2015

The Effect of Processing Method of Distiller's Dried Grains with Solubles on Hen Egg Production, Egg Quality, and Yolk Color

Lindsay Renee Brunet
Louisiana State University and Agricultural and Mechanical College

THE EFFECT OF PROCESSING METHOD OF DISTILLER'S DRIED GRAINS WITH SOLUBLES ON HEN EGG PRODUCTION, EGG QUALITY, AND YOLK COLOR

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Animal Sciences

by
Lindsay R. Brunet
B.S., Louisiana State University, 2013
August 2015

ACKNOWLEDGEMENTS

I would like to thank Dr. Theresia Lavergne for introducing me to the poultry industry and giving me the opportunity to conduct my own undergraduate research which led to my interest in furthering my academic career. Your continual support and encouragement was pivotal in my success, without it, I would not be where I am. I would like to thank Dr. Jack Losso for serving on my committee, but even more for your unfailing patience with me in my lab work. I truly appreciate all of the time you have committed to my research. I would also like to thank Dr. Cathleen Williams for serving on my committee and for her continued patience throughout this process.

I would like to thank all of my fellow graduate students, particularly Amanda and Kandice for their support and encouragement throughout this process. I would especially like to thank Dr. Jose Charal and Katherine Rubio Zapata for being amazingly warm and supportive lab mates who I now consider lifelong friends. Special thanks to James Maynard for all of his help during my project.

I would also like to thank Mr. Gerry, James, and all of the rest of the poultry farm crew for all of their help throughout the past two years.

I would like to thank my biggest supporters, my parents, without whom I certainly would not be where I am today. Your support, encouragement, and love have shaped my whole being and have been instrumental in all my successes in academia, as well as life. I want to thank my brothers for being supportive and encouraging, especially Robert, without whom I would be lost. I would also like to

thank my step father for his unconditional love and support even though we've only known each other for a short time, you have had a tremendous impact on my life. I would also like to thank my friends who have played such a pivotal part in my happiness and success, Emily, Brittany, and Julie. Words cannot express the love and appreciation I have for all of the wonderful people in my life. And last but certainly not least, I would like to thank God for the strength and blessings he has bestowed upon me. Without Him, nothing would be possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

ABSTRACT

Distiller's dried grains with solubles (DDGS) is a common byproduct of the ethanol industry and is used in animal feeds. Carotenoids (xanthophylls) are already present in the human eye, and increasing the amount of carotenoids in the eye can help prevent eye diseases. The purpose of this research was to confirm that adding DDGS to standard corn and soybean meal hen diets may increase the amount of lutein available in egg yolks. An experiment was conducted with Hy-Line W-36 hens to evaluate the effects of DDGS in corn-soybean meal diets. Three hundred fifteen hens were fed one of seven treatment diets with five replications of nine hens per replicate in a completely randomized design. This was a 56-d trial. The treatment diets were: 1) Control (no DDGS), 2) 10% DDGS processed with heat treatment (DDGS+H), 3) 10% DDGS processed without heat treatment (DDGS-H), 4) 20% DDGS+H, 5) 20% DDGS-H, 6) 30% DDGS+H, and 7) 30% DDGS-H. Average daily feed intake, feed efficiency, egg specific gravity, egg mass, yolk color, and Haugh units were determined on three consecutive days at the end of each 28-d period. The eggs collected on the last three days of each 28-day period were stored either at room temperature or under refrigeration. Half of the stored eggs were broken out after three days of storage while the other half were broken out on day seven of storage, and measurements were collected. Throughout the trial, there was no effect of dietary treatment on average daily feed intake, feed efficiency, hen day production, egg weight, specific gravity, or hen weight. At the end of both 28-d periods, yolk redness (a*) was increased in eggs from hens fed DDGS-H or DDGS+H. Yolk

yellowness (b*) was increased in hens fed diets with 20% of either DDGS+H or DDGS-H at the end of the second 28-d period. Storage method did affect egg quality. Eggs stored in refrigeration were higher in quality. The inclusion of any level of DDGS in hen diets did not affect hen egg production or egg quality but did increase yolk redness (a*) and yellowness (b*) which could be an indicator of increased lutein content.

CHAPTER 1

INTRODUCTION

Distiller's dried grains with solubles (DDGS) has been a feed ingredient for livestock and poultry for many years. The use of DDGS in animal feeds has increased with growth of the ethanol industry. The effects of adding DDGS to poultry diets has been a research interest, particularly considering the positive effects that it has on meat and eggs. DDGS adds pigmentation to the skin, as well as to the yolk. With the pigmentation, DDGS also deposits lutein into the yolk which is beneficial to human eye health. Research has been conducted in recent years to determine to what extent this deposition occurs, as well as the optimum dietary inclusion rate.

It is known that protein can be denatured by heat, reducing its quality. Thus, a study was conducted in which two different sources of DDGS, one processed with heat and one without, were fed at a 20% inclusion rate to laying hens (Brunet et al., 2013). Results indicated that lutein and yolk color were increased with either source of DDGS over the control, and that DDGS processed with heat had numerically higher yolk color and lutein values, contrary to the hypothesis.

Considering the results from this previous study, the objectives of this research were to determine the optimal level of inclusion of DDGS in laying hen diets, to determine if a lower inclusion level would increase yolk lutein content, and to determine if increasing DDGS inclusion beyond 20% also increases lutein

deposition. Additionally, the effect of egg storage method and storage length on the quality of eggs from hens fed DDGS was evaluated.

CHAPTER 2

REVIEW OF LITERATURE

INTRODUCTION

The U.S. and Brazil have been the two leading producers of ethanol since the 1970's. Government support, along with tax exemptions, has made ethanol production from corn and sugarcane a desirable venture (Solomon et al., 2007). The U.S. produced 14.34 billion gallons of ethanol in 2014 (U.S. Energy Information Administration). Brazil, the world's leader in sugarcane ethanol, has successfully replaced 42% of their gasoline consumption with sugarcane ethanol. In the 2013-2014 crop year, 634 billion gallons of sugarcane ethanol were produced in Brazil. In the 2014-2015 crop year, 5.2 billion bushels of corn were used to produce ethanol in the U.S., which in turn yielded 1.2 billion bushels of DDGS to be used in animal feed (Wisner, 2015). On average, 8.2 kg of distiller's dried grains with solubles (DDGS) are produced per bushel of corn used for ethanol production (Shurson, 2013). Population and ethanol use have increased over the years, while crop land has decreased. Because of this, it has been a goal for the past decade or so to find alternatives for corn in animal feed.

DISTILLER'S DRIED GRAINS WITH SOLUBLES

Distiller's dried grains with solubles is a by-product of the ethanol industry. During fermentation of corn, ethanol is produced from the starch. Ethanol manufacturing from corn grain results in three main products: bioethanol, the primary end product; residual nonfermentable corn kernel components, which are typically marketed as the coproduct known as DDGS; and carbon dioxide

(Rosentrater, 2006). The DDGS has a high nutrient quality, due to the concentration of product from its predecessor, but the actual nutrient content is quite variable. This variability is a problem when formulating diets for use in livestock and poultry feed (Swiatkiewicz and Koreleski, 2008). Many research trials have been conducted to help evaluate the nutritional value of DDGS and create a standard that can be used in formulating diets. Findings have confirmed that the nutritional value of DDGS is greatly influenced by processing techniques used (Martinez-Amezcua et al., 2007).

DISTILLER'S DRIED GRAINS WITH SOLUBLES PROCESSING

The method used to process DDGS (Figure 1.1) has an effect on nutrient content and availability of nutrients in the DDGS. The production process consists of several steps, including grinding, cooking, liquefying, saccharifying, fermenting, and distilling the corn grain (Rosentrater, 2006). The nonfermentable residual materials following fermentation are removed from the process stream during the distillation stage in the form of stillage. After removing excess water via centrifugation, these wet grains are combined with condensed distiller's solubles, dried to ensure a substantial shelf life, and then sold as DDGS (Rosentrater, 2006).

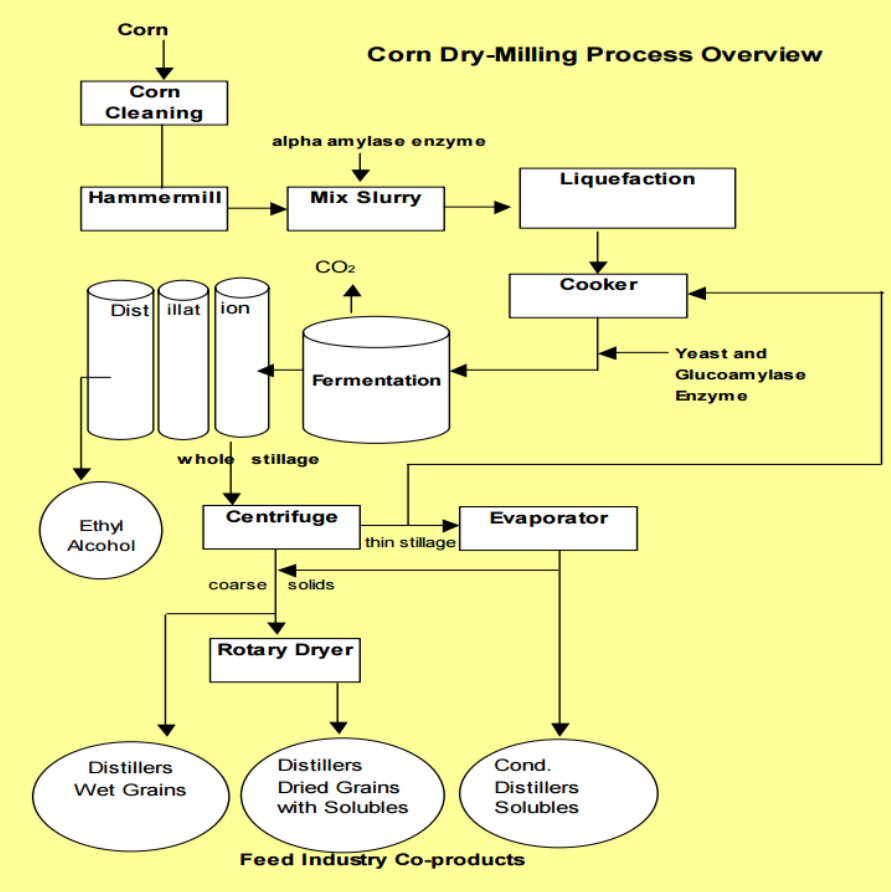

Figure 1.1 Photo courtesy of Dr. Jerry Shurson, UMN

Dryer temperatures vary widely from processing plant to processing plant and have been reported to range from as low as 127 degrees Celsius to 621 degrees Celsius (Doppenberg and van der Aar, 2007). Amezcua and Parsons (2007) reported that excessive heat processing has a negative effect on protein solubility and amino acid content and digestibility, particularly for lysine, for several feedstuffs, such as soybean meal, canola meal, and meat and bone meal. Amezcua and Parsons (2007) reported that the effect of heat on DDGS was consistent with their previous study. Amezcua and Parsons (2007) reported that increased heat processing of DDGS increased the bioavailability of phytate phosphorus but decreased digestibility of amino acids, particularly lysine, while

bioavailability of phosphorus was not affected by particle size. Rosentrater (2006) claims that data on the physical properties of DDGS are not only essential for livestock and poultry diet formulation, but also for the design of equipment and processing facilities, and the optimization of unit operations, storage, and material handling systems. Their results also stated that to be effectively utilized as feed materials, especially during rail shipping across the United States, it is crucial that DDGS be consistently dried to approximately 12% moisture content, or even slightly lower. Their results also indicated that the effects of excessive heating were greater for DDGS than for the other feedstuffs evaluated. The greater effects of heating on DDGS are probably associated with the formation of Maillard reaction products due to the higher concentration of reducing sugars from the solubles fraction of DDGS (Amezcua and Parsons, 2007).

DISTILLER'S DRIED GRAINS WITH SOLUBLES COMPOSITION

The variability of DDGS products produced in different plants is a problem when trying to formulate diets. Rosentrater (2006) reported that all physical properties (of DDGS) exhibited statistically significant differences between processing plants. Different ethanol processing methods used in each plant, such as fermentation method, distillation and drying method, and/or feedstock grain used in each plant affect chemical profiles (Ortin and Yu, 2009). The DDGS contain high levels of protein (27.3%), fiber, and fat (10.67%), and also contain considerable amounts of other important nutrients, such as lutein, choline, and long-chain unsaturated fatty acids (Sun et al., 2013). The energy values of corn DDGS were significantly higher than in corn, indicating that corn DDGS is

superior to original corn used in diets (Ortin and Yu, 2009). The DDGS has a higher non-phytate phosphorous content and higher relative bioavailability of phosphorous than the original corn source (Amezcua and Parsons, 2007). They also reported that increased heating of DDGS increased bioavailability of phosphorous but decreased digestibility of amino acids, particularly lysine, and that bioavailability of phosphorous was not affected by particle size. DDGS are often used at low concentrations (10 or 15%) as a feed ingredient for laying hens without affecting laying performance and egg quality (Roberson et al., 2005; Świątkiewicz and Koreleski, 2008; Masa'deh et al., 2011; Sun et al., 2012; Jiang et al., 2013; Sun et al., 2013; Hahn-Didde and Purdum, 2014). Sun et al. (2013) also stated that high dietary fiber content in DDGS diets may have a positive effect on controlling cholesterol levels in eggs, since many researchers have found a positive relationship between high-fiber diets and low serum cholesterol in humans.

DISTILLER'S DRIED GRAINS WITH SOLUBLES EFFECT ON EGGS

Since eggs are a product of laying hens and are primarily used for human consumption, it is important to evaluate the effects of feedstuffs on egg quality. Sun et al. (2013) state that the differences in component and nutrient concentration of DDGS diets may influence the chemical composition and nutrient content of eggs, especially when DDGS are used at high levels in the diet. Although, Świątkiewicz and Koreleski (2006) found that eggs from hens fed diets with DDGS had a higher yolk color score, and even 5% dietary inclusion of DDGS was sufficient to improve this parameter. Research from this point of view

is largely undeveloped. Yolk is the most nutritive part of the egg and contains many functional nutrients such as choline and lutein (Sun et al., 2013). Sun et al. (2013) report that the 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 a high level of DDGS in the diet. In their trial, Sun et al. (2013) fed hens diets containing corn DDGS at 0, 17, 35, or 50%. Fat content of egg yolk from hens fed diets with 50% DDGS was significantly higher than egg yolk from hens fed any of the other dietary treatments, and protein content of egg yolk from hens fed 50% DDGS in their diet was significantly lower than that of hens fed any of the other dietary treatments. All fatty acids, except margaroleic acid and docosahexaenoic acid (DHA), in egg yolk were influenced by DDGS inclusion in hen diets. The lutein content of the hen diets increased as the level of DDGS increased in the diet. Thus, Sun et al. (2013) concluded that 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. Similarly, Brunet et al. (2013) found that lutein content was increased in yolks from hens fed either DDGS without heat treatment (DDGS-H) or DDGS with heat treatment (DDGS+H) during processing, compared to hens fed no DDGS.

LUTEIN

Egg yolk carotenes are classified as xanthophylls. Xanthophylls include lutein, zeaxanthin, and cryptoxanthin (Sun et al., 2013). Especially in European countries, yellow skin and yolk color of poultry and eggs is a consumer preference. Sun et al. (2013) report that lutein has been used in the poultry diet

for a long time; this pigment can provide desirable yellow color in egg yolk and chicken skin, which consumers prefer. Birds cannot synthesize these pigments and, therefore, must rely on dietary sources for color absorption. According to Sun et al. (2013), DDGS contain high levels of xanthophyll, and diets containing DDGS should increase lutein content in egg yolk. Lutein, however, does not just supply the consumer demanded color to skin and yolk, but also poses great health benefits to the consumer. According to Moeller et al. (2000), the carotenoid xanthophylls, lutein and zeaxanthin, accumulate in the human eye lens and macular region of the retina. Studies have shown that generous intakes of lutein and zeaxanthin, particularly from certain xanthophyll-rich foods like spinach, broccoli and eggs, are associated with a significant reduction in the risk for cataracts (up to 20%) and for age-related macular degeneration (up to 40%) (Moeller et al., 2000). A large egg yolk contains 1.2 mg/100 g of xanthophylls (Perry et al., 2008). The National Eye Institute reports that 10 mg of lutein and 2 mg zeaxanthin taken every day for five years reduces the risk of macular degeneration progression by 10 to 25% (Hobbs and Bernstein, 2014)

CHAPTER 3

THE EFFECT OF PROCESSING METHOD OF DISTILLER'S DRIED GRAINS WITH SOLUBLES ON HEN EGG PRODUCTION, EGG QUALITY, AND YOLK COLOR

INTRODUCTION

The increased use of corn for ethanol production has resulted in an increased quantity of distiller's dried grains with solubles (DDGS) entering the feed market in recent years (Swiatkiewicz and Koreleski, 2006). The increase in availability along with high quality and low variability of nutrients makes DDGS an attractive feed ingredient in animal feeds in the U.S. In recent years, the poultry industry has included DDGS in most commercial poultry diets.

Leeson and Caston (2004) stated that over the last 10 years there has been increased awareness of the role of xanthophylls in human health, and in particular the roles of lutein and zeaxanthin in prevention of certain eye disorders. Carotenoids already are present in the human eye, and increasing the amount of carotenoids in the eye can help prevent disease such as macular degeneration and cataracts (Heiting and Jegtvig, 2012). DDGS contains xanthophylls which are a part of the carotenoid group.

Thus, it would be valuable to determine if feeding DDGS to laying hens increases the lutein (a type of xanthophyll) content of eggs. It is thought that adding DDGS to the standard corn and soybean meal diets may increase the amount of lutein available in egg yolks. Since a typical corn and soybean meal based commercial poultry diet does not supply the necessary amount and type of xanthophylls to produce the deep yellow color in the egg yolk and skin, DDGS

can be a good source of these pigments as long as they have not been overheated during the production process (Salim et al., 2010). Masa'deh (2011) stated that distiller's dried grains with solubles provide more xanthophylls than corn with approximately 34 mg/kg, which is three times the corn xanthophyll content.

There is very little reported research on this topic. Most egg layer research on the inclusion of DDGS in laying hen diets has reported the effects on egg yolk as a secondary finding. Research has shown that DDGS inclusion increases, to some extent, the color of egg yolks. Published research reports that the inclusion of up to 20% DDGS in laying hen diets does not negatively affect hen feed intake, laying rate, total egg mass, mean egg weight, or feed conversion ratio. The color and breaking strength of eggshell, as well as the albumen height (Haugh units) are not affected by the inclusion of DDGS up to 20% in the laying hen diet (Wu-Haan et al., 2010; Masa'deh et al., 2011; Purdum et al., 2014). Also, it has been reported that yolk color is significantly increased by DDGS inclusion (Cheon et al., 2008) in laying hen diets.

The goals of this research were to further evaluate the use of DDGS in laying hen diets to determine if different DDGS processing methods affect the color intensity of the egg yolk, to determine if there was a significant increase in egg yolk color when laying hens were fed DDGS, to determine if there were any negative effects of feeding DDGS to laying hens, and to determine if the inclusion of DDGS in the hen diet increased the lutein content in their eggs.

MATERIALS AND METHODS

All experimental animal use was in compliance with the Louisiana State University Agricultural Center Animal Care and Use Committee.

An experiment was conducted with 315 Hy-Line W-36 hens at 61 weeks of age. The Hy-Line W-36 is considered the world's most efficient egg layer that produces dozens of eggs with minimum feed consumption which makes her the industry's lowest cost producer of eggs and generates maximum profits for producers (Hyline, 2015). Hens were housed in a tunnel-ventilated caged layer house at the LSU AgCenter Central Stations Poultry Farm. Each replicate consisted of three adjoining cages with three hens per individual cage for a total of nine hens per replicate. The cages were metal wire (52x34x30 cm) in double-decker rows providing 520 cm^2 per hen. Each cage had one nipple waterer. Metal feed troughs were divided by replicate to insure that the hens were not able to consume feed assigned to adjoining replicates. A divider was inserted into the egg collection area to prevent mixing of eggs from separate replicates. Hens were provided mash form feed ad libitum. On days 0, 28, and 56 of the trial, all hens were weighed individually, with weights grouped by pen to insure no growth differences were observed.

Hens were fed one of seven dietary treatments with five replications of each treatment diet. Diets were corn-soybean meal based and formulated to meet the dietary requirements suggested in the Hy-Line W-36 management guide (Hy-Line International, 2012). Diets were formulated to contain 2,282 kcal ME/kg. The dietary treatments were: 1) control (C) diet containing no DDGS, 2) C with a 10% inclusion rate of heat processed DDGS (DDGS+H), 3) C with a

10% inclusion rate of non-heat processed DDGS (DDGS-H), 4) C with a 20% inclusion of DDGS+H, 5) C with a 20% inclusion of DDGS-H, 6) C with a 30% inclusion of DDGS+H, and 7) C with a 30% inclusion of DDGS-H. The composition and calculated nutrient contents of the treatment diets are in Table 3.1.

Weekly hen-day egg production was recorded. Average daily feed intake, feed efficiency, egg specific gravity, egg mass, yolk color, hen weight, and Haugh units were determined on three consecutive days at the end of each 28 day period. All eggs from each pen on the three consecutive days were collected and labeled accordingly. Egg weight and specific gravity was determined for each egg prior to break out. Eggs were then broken out to determine albumen height and yolk color. Albumen height was determined using a tripod micrometer (Baxlo Precision, Barcelona, Spain). Yolk color values were determined using the Minolta CM-508d spectrophotometer (Minolta Co Ltd, Mississauga, ON, Canada). On the second day of the three consecutive days of collection, the yolks were separated from the whites, pooled together, and frozen for further analysis of lutein content.

All data were analyzed by ANOVA as a completely randomized design using the GLM procedures in SAS (SAS Inst. Inc., Cary, NC). The three adjoining cages containing nine layers was the experimental unit. Treatment means were separated by the LSD option of SAS at α level of $P < 0.05$.

Table 3.1 Percentage composition of diets fed to laying hens, as fed basis.

Ingredient, %	Control	10% DDGS +Heat	10% DDGS -Heat	20% DDGS +Heat	20% DDGS -Heat	30% DDGS +Heat	30% DDGS -Heat
Corn	59.60	50.90	51.53	46.45	46.64	42.44	42.73
Soybean meal, 48%	23.30	21.48	20.91	15.74	15.55	9.62	9.35
Limestone	11.58	11.69	11.72	11.81	11.86	11.94	12.01
DDGS-Heat[1]			10.00		20.00		30.00
DDGS+Heat[2]		10.00		20.00		30.00	
Poultry Fat	2.75	3.46	3.38	3.50	3.50	3.50	3.50
Monocalcium phosphate	1.55	1.35	1.35	1.17	1.17	0.99	0.99
Salt	0.43	0.38	0.38	0.34	0.32	0.29	0.27
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.19	0.14	0.14	0.20	0.20	0.20	0.20
Choline Chloride[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated composition							
ME (kcal/kg)	2822	2822	2822	2822	2822	2822	2822
Ca, %	4.84	4.84	4.84	4.84	4.84	4.84	4.84
P, %	0.65	0.66	0.66	0.66	0.65	0.65	0.64
Non-phytate P, %	0.43	0.43	0.43	0.43	0.43	0.43	0.43
Total amino acids							
Lysine, %	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Methionine, %	0.43	0.41	0.4	0.48	0.48	0.48	0.49
Methionine + Cysteine, %	0.69	0.69	0.69	0.76	0.78	0.77	0.79

(Table 3.1 continued)

Ingredient, %	Control	10% DDGS +Heat	10% DDGS -Heat	20% DDGS +Heat	20% DDGS -Heat	30% DDGS +Heat	30% DDGS -Heat
Threonine, %	0.6	0.64	0.63	0.62	0.62	0.59	0.60
Tryptophan, %	0.18	0.19	0.18	0.17	0.17	0.15	0.14

[1]Distiller's dried grains with solubles (DDGS) obtained from Poet Nutrition (Dakota Gold, Poet Nutrition, Sioux Falls, SD).

[2] DDGS obtained from Greenplains Renewable Energy.

[3]Provided per kilogram of diet: vitamin A, 8,002.78 IU; vitamin D_3, 3003.8 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

[4] Provided per kilogram of diet: Cu (copper sulfate), 7 mg; I (calcium iodate), 1 mg; Fe (ferrous sulfate•H_2O), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.15 mg; Zn (zinc sulfate), 44 mg.

[5] Contains 750,000 mg/kg of choline.

RESULTS

The addition of DDGS at any inclusion level did not affect (P>0.05) average daily feed intake, feed efficiency, hen day production, egg weight, specific gravity, or hen weight (Tables 3.2 and 3.3). The number of eggs produced ranged from 200.40 to 205.40 during the first 28 day collection period, and ranged from 200.20 to 204.00 during the second 28 day collection period. Eggs produced per kilogram of feed ranged from 8.32 to 8.77 during the first 28 day collection period, and ranged from 7.74 to 8.13 during the second 28 day collection period. Also, hen day production (%) ranged from 79.52 to 81.51 and from 79.44 to 80.98 during the first and second 28 day collection periods, respectively.

Egg quality data are presented in Table 3.2. Yolk redness, (a*), was increased (P<0.01) in hens fed DDGS-H or DDGS+H at any inclusion level when measured at the end of both collection periods. Yolk redness (a*) ranged from -0.96 to 0.67 and from -0.62 to 0.37 for the first and second 28 day collection periods, respectively. Yolk yellowness, (b*), was increased (P<0.01) in hens fed DDGS+H or DDGS-H at a 20% inclusion level. However, this increase was only observed at the end of the trial and not at the first 28 day collection period. Yolk yellowness (b*) ranged from 28.51 to 29.83 and from 27.77 to 31.22 for the first and second 28 day collection periods, respectively.

The inclusion of any level of DDGS in hen diets did not affect (P>0.05) hen egg production or egg quality. Yolk redness (a*) and yellowness (b*) were increased in hens fed diets containing DDGS.

Table 3.2 The effect of distiller's dried grains with solubles on hen performance and egg quality.

Treatment	Response criteria[1,2]									
	Num. of eggs	ADFI, g	Eggs/ kg of feed	HDP, %	Egg wt, g	Sp. Gr.	L[3]	a[4]	b[5]	Haugh Units[6]
First 28 Days										
No DDGS	205.40	93.81	8.71[a]	81.51	64.68[a]	1.0796[a]	53.68[ab]	-0.96[c]	28.51[b]	95.93[ab]
10% DDGS+H[7]	202.00	94.74	8.46[a]	80.16	63.32[a]	1.0796[a]	53.85[a]	-0.28[b]	29.75[a]	95.26[abc]
10% DDGS-H[8]	201.00	94.44	8.45[a]	79.76	64.20[a]	1.0796[a]	54.06[a]	-0.31[bc]	29.56[ab]	94.77[abc]
20% DDGS+H	203.60	92.29	8.77[a]	80.79	64.06[a]	1.0791[a]	53.13[ab]	0.11[ab]	29.74[a]	95.66[abc]
20% DDGS-H	203.60	92.44	8.74[a]	80.79	63.59[a]	1.0791[a]	52.68[ab]	0.23[ab]	29.83[a]	93.40[bc]
30% DDGS+H	200.40	95.87	8.32[a]	79.52	64.53[a]	1.0792[a]	52.16[b]	0.67[a]	28.71[ab]	92.86[c]
30% DDGS-H	202.20	93.97	8.54[a]	80.24	64.38[a]	1.0802[a]	52.48[ab]	0.34[ab]	29.10[ab]	97.56[a]
SEM	2.23	1.52	0.18	0.88	0.50	0.0008	0.57	0.23	0.42	0.97
P-value	0.73	0.66	0.52	0.73	0.46	0.95	0.17	0.01	0.17	0.04
Second 28 Days										
No DDGS	203.60	101.77	7.94[ab]	80.79	63.58[ab]	1.0742[a]	52.89[ab]	-0.62[c]	27.77[d]	95.92[a]

(Table 3.2 continued)

	Num. of eggs	ADFI, g	Eggs/ kg of feed	HDP, %	Egg wt, g	Sp. Gr.	L[3]	a[4]	b[5]	Haugh Units[6]
10% DDGS+H	203.80	102.53	7.89^{ab}	80.87	64.26^{ab}	1.0719^{ab}	53.32^{ab}	-0.42^{c}	27.86^{d}	94.01^{ab}
10% DDGS-H	200.40	102.91	7.74^{b}	79.52	65.09^{ab}	1.0736^{ab}	54.50^{a}	-0.07^{b}	29.41^{bc}	95.22^{a}
20% DDGS+H	203.80	99.66	8.13^{a}	80.87	65.45^{a}	1.0727^{ab}	53.73^{ab}	0.37^{a}	30.34^{ab}	91.60^{bc}
20% DDGS-H	204.00	103.75	7.81^{ab}	80.95	63.12^{b}	1.0704^{b}	54.54^{a}	0.32^{a}	31.22^{a}	91.06^{c}
30% DDGS+H	200.20	101.53	7.83^{ab}	79.44	64.41^{ab}	1.0722^{ab}	52.76^{ab}	0.19^{a}	28.35^{cd}	93.86^{ab}
30% DDGS-H	202.20	100.35	8.00^{ab}	80.24	64.50^{ab}	1.0737^{ab}	52.41^{b}	0.23^{a}	28.81^{cd}	95.33^{a}
SEM	1.77	1.44	0.13	0.70	0.71	0.0013	0.70	0.08	0.52	0.93
P-value	0.52	0.45	0.45	0.52	0.30	0.44	0.23	0.01	0.01	0.01

Overall			
No DDGS	97.79	8.31^{a}	81.15
10% DDGS+H	98.63	8.16^{a}	80.52
10% DDGS-H	98.67	8.07^{a}	79.64
20% DDGS+H	95.97	8.43^{a}	80.83

(Table 3.2 continued)

	Num. of eggs	ADFI, g	Eggs/ kg of feed	HDP, %	Egg wt, g	Sp. Gr.	L[3]	a[4]	b[5]	Haugh Units[6]
20% DDGS-H		98.09	8.25[a]	80.87						
30% DDGS+H		98.70	8.06[a]	79.48						
30% DDGS-H		97.16	8.26[a]	80.24						
SEM		1.53	0.13	0.68						
P-value		0.61	0.44	0.53						

[1] Response criteria: Number of eggs, Average Daily Feed Intake (ADFI), Eggs produced per kilogram of feed, Hen day production (HDP), Average egg weight (Egg Wt.), Specific Gravity (Sp, Gr.), colorimeter values (L,a,b) (Minolta CM-508d spectrophotometer), Haugh Units

[2] Data are means of 5 replicates of 9 layers per replicate.

[3] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

[4] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

[5] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

[6] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.

[7] DDGS processed with heat

[8] DDGS processed without heat

[abcd] Means with different superscripts within a column are different (P<0.05).

Table 3.3 Body weight of hens fed distiller's dried grains with solubles.

	Weights, kg[1]		
Treatment	Initial	First 28 Days	Second 28 Days
No DDGS	1.66	1.51	1.58
10% DDGS+H[2]	1.66	1.49	1.60
10% DDGS-H[3]	1.65	1.56	1.63
20% DDGS+H	1.67	1.58	1.61
20% DDGS-H	1.63	1.54	1.60
30% DDGS+H	1.61	1.50	1.58
30% DDGS-H	1.66	1.51	1.57
SEM	0.03	0.06	0.03
P-value	0.69	0.91	0.90

[1]Data are means of 5 replicates of 9 layers per replicate.
[2]Distiller's dried grains with solubles (DDGS) processed with heat
[3] DDGS processed without heat

DISCUSSION

Roberson et al. (2005) reported a linear decrease in egg production parameters with an increase in DDGS in hen diets. Our results differ from these findings and suggest that hen diets with up to 30% DDGS did not decrease egg production and are practical to use in laying hen diets. Roberson et al. (2005) did, however, report that yolk color increased linearly with increased dietary DDGS inclusion, and was evident after only a month of feeding. Masa'deh et al. (2011) also reported a linear increase in yolk color with increasing levels of dietary DDGS. These results support our findings. Rosentrater (2006) explains that correlations involving the color (L*a*b*) values are especially appealing for

further study because they hold potential for developing prediction relationships between product color and other variables with which they are correlated. This statement is in agreement with our hypothesis that the increase in yolk redness and yellowness in hens fed diets containing DDGS suggests that DDGS increased pigment deposition in the yolk. Yolk redness would be characterized as having an a* value that is positive, i.e. the higher the number, the more red the sample is; similarly, yolk yellowness would be characterized as having a b* value that is positive (Hunter Lab application note, 2012). The increase in pigment means an increase in xanthophyll content, which includes lutein. Thus, the inclusion of DDGS in hen diets may increase lutein content of the egg yolk.

CHAPTER 4

THE EFFECT OF STORAGE METHOD AND STORAGE LENGTH ON THE QUALITY OF EGGS FROM HENS FED DISTILLER'S DRIED GRAINS WITH SOLUBLES

INTRODUCTION

In the United States, the majority of eggs reach store shelves within one week of being laid and are refrigerated from the point of packaging, so eggs are stored only for short periods of time (Meunier and Latour, 2000). However, in other countries, egg storage is very different, and is critical to food safety and shelf life. Many countries do not use refrigeration due to lack of availability and erratic power supply. Therefore, shelf life is reduced compared to that of refrigerated eggs (Eke et al., 2013). Eggs are perishable and can rapidly undergo weight loss and interior quality deterioration during storage, causing a major economic loss to the poultry industry. Losses to the egg industry as a result of interior egg and egg shell quality have been estimated to be in excess of $10 million annually (Sert et al., 2011).

Egg washing is a practice that is mainly concentrated in the U.S. Bacteria are a concern in other countries because of this. An alternative to egg washing is coating eggs with a protective oil layer. Eke et al. (2013) reported that bacteria, yeast, and mold counts on eggs are higher when they are stored at ambient temperature compared to eggs that are oiled or refrigerated.

The implementation of feeding DDGS to laying hens to increase lutein content in the egg yolk is a new practice in the field of designer eggs. Designer eggs are simply eggs enriched with beneficial health supplements. Lutein has

been shown to improve eye health by reducing macular degeneration and cataracts. Increasing lutein intake levels is the most effective prevention to date to fight macular degeneration (Leeson and Caston, 2004). Surai and Sparks (2001) explain that eggs, which are consumed regularly by most of the population, when enriched with DHA, vitamin E, lutein and selenium, are capable of substantially improving the diet quality of humans. Many of the designer egg combinations have been studied in detail and are readily available for consumers. However, lutein enriched eggs are still somewhat of an unknown and still in the experimental stages. Wenzel et al. (2011) claim there have been no storage studies considering the influence of time, temperature, and prior pasteurization on the content of xanthophylls in freeze-dried egg yolk. Since macular degeneration is the leading cause of blindness in many countries, it would be beneficial to determine how lutein is affected by egg storage practices in other countries (Leeson and Caston, 2004).

Wenzel et al. (2011) conducted a study in which freeze-dried egg yolks were evaluated for xanthophyll content. They concluded that the retention of xanthophyll content depends more on exposed light and pigments contained in the yolk than on the storage temperature. Based on the findings of the above experiment, it is worth further exploration to determine lutein content of eggs stored in refrigeration and eggs stored at ambient temperature.

MATERIALS AND METHODS

An experiment was conducted with 315 Hy-Line W-36 hens at 61 weeks of age. The Hy-Line W-36 is considered the world's most efficient egg layer that

produces dozens of eggs with minimum feed consumption which makes her the industry's lowest cost producer of eggs and generates maximum profits for producers (Hyline, 2015). Hens were housed in a tunnel-ventilated caged layer house at the LSU AgCenter Central Stations Poultry Farm. Each replicate consisted of three adjoining cages with three hens per individual cage for a total of nine hens per replicate. The cages were metal wire (52x34x30 cm) in double-decker rows providing 520 cm^2 per hen. Each cage had one nipple waterer. Metal feed troughs were divided by replicate to insure that the hens were not able to consume feed assigned to adjoining replicates. A divider was inserted into the egg collection area to prevent mixing of eggs from separate replicates. Hens were provided mash form feed ad libitum. On days 0, 28, and 56 of the trial, all hens were weighed individually, with weights grouped by pen to insure no growth differences were observed.

Hens were fed one of seven dietary treatments with five replications of each treatment diet. Diets were corn-soybean meal based and formulated to meet the dietary requirements suggested in the Hy-Line W-36 management guide (Hy-Line International, 2012). Diets were formulated to contain 2,282 kcal ME/kg. The dietary treatments were: 1) control (C) diet containing no DDGS, 2) C with a 10% inclusion rate of heat processed DDGS (DDGS+H), 3) C with a 10% inclusion rate of non-heat processed DDGS (DDGS-H), 4) C with a 20% inclusion of DDGS+H, 5) C with a 20% inclusion of DDGS-H, 6) C with a 30% inclusion of DDGS+H, and 7) C with a 30% inclusion of DDGS-H. The

composition and calculated nutrient contents of the treatment diets are in Table 3.1.

Egg specific gravity, egg mass, yolk color, and Haugh units were determined on three consecutive days at the end of each 28 day period. All eggs from each pen on the three consecutive days were collected and labeled accordingly. Egg weight and specific gravity was determined for each egg prior to break out. Eggs were then broken out to determine albumen height and yolk color. Albumen height was determined using a tripod micrometer (Baxlo Precision, Barcelona, Spain). Yolk color values were determined using the Minolta CM-508d spectrophotometer (Minolta Co Ltd, Mississauga, ON, Canada). At the end of each 28-day collection period, an additional egg collection was made to evaluate the effect of storage length and storage temperature. Approximately eight to 12 eggs were collected and stored for each replication of each dietary treatment group. Eggs were randomly allotted to refrigeration storage or ambient temperature storage. The refrigerated eggs were stored at 7.22 degrees Celsius, while the eggs stored at ambient temperature were stored at 23.89 degrees Celsius.

On day three of storage, half of the eggs from both groups were broken out and all the same measurements were taken as described above. The egg yolks were separated and frozen for further analysis. The same procedure was followed for the remainder of the stored eggs on day seven of storage.

All data were analyzed by ANOVA as a completely randomized design using the GLM procedures in SAS (SAS Inst. Inc., Cary, NC). The three adjoining

cages containing nine layers was the experimental unit. Treatment means were separated by the LSD option of SAS at α level of $P < 0.05$.

RESULTS

The data for egg storage length and method are in Tables 4.1 to 4.6. For the first 28 days of the trial, egg weight was greater ($P<0.05$) for eggs at day zero of storage than for eggs at day three or day seven of storage. Specific gravity and Haugh units decreased ($P<0.05$) linearly with increased storage time. The L* values increased ($P<0.01$) linearly with increased storage time. The a* and b* values were not affected ($P>0.05$) by storage length. Specific gravity and Haugh units were decreased ($P<0.05$) for eggs stored at room temperature compared to eggs stored in refrigeration. The L* values were lower ($P<0.01$) for eggs stored in refrigeration compared to eggs stored at room temperature. Egg weight and a* and b* values were not affected ($P>0.05$) by storage conditions.

For the second 28 days of the trial, egg specific gravity and Haugh units decreased ($P<0.05$) linearly with increased storage time. The L* and b* values increased ($P<0.01$) with increased storage time. Egg weight and a* values were not affected ($P>0.05$) by storage length. Specific gravity and Haugh units were lower for eggs that were stored at room temperature compared to eggs that were refrigerated. The L*, a*, and b* values were decreased ($P<0.02$) in eggs that were refrigerated compared to eggs that were stored at room temperature. Egg weight was not affected ($P>0.05$) by storage conditions.

Table 4.1 Effect of egg storage method (room temperature vs. refrigerated) on quality of eggs stored for three or seven days from hens fed distiller's dried grains with solubles (DDGS) (first 28 days)[1]

Treatment	Egg weight, g	Specific gravity	L[2]	a[3]	b[4]	Haugh Units[5]
3 days						
Room temperature[6]						
No DDGS	62.44	1.072^{bc}	60.29^{a}	-1.23^{a}	31.59^{b}	87.89^{cdef}
10% DDGS+H[7]	60.21	1.070^{c}	60.21^{a}	-0.55^{a}	34.13^{b}	92.77^{abc}
10% DDGS-H[8]	62.72	1.075^{abc}	59.81^{a}	-0.51^{a}	33.78^{b}	87.01^{defg}
20% DDGS+H	63.34	1.072^{bc}	59.56^{ab}	-0.50^{a}	34.36^{b}	82.93^{fgh}
20% DDGS-H	62.70	1.072^{bc}	58.55^{abc}	-0.09^{a}	33.77^{b}	83.75^{efgh}
30% DDGS+H	63.24	1.073^{abc}	57.73^{bcd}	0.37^{a}	34.78^{b}	78.88^{h}
30% DDGS-H	61.74	1.072^{bc}	57.66^{cd}	0.25^{a}	36.34^{b}	83.75^{efgh}
Refrigerated[9]						
No DDGS	61.94	1.075^{abc}	57.53^{cd}	-0.99^{a}	31.03^{a}	94.47^{ab}
10% DDGS+H	62.76	1.078^{a}	57.32^{cde}	-0.76^{a}	32.17^{b}	97.66^{a}
10% DDGS-H	64.20	1.076^{ab}	57.31^{cde}	-0.55^{a}	32.97^{b}	92.58^{abc}
20% DDGS+H	61.28	1.077^{ab}	55.99^{def}	-0.70^{b}	32.85^{b}	92.72^{abc}
20% DDGS-H	61.58	1.074^{abc}	56.96^{cdef}	-0.24^{a}	30.78^{b}	90.74^{bcd}
30% DDGS+H	63.59	1.077^{ab}	55.14^{f}	0.34^{a}	31.94^{b}	88.43^{cde}
30% DDGS-H	64.74	1.076^{ab}	55.50^{ef}	0.44^{a}	34.56^{b}	82.44^{gh}
7 days						
Room temperature						
No DDGS	62.44^{ab}	1.072^{cd}	60.29^{a}	-1.23^{c}	31.59^{ef}	67.42^{d}
10% DDGS+H	60.21^{b}	1.070^{de}	60.21^{ab}	-0.55^{ab}	34.13^{abc}	76.18^{b}
10% DDGS-H	62.72^{a}	1.075^{cde}	59.81^{bc}	-0.51^{bc}	33.78^{cd}	73.07^{bcd}
20% DDGS+H	63.34^{ab}	1.072^{e}	59.56^{bc}	-0.50^{a}	34.36^{abc}	73.76^{bc}
20% DDGS-H	62.70^{a}	1.072^{de}	58.55^{c}	-0.09^{a}	33.77^{ab}	71.25^{bcd}
30% DDGS+H	63.24^{ab}	1.073^{de}	57.73^{bc}	0.37^{a}	34.78^{ab}	67.77^{cd}
30% DDGS-H	61.74^{a}	1.072^{cd}	57.66^{bc}	0.25^{a}	36.34^{a}	67.71^{cd}
Refrigerated						
No DDGS	63.54^{a}	1.072^{ab}	58.57^{d}	-1.17^{c}	29.91^{f}	88.07^{a}
10% DDGS+H	62.82^{ab}	1.072^{ab}	57.62^{def}	-0.82^{bc}	31.57^{def}	88.03^{a}
10% DDGS-H	62.45^{ab}	1.073^{ab}	57.97^{de}	-0.86^{bc}	31.82^{def}	87.36^{a}
20% DDGS+H	63.80^{a}	1.073^{a}	57.23^{def}	-0.21^{ab}	33.91^{cde}	90.06^{a}
20% DDGS-H	62.27^{ab}	1.074^{a}	56.24^{ef}	-0.31^{ab}	33.55^{cde}	86.15^{a}
30% DDGS+H	61.99^{ab}	1.069^{bc}	56.77^{ef}	-0.07^{a}	33.98^{cde}	87.01^{a}
30% DDGS-H	62.33^{ab}	1.074^{a}	56.01^{f}	0.30^{a}	34.84^{bcd}	88.99^{a}

(Table 4.1 continued)

Treatment	Egg weight, g	Specific gravity	L[2]	a[3]	b[4]	Haugh Units[5]
P-values (P<)						
Overall treatment	0.04	0.01	0.01	0.10	0.41	0.01
Storage method	0.58	0.01	0.01	0.16	0.38	0.01

[1]Data are means of 5 replicates with 9 layers per replicate.
[2] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.
[3] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.
[4] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.
[5] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[6] Approximately 23.89 degrees Celsius
[7] DDGS processed with heat
[8] DDGS processed without heat
[9] Approximately 7.22 degrees Celsius
[abcdefgh] Means with different superscripts within a column are different (P<0.05).

Table 4.2 Effect of egg storage method (room temperature vs. refrigerated) on quality of eggs stored for three or seven days from hens fed distiller's dried grains with solubles (DDGS) (second 28 days)[1]

Treatment	Egg weight, g	Specific gravity	L[2]	a[3]	b[4]	Haugh Units[5]
Day 3						
Room temperature[6]						
No DDGS	61.34^{b}	1.067^{cde}	63.81^{a}	-0.33^{cd}	33.65^{abcd}	88.87^{bc}
10% DDGS+H[7]	65.60^{a}	1.066^{e}	59.41^{b}	-0.38^{de}	34.53^{a}	87.38^{cd}
10% DDGS-H[8]	62.03^{ab}	1.069^{abcde}	59.60^{ab}	0.13^{abcd}	33.85^{abc}	87.52^{cd}
20% DDGS+H	64.51^{ab}	1.068^{bcde}	58.63^{bc}	0.28^{abc}	34.02^{ab}	84.67^{cd}
20% DDGS-H	63.62^{ab}	1.067^{cde}	56.43^{bcd}	0.40^{ab}	32.26^{abcde}	83.88^{cd}
30% DDGS+H	65.19^{ab}	1.066^{e}	53.44^{de}	0.41^{ab}	30.01^{ef}	81.48^{d}
30% DDGS-H	64.91^{ab}	1.070^{de}	55.72^{bcde}	0.47^{ab}	30.98^{bcdef}	82.07^{d}
Refrigerated[9]						
No DDGS	65.05^{ab}	1.070^{abcd}	57.73^{bc}	-0.98^{e}	30.36^{cdef}	98.50^{a}
10% DDGS+H	62.53^{ab}	1.068^{bcde}	56.64^{bcd}	-0.30^{cd}	30.33^{def}	98.29^{a}
10% DDGS-H	65.68^{a}	1.071^{ab}	56.53^{bcd}	-0.10^{bcd}	30.50^{cdef}	97.75^{a}
20% DDGS+H	64.17^{ab}	1.070^{abcd}	54.99^{cde}	0.66^{a}	31.77^{abcdef}	94.23^{ab}
20% DDGS-H	64.35^{ab}	1.071^{abc}	55.94^{bcde}	0.19^{abcd}	32.26^{abcde}	89.52^{bc}
30% DDGS+H	64.52^{ab}	1.070^{abcd}	51.87^{e}	0.24^{abcd}	31.51^{abcdef}	88.40^{bc}
30% DDGS-H	64.58^{ab}	1.072^{a}	53.27^{de}	0.27^{abc}	28.33^{f}	89.06^{bc}
Day 7						
Room temperature						
No DDGS	67.03^{a}	1.061^{c}	62.37^{a}	-0.85^{d}	33.81^{bcd}	70.60^{bc}
10% DDGS+H	63.32^{ab}	1.060^{c}	60.86^{ab}	-0.20^{c}	36.42^{abcd}	70.44^{bc}
10% DDGS-H	63.30^{ab}	1.061^{c}	61.89^{ab}	0.46^{ab}	36.87^{abcd}	74.70^{b}
20% DDGS+H	63.77^{ab}	1.062^{bc}	61.69^{ab}	0.45^{ab}	37.26^{abcd}	68.99^{bc}
20% DDGS-H	66.47^{ab}	1.061^{c}	60.11^{abc}	0.25^{abc}	36.70^{abcd}	67.78^{bc}
30% DDGS+H	63.69^{ab}	1.060^{c}	61.86^{ab}	0.34^{ab}	38.82^{abc}	67.58^{c}
30% DDGS-H	64.35^{ab}	1.063^{bc}	59.55^{bcd}	0.55^{a}	39.63^{ab}	74.79^{b}
Refrigerated						
No DDGS	61.87^{ab}	1.069^{a}	57.50^{cde}	-0.71^{d}	32.01^{d}	90.56^{a}
10% DDGS+H	64.22^{ab}	1.068^{c}	57.49^{cde}	-0.69^{d}	32.97^{cd}	89.70^{a}
10% DDGS-H	63.78^{ab}	1.068^{c}	57.07^{de}	-0.75^{d}	32.07^{d}	90.50^{a}
20% DDGS+H	63.87^{ab}	1.069^{a}	56.64^{e}	0.12^{abc}	33.02^{cd}	88.70^{a}
20% DDGS-H	62.59^{ab}	1.069^{a}	56.45^{e}	0.12^{abc}	33.17^{cd}	90.23^{a}
30% DDGS+H	61.22^{b}	1.064^{b}	56.45^{e}	0.01^{bc}	32.66^{d}	87.83^{a}
30% DDGS-H	63.20^{ab}	1.069^{a}	57.62^{cde}	0.16^{abc}	40.04^{a}	89.62^{a}

(Table 4.2 continued)

Treatment	Egg weight, g	Specific gravity	L[2]	a[3]	b[4]	Haugh Units[5]
P-values (P<)						
Overall treatment	0.28	0.01	0.01	0.03	0.01	0.01
Storage method	0.41	0.01	0.01	0.02	0.01	0.01

[1]Data are means of 5 replicates with 9 layers per replicate.
[2] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.
[3] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.
[4] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.
[5] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[6]Approximately 23.89 degrees Celsius
[7] DDGS processed with heat
[8] DDGS processed without heat
[9] Approximately 7.22 degrees Celsius
[abcdef] Means with different superscripts within a column are different (P<0.05).

Table 4.3 Effect of length of egg storage (0, 3, or 7 days) at room temperature[1] on quality of eggs from hens fed distiller's dried grains with solubles (DDGS) (first 28 days)[2]

Treatment	Egg weight, g	Specific gravity	L[3]	a[4]	b[5]	Haugh Units[6]
Day 0						
No DDGS	64.68	1.0796	53.68	-0.96	28.51	95.93
10% DDGS+H[7]	63.32	1.0796	53.85	-0.28	29.75	95.26
10% DDGS-H[8]	64.20	1.0796	54.06	-0.31	29.56	94.77
20% DDGS+H	64.06	1.0791	53.13	0.11	29.74	95.66
20% DDGS-H	63.59	1.0791	52.68	0.23	29.83	93.40
30% DDGS+H	64.53	1.0792	52.16	0.67	28.71	92.86
30% DDGS-H	64.38	1.0802	52.48	0.34	29.10	97.56
Day 3						
No DDGS	62.44	1.072	60.29	-1.23	31.59	87.89
10% DDGS+H	60.21	1.070	60.21	-0.55	34.13	92.77
10% DDGS-H	62.72	1.075	59.81	-0.51	33.78	87.01
20% DDGS+H	63.34	1.072	59.56	-0.50	34.36	82.93
20% DDGS-H	62.70	1.072	58.55	-0.09	33.77	83.75
30% DDGS+H	63.24	1.073	57.73	0.37	34.78	78.88
30% DDGS-H	61.74	1.072	57.66	0.25	36.34	83.75
Day 7						
No DDGS	61.74	1.066	63.35	-1.18	30.78	67.42
10% DDGS+H	58.82	1.064	62.33	-0.41	35.55	76.18
10% DDGS-H	63.90	1.066	60.79	-0.83	34.45	73.07
20% DDGS+H	62.91	1.062	60.86	0.21	36.55	73.76
20% DDGS-H	63.68	1.064	60.41	0.11	37.95	71.25
30% DDGS+H	63.28	1.063	61.34	0.06	37.82	67.77
30% DDGS-H	63.89	1.066	60.57	0.29	38.57	67.71
P-values (P<)						
Overall treatment	0.04	0.01	0.01	0.10	0.41	0.01
Storage length	0.01	0.01	0.01	0.14	0.31	0.01

[1] Approximately 23.89 degrees Celsius

[2]Data are means of 5 replicates with 9 layers per replicate.

[3] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

[4] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

[5] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

(Table 4.3 continued)
[6] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[7]DDGS processed with heat
[8] DDGS processed without heat

Table 4.4 Effect of length of egg storage (0, 3, or 7 days) in refrigeration[1] on quality of eggs from hens fed distiller's dried grains with solubles (DDGS) (first 28 days)[2]

Treatment	Egg weight, g	Specific gravity	L[3]	a[4]	b[5]	Haugh Units[6]
Day 0						
No DDGS	64.68	1.0796	53.68	-0.96	28.51	95.93
10% DDGS+H[7]	63.32	1.0796	53.85	-0.28	29.75	95.26
10% DDGS-H[8]	64.20	1.0796	54.06	-0.31	29.56	94.77
20% DDGS+H	64.06	1.0791	53.13	0.11	29.74	95.66
20% DDGS-H	63.59	1.0791	52.68	0.23	29.83	93.40
30% DDGS+H	64.53	1.0792	52.16	0.67	28.71	92.86
30% DDGS-H	64.38	1.0802	52.48	0.34	29.10	97.56
Day 3						
No DDGS	61.94	1.075	57.53	-0.99	31.03	94.47
10% DDGS+H	62.76	1.078	57.32	-0.76	32.17	97.66
10% DDGS-H	64.20	1.076	57.31	-0.55	32.97	92.58
20% DDGS+H	61.28	1.077	55.99	-0.70	32.85	92.72
20% DDGS-H	61.58	1.074	56.96	-0.24	30.78	90.74
30% DDGS+H	63.59	1.077	55.14	0.34	31.94	88.43
30% DDGS-H	64.74	1.076	55.50	0.44	34.56	82.44
Day 7						
No DDGS	63.54	1.072	58.57	-1.17	29.91	88.07
10% DDGS+H	62.82	1.072	57.62	-0.82	31.57	88.03
10% DDGS-H	62.45	1.073	57.97	-0.86	31.82	87.36
20% DDGS+H	63.80	1.073	57.23	-0.21	33.91	90.06
20% DDGS-H	62.27	1.074	56.24	-0.31	33.55	86.15
30% DDGS+H	61.99	1.069	56.77	-0.07	33.98	87.01
30% DDGS-H	62.33	1.074	56.01	0.30	34.84	88.99
P-values (P<)						
Overall treatment	0.04	0.01	0.01	0.10	0.41	0.01
Storage length	0.01	0.01	0.01	0.14	0.31	0.01

[1] Approximately 7.22 degrees Celsius

[2]Data are means of 5 replicates with 9 layers per replicate.

[3]L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

[4] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

[5] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

(Table 4.4 continued)
[6]The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[7] DDGS processed with heat
[8] DDGS processed without heat

Table 4.5 Effect of length of egg storage (0, 3, or 7 days) at room temperature[1] on quality of eggs from hens fed distiller's dried grains with solubles (DDGS) (second 28 days)[2]

Treatment	Egg weight, g	Specific gravity	L[3]	a[4]	b[5]	Haugh Units[6]
Day 0						
No DDGS	63.58	1.0742	52.89	-0.62	27.77	95.92
10% DDGS+H[7]	64.26	1.0719	53.32	-0.42	27.86	94.01
10% DDGS-H[8]	65.09	1.0736	54.50	-0.07	29.41	95.22
20% DDGS+H	65.45	1.0727	53.73	0.37	30.34	91.60
20% DDGS-H	63.12	1.0704	54.54	0.32	31.22	91.06
30% DDGS+H	64.41	1.0722	52.76	0.19	28.35	93.86
30% DDGS-H	64.50	1.0737	52.41	0.23	28.81	95.33
Day 3						
No DDGS	61.34	1.067	63.81	-0.33	33.65	88.87
10% DDGS+H	65.60	1.066	59.41	-0.38	34.53	87.38
10% DDGS-H	62.03	1.069	59.60	0.13	33.85	87.52
20% DDGS+H	64.51	1.068	58.63	0.28	34.02	84.67
20% DDGS-H	63.62	1.067	56.43	0.40	32.26	83.88
30% DDGS+H	65.19	1.066	53.44	0.41	30.01	81.48
30% DDGS-H	64.91	1.070	55.72	0.47	30.98	82.07
Day 7						
No DDGS	67.03	1.061	62.37	-0.85	33.81	70.60
10% DDGS+H	63.32	1.060	60.86	-0.20	36.42	70.44
10% DDGS-H	63.30	1.061	61.89	0.46	36.87	74.70
20% DDGS+H	63.77	1.062	61.69	0.45	37.26	68.99
20% DDGS-H	66.47	1.061	60.11	0.25	36.70	67.78
30% DDGS+H	63.69	1.060	61.86	0.34	38.82	67.58
30% DDGS-H	64.35	1.063	59.55	0.55	39.63	74.79
P-values (P<)						
Overall treatment	0.28	0.01	0.01	0.03	0.01	0.01
Storage length	0.54	0.01	0.01	0.41	0.01	0.01

[1] Approximately 23.89 degrees Celsius

[2]Data are means of 5 replicates with 9 layers per replicate

[3] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

[4] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

(Table 4.5 continued)
[5] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.
[6] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[7] DDGS processed with heat
[8] DDGS processed without heat

Table 4.6 Effect of length of egg storage (0, 3, or 7 days) in refrigeration[1] on quality of eggs from hens fed distiller's dried grains with solubles (DDGS) (second 28 days)[2]

Treatment	Egg weight, g	Specific gravity	L[3]	a[4]	b[5]	Haugh Units[6]
Day 0						
No DDGS	63.58	1.0742	52.89	-0.62	27.77	95.92
10% DDGS+H[7]	64.26	1.0719	53.32	-0.42	27.86	94.01
10% DDGS-H[8]	65.09	1.0736	54.50	-0.07	29.41	95.22
20% DDGS+H	65.45	1.0727	53.73	0.37	30.34	91.60
20% DDGS-H	63.12	1.0704	54.54	0.32	31.22	91.06
30% DDGS+H	64.41	1.0722	52.76	0.19	28.35	93.86
30% DDGS-H	64.50	1.0737	52.41	0.23	28.81	95.33
Day 3						
No DDGS	65.05	1.070	57.73	-0.98	30.36	98.50
10% DDGS+H	62.53	1.068	56.64	-0.30	30.33	98.29
10% DDGS-H	65.68	1.071	56.53	-0.10	30.50	97.75
20% DDGS+H	64.17	1.070	54.99	0.66	31.77	94.23
20% DDGS-H	64.35	1.071	55.94	0.19	32.26	89.52
30% DDGS+H	64.52	1.070	51.87	0.24	31.51	88.40
30% DDGS-H	64.58	1.072	53.27	0.27	28.33	89.06
Day 7						
No DDGS	61.87	1.069	57.50	-0.71	32.01	90.56
10% DDGS+H	64.22	1.068	57.49	-0.69	32.97	89.70
10% DDGS-H	63.78	1.068	57.07	-0.75	32.07	90.50
20% DDGS+H	63.87	1.069	56.64	0.12	33.02	88.70
20% DDGS-H	62.59	1.069	56.45	0.12	33.17	90.23
30% DDGS+H	61.22	1.064	56.45	0.01	32.66	87.83
30% DDGS-H	63.20	1.069	57.62	0.16	40.04	89.62
P-values (P<)						
Overall treatment	0.28	0.01	0.01	0.03	0.01	0.01
Storage length	0.54	0.01	0.01	0.41	0.01	0.01

[1] Approximately 7.22 degrees Celsius

[2]Data are means of 5 replicates with 9 layers per replicate.

[3] L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

[4] a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

[5] b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

(Table 4.6 continued)
[6] The Haugh unit is a measure of egg protein quality based on the height of its albumen. The formula for calculating the Haugh unit is: HU = 100 * log(h-1.7w^{0.37} + 7.6), where: HU = Haugh unit, h = observed height of the albumen in millimeters, and w = weight of egg in grams.
[7]DDGS processed with heat
[8] DDGS processed without heat

DISCUSSION

The objective of this experiment was to determine the effects that storage length and conditions have on egg quality and yolk color. Scott and Silversides (2000) conducted an experiment where samples of eggs were stored for periods of 1, 3, 5, and 10 days at room temperature. They report that the principal changes with storage were decreasing albumen and egg weights. Our results partly agree with these findings. While egg weight during the first 28 days of the trial was lower for stored eggs, storage time did not affect egg weight during the second 28 days of the trial. Specific gravity and Haugh units were decreased linearly for both collection periods which indicated a decrease in egg quality. These results confirm our hypothesis. It was hypothesized that storage time would not affect egg yolk color, however, yolk lightness (L* value) increased with storage time for both collection periods. Yolk redness (a* value) was not affected by storage length, while yolk yellowness (b* value) increased only in the second 28 day period of the trial. These results indicate a need for further study, and possibly a chemical analysis of the eggs yolks to determine the effects of storage length on lutein content.

Specific gravity and Haugh units were decreased for the eggs that were stored at room temperature compared to eggs stored in refrigeration. These

results also agree with our hypothesis. Yolk lightness (L*) was decreased in eggs stored in refrigeration for both collection periods, while yolk redness (a*) and yellowness (*b) were not affected in the first 28 days of the trial and were decreased in eggs refrigerated in the second 28 days of the trial. This also indicates a need for further analysis of egg yolks for lutein content.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The purpose of this research was to determine the optimal inclusion rate of DDGS in laying hen diets, as well as to determine the effect of DDGS on hen egg production and egg quality characteristics. Additionally, an objective was to determine the effect of storage length and conditions on egg quality and egg yolk color of eggs from hens fed DDGS.

Determining the optimal inclusion rate of DDGS was evaluated with three inclusion levels. A 20% inclusion rate of DDGS is widely thought to be the most suitable inclusion rate, but with cost being an important factor in industry when determining what ingredients go into feed, it is important to know if a lower inclusion rate still provides positive results on yolk color and content. Results indicated that yolk color is still increased at a 10% DDGS inclusion rate. A 30% DDGS inclusion rate also was evaluated to determine if the increase in yolk color and content plateaued, or if higher DDGS inclusion levels deposited more xanthophyll in the yolk. Results indicated that 30% inclusion level did increase yolk color compared to eggs from hens fed diets containing 10% or 20% DDGS.

Two different sources of DDGS were used to determine if the conventional heat processing of DDGS has a lower feeding value than DDGS processed without heat. Based on our results, no significant differences were observed between hens fed either of the two sources of DDGS.

Increased storage time and temperature did decrease egg quality as expected. Yolk lightness (L*) increased with storage time and decreased with

refrigeration. Yolk redness (*a) remained unchanged during storage except for a decrease in refrigerated eggs during the second 28 days of the trial. Yolk yellowness (b*) was not affected during the first 28 days of the trial, but was affected during the second 28 days of the trial. Yolk yellowness was increased with increased storage time, but decreased with refrigeration. These results were unexpected as the hypothesis was that yolk color would remain unchanged.

Based on the results of these experiments, further research is needed to determine the most efficient and cost effective inclusion level of DDGS in laying hen diets, as well as to use chemical analysis to determine the effects of storage time and temperature on the level of xanthophyll deposition in the egg yolk.

REFERENCES

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

Brunet, L.R., D.R. Ingram, J.N. Losso, and T.A. Lavergne. 2013. The effects of distillers dried grains with solubles on egg production and yolk color. Poult. Sci. 92 (E-Suppl.1): 137.

Choen, Y.J., H.L. Lee, M.H. Shin, A. Jang, S.K. Lee, J.H. Lee, B.D. Lee, and C.K. Son. 2008. Effects of corn distiller's dried grains with solubles on production and egg quality in laying hens. Asian-Australasian Journal of Animal Sciences. 21, 9:1318.

Doppenberg, J. and P. van der Aar. 2007. Biofuels: implications for the feed industry. Wageningen Academic Publishers, The Netherlands.

Eke, M.O., N.I. Olaitan, and J.H. Ochefu. 2013. Effect of storage conditions on the quality attributes of shell (table) eggs. NIFOJ Vol. 31 No. 2, pages 18 – 24.

Hahn-Didde, D., and S.E. Purdum. 2014. The effects of an enzyme complex in moderate and low nutrient-dense diets with dried distillers grains with solubles in laying hens. J. Appl. Poult. Res. 23:23–33.

Heiting, G. and S. Jegtvig. 2012. Lutein and Zeaxanthin: Eye and Vision Benefits. http://www.allaboutvision.com/nutrition/lutein.htm

Hobbs, R.P. and P.S. Bernstein. 2014. Nutrient supplementation for age-related macular degeneration, cataract, and dry eye. J Ophthalmic Vis Res. 9(4): 487–493.

Hunter Associates Laboratory Inc. 2012. Measuring Color using Hunter L, a, b. versus CIE 1976 L*a*b*. http://www.hunterlab.com/an-1005b.pdf?r=false

Hy -Line International. 2012. Hy-Line W-36 Performance Standards Manual. Hy-Line International, West Des Moines, IA.

Hy -Line International. 2015. Product Information: Hy-Line W-36. http://www.hyline.com/aspx/products/productinformation.aspx

Jiang, W., L. Zhang, and A. Shan. 2013. The effect of vitamin E on laying performance and egg quality in laying hens fed corn dried distillers grains with solubles. Poultry Science 92:2956–2964.

Leeson, S. and L. Caston. 2004. Enrichment of eggs with lutein. Poultry Science 83:1709–1712.

Martinez-Amezcua, C., C.M. Parsons, V. Singh, R. Srinivasan, and G.S. Murthy. 2007. Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. Poultry Science 86:2624–2630.

Masa'deh, M.K., S.E. Purdum, and K.J. Hanford. 2011. Dried distillers grains with solubles in laying hen diets. Poultry Science 90:1960-1966.

Meunier, R.A., and M.A. Latour. 2000. Commercial Egg Production and Processing. Purdue University. Pub. AS-545-W.

Moeller, S.M., P.F. Jacques, and J.B. Blumberg. 2000. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. Journal of the American College of Nutrition, Vol. 19, No. 5, 522S–527S.

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

Perry, A., H. Rasmussen, and E.J. Johnson. 2008. Xanthophyll (lutein, zeaxanthin) content in fruits, vegetables and corn and egg products. Journal of Food Composition and Analysis 22:9-15.

Purdum, S., K. Hanford, and B. Kreifels. 2014. Short-term effects of lower oil dried distillers grains with solubles in laying hen rations. Poultry Science 93:2592–2595.

Roberson, K.D., J.L. Kalbfleisch, W. Pan, and R.A. Charbeneau. 2005. Effect of corn distiller's dried grains with solubles at various levels on performance of laying hens and egg yolk color. International Journal of Poultry Science 4 (2): 44-51.

Rosentrater, K. A. 2006. Some physical properties of distillers dried grains with solubles (DDGS). Appl. Eng. Agric. 22(4): 589-595.

Salim, H.M., Z.A. Kruk, and B.D. Lee. 2010. Nutritive value of corn distillers dried grains with solubles as an ingredient of poultry diets: A review. World's Poultry Science Journal, Vol. 66:411.

Scott, T.A. and F.G. Silversides. 2000. The effect of storage and strain of hen on egg quality. Poultry Science 79:1725–1729.

Sert, D., A. Aygun, and M.K. Demir. 2011. Effects of ultrasonic treatment and storage temperature on egg quality. Poultry Science 90:869–875.

Shurson, J. 2013. Production, nutritional value, and physical characteristics of high quality u.s. corn ddgs. http://www.ddgs.umn.edu/prod/groups/cfans/@pub/@cfans/@ddgs/documents/asset/cfans_asset_414238.pdf.

Solomon, B.D., J. R. Barnes, and K. E. Halvorsen. 2007. Grain and cellulosic ethanol: history, economics, and energy policy. Biomass and Bioenergy 31:416-425.

Sun, H., E.J. Lee, H. Samaraweera, M. Persia, and D.U. Ahn. 2013. Effects of increasing concentrations of corn distillers dried grains with solubles on chemical composition and nutrient content of egg. Poultry Science 92:233–242.

Sun, H., E.J. Lee, H. Samaraweera, M. Persia, H.S. Ragheb, and D.U. Ahn. 2012. Effects of increasing concentrations of corn distillers dried grains with solubles on the egg production and internal quality of eggs. Poultry Science 91:3236–3246.

Surai, P.F., and N.H.C. Sparks. 2001. Designer eggs: from improvement of egg composition to functional food. Trends in Food Science & Technology 12:7–16.

Swiatkiewicz, S. and J. Koreleski. 2006. Effect of maize distillers dried grains with solubles and dietary enzyme supplementation on the performance of laying hens. Journal of Animal and Feed Sciences 253–260.

Swiatkiewicz, S. and J. Koreleski. 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World's Poultry Science Journal 64: 257-266.

U.S. Department of Energy. 2014. How much ethanol is produced, imported, and consumed in the United States? http://www.eia.gov/tools/faqs/faq.cfm?id=90&t=4

Wenzel, M., I. Seuss-Baum, and E. Schlich. 2011. Influences of storage time and temperature on the xanthophyll content of freeze-dried egg yolk. Food Chemistry 124:1343–1348.

Wisner, R. 2015. Ethanol usage projections & corn balance sheet. Agricultural Marketing Resource Center.

Wu-Haan, W., W. Powers, R. Angel, and T.J. Applegate. 2010. The use of distillers dried grains plus solubles as a feed ingredient on air emissions and performance from laying hens. Poultry Science 89:1355–1359.

APPENDIX

NUTREINT ANALYSIS OF TREATMENT DIETS

Treatment diet	Dry matter, %	Ash, %	Crude protein, %
First mixing			
No DDGS	90.00	12.10	16.33
10% DDGS+H	89.65	11.35	16.75
10% DDGS-H	89.75	12.65	15.97
20% DDGS+H	89.30	11.30	17.44
20% DDGS-H	89.60	13.05	17.10
30% DDGS+H	88.60	11.40	19.39
30% DDGS-H	89.65	11.40	17.83
Second mixing			
No DDGS	89.35	10.00	15.39
10% DDGS+H	82.90	9.40	16.83
10% DDGS-H	89.00	8.10	16.55
20% DDGS+H	89.15	9.05	15.41
20% DDGS-H	89.35	12.45	17.59
30% DDGS+H	88.85	12.45	17.62
30% DDGS-H	89.55	12.35	17.77

Vita

Lindsay Renee Brunet, daughter of Bob and Debby Brunet, was born in south Louisiana, in November of 1990. Lindsay is the youngest of four children. She attended a college preparatory high school at Sacred Heart High School in Ville Platte, Louisiana. After graduating in 2009, she attended Louisiana State University at Eunice for one year before transferring to Louisiana State University in Baton Rouge, Louisiana. In May of 2013, she completed a Bachelor of Science degree with a concentration in animal sciences. In June of 2013, she began her pursuit of a Master of Science degree with a concentration in animal sciences at Louisiana State University.