.687	0.608	-
Sodium chloride	0.242	0.154	0.248	-
Premix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.213	0.05	0.241	0.239
L-Lysine	0.061	0.28	0.102	0.376
Coccidiostat[3]	0.05	0.05	0.05	0.05
L-Threonine	-	-	0.025	0.052
Calculated				
CP (%)	23.19	23.19	23.87	24.76
TME_n (kcal/kg)	-	-	3,005	3,005
AME (kcal/kg)	3,125	3,125	-	-
Ca (%)	0.9	0.9	0.9	1.1
Available P (%)	0.45	0.45	0.45	0.45
Na (%)	0.2	0.2	0.2	0.24
Choline (mg/kg)	1,638.727	2,105.936	1,649.063	2,190.49
Digestible TSAA	0.90	0.90	0.90	0.90
Digestible Lys	1.25	1.25	1.25	1.25
Digestible Thr	0.84	0.88	0.81	0.81

[1] Animal protein blend, with a guaranteed CP value of 60% (H.J. Baker & Bro., Inc.; Little Rock, AR)

[2] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.3 mg.

[3] Dietary inclusion of coccidiostat provides 60 g salinomycin sodium per 907.2 kg of feed.

Based on the results from this laboratory, which came back negative for these fungal metabolites, samples of DDGS were sent for analysis of true digestible AA and TME_n, done via the use of precision-fed cecectomized roosters at the University of Illinois, in methods described by Parsons (1986). Upon receiving the analyses for these nutrients, the nutrient matrix for this ingredient was updated, and the diets for study two were then formulated accordingly.

All birds in each pen were weighed collectively at the beginning and at the end of each study. Feed consumption and mortality were monitored throughout the studies and feed conversion was corrected for the weight of mortality, and represents: (g of feed consumed by all birds in a pen) / (g of BW per pen + g of weight of dead birds).

Data in these experiments were evaluated using analysis of variance in a randomized complete block design with the pen representing this experimental unit. Percentage data for mortality were transformed to arcsine √% for analysis. In study one, DDGS x gender were tested for two-way interactions, and then main effects. All data were analyzed by the GLM procedure of SAS (2004) and treatment effects ($P \leq 0.05$) were separated using Fisher's Least Significant Difference test option of SAS (2004) using an α of 0.05.

Results and Discussion

Results from study one are shown in table 5.2. The 24 and 32% DDGS treatments exhibited significantly lower BWG values compared to all other dietary treatments and BWG results exhibited a strongly linear trend overall. This agrees with the results of previous research, showing that DDGS in the diet did not affect BWG at levels of up to 20% of the diet (Wang et al. 2008).

Table 5.2 Results (Study I, 0-14 days)

Treatment	BW gain[1]	Feed intake[2]	CFCR[3]	Mortality[4]
0% DDGS	384 ^{a}	495 ^{ab}	1.29 ^{d}	0.4
8% DDGS	385 ^{a}	502 ^{a}	1.30 ^{cd}	3.3
16% DDGS	387 ^{a}	512 ^{a}	1.32 ^{c}	1.7
24% DDGS	367 ^{b}	497 ^{ab}	1.35 ^{b}	5.8
32% DDGS	355 ^{b}	483 ^{b}	1.41 ^{a}	4.9
Males	379 ^{a}	500	1.32 ^{b}	2.6
Females	372 ^{b}	496	1.34 ^{a}	3.8
SEM	4.6	6.0	0.006	1.59
ANOVA P-Value				
DDGS	0.0001	0.02	0.0001	0.13
Sex	0.045	0.38	0.0002	0.38
DDGS x Sex	0.13	0.044	0.92	0.36
DDGS Linear	<0.0001	0.16	<0.0001	0.03
DDGS Quadratic	0.009	0.006	0.23	0.77

[1] Values represent the BW gained expressed as grams/bird.
[2] Values represent the feed consumption expressed as grams/bird.
[3] Values represent the feed conversion after being corrected for mortality weight.
[4] Values represent the incidence of mortality expressed as a percentage of the population.
[a-d] Means within a column not sharing a common superscript differ ($P \leq 0.05$).

Feed intake exhibited an interaction which resulted from the females consuming significantly more than the males at the 16% DDGS level. Males and females did not consume significantly different amounts for any other treatment. Also, a quadratic trend was observed where feed consumption increases until reaching the 16% DDGS level, at which point consumption then drops off for the birds in the 24 and 32% DDGS treatments. These results do not agree with those of previous research that showed no differences in feed intake when feeding levels of 0, 6, 12, and 18% DDGS during a

starter phase (Lumpkins et al. 2004). They are also in disagreement with those reported by Wang et al. (2008) that showed a tendency for consumption to increase as DDGS content of the diet increased during the starter phase. Due to this unexpected drop in feed consumption exhibited by the birds, the researchers decided to analyze the diets and DDGS used in study one for a possible contamination of common mycotoxins. The results of these analyses were a negative presence of these metabolites in the DDGS source as well as the diet. Therefore, the DDGS were sent for analysis of true AA digestibility and TME_n content, in preparation for study two.

Results for FCR showed the 32% DDGS treatment to have the significantly greatest value. The control treatment had the lowest FCR numerical value, but was not significantly less than that of the 8% treatment. The data shows a strongly linear trend towards increasing FCR as DDGS content of the diet increases, and this does closely mirror the results seen by Wang and colleagues (2008). No differences were observed for mortality.

In study two (Table 5.3), there is once again a strongly linear trend observed for the parameter of BWG with the 32% DDGS treatment exhibiting the significantly lowest value, while the control treatment had the greatest value. These results are in fairly close agreement with those of the first study and with those of previous research (Wang et al. 2008), although they are numerically lower in study two than in the first study. FCR results for study two showed a similar, strongly linear, trend to those results seen in study one and in previous research (Wang et al. 2008). The 32% treatment had the greatest value and there was a linear decrease to the control treatment value for FCR, which was significantly less than all other treatments, except for the 8% treatment. The 16% treatment was intermediate, not being significantly greater than the 8% treatment or

significantly less than the 24% treatment. In study two there were no statistical differences among any of the treatments for the parameters of mortality and feed intake.

Table 5.3 Results (Study II, 0-14 days)

Treatment	BW gain[1]	Feed intake[2]	CFCR[3]	Mortality[4]
0% DDGS	322 ^{a}	436	1.34 ^{d}	2.9
8% DDGS	310 ^{ab}	431	1.36 ^{cd}	2.2
16% DDGS	316 ^{ab}	444	1.39 ^{bc}	4.8
24% DDGS	300 ^{b}	433	1.44 ^{ab}	4.8
32% DDGS	277 ^{c}	423	1.47 ^{a}	4.0
SEM	7.2	8.2	0.02	2.15
ANOVA P-Value	0.0018	0.5931	0.0001	0.8992
DDGS Linear	0.0003	0.48	<0.0001	0.46
DDGS Quadratic	0.11	0.29	0.58	0.74

[1] Values represent the BW gained expressed as grams/bird.
[2] Values represent the feed consumption expressed as grams/bird.
[3] Values represent the feed conversion after being corrected for mortality weight.
[4] Values represent the incidence of mortality expressed as a percentage of the population.
[a-d] Means within a column not sharing a common superscript differ ($P \leq 0.05$).

The researchers feel that the fact that there were no differences for feed intake in study two was the direct result of the analysis, and thus re-evaluation of the nutrient levels, of the DDGS product via the use of the cecectomized roosters. As for the results seen for the parameters of BWG and FCR, the same trends were seen in both study one and study two, and agree with those results seen in research done in the very recent past by Wang et al. (2008). As a result the researchers feel that DDGS can be included in a diet from 0 to 14d of age, for broiler chicks, at levels up to 8% without any detrimental effects on BWG, FCR or feed intake.

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CHAPTER VI

EFFECTS OF FEEDING DISTILLERS DRIED GRAINS WITH SOLUBLES TO BROILERS FROM 0 TO 28 DAYS POSTHATCH ON BROILER PERFORMANCE, FEED MANUFACTURING EFFICIENCY AND SELECTED INTESTINAL CHARACTERISTICS

Abstract

A study evaluated the effects of feeding two levels (0 vs. 8%) of distillers dried grains with solubles (DDGS) in a starter broiler diet (0-14 d; 45 replicates/trt) and subsequently feeding a grower diet (14-28 days) with either 0, 7.5, 15, 22.5, or 30% DDGS (9 replicates/trt). Ross × Ross 308 male broilers were used in this experiment, and evaluation criteria consisted of feed mill parameters, broiler growth, relative liver weight, ileal viscosity, and cecal content count of *Clostridium perfringens* and *Escherichia coli* analyzed by both selective media and real-time PCR. Increased inclusion of DDGS resulted in non-linear response for production rate ($P<0.05$), conditioner energy usage ($P<0.01$), and pellet mill energy usage ($P<0.05$). Increasing DDGS resulted in a linear decrease in pellet quality ($P<0.001$), and an increase ($P<0.01$) in total fines. Inclusion of DDGS decreased ($P<0.001$) energy usage at the pellet mill and decreased ($P<0.05$) bulk density of the diets. The DDGS levels fed during the starter phase (0 vs. 8%) had no impact on the broilers at 14 or at 28 days of age. Increasing DDGS inclusion levels during the grower phase resulted in a linear decrease ($P<0.001$) in BW gain and liver relative weight ($P<0.001$). A DDGS starter × grower interaction ($P<0.05$) was observed

for feed consumption, where birds that consumed no DDGS during the starter phase exhibited a decrease in feed consumption with the higher inclusion levels of DDGS during the grower phase, while birds that received 8% DDGS during the starter phase were unaffected by DDGS inclusion level in the grower phase. Feed conversion, mortality, ileal viscosity, and cecal *C. perfringens* and *E. coli* concentrations were unaffected by DDGS level in the grower diet. The feed intake response suggests a beneficial effect of exposing broiler chicks to DDGS if inclusion levels of 22.5% or higher is to be fed after 14 days of age. However, the data suggests that the young broiler can be negatively impacted with inclusion levels of 15% DDGS or higher up to 28 d of age.

Introduction

The production of ethanol continues to increase dramatically. There is also an increased interest in the primary by-product associated with its production, distillers dried grains with solubles (DDGS). There have been major increases in the production of ethanol feed co-products, with DDGS representing the majority, and estimates are that the increases will continue (Renewable Fuels Association, 2008). While it is not certain how long this trend will continue it is only reasonable to take advantage of this economic and readily available feedstuff. With any new product there are limitations in its use. An initial limitation is associated with feed throughput and pellet quality. Behnke (2007) suggested that once DDGS in the diet exceeds levels of 5-7%, pellet throughput, as well as pellet quality may be negatively affected. However, little data exists to support these observations. Beyond the simple physical factors of pellet quality and throughput, it has been well documented that nutritional variability among DDGS sources can become a

major issue when formulating diets of growing broilers (Cromwell et al., 1993, Batal and Dale, 2006, Fastinger et al., 2006).

Food safety is a major issue throughout all aspects of the food industry and is constantly on the mind of any major integrator. A major portion of food safety is concerned with the presence of pathogenic bacteria and the prevention of these bacteria in different products. Two pathogens of particular interest to the broiler industry are *E. coli* and *C. perfringens*. While there is little or no research as to the effects that DDGS inclusion in the diet can have on the colonization of these bacteria in poultry, the same is not true of beef cattle. Jacob et al. (2008) reported a positive association between the use of DDGS in the diet of feedlot cattle and the prevalence of *E.coli O157* in fecal material.

It is of utmost importance that we understand the potential limitation and challenges of using high inclusion levels of DDGS in broiler diets. This study was designed to address some of those concerns: 1) evaluate the effects of varying levels of DDGS on pelleting characteristics and feed mill efficiency; 2) observe the effects on performance caused by feeding increasing levels of DDGS (0, 7.5, 15, 22.5 and 30%) to growing broilers from 14 to 28 days of age after being fed 0 or 8% DDGS during the starter phase; and 3) determine the effects that various levels of DDGS may have in the grower diet on intestinal viscosity and cecal populations of *E .coli* and *C. perfringens* of young broilers.

Materials and Methods

Feed Milling

This portion of the study was conducted over a 4 d period at the West Virginia University pilot feed mill in Morgantown, WV. Equipment used included: Weigh-tronix

stationary feed mill SFM-2000: integrated hammer mill, scale, microingredient mixer; 15-horsepower horizontal shaft hammer mill (screen size: 1/8in.); 907.2 kg capacity single-screw vertical mixer in series with a pellet mill; CPM 2288A master model pellet mill with a 40-horsepower main drive motor, 12 inch diameter, 3/16 × 1.77 inch die. There were 4 separate grower-phase diets that varied in DDGS inclusion level: 0, 15, 30, and 30 plus 2% sand. This study was designed as a Latin-square and treatments were blocked by day of production and run order. Each treatment was replicated 4 times, with each batch being 453.6 kg and representing an experimental unit. The treatment utilizing the 2% sand addition was included to see if the sand could act similar to rock phosphate sources by providing a pellet die scouring effect. Initially the 0 and 30% DDGS diets were made and the 15% DDGS diet was the result of blending. The 0 and 30% DDGS diets manufactured are shown in Table 6.1. These same diets would later be used to manufacture the diets that would be fed during the growout portion of the study. All diets were mixed for 15 min in a single screw vertical mixer. Prior to pelleting, all diets were batched into their 453.6 kg aliquots in mash form. For the 30% DDGS plus sand treatment, sand was included at the expense of the total diet. The sand used had an average particle size of 450 microns. Once all feed was batched, each individual batch was transferred back into the mixer where it was then conveyed to the conditioner-pellet mill.

Table 6.1 Experimental diet composition (% as-is)

Ingredients	Starter diets (0-14 d)		Grower diets (14-28 d)	
	0% DDGS	8% DDGS	0% DDGS	30% DDGS
Corn	58.0	53.0	63.7	38.7
Soybean meal (48%CP)	35.1	32.2	28.9	22.1
DDGS [1]	-	8.0	-	30.0
ProPlus [2]	1.50	1.50	2.50	2.50
Poultry fat	2.99	2.57	1.90	3.88
Deflourinated	1.52	1.36	1.37	0.73
Calcium carbonate	0.04	0.29	0.63	1.19
Premix [3]	0.25	0.25	0.25	0.25
NaCl	0.24	0.09	0.22	0.12
DL-Methionine	0.21	0.30	0.21	0.14
L-Lysine HCl	0.06	0.30	0.18	0.26
Coccidiostat [4]	0.05	0.05	0.05	0.05
L-Threonine	-	0.08	0.05	0.03
Calculated Composition				
AME (kcal/kg)	3,100	3,100	3,125	3,125
Available P (%)	0.46	0.46	0.45	0.45
Ca (%)	0.92	0.92	0.90	0.90
CP (%)	23.2	23.2	21.4	24.0
Dig. TSAA (%)	0.90	0.90	0.82	0.82
Dig. Lysine (%)	1.25	1.25	1.14	1.14
Dig. Threonine (%)	0.84	0.88	0.74	0.74

[1]Distillers Dried Grains with Solubles.
[2]Animal protein blend, with a CP value of 60% (H.J. Baker & Bro., Inc.; Little Rock, AR)
[3] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.3 mg.
[4] Dietary inclusion of coccidiostat provides 60 g salinomycin sodium per 907.2 kg of feed.

Mash was conditioned to a steady-state temperature of 82°C (180°F). Steam pressure at the gauge was 262 kPa (38 psi) through use of a globe valve. Feed temperature was monitored with a digital thermometer inserted directly into the stream of conditioned mash, and was controlled by throttling steam into the conditioner using a ball valve. Rate of feed entering the conditioner was held constant across all treatments. Pellets were formed using a California Pellet Mill (4.25-ft length, 1.02-ft diameter short-term CPM conditioner (3 steam inlet ports), 429 rpm shaft speed; 21 picks; 10-s feed retention time) and were cooled on a horizontal belt cooler using forced ambient air. Relative electrical energy usage at both the conditioner and pellet mill were determined using Powerlogic power meters attached to the 3-phase leads of the pellet mill main drive and conditioner motor (Square D). Production rate, percentage of fines and bulk density were also estimated. One representative bag from each manufacturing run was reserved for determination of pellet quality, as measured by pellet durability index (PDI) and modified pellet durability index (MPDI). Pellet quality was assessed on the day of manufacture via a tumbling box according to ASAE standard S269.4 (ASAE, 1997). Because of the use of a 3/16 × 1.77 in. die pellets were sifted in a No. 6 American Society for Testing and Materials (ASTM) screen. The MPDI was determined in a similar manner, with the exception of adding five 13-mm hex nuts to the pre-tumbled sample to obtain added pellet agitation (ASAE, 1997).

Grow-out

The grow-out portion of the study encompassed the period between 0 to 28 days of age using Ross × Ross 308 males obtained from a commercial hatchery. Day-old chicks were randomly placed in each of 90 floor pens (15 birds/pen; 1350 birds total;

0.07m^2/bird). The close-sided house had thermostatically controlled heating, cool cells and cross ventilation. Each pen contained built-up litter, a hanging feeder (22.5kg capacity) and nipple drinkers (3 nipples/pen). The lighting program was 23 hr light and 1 hr dark and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via in ovo administration at day 18), as well as Newcastle disease and infectious bronchitis (via coarse spray at hatch).

To ensure accurate formulation of the experimental diets, samples of DDGS, corn, soybean meal, and ProPlus were analyzed for total amino acids and crude protein composition (AOAC International, 2006). Digestible amino acid (AA) values were calculated from published digestible coefficients (Ajinomoto, 2004) by using the analyzed total AA content of the ingredients. Crude protein was not assigned a minimum value during formulation, and essential digestible AA were maintained in all dietary treatments by setting minimum formulation ratios relative to digestible Lys as follows: TSAA 75, Thr 65, Val 78, Ile 68, Trp 17, and Arg 105, and following previously published recommendations (Lemme et al., 2004). All other essential nutrients were formulated to meet or exceed nutrient recommendations (NRC, 1994). Upon receiving the results for CP and amino acid analysis of the feed ingredients, the nutrient matrix was updated and the feed formulas were solved using linear programming (Table 6.1).

During the starter phase (0-to-14 days) two DDGS inclusion levels (0 vs. 8%) were fed to the 90 floor pens (2 treatments; 45 replicates/trt). Subsequently, each of these two DDGS levels provided during the starter phase was fed 0, 7.5, 15, 22.5, or 30% DDGS during the grower phase (14-to-28 days), resulting in a 2 × 5 factorial study (2 DDGS in starter × 5 DDGS in grower) and was replicated 9 times for a total of 90 experimental units. The feed was provided to the birds from 0 to 14 days of age in

crumbles, and from 14 to 28 days as pellets (Table 6.1). Treatments were blocked completely, according to location within the house. Table 6.1 shows the 0 and 30% DDGS grower diets used in both the feed milling and grow-out portions of the study. The 7.5, 15 and 22.5% DDGS grower diets were the result of blending between the 0 and 30% grower diets. Feed and water were provided for ad libitum consumption.

All birds in each pen were weighed collectively at the beginning and end of each feed phase. Feed consumption and mortality were monitored throughout the study and feed conversion was corrected for the weight of mortality and represents: (g of feed consumed by all birds in a pen) / (g of BW per pen + weight of dead birds). All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Bacterial Quantification

The *E. coli* strain 25922 and the *C. perfringens* strain 13124 were acquired through the American Type Culture Collection (Manassas, VA). *E. coli* was cultured on Trypticase Soy Agar (TSA) at 37°C under aerobic conditions and *C. perfringens* was cultured on Reinforced Clostridial medium at 37°C under anaerobic conditions. Anaerobic conditions were established using a Mitsubishi Anaeropak jar and anaerobic indicators were used to ensure these conditions were maintained during the incubation periods.

At 28 days of age, one bird per pen was randomly selected and euthanized via cervical dislocation. Ceca were removed and weighed pre and post extraction of contents. Cecal contents were diluted 1:10 in sterile PBS (1mL total volume) and vortexed for 1 min at maximum setting to homogenize the sample. Dilutions of the samples were then

plated on selective media. For detection of *E. coli* in the samples, dilutions of the cells were plated on MacConkey agar supplemented with MUG (Remel) and incubated for 24 hr at 37°C. For detection of *C. perfringens* in the samples, dilutions of the cells were plated on Perfringens agar base with TSC selective supplement (Oxoid) for 24 hr at 37°C under anaerobic conditions. Anaerobic conditions were established and monitored as previously described. *C. perfringens* colonies were counted based on the appearance of black and opaque colonies. *E. coli* colonies were counted after examination with a long-wave UV lamp for confirmation.

DNA was isolated from 24 hr cultures of *E. coli* 25922 and *C. perfringens* 13124 for standardizations using the DNeasy tissue kit from Qiagen. DNA from the cecal samples collected and analyzed above by selective media was isolated using the QIAamp DNA stool mini kit (Qiagen). Briefly, intestinal samples diluted 1:10 in sterile PBS were treated with 0.5% Tween 20, vortexed for 1 min, and incubated at room temperature for 10 min. Following this initial lysis period, 200µL of sample was used for DNA isolation following the manufacturer's protocol (Qiagen). Primer and MGB probe sets for *C. perfringens* and *E. coli* were designed against the 16S rDNA of each strain using the Applied Biosystems Custom Taqman Assay Design Tool. Sequences for each set are listed in Table 6.2. qPCR was performed separately for *C. perfringens* and *E. coli* assay sets for all cecal samples and bacterial standards.

Table 6.2 Primers used for the detection of *E.coli* and *C.perfringens* by real-time PCR

Primer or	Target Gene	Sequence (5' → 3')
Primers		
APEC_R	*E.coli* 16S RNA	GTGGACTACCAGGGTATCTAATCCT
APEC_F		CCCCCTGGACGAAGACTGA
CPERF_R	*C.perfringens* 16S	GTGGACTACCAGGGTATCTAATCCT
CPERF_F		GCGACTCTCTGGACTGTAACTG
Probes		
APEC_M	*E.coli* 16S RNA	TCCCCACGCTTTCG
CPERF_M	*C.perfringens* 16S	CTCCCCACGCTTTCG

Each reaction contained the following: 9µL of genomic DNA, 1µL of assay mix (specific for either *C. perfringens* or *E. coli*) and 10µL of Taqman Universal PCR Master Mix (Applied Biosystems). Standard curves were generated for *C. perfringens* and *E. coli* using DNA from pure cultures diluted 1:2, starting with 100ng concentrations. Reactions were performed with a StepOne system from Applied Biosystems under standard cycling conditions: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 95°C for 15 sec and 60°C for 1 min. Quantitations of *C. perfringens* and *E. coli* present in cecal samples were calculated by applying the Ct values generated to the standard curve of the corresponding target using the software associated with the StepOne system. It should be noted that Ct values represent an inverse response, so a higher Ct value is not indicative of a higher concentration but rather a lower one. The experimental unit for bacterial quantification by either selective media or qPCR corresponded to the bird that was randomly selected from each replicate pen.

Viscosity and Liver Measurements

At 28 days of age, 2 birds per pen were randomly selected. Each bird was weighed individually and euthanized via cervical dislocation before having the liver removed and individually weighed to determine the relative liver weight. At this time each bird also had the contents of the ileum (meckel's diverticulum to ileocecal junction) removed and the ileal contents of both birds were placed in a common container and homogenized via manual mixing. After thorough mixing, two 1.5 mL eppendorf tubes were filled with the digestive contents and centrifuged at 14,500 × g for 2 min. After centrifugation was complete, 250 µL of the supernatant, from each eppendorf tube, was transferred to the sample cup of a Brookfield digital viscometer (Model LVDV-II+P CP, Brookfield Engineering Laboratories Inc., Middleboro, MA) for a total sample volume of 0.5 mL. Based on previous research by Bedford and Classen (1993) viscosity (in centipoise, cp = 1/100 dyne second per cm2) was determined at a shear rate of 60 sec-1 at 37°C.

Statistical Analysis

The feed mill portion of the study was analyzed using the proc GLM option of SAS software (SAS Institute, 2004) with a *P*-value $\leq$ 0.05 indicating significance. The data were tested for linear and quadratic contrasts using the 3 DDGS inclusion levels (0, 15, and 30%) and without the 30% DDGS + sand treatment. When overall significant differences ($P < 0.05$) existed among all 4 treatments, the Fisher's least significant difference option of SAS was used to separate treatment means (SAS Institute, 2004). Treatments were blocked by day of production and run order, thus replicating each treatment 4 times with each 453.6 kg batch representing an experimental unit. Data generated from the grow-out and sub-sample collection for analysis of *E. coli*, *C.*

perfringens, and ileal viscosity, were evaluated using a two-way analysis of variance (DDGS in starter phase vs. DDGS in grower phase) in a randomized complete block design with the pen representing an experimental unit, and using the proc GLM option of SAS software (SAS Institute, 2004) with a *P*-value $\leq$ 0.05 indicating significance. The data was also tested for linear and quadratic contrasts with incremental levels of DDGS during the grower phase (0, 7.5, 15, 22.5, 30%).

Results

To prevent confusion for the reader in the results, discussion and tables we would like to further define some aspects of the experimental methods. The data presented and discussed in Figure 6.1 and Tables 6.4 and 6.5 correspond to data obtained at 28 days of age. However, one of the aims of this study was to explore potential carryover effects of feeding 0 vs. 8% DDGS levels from 0 to 14 days. Therefore, a main effect noted in the text and illustrations as "pre-grower" will correspond to the effect that feeding 0 vs. 8% DDGS during the starter phase (0-14 days) had on the performance of the broilers during the grower phase (14-28 days). As a reminder, grower-phase levels of DDGS consisted of 0, 7.5, 15, 22.5 or 30%.

Significant results were obtained for all milling variables measured (Table 6.3). Quadratic contrast analysis showed that increasing levels of DDGS resulted in a non-linear response for production rate ($P < 0.05$), conditioner energy usage ($P < 0.01$), and pellet mill energy usage ($P < 0.05$). Inclusion levels of 30% DDGS resulted in a decrease in pellet quality ($P < 0.001$) and pellet mill energy usage ($P < 0.001$), and an increase ($P < 0.05$) in conditioner electrical energy usage and amount of total fines ($P < 0.001$). The control diet resulted in greater pellet mill relative energy ($P < 0.001$) usage and diet bulk

density ($P < 0.05$) compared to the diets containing 15 and 30% DDGS. Analysis of the data suggests that the use of sand did not have any impact when added to a diet with 30% DDGS when compared to the diet with 30% devoid of sand (Table 6.3).

At the end of the starter phase (14 days), analysis of the data showed that BW (0% DDGS = 362 g; 8% = 363 g; SEM = 4.0), feed consumption (0% DDGS = 455 g; 8% = 464 g; SEM = 5.0), and feed conversion (0% DDGS = 1.25; 8% = 1.27; SEM = 0.007) were not different ($P > 0.05$) in chicks that were fed either 0 or 8% DDGS.

Table 6.3 Effects of various levels of DDGS[1] on feed mill efficiency and pellet quality of grower phase broiler diets

DDGS (%) [1]	Production Rate (MT/hr)	Conditioner Relative Energy Usage (KWH/MT)	Pellet Mill Relative Energy Usage (KWH/MT)	PDI (%)[2]	MPDI (%)[3]	Bulk Density (kg/m^3)	Total Fines (%)[4]
0	1.211	0.659 ^{bc}	6.531 ^{a}	74.4 ^{a}	56.3 ^{a}	631.8 ^{a}	30.8 ^{c}
15	1.266	0.646 ^{c}	5.127 ^{b}	66.8 ^{b}	43.5 ^{b}	622.8 ^{b}	41.7 ^{b}
30	1.143	0.749 ^{a}	4.775 ^{c}	62.1 ^{c}	34.1 ^{c}	618.3 ^{b}	54.2 ^{a}
30+sand [5]	1.149	0.723 ^{ab}	5.019 ^{bc}	62.3 ^{c}	37.5 ^{c}	616.9 ^{b}	54.5 ^{a}
SEM	0.013	0.007	0.0798	0.54	1.50	2.60	1.52
P-value	0.07	0.02	0.001	0.001	0.001	0.02	0.001
Linear contrast [6]	0.039	0.003	0.001	0.001	0.002	0.001	0.002
Quadratic contrast [6]	0.013	0.007	0.013	0.12	0.42	0.07	0.69

[a-c]Means within a column not sharing a common superscript differ ($P \leq 0.05$).
[1]Distillers dried grains with solubles.
[2]Pellet durability index.
[3]Modified pellet durability index.
[4]Percent of total feed produced that was fines.
[5]Sand was included at the expense of all ingredients in the diet at a rate of 2%. The p value corresponds to an orthogonal contrast between the treatments with 30% ddgs and 30% ddgs + sand.
[6]Linear and quadratic orthogonal contrasts were tested using the incremental dietary DDGS treatments (0, 15 and 30%) except the “30% DDGS + sand” treatment.

With one exception of feed consumption, the results indicate that the different DDGS levels fed during the starter phase did not affect how birds performed from 14 to 28 days of age. At 28 days of age, increasing dietary DDGS linearly decreased BW gain (P<0.001) and liver relative weight (P<0.001) (Table 6.4). There was also a linear trend (P = 0.05) suggesting an increase in feed conversion with incremental DDGS levels in the grower phase diet. Mortality and viscosity were unaffected by DDGS grower phase level. The only pre-grower DDGS × grower DDGS interaction (P<0.05) observed during the study was for feed consumption, where birds that consumed no DDGS during the starter phase had a decrease in feed consumption at 22.5 and 30% DDGS levels during the grower phase when compared to birds that did consume DDGS in the starter phase (Figure 6.1). Bacterial levels of both *E. coli* and *C. perfringens*, measured through selective media or qPCR, were shown to be unaffected by dietary DDGS (Table 6.5). However, a marginal linear (P=0.07) trend was observed for *C. perfringens* with increasing levels of DDGS when analyzed via qPCR, showing a Ct value that decreased with increasing inclusion of DDGS, therefore suggesting that the presence of cecal *C. perfringens* linearly increased with DDGS (higher Ct values correspond to lower DNA concentration). Conversely, this effect was not supported by the values from selective media bacterial growth.

Table 6.4 Live production, relative liver weight, and ileal viscosity at 28 days of age

Grower DDGS (%)	BWG from 14 to 28 d (g)[2]	Feed intake from 14 to 28 d (g)	Feed conversion from 14 to 28 d	Mortality from 14 to 28 d (%)	Relative liver wt (%)[3]	Viscosity (cp)[4]
0	1,073 [ab]	1,661[ab]	1.55	0.78	2.49[a]	1.89
7.5	1,077 [a]	1,671[a]	1.55	0.78	2.46[ab]	1.99
15	1,053 [bc]	1,643[abc]	1.56	1.81	2.41[abc]	1.99
22.5	1,048 [c]	1,632[bc]	1.56	0.00	2.37[bc]	2.07
30	1,026 [d]	1,629[c]	1.57	0.78	2.36[c]	2.13
SEM	7.7	11.7	0.006	0.695	0.033	0.090
Pre-grower						
No (0%)	1,053.4	1,644	1.55	0.50	2.41	2.02
Yes (8%)	1,058.5	1,652	1.56	1.19	2.43	2.02
SEM	4.9	7.4	0.004	0.437	0.021	0.056
Analysis of Variance (P)						
Pre-grower DDGS	0.29	0.25	0.09	0.29	0.57	0.95
Grower DDGS	<0.0001	0.03	0.20	0.56	0.02	0.44
Pre-grower × Grower	0.08	0.04	0.28	0.85	0.76	0.64
DDGS Grower Linear	<0.0001	0.008	0.05	0.76	0.0004	0.07
DDGS Grower	0.25	0.83	0.84	0.63	0.64	0.96

[1]Distillers dried grains with solubles
[2] Body weight gain
[3] Expressed as percent of total body weight
[4] Centipoise, cp = 1/100 dyne second per cm^2
[a-d] Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 6.5 *C.perfringens* and *E.coli* present at 28 days of age

	E. coli (log_{10} cfu/g cecal contents)	*C. perfringens* (log_{10} cfu/g cecal contents)	*E. coli* (Ct values)	*C. perfringens* (Ct values)
Grower DDGS (%)				
0	6.33	4.46	23.09	24.29
7.5	5.61	4.28	23.78	24.47
15	5.99	4.04	22.31	23.66
22.5	6.25	4.44	24.14	23.81
30	5.93	4.19	23.54	23.60
SEM	0.23	0.21	0.59	0.37
Pre-grower				
No (0%)	5.87	4.17	23.27	23.98
Yes (8%)	6.21	4.41	23.44	23.97
SEM	0.15	0.13	0.37	0.23
Analysis of Variance				
Pre-grower DDGS	0.11	0.23	0.72	0.99
Grower DDGS	0.20	0.57	0.24	0.35
Pre-grower × Grower	0.24	0.23	0.92	0.78
DDGS Linear	0.76	0.59	0.49	0.07
DDGS Quadratic	0.45	0.56	0.69	0.89

[a-b] Means within a column not sharing a common superscript differ ($P < 0.05$).
[1] Distillers dried grains with solubles.

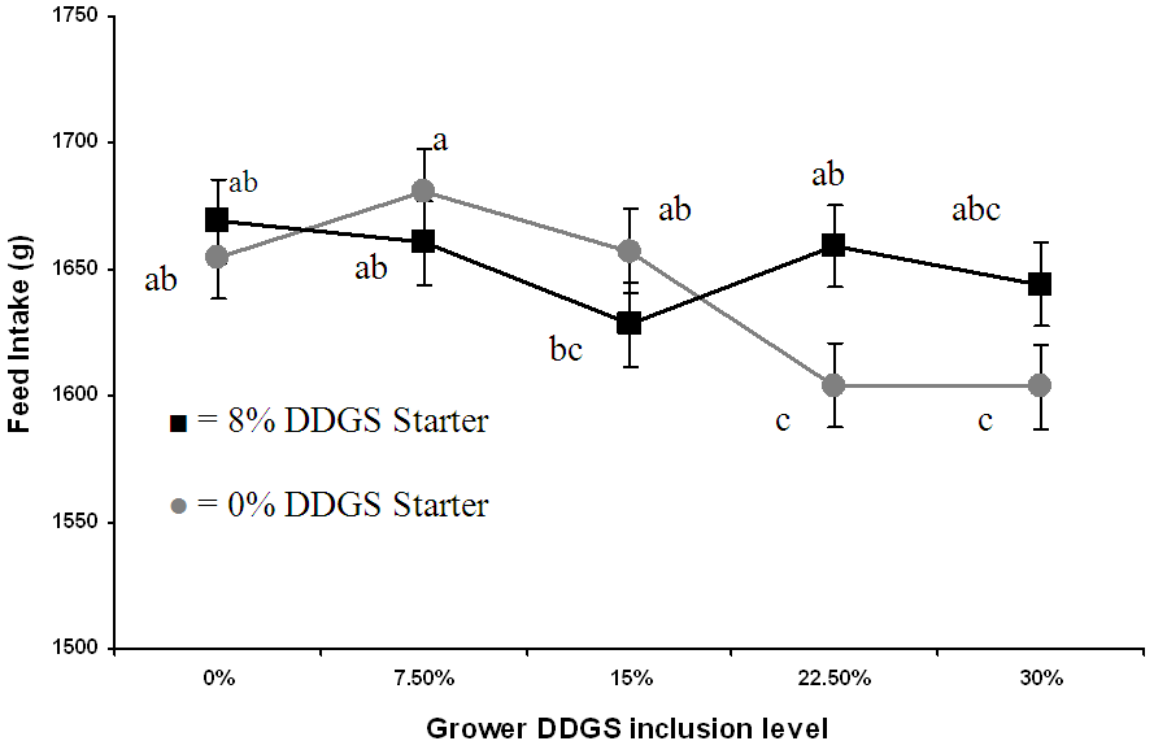

Figure 6.1 Interactive effects of feed consumption during the grower phase (from 14 to 28 days)

Discussion

Feed Milling

There is some agreement, mostly in the form of "popular belief" of the effects that DDGS play in feed manufacturing, but to the authors' knowledge no supportive scientific evidence exists. On the other hand, it has been well documented that feeding high quality pellets can result in improved gain and conversion in broilers (Nir et al., 1994 and Jensen et al., 1962). Production rate was shown to be affected primarily by the 30% DDGS inclusion level in the diet (Table 6.3). This effect could be attributed to the decrease in the level of inorganic phosphate which is known to have a "scrubbing" effect inside the die, although this effect also lacks scientific validation and warrants further research. Dietary DDGS inclusion resulted in decreased pellet quality, likely because this

ingredient has a reduced starch component in comparison to ground corn, which could result in less starch gelatinization and decreased pellet binding. However, when looking at the composition of the grower diets (Table 6.1) higher DDGS inclusion levels resulted in higher amounts of added fat. Salmon (1985) added 3, 6 and 9% of added fat to pellets and reported a decrease with increased fat levels, and in this study all fat was added at the mixer. If fat level is indeed a primary cause of decreased pellet quality, then one could recommend application of a portion of the fat using post-pellet application when diets contain elevated levels of DDGS.

In this study, bulk density declined with DDGS addition to the diet. This decline is most likely due to the lower bulk density that DDGS has compared to regular corn. As DDGS increased in the diet, the amount of fines also increased, in agreement with previous reports by Min et al. (2009); this trend is typically a proportional response of pellet quality and is in complete agreement with the current study (Table 6.3).

Inclusion of 30% DDGS resulted in an increased energy usage at the conditioner, while the use of no DDGS resulted in an increase in energy usage at the pellet mill. Arguably, this increase in energy usage at the conditioner is of less interest than the decrease seen at the pellet mill, considering that it is the pellet mill that traditionally has much larger motors and consequently higher energy inputs. It is possible that in both cases the changes observed are the result of added fat in the diet. It is widely believed that a diet with a higher oil inclusion will have increased pellet mill throughput compared to a diet with less oil, mostly due to the lubricating action of fat at the mash die interface (Thomas et al., 1998). As for the increased energy used at the conditioner, it can be hypothesized that higher supplemental fat amounts create a thicker, more viscous, mash that may requires more energy to be augered through the conditioner.

Another recent "popular belief" is that sand aids in overall mill performance when manufacturing pelleted diets that are low in rock phosphates, such as those diets with high levels of DDGS. It is believed that incorporating sand in a diet could create a "die scouring" effect during pelleting, by reducing friction through "scrubbing the die" thus easing overall diet throughput and decreasing energy usage. However, as observed in the current results, the 30% + sand diet did not affect any of the parameters measured when compared to its counterpart devoid of sand.

Grow-out and Liver Weight

Absence of a pre-grower main effect for the performance of broilers at 28 days of age (Table 6.4) was expected based on previous research conducted by Lumpkins et al. (2004) where DDGS were fed at a rate of 15% of the starter diet. These results agree with Parsons et al. (1983) who reported that up to 20% of the soybean meal in a chick diet could be replaced by DDGS without any detrimental effect on growth rate. The birds were impacted by the different levels of DDGS fed during the grower phase. A linear trend was observed towards decreasing BW gain. Inclusion of DDGS past 7.5% of the diet resulted in lower BW gain values. However, it is important to note that the birds receiving 15% DDGS in the grower phase did not exhibit a significantly lower BWG compared to the control birds. This is in close agreement with the suggestion made by Lumpkins et al. (2004) that DDGS from modern ethanol plants can be safely used at levels from 12 to 15% in the grower period. Wang et al. (2008) also reported that once DDGS in the diet exceeded 20% there was a significant decline in BWG in the grower period. In each of the two studies mentioned previously, the level of DDGS in the diet changed at different increments from the current study and thus it is hard to discern the

line of maximum inclusion for DDGS in the grower phase. Wang et al. (2008) reported an increase in FCR as DDGS in the diet increased from 20 to 30% of the diet in the grower phase, while Lumpkins et al. (2004) reported similar results to our study with no difference in FCR with DDGS comprising up to 18% of the diet. While not as prominent as the results of the previous research mentioned, the current study showed a linear trend for increased FCR when DDGS inclusion was increased in the grower phase diet. This current trend could be due to different factors. Two such factors are changes in gut viscosity as well as a possible toxicity as a result of the high DDGS inclusion, and both are factors that were evaluated in the current study.

Feed intake exhibited a linear trend towards decreased consumption as DDGS increased in the diet, but an interaction observed between the pre-grower and grower DDGS inclusion levels demonstrated how birds that consumed no DDGS during the starter phase exhibited a decrease in feed consumption at 22.5 and 30% during the grower phase, while birds that received 8% DDGS during the starter phase were unaffected by DDGS grower level (Figure 6.1). This feed consumption pattern exhibited by the birds that received no DDGS during the starter phase suggests an inability to adapt to the presence of DDGS in the grower diet and thus the relatively high levels of 22.5 and 30% may have caused that decrease in consumption. There was also a linear trend seen for relative liver weight (Table 6.4), whereas DDGS increased there was a decrease in relative liver weight. The liver was chosen to be weighed in this study as an evaluator for any metabolic challenge that could perhaps be associated with feeding high levels of DDGS. This linear decrease in the relative weight of the liver associated with feeding increasing dietary levels of DDGS may have been caused by a marginal toxicity resulting

in atrophy of the liver, but until biochemical and histological analyses are conducted it is difficult to ascertain.

Microflora and Viscosity

Past research performed by Jacob et al. (2007) has shown a positive association between dietary DDGS and fecal prevalence of *E. coli* in cattle. No changes in *E. coli* presence were observed in this study in cecal contents of these birds. A likely explanation for this difference may lie in the difference between the two species' gastrointestinal tracts. The digestive system of cattle widely differs from the monogastric system of the broiler, and therefore too many factors could have resulted in the bacterial increase reported in cattle but not seen in broilers. While there is little research into effects that different feedstuffs have on *E. coli* levels of the broiler digestive system, Rubio et al. (1998) reported that *E. coli* counts were unaffected by the inclusion of sweet lupin seed meal in the diet, compared to a commercial type, wheat and soybean meal based diet.

Because of potential economic impact to broiler operations throughout the world, the presence of *C. perfringens* and *E. coli* was evaluated in the present study to determine if varying concentrations of DDGS present in feed would impact the colonization of these microbes in broilers. Our results indicate that no significant differences were observed. There may be some indication (P=0.07) that *C. perfringens* could possibly be increasing in cecal concentration as the percent of DDGS increased per our qPCR results. In contrast, this *C. perfringens* result was unsupported by the selective media counts, thus warranting further investigation. The variation between the qPCR and viable plate counts could be attributed to the presence of spore DNA and/or dead cells present in our samples analyzed by qPCR. Slight decreases were also observed in the presence of *E. Coli* in the

cecal samples, though these changes were also not significant. To our knowledge research evaluating the use of DDGS in the diet and its effect on cecal concentration of *C. perfringens* in broilers is not readily available. Annett et al. (2002) reported that *C. perfringens* is more prevalent in broilers fed a wheat or barley based diet, as compared to those fed a corn based diet. Changes in the levels of *C. perfringens* have also been associated with changes in protein source and level as shown by Drew et al. (2004). These authors established that the level of dietary CP did have an effect on the *C. perfringens* levels at 28 d of age in broilers. However, Drew et al. (2004) promoted *C. perfringens* colonization by inoculating this microorganism in the feed and not including an antibiotic or coccidiostat in the experimental diets. Furthermore, diets fed by Drew et al. (2004) were not pelleted. Although there was a measurable difference in the CP levels of the experimental diets used in the current study, these feeds were steam pelleted and were not inoculated with *C. perfringens*. Therefore, this potential increase in viable *C. perfringens* present with increasing dietary DDGS levels observed herein are the result of an effect that simulates commercial conditions closer and thus should be further evaluated for its practical commercial implication.

It has long been accepted that viscous grains are known to increase the viscosity of the digestive contents in broilers (Bedford, 1996, Jozefiak et al., 2006, Jia et al. 2009) when compared to corn based diets. The marginal ($P = 0.07$) increase in viscosity with DDGS seems to suggest that a trend may be emerging and could possibly become significant when birds are fed DDGS for longer periods. Lee et al. (2003) observed a relationship where increasing intestinal viscosity corresponded with growth depression in broilers fed guar meal germ and hull fractions in the diet. This response should be further

evaluated, particularly in older broilers with a more functional and developed small intestine.

In conclusion, DDGS seem to be safe at inclusion levels of 8% from 0 to 14 days of age. During the grower phase, it seems clear that high levels of DDGS in the diet resulted in pellet quality and bulk density decreasing while reducing energy usage at the pellet mill. The marginal trends observed for increased intestinal viscosity and cecal *C. perfringens* viability with increasing DDGS in the diet warrant further investigation. When feeding the grower diets to broilers from 14 to 28 days of age, it appears that the limit of inclusion may be between 7.5 and 15% of the diet. It may be advantageous to the bird to have DDGS in the starter diet in order to condition the digestive system to this byproduct before being exposed to even higher levels in the grower phase.

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CHAPTER VII

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

Abstract

A study evaluated the effects of feeding two levels (0 vs. 8%) of distillers dried grains with solubles (DDGS) in a broiler diet during the starter and grower phases (0-14-28 days; 40 replicates/trt) and subsequently feeding each of these a finisher diet (28-42days) with either 0, 7, 14, 21, or 28% DDGS (8 replicates/trt). During the starter phase (0-14 days) birds receiving DDGS in the diet exhibited significantly ($P \leq 0.05$) higher feed conversion (FCR) than those that received no DDGS. The same effect occurred in the 0-28 day period in addition to a significant decrease in weight gain (BWG) among the birds receiving DDGS versus those without. During the finisher phase, as DDGS increased in the diet, past 14%, a significant decrease in BWG and feed intake was observed versus the control, both decreasing in a linear manner. Cumulative BWG and feed intake exhibited a similar response to that observed for BWG and feed intake during the finisher phase, by showing a linear decrease with increasing DDGS. Dressing and breast meat yield declined in a linear manner. Increasing DDGS in the finisher phase diet led to an increase in relative gizzard and large intestine weight, but did not have an effect on digesta viscosity. High levels of DDGS linearly decreased the presence of *Escherichia*

coli in the ileum based on selective media analysis, but showed a quadratic response according to quantitative PCR. According to selective media analysis, but not quantitative PCR, pre-finisher × finisher inetarctions were observed for ileal *E. Coli* and *Listeria monocytogenes* as well as *L .monocytogenes* in the ceca. Interactions showed that when birds were fed no DDGS in the pre-finisher, ileal *E Coli* concentration was increased at 0% DDGS in the finisher versus birds that were fed DDGS in the pre-finisher and then received the 0% DDGS finisher. Interactions for ileal and cecal counts of *L. monocytogenes* were somewhat irregular but seem to suggest a difference in ileal counts when 21% DDGS were being fed and differences in cecal counts when 14% DDGS were being offered. DDGS can be effectively incorporated into commercial broiler diets fed from placement till a 42 day slaughter age up to levels of 14% of the diet. Potential beneficial results on *E. coli* ileal counts warrant further investigation. However, care and attention must be given to the inclusion levels and age of the birds as well as to the economics of a given situation.

Introduction

Distillers dried grains with solubles (DDGS), the primary by-product of corn to ethanol fermentation, can be a good choice for producers to include into their poultry rations, especially during times of economic hardship. Ethanol production continues to increase and while someday this trend may reach a plateau, it is only reasonable to take advantage of the large amounts of DDGS being produced now. DDGS are not without their limitations though, as problems have been noted with nutritional variability (Cromwell et al., 1993, Batal and Dale, 2006, Fastinger et al., 2006). It has also been shown that high levels of DDGS in a pelleted broiler diet can lead to decreased bulk

density and pellet durability (Loar et al., 2010). Many issues associated with nutritional variability can be avoided if a producer gets a complete analysis of the DDGS product and their supplier produces a quality and consistent product. Dealing with the effects of DDGS on feed milling parameters becomes a tradeoff between the decreased diet cost and the decreased physical quality of the diet, seen with the usage of higher levels in the formulation.

In an age where food safety issues gain more prominence every day, any dietary component that may affect microflora levels in food animals deserves thorough investigation. Little research has been done as to the effect of DDGS on the colonization of potentially harmful bacteria in the gastrointestinal (GI) tract of broilers. In a previous study, the current authors observed the effects of varying levels of DDGS on cecal colonization of *Escherichia coli* and *Clostridium perfringens* and discovered that DDGS level in the diet had no significant effect on either microbe (Loar et al., 2010). However, the researchers did observe a marginal linear (P=0.07) trend for *C. perfringens* with increasing levels of DDGS when analyzed via qPCR suggesting that the presence of cecal *C. perfringens* linearly increased with DDGS. This previous study was conducted until the birds reached 28d of age, and it is possible that given more growth time on DDGS containing diets, differences in bacterial levels may emerge. Loar et al. (2010) also tested ileal viscosity at 28d of age, and while there were no significant differences, the researchers felt that further investigation into DDGS effects on gut viscosity was necessary with older birds. This decision was based on the presence of another marginal linear trend (P=0.07) where ileal viscosity increased as DDGS level in the grower phase increased.

It is vital to understand the limitations associated with feeding higher levels of DDGS and how those limitations may change based on the age of the bird. This particular study was designed to help elucidate some of those limitations: 1) evaluate the effects of increasing levels of DDGS (0, 7, 14, 21 and 28%) from 28 to 42 days on broiler growth and processing yields after the birds received either 0 or 8% DDGS in the starter and grower phases; 2) determine the effects of varying levels of DDGS on intestinal, liver, and gizzard weight, ileal viscosity, and liver glycogen content at 42 days of age; 3) determine the effects that various levels of DDGS in the finisher diet may have on cecal and ileal populations of *Listeria monocytogenes*, *E. coli* and *C. perfringens* in broilers.

Materials and Methods

Grow-out

The grow-out portion of the study encompassed the period between 0 to 42 days of age, using Ross × Ross 708 males obtained from a commercial hatchery. Day-old chicks were randomly placed in each of 80 floor pens (12 birds/pen; 960 birds total; 0.09m^2/bird). The close-sided house had thermostatically controlled heating, cool cells and cross ventilation. Each pen contained built-up litter, a hanging feeder (22.5kg capacity) and nipple drinkers (3 nipples/pen). The lighting program was 23 h light and 1 h dark, and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via *in ovo* administration at day 18), as well as Newcastle disease and infectious bronchitis (via coarse spray at hatch).

During the starter and grower phases (0-14 and 14-28 days), two DDGS inclusion levels (0 vs. 8%) were fed to the 80 floor pens (2 treatments; 40 replicates/trt). Subsequently, each of these two DDGS levels provided during the pre-finisher feed

phases was fed either 0, 7, 14, 21, or 28% DDGS during the finisher phase (28-42 days), resulting in a 2 × 5 factorial study (2 DDGS in pre-finisher × 5 DDGS in finisher) and was replicated 8 times for a total of 80 experimental units. The feed was provided to the birds from 0 to 14 days of age in crumbles, and from 14 to 28 days and 28 to 42 days as pellets (Table 7.1). Treatments were blocked completely, according to location within the house. Table 7.1 shows the 0 and 8% DDGS diets fed during the starter and grower periods, as well as the 0 and 28% DDGS finisher diets. The 7, 14 and 21% DDGS finisher diets were the result of blending between the 0 and 28% finisher diets. Crude protein was not assigned a minimum value during formulation, and essential digestible amino acids were maintained in all dietary treatments by setting minimum formulation ratios relative to digestible Lys as follows: TSAA 75, Thr 65, Val 78, Ile 68, Trp 17, and Arg 105, and following previously published recommendations (Lemme et al., 2004). All other essential nutrients were formulated to meet or exceed nutrient recommendations (NRC, 1994). Feed and water were provided for ad libitum consumption.

All birds in each pen were weighed collectively at the beginning and end of each feed phase. Feed consumption and mortality were monitored throughout the study and feed conversion (FCR) was corrected for the weight of mortality and represents: (g of feed consumed by all birds in a pen) / (g of BW per pen + weight of dead birds). All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Table 7.1 Experimental diet composition (% as is)

Ingredients	Starter diets (0-14 d)		Grower diets (14-28 d)		Finisher diets (28-42 d)	
	0% DDGS	8% DDGS	0% DDGS	8% DDGS	0% DDGS	28% DDGS
Corn	57.4	53.1	64.4	60.1	71.4	50.2
SBM (48%CP)	35.6	31.9	29.5	25.8	23.4	15.8
DDGS [1]	-	8.0	-	8.0	-	28.0
ProPlus [2]	2.0	2.0	2.0	2.0	2.0	2.0
Poultry fat	2.33	2.42	1.49	1.59	0.73	2.22
Deflourinated PO_4	0.92	0.97	0.79	0.83	0.71	0.84
Calcium carbonate	0.82	0.62	0.81	0.61	0.80	0.11
Premix [3]	0.25	0.25	0.25	0.25	0.25	0.25
NaCl	0.25	0.17	0.24	0.16	0.24	0.05
DL-Methionine	0.24	0.24	0.21	0.21	0.19	0.15
L-Lysine HCl	0.12	0.19	0.18	0.26	0.19	0.31
Coccidiostat [4]	0.05	0.05	0.05	0.05	0.05	0.05
L-Threonine	0.03	0.04	0.04	0.06	0.04	0.01
Phytase enzyme[5]	0.02	0.02	0.02	0.02	0.02	0.02
Calculated Composition						
AME (kcal/kg)	3,075	3,075	3,100	3,100	3,125	3,125
Available P (%)	0.45	0.45	0.42	0.42	0.40	0.40
Ca (%)	0.90	0.90	0.84	0.84	0.80	0.80
CP (%)	23.06	23.21	20.75	20.89	18.39	20.68
Dig. TSAA (%)	0.90	0.90	0.82	0.82	0.75	0.75
Dig. Lysine (%)	1.25	1.25	1.14	1.14	1.0	1.0
Dig. Threonine (%)	0.81	0.81	0.74	0.74	0.65	0.65

[1]Distillers Dried Grains with Solubles.
[2]Animal protein blend, with a CP value of 60% (H.J. Baker & Bro., Inc.; Little Rock, AR)
[3] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.3 mg.
[4] Dietary inclusion of coccidiostat provides 60 g salinomycin sodium per 907.2 kg of feed.
[5]DSM Nutritional Products (Parsippany, New Jersey)

Bacterial Quantification

The *E. coli* strain 25922, the *C. perfringens* strain 13124, and the *L. monocytogenes* strain EGD-e were all acquired through the American Type Culture Collection (Manassas, VA). *E. coli* 25922 were cultured on Trypticase Soy Agar (TSA) at 37°C under aerobic conditions. *C. perfringens* 13124 were cultured on Perfringens agar base with TSC selective medium at 37°C under anaerobic conditions. Anaerobic conditions were established using a Mitsubishi Anaeropak jar. Anaerobic indicators were used to ensure anaerobic conditions were maintained.

At 42 days of age, two birds per pen were randomly selected and euthanized via cervical dislocation. Freshly harvested ceca and ilea were collected and contents were extracted and combined for the two birds in two separate whirl pak bags, one for cecal samples and one for ileal samples. Samples were homogenized in a Seward 400 circulator stomacher (Fisher Scientific, Pittsburgh, PA). Contents were weighed and samples were diluted in sterile 1X PBS prior to plating on selective media. For detection of *E. coli* in the samples, dilutions of the cells were plated on MacConkey agar supplemented with MUG (Remel) and incubated for 24 hr at 37°C. Purple colonies with fluorescence under a long-wave UV lamp were scored as positive for *E. coli*. For detection of *C. perfringens* in the samples, dilutions of the cells were plated on Perfringens agar base with TSC selective supplement (Oxoid) for 24 hr at 37°C under anaerobic conditions. Anaerobic conditions were established and monitored as described above. *C. perfringens* colonies were counted based on the appearance of black and opaque colonies. For the detection of *L. monocytogenes*, samples were plated onto Brilliance Listeria agar base supplemented with Brilliance Listeria selective and differential supplements at 37°C (Oxoid). *Listeria* sp. were detected by the presence of blue colonies.

Pure cultures of *E. coli* 25922, *C. perfringens* 13124, and *L. monocytogenes* EGD-e were grown for 24 hr prior to isolation of DNA using DNeasy tissue kit from Qiagen. DNA from the cecal and ileum samples collected and analyzed above was isolated using the QIAmp DNA stool mini kit with minor modifications. The samples were diluted 1:10 in sterile PBS, treated with 0.5% Tween 20, vortexed for 1 min, and incubated at room temperature for 10 min. Following this initial lysis period, 200ul of sample was used for DNA isolation using the manufacturer's protocol.

Table 7.2 Primers used for the detection of *E. coli*, *C. perfringens*, and *L. monocytogenes* by real-time PCR

Primer or Probe	Target gene	Sequence (5' – 3')
Primers		
APEC_R	*E. coli* 16S RNA	GTGGACTACCAGGGTATCTAATCCT
APEC_F		CCCCCTGGACGAAGACTGA
CPERF_R	*C. perfringens* 16S RNA	GTGGACTACCAGGGTATCTAATCCT
CPERF_F		GCGACTCTCTGGACTGTAACTG
LIST_R	*L. monocytogenes actA*	CACTGCATCTCCGTGGTATACTAA
LIST_F		TGCAAGTCCTAAGACGCCA
Probes		
APEC_P FAM	*E. coli* 16S RNA	TCCCCACGCTTTCG
CPERF_P FAM	*C. perfringens* 16S RNA	CTCCCCACGCTTTCG
LIST_P FAM	*L. monocytogenes actA*	CGATTTCATCCGCGTGTTTCTTTTCG

Primer and FAM-labeled probe sets for *C. perfringens*, *E. coli*, and *L. monocytogenes* were purchased through IDT DNA. The assays for *C. perfringens* and *E. coli* were designed against the 16S rRNA, while the assay for *L. monocytogenes* detection was designed against the *actA* gene as previously described (Oravcova et al., 2007). Sequences for each set are listed in (Table 7.2). qPCR was performed separately for *C.*

perfringens, *E. coli*, and *L. monocytogenes* assay sets for all cecal and ileum samples and bacterial standards. Each reaction contained the following: 9µl of genomic DNA, 1µl of assay mix (specific for either *E. coli* or *C. perfringens*), 10µl of Taqman Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA). Standard curves were generated for *E. coli* and *C. perfringens* using DNA from pure cultures diluted 1:2, starting with 100ng concentrations. Reactions were performed with a StepOne system from Applied Biosystem under standard cycling conditions: 2 min 50°C, 10min at 95°C, and 40 cycles of 95°C 15 sec and 60°C 1 min.

Viscosity and Organ Measurements

At 42 days of age, 2 birds per pen were randomly selected. One bird was weighed individually and euthanized via cervical dislocation before having the small intestine, large intestine and gizzard removed, cleaned out and individually weighed to determine their relative weights. The contents of the ileum (meckel's diverticulum to ileocecal junction) were emptied into a separate container. The second bird was also euthanized in the same manner and at this time had the contents of the ileum removed and combined with those of the first bird and homogenized via manual mixing. After thorough mixing, two 1.5 mL eppendorf tubes were filled with the digestive contents and centrifuged at 14,500 × *g* for 2 min. After centrifugation was complete, 250 µL of the supernatant from each eppendorf tube was transferred to the sample cup of a Brookfield digital viscometer (Model LVDV-II+P CP, Brookfield Engineering Laboratories Inc., Middleboro, MA) for a total sample volume of 0.5 mL. Based on previous research by Bedford and Classen (1993) viscosity (in centipoise, cp = 1/100 dyne second per cm^2) was determined at a shear rate of 60 sec^{-1} at 37^oC.

One bird per pen was randomly selected for treatments 1, 6, 7, 8, 9 and 10 and euthanized via cervical dislocation before having the entire liver removed and weighed. This was done so as to obtain relative liver weight. A small portion of the liver was immediately preserved in 10% perchloric acid for later analysis of the glycogen content. Glycogen content analysis was performed via the method described by Bennett, et al. (2007). The researchers chose to analyze only treatments 1 and 6 thru 10 because of sample size preparation limitations. Birds in treatment 1 received no DDGS containing diets at any point in the study while birds in treatment 6, 7, 8, 9, and 10 all received 8% DDGS in the pre-finisher phase and then 0, 7, 14, 21, and 28% DDGS in the finisher, respectively.

Processing Measurements

At 42 days of age, 6 birds per pen were randomly selected, tagged, individually weighed and cooped 12 hr before processing. Birds were processed at a pilot processing plant. Electrical stunning was performed by applying 11.5 volts (<0.05 mA, AC to DC current), for 30 sec to each broiler, and broiler carcasses were scalded, picked and eviscerated automatically using commercial prototype equipment. Carcass and abdominal fat weights were obtained as birds were manually removed from the line. Birds were then chilled for 4 hr at which point all birds were manually deboned and weights were obtained for breasts and back halves of all birds. Absolute and relative weights (% of live weight) were determined for abdominal fat, back half, carcass and boneless-skinless breast meat. Occurrence of deep pectoral myopathy in *Pectoralis minor* muscles was monitored and recorded.

Statistical Analysis

Data generated from the grow-out, processing and sub-sample collection for analysis of *E. coli*, *C. perfringens*, *L. monocytogenes*, digestive organ weight and ileal viscosity, were evaluated using a two-way analysis of variance (DDGS in pre-finisher phases vs. DDGS in finisher phase) in a randomized complete block design with the pen representing an experimental unit. Percentage data for mortality were transformed to arcsine √% for analysis. Data were tested for interactions (pre-finisher x finisher), and then main effects. The data were also tested for linear and quadratic contrasts with the incremental levels of DDGS during the finisher phase.

Data from the liver weight and glycogen concentration portions were analyzed using analysis of variance in a randomized complete block design with the pen representing experimental unit. All data in the study were analyzed by the GLM procedure of SAS (2004) and treatment effects ($P \leq 0.05$) were separated using Fisher's Least Significant Difference test option of SAS (2004) using an α of 0.05.

Results

One of the objectives of this study was to observe any carryover effects of feeding 0 vs. 8% DDGS levels from placement up to 28 days (Table 7.3). Therefore, a main effect noted in the text and illustrations as "pre-finisher" will correspond to the effect that feeding 0 vs. 8% DDGS during the starter and grower feed phases (0-28 days) had on the performance of the broilers during the finisher phase (28-42 days). As a reminder, finisher-phase levels of DDGS consisted of 0, 7, 14, 21 or 28% and the performance data for 0-42 days as well as for 28-42 d are presented in Table 7.4.

In Table 7.3 the live production results from placement to 14 days and placement to 28 days are displayed. Results show that 8% DDGS in the pre-finisher resulted in a

significantly higher FCR (P=0.006) at 14 d of age compared to no DDGS in the pre-finisher. Furthermore, when data were analyzed for the 0 to 28 day period a significant decrease in BWG (P=0.03) accompanies another significant increase in FCR (P<0.0001) for birds fed 8% DDGS in the pre-finisher versus those that received no DDGS.

Effects of DDGS in pre-finisher were not observed to influence the finisher phase performance, but did have an impact on cumulative BWG and feed conversion (Table 7.4). Birds that received 8% DDGS during the pre-finisher periods of the study had decreased (P<0.05) BW gain and increased (P<0.0001) FCR compared to those that received no DDGS. Main effects of DDGS in the pre-finisher feed were not observed for any of the other evaluation criteria, thus from this point forward only the effect of finisher DDGS levels will be addressed.

Table 7.3 Live production results of broilers fed either 0 or 8% DDGS[1] during the pre-finisher period from 0 to 28 days of age

	0-to-14 d of age			0-to-28 d of age		
	BWG (g)[2]	Feed Conversion	Feed intake (g)	BWG (g)[2]	Feed Conversion	Feed intake (g)
Pre-Finisher						
No (0%)	331.9	1.37[b]	457.0	1412.8[a]	1.49[b]	2115.6
Yes (8%)	328.5	1.39[a]	460.4	1386.5[b]	1.52[a]	2119.2
SEM	2.76	0.005	3.07	8.61	0.004	10.9
Analysis of Variance (P)						
Pre-finisher DDGS	0.37	0.006	0.43	0.03	<0.0001	0.81

[1]Distillers dried grains with solubles
[2] Body weight gain
[a-c] Means within a column not sharing a common superscript differ (P < 0.05).

Table 7.4 Live production results of broilers fed either 0 or 8% DDGS[1] during the pre-finisher period and various levels of DDGS from 28 to 42 days of age

	0-to-42 d of age			28-to-42 d of age		
	BWG (kg)[2]	Feed Conversion	Feed intake (kg)	BWG (kg)[2]	Feed conversion	Feed intake (kg)
Finisher DDGS (%)						
0	2.66[ab]	1.62	4.39[a]	1.24[ab]	1.92	2.42[a]
7	2.68[a]	1.62	4.35[ab]	1.26[a]	1.91	2.40[a]
14	2.62[abc]	1.63	4.29[ab]	1.22[bc]	1.93	2.35[ab]
21	2.59[bc]	1.63	4.23[b]	1.19[c]	1.93	2.31[b]
28	2.57[c]	1.64	4.23[b]	1.19[c]	1.94	2.29[b]
SEM	0.025	0.007	0.044	0.017	0.012	0.028
Pre-Finisher						
No (0%)	2.65[a]	1.62[b]	4.33	1.23	1.92	2.37
Yes (8%)	2.59[b]	1.64[a]	4.27	1.20	1.94	2.33
SEM	0.016	0.004	0.027	0.011	0.007	0.018
Analysis of Variance (P)						
Pre-finisher DDGS	0.04	<0.0001	0.21	0.06	0.26	0.16
Finisher DDGS	0.04	0.42	0.03	0.01	0.47	0.005
Pre-finisher × Finisher	0.87	0.15	0.77	0.67	0.19	0.96
DDGS Linear	0.002	0.11	0.0009	0.002	0.04	<0.0001
DDGS Quadratic	0.59	0.90	0.65	0.65	0.96	0.78

[1]Distillers dried grains with solubles
[2] Body weight gain
[a-c]Means within a column not sharing a common superscript differ (P < 0.05).

BW gain from 28-42 days of age was significantly lowered (P = 0.01) in the birds that received either 21 or 28% DDGS in the diet compared to those that received the control or 7% DDGS (Table 7.4). Those fed the 14% DDGS diet were intermediate in BW gain for the same period. For feed intake from 28 to 42 days, and in parallel with BW gain, birds that received either 21 or 28% DDGS exhibited a significantly lower (P=0.005) intake compared to either the control or 7% DDGS diet. Both of the aforementioned parameters were observed to decrease in a linear manner as DDGS inclusion in the finisher phase increased (Table 7.4). Intake for birds receiving the 14% DDGS diet was intermediate. Data for cumulative BW gain and feed intake agrees with that of the finisher phase, and showed that BW gain and feed consumption were linearly reduced (P<0.01) with increasing levels of DDGS. Feed intake from 0 to 42 days was significantly less (P<0.05) with the 21 and 28% DDGS diets compared to the control. Feed conversion was shown to be linearly affected (P<0.05) in a minor way by a linear increase with DDGS during finisher feed phase.

Carcass yield, breast meat yield, and breast meat weight were observed to be linearly reduced (P<0.01) with increasing finisher DDGS levels (Table 7.5). A response observed for abdominal fat weight and percentage was such that birds receiving 14% DDGS exhibited significantly lower values (P<0.01) than all other treatments. No significant differences were observed for back half weight or percentage (Table 7.5).

Table 7.5 Processing results of broilers fed either 0 or 8% DDGS[1] during the pre-finisher period and various levels of DDGS from 28 to 42 days of age

	Dress (%)[2]	Carcass (kg)	Fat (g)	Fat (%)[2]	Back (g)	Back (%)[2]	Breast (g)	BMY (%)[2]
Finisher DDGS (%)								
0	68.94[a]	1.93	38[a]	1.36[a]	803	28.69	618	22.11[a]
7	68.72[ab]	1.94	37[a]	1.30[a]	824	28.89	620	22.06[a]
14	68.57[ab]	1.91	31[b]	1.14[b]	798	28.70	611	21.92[a]
21	68.37[b]	1.88	35[a]	1.27[a]	795	28.82	589	21.37[b]
28	67.81[c]	1.86	35[a]	1.27[a]	787	28.44	589	21.67[ab]
SEM	0.153	0.024	1.1	0.040	9.6	0.148	9.8	0.168
Pre-Finisher								
No (0%)	68.39	1.92	35	1.25	809	28.79	611	21.83
Yes (8%)	68.57	1.89	35	1.29	793	28.63	599	21.81
SEM	0.098	0.015	.7	0.025	6.1	0.093	6.2	0.105
Analysis of Variance (P)								
Pre-finisher DDGS	0.21	0.18	0.69	0.26	0.09	0.27	0.21	0.88
Finisher DDGS	<0.0001	0.17	0.001	0.005	0.17	0.30	0.06	0.01
Pre-finisher × Finisher	0.24	0.91	0.59	0.45	0.71	0.63	0.96	0.76
Finisher DDGS Linear	<0.0001	0.01	0.06	0.16	0.06	0.23	0.004	0.004
Finisher DDGS Quadratic	0.22	0.51	0.02	0.01	0.38	0.13	0.64	0.63

[1]Distillers dried grains with solubles

[2] Percent of live weight

[a-c] Means within a column not sharing a common superscript differ (P < 0.05).

Gizzard absolute and relative weight, and large intestine relative weight, were all linearly increased as DDGS rose in the finisher phase (Table 7.6). Birds fed the 28% DDGS diet were found to have gizzards significantly heavier ($P<0.01$) than those of the control, 7 or 14% DDGS-fed birds. The percent of live weight of the bird represented by the gizzard was also shown to be significantly greater ($P<0.001$) for the 21 and 28% treatments compared to the control diet. Small intestine, and ileal viscosity were found to be unaffected by the DDGS treatments imposed (Table 7.6). The total, but not the relative weight of the liver, was found to linearly decrease ($P<0.05$) as DDGS increased in the finisher phase (Table 7.7). In agreement with relative liver weight, no effects of DDGS in glycogen concentrations were observed (Table 7.7).

Table 7.6 Gastrointestinal organ results of broilers fed either 0 or 8% DDGS[1] during the pre-finisher period and various levels of DDGS from 28 to 42 days of age

	Small Intestine (kg)	Small Intestine (%)[2]	Large Intestine (kg)	Large Intestine (%)[2]	Gizzard (kg)	Gizzard (%)[2]	Viscosity (cp)[3]
Finisher DDGS (%)							
0	0.092	2.96	0.014	0.45	0.038[c]	1.24[c]	2.32
7	0.084	2.88	0.014	0.46	0.040[bc]	1.38[bc]	2.29
14	0.090	3.02	0.014	0.47	0.041[bc]	1.37[bc]	2.30
21	0.093	3.02	0.014	0.49	0.045[ab]	1.53[a]	2.04
28	0.091	3.07	0.015	0.49	0.046[a]	1.51[ab]	2.34
SEM	0.0027	0.079	0.0005	0.016	0.0017	0.051	0.121
Pre-Finisher							
No (0%)	0.092	3.04	0.014	0.47	0.043	1.43	2.28
Yes (8%)	0.088	2.94	0.014	0.48	0.041	1.37	2.24
SEM	0.0017	0.049	0.0003	0.01	0.0011	0.032	0.076
Analysis of Variance (P)							
Pre-finisher DDGS	0.12	0.17	0.89	0.48	0.19	0.15	0.70
Finisher DDGS	0.12	0.47	0.36	0.36	0.006	0.0007	0.44
Pre-finisher × Finisher	0.47	0.83	0.86	0.93	0.18	0.08	0.83
Finisher DDGS Linear	0.42	0.14	0.11	0.03	0.001	0.0003	0.58
Finisher DDGS Quadratic	0.48	0.66	0.22	0.80	0.77	0.49	0.43

[1]Distillers dried grains with solubles

[2] Percent of live weight

[3] Centipoise, cp = 1/100 dyne second per cm^2

[a-c] Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 7.7 Glycogen and liver results of broilers fed either 0 or 8% DDGS[1] during the pre-finisher period and various levels of DDGS from 28 to 42 days of age

	Liver Weight (kg)	Relative Liver (%)[2]	Glycogen Concentration (%)[3]
Treatment (pre-finisher – finisher)[4]			
1 (0 - 0% DDGS)	0.0655	2.06	4.20
6 (8 - 0% DDGS)	0.0625	2.05	3.46
7 (8 - 7% DDGS)	0.0629	2.01	3.86
8 (8 - 14% DDGS)	0.0609	2.02	3.67
9 (8 - 21% DDGS)	0.0575	1.96	3.71
10 (8 - 28% DDGS)	0.0594	1.98	3.90
SEM	0.00288	0.056	0.418
Analysis of Variance (P)			
Treatment	0.43	0.79	0.89
Linear	0.04	0.21	0.53
Quadratic	0.66	0.52	0.35

[1]Distillers dried grains with solubles
[2] Percent of live weight
[3]Percent of liver
[4]First number in parentheses following treatment is DDGS level fed during pre-finisher and second number is DDGS level fed in finisher phase

When observing the results of the bacterial quantification, as measured by physical plate count (Table 7.8) on selective media, no significant results were seen for *E. coli* or *C. perfringens* colonization in the ceca or for *C .perfringens* levels in the ileum. Pre-finisher × finisher interactions ($P<0.05$) were observed for *E. coli* colonization in the ileum (Fig. 7.1) as well as for *L. monocytogenes* in both the ileum (Fig. 7.2) and ceca (Fig. 7.3). In the case of the interaction for *E. coli*, only at 0% DDGS in the finisher did the ileal *E.coli* levels differ between birds that received DDGS in the pre-finisher and those that received none with the birds that received DDGS in the pre-finisher exhibiting

decreased *E.coli* counts versus those that didn't receive DDGS (Fig 7.1). For both interactions concerning *L. monocytogenes* there appears to be a similar result. Birds that received no DDGS in the pre-finisher feed phases exhibit colonization levels that are not different from those of birds that received 8% DDGS in the pre-finisher phase at all levels of DDGS in the finisher diet, except for one. At 21% DDGS in the finisher *L. monocytogenes* colonization in the ileum was significantly greater for birds that received DDGS in the pre-finisher phase vs. those that did not (Fig. 7.2). The same trend is observed for *L. monocytogenes* colonization in the ceca, only this time the significant difference exists at the 14% DDGS finisher ration (Fig. 7.3).

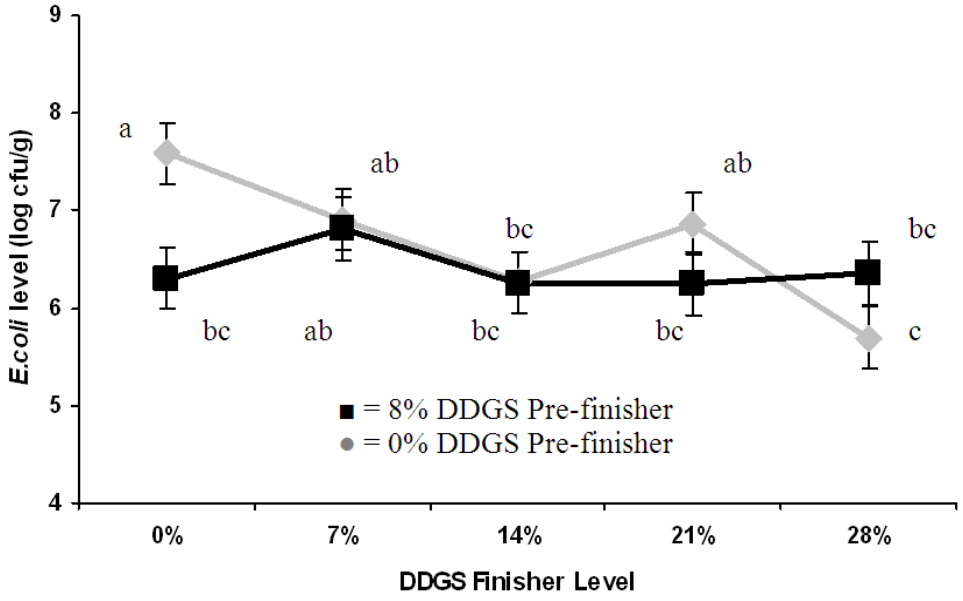

Figure 7.1 Interactive effects of *E. coli* levels in the ileum, based on physical plate counts, during the finisher phase (from 28 to 42 days)

Results for bacterial quantification via the use of qPCR (Table 7.9) showed that birds receiving the 14% DDGS diet had the highest cycle threshold (Ct) value, and thus the lowest level of *E. coli* in the ileum. However, the 14% DDGS Ct value was not

significantly different from that of the 28% or 7% DDGS treatment Ct values. The birds that received the control diet exhibited the lowest Ct value for *E. coli* levels in the ileum, but were not significantly different from the 7% or 21% DDGS treatments. No other effects were observed for *E. coli* levels in the ceca, or for *C. perfringens* and *L. monocytogenes* in either the ceca or the ileum, as measured via qPCR.

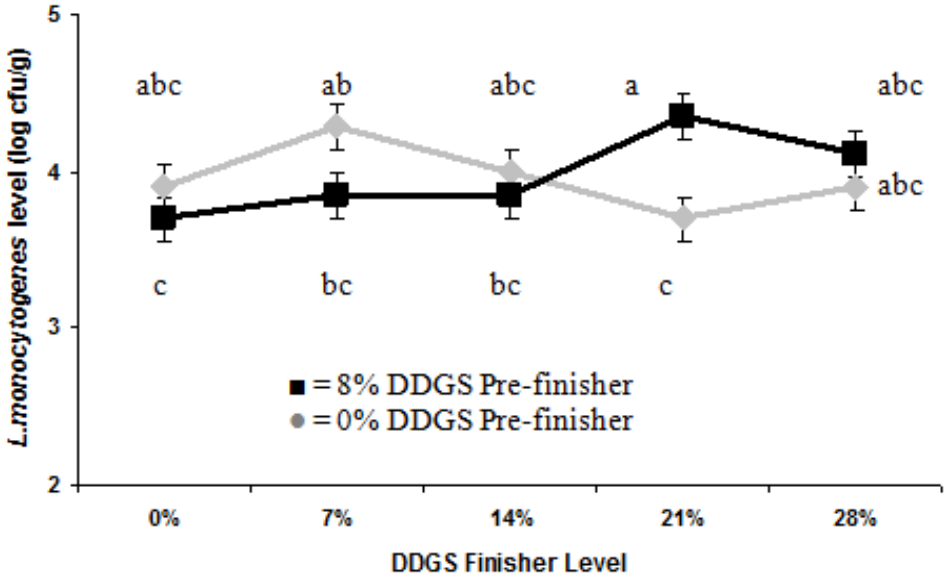

Figure 7.2 Interactive effects of *L. monocytogenes* levels in the ileum, based on physical plate counts, during the finisher phase (from 28 to 42 days)

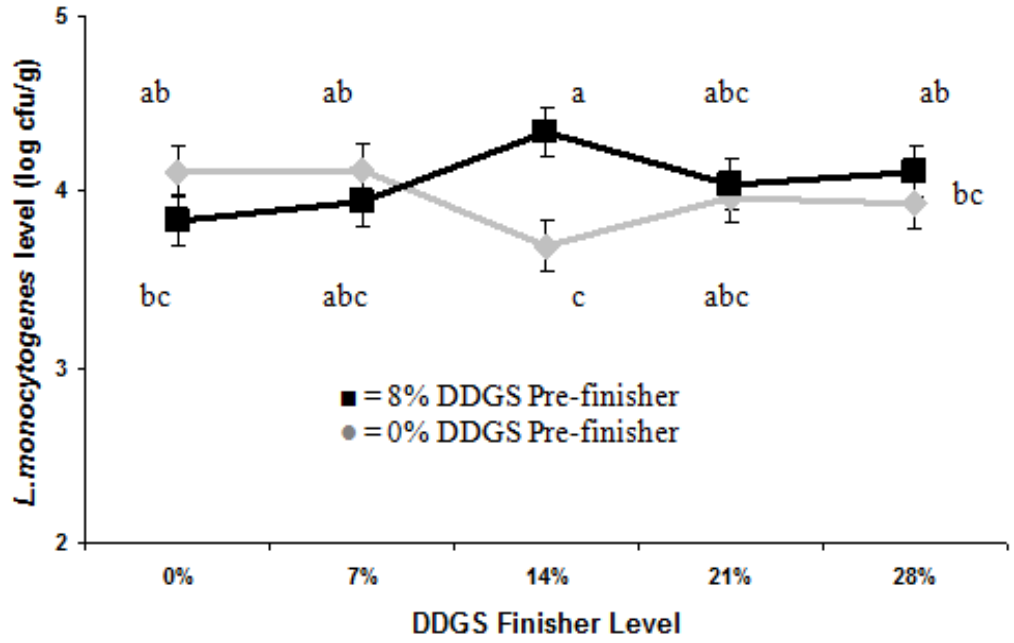

Figure 7.3 Interactive effects of *L. monocytogenes* levels in the cecum, based on physical plate counts, during the finisher phase (from 28 to 42 days)

Table 7.8 *C. perfringens, E. coli* and *L. monocytogenes* present at 42 days of age in ileum and ceca, plate count results

	E.coli ileum (log_{10} cfu/g cecal contents)	*C.perfringens* ileum (log_{10} cfu/g cecal contents)	*L.monocytogenes* ileum (log_{10} cfu/g cecal contents)	*E.coli* ceca (log_{10} cfu/g cecal contents)	*C.perfringens* ceca (log_{10} cfu/g cecal contents)	*L.monocytogenes* ceca (log_{10} cfu/g cecal contents)
Finisher DDGS (%)						
0	6.99[a]	5.88	3.85	8.05	6.13	3.97
7	6.86[ab]	5.96	4.07	7.69	5.73	4.04
14	6.26[bc]	5.72	3.89	7.77	6.07	4.02
21	6.54[abc]	5.83	4.09	7.93	6.12	4.00
28	6.05[c]	5.75	3.99	7.77	6.30	4.02
SEM	0.226	0.259	0.102	0.149	0.275	0.099
Pre-Finisher						
No (0%)	6.68	5.89	3.94	7.75	5.98	3.97
Yes (8%)	6.39	5.77	4.05	7.93	6.15	4.05
SEM	0.142	0.164	0.064	0.094	0.174	0.063
Analysis of Variance (P)						
Pre-finisher DDGS	0.19	0.62	0.46	0.18	0.50	0.33
Finisher DDGS	0.03	0.97	0.47	0.41	0.68	0.99
Pre-finisher × Finisher	0.05	0.95	0.02	0.69	0.64	0.02
Finisher DDGS Linear	0.004	0.61	0.50	0.52	0.38	0.82
Finisher DDGS Quadratic	0.83	0.98	0.49	0.37	0.38	0.79

[1]Distillers dried grains with soluble

[a-c] Means within a column not sharing a common superscript differ ($P < 0.05$).

Table 7.9 *C. perfringens*, *E. coli* and *L. monocytogenes* present at 42 days of age in ileum and ceca - PCR results

	E.coli Ileum (Ct^2 values)	*C.perfringens* Ileum (Ct values)	*L.monocytogenes* Ileum (Ct values)	*E.coli* Ceca (Ct values)	*C.perfringens* Ceca (Ct values)	*L.monocytogenes* Ceca (Ct values)
Finisher DDGS (%)						
0	18.19[c]	33.82	33.92	19.93	25.61	34.32
7	19.39[abc]	33.96	34.19	20.89	25.94	34.18
14	20.37[a]	33.95	33.85	21.36	25.68	34.45
21	19.02[bc]	34.63	34.42	20.59	25.35	34.35
28	19.62[ab]	34.18	33.87	21.52	25.57	35.06
SEM	0.454	0.393	0.458	0.526	0.344	0.385
Pre-Finisher						
No (0%)	19.42	34.01	34.24	20.81	25.68	34.32
Yes (8%)	19.21	34.21	33.84	20.88	25.57	34.66
SEM	0.289	0.249	0.292	0.334	0.216	0.242
Analysis of Variance (P)						
Pre-finisher DDGS	0.62	0.58	0.27	0.98	0.66	0.33
Finisher DDGS	0.02	0.60	0.79	0.21	0.77	0.53
Pre-finisher × Finisher	0.88	0.98	0.52	0.65	0.33	0.23
Finisher DDGS	0.09	0.27	0.95	0.08	0.52	0.16
Finisher DDGS	0.04	0.75	0.66	0.54	0.82	0.35
Quadratic						

[1]Distillers dried grains with solubles

[2]Threshold cycle

[a-c] Means within a column not sharing a common superscript differ ($P < 0.05$).

Discussion

Grow-out

In previous research conducted in the same facilities under similar conditions, Loar et al. (2010) observed no main effects for a pregrower period where birds were fed either 0 or 8% DDGS from 0 to14 days of age before receiving varying levels of DDGS in the grower phase from 14 to 28 days. In the current study, 0 or 8% DDGS was fed during the starter and grower phases, for what is being called the "pre-finisher" feed phase (0 to 28 days). The significant main effects for BW gain and FCR from 0-42 days seen during the pre-finisher phase (Table 7.4) are believed to be the result of feeding either 0 or 8% DDGS not just in the starter phase, but also through the grower phase. The researchers feel that these results represent carryover effects of feeding 0 vs. 8% DDGS from 0 to 28 days. This can be partly explained by the effects observed prior to the finisher phase, where birds consuming 8% DDGS had inferior BWG and FCR compared to those fed diets devoid of this ingredient. So, while previous research conducted by Lumpkins et al. (2004) and Parsons et al. (1983) indicates that DDGS levels in excess of 8% of the starter ration had no deleterious effects on growth, the lack of a significant effect does not carry through the grower phase as well.

Varying levels of DDGS in the finisher phase did have effects on BW gain and feed intake from 28 to 42 days as well as from 0 to 42 days. Body weight gain during these two periods exhibited a linear decrease ($P<0.01$) as DDGS increased in the diet past 7% of the ration (Table 7.4). Feed intake for both periods exhibited a linear decrease ($P<0.001$) as DDGS increased in the diet (Table 7.4). These results differ from those of Waldroup et al. (1981) where the researchers found no differences in BW gain or FCR at

42 days of age, in a study where birds were fed DDGS up to 25% of the diet and energy levels were constant. The results for BWG and feed intake are in close agreement with the suggestion of Lumpkins et al. (2004) that DDGS from modern ethanol plants can be safely incorporated into a diet at 12 to 15% for the grower and finisher phases. In the case of feed intake and BW gain, for both periods mentioned, our 14% ration did not produce results significantly different from those of the control diet.

Processing Yields

Lumpkins et al. (2004) reported that feeding up to 18% DDGS for a period of 42 days resulted in no significant differences in processing weights or yields, including carcass, breast, wings, front half, and back half, while results for fat weight and percentage were not reported. The current research partially supports these findings as there were significant differences for the parameters of dressing percentage and breast meat yield (Table 7.5), but these differences did not occur until DDGS exceeded 14% of the diet. That is the 0, 7, and 14% DDGS diets did not differ significantly for the parameters mentioned and the next level of DDGS fed was 21%, which is in excess of the level fed by Lumpkins et al. (2004). When observing the results for fat weight and percentage in the current study, only one diet yielded results significantly different from the other diets. In both cases, the birds that received 14% DDGS had significantly less fat than the others, and this is further confirmed by the quadratic response observed for both of these parameters. Currently the researchers are unsure what caused the birds that received 14% DDGS to respond in such a manner.

Viscosity and Organ Measurements

Data from the weights of the large intestine, small intestine, and gizzard (Table 7.6), reveal that the only significant results were observed for gizzard weight. As the DDGS content of the diet increased a linear increase in gizzard weight, as well as percent of live weight, was observed. This result is most likely due to the insoluble fiber content of the DDGS, as Hetland et al. (2005) suggested that insoluble fiber can modulate gizzard activity and thus size, as a more active gizzard will be a larger gizzard.

As has long been noted in previous research (Bedford, 1996, Jozefiak et al., 2006, Jia et al. 2009), certain grains can lead to changes in the viscosity of the digestive contents of broilers when compared to a standard corn-based diet. In a previous trial completed by Loar et al. (2010), a marginal increase in viscosity at 28 days of age was observed with the inclusion of DDGS. The current study observed the viscosity at 42 days of age with similar inclusion levels of DDGS as those used by Loar et al. (2010) and observed no significant differences between the treatments. It appears that any marginal results seen at 28 days of age may dissipate as the bird ages and the GI tract matures, although birds were exposed to DDGS for a longer period of time. Loar et al. (2010) also reported a linear decrease in relative liver weight observed as DDGS increased in the diet. Without biological or histological analysis, it was hypothesized that DDGS could have led to hepatic atrophy of the liver or under accumulation of glycogen per unit of liver area. In the current study, no relative liver weight differences between the treatments were observed, therefore leading to no changes in liver glycogen concentration (Table 7.7). The authors believe, that as in the case of ileal viscosity, response for relative liver weight or glycogen content may have dissipated due to an increase in feed intake

and the higher starch consumption from higher corn inclusion levels in latter feeding phases.

Bacterial Quantification

No differences were observed for the colonization levels of *C. perfringens* in either the ceca or the ileum, as measured by both physical plate count and qPCR analysis. This is in close agreement with past research performed in similar conditions and reported by Loar et al. (2010). The past research revealed that *C. perfringens* colonization in the ceca of 28 day broilers was unchanged by DDGS inclusion in the diet during the grower phase when feeding inclusion levels up to 30% when measured by physical plate count. However, Loar et al. (2010) did report a marginal linear trend towards increasing *C. perfringens* levels in the ceca as DDGS increased in the diet, as evaluated by qPCR analysis. Once again the researchers hypothesize that in the current study the increased age of the bird (42 days versus 28 days in the previous research) allowed time for the gut, and consequently the microflora, to become accustomed to the DDGS and thus the trend seen at 28 d in the previous study, is currently absent. The same study showed no changes in the levels of *E. coli*. In agreement with the past research of Loar et al. (2010), the current study showed no significant differences for the colonization of *E. coli* in the ceca. However, a pre-finisher × finisher interaction was observed for *E. coli* levels in the ileum, as measured by plate count (Fig. 7.1). The interaction showed how birds receiving 8% DDGS during the pre-finisher feeding period had unchanged *E. coli* levels versus birds that received no DDGS in the pre-finisher period at all levels of DDGS in the finisher with the exception of 0% DDGS in the finisher. It appears that exposure to DDGS may result in an environment more unfavorable for *E. coli* colonization in the ileum,

compared to the environment created by a diet devoid of DDGS. This is further supported by the results obtained from qPCR analysis of *E. coli* levels in the ileum, as both the 14 and 28% DDGS diets exhibited significantly higher Ct values, and thus lower *E. coli* levels, than birds that received the 0% diet. While there is little research concerning the effects of DDGS on *E. coli* levels in the digestive tract of broilers, past research concerning other feedstuffs may be found. Rubio et al. (1998) reported that *E. coli* levels were unaffected by the inclusion of sweet lupin seed meal in the diet when compared to a wheat and soybean meal based diet. In more recent research, Giannenas et al. (2010) found that inclusion of a dietary mushroom had no effect on the levels of *E. coli* in either the ileum or cecum of broilers.

The interactions observed via physical plate count results, but not qPCR, for *L. monocytogenes* levels in both the ceca and ileum (Fig. 7.2 and 7.3) appear to show very similar results. For *L. monocytogenes* levels there were no differences between birds that received 0% and those that received 8% DDGS in the pre-finisher phase at all levels of the finisher diet, with one exception. In the ileum that exception was at the 21% DDGS finisher diet while in the cecum it was at the 14% DDGS finisher diet. In each case, the birds that received the 8% DDGS pre-finisher exhibited higher *L. monocytogenes* levels. This seems to suggest that there is an inclusion point in the diet at which DDGS are favorable to the growth and proliferation of *L. monocytogenes*. However, if the DDGS inclusion is too low or high, the *L. monocytogenes* growth effect is not observed.

The results for *L. monocytogenes* colonization were in disagreement with past research in general. In more than one case, researchers have reported similar challenges as those reported by Cox et al. (1997), where they were unable to isolate any *L. monocytogenes* from the cecal samples of 115 birds entering a processing facility. As a

result, Cox et al. (1997) suggested that *L. monocytogenes* contamination occurred on an infrequent basis on the farm and that when it was carried into a processing facility, the equipment became contaminated reservoirs for the bacteria. In the current study, the authors suggest that the use of an extensive built-up litter program led to the presence of *L. monocytogenes* in the birds, although not all tested positive for the microbe. Also, while the qPCR analysis of *L. monocytogenes* colonization did show the presence of the microbe, it failed to support the interactions described when using selective media.

In conclusion it appears that the limit of DDGS inclusion in the finisher phase of a broiler diet is approximately 14%. Levels higher than this resulted in negative effects on growth, feed intake, carcass yield and breast meat yield. It also appears that while it may not be a dramatic effect, DDGS in the diet can possibly create an unfavorable environment for *E. coli* growth and proliferation, although further research is warranted to help clarify this response.

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CHAPTER VIII

EFFECT OF VARYING CONDITIONING TEMPERATURE AND FAT APPLICATION ON A DIET CONTAINING DISTILLERS DRIED GRAINS WITH SOLUBLES: FEED MANUFACTURING AND BROILER PERFORMANCE EFFECTS

Abstract

A study was performed to evaluate the effects of different conditioning temperatures and alternate fat application methods on a standard broiler finisher diet containing distillers dried grains with solubles (DDGS). Data were collected to determine feed mill efficiency as well as broiler growth and yield. There were three conditioning temperatures, 165, 185, and 205 degrees Fahrenheit and two methods of fat application, pre and post pellet, which resulted in a 3 x 2 factorial and a total of 6 treatments. Ross x Ross 708 females were used in this experiment and all birds received an identical, industry standard starter and grower ration from 0 to 14 days and 14 to 28 days respectively. Treatments were fed starting on day 28 when the grower diet was removed. Increased temperature and pre pellet fat application both resulted in decreased energy usage at the pellet mill ($P<0.001$). Post pellet fat application also resulted in greater bulk density compared to pre pellet application ($P<0.001$). Diets conditioned at 205 degrees yielded a bulk density significantly greater than that of diets conditioned at 185 degrees ($P=0.03$) while the 165 degree conditioning temperature was intermediate. As conditioning temperature increased, total fines decreased ($P<0.001$) and post pellet fat

application resulted in a greater percent of total fines compared to pre pellet application (P<0.001). A temperature x fat interaction was seen for both pellet durability index (PDI) and modified pellet durability index (MPDI). The interaction was essentially the same for both parameters and it was such that as conditioning temperature increased the difference between PDI and MPDI values for pre and post pellet fat application decreased (P<0.001). In the broiler growth data there was a temperature x fat interaction observed for feed conversion (P=0.07). It is shown that no difference exists between the fat applications when diets were conditioned at 165 or 205 degrees, but when diets were conditioned at 185 degrees the post pellet fat application resulted in a significantly greater feed conversion compared to pre pellet fat application. This research suggests that at 185 degrees the fat being added pre pellet aids in protecting the nutrients from heat related degradation via a coating action by the fat. The 205 degree temperature creates such stressful heat conditions that the fat is unable to protect the nutrients while the 165 degree temperature is not hot enough to cause harm to the nutrients in either fat application method. No significant results were obtained for any processing or yield data.

Introduction

Peer reviewed research concerning the effects of diets containing DDGS on feed manufacturing is scarce to say the least. Over the past decade there has been an enormous increase in the production of DDGS as a result of domestic ethanol production as reported by the Renewable Fuels Association (2010). While the future of this coproduct relies heavily on the use of ethanol in the automobile fuel supply, for now there is a good supply of DDGS for use in animal feeds. DDGS can be a very economically sound choice for inclusion in a poultry diet provided certain details are properly addressed. Nutritional

variability is often an issue depending on the supplier of the DDGS (Cromwell et al., 1993, Batal and Dale, 2006, Fastinger et al., 2006) and there are also issues associated with the feed manufacturing process, as shown by Loar et al. (2010). Many issues associated with nutritional variability can be avoided if a poultry producer has a supplier that produces a consistent product, although this is typically more costly on both ends. If a producer simply looks for the cheapest DDGS supply and has several sources, most researchers recommend a complete analysis of the DDGS before including it in a ration. Product variability seems to be improving though, as many DDGS suppliers have taken the steps to insure a consistent and quality product as a result of demand. The issues associated with feed manufacturing arise as a result of the physical characteristics of DDGS and cannot be avoided for the time being if a poultry producer wishes to include high levels of DDGS in the diet. It simply becomes a calculation of the decreased diet cost versus the decreased physical quality of the diet when DDGS are fed at high inclusion rates.

Since it is now well known and accepted that DDGS can be an economical and nutritionally effective dietary component for poultry, it becomes necessary to research the feed manufacturing issues. This study was designed to observe the effects that varying conditioning temperatures and fat application method would have on the feed manufacturing variables of an industry standard DDGS containing diet. The study also goes on to observe the effects of feeding these diets to broilers on growth and yield of the birds.

Materials and Methods

Feed Milling

This portion of the study was conducted over a 6 d period at the West Virginia University pilot feed mill in Morgantown, WV. Equipment used included: Weigh-tronix stationary feed mill SFM-2000: integrated hammer mill, scale, microingredient mixer; 15-horsepower horizontal shaft hammer mill (screen size: 1/8in.); 907.2 kg capacity single-screw vertical mixer in series with a pellet mill; CPM 2288A master model pellet mill with a 40-horsepower main drive motor, 12 inch diameter, 3/16 × 1.5 inch die. There were 6 separate finisher-phase diets as a result of a 3 x 2 factorial design with 3 conditioning temperatures (165, 185, and 205°F) and 2 fat application methods (pre or post pellet). This study was designed as a Latin-square and treatments were blocked by day of production and run order. Each treatment was replicated 6 times, with each batch being 272.2 kg and representing an experimental unit. All treatments were identical in formulation (Table 8.1), but initially two large batches were made for each day where 1 batch was nutritionally complete and would be used to produce the pre pellet fat application diets at all 3 conditioning temperatures. The other batch only contained 1% of the 2.18% poultry oil that was required in the formulation as this batch was used to manufacture the post pellet fat application diets at all 3 conditioning temperatures. All diets were mixed for 15 min in a single screw vertical mixer.

Table 8.1 Experimental diet composition (% as-is)

Ingredients	Finisher Diet (28-42 d)
Corn	64.4
Soybean meal (48% CP)	23.9
DDGS[1]	4.0
Meat and bone meal	4.0
Poultry oil[2]	2.18
Calcium carbonate	0.42
Premix[3]	0.25
NaCl	0.28
DL-Methionine	0.23
L-Lysine	0.19
L-Threonine	0.03
Coccidiostat[4]	0.05
Choline Chloride (60%)	0.02
Phytase enzyme[5]	0.02
Dicalcium phosphate	0.003
Calculated Composition	
AME (kcal/kg)	3,150
Available P (%)	0.41
Ca (%)	0.82
CP (%)	19.1
Dig. TSAA (%)	0.75
Dig. Lysine (%)	1.0
Dig. Threonine (%)	0.65

[1]Distillers Dried Grains with Solubles
[2]Pre pellet application diets - all fat added at mixer
post pellet application diets - 1% added at the mixer, 1.18% post pellet
[3] The vitamin and mineral premix contained per kg of diet: retinyl acetate, 2,654 µg; cholecalciferol, 110 µg; dl-α-tocopherol acetate, 9.9 mg; menadione, 0.9 mg; B_{12}, 0.01 mg; folic acid, 0.6 µg; choline, 379 mg; d-pantothenic acid, 8.8 mg; riboflavin, 5.0 mg; niacin , 33 mg; thiamin, 1.0 mg; d-biotin, 0.1 mg; pyridoxine, 0.9 mg; ethoxiquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper, 4 mg; iodine, 0.5 mg; selenium, 0.3 mg.
[4] Dietary inclusion of coccidiostat provides 60 g salinomycin sodium per 907.2 kg of feed.
[5]DSM Nutritional Products (Parsippany, New Jersey)

Prior to pelleting, all diets were batched into their 272.2 kg aliquots in mash form. The missing poultry oil (1.18%) in 3 of the diets, would be added after manufacture to each of the post pellet diets via pouring the pelleted diets back into the vertical mixer and adding the liquid fat through a micro ingredient chute on the mixer. Once all feed was batched, each individual batch was transferred back into the mixer where it was then conveyed to the conditioner-pellet mill.

Mash was conditioned to a steady-state temperature of 165, 185, or 205°F depending on treatment. Steam pressure at the gauge was 262 kPa (38 psi) through use of a globe valve. Feed temperature was monitored with a digital thermometer inserted directly into the stream of conditioned mash, and was controlled by throttling steam into the conditioner using a ball valve. Rate of feed entering the conditioner was held constant across all treatments. Pellets were formed using a California Pellet Mill (4.25-ft length, 1.02-ft diameter short-term CPM conditioner (3 steam inlet ports), 429 rpm shaft speed; 21 picks; 10-s feed retention time) and were cooled on a horizontal belt cooler using forced ambient air. Relative electrical energy usage at both the conditioner and pellet mill were determined using Powerlogic power meters attached to the 3-phase leads of the pellet mill main drive and conditioner motor (Square D). Production rate, percentage of fines and bulk density were also estimated. One representative bag from each manufacturing run was reserved for determination of pellet quality, as measured by pellet durability index (PDI) and modified pellet durability index (MPDI). Pellet quality was assessed on the day of manufacture via a tumbling box according to ASAE standard S269.4 (ASAE, 1997). Because of the use of a 3/16 × 1.5 in. die, pellets were sifted in a No. 6 American Society for Testing and Materials (ASTM) screen. The MPDI was

determined in a similar manner, with the exception of adding five 13-mm hex nuts to the pre-tumbled sample to obtain added pellet agitation (ASAE, 1997).

Grow-out

The grow-out portion of the study encompassed the period between 28 to 42 days of age using Ross × Ross 708 females obtained from a commercial hatchery. Day-old chicks were randomly placed in each of 60 floor pens (13 birds/pen; 780 birds total; 0.08m^2/bird). The close-sided house had thermostatically controlled heating, cool cells and cross ventilation. Each pen contained built-up litter, a hanging feeder (22.5kg capacity) and nipple drinkers (3 nipples/pen). The lighting program was 23 hr light and 1 hr dark and ventilation was accomplished by negative air pressure. Chicks were vaccinated for Marek's disease (via *in ovo* administration at day 18), as well as Newcastle disease and infectious bronchitis (via coarse spray at hatch).

To ensure accurate formulation of the experimental diets, samples of DDGS, corn, soybean meal, and ProPlus were analyzed for total amino acids and crude protein composition (AOAC International, 2006). Digestible amino acid values were calculated from published digestible coefficients (Ajinomoto, 2004) by using the analyzed total amino acid content of the ingredients. Crude protein was not assigned a minimum value during formulation, and essential digestible amino acids were maintained in all dietary treatments by setting minimum formulation ratios relative to digestible Lys as follows: TSAA 75, Thr 65, Val 78, Ile 68, Trp 17, and Arg 105, and following previously published recommendations (Lemme et al., 2004). All other essential nutrients were formulated to meet or exceed nutrient recommendations (NRC, 1994). Upon receiving

the results for CP and amino acid analysis of the feed ingredients, the nutrient matrix was updated and the feed formulas were solved using linear programming (Table 8.1).

All birds were fed a common starter and grower ration from 0 – 28 days. On day 28 the grower feed was removed, all pens were equalized (12 birds/pen; 720 birds total; 0.09m^2/bird), and feeding of the 6 experimental finisher diets (3 temperatures x 2 fat application methods) commenced. Each treatment was replicated 10 times for a total of 60 experimental units. The feed was provided to the birds from 0 to 14 days of age in crumbles, and from 14 to 28 days as pellets. The experimental diets that were fed from 28 to 42 days were fed in crumble form also so as to eliminate any pellet quality effects on bird performance as the researchers wished to focus only on the effects of conditioning temperature and fat application method. Treatments were blocked completely, according to location within the house. Table 8.1 shows the finisher diet containing 4% DDGS used in both the feed milling and grow-out portions of the study. Feed and water were provided for ad libitum consumption.

All birds in each pen were weighed collectively at the beginning and end of the finisher phase. Feed consumption and mortality were monitored throughout the finisher phase and feed conversion was corrected for the weight of mortality and represents: (g of feed consumed by all birds in a pen) / (g of BW per pen + weight of dead birds). All procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee.

Processing Measurements

At 42 days of age, 5 birds per pen were randomly selected, tagged, individually weighed and cooped 12hr before processing. Birds were processed at a pilot processing

plant. Electrical stunning was performed by applying 11.5 volts (<0.05 mA, AC to DC current), for 3 sec for each broiler, and broiler carcasses were scalded, picked and eviscerated automatically using commercial prototype equipment. Carcass and abdominal fat weights were obtained as birds were manually removed from the line. Birds were then chilled for 4 h at which point all birds were manually deboned and weights were obtained for breasts, wings, and back halves of all birds. Absolute and relative weights (% of live weight) were determined for abdominal fat, back half, carcass, wings, and boneless-skinless breast meat. Occurrence of deep pectoral myopathy in *Pectoralis minor* muscles was monitored for and recorded.

Statistical Analysis

The feed mill portion of the study was analyzed using the proc GLM option of SAS software (SAS Institute, 2004) with a *P*-value $\leq$ 0.07 indicating significance. Data were first tested for temperature x fat application interactions before testing for temperature and fat application main effects. When overall significant differences ($P \leq$ 0.07) existed among all 6 treatments, the Fisher's least significant difference option of SAS was used to separate treatment means (SAS Institute, 2004). Treatments were blocked by day of production and run order, thus replicating each treatment 6 times with each 272.2 kg batch representing an experimental unit. Data generated from the grow-out portion of the study were evaluated as a randomized complete block design with the pen representing an experimental unit, and using the proc GLM option of SAS software (SAS Institute, 2004) with a *P*-value $\leq$ 0.07 indicating significance. Percentage data for mortality were transformed to arcsine $\sqrt{\%}$ for analysis. Data were tested for interactions (temperature x fat application), and then main effects.

Results and Discussion

Feed Milling

Significant results were obtained for all feed milling parameters with the exception of production rate and conditioner energy usage. The 185 and 205°F conditioning temperatures significantly decreased energy usage at the pellet mill ($P<0.0001$) compared to the 165°F temperature (Table 8.2). This is most likely due to the increased steam in the hotter temperatures producing a lubricating action at the die, thus reducing the energy needed to propel the mash through. This lubricating effect of steam has been noted before by Briggs et al. (1999). A decrease in energy usage at the pellet mill was also seen with the pre pellet fat application method ($P<0.0001$) compared to the post pellet fat application (Table 8.2). Once again this decrease in energy usage is attributed to the increased fat level added at the mixer in the pre pellet treatment creating a lubricating effect, and is supported by past research (Loar et al. 2010, Thomas et al., 1998). A temperature x fat interaction was observed for both PDI and MPDI (Figure 8.1 and 8.2) and was basically the same in both cases. For both parameters, as conditioning temperature increased the difference seen between the physical quality of pellets produced via the different fat application methods decreased ($P<0.001$). Although, each conditioning temperature that had post pellet fat application had a significantly greater PDI and MPDI value versus the diet with pre pellet fat application at the same conditioning temperature.

Table 8.2 Effects of various conditioning temperatures and fat application method on feed mill efficiency and pellet quality of finisher phase broiler diets

Temperature	Production Rate (MT/hr)	Conditioner Relative Energy Usage (KWH/MT)	Pellet Mill Relative Energy Usage (KWH/MT)	PDI (%)[2]	MPDI (%)[3]	Bulk Density (kg/m^3)	Total Fines (%)[4]
165	0.759	0.961	9.883^{a}	73.3^{c}	59.7^{c}	42.55^{ab}	38.5^{a}
185	0.759	0.952	8.386^{b}	82.7^{b}	72.4^{b}	42.21^{b}	30.1^{b}
205	0.772	0.973	8.284^{b}	93.8^{a}	90.4^{a}	42.84^{a}	17.3^{c}
SEM	0.006	0.009	0.068	0.48	0.84	0.146	1.21
Fat							
Pre	0.764	0.957	8.407^{b}	76.7^{b}	66.8^{b}	40.35^{b}	23.3^{b}
Post	0.764	0.967	9.295^{a}	89.7^{a}	81.5^{a}	44.72^{a}	33.9^{a}
SEM	0.005	0.008	0.055	0.39	0.69	0.119	0.99
Analysis of Variance (*P*)							
Temperature	0.21	0.30	<0.0001	<0.0001	<0.0001	0.03	<0.0001
Fat App.	0.99	0.37	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Temp. × Fat	0.59	0.65	0.99	<0.0001	<0.0001	0.67	0.52

[a-c] Means within a column not sharing a common superscript differ ($P \leq 0.05$).
[2] Pellet durability index.
[3] Modified pellet durability index.
[4] Percent of total feed produced that was fines.

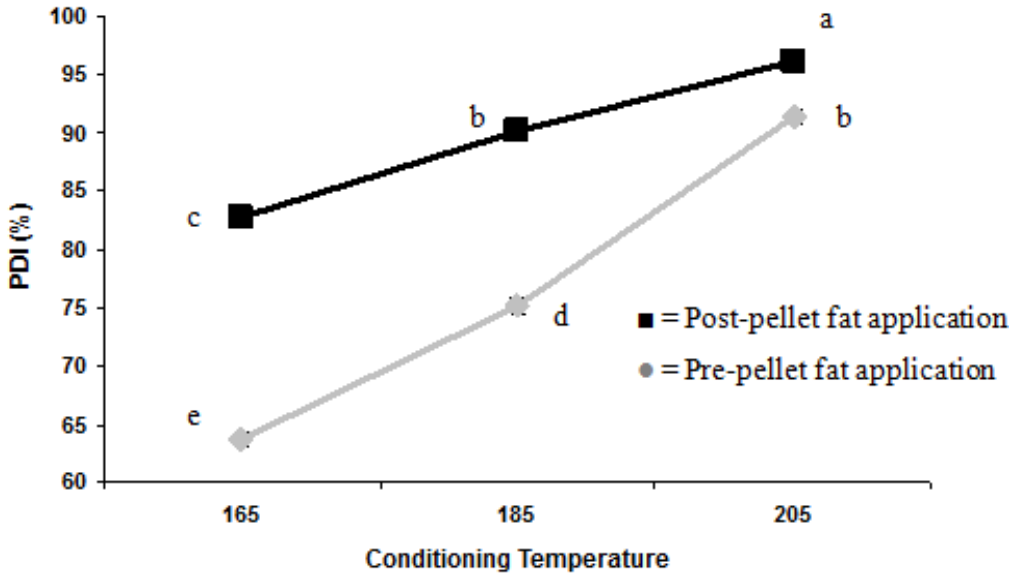

Figure 8.1 Interactive effects of pellet durability index

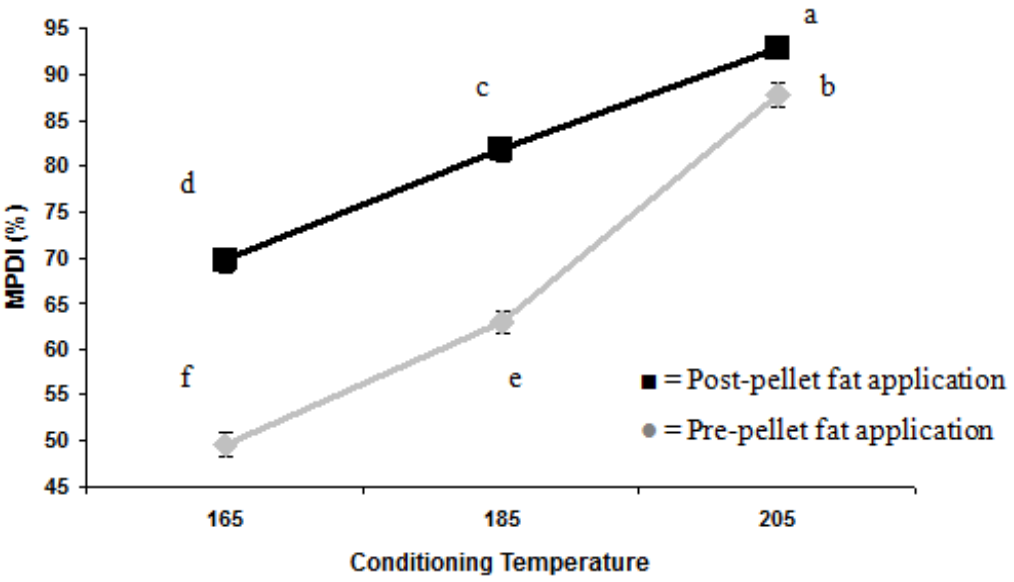

Figure 8.2 Interactive effects of modified pellet durability index

These results are in agreement with past reports by Salmon (1985) that increased fat at the mixer will decrease pellet quality, and Cutlip et al. (2008) that increased steam temperature will increase pellet quality.

Results for bulk density (Table 8.2) show that the 205°F treatment had significantly greater bulk density when compared to the 185°F conditioned diets (P=0.03), while the 165°F treatment was not different from either of the other treatments. These results do not necessarily agree with the other findings. Moritz et al. (2002) reports that high quality pellets result in more air space in a bulk density sample as a result of more whole pellets and decreased fines, thus resulting in a lower bulk density. According to Cutlip et al. (2008), the increased steam temperature will increase pellet quality. If the bulk density results of the current study were to agree with past research, the 165°F treatment would not have been statistically the same as both the other treatments. However, even the most extreme difference observed, the difference between the bulk densities of the 185 and 205°F treatments, was around .64kg/m^3. As a result, the researchers feel that these unexplainable bulk density results are simply the product of experimental error. In more bulk density data, it is shown that the post pellet fat application resulted in significantly greater bulk density (P<0.0001) compared to the pre pellet fat application method (Table 8.2). This result is attributed to the fact that the bulk density of the post pellet fat application treatments was measured after the fat was added post pellet, via placing the pelleted feed back into the mixer and adding the fat thru a micro ingredient chute. As a result the pellets from the post pellet fat application treatments underwent significantly more stress and handling than the pre pellet fat application pellets and thus had more physical damage. Consequently, they were smaller pellets and thus left less air space in each bulk density sample, resulting in a heavier

sample. Results for total fines (Table 8.2) were what were expected based on the previous explanation for the bulk density results where fat application was concerned. The post pellet fat application method resulted in significantly more fines ($P<0.0001$) than the pre pellet fat application method. Once again, the researchers attribute this result to the increased handling and stress the post pellet fat application pellets endured. In agreement with Cutlip et al. (2008) that increased steam temperature will increase pellet quality, the 185°F treatment had significantly less fines ($P<0.0001$) than the 165°F treatment while the 205°F treatment had significantly less fines than the 185°F treatment.

Grow-out

The only significant result observed for the grow-out portion (Table 8.3) of the study was a temperature x fat application interaction for feed conversion ($P=0.07$). When observing the graphical representation of the interaction (Figure 8.3) it becomes evident that fat added at the mixer may have a "protective" effect on nutrients. At both the 165 and 205°F conditioning temperatures there were no significant differences between the two different fat application methods. However, at the 185°F conditioning temperature diets that were produced utilizing the post pellet fat application method yielded significantly higher feed conversion values than those manufactured using the pre pellet fat application method. The researchers suggest that at a conditioning temperature of 165°F the heat stress the nutrients are exposed to is minimal, and results in no noticeable effects on feed conversion. At a conditioning temperature of 205°F the heat stress is so great that the nutrients are degraded comparably, regardless of whether the pre or post pellet fat application method is utilized. But, at 185°F the heat stress the nutrients endure can be ameliorated via the use of pre pellet fat application and thus the reason the post

pellet fat application diets yielded significantly higher feed conversion values for this conditioning temperature. Reducing added fat at the mixer has been suggested as a cause of nutrient degradation via an increase in frictional heat at the mash die interface by previous researchers as well (Gehring et al. 2009).

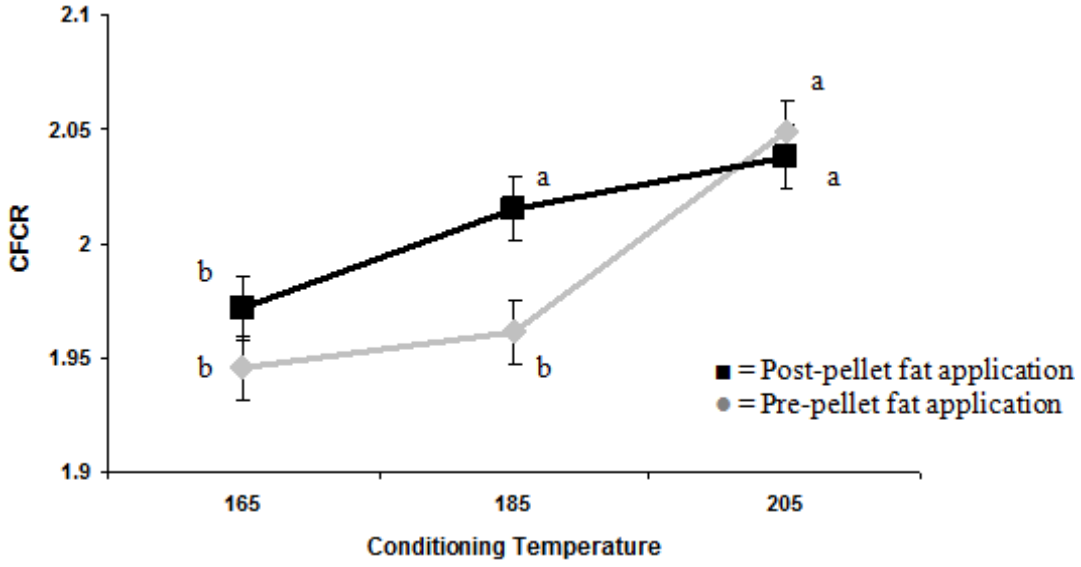

Figure 8.3 Interactive effects of feed conversion

Processing Measurements

No significant results were observed for any of the processing parameters measured (Table 8.4). It may seem odd that with all the effects found in the feed milling portion of the study that no effects were seen for the processing parameters, but it is necessary to remember that all feed was crumbled before being fed. The researchers did not wish to observe any effects in growth or yield of the birds as a result of feed form and physical quality. The researchers wanted any significant results observed for bird growth and yield to be the result of nutritional changes in the diet and not physical feed quality.

Table 8.3 Live production at 42 days of age

	BWG 28 to 42 d (kg)[1]	Feed intake 28 to 42 d (kg)	Feed conversion 28 to 42 d	Mortality 28 to 42 d (%)
Temperature				
165	1.08	2.14	1.96[c]	0.42
185	1.09	2.16	1.99[b]	0.42
205	1.07	2.19	2.04[a]	0.42
SEM	0.011	0.021	0.009	0.42
Fat Application				
Pre	1.09	2.16	1.99[b]	0.56
Post	1.08	2.16	2.01[a]	0.28
SEM	0.009	0.017	0.008	0.34
Analysis of Variance (P)				
Temperature	0.57	0.19	<0.0001	1.0
Fat Application	0.32	0.98	0.05	0.57
Temp × Fat	0.09	0.94	0.07	0.28

[a-c]Means within a column not sharing a common superscript differ ($P \leq 0.05$).
[1]Body weight gain

So, there is the possibility that only feed conversion as discussed previously was affected, and perhaps the diets were not altered enough by the conditioning temperatures and fat application methods to cause other measurable responses in the birds. However, as is well known, male broilers will grow faster than females and thus require more nutrients. This usually means that a slight effect as a result of different dietary treatments would be more evident and significant in male birds. The researchers feel that there may have been more pronounced results in some of the parameters measured had the study utilized males instead of females.

Table 8.4 Processing results

	Dress (%)[1]	Carcass (kg)	Fat (kg)	Fat (%)[1]	Wings (kg)	Wings (%)[1]	Back (kg)	Back (%)[1]	Breast (kg)	BMY[2] (%)[1]
Temperature										
165	69.1	1.69	0.039	1.61	0.18	7.42	0.69	28.38	0.55	22.54
185	69.0	1.69	0.042	1.70	0.18	7.36	0.69	28.05	0.55	22.60
205	69.1	1.71	0.041	1.65	0.18	7.42	0.69	28.06	0.56	22.59
SEM	0.11	0.011	0.0009	0.039	0.001	0.031	0.005	0.116	0.005	0.116
Fat Application										
Pre	69.1	1.71	0.042	1.64	0.18	7.39	0.69	28.09	0.56	22.61
Post	69.1	1.69	0.041	1.67	0.18	7.40	0.69	28.22	0.55	22.54
SEM	0.09	0.009	0.0008	0.032	0.0008	0.026	0.004	0.094	0.004	0.096
Analysis of Variance (P)										
Temperature	0.87	0.57	0.18	0.25	0.12	0.20	0.80	0.12	0.75	0.94
Fat Application	0.94	0.24	0.42	0.39	0.89	0.79	0.48	0.38	0.20	0.54
Temp × Fat	0.67	0.59	0.87	0.83	0.77	0.48	0.99	0.31	0.89	0.44

[1] Percent of live weight
[2] Breast meat yield

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CHAPTER IX

CONCLUDING STATEMENTS

Conclusions

Distillers dried grains with solubles (DDGS) are a more than feasible option for inclusion into today's commercial poultry rations. The research presented herein, along with the research cited, clearly proves the suitability of this ingredient. Of course markets will always fluctuate and prices will vary, but it is unlikely that DDGS will ever disappear from the agricultural scene. We have shown that if the appropriate steps are taken, DDGS can be fed to commercial broilers at any stage of the growth cycle. We have also shown that commercial laying hens may be able to adequately perform on diets containing up to 30% DDGS. If a producer wishes to make an even stronger DDGS product, nutritionally speaking, we have shown the option of the "Elusieve" process.

DDGS does not in any way harm consumer sensory perception of the food products obtained from animals that consume DDGS. In fact, eggs from DDGS hens may be preferred by consumers in some cases. It also resulted in darker egg yolks, and it is unclear currently how consumers feel about that characteristic. Contrary to former beliefs, the research just presented also shows that DDGS in no way aids in the growth and/or proliferation of pathogenic bacteria. In fact, we were able to show in one case that DDGS may create an unfavorable enteric environment for the bacteria *E. coli.* While DDGS inclusion may have a negative effect on the physical quality of a commercial

poultry diet, those negative effects may be ameliorated with changes in conditioning temperature or fat application method.

Overall, DDGS is a product that was once considered almost a waste product of the beverage and ethanol industries, suitable only for ruminant animals. Nowadays, it has been shown that it can be safely used in the poultry industry, and has become a significant agricultural export for North America. I feel its use in poultry rations will only increase. Undoubtedly, the price of DDGS is bound to increase as a result of increased use but it will prove to be a valuable ingredient in commercial diets as more and more research is conducted and poultry producers will pay for it just as for any other dietary constituent. Further, more companies are realizing the potential of DDGS and are working towards alternative forms of the product and ways to increase its nutritional, and thus financial value. This will only aid in creating a stronger market for the product. I project that in the following decade, DDGS will be as common an ingredient in a poultry diet as corn or soybean meal. This is only a reasonable assumption because as the government approved the use of E15 in 2007 and newer vehicles, they in turn gave a reason for increased production of DDGS. As long as ethanol is made, DDGS will remain a byproduct that agriculture should take advantage of due to its economic and nutritional benefits. This research has hopefully aided in the push to feed more DDGS to poultry on a more consistent basis by showing that it is a nutritionally sound decision.