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Effects of increasing concentrations of corn distiller's dried grains with solubles on the egg production, internal quality of eggs, chemical composition and nutrients content of egg yolk

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Effects of increasing concentrations of corn distiller’s dried grains with solubles on the egg production, internal quality of eggs, chemical composition and nutrients content of egg yolk

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Iowa State University
Ames, Iowa
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ABSTRACT

The objective of this study was to determine the effects of feeding high levels of corn distiller’s dried grains with solubles (DDGS) on egg production, internal quality of eggs, chemical composition and important nutrients content of egg yolk. Four diets were formulated to contain 0, 17, 35 or 50% corn DDGS in a corn and soybean meal base. A total of 240 54-week-old single-comb White Leghorn laying hens were randomly allotted with 2 birds per cage with three consecutive cages representing an experimental unit (EU). Each EU was assigned to one of the four dietary treatments according to a completely randomized design. Hens were fed for a 24-week experimental period after transition feeding to gradually increase corn DDGS inclusion over a four-week period. After the first 12-wk period, the diets were reformulated to meet amino acid requirements.

Egg production was recorded daily and feed consumption was measured weekly. Egg component, yolk color, Haugh unit during storage, and shell breaking strength were measured every two weeks. Chemical composition and nutritional components in egg yolk were measured every two weeks. Chemical composition of egg yolk including protein, lipids, and moisture was determined. The nutritional components in egg yolk, including fatty acid composition, and the content of cholesterol, lutein, and choline were measured.

Egg production, egg weight, egg mass, feed intake, and feed efficiency were adversely affected by the highest level of DDGS (50%) in the diet before diet reformulation. Once diets were reformulated with increased concentrations of lysine and
methionine, differences among the dietary treatments were reduced and the performance of the 50% DDGS diets was improved significantly, and no differences in egg production, egg weight and feed intake among DDGS treatments were found during the last 6 weeks of study. DDGS diets positively affected the internal quality of eggs during storage. Yolk color increased linearly as DDGS concentration increased, and Haugh unit was improved from 50% DDGS diet treatment group. Shell breaking strength was not influenced by DDGS diets. Shell weight percentage increased at 50% dietary DDGS level. Egg yolk from hens fed highest DDGS-containing diet tended to have higher fat content and lower protein content. Total polyunsaturated fatty acids were significantly increased by DDGS diet. The contents of choline and cholesterol were initially higher in 50% DDGS treatment group, but the difference among four DDGS treatments reduced in the later period, especially no difference was found during the last 4 weeks. Lutein content increased linearly as DDGS levels increased.

It was concluded that up to 50% of DDGS could be included in the layer’s diet without affecting egg weight, feed intake, egg production, and egg internal quality as long as digestible amino acids were sufficient in DDGS-added diets. Moreover, this study indicated that feeding high levels of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not influence the content of cholesterol and choline.
CHAPTER 1. INTRODUCTION

GENERAL INTRODUCTION

Distillers dried grains with solubles (DDGS) are by-product of ethanol and beverage industry. DDGS have been available to livestock feed industry for many years. As a high nutritious and economical feed source, DDGS are being used at extensive levels for animals (Kalscheur et al., 2006; Noll, 2008). For example, DDGS can be used up to 30% for lactating dairy cattle (Kalscheur et al., 2006). Currently in the U.S., DDGS are predominantly used in ruminants. Dairy cattle consume around 42% of DDGS, followed by beef (38%), swine (14%) and poultry (5%).

The production of bioethanol increased rapidly in recent years, and continuous expansion is expected in the future (F.O. Licht, 2008, 2009, and 2010). As the production of ethanol increased rapidly, increased quantities of DDGS are available to livestock feed industry, and this has rekindled the interest of increasing DDGS incorporation in animal diets. In poultry diets, DDGS are usually used at a low level (5 or 10%) (Day et al., 1973; Couch et al., 1957). Recently, many researchers have found that up to 25% DDGS in layer diet would not affect egg production and egg quality. Pineda et al. (2008) used 69% DDGS inclusion rate in layer diets and found that there was no significant different on egg mass and egg internal quality. However, it was a short-term study (8 weeks), and the effects of long-term feeding high concentrations of DDGS to laying hens are unknown.

DDGS are good source of fiber and fat, and contain several nutrients of which are important to human health, such as lutein and choline. The differences in DDGS diets may result in nutritional differences in egg yolk, such as fat content, fatty acid composition,
cholesterol content, choline content, and lutein content. Eggs are very important human food, and their compositions are of great influence for our nutrients intake. The higher inclusion rates of DDGS for laying hens may change the nutritional value of eggs, and that changes may have positive effect on human health.

Virginiamycin is usually added in ethanol production processes to control the growth of lactic acid bacteria and to increase ethanol production. Therefore, there is a possibility of virginiamycin residues present in DDGS as well as diets containing DDGS. Virginiamycin residues are restricted to 0.1 ppm in poultry products (Food Safety and Inspection Service, 1998). The possibility of virginiamycin residues in DDGS feed and eggs is unknown, especially when high DDGS concentration is used in layer diet.

With the increased quantity of DDGS available for feed usage, there is a great potential to increase DDGS usage in poultry diet. However, maximum level of DDGS in layer diet is still unclear, and the effects of long-term feeding high levels of DDGS on egg production, egg internal quality and egg nutritious content are unknown.

**RESEARCH OBJECTIVES**

Based on the previous study (Pineda et al., 2008), we hypothesized that long-term feeding of laying hens with diets containing high levels of DDGS (17%, 35%, and 50%) would not adversely affect egg production, egg internal quality, and egg shell quality. Moreover, high DDGS diets would not adversely affect nutritional value of egg and its chemical composition.

The major objectives of the present study were 1) to investigate the effects of long-term feeding of high-level dietary corn DDGS (up to 50%) on the laying performance (egg
production, egg weight, feed intake, and feed efficiency), egg components (albumen, yolk, and shell) and egg shell quality, yolk color, and Haugh unit during storage. The possibility of virginiamycin residues in DDGS diets was also determined. 2) To investigate the changes of chemical composition and nutrients content of egg yolk, including protein, lipids, moisture, fatty acid composition, cholesterol, lutein, and choline by high DDGS diets.

LITERATURE REVIEW

Bioethanol and DDGS

Distillers dried grains with solubles are by-product of ethanol industry and beverage industry. DDGS have been widely used as high nutrient and economical feed ingredient for livestock (Kalscheur et al., 2006; Noll, 2008). DDGS were mainly produced from beverage industry during the fermentation process before 1990. The demands of fuel ethanol together with government encouragement have given a boost to the production of DDGS from non-beverage ethanol plants since late 1990s. Currently 98% of DDGS produced in the U.S. come from fuel-ethanol plants, with only 2% from beverage industry (University of Minnesota, 2008a). Ethanol production generates three parts: ethanol, the major product; non-fermentable corn residues (DDGS) which are marketed to feed industry; and carbon dioxide which is usually released to atmosphere because of logistic difficulties to specific compressed gas markets (U.S. Grains Council, 2008).

Currently, Brazil and United States are the two countries that produce and utilize the largest amounts of fuel ethanol in the world. The incorporation rate of ethanol in global gasoline fuel reached to 5.4% in 2008 (United Nations Environment Programme, 2009). In the U.S., most of the cars can run with up to 10% ethanol in the blends (Worldwatch Institute
and Center for American Progress, 2006). Many other countries in Europe and Asia also realize the huge economic benefits of bioethanol, and encourage the establishment of new ethanol plants, in addition to promoting the use of ethanol and reducing the dependency on oil. The European Union has set the goal to get biofuel share of all the transport fuels at 5.75% by 2010 (Official Journal of the European Union, 2003).

The production of bioethanol increased rapidly in recent years. It expended from 17 billion liters in 2000 to more than 52 billion liters in 2007. Moreover, the worldwide ethanol fuel production has reached to 73.9 billion liters in 2009 (F.O.Licht, 2008, 2009, and 2010). As the production of ethanol increased rapidly, increased quantities of DDGS are available to livestock feed industry. Many modern ethanol plants adopt new technologies (such as gentler drying process) to improve the quality of DDGS, thus DDGS can be used at higher level in monogastric animals (Lumpkins et al., 2003). Therefore, the higher inclusion rates of DDGS in animal feed may become possible, and this may in addition boost the development of ethanol industry.

Bioethanol as a renewable energy can be produced from many kinds of grains and plants. Corn is the major source used in fuel ethanol production, but other grains, such as sorghum, wheat, rye and barley malt or their combinations are also used in certain regions depending on geography and availability. Although DDGS can be made from many grains, only corn-ethanol production line has been successfully industrialized to economically convert corn grain into ethanol on a large scale so far (Rosentrater, 2005). Thus corn DDGS become one of the predominant feed ingredients for livestock, and its nutritive value is very important for animal diet formulation.
During corn DDGS production, corn is first ground using a hammer mill, and then water is added to make slurry. Enzymes and yeast are usually added in the process to help ethanol fermentation. After the starch of corn is fermented into ethanol and carbon dioxide, the remaining part is whole stillage, which contains 90% water (U.S. Grains Council, 2008). The whole stillage is then centrifuged to produce distillers’ grains and thin stillage. The distillers’ grains contain primarily unfermented corn components (protein, fiber, fat). The thin stillage is often called distillers soluble, which contains yeast cells, soluble nutrients and fine corn particles. Corn DDGS is finally made by adding some distillers solubles back to the distillers grains followed by gentle drying procedure. Finally, corn DDGS contains at least 75% solids from the whole stillage, and the solubles are added to the thin stillage (AAFCO, 2007). The soluble addition can influence the particle size, color, and content of fat and minerals of DDGS (Noll et al., 2007).

**DDGS nutrient variability**

The high variability of nutrient concentration and quality is the main concern for the use of DDGS from different resources. Depending on the specific regions and ethanol plants, the grains used in ethanol production process would be different: some plants use wheat and corn mixture, some use other mixed grains, some remove the fiber-rich hulls before fermentation, several plants skip the jet-cooking procedure, and so on (Babcock et al., 2008). Compared with wheat DDGS, corn DDGS contains higher levels of fiber and fat, but lower level of protein (Fathi and Aflfl, 2008). However, the contents of lysine and methionine in wheat and corn DDGS were not significantly different.

Belyea et al. (2004) indicated that the high variation of DDGS was due to the variations in processing technologies and raw materials. Due to these differences, the levels
of most nutrients in DDGS have high variations, especially in lysine, methionine, vitamins, minerals, and phosphorus content. Therefore, complete chemical analysis of each DDGS source is very important before diet formulation, especially when high levels of DDGS are used in animal diets.

Modern ethanol plants adopt new technologies to improve the quality of DDGS. Some plants use a technology to separate fibers from DDGS to improve DDGS quality and to be more suitable for non-ruminant animal usage (Loar et al., 2008). A new bio-refining production technology has been applied to separate bran, germ and endosperm before ethanol production, and endosperm is used to produce a high protein DDG. This new high protein by-product can be used at 12% level for laying hens without adverse effects (Jung et al., 2008).

The nutrient variability of DDGS is common even in the same ethanol plant among different production years. The differences in the same plant could be due to several reasons: materials (corn quality variation from different sources), the production process (temperatures, distillation process, mash times, etc.) (Carpenter, 1970; Olentine, 1986), and the amount of solubles added back to the wet grains (Martinez-Amezcua et al., 2007).

**Metabolizable Energy of DDGS**

Several studies have investigated the metabolizable energy (TMEn) content in DDGS for poultry. Lumpkins et al. (2004) reported that the metabolizable energy content of a single DDGS source was 2,905 kcal/kg. In a later study, the same group examined the metabolizable energy content of 17 DDGS samples from 6 different ethanol plants and found that metabolizable energy contents ranged from 2,490 to 3,190 kcal/kg with a mean of 2,820 kcal/kg and an associated coefficient of variation of 6.4% (Batal and Dale, 2006). Fastinger
et al. (2006) concluded that the metabolizable energy content of DDGS averaged 2,871 kcal/kg with considerable variations among the five samples. Parsons et al. (2006) also reported a large variation in metabolizable energy values of 20 DDGS samples with a mean 2,863 kcal/kg and a range spanning 447 kcal/kg. Based on a published metabolizable energy values, Waldroup et al. (2007) suggested nutritionist to use a metabolizable energy value of 2,851 kcal/kg for DDGS.

The variation of corn DDGS can be minimized if similar production technologies and corn from a narrow geographical area are used, but Stein (2009) tested four DDGS samples from ethanol plants which were located within 250 km from each other, and found that metabolizable energy content varied between 3,575-3,975 kcal/kg. They concluded that there should be other factors contributing to the variability of energy in DDGS.

Several studies have used chemical composition and gross energy for the estimation of metabolizable energy content in corn DDGS. Batal and Dale (2006) predicted the metabolizable energy using an equation: metabolizable energy = 2,732.7 + 36.4 (fat) – 76.3 (fiber) + 14.5 (protein) – 26.2 (ash) with accuracy of R²=0.45. Fastinger et al. (2006) found that the metabolizable energy of DDGS is close to 60% of its gross energy content, and this is similar to other ingredients with high protein content, such as soybean meal (Leske et al., 1991). Although, the determination of metabolizable energy from gross energy is simple and easy, it is not always accurate and reliable (Fastinger et al., 2006).

Since oil content is the best single predictor of metabolizable energy content in DDGS with r²=0.29 (Batal and Dale, 2006), the amount of solubles added back to wet grains is critical for metabolizable energy content. Noll et al. (2007) found that the solubles contain three times more fat than wet grains, and the rate of solubles that are added back to wet
grains in the DDGS manufacturing process is directly related to metabolizable energy content of DDGS ($r^2 = 0.88$). The oil content in corn DDGS varies from 2.5% to 16%, and thus DDGS have a potential large variation in metabolizable energy (Batal and Dale, 2006; Parsons et al., 2006; University of Minnesota, 2008b).

The color of DDGS (lightness) was reported to have a strong inverse correlation ($r = -0.98$) with the added back rates of solubles in DDGS from a single ethanol plant, and darker DDGS was assumed to have higher metabolizable energy content (Noll et al., 2007). However, Fastinger et al. (2006) used DDGS samples from several ethanol plants and found a moderate linear relationship ($r^2 = 0.52$) between lightness and DDGS metabolizable energy content with bigger variations compared with Noll’s study. Different DDGS source is probably the reason for the difference in the relationships between lightness and metabolizable energy values in these studies. This difference suggests that lightness and its linear relationship with metabolizable energy is not a reliable indicator for energy level in DDGS (Babcock et al., 2008).

**Protein and amino acid content**

DDGS are a good source of protein, and is composed of around 27% protein. However, variations in protein content were observed in many studies (Batal and Dale, 2006; Fastinger et al., 2002), and the protein content in those studies ranged from 23% to 32%. Protein content of DDGS samples from 10 new ethanol plants located in Minnesota and South Dakota was reported for 30.2%, and lysine and methionine digestibility were 0.85% and 0.55%, respectively (Spyihs et al., 2002). Amino acids, especially lysine and methionine, which are two important amino acids for poultry, are highly susceptible to heat damage.
during drying processes. The content and digestibility of these amino acids are the main concerns when DDGS are used in monogastric animal feed (Warnick and Anderson, 1968).

Lighter color DDGS are assumed to have more total and digestible amino acids, especially lysine, while darker color DDGS have lower amino acid content. Fastinger et al. (2006) examined the lysine content of DDGS in 5 samples, and observed a range of 0.48 - 0.76% lysine with the lowest in the darkest DDGS sample. Moreover, with the darkest DDGS sample, apparent and true lysine digestibility was the lowest for adult caecectomised roosters. Other essential amino acids also showed differences in content and digestibility among samples with variations. Ergul et al. (2003) found that there was a positive correlation between lysine, cysteine and threonine digestibility, and lightness (L*) and yellowness (b*) values of DDGS. Batal and Dale (2006) proved this relationship in another study, and observed higher total and digestible amino acids in the more yellow and lighter DDGS samples. Considerable variations were found in DDGS samples with different colors. The authors suggested that the color of DDGS could be used as a good indicator of amino acid content and digestibility, and the color analysis is an easy and fast way to get quick results.

Lumpkins and Batal (2005) evaluated the bioavailability of lysine in DDGS from a modern fuel ethanol plant. They found the true digestibility of lysine was 75% on the precision-fed cecectoized rooster assay, and higher digestibility of lysine (80%) in slope-ratio chick growth experiments. These values were slightly higher than that of an earlier study in which average lysine digestibility was 71% among 4 DDGS samples (Ergul et al., 2003). Several studies used DDGS samples from beverage industry and reported lysine digestibility of these DDGS samples to be 66-90% (Combs and Bossard, 1969; Parsons et al., 1983). Parsons et al. (2006) reported a range between 59 and 84% for lysine digestibility. The lysine
digestibility of DDGS from modern ethanol plant was not significantly different from that of corn (81%). Thus Lumpkins and Batal (2005) concluded that lysine digestibility of DDGS was not largely influenced by drying process. However, more recently, Pahm et al. (2009) reported 61.4% mean lysine digestibility from seven different DDGS sources for Single Comb White Leghorn roosters, and this value was much lower than that of the corn.

The variations of amino acid content and digestibility among DDGS sources are likely due to the differences in protein content of corn, the amount of solubles in DDGS, and processing technologies (Belyea et al., 2004; Martinez-Amezcua et al., 2007; Babcock et al., 2008). The corn harvested in Nebraska in 1988 contained 7.8% to 10% protein, and 0.22 to 0.32% lysine (Reese and Lewis, 1989). Since the nutrient concentration is different in the solubles and wet grains, the portion of the solubles influences the final protein content of DDGS. The add-back rates of the solubles also affect the color, and darker color has higher portion of the solubles. Color was suggested as an indicator of amino acid digestibility (Cromwell et al., 1983). Higher portion of the solubles needs longer drying procedure and higher temperature, and in turn affects amino acid digestibility (Noll et al., 2007). Processing temperature is crucial for amino acid digestibility, and the temperature can range from 260 to 1150 °F depending on specific ethanol plant (US Grains Council, 2008). Heat damage to lysine digestibility has been well recognized (Warnick and Anderson, 1968; Stein et al., 2006; Fontaine et al., 2007). The Maillard reaction between reducing sugars and the ε-amino group of lysine affects amino acid digestibility, because poultry do not have enzymes to break down the bond between lysine and sugar residues. Therefore, the Maillard-reaction products cannot be digested in poultry, and eventually are excreted from body.
**Phosphorous**

DDGS are a good source of bioavailable phosphorus. Corn grains contain 0.3% phosphorus (P) but most of the phosphorus is in phytate form. Since poultry do not have phytase to free the phytate phosphorus, most of the phosphorus in corn cannot be used by poultry. In contrast, DDGS contain 0.72% phosphorus (Martinez-Amezcua et al., 2004), most of which is bioavailable. The phosphorous content varies by DDGS sources, and can range from 0.59 to 0.95% (Spiehs et al., 2002; Batal and Dale, 2003; Martinez-Amezcua et al., 2004; Stein et al., 2006). Same as the variations in amino acids, variations in phosphorous content would be due to the differences in phosphorous content of corn, the rates of solubles added back in DDGS, and processing technologies (Martinez-Amezcua et al., 2007; Noll et al., 2007; Noll, Parsons, and Dozier, 2007).

The bioavailability of phosphorous in corn grain is only 30%, but that in DDGS is much higher because of phytate destruction during drying procedure (Martinez-Amezcua et al., 2004; Martinez-Amezcua and Parsons, 2007). Two “good quality” DDGS samples from commercial feed mills had a relative bioavailability of 69% and 75%, which is very close to the reference data (NRC, 1994), while the phosphorus in dipotassium hydrogen phosphoric acid (K$_2$HPO$_4$) is 100% bioavailable (Martinez-Amezcua et al., 2004). The authors also found that phosphorus variability ranged from 69% to 102% with a mean of 82%. The bioavailability of P in DDGS was reported at level of 77% (NRC, 1998).

Phosphorus bioavailability appears to increase with heat processing and the fermentation process during ethanol production. Phytase is widely added in poultry feed to release phosphate from phytic acid to improve the nutritive value. Phytase is produced by yeast, and it could change phosphorous to a more available form, which could increase the
bioavailability of P in DDGS (Dale and Batal, 2005). Similarly, Martinez-Amezcua et al., (2006) found that phytase released 0.049-0.0072% more P, which represented approximately 20-28% of the non-bioavailable P in DDGS. Same researchers also found increased bioavailability of P in the diet with 3% citric acid. Martinez-Amezcua et al. (2007) investigated the effect of heat processing on bioavailability of phosphorous in DDGS, and found that phosphorus bioavailability increased from 69% in the control samples to 91% in the oven dried samples. The authors concluded that heating process had a positive relationship with P bioavailability. Therefore, dark color DDGS that come from high temperature heating are assumed to have greater phosphorus content.

**Other minerals**

Corn grains have very low levels of calcium, potassium, sulfur, and sodium (Babcock et al., 2008). In DDGS, calcium and potassium are concentrated about three folds, and the contents of sulfur and sodium are much greater than the expected values (three folds). Again, variations of mineral content have been observed in several studies. Spiehs et al. (2002) reported year-to-year differences in Mn, Zn and Cu content, and pointed out that this variability would be due to the differences in corn sources and ethanol processing. Minerals have important role in physiology and biochemical processes of livestock, and deficiency in mineral can cause low production performance, low growth rate and loss of weight and appetite (Underwood and Suttle, 1999). Thus prior to diet formulation, mineral analysis should be done to avoid any adverse effect on animal and production.

Batal and Dale (2003) reported that Na content from 12 DDGS samples ranged from 0.09 to 0.44% with a mean value of 0.23%. The authors pointed out that the sources for the extra sodium are not clear so far, and water quality of ethanol plant would be one of the
reasons. Although poultry can tolerate high levels of sodium in the diet (Klasing and Austic, 2003), the level of sodium should be monitored when large amounts of DDGS are used in the diet. High levels of sodium can cause higher consumption of water, and consequently result in dirty eggs (Leeson et al., 1995; Klasing and Austic, 2003).

High amount of sulfur in DDGS comes from the sulfur in the yeast, well water, and sulfuric acid (H\textsubscript{2}SO\textsubscript{4}), which is added at several stages in the processing for pH adjustments. The sulfur content of DDGS varies from 0.3% to over 1% (Spiehs et al., 2002; Batal and Dale, 2003; University of Minnesota, 2008b). In cattle diets, sulfur level is limited to 0.4% due to its toxic effects (NRC, 1980). Broiler can tolerate sulfur up to 0.5%, and the level can be even higher for laying hens (Leeson and Summers, 2005). However, sulfur may interfere with the absorption of calcium and trace minerals in small intestines and affects the strength of bone and eggshell (Pineda et al., 2008).

**Carotenoid pigments**

Carotenoids are naturally occurring pigments in plants and organisms, and the color ranges from yellow to red. Xanthophylls (which contain oxygen) and carotenes (which contain no oxygen) are the main two classes of over 600 carotenoides. Corn grains contain xanthophylls pigments (zeaxanthin and lutine), which are very susceptible to light and heat damage (Kerrer and Jucker, 1950). Corn grains contain about 20 ppm of xanthophylls (Leeson and Summers, 2005), and DDGS are expected to be a good source of xanthophylls since xanthophylls are usually concentrated around three folds after ethanol processing. Due to light and heat damages, however, the actual xanthophylls content may be lower than expected.
Roberson et al. (2005) measured the xanthophylls content in two DDGS samples, and found darker sample had very low level (only 3.48 ppm) of xanthophylls with another lighter sample having 29.75 ppm of xanthophylls. Xanthophylls content from 16 DDGS samples was averaged at 36.72 ppm, which is higher than that in Roberson’s study (Salim et al., 2010). The variability in xanthophylls content may bring non-continuous effects on yolk and skin color. However, DDGS are still a good source of these pigments if care is taken during the heating procedure.

Egg yolk carotenes are classified as xanthophylls and carotenes (Karrer and Schopp, 1934). Xanthophils include lutein, zeaxanthin, and cryptoxanthin at level of 0.1, 0.2 and 0.03% of egg yolk, respectively (Romanoff and Romanoff, 1949). Lutein has been used in poultry diet for long time. This pigment can provide desirable yellow color in egg yolk and chicken skin, which consumers prefer (Perez-Vendrell et al., 2001; Leeson and Caston, 2004). Avian and mammals cannot synthesize xanthophyll pigments, and thus fully depend on sources of the diet for color absorption (Goodwin, 1984). Moreover, lutein has an important role in preventing age-related macular degeneration (Moeller et al., 2000). DDGS are good source of xanthophyll, thus DDGS diet may improve the lutein content in egg yolk.

**Other nutrients**

DDGS can be a rich source of water-soluble vitamins, specially thiamine and riboflavin (D’Ercole et al., 1939). Most of the riboflavin in DDGS comes from the solubles (Sloan, 1941). DDGS also contain several biologically active substances, such as nucleotides, mannanooligosacharids, beta-1,3/1,6-glucan, inositol, glutamine and nucleic acids. Tsang and Schaible (1960) identified growth factors in DDGS that can promote animal growth and hatchability in poultry. However, these factors as well as their functions are not fully
recognized and understandable. In another study, young growing pigs were fed with 10% DDGS, and 10% DDGS diet showed positive effects on health and growth (Whitney et al., 2006).

**Virginiamycin**

Virginiamycin is usually added in ethanol production process to control the growth of lactic acid bacteria and to increase ethanol production. Therefore, there is a high possibility of virginiamycin residues present in DDGS and DDGS diets. Virginiamycin residue is restricted at level of 0.1 ppm in poultry products (Food Safety and Inspection Service, 1998). However, the amount of virginiamycin in diet may be very small and that deposited in egg yolk may be at undetectable ranges.

**DDGS in animal feed**

DDGS was first introduced to beef cattle because of their relatively high fiber content. Later, DDGS have been widely used in other animal feeds, such as swine and poultry. Currently in the U.S., DDGS are predominantly used in ruminant. Dairy cattle consume around 42% of DDG, followed by beef (38%), swine (14%) and poultry (5%). There are great opportunities for potential DDGS usage in swine and poultry. According to recent studies, DDGS can be used up to 30% for lactating dairy cattle (Kalscheur et al., 2006); up to 40% for beef feedlot cattle (Klopfenstein et al. 2008); up to 30% for grower-finisher swine (Stein and Shurson, 2008); up to 15% for poultry (Noll, 2008).

**DDGS for broilers**

DDGS have been used for broilers for many years, but very low level (5%) of DDGS has been added in the broiler diet. Improvements in body growth rate and other characteristics were observed in early studies that incorporated DDGS to broiler and turkey
diet (Day et al., 1973; Couch et al., 1957). Later, Waldroup et al. (1981) found that DDGS can be included at 25% level without any adverse effects on body weight gain and feed conversion as long as metabolizable energy level was consistent. DDGS can replace up to 40% soybean if lysine level in broiler diet can meet the requirement (Parsons et al., 1983). Due to high fiber content and variability of amino acids, Batal and Parsons (2002 a, b) recommended that 25% to 30% DDGS were too high for first two weeks chickens. Lumpkins et al. (2004) reported that up to 6% DDGS could be used in the starter diet after which higher levels of DDGS (12-15%) could be safely fed to broilers in the growing period.

DDGS produced from modern ethanol plants are considered to have better quality in terms of amino acid content and its nutrients bioavailability. Lumpkins et al. (2004) evaluated the low and high density diets incorporated with 0 or 15% DDGS for broilers, and found no differences in performance parameters between 0 and 15% DDGS in high density diets. However, lower feed efficiency was found in the low density diet with 15% DDGS incorporation. In another experiment, the same authors reported no differences in body weight and feed conversion among 4 levels of DDGS inclusion rate (0, 6, 12, and 18%), except the starter period with 18% DDGS.

Based on digestible amino acid levels, Wang et al. (2007) evaluated the effect of DDGS with different inclusion levels (0, 5, 10, 15, 20, and 25%) on broiler performance. They reported that growth rate was not affected by DDGS, but feed conversion was poorer in 25% DDGS diet than control group. In addition, diets with 15 and 25% had lower dressing percentage, and 25% DDGS diet had lower breast weight. The authors concluded that DDGS could be added at level of 15 or 20% with little adverse effects on performance. Thacker and Widyaratne (2007) found that the diet with 20% wheat DDGS had a decreasing trend on
performance, and suggested to use 15% wheat DDGS for broilers without negative effect on performance. Wang et al. (2007c) showed that high inclusion rate of DDGS can affect broiler performance due to amino acid deficiency.

There are several studies focusing on the effect of DDGS on carcass quality and meat quality of poultry. Lumpkins et al. (2004) reported that breast weight and other meat cuts were not affected by different inclusion rates of DDGS. Wang et al. (2007a, b) found that there was no DDGS effect on carcass quality until the diet was incorporated with 15% DDGS. However, when diet with 30% DDGS was fed to broilers, breast meat yield tended to decrease due to amino acid deficiency. More recently, Corzo et al. (2009) found that color, pH, cooking loss, shear force, and consumer palatability of thigh and breast meat were not influenced by the supplementation of DDGS. Moreover, fatty acid composition differed between 0 and 8% DDGS diets. There were more linoleic and total polyunsaturated fatty acids in meat as the addition of DDGS increased. Similarly, Choi et al. (2008) found increased unsaturated fatty acids in meat with increased DDGS inclusion rates. They concluded that 15% DDGS in broiler diet would not bring any adverse effect on performance and meat quality. However, further study is needed to investigate the optimum level of DDGS that could be incorporated in broiler diet.

**DDGS for laying hens**

Early studies showed that DDGS could be used at 5-20% levels in diets for laying hens without adverse effects on egg production and egg weight (Morrison, 1954; Matterson et al., 1966; Harms et al., 1969; Jensen et al., 1974). DDGS have been used at conservative levels (5%) for many years due to limited supply, cost and nutrient variability (Waldroup et al., 1981; Noll et al., 2001). Alenier and Combs (1981) reported increased feed intake in
laying hens with up to 10% DDGS inclusion rate, but this effect was not shown in broiler chickens (Cantor and Johnson, 1983). Compared to the hens fed with control corn-soybean diets, body weight, liver weight, plasma lipids, T3 and estradiol levels were significantly reduced when hens were fed with 20% DDGS diets (Lilburn and Jensen, 1984; Akiba et al., 1982).

Lumpkins et al. (2005) used DDGS from a new generation plant, and reported that there were no DDGS-related differences on egg production, egg quality, eggshell strength or yolk color at DDGS incorporation rate of 15%. More recently, Roberson et al. (2005) used four levels of DDGS (0, 5, 10, and 15%) on Hy-line W36 laying hens for 20 weeks. They found inconsistent DDGS treatment effects at certain periods, during those period egg production, egg weight, egg mass, and specific gravity decreased linearly as DDGS level increased. They concluded that up to 15% DDGS can be used in laying hens diet, but lower levels of DDGS should be used at the beginning when introducing DDGS diet to layers. Similarly, no significant differences on egg production and egg quality were found when 0 and 15% of DDGS were added in diets (Swiatkiewicz and Koreleski, 2006). Recently, Scheideler et al. (2008) concluded that inclusion of up to 25% DDGS had no adverse effects in egg production, feed consumption, and body weight, but egg weights were reduced in diets with 20% and 25% DDGS because of amino acid deficiency. Cheon et al. (2008) also reported that 20% DDGS diet did not influence the egg production, total egg mass, mean egg weight, and feed intake.

The effect of DDGS diets on internal quality of eggs, including eggshell strength, yolk color, Haugh unit, and chemical composition of egg yolk, has been investigated. Yolk color was intensified rapidly when laying hens were fed with 10% DDGS diet (Roberson et
al., 2005). However, Lumpkins et al. (2005) reported that there was no relationship between the levels of DDGS and yolk color in diets with up to 15% DDGS. Roberts et al. (2007b) noted that 10% DDGS diet had no effect on yolk color. However, Cheon et al. (2008) reported significant increase in yolk color by DDGS diet. Swiatkiewicz and Koreleski (2006) also found that yolk color increased significantly with increased DDGS inclusion rate, but no differences were found in Haugh unit and eggshell strength. Other researchers also reported that Haugh unit and eggshell strength were not affected by DDGS incorporation (Lumpkins et al., 2005; Roberson et al., 2005). However, Lilburn and Jensen (1984) observed increased Haugh unit from diet with addition of 20% DDGS.

**DDGS for turkeys**

Couch et al. (1957) reported that inclusion of 5% DDGS in turkey diet improved turkey growth performance by 17-32%. In an early study, Potter (1996) found that up to 20% of DDGS in turkey diets would not induce any adverse effect if lysine and energy levels were adjusted. Manley et al. (1978) found a positive effect on egg production of turkey hens when 3% DDGS was included in the diet. Roberson (2003) pointed out that 10% DDGS could be used in finishing diet of large white female turkey hens without any adverse effect on weight gain and feed conversion, if diet formulation was properly adjusted. More recently, Noll et al. (2004) reported that DDGS could be fed to turkey toms at up to 20% level without negative effects on body weight and feed conversion. They also found 10 or 15% DDGS in high protein diets can improve body weight gains.

Deficiency in amino acid is likely to be the reason for the decreased body weight when turkeys were fed with 27% DDGS diet (Roberson, 2003). The actual digestibility of lysine in DDGS source used in that study was lower than the predicted value. Similarly, Noll
et al. (2002) found that higher incorporation of DDGS had no negative effects on breast meat yield if all the diets had balanced amino acid levels.

**DDGS effect on egg production and egg quality**

Since DDGS can be used as a nutritive feed ingredient for poultry, and quality of DDGS has been greatly improved by modern processing technique, the inclusion rate of DDGS in poultry diet would be elevated without adverse effects on egg production and egg quality. Many researchers have shown great interest in investigating the maximum level of DDGS in laying hen diet. Recently, Pineda et al. (2008) fed layers with diets containing 0, 23, 46, and 69% corn DDGS for 8 weeks and found that egg production increased linearly, while egg weight decreased linearly as DDGS level increased. As a result, egg mass was not influenced by the DDGS levels in the diet. They also found that yolk color was improved as DDGS level increased, but Haugh unit, eggshell weight, and egg components were not influenced by the DDGS concentration in the diets. This study indicated that feeding laying hen with diets containing high levels of DDGS is possible and would not bring adverse effects on egg production and egg quality.

DDGS are also a special feed ingredient for poultry because DDGS contain considerable amounts of several important functional nutrients such as lutein, choline, and long chain unsaturated fatty acids, which are not abundant in other feed ingredients. The content of these nutrients in DDGS can influence the chemical composition and nutrient content of eggs, especially when DDGS are used at high levels in the diet.

Egg yolk is the most nutrient-rich part of egg, and contains high levels of functional nutrients such as choline and lutein. Choline is very important for brain development, liver function and cognitive function for animals (Shaw et al., 2004). Deficiency in choline may
result in higher risk to cancer (Xu et al., 2008 & 2009) and neural tube defects (Shaw et al., 2004). Small amount of cholesterol in egg yolk may be originated from feed, but major part of yolk cholesterol is synthesized inside the body when egg yolk is formed. High dietary fiber content in DDGS diets may have positive effect in controlling cholesterol levels in eggs, since many researchers have found a positive relationship between higher fiber diet and lower serum cholesterol (Lairon et al., 2005; Bruckert and Rosenbaum, 2011). In addition, fatty acid composition may have effect on cholesterol level. Several studies found that feeding polyunsaturated fatty acids would increase yolk cholesterol level (Summers, Slinger and Anderson, 1966; Weiss, Naber, and Johnson, 1964). The higher inclusion rate of DDGS in laying hen diet may improve the nutritional value of eggs with lower production cost, and thus may not only support the development of ethanol industry, but also benefit the poultry industries.

Environmental issues

Atmospheric NH₃ emission is a major pollution problem with domestic animal industry (Aneja et al., 2006), and poultry industry is the largest contributor of NH₃ emission in the United States (EPA, 2004). Nitrogen is excreted from manure and converted to ammonia by manure microbes (Pineda et al., 2008). With high inclusion level of DDGS (around 50%), diets may contain over 20% crude protein, compared with 15-17% CP in the normal diets. Roberts et al. (2007b) found a positive correlation between the amount of DDGS in the diets and N excretion with a high correlation value ($r^2 = 0.91$). NH₃ can reduce air quality, lower egg production and body weight, cause eutrophication of water resources, and adversely impact lung function as well as workers’ health (Carlile, 1984; Deaton et al., 1984; Omland, 2002; Nagaraja et al., 1983; Ritz et al., 2004; Pineda et al., 2008).
However, NH₃ emission could be reduced or inhibited by high content of fiber in poultry diet. Microbes in the large intestine can ferment the undigested fiber from DDGS diet to short chain fatty acids, and the fatty acids can lower manure pH, which results in the production of less volatile ammonium form of N. Thus, total evaporation of NH₃ can be reduced, and therefore less negative effect on air pollution can be achieved by DDGS diets (Babcock et al., 2008; Bregendahl et al., 2008; Roberts et al., 2007a).

Corn DDGS may also have a relatively high content of sulfur, which can cause elevated hydrogen sulphide emission when sulfur is excreted from poultry. Hydrogen sulphide can adversely affect egg production and air quality (Pineda et al., 2008).

**REFERENCES**


Scheideler, S.E., Masa’dah, H., and Roberson, K. 2008. Dried distillers grains with solubles in laying hens ration and notes about mycotoxins in DDGS. International pre-show nutrition symposium, Midwest Poultry Federation Convention, March 18-20, St. Paul, MN.


Worldwatch Institute and Center for American Progress. 2006. American energy: The renewable path to energy security.


CHAPTER 2. EFFECTS OF INCREASING CONCENTRATIONS OF CORN DISTILLER’S DRIED GRAINS WITH SOLUBLES ON THE EGG PRODUCTION AND INTERNAL QUALITY OF EGGS OF LAYING HENS

ABSTRACT

The objective of this study was to determine the effects of feeding high concentrations of corn distiller’s dried grains with soluble (DDGS) on egg production and the internal quality of eggs from laying hens. Four diets were formulated to contain 0, 17, 35 or 50% corn DDGS. A total of 240 54-week-old single-comb White Leghorn laying hens were randomly allotted to 2 birds per cage with three consecutive cages representing an experimental unit (EU). Each EU was assigned to one of four dietary treatments according to a completely randomized design. Hens were fed for a 24-week experimental period after transition feeding to gradually increase corn DDGS inclusion over a four-week period. Two sets of experimental diets were formulated to meet or exceed the National Research Council (1994) nutrient recommendations for laying hens. Each diet formula was fed for 12 weeks. Egg production was recorded daily and feed consumption was measured weekly. Egg component, yolk color, Haugh unit during storage times, and shell breaking strength were measured every two weeks. Egg production, egg weight, egg mass, feed intake, and feed efficiency were adversely affected by the highest level of DDGS in the diet (50%) during the first 12-wk period. Once diets were reformulated to include an increased concentration of both lysine and methionine, differences among the dietary treatments were reduced, as the
performance of the 50% DDGS diets was greatly improved. Over the last 6 weeks of study, no differences in egg production, egg weight and feed intake among DDGS treatments were found. DDGS diets positively affected the internal quality of eggs during storage. Improved yolk color and Haugh unit were observed as the dietary DDGS levels increased. Shell weight percentage was increased in 50% DDGS diet, but no differences in yolk and albumen percentage were observed. It was concluded that up to 50% of DDGS could be included in the layer’s diet without affecting egg weight, feed intake, and egg production as long as digestible amino acids were sufficient in DDGS-added diets.

**Key words:** corn distiller’s dried grains with solubles, laying hen, egg production, egg internal quality
INTRODUCTION

Distiller’s dried grains with solubles (DDGS) are a by-product of ethanol industry, and are often used at lower concentrations as a feed ingredient for laying hens. Corn DDGS are widely used in the US as an economical alternative source for protein, energy, and available phosphorus (Weigel et al., 1997; Creswell, 2006). After starch is utilized during the fermentation process of ethanol production, all the non-fermentable nutrients and components of corn are concentrated to around three-folds those of corn in the final DDGS product (Spiehs et al., 2002). As the production of bioethanol has increased rapidly in recent years, increased quantity of DDGS becomes available for feed industry (F.O. Licht, 2008, 2009, and 2010).

Distiller’s dried grains with solubles have been safely added up to 10 or 15% in laying hen diets without adversely affecting laying performance and egg production (Lumpkins et al., 2005; Roberson et al., 2005; Swiatkiwicz and Koreleski, 2006). Several researchers have investigated increasing the concentration of DDGS in laying hen diets in an attempt to identify feeding maximums that do not result in any harmful effect on egg production and egg quality, which including haugh unit, yolk color and eggshell strength. Haugh unit is a measurement based on egg weight and the height of thick egg white surrounding the egg yolk, which was introduced by Raymond Haugh in 1937. Egg white protein can maintain their structure and strongly hold the water when eggs are fresh, thus when eggs are broke, the egg white can stand high and be close to the egg yolk. However after storage, egg white protein start to lose their structure and function, thus when eggs are broke, the egg white spreads out and becomes watery. Cheon et al. (2008) reported that addition of 20% DDGS in laying hen diets had no harmful effects on egg quality and egg
production. Pineda et al. (2008) fed layers diets containing 0, 23, 46, and 69% corn DDGS for 8 weeks and found that egg production increased linearly, while egg weight decreased linearly as DDGS level increased. As a result, egg mass was not influenced by DDGS concentration in the diet. They also found that yolk color was improved as DDGS level increased, but Haugh unit, eggshell weight, egg components were not influenced by the DDGS concentration in the diets. This study indicated that feeding high concentrations of DDGS to laying hen diets would not produce adverse effects on egg production and egg quality. However, it was a short-term study (8 weeks), and the effects of long-term feeding high concentrations of DDGS to laying hens are unknown.

An additional unknown in the use of DDGS in higher dietary concentrations is the possibility of virginiamycin as a residue in the feed ingredient. Virginiamycin is usually added in ethanol production processes to control the growth of lactic acid bacteria, therefore to prevent potential yield loss of ethanol (Hynes et al., 1997). Therefore, there is a possibility that virginiamycin residues are present in DDGS themselves and diets containing DDGS. Virginiamycin residues are restricted to concentrations of 0.1 PPM in poultry products (Food Safety and Inspection Service, 1998).

The objectives of this study were to investigate the effects of long-term feeding of high-level dietary corn DDGS (up to 50%) on the laying performance (egg production, egg weight, feed intake, and feed efficiency), egg components (albumen, yolk, and shell) and egg shell quality, yolk color, and Haugh unit during storage and to explore to possibility of virginiamycin residue in diet containing higher concentrations of DDGS.
MATERIALS AND METHODS

DDGS Diets

The corn DDGS used in this study was obtained from a local ethanol plant (Lincolnway Energy, Nevada, IA). Corn DDGS diets for laying hens were formulated based on National Research Council nutrient recommendations for laying hens (NRC, 1994) and the current DDGS nutrient profiles on the website of University of Minnesota (http://www.ddgs.umn.edu/profiles-current.htm). Diets were formulated having four levels of corn DDGS (0, 17, 35, and 50%) included by replacing increasing amounts of corn, soybean meal, dicalcium phosphate, and DL-methionine, with DDGS, animal vegetable blended fat, limestone and lysine. The highest inclusion level of corn DDGS was chosen on the basis of the results of Pineda et al. (2008). The diets were formulated to be isocaloric based on energy values for feed ingredients published by the National Research Council. The diets were formulated on a total amino acid basis and total crude protein of diets increased with increasing concentrations of DDGS.

After the first 12-week experiment period, diet formulas were modified by addition of lysine and methionine in an attempt to meet the production requirements of laying hens fed the 50% DDGS diets. The composition and nutrients content of the two DDGS diet formulas are shown in Tables 2.2 and 2.3. The two formulas had the same concentrations of ME, Ca, P, most amino acids except lysine and methionine, and very similar protein level in the treatment with the same DDGS level.

DDGS samples were prepared for proximate and amino acid content. The nutrient composition of corn DDGS used in this study was analyzed at the Poultry Science Center of University of Arkansas (Table 2.1).
**Virginiamycin Residues in the DDGS Diets**

DDGS samples and DDGS diets were sent to Purdue University for virginiamycin residue analysis (Office of Indiana State Chemist, Purdue University, Microbiology Department, Dr. Ragheb). Plate method and bio-autography method were used for the analysis (Table 2.4). Both methods can detect active virginiamycin residue in DDGS and diets containing DDGS. The plate method is more accurate, but bio-autography has lower testing limit (unpublished method, kindly provided by Dr. Ragheb, Purdue University, IN).

**Birds and Experimental Design**

The experiment was conducted in the poultry research and teaching farm at Iowa State University. The study was approved by the Institutional Animal Care and Use Committee at Iowa State University (Approval # 4-09-6732-G). A total of 240 54-week-old Single-comb White Leghorn laying hens (Hy-Line W-36) were placed in 128 cages (2 birds/cage) with 96 square inches per bird. Three consecutive cages were considered an experiment unit, and each EU was assigned to one of four diets containing 0, 17, 35, or 50% DDGS using completely randomized design methodology. Each diet treatment was fed to 10 experimental units of six hens resulting in a total of 240 hens for the four diets. Hens had free access to the diets, and were provided with 16 hours of light and 8 hours of darkness per day. The ambient temperature was maintained at 26 °C throughout the study.

Prior to the experiment, the hens (except for control birds) were fed a corn-soybean meal-based diet containing 10% corn DDGS for 2 weeks and the hens for high DDGS treatments were acclimated to the high inclusion rates of corn DDGS by gradually introducing the treatment diets over the next 4-week period. The birds were 42-week-old at
the beginning of experiment and the average egg production rate was 85% when the experiment began.

**Performance and Egg Quality Measurements**

**Production Performance.** Hens were individually weighed at the beginning of the experiment and every 4 weeks throughout the study. Individual hen weights were averaged over the EU for statistical analysis. Egg production and egg weight were recorded daily and averaged per EU during the study. Egg mass was calculated as egg production × egg weight expressed as g of egg produced per day. Feed consumption of each pen was determined once a week during the experiment, and was calculated by subtracting the residual feed from total feed at the beginning of each week. Feed efficiency was calculated by dividing feed intake by egg mass production.

**Internal Quality of Egg.** Yolk color, Haugh unit, egg components, and shell breaking strength were determined every two weeks over the experimental period. Eggs were evaluated for internal quality by measuring Haugh units and yolk color. Yolk color was measured subjectively with an improved Roche yolk color fan by matching the yolk color on a glass plate with the 10 bands of the color fan. Standard color score was from 1 (extremely pale) to 10 (intensive yellow-orange). The selected colors were characterized by tristimulus values of the CIE (1993) standard colorimetric system. Five eggs from each EU were randomly selected from a two-day egg production every two weeks, and if egg production of a pen in those two days was smaller than five, then four eggs were utilized. Haugh unit was measured at four storage times (0, 1, 2, and 3 weeks) to better understand the effects of DDGS inclusion on egg white quality. The height of the thick albumen of each egg was measured three times by an electronic tripod albumen-height gauge (Ames Inc., Melrose,
MA), and the Haugh unit was calculated by the average albumen height and egg weight following the equation

$\text{Haugh unit} = 100 \times \log (\text{albumen height} - 0.01 \times 5.6745 \times (30 \times \text{egg weight}^{0.37} - 100) + 1.9).$

Five eggs from each pen were randomly selected for each storage time (two days’ eggs for each storage time), and if egg production of a pen in any two selected days was smaller than five, then four eggs were utilized.

For egg component measurement, five eggs from each pen were randomly chosen from a two-day egg collection every two weeks. Eggs were broken to separate the shell, and the yolk and albumen using an egg yolk separator. Before measuring, eggshell was cleaned using paper towels to remove the remaining albumen. Yolk and shell weight were measured directly, albumen weight was calculated by subtracting yolk and shell weight from overall egg weight. The percentage of each component was calculated by dividing each component weight by overall egg weight. Shell breaking strength was determined using the TA.XT2i® Texture Analyzer (Texture Technologies Corp., New York). The egg was placed on A/ES stable micro systems egg support, and the force from an aluminum compression platen (7cm in diameter) to break the eggshell was measured. Ten eggs were randomly selected from each treatment for shell strength measurement.

**Statistical Analyses**

This experiment used a completely randomized design. The data were analyzed by one-way ANOVA with the GLM procedure of SAS institute by one variation (treatment or week) (SAS 9.2, 2008), and Duncan’s multiple range test was used to separate means. In addition, data were analyzed as repeated measure, employing split-plot Mixed procedure of SAS sliced by week, with main effects (treatment and week) and random effect (treatment x
week interaction). Statistical significance was assumed at \( p < 0.05 \) to determine the differences among four treatments.

**RESULTS AND DISCUSSION**

*Nutrient Composition of DDGS and Diets*

Nutrient composition of DDGS sample is shown in Table 2.1. The DDGS used in this study contained 27.3% CP, 10.67% fat, 0.91% lysine, 0.81% methionine, and 4.35% fiber, which are similar to those reported in NRC (1994) and DDGS website of University of Minnesota. Due to variations in the processing and raw materials, protein level in DDGS can range from 23% to 32%, and fat level can vary from 2.5% to 16% (Fastinger et al., 2002; Batal and Dale, 2006; Parsons et al., 2006; University of Minnesota, 2008). Variation in nutrients is a major concern when introducing DDGS to animal feed because it directly affects the diet formulation.

*Virginiamycin*

Both plate and bio-autography methods can detect the presence of active virginiamycin residue among four DDGS diets, but the active virginiamycin residues from all the DDGS diet treatments were below the restriction limit of 0.1ppm (Table 2.4) (Food Safety and Inspection Service, 1998). Thus the possibility of virginiamycin being present in egg yolk is negligible. The plate method is common for detecting virginiamycin with great accuracy. Bio-autography method with lower detective limits was also used in our study to ensure that low levels of virginiamycin in the diets could be detected. The detection limit for the plate assay is 0.05 ppm. The virginiamycin results in Table 2.4 are between 0.05 ppm and 0.1 ppm, which are close to the detection limit for the virginiamycin. Also, the variations at
the levels were large due to interference by other ingredients. Therefore, virginiamycin in DDGS can be considered negligible. However, caution should be used in interpreting these results as these data were generated using one sample of DDGS from one plant and would not reflect variation in processes due to multiple production methods.

**Laying Hen Performance**

The egg production of 50% DDGS diet was much lower than that of the other three DDGS treatments before the diet reformulation (Figure 2.1). Overall egg production of control and 35% DDGS treatments were highest (Table 2.5). The egg production of control and 35% DDGS treatment groups was similar over the whole experimental period, and that of 17% DDGS treatment was reduced but due to the unexplained reduction at 17 and 18 weeks. Egg production rates decreased slightly in all DDGS treatment groups as the age of laying hens increased. Overall the 17% DDGS fed birds resulted in slightly but significantly reduced egg production, this drop is most likely due to the significant drop in egg production, feed intake, egg weight, and egg mass, which was observed in hens fed 17% DDGS diet at 17\textsuperscript{th} and 18\textsuperscript{th} week. Two of the three total mortalities for this treatment were noted over this time again suggesting a non-DDGS issue. When additional lysine and methionine were added, egg production of the 50% DDGS treatment group was greatly improved resulting in similar production to the corn-soybean meal control diet. The results of current study before diet reformulation agree with previous studies, in which egg production was adversely affected by DDGS diets (Swiatkiwicz and Koreleski, 2006; Pineda et al., 2008; Shalash et al., 2010). However, after diet reformulation, the egg production among DDGS treatments was not significantly different during 19-24 week in this study. This suggests that high concentration DDGS may not affect egg production with balanced nutrients in the diet.
Feed intake of the hens followed similar pattern to egg production during the whole study period (Table 2.5 and Figure 2.2). Lowest feed intake was observed in 50% DDGS treatment but no significant difference among other three treatment groups were observed before the diet formulation change. Feed intake of 50% DDGS treatment group increased dramatically during the first four weeks after the change of the diet formula, and was the highest from 15 to 18 wk among all treatments. In the last 6 weeks of the study, the feed intake among four DDGS diet treatments was not significantly different (p > 0.05). Again, a reduction in feed intake was noted over the 15 to 18 week period for the birds fed the 17% DDGS diets corresponding to reduced egg production, reduced egg mass and increased mortality. These data disagree with the report of Alenier and Comb (1981) who found increased feed intake when 10% DDGS diet was fed. However, our results agree with other recent reports in which no significant difference in feed intake was observed when 15, 20 or 25% DDGS was included in laying hen diets (Roberson et al., 2005; Cheon et al., 2008; Scheideler et al., 2008).

The overall egg weight of 50% DDGS treatment was lower than that of control and 35% DDGS treatments (Table 2.5). Egg weight of 50% DDGS treatment decreased significantly before diet reformulation (Figure 2.3). After diet reformulation, 50% DDGS treatment showed a great increase in egg weight from 60.53g to 65.46g, and the other three DDGS treatments had no apparent changes except for a drop in egg weight with 17% DDGS treatment from 15 to 18 week period. All DDGS treatments had the same egg weight for the last 6 weeks of study (P > 0.05). The results after diet reformulation disagree with the studies of Cheon et al. (2008), Pineda et al. (2008), and Shalash et al. (2010), who observed decreased egg weight by DDGS inclusion.
The highest overall egg mass was observed with control treatment and 35% DDGS treatment, and the lowest was observed with 50% DDGS treatment (Table 2.5 and Figure 2.4). The difference in egg mass between 50% DDGS treatment and the other three treatments decreased after the change of diet formula. At 15 to 18 wk, the egg mass of 17% DDGS treatment was reduced in conjunction to the reduced egg production and feed intake over the same period. The decreased egg mass of this study agrees with the study of Shalash et al. (2010), in which decreased egg mass was reported when 15 or 20% DDGS was used in laying hens diet. However, it disagrees with the studies of Cheon et al. (2008) and Pineda et al. (2008) in which DDGS concentration was found to have no influence egg mass.

Feed efficiency was significantly reduced in all three DDGS treatment in comparison to the corn-soybean meal diet (Table 2.5). The best feed efficiency was observed with control diet treatment (531.6g egg/kg feed) followed by 35% DDGS treatment (501.9g egg/kg feed), 17% DDGS treatment (487.6g egg/kg feed), and 50% DDGS treatment (431.8g egg/kg feed). These data might indicate that the hens are increasing feed intake to maintain egg production due to the elevated dietary fiber found in the DDGS treated diets.

Body weight of laying hens in four DDGS diet treatments (0, 17, 35, and 50%) before the experiment was 1.56 kg, 1.50 kg, 1.62 kg, and 1.51 kg respectively (Table 2.5). At the end of the study, body weight of laying hens in four treatments was 1.57 kg, 1.50 kg, 1.62 kg, and 1.56 kg. There was no significant difference of laying hen body weight change among four DDGS treatments during the study (p>0.05) (Table 2.5). There was a significant decrease in hens’ body weight with 50% DDGS treatment from 1.50 kg to 1.35 kg before diet reformulation to increase amino acids (data not shown), but the body weight increased from 1.35 kg to 1.56 kg after supplemental amino acids were added to the diets. The body weight
of 17% DDGS treatment were reduced at the fourth measurement (13 to 16 wk), again, corresponding to reduced feed intake, egg production, egg mass and increased mortality over this time period.

The significant reduction in feed intake, egg production and egg weight of the hens fed the 50% DDGS diets are most likely due to an amino acid deficiency as these values dramatically increased once diets were reformulated with increased methionine and lysine. This is most likely due to the reduced digestible lysine and methionine in DDGS. Hughes and Hauges (1945) reported that when DDGS was used as the main protein source, there would be a slight deficiency in lysine and tryptophan that would cause decreased broiler performance. Soybean meal when used in traditional ratios with corn has favorable amino acid composition for birds, but adding more corn protein by the addition of high concentrations of corn DDGS can result in changes in dietary amino acid ratios. As dietary DDGS level increases, soybean meal decreases, soy makes up approximately 30% of the protein in 35% DDGS diet treatment, but it makes up less than 15% of the protein in 50% DDGS diet. Therefore, the differences in amino acid composition and their availability among diets could have significant impact on egg production if not accounted for during formulations. Shalash et al. (2010) pointed out that high content of crude fiber and sulfur, and low palatability of DDGS could be the reason for decreased egg production, egg weight, and egg mass. In addition, high protein and low starch content in DDGS-containing diets would have changed gluconeogenesis pathway to solely rely on converting amino acids to glucose, and high fat content would become major way of supplying energy through fatty acid oxidation. These changes could have influenced poultry metabolism over time, and ultimately might have affected laying performance (Shalash et al., 2010).
In summary, egg production, egg mass, feed efficiency, and egg weight were affected by the levels of corn DDGS in the diets before the diet reformulation. The highest DDGS level (50%) had the lowest egg production, egg mass, feed efficiency and egg weight, and control diet (0% DDGS) had the best results for all the parameter listed above. The low laying hen performance before diet reformulation was probably due to overestimation of lysine and methionine in DDGS. However, after diet reformulation, the differences of laying hen performance of hens among the four DDGS treatments gradually decreased and disappeared after 4 weeks. Egg production, feed intake, and egg weight were not significantly affected by DDGS level for the last 6 weeks of the experimental period. Laying hens responded to high-protein, high-fat, and high-fiber corn DDGS by decreasing egg production and egg mass, but not by changing egg weight. No difference in egg weight among treatments during last 6-wk period indicated that when amino acids (lysine and methionine) in corn DDGS diets are sufficient, egg weight may not be affected by DDGS effect, but if diet is not balanced for nutrient content, a decrease in egg production and egg mass can occur in the diet with high DDGS inclusion.

**Internal Quality of Egg**

Table 2.6 indicates that increasing DDGS level from 0 to 50% had significant effects on yolk color and Haugh units over various storage times. Yolk color was enhanced very quickly (within two weeks) after receiving DDGS diets. As DDGS level increased, the yolk color score increased linearly (50% > 35% > 17% > 0%), and this treatment effect was continued during the 24-wk study. Increased yellowness of yolk was expected because DDGS contains high concentrations of xanthophylls, which is responsible for the yellow color of yolk. These results agree with the report of Roberson et al. (2005). Although
Lumpkins et al. (2005) reported that there was no improvement in yolk color when 15% DDGS was used in the diet and Roberts et al. (2007b) noted that 10% DDGS had no effect on yolk color, however, Cheon et al. (2008) reported significant improvement in yolk color with 10, 15 and 20% DDGS addition in the diet. Swiatkiewicz and Koreleski (2006) also found that yellow color intensity in yolk increased significantly with 5, 10, 15 and 20% DDGS in the diet. Xanthophylls pigments are susceptible to light and heat damage, thus the observed different effects on yolk color from different research groups might have been related to the different xanthophylls content in DDGS sources. Moreover, as DDGS level increased in the diet, xanthophylls concentration in the diet increased which resulted in linear increased yolk color intensity.

Egg albumen quality was improved in 50% DDGS treatment with the highest Haugh unit at all storage times, and there was no difference among the other three DDGS treatments during the whole period (Table 2.6). Haugh unit decreased with storage time in all DDGS treatments. Swiatkiewicz and Koreleski (2006) found no differences in Haugh unit and eggshell strength of eggs from hens fed diets containing 0, 5, 10, 15, and 20% DDGS. Pineda et al. (2008) reported no difference in Haugh unit when high level of DDGS was used. Other researchers also reported that Haugh unit and eggshell strength were not affected by DDGS incorporation (Jensen et al., 1978; Lumpkins et al., 2005; Roberson et al., 2005). However, Lilburn and Jensen (1984) observed increased Haugh unit with the addition of 20% DDGS, and Jensen et al. (1978) also reported increased Haugh unit with 10% DDGS treatment. In the current study, Haugh unit of eggs from the hens fed 50% DDGS had higher Haugh units than other dietary treatments during all storage times, suggesting that high DDGS in the diet may have positive effects in maintaining the physical state of egg albumin.
Table 2.7 shows that the effect of DDGS treatment on egg shell breaking strength was only found over 7-8 week and 15-16 week during which time 50% DDGS treatment had higher shell breaking strength force than the other three DDGS treatments. Aside from the two previous mentioned intervals, the difference in eggshell strength among four DDGS treatments was not significant (p > 0.05). Lumpkins et al. (2003) reported no DDGS effect on shell strength when 15% DDGS was added in the diet. Shalash et al. (2010) reported increased shell thickness, but Pineda et al. (2008) found no change in eggshell quality by DDGS inclusion. In this study, significant differences in eggshell strength among DDGS treatments were shown only over 7-8 week and 15-16 week (p < 0.05), which might due to smaller eggs were selected in 50% DDGS treatment group. Because the effect of DDGS on shell breaking strength was not consistent and occurred infrequently, it is difficult to say that DDGS treatments affect shell strength.

No significant difference in overall albumen and yolk percentage was detected among four DDGS treatments during the whole 24-wk period (Table 2.6). Difference in shell percentage among four DDGS treatments was observed at 9-12 week, 15-16 week and 19-20 week, and difference in yolk percentage was observed at 11-12 week. The overall shell percentage was the highest with 50% DDGS treatment with no difference among the other three DDGS treatments (Table 2.6). Roberson et al. (2007) observed increased albumen content with diets containing corn DDGS compared with control. Shalash et al. (2010) reported that weight percentage of egg components (albumen, yolk, and shell) was not influenced by up to 20% DDGS in diet rations. In this study, overall shell percentage was affected by DDGS treatments. However, during last four weeks of study, the shell percentage among four DDGS treatments was not different. The larger shell percentage might be due to
smaller egg size with 50% DDGS treatment compared with other three DDGS groups. Small eggs may tend to have thicker eggshell than larger eggs when the same amount of calcium is deposited (Elaroussi et al., 1994).

In conclusion, it appears that high level of DDGS (up to 50%) can be fed to laying hens without significant affect on egg production, feed intake and egg weight with sufficient digestible amino acid. Once the 50% DDGS diet was further supplemented with lysine and methionine, there were no differences in egg production, feed intake and egg weight during last 6 weeks between the high DDGS diet and the positive control. The decreased egg production, feed intake, egg mass and egg weight observed before the diet reformulation is most likely due to an amino acid deficiency, as all performance parameters were improved after additional amino acids were supplied in the diets. DDGS positively affected Haugh units in the 50% DDGS diet, and yolk color was improved linearly as DDGS level increased. Eggshell strength and egg component were inconsistently affected by DDGS treatments.

ACKNOWLEDGEMENTS

This study was supported by Power Funds of Iowa. Hens were donated by Sparboe Company, DDGS feed was provided by Lincoln Way Energy (Nevada, IA), blended fat was donated by Feed Energy Company (Des Moines, IA), and limestone was donated by Iowa Limestone Company (Des Moines, IA). The assistance provided by personnel in laboratories of Ahn, Sebranek, and Persia and Iowa State University poultry farm is greatly appreciated.
Table 2.1 Nutrient analysis of corn distiller’s dried grains with solubles (DDGS) used in the study (% as is)

<table>
<thead>
<tr>
<th>Amino Acid Profile</th>
<th>%</th>
<th>Nutrients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.91</td>
<td>Dry Matter</td>
<td>87.60</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.97</td>
<td>Protein</td>
<td>27.30</td>
</tr>
<tr>
<td>Serine</td>
<td>1.13</td>
<td>Ash</td>
<td>4.35</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.46</td>
<td>Fat</td>
<td>10.67</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.13</td>
<td>Minerals</td>
<td>ppm</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.01</td>
<td>P</td>
<td>9296</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.76</td>
<td>K</td>
<td>11578</td>
</tr>
<tr>
<td>Valine</td>
<td>1.46</td>
<td>Ca</td>
<td>312</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.81</td>
<td>Mg</td>
<td>3532</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.08</td>
<td>S</td>
<td>4871</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.19</td>
<td>Na</td>
<td>2171</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.89</td>
<td>Fe</td>
<td>68.90</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.32</td>
<td>Mn</td>
<td>8.97</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.91</td>
<td>Zn</td>
<td>62.60</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.75</td>
<td>Cu</td>
<td>6.24</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.16</td>
<td>Al</td>
<td>30.70</td>
</tr>
</tbody>
</table>

1 Analyzed at Central Analytical Lab, Poultry Science Center, University of Arkansas
2 Nutrient values expressed as grams per 100 gram sample on weight/weight percentage
**Table 2.2** Composition and calculated analysis of experimental diet (1-12 week) with various levels of corn distiller’s dried grains with soluble (DDGS) (% as is)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Corn</td>
<td>59.20</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>25.00</td>
</tr>
<tr>
<td>DDGS1</td>
<td>0.00</td>
</tr>
<tr>
<td>Blended fat2</td>
<td>2.70</td>
</tr>
<tr>
<td>Limestone3</td>
<td>11.02</td>
</tr>
<tr>
<td>Dical phosphate</td>
<td>1.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.38</td>
</tr>
<tr>
<td>V and M Mix4</td>
<td>0.50</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0.01</td>
</tr>
<tr>
<td>Bio-Lys5</td>
<td>0.00</td>
</tr>
<tr>
<td>DL-Met</td>
<td>0.14</td>
</tr>
<tr>
<td>Calculated (%)</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>16.70</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2,825</td>
</tr>
<tr>
<td>Ca</td>
<td>4.50</td>
</tr>
<tr>
<td>P</td>
<td>0.30</td>
</tr>
<tr>
<td>Na</td>
<td>0.17</td>
</tr>
<tr>
<td>Arg</td>
<td>1.10</td>
</tr>
<tr>
<td>His</td>
<td>0.46</td>
</tr>
<tr>
<td>Ile</td>
<td>0.70</td>
</tr>
<tr>
<td>Leu</td>
<td>1.53</td>
</tr>
<tr>
<td>Lys</td>
<td>0.89</td>
</tr>
<tr>
<td>SAA</td>
<td>0.70</td>
</tr>
<tr>
<td>Phe+Tyr</td>
<td>1.35</td>
</tr>
<tr>
<td>Thr</td>
<td>0.63</td>
</tr>
<tr>
<td>Val</td>
<td>0.71</td>
</tr>
<tr>
<td>Trp</td>
<td>0.22</td>
</tr>
</tbody>
</table>

1. DDGS was donated by LincolnWay Energy, Nevada, IA
2. Blended fat contained vegetable and animal fat, donated by Feed Energy Company, Des Moines, IA
3. Limestone was donated by Iowa Limestone Company, Des Moines, IA
4. V and M Mix = vitamin and mineral premix: contained the followings per kilogram diet: Selenium, 0.2 ppm; vitamin A, 6608 IU; vitamin D3, 2203 ICU; vitamin E, 14 IU; menadione, 0.88 mg; vitamin B12, 9.35 µg; biotin, 33 µg; choline, 358 mg; folic acid, 1.1 mg; niacin, 33 mg; pantothenic acid, 8.8 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg.
5. Bio-Lys (78.8% Lysine)
Table 2.3 Composition and calculated analysis of experimental diet (13-24 week) with various levels of corn distiller’s dried grains with soluble (DDGS) (% as is)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% DDGS</th>
<th>0</th>
<th>17</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>59.13</td>
<td>50.87</td>
<td>36.38</td>
<td>26.62</td>
</tr>
<tr>
<td>Soybean meal</td>
<td></td>
<td>25.00</td>
<td>15.00</td>
<td>10.00</td>
<td>3.50</td>
</tr>
<tr>
<td>DDGS(^1)</td>
<td></td>
<td>0.00</td>
<td>17.00</td>
<td>35.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Blended fat(^2)</td>
<td></td>
<td>2.72</td>
<td>3.88</td>
<td>5.75</td>
<td>7.07</td>
</tr>
<tr>
<td>Limestone(^3)</td>
<td></td>
<td>11.02</td>
<td>11.22</td>
<td>11.46</td>
<td>11.65</td>
</tr>
<tr>
<td>Dical phosphate</td>
<td></td>
<td>1.05</td>
<td>0.80</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td>0.38</td>
<td>0.29</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>V and M Mix(^4)</td>
<td></td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>L-Thr</td>
<td></td>
<td>0.01</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Bio-Lys(^5)</td>
<td></td>
<td>0.00</td>
<td>0.24</td>
<td>0.26</td>
<td>0.41</td>
</tr>
<tr>
<td>DL-Met</td>
<td></td>
<td>0.19</td>
<td>0.15</td>
<td>0.04</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Calculated (%)

<table>
<thead>
<tr>
<th></th>
<th>CP</th>
<th>ME (kcal/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16.70</td>
<td>2,825</td>
</tr>
<tr>
<td></td>
<td>16.60</td>
<td>2,825</td>
</tr>
<tr>
<td></td>
<td>18.30</td>
<td>2,825</td>
</tr>
<tr>
<td></td>
<td>19.00</td>
<td>2,825</td>
</tr>
</tbody>
</table>

Fat 5.16 | 7.26 | 10.22 | 12.46 |
Fiber 2.28 | 3.26 | 4.38 | 5.28 |
Ca 4.50 | 4.50 | 4.50 | 4.50 |
P 0.30 | 0.30 | 0.30 | 0.30 |
Na 0.17 | 0.18 | 0.17 | 0.17 |
Arg 1.10 | 0.95 | 0.97 | 0.91 |
His 0.46 | 0.45 | 0.51 | 0.53 |
Ile 0.70 | 0.67 | 0.73 | 0.75 |
Leu 1.53 | 1.68 | 2.00 | 2.20 |
Lys 0.89 | 0.86 | 0.86 | 0.87 |
SAA 0.75 | 0.76 | 0.75 | 0.78 |
Phe+Tyr 1.34 | 1.38 | 1.59 | 1.70 |
Thr 0.63 | 0.65 | 0.68 | 0.71 |
Val 0.71 | 0.75 | 0.88 | 0.95 |
Trp 0.22 | 0.18 | 0.18 | 0.16 |

\(^1\) DDGS was donated by LincolnWay Energy, Nevada, IA
\(^2\) Blended fat contained vegetable and animal fat, donated by Feed Energy Company, Des Moines, IA
\(^3\) Limestone was donated by Iowa Limestone Company, Des Moines, IA
\(^4\) V and M Mix = vitamin and mineral premix: contained the followings per kilogram diet: Selenium, 0.2 ppm; vitamin A, 6608 IU; vitamin D3, 2203 ICU; vitamin E, 14 IU; menadione, 0.88 mg; vitamin B12, 9.35 µg; biotin, 33 µg; choline, 358 mg; folic acid, 1.1 mg; niacin, 33 mg; pantothenic acid, 8.8 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg.
\(^5\) Bio-Lys (78.8% Lysine)
Table 2.4 Virginiamycin residues in the diets of laying hens with various DDGS levels (ppm) by plate assay and bio-autography estimation

<table>
<thead>
<tr>
<th>DDGS diets (%)</th>
<th>Plate assay</th>
<th>Bio-autography estimation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-12 week</td>
<td>13-24 week</td>
</tr>
<tr>
<td>0</td>
<td>0.064</td>
<td>0.075</td>
</tr>
<tr>
<td>17</td>
<td>0.032</td>
<td>0.079</td>
</tr>
<tr>
<td>35</td>
<td>0.065</td>
<td>0.085</td>
</tr>
<tr>
<td>50</td>
<td>0.090</td>
<td>0.064</td>
</tr>
<tr>
<td>DDGS sample</td>
<td>0.031</td>
<td>0.027</td>
</tr>
</tbody>
</table>

\(^1\)Values are means of 2 replications, n=2.
Table 2.5 Effect of diets with various levels of corn distiller’s dried grains with solubles (DDGS) on laying rate, egg mass, egg weight, feed intake, feed efficiency, and body weight change during the 24-week-long period⁴

<table>
<thead>
<tr>
<th>Item</th>
<th>DDGS diets (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Egg production, %</td>
<td>87⁷</td>
<td>83⁶</td>
</tr>
<tr>
<td>Egg mass, g/hen/day</td>
<td>56.0¹</td>
<td>51.8²</td>
</tr>
<tr>
<td>Egg weight, g/egg</td>
<td>64.7¹</td>
<td>63.3³</td>
</tr>
<tr>
<td>Feed intake, g/hen/day</td>
<td>104.4²</td>
<td>104.2²</td>
</tr>
<tr>
<td>Feed efficiency, g egg/kg feed</td>
<td>531.6¹</td>
<td>487.6³</td>
</tr>
<tr>
<td>Start</td>
<td>1.56</td>
<td>1.50</td>
</tr>
<tr>
<td>End</td>
<td>1.57</td>
<td>1.50</td>
</tr>
<tr>
<td>Change</td>
<td>0.02</td>
<td>0.00</td>
</tr>
</tbody>
</table>

a,b,c Means with no common superscript in the same row differ significantly (P < 0.05)

⁴Values are means of 10 replications.

⁵Means with no common superscript in the same row differ significantly (P < 0.05)
Table 2.6 Effects of corn distiller’s dried grains with solubles on egg internal quality (yolk color, Haugh unit, and egg composition) during 24-week-long period

<table>
<thead>
<tr>
<th>Treatments</th>
<th>yolk color¹</th>
<th>Haugh unit²</th>
<th>Components³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 wk</td>
<td>1 wk</td>
</tr>
<tr>
<td>0% DDGS</td>
<td>5.5abc</td>
<td>80.5b</td>
<td>76.4b</td>
</tr>
<tr>
<td>17% DDGS</td>
<td>7.0bc</td>
<td>81.8b</td>
<td>78.0b</td>
</tr>
<tr>
<td>35% DDGS</td>
<td>7.9bc</td>
<td>82.3b</td>
<td>78.3b</td>
</tr>
<tr>
<td>50% DDGS</td>
<td>8.7a</td>
<td>85.3a</td>
<td>82.3a</td>
</tr>
<tr>
<td>SEM</td>
<td>0.12</td>
<td>0.77</td>
<td>0.79</td>
</tr>
</tbody>
</table>

a,b,c,d means in the same column with different letters differ significantly (P < 0.05).

¹Yolk color score ranges from 1 to 10, n = 10

²Haugh unit equation: 100*log (height-0.01*5.6745*(30*weight^0.37-100) +1.9), n=10

³Values are means of 10 replications, each containing 6 hens
Table 2.7 Effects of corn distiller’s dried grains with solubles on shell breaking strength (g) during 24-week-long period

<table>
<thead>
<tr>
<th>Week</th>
<th>% DDGS</th>
<th>0</th>
<th>17</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>3958</td>
<td>3920</td>
<td>4295</td>
<td>4270</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>4057</td>
<td>4100</td>
<td>4171</td>
<td>4424</td>
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<tr>
<td>5</td>
<td></td>
<td>4221</td>
<td>4090</td>
<td>3759</td>
<td>3902</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>4137b</td>
<td>3979b</td>
<td>4166b</td>
<td>4824a</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>4309</td>
<td>3932</td>
<td>3782</td>
<td>4283</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>3974</td>
<td>3977</td>
<td>3883</td>
<td>4339</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>3765</td>
<td>4016</td>
<td>3941</td>
<td>4123</td>
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\(^{a,b,c}\) means in the same row with different letters differ significantly (P < 0.05).

\(^1\)Values represent the mean of shell breaking force per treatment every 2 week, n = 10.
Figure 2.1 Effect of diets with various levels of corn distiller’s dried grains with solubles\textsuperscript{1} on egg production rate\textsuperscript{2} during the 24-week-long period\textsuperscript{3}. \textsuperscript{*4}Denotes that 50% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment (p<0.05). \textsuperscript{2,4}Denotes that 17 and 50% DDGS dietary treatments are significantly lower than the 0% DDGS dietary treatment (p<0.05). \textsuperscript{1}The ratio of corn and soybean meal base in the diet reduced as DDGS level increased. \textsuperscript{2}Values are means of 10 replications, each containing 6 hens. \textsuperscript{3} Each data point means averaged egg production rate on a two-week basis.
Figure 2.2 Effect of diets with various levels of corn distiller’s dried grains with solubles\(^1\) on feed intake\(^2\) during the 24-week-long period\(^3\). \(^*\)^2 Denotes that 17% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment \((p<0.05)\). \(^*\)^4 Denotes that 50% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment \((p<0.05)\).

\(^1\)The ratio of corn and soybean meal base in the diet reduced as DDGS level increased. 
\(^2\)Values are means of 10 replications, each containing 6 hens. 
\(^3\)Each data point means averaged feed intake on a two-week basis.
Figure 2.3 Effect of diets with various levels of corn distiller’s dried grains with solubles\(^1\) on egg weight\(^2\) during the 24-week-long period\(^3\). *\(^2\)Denotes that 17% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment (p<0.05). *\(^4\)Denotes that 50% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment (p<0.05). *\(^2,4\)Denotes that 17 and 50% DDGS dietary treatments are significantly lower than the 0% DDGS dietary treatment (p<0.05). \(^1\)The ratio of corn and soybean meal base in the diet reduced as DDGS level increased. \(^2\)Values are means of 10 replications, each containing 6 hens. \(^3\)Each data point means averaged egg weight on a two-week basis.
**Figure 2.4** Effect of diets with various levels of corn distiller’s dried grains with solubles on egg mass during the 24-week-long period. *2Denotes that 17% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment (p<0.05). *4Denotes that 50% DDGS dietary treatment is significantly lower than the 0% DDGS dietary treatment (p<0.05). *2,4Denotes that 17 and 50% DDGS dietary treatments are significantly lower than the 0% DDGS dietary treatment (p<0.05). ¹The ratio of corn and soybean meal in the diet reduced as DDGS level increased. ²Values are means of 10 replications, each containing 6 hens. ³Each data point means averaged egg mass on a two-week basis.
REFERENCES


CHAPTER 3. EFFECTS OF INCREASING CONCENTRATIONS OF CORN DISTILLER’S DRIED GRAINS WITH SOLUBLES ON CHEMICAL COMPOSITION AND NUTRIENTS CONTENT OF EGG

ABSTRACT

The objective of this study was to determine the effects of feeding high concentrations of corn distiller’s dried grains with soluble (DDGS) on chemical composition and important nutrients content of egg yolk. Four diets were formulated to contain 0, 17, 35 or 50% corn DDGS. A total of 240 54-week-old single-comb White Leghorn laying hens were randomly allotted to 2 birds per cage with three consecutive cages representing an experimental unit (EU). Each EU was randomly assigned to one of four dietary treatments according to a completely randomized design. Hens were fed for a 24-week experimental period after transition feeding to gradually increase corn DDGS inclusion over a four-week period. Two sets of experimental diets were formulated to meet or exceed the National Research Council nutrient recommendations for laying hens. Each diet formula was fed for 12 weeks. Chemical composition and nutritional components in egg yolk were measured every two weeks. The results showed that egg yolk from hens fed higher DDGS-containing diet tended to have higher fat content and lower protein content. Total polyunsaturated fatty acids were significantly increased by DDGS diet. The contents of choline and cholesterol were initially higher in 50% DDGS treatment group, but were not different in the later period, especially during last 4 weeks. Lutein content increased linearly as DDGS level increased. The results indicated that feeding high level of DDGS can increase the content of
lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.

**Key words**: corn distiller’s dried grains with soluble, egg yolk, choline, lutein, cholesterol
INTRODUCTION

Distiller’s dried grains with solubles (DDGS) have been available for feed industry for many years, and have been considered a nutritional and economical feed ingredient. As a by-product of ethanol industry, DDGS are the leftover product after grains fermentation process and ethanol distillation. After starch is utilized during the fermentation process of ethanol production, other non-fermentable nutrients (protein, fiber, fat, vitamins and minerals) are concentrated around three-folds in the final DDGS product (NRC, 1994; Weigel et al., 1997; Spiehs et al., 2002). As the production of bioethanol has increased rapidly in recent years, increased quantity of DDGS becomes available for feed industry (F.O. Licht, 2008, 2009, 2010).

DDGS are often used at low concentrations (10 or 15%) as a feed ingredient for laying hens without affecting laying performance and egg quality (Lumpkins et al., 2005; Roberson et al., 2005; Swiatkiwicz and Koreleski, 2006). As large amounts of DDGS become available in feed market, the possibility of using higher DDGS inclusion rate in poultry feed has become an interest for many researchers and poultry producers. Previous study (Pineda et al., 2008) showed that DDGS could be incorporated at 69% level in laying hen diet for short-term without negative effects on egg production, metabolic conditions, and egg quality.

DDGS contain high levels of protein, fiber and fat, and also contain considerable amounts of other important nutrients, such as lutein, choline, and long chain unsaturated fatty acids. The differences in component and nutrient concentration of DDGS diet may influence the chemical composition and nutrient content of eggs, especially when DDGS are used at high levels in the diet. Yolk is the most nutritive part of egg, and contains many functional
nutrients such as choline and lutein. Phosphatidylcholine (PC) constitutes around 80% of the total phospholipids in the egg yolk, and has many physicochemical functions. Choline is very important for brain development, liver function and cognitive function for cellular membranes (Shaw et al., 2004). Deficiency in choline may result in higher risk of cancer (Xu et al., 2008 & 2009) and neural tube defects (Shaw et al., 2004).

Egg yolk carotenes are classified as xanthophylls and carotenes (Karrer and Schopp, 1934). Xanthophylls include lutein, zeaxanthin and cryptoxanthin, and present at level of 0.1, 0.2 and 0.03% of egg yolk, respectively (Romanoff and Romanoff, 1949). Lutein has been used in poultry diet for a long time. This pigment can provide desirable yellow color in egg yolk and chicken skin, which consumers prefer (Perez-Vendrell et al., 2001; Leeson and Caston, 2004). Moreover, lutein plays an important role in preventing age-related macular degeneration (Moeller et al., 2000). Avian and mammalian cannot synthesize xanthophyll pigments, and thus fully depend on dietary sources for color absorption (Goodwin, 1984). DDGS contain high levels of xanthophyll, and thus diets containing DDGS can increase lutein content in egg yolk.

Cholesterol content of egg yolk can range from 11-15 mg/g yolk, which is around 5% of total yolk lipids (Vargas et al., 1986; Kodchodkar et al., 1976). Free cholesterol is about 84% of the total cholesterol and the remaining 16% is cholesterol esters (Karrer and Schopp, 1934). The cholesterol of egg yolk can come from the feed ingredient, such as animal fat (Basmacioglu and Ergul, 2005), with the major part synthesized when egg yolk is formed inside the body (Griffin, 1992). High dietary fiber content in DDGS diets may have positive effect on controlling cholesterol levels in eggs, since many researchers have found a positive
relationship between high fiber diets and low serum cholesterol (Lairon et al., 2005; Bruckert and Rosenbaum, 2011).

Egg is a very important component of human food, and it is important to evaluate its chemical composition and the content of important nutrients in egg yolk from high level DDGS diet. Interest of increasing DDGS content in laying hen diet has been rekindled; however, little work has been done to determine the effect of high inclusion rate of DDGS on egg yolk composition and nutrients content of egg yolk. The objective of this study is to investigate the changes of chemical composition and nutrients content of egg yolk by diets with high DDGS inclusion rates.

**MATERIALS AND METHODS**

**DDGS Diets**

The corn DDGS used in this study was obtained from a local ethanol plant (Lincolnway Energy, Nevada, IA). Corn DDGS diets for laying hens were formulated based on National Research Council nutrient recommendations for laying hens (NRC, 1994) and the current DDGS nutrient profiles on the website of University of Minnesota (http://www.ddgs.umn.edu/profiles-current.htm). Diets were formulated with four levels of corn DDGS (0, 17, 35, and 50%) by replacing increasing amounts of corn, soybean meal, dicalcium phosphate, and DL-methionine, with DDGS, animal vegetable blended fat, limestone and lysine. The highest inclusion level of corn DDGS was chosen on the basis of the results of Pineda et al. (2008). The diets were formulated to be iso-caloric based on energy values for feed ingredients published by the National Research Council. The diets
were formulated on a total amino acid basis and total crude protein of diets increased with increasing amount of DDGS.

After the first 12-week experiment period, diet formulas was modified by adding additional amounts of lysine and methionine in an attempt to meet the production requirements of laying hens fed the 50% DDGS diet. The composition and nutrients content of the two DDGS diet formulas are shown in Tables 3.2 and 3.3. The two formulas had the same concentrations of ME, Ca, P, most amino acids except lysine and methionine, and very similar protein levels in the treatment with the same DDGS level.

DDGS samples were prepared for proximate and amino acid content. The nutrient composition of corn DDGS used in this study was analyzed at the Poultry Science Center of University of Arkansas (Table 3.1).

**Birds and Experimental Design**

The experiment was conducted in the poultry research and teaching farm at Iowa State University. The study was approved by the Institutional Animal Care and Use Committee at Iowa State University (Approval # 4-09-6732-G). A total of 240 54-week-old Single-comb White Leghorn laying hens (Hy-Line W-36) were placed in 120 cages (2 birds/cage) with 96 square inches per bird. Three consecutive cages were considered an experiment unit, and were assigned to one of four diets containing 0, 17, 35, or 50% DDGS using completely randomized design. Every diet was fed to 10 experimental units of hens. Hens had free access to the diet, and were provided with 16 hours of light and 8 hours of darkness per day. The temperature was maintained at 26 °C throughout the study.

Prior to the experiment, the hens (except for control birds) were fed a corn-soybean meal-based diet containing 10% corn DDGS for 2 weeks and the hens were acclimated to the
inclusion rates of corn DDGS by gradually introducing the treatment diets over the next 4-week period.

**Chemicals and Reagents**

For choline analysis, choline standard (choline bitartrate) and enzymes, including phospholipase D, choline oxidase and peroxidase, were purchased from Sigma Chemicals Co. (St. Louis, MO). Phospholipase D (type VI, 4750 units/mg) was produced from *Streptomyces chromofusus* and one unit was defined to liberate 1.0 µmol choline from L-α-phosphatidylcholine (egg yolk) per hour at pH 5.0 at 30 °C. Choline oxidase (12 units/mg) was produced from *Alcaligenes* species and the definition of one unit was to form 1.0 µmol choline from betaine aldehyde per minute at pH 8.0 at 37 °C. Peroxidase (type I, 116 purpurogallin units/mg) was produced from horseradish and the definition of one unit was to form 1.0 mg purpurogallin from pyrogallol in 20 s at pH 6.0 at 20 °C. Chromogenic reagent was prepared by dissolving 100 units of phospholipase D, 120 units choline oxidase, 280 units peroxidase, 15 mg 4-aminoantipyrine, and 50 mg phenol added in 100-mL of 0.05 M Trizma buffer (pH 8.0). The chemicals used for lutein extraction were reagent grades. Lutein (Xanthophyll) standard was purchased from Sigma. Folch’s solution (chloroform: methanol = 2:1) for fat extraction was prepared using reagent grade chloroform and methanol, and BF₃-methanol (boron-trifluoride methanol, 14%, Sigma Chemical Co.) was used as a methylating reagent for fatty acids. For cholesterol analysis, ascorbic acid and cholestane were purchased from Sigma. Pyridine was from Fisher Scientific. Sylon BFT (BSTFA+TMCS, 99:1) was purchased from Sigma-Aldrich-Supelco chemical.
Sample Preparation

Every 2 weeks, a total of 80 eggs (20 eggs for each treatment) were randomly selected to prepare yolk samples. Each treatment had four replications (5 eggs for each replication). The eggs from each treatment were broken to separate yolk and albumen, and then five yolks from the same treatment were mixed in a beaker, transferred to scintillation vials and used as a replication. All the eggs and samples were stored at refrigerated temperature until use.

Analyses of Yolk Composition (lipid, moisture, and protein contents)

Lipid Extraction. Yolk lipids were extracted using Folch’s solution (Folch et al., 1957). Three grams yolk and 30 ml Folch’s solution (CHCl₃: CH₂OH = 2:1) were added in a 50-mL test tube and homogenized at high speed for 30 s using a Polytron (Type PT 10/35; Brinkman Instruments Inc., Westbury, N.Y., U.S.A.). After standing overnight, the sample was filtered through a Whatman #1 filter paper into a 100-mL graduated cylinder (with glass stopper). Test tube was washed twice with 8 ml (4+4 each side) and 4 ml (2+2 each side) Folch’s solution, and the filter paper was washed with 10 ml (5+5 each side) of Folch’s solution.

Ten ml (equal to one quarter of filtrate volume) of 0.88% NaCl solution was added to the filtrate and mixed well. The inside of the cylinder was washed with 2 mL of Folch solution. After overnight standing for phase separation, the lower layer (lipid and chloroform) volume was recorded, and the top layer (methanol and water) of the solution was carefully siphoned off to avoid contaminating the CHCl₃ layer. Ten ml CHCl₃ layer were transferred to a pre-weighted weighing dish (42 ml low form aluminum, Fisher Scientific) and weighed after chloroform was evaporated in a fume hood. Total crude lipids were
calculated. The remaining CHCl$_3$ layer was transferred into a glass scintillation vial and used for other analysis.

**Moisture and Protein Measurements.** Moisture was determined using the AOAC method (AOAC, 1980). One gram of yolk sample was transferred to a pre-weighted weighing dish and kept in an oven at 105-110°C for 12 hours. After cooling, the weight loss was determined and used to calculate moisture content. Yolk protein content was measured using the micro-Kjeldahl method 990.03 (AOAC, 2006).

**Fatty Acid Composition.** One milliliter methylating reagent (boron-trifluoride methanol, Sigma Chemical Co.) and 0.4 mL lipid extract were added into a 20-mL test tube, capped tightly and incubated in a 90 °C water-bath for 1 h. After cooling to room temperature, 3 ml hexane and 5 ml water were added, mixed thoroughly, and left at room temperature overnight for phase separation. The top hexane layer containing methylated fatty acids (1.5 mL) was collected in a GC vial and analyzed using a GC (HP 6890, Hewlett Packard Co.). HP-wax column (30 m x 0.25 mm i.d., 0.25 µm nominal) was used to separate fatty acids methylates. A ramped oven temperature conditions (from 180 °C, increased to 200 °C at 5 °C/min, held at 200 °C for 6 min, to 220 °C at 10 °C/min, to 230 °C at 5 °C/min, and then held at 230 °C for 7.0 min) were used. Temperatures of the inlet and detector were 230 °C and 280 °C, respectively. Helium was the carrier gas at linear flow of 0.9 mL/min. Detector (FID) air, H$_2$, and make-up gas (He) flows were 350, 35, and 42.1 ml/min, respectively. Fatty acids were identified by comparing the retention times of known standards. Relative quantities were expressed as weight percent of total fatty acids (Nam et al., 2001).
Cholesterol. Five ml chloroform layer from lipid extraction were transferred to a 50 ml test tube and evaporated under Nitrogen gas. Ten ml saponification reagent (ethanol: 33% KOH=94:6), 0.5 ml 20% ascorbic acid, and 100 µl 5 α-cholestane solution (10 mg/µl in chloroform) were added to the sample, and the sample was incubated in a waterbath at 50°C for 1 hour. After cooling to room temperature, 8 ml water and 3 ml hexane were added to the sample, mixed thoroughly by vigorous shaking, and kept overnight for phase separation. One ml of the top layer (hexane) was transferred to a scintillation vial, dried under nitrogen flow, and then added with 200µl pyridine and 100µl Sylon BFT (99%BSTFA+1% TMCS). After keeping overnight at room temperature, the sample was transferred to a GC vial for GC analysis. Cholesterol was analyzed using a HP 6890 GC equipped with an autosample injector and a flame ionization detector (FID; Hewlett Packard Co., Wilmington, Del., U.S.A.). A 0.25 mm i.d. 30 m HP-5MS column with 0.25µm film thickness was used. A splitless inlet (5µl) was used to inject samples into the capillary column using an autosampler (model 7683; Hewlett Packard Co.). A ramped oven temperature was used (from 180 °C increased to 260 °C at 8 °C/min, increased to 280 °C at 2 °C/min, and held for 13 min). Temperature of the inlet was 290 °C, and detector temperature was 320 °C. Helium was the carrier gas at a constant flow of 1.2 mL/min. Detector (FID) air, H2, and make-up gas (He) flow rates were 400 mL/min, 35 mL/ min, and 40 mL/min, respectively. The area of cholesterol peak (pA*s) was integrated using the ChemStation software (Hewlett Packard Co.) and the amount of cholesterol was calculated using an internal standard (5-cholestane).

Lutein. 0.5 g of yolk sample was weighed in a 50-mL conical centrifuge tube and homogenized with 10 mL 30% methanolic potassium hydroxide (w/v) and 50-µL 10% methanolic butylated hydroxytoluene (BHT) using a Brinkman Polytron (Type PT 10/35,
Brinkman Instrument Inc., Westbury, N.Y., U.S.A.) for 15 s at high speed. The homogenate was heated for 1 h at 50 °C to saponify the lipids and hydrolyze the carotenol esters. After heating, the sample was kept in the dark. The carotenoids were extracted with 10 ml ether: hexane (1:1, vol: vol) by homogenizing with a Brinkman Polytron. After that 10 mL of water was added to the sample and homogenized again. The mixture was centrifuged at 4,000 x g for 30 min at 4 °C for phase separation. Three ml of the top layer (hexane-ether layer) were collected in scintillation vial and the solvents were evaporated to dryness using N₂ gas. The dried carotenoid residue was dissolved in 1 mL methanol, filtered through a 0.45 µm polytetrafluoroethylene (PTFE) membrane filter, and then analyzed using an HPLC. An Agilent 1100 Series HPLC equipped with binary pump, microvacuum degasser, microautosampler, column compartment with temperature controller, and diode-array detector (DAD) was used. A reversed-phase HPLC column (Zobax Eclipse XDB-C18, 2.1 mm x 100 mm, 3.3–µm particle size) was used to separate lutein. The column flow rate and injection volume were 0.5 mL/min and 10 µL, respectively. Two mobile phases (solvent A: prepared with 75% acetonitrile, 15% methanol, 10% water, 0.04% ammonium acetate and solvent B: 85% methanol with 2% 1 M ammonium acetate pH 4.6, 15% methyl-tert-buty leth e) were used with a step-gradient from 100% solvent A to 100% solvent B at 15 min. Column temperature was maintained at 15 °C (Handelman et al., 1999; Bonora et al., 2000).

**Choline.** Choline was determined using the AOAC official method 999.14 (Rader et al., 2004). Sample (1 g) in a 50-ml tube was homogenized with 15 ml of 1 N sodium hydroxide (NaOH) solution using a Brinkman Polytron (Type PT 10/35, Brinkman Instrument Inc., Westbury, N.Y., U.S.A.) for 15 s at high speed. The homogenate was heated
for 3 h at 70 °C, cooled to room temperature, pH adjusted to pH 3.5-4.0 with 6 N HCl, net volume adjusted to 25 ml, and then homogenized using a Polytron for 5 sec at high speed. The homogenate was filtered through a Whatman #41 filter paper and the filtrate was collected. The collected filtrate was re-filtered through a glass microfiber syringe filter (25 mm, Whatman).

For each sample, two 10-mL test tubes were labeled as sample test solution (tube 1) and sample blank (tube 2). An aliquot (100 µL) of the final filtrate was transferred to each tube. Water (3 mL) was added to each sample blank (tube 2) and 3 mL chromogenic reagent was added to each sample test solution (tube 1), the standards, and the reagent blank. All test tubes were incubated in covered water bath at 37 °C for 15 min to develop color. The absorbance of samples was measured at 505 nm against a reagent blank containing 100 µL water and 3 mL chromogenic reagent. The amount of choline was expressed as milligrams of choline hydroxide per 100 gram of sample.

**Statistical Analyses**

This experiment used a completely randomized design. The data were analyzed by one-way ANOVA with the GLM procedure of SAS institute by one variation (treatment or week) (SAS 9.2, 2008), and Duncan’s multiple range test was used to separate means. In addition, data were analyzed as repeated measure, employing split-plot mixed procedure of SAS sliced by week, with main effects (treatment and week) and random effect (treatment x week interaction). Statistical significance was assumed at p < 0.05 to determine the differences among four treatments.
RESULTS AND DISCUSSION

During the 24-week experiment period, no difference in moisture content of egg yolk among four DDGS treatments was found, and the moisture content of egg yolk ranged from 48.54 to 48.74% (Table 3.4). However, the diet with highest DDGS inclusion rate (50%) affected the fat and protein content of egg yolk. Fat content of egg yolk in 50% DDGS group was significantly higher than that of the other three groups, and protein content of egg yolk in 50% DDGS group was significantly lower than that of the other three groups (Table 3.4). Fat and protein levels of DDGS diets increased as DDGS inclusion rate increased, and fat content of DDGS diets differed greatly ranging from 5.16 to 12.46%. The diet with higher fat content resulted in greater levels of yolk lipids. The difference in protein and fat content of egg yolk may come from the different protein and fat level in the DDGS diets. But this effect was only observed in the diet with 50% DDGS concentration.

The fatty acid composition of four DDGS diets was different, and resulted in the difference in fatty acid composition of egg yolk (Table 3.5). All fatty acids but margaroleic acid and DHA in egg yolk were influenced by DDGS diets. Palmitoleic acid and oleic acid decreased linearly as DDGS level increased. On contrary, margaric acid and linoleic acid increased linearly as DDGS levels increased as reported by Cheon et al. (2008). Total saturated and unsaturated fatty acids among four DDGS treatments were not significantly different; however total polyunsaturated fatty acids increased linearly as dietary DDGS level increased. This result agrees with the study of Rew et al. (2009) who reported that 20% DDGS diet resulted in increased polyunsaturated fatty acids in egg yolk. Schilling et al. (2010) reported a linear increase in linoleic and polyunsaturated fatty acids in breast and thigh meat as dietary DDGS increased.
Dietary fatty acid composition was considered as the most important factor that influence the fatty acid composition of broiler meat and hen eggs (Cortinas et al., 2004). The current study shows that different DDGS inclusion rates can influence the fatty acid composition of egg yolk due to the differences in fatty acid composition in DDGS diets. Among those polyunsaturated fatty acids in egg yolk, linoleic and arachidonic acids belong to omega-6 fatty acids, and linolenic acid, DPA and DHA belong to omega-3 fatty acids. Both omega-3 fatty acids (linolenic acid and EPA) and omega-6 fatty acids (linoleic acid) were influenced by DDGS diets.

All diets (0, 17, 35, and 50% DDGS) contained similar levels of choline (77.7, 77.4, 76.9, and 77.3 mg/100 g, respectively). As shown in Figure 3.1a, choline content in yolk (mg choline/100g egg yolk) of 50% DDGS treatment was higher than that of the other three groups (0, 17, and 35%) most of the times during the study. The differences in choline content in egg yolk at 3, 7, 11, 13, 15, and 17 wk of study were significant (p < 0.05), and the differences were mainly due to higher choline content in 50% DDGS group. However, choline content among the four DDGS treatments was not different during the last 6-week period. The higher choline content in eggs fed with 50% DDGS diet could be related to smaller egg size in that group, so the choline content was more condensed when expressed as mg choline/ 100 g yolk. When the egg size was not different among four treatments during the last 6 weeks, the choline content among four treatments was not significantly different. As shown in Figure 3.1b, when choline content is expressed as mg choline per egg, there were only differences at week 7 and week 15 (p<0.05) among four DDGS treatments. Choline content in each egg from four treatments was not influenced by DDGS addition. When choline content is expressed as mg/hen/day (Figure 3.1c), the choline pattern is very
similar to that of egg production, feed intake, and egg weight shown in Chapter 2. The differences were mainly found in 50% DDGS treatment group before the diet reformulation. Dietary reference intake for choline recommends 425 mg/day for adult women and 550 mg/day for adult men and children (Institute of medicine, 1997). In this study, choline content in eggs from DDGS treatments was very similar, and DDGS diets did not affect the choline content in egg yolk especially when the egg size was the same among the four DDGS treatments.

Cholesterol level in egg yolk from hens fed with 50% DDGS diet was continuously higher than that of the other three DDGS treatment groups during 5 to 13 week feeding period, but the difference became small at the later period. The cholesterol content of eggs from hens fed with 50% DDGS diet showed an inconsistent response, which could be related to the size of eggs that were selected. Larger eggs tend to contain lower cholesterol concentration than smaller eggs. The inconsistent response observed in 50% DDGS treatment may also be related to its lower egg production, since lower egg production allows cholesterol to be condensed more in the yolk. When the egg production and egg size of four DDGS treatments became similar during the last 6 weeks, the difference in yolk cholesterol from four DDGS groups was not significant. A slight increase of cholesterol content in 0, 17, and 35% DDGS groups was observed over time, and that could be due to the decreased egg production as hen’s age increased.

Cholesterol content in yolk is relatively resistant to change, and only slightly differ by cholesterol levels in the feed (Fenton and Sim, 1991). Increased cholesterol content in yolk was observed when high cholesterol diets were fed (Harris and Wilcox, 1963; Weiss, Johnson, and Naber, 1967), especially when total fat in a diet was high. In current study, fat
level in the diets increased as DDGS level increased (Table 2.3). Since the fat in the DDGS diet was from vegetable and animal sources, the cholesterol content from animal fat in the diet would be higher in the DDGS diet with higher fat levels. Cholesterol intake might be higher in 50% DDGS group and may have resulted in higher cholesterol content in egg yolk. In addition, fatty acid composition in the diet may have some effect on cholesterol level. Several studies found that feeding polyunsaturated fatty acids would increase yolk cholesterol level (Summers et al., 1966; Weiss et al., 1964). The content of polyunsaturated fatty acids in egg yolk was the highest with 50% DDGS treatments in this study, which may have contributed to the increased cholesterol level in egg yolk. Furthermore, dietary fiber has been found to have cholesterol-lowering effect (Kirby et al., 1981), and soluble fiber is the main component to have this effect (Glassman et al., 1990; Williams et al., 1991). Bruckert and Rosenbaum (2011) found that increased fiber intake can significantly lower cholesterol concentration of serum. Higher dietary fiber consumption is often associated with lower total serum cholesterol (Lairon et al., 2005). However, high fiber content in DDGS diets did not show any cholesterol-lowering effect, which would be due to the low amount of soluble fiber present in the DDGS diets. Since multiple factors would influence the cholesterol content in egg yolk, the effect of DDGS diet on cholesterol content would be compromised, and did not show significant difference.

Egg yolk is rich in color pigments, such as carotenes and xanthophylls, which are responsible for yolk color. They cannot be synthesized by hen, and must be obtained from feed. Xanthophylls include lutein, zeaxanthin and cryptoxanthin, and present at level of 0.1, 0.2 and 0.03% of egg yolk, respectively (Romanoff and Romanoff, 1949). The lutein content of DDGS diets increased as DDGS level increased, and was 4.86, 6.80, 8.15, and 9.10 µg/g
feed, respectively. The lutein content of egg yolk from four DDGS treatments were significantly different (p<0.05), and increased linearly as DDGS level increased. This suggests that dietary lutein can be digested and absorbed very well by layers. DDGS, as a good source of xanthophylls, was reported to contain 2.37 to 34.00 mg/kg in several DDGS products (NRC, 1981; Sauvant and Tran, 2004). Moeller et al. (2000) found that lutein from food sources can reduce the risk of age-related macular degeneration (AMD) up to 40% and reduce the risk of cataract up to 20%. Seddon et al. (1994) reported that lutein and zeaxanthin are prominent macular pigments that can reduce the risk of AMD. Even though egg is not the richest source of xanthophylls, the xanthophylls in egg are highly digestible and absorbable (Moeller et al., 2000). Thus, DDGS could be used as a good lutein source for eggs, and lutein-enriched eggs could have great potential to lower the risk of eye diseases.

In summary, DDGS diet can influence egg yolk composition and other important nutrients content in egg yolk. Fat and protein content of egg yolk was affected by 50% DDGS treatment. The proportion of polyunsaturated fatty acids in egg yolk increased significantly by DDGS diet. The contents of choline and cholesterol were initially higher with 50% DDGS treatment, but were not affected by the DDGS treatments in the later period, especially during last 4 weeks. Lutein content increased linearly as DDGS level increased. These results indicated that feeding high levels of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.
ACKNOWLEDGEMENTS

This study was supported by Power Funds of Iowa. Hens were donated by Sparboe Company, DDGS feed was provided by Lincoln Way Energy (Nevada, IA), blended fat was donated by Feed Energy Company (Des Moines, IA), and limestone was donated by Iowa Limestone Company (Des Moines, IA). The assistance provided by personnel in laboratories of Ahn, Sebranek, and Persia and Iowa State University poultry farm is greatly appreciated.
Table 3.1 Nutrient analysis of corn distiller’s dried grains with solubles (DDGS) used in the study (% as is)

<table>
<thead>
<tr>
<th>Amino Acid Profile</th>
<th>%</th>
<th>Nutrients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>1.91</td>
<td>Dry Matter</td>
<td>87.60</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.97</td>
<td>Protein</td>
<td>27.30</td>
</tr>
<tr>
<td>Serine</td>
<td>1.13</td>
<td>Ash</td>
<td>4.35</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>4.46</td>
<td>Fat</td>
<td>10.67</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.13</td>
<td>Minerals</td>
<td>ppm</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.01</td>
<td>P</td>
<td>9296</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.76</td>
<td>K</td>
<td>11578</td>
</tr>
<tr>
<td>Valine</td>
<td>1.46</td>
<td>Ca</td>
<td>312</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.81</td>
<td>Mg</td>
<td>3532</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.08</td>
<td>S</td>
<td>4871</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.19</td>
<td>Na</td>
<td>2171</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.89</td>
<td>Fe</td>
<td>68.90</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.32</td>
<td>Mn</td>
<td>8.97</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.91</td>
<td>Zn</td>
<td>62.60</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.75</td>
<td>Cu</td>
<td>6.24</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.16</td>
<td>Al</td>
<td>30.70</td>
</tr>
</tbody>
</table>

1 Analyzed at Central Analytical Lab, Poultry Science Center, University of Arkansas
2 Nutrient values expressed as grams per 100 gram sample on weight/weight percentage
Table 3.2 Composition and calculated analysis of experimental diet (1-12 week) with various levels of corn distiller’s dried grains with soluble (DDGS) (% as is)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% DDGS</th>
<th>0</th>
<th>17</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td></td>
<td>59.20</td>
<td>51.37</td>
<td>36.62</td>
<td>26.80</td>
</tr>
<tr>
<td>Soybean meal</td>
<td></td>
<td>25.00</td>
<td>15.00</td>
<td>10.00</td>
<td>3.50</td>
</tr>
<tr>
<td>DDGS1</td>
<td></td>
<td>0.00</td>
<td>17.00</td>
<td>35.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Blended fat2</td>
<td></td>
<td>2.70</td>
<td>3.68</td>
<td>5.65</td>
<td>7.00</td>
</tr>
<tr>
<td>Limestone3</td>
<td></td>
<td>11.02</td>
<td>11.22</td>
<td>11.46</td>
<td>11.65</td>
</tr>
<tr>
<td>Dical phosphate</td>
<td></td>
<td>1.05</td>
<td>0.80</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td>0.38</td>
<td>0.29</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>V and M Mix4</td>
<td></td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>L-Thr</td>
<td></td>
<td>0.01</td>
<td>0.05</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Bio-Lys5</td>
<td></td>
<td>0.00</td>
<td>0.03</td>
<td>0.16</td>
<td>0.30</td>
</tr>
<tr>
<td>DL-Met</td>
<td></td>
<td>0.14</td>
<td>0.06</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Calculated (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td></td>
<td>16.70</td>
<td>16.50</td>
<td>18.30</td>
<td>19.00</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td></td>
<td>2,825</td>
<td>2,825</td>
<td>2,825</td>
<td>2,825</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
<td>4.50</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Na</td>
<td></td>
<td>0.17</td>
<td>0.18</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Arg</td>
<td></td>
<td>1.10</td>
<td>0.95</td>
<td>0.97</td>
<td>0.91</td>
</tr>
<tr>
<td>His</td>
<td></td>
<td>0.46</td>
<td>0.45</td>
<td>0.51</td>
<td>0.53</td>
</tr>
<tr>
<td>Ile</td>
<td></td>
<td>0.70</td>
<td>0.67</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>Leu</td>
<td></td>
<td>1.53</td>
<td>1.69</td>
<td>2.00</td>
<td>2.20</td>
</tr>
<tr>
<td>Lys</td>
<td></td>
<td>0.89</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
<tr>
<td>SAA</td>
<td></td>
<td>0.70</td>
<td>0.70</td>
<td>0.72</td>
<td>0.78</td>
</tr>
<tr>
<td>Phe+Tyr</td>
<td></td>
<td>1.35</td>
<td>1.38</td>
<td>1.60</td>
<td>1.70</td>
</tr>
<tr>
<td>Thr</td>
<td></td>
<td>0.63</td>
<td>0.62</td>
<td>0.68</td>
<td>0.71</td>
</tr>
<tr>
<td>Val</td>
<td></td>
<td>0.71</td>
<td>0.75</td>
<td>0.88</td>
<td>0.95</td>
</tr>
<tr>
<td>Trp</td>
<td></td>
<td>0.22</td>
<td>0.18</td>
<td>0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

1 DDGS was donated by LincolnWay Energy, Nevada, IA
2 Blended fat contained vegetable and animal fat, donated by Feed Energy Company, Des Moines, IA
3 Limestone was donated by Iowa Limestone Company, Des Moines, IA
4 V and M Mix = vitamin and mineral premix: contained the followings per kilogram diet: Selenium, 0.2 ppm; vitamin A, 6608 IU; vitamin D3, 2203 ICU; vitamin E, 14 IU; menadione, 0.88 mg; vitamin B12, 9.35 µg; biotin, 33 µg; choline, 358 mg; folic acid, 1.1 mg; niacin, 33 mg; pantothenic acid, 8.8 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg.
5 Bio-Lys (78.8% Lysine)
Table 3.3 Composition and calculated analysis of experimental diet (13-24 week) with various levels of corn distiller’s dried grains with soluble (DDGS) (% as is)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Corn</td>
<td>59.13</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>25.00</td>
</tr>
<tr>
<td>DDGS(^1)</td>
<td>0.00</td>
</tr>
<tr>
<td>Blended fat(^2)</td>
<td>2.72</td>
</tr>
<tr>
<td>Limestone(^3)</td>
<td>11.02</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.05</td>
</tr>
<tr>
<td>Salt</td>
<td>0.38</td>
</tr>
<tr>
<td>V and M Mix(^4)</td>
<td>0.50</td>
</tr>
<tr>
<td>L-Thr</td>
<td>0.07</td>
</tr>
<tr>
<td>Bio-Lys(^5)</td>
<td>0.00</td>
</tr>
<tr>
<td>DL-Met</td>
<td>0.19</td>
</tr>
<tr>
<td>Calculated (%)</td>
<td></td>
</tr>
<tr>
<td>CP (g/kg)</td>
<td>16.70</td>
</tr>
<tr>
<td>ME (kcal/kg)</td>
<td>2,825</td>
</tr>
<tr>
<td>Fat</td>
<td>5.16</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.28</td>
</tr>
<tr>
<td>Ca</td>
<td>4.50</td>
</tr>
<tr>
<td>P</td>
<td>0.30</td>
</tr>
<tr>
<td>Na</td>
<td>0.17</td>
</tr>
<tr>
<td>Arg</td>
<td>1.10</td>
</tr>
<tr>
<td>His</td>
<td>0.46</td>
</tr>
<tr>
<td>Ile</td>
<td>0.70</td>
</tr>
<tr>
<td>Leu</td>
<td>1.53</td>
</tr>
<tr>
<td>Lys</td>
<td>0.89</td>
</tr>
<tr>
<td>SAA</td>
<td>0.75</td>
</tr>
<tr>
<td>Phe+Tyr</td>
<td>1.34</td>
</tr>
<tr>
<td>Thr</td>
<td>0.63</td>
</tr>
<tr>
<td>Val</td>
<td>0.71</td>
</tr>
<tr>
<td>Trp</td>
<td>0.22</td>
</tr>
</tbody>
</table>

1 DDGS was donated by LincolnWay Energy, Nevada, IA  
2 Blended fat contained vegetable and animal fat, donated by Feed Energy Company, Des Moines, IA  
3 Limestone was donated by Iowa Limestone Company, Des Moines, IA  
4 V and M Mix = vitamin and mineral premix: contained the followings per kilogram diet: Selenium, 0.2 ppm; vitamin A, 6608 IU; vitamin D3, 2203 IU; vitamin E, 14 IU; menadione, 0.88 mg; vitamin B12, 9.35 µg; biotin, 33 µg; choline, 358 mg; folic acid, 1.1 mg; niacin, 33 mg; pantothenic acid, 8.8 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg.  
5 Bio-Lys (78.8% Lysine)
Table 3.4 Fat, moisture and protein content of egg yolk from hens fed diets with various levels of corn distiller’s dried grains with soluble (DDGS)\(^1\)

<table>
<thead>
<tr>
<th>Chemical component (%)</th>
<th>DDGS diets (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.13(^b)</td>
<td>32.53(^b)</td>
</tr>
<tr>
<td>Moisture</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48.54</td>
<td>48.74</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.89(^a)</td>
<td>16.88(^a)</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\) Means with no common superscript in the same row differ significantly (P < 0.05)

\(^1\)Values are means of 4 replications.
Table 3.5 Fatty acids composition of diet formula 1 and egg yolks from hens fed diets with various levels of corn distiller's dried grains with soluble (DDGS)

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>DDGS Diet 1</th>
<th>Egg yolk 2</th>
<th>SEM 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% DDGS</td>
<td>17% DDGS</td>
<td>35% DDGS</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>0.80</td>
<td>0.50</td>
<td>0.55</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>22.80</td>
<td>18.25</td>
<td>17.55</td>
</tr>
<tr>
<td>Plamitoleic acid</td>
<td>0.65</td>
<td>0.55</td>
<td>0.60</td>
</tr>
<tr>
<td>Margaric acid</td>
<td>0.40</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Margaroleic acid</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>8.20</td>
<td>6.20</td>
<td>5.95</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>28.15</td>
<td>25.65</td>
<td>26.40</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>36.20</td>
<td>45.40</td>
<td>45.15</td>
</tr>
<tr>
<td>Linolenic acid</td>
<td>2.50</td>
<td>2.90</td>
<td>3.35</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>0.20</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>EPA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DHA</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>SFA</td>
<td>32.10</td>
<td>25.25</td>
<td>24.35</td>
</tr>
<tr>
<td>USFA</td>
<td>67.90</td>
<td>74.75</td>
<td>75.65</td>
</tr>
<tr>
<td>PUFA</td>
<td>38.95</td>
<td>48.45</td>
<td>48.60</td>
</tr>
</tbody>
</table>

SFA: saturated fatty acid, USFA: unsaturated fatty acid, PUFA: polyunsaturated fatty acid

Means with no common superscript in the same row differ significantly (P < 0.05)

1 Fatty acid composition of DDGS diets, values are means of 4 replications

2 Fatty acid composition of egg yolk from DDGS diets, values are means of 4 replications

3 Standard error of mean of fatty acid composition of egg yolk from laying hens fed DDGS diets
Figure 3.1 Effect of diets with various levels of corn distiller’s dried grains with solubles\textsuperscript{1} on choline\textsuperscript{2} of egg yolk during the 24-week-long period\textsuperscript{3}. Figure 3.1a is based on choline content of 100 g egg yolk, Figure 3.1b is based on choline content per egg, and Figure 3.1c is based on choline content per hen per day. \textsuperscript{*4}Denotes that 50\% DDGS dietary treatment is significantly higher than the 0\% DDGS dietary treatment (p<0.05). \textsuperscript{*2}Denotes that 17\% DDGS dietary treatment is significantly different from the 0\% DDGS dietary treatment (p<0.05). \textsuperscript{*2,4}Denotes that 17 and 50\% DDGS dietary treatments are significantly different from the 0\% DDGS dietary treatment (p<0.05). \textsuperscript{1}The ratio of corn and soybean meal base in diet reduced as DDGS level increased. \textsuperscript{2}Values are means of 4 replications. \textsuperscript{3}Each data point means averaged choline content on a two-week basis.
Figure 3.1b

Figure 3.1c
Figure 3.2 Effect of diets with various levels of corn distiller’s dried grains with solubles\(^1\) on cholesterol\(^2\) of egg yolk during the 24-week-long period\(^3\). *\(^4\)Denotes that 50% DDGS dietary treatment is significantly higher than the 0% DDGS dietary treatment (\(p<0.05\)). \(^2\)*\(^4\)Denotes that 17 and 50% DDGS dietary treatments are significantly higher than the 0% DDGS dietary treatment (\(p<0.05\)). \(^3\)*\(^4\)Denotes that 35 and 50% DDGS dietary treatments are significantly higher than the 0% DDGS dietary treatment (\(p<0.05\)). \(^1\)The ratio of corn and soybean meal base in diet reduced as DDGS level increased. \(^2\)Values are means of 4 replications. \(^3\)Each data point means averaged cholesterol content of egg yolk on a two-week basis.
Figure 3.3 Effect of diets with various levels of corn distiller’s dried grains with solubles\(^1\) on lutein\(^2\) of egg yolk during the 24-week-long period\(^3\). *\(^2\,3\,4\) Denotes that 17, 35 and 50% DDGS dietary treatments are significant higher than 0% DDGS dietary treatment (p < 0.05). \(^1\) The ratio of corn and soybean meal base in diet reduced as DDGS level increased. \(^2\) Values are means of 4 replications. \(^3\) Each data point means averaged lutein content of egg yolk on a two-week basis.
REFERENCES


Wiley & Sons, New York, P311.


CHAPTER 4. GENERAL CONCLUSIONS

Due to high variability of nutrients in DDGS from different sources, deficiency in amino acids is likely to occur in DDGS diet, especially when DDGS is used at high levels. In this study, the contents of digestible lysine and methionine were not sufficient in 50% DDGS diet in the first 12-wk period, then the diets were reformulated to meet the requirement of amino acid for laying hens in the second 12-wk period. It is important to measure the nutrient content of DDGS before the diet formulation to meet all the production needs of laying hens.

With sufficient digestible amino acids, it appears that high levels of DDGS (up to 50%) can be fed to laying hens without significant effect on egg production, feed intake and egg weight. Once the 50% DDGS diet was further supplemented with lysine and methionine, there were no differences in egg production, feed intake, and egg weight during the last 6 weeks between the high DDGS diet and the control. The decreased egg production, feed intake, egg mass and egg weight observed before the diet reformulation is most likely due to the amino acid deficiency as all performance parameters were improved after additional amino acids (lysine and methionine) were supplied in the diets. DDGS positively affected Haugh units and yolk color, which were improved linearly as the DDGS levels in diet increased. Eggshell strength and egg component were not affected by DDGS treatments.

Moreover, DDGS diet can influence egg yolk composition and other important nutrients content in egg yolk. Fat and protein contents of egg yolk were influenced by 50% DDGS treatment. The proportion of polyunsaturated fatty acids in egg yolk increased significantly by DDGS diet. The contents of choline and cholesterol were initially higher with 50% DDGS treatment, but were not affected by the DDGS treatments in the later
period, especially during last 4 weeks. Lutein content of egg yolk increased linearly as DDGS level increased. These results indicated that feeding high levels of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.

In conclusion, DDGS as a nutritional and economical feed ingredient, can be included in laying hen diet at higher levels. Up to 50% DDGS inclusion rate would not negatively affect egg production, egg weight, feed intake, feed efficiency and egg mass. Egg quality such as yolk color and Haugh unit can be improved by adding high levels of DDGS in layer diets. Other valuable nutrients, such as lutein and polyunsaturated fatty acids, can also be improved by DDGS diets. In brief, feeding high levels of DDGS (up to 50%) to laying hens is possible and applicable as long as amino acids are sufficient in the diet.
I would like to express my sincerest appreciation to my major professor, Dr. Dong U. Ahn (Department of Animal Science, Meat Science Program), for his guidance, support and constructive criticism throughout this research. I would also like to thank Dr. Michael Persia (Department of Animal Science, Animal Nutrition Program) and Dr. Joseph Sebranek (Department of Animal Science, Meat Science Program) for kindly serving as a committee member and consultants. Thanks for sharing their knowledge throughout my research project. Special thanks to Martha Jeffrey and Elaine Larson for their help during the project.

I would give my sincere thanks to all the members of the Ahn lab, past and present. Special thanks to Dr. Eun Joo Lee for her generous help and guidance. Thank Himali Samaraweera, Wangang Zhang, Shan Xiao, Marwan Al-Hijazeen for their great help and friendship. I would also sincerely thank my family for their love and support. Thank you for always encouraging to pursuit my dreams.