EFFECTS OF A PROPRIETARY PREMIX ON PRODUCTIVE PERFORMANCE AND EGG QUALITY OF WHITE AND BROWN EGG LAYING HENS FED DIETS HIGH IN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS)

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THESIS

A thesis submitted in partial fulfillment of the
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By

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2013

ABSTRACT OF THESIS

EFFECTS OF A PROPRIETARY PREMIX ON PRODUCTIVE PERFORMANCE AND EGG QUALITY OF WHITE AND BROWN EGG LAYING HENS FED DIETS HIGH IN DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS)

Abstract

The objective of this thesis was to evaluate inclusion of up to 35% by-product DDGS (with reduced Ca and P) with and without addition of a proprietary premix (enzyme and antioxidant; Alltech Inc.) on productive performance and egg quality of white and brown egg laying hens. A total of 288 white or brown hens were randomly allocated to one of the following treatments: 1) corn-soybean meal (control), 2) 25% DDGS, 3) 25% DDGS plus premix, 4) 35% DDGS, and 5) 35% DDGS plus premix. Hen body weight values were impaired with addition of 25 and 35% DDGS when compared to the control. Premix helped maintain body weight comparable to control in the brown hens; however was not noted in white hens. In the second dietary phase, addition of DDGS increased feed intake in white egg laying hens and was maintained with the premix. Brown hens on premix came into lay faster than the control and DDGS diets, but DDGS reduced overall egg production. Haugh units, yolk color, and purchase intent scores were increased in brown eggs with DDGS diets. White eggs had increased yolk color with DDGS. In conclusion, up to 35% DDGS is acceptable in white laying hen diets.

KEYWORDS: enzyme and antioxidant, distillers dried grains with solubles, laying hens, production, egg quality

Megan van Benschoten

November 19, 2013

PROGRAMMED NUTRITION STRATEGY ON PERFORMANCE AND EGG QUALITY OF BROWN AND WHITE LAYING HENS FED DISTILLERS DRIED GRAINS WITH SOLUBLES (DDGS) THROUGH A FULL PRODUCTION CYCLE

By

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November 19, 2013

Date

In dedication to my loving grandfathers George McNabb and Harvey van Benschoten for this would not have been possible without their exemplarily work ethic and unwavering support. I am forever grateful for your infinite love.

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CHAPTER 1. Literature review

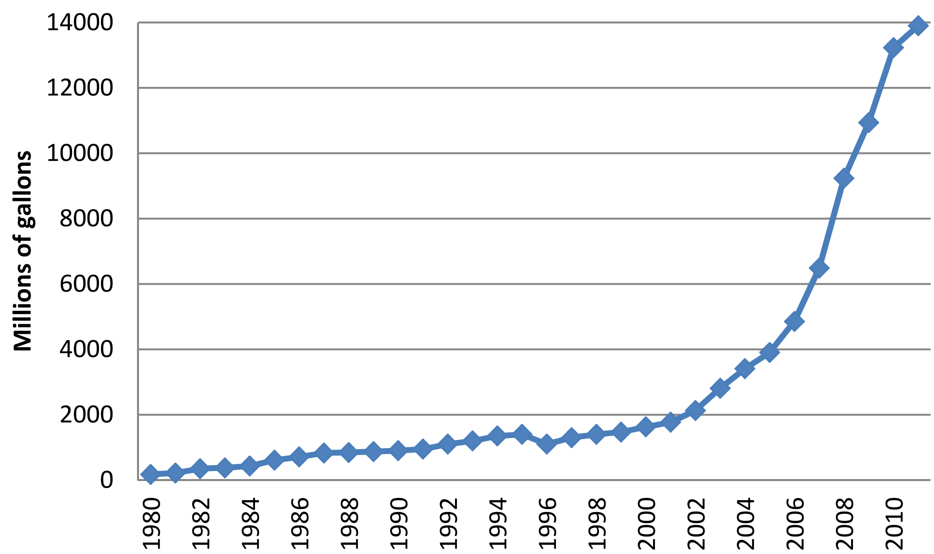
1.1 Introduction

A by-product of the ethanol industry, distillers dried grains with solubles (DDGS), has been a feed ingredient in many commercial poultry diets for the past decade. However, the literature is still inconsistent as the upper limit that can be included without negative effects on production. Inconsistency exists in the nutrient values of DDGS due to the quality of the corn and variations in processing and drying methods. Increasing demands for grains in both feed and ethanol industries have increased the supply of corn DDGS while limiting the availability of high quality grains for animal production. Feed additives have been developed to help include higher levels of these less digestible by-product feeds without sacrificing production and while attempting to lower costs for producers.

1.1.1 History of DDGS

Distillers dried grains with solubles from both fuel and alcohol production, have been researched as a feed ingredient in animal diets since the early 1970's. Yet ethanol production for fuel dates back much farther as Henry Ford fueled the Model T car with ethanol in 1908. The push for commercial use of DDGS in livestock and poultry diets occurred with the Energy Policy Act of 2005 and the Energy Independence and Security Act of 2007, making the United States the world's largest producer of ethanol [1]. These policies were enacted to both provide fuel for our domestic needs and produce a fuel with clean air emissions. These acts increased the demand for grains for fuel (Figure 1.1) around 2005 and subsequently increased the price of high quality grains and availability of by-product feedstuffs, such as DDGS, for animal consumption. In 2011, the estimated fuel ethanol production reached approximately 14,000 million gallons [1].

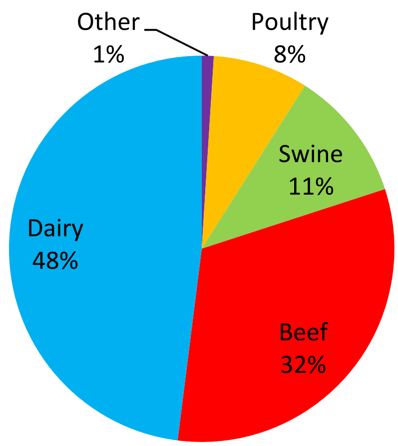
Figure 1.1 Estimated Historic U.S. Fuel Ethanol Production[1].



[1]Adapted from [1].

A steady demand of corn for ethanol production from 2011-2040 was anticipated in the Annual Energy Outlook 2013, with an annual growth of -0.1% for renewable energy consumption [2]. Therefore, over the next 29 years, not much change is expected in the demand for corn for fuel ethanol. Using the 2011 records to predict the need of corn in the future, roughly five billion bushels of corn will be needed each year for ethanol production (40% of total U.S. corn crop in 2011); creating approximately 39.4 million metric tons of by-product DDGS for livestock use [3]. Poultry used roughly eight percent of the total U.S. DDGS supply in 2011 (Figure1.2) [1]. The wide use of increased levels of DDGS in poultry diets is partially limited due to the quality and variability among nutrient composition of DDGS sources. However, recent technology has improved the digestibility of DDGS and increased the amount of DDGS that can be added to poultry diets.

Figure 1.2 Estimated Consumption of DDGS by Species in 2011[1].



[1]Reproduced from [1].

This thesis will focus on the effects of feeding higher levels (25 and 35%) of DDGS through a full laying cycle using white and brown shell producing laying hens with and without the addition of a proprietary premix [4]. The proprietary premix utilized will herein be referred to as premix and contains enzyme and antioxidant technologies said to release more nutrients from less digestible feedstuffs. Chapter 2 describes the evaluation of the proprietary premix on the productive performance and egg quality of white egg shell laying hens fed DDGS diets through 59 weeks of production. The effects of the proprietary premix on the productive performance and egg quality of brown shell laying hens fed DDGS diets through 60 weeks of production is described in Chapter 3. Chapter

4 of this thesis will summarize the effects of high levels of DDGS in both white and brown laying hen diets and provided conclusions.

1.2 Ethanol Production and DDGS

1.2.1 Process of making ethanol

There are two main ways to make ethanol from corn and other grains: dry-milling and wet-milling. The wet-milling process produces by-product feedstuffs like corn gluten meal and corn germ meal. The dry-milling process produces the by-product DDGS and will be focused on in this thesis. It is estimated that the production of 2.8 gallons of ethanol requires one bushel of corn (56 pounds) and produces approximately 17.5 pounds of DDGS when the dry-mill process is used [3]. Dry-milling is a multistep process that includes grinding, cooking and saccharification, fermentation, distillation, oil extraction, and co-product production [5].

The dry-milling process starts with the grinding of whole kernel through a screen usually ranging from five to eight millimeters in diameter [6]. Water and recycled stillage are then mixed and heated with the finely ground corn and amylolytic enzymes are added to aid in the release of starch and glucose. During the fermentation process, yeast (namely *Saccharomyces cerevisiae*) converts glucose into ethanol and carbon dioxide [7]. The ethanol is then collected via a distillation process to maintain its purity. After distillation, water is then removed from the mash and the stillage is then heated and centrifuged during the oil extraction process. Lastly, an evaporator removes the final amount of moisture and the DDGS by-product is available for transport and use [5].

1.2.2 Distillers dried grains with solubles as a feed ingredient

Although much of the starch is extracted from the corn during the fermentation process, DDGS are still considered a good source of energy, protein, water-soluble vitamins, and minerals [8]. As corn starch is fermented into ethanol and CO_2, the concentration of the remaining nutrients in DDGS increase by two-to-three-fold [9]. Therefore, some of the more costly components required in poultry diets (i.e. energy, protein, and phosphorus) are found in DDGS that can be partially substituted for corn, soybean-meal, and supplemental phosphorus in the diet to help decrease some of costs in laying hen rations.

According to the Nutrient Requirements of Poultry (NRC), DDGS contain: metabolizable energy (2,480 kcal/kg), protein (27.4%), fat (9%), crude fiber (9.1%), phosphorus (0.72%), [10]. The NRC (1994) also states that corn grain contains: metabolizable energy (3,350 kcal/kg), protein (8.5%), fat (3.8%), crude fiber (2.2%), phosphorus (0.28%). Both DDGS and corn grain provide minerals and vitamins; however, DDGS are more concentrated (except pyridoxine and thiamin) in the following: calcium (0.02 vs. 0.17%), potassium (0.65 vs.0.30%), chlorine (0.17 vs. 0.04%), iron (290 vs. 45 mg/kg), magnesium (0.19 vs. 0.12%), manganese (24 vs. 7 mg/kg), sodium (0.48 vs. 0.02%), sulfur (0.30 vs. 0.08%), copper (57 vs. 3 mg/kg), selenium (0.39 vs. 0.03 mg/kg), zinc (80 vs. 18 mg/kg), biotin (0.78 vs. 0.06 mg/kg), choline (4,842 vs.600 mg/kg), folacin (0.9 vs.0.4 mg/kg), niacin (71 vs. 24 mg/kg), pantothenic acid (11.0 vs. 4.0 mg/kg), pyridoxine (2.2 vs. 7.0 mg/kg), riboflavin (8.6 vs.1.0 mg/kg), thiamin (2.9 vs. 3.5 mg/kg), and vitamin E (40 vs. 22 mg/kg).

Table 1.1 Compositions of DDGS and Corn[1]

Composition	***Percent of Diet (%)****	
	Distillers' grains with solubles, dehydrated	**Corn grain**
Dry Matter	92.0	89.0
ME, kcal/kg	2,480	3,350
Protein	27.4	8.5
Ether Extract	9.0	3.8
Crude Fiber	9.1	2.2
Calcium	0.17	0.02
Total Phosphorus	0.72	0.28
Non-phytate Phosphorus	0.39	0.08
Sodium	0.48	0.02

[1]Adapted from [10].
*As-fed basis.

When compared to corn grain, there is less metabolizable energy in the by-product DDGS (2,480 vs. 3,350 kcal/kg) due to removal of starch during the ethanol production process. The decrease in energy due to the removal of starch concentrates the remaining nutrients two-to-three-fold. Therefore compared with corn, DDGS contains more fat (9.0 vs. 3.8%), fiber (9.1 vs. 2.2%), calcium (0.17 vs. 0.02%), and sodium (0.48 vs. 0.02). More importantly, there is greater availability of phosphorus and increased protein in the DDGS by-product. The increased phosphorus in DDGS (0.39 vs. 0.08%) [10] is partially due to the reduction in phytic acid content during the fermentation process [11]. Exogenous enzymes like phytase also have the potential to further increase the availability of phosphorus by releasing more of the phytate-bound phosphorus from the total phosphorus (0.72%) in DDGS. The protein content of DDGS is also much greater than that of corn (27.4 vs. 8.5%). Distillers dried grains with solubles are more concentrated than corn in the following amino acids: arginine, glycine, serine, histdine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan and valine, however the availability in DDGS could be a concern [10].

1.2.3 Variability in DDGS

Distillers dried grains with solubles are a highly variable feed ingredient causing some negative impacts on the market value of DDGS [12]. It was originally thought that differences in corn caused much of the variation in the DDGS. However, Belyea et al. [13] disputed this by taking samples of both corn and DDGS from a plant for a five year period. There was little to no variation in the corn prior to processing, suggesting that most of the variability in DDGS comes from other sources. Instead, most of the variability in DDGS can be attributed to corn fractioning prior to processing and processing conditions like the duration of processing [12], the poorly controlled mixing of distillers grains and wet solubles near the end of processing [13, 14] and the variability of temperatures used to dry the DDGS [12, 15].

Several studies have looked at the nutrient composition of DDGS from different sources- both brewers and ethanol plants. Cromwell et al. [8] looked at seven different samples from the beverage industry and two different samples from the fuel industry. They found similar values in DM (90.5%), CP (26.9%), and ether extract (9.7%) to the 1994 NRC for poultry [10]. However, the ranges for CP (23.4 to 28.7%) and ether extract (9 to 12%) were quite different. The average value for ether extract was 9.7%: yet, one sample contained only 2.9%. Lysine concentrations were also highly variable (0.43 to 0.89%) [8] and thought to be related to the color of the DDGS, as the lowest lysine concentrations were correlated to the darkest colored DDGS samples. It is thought that the darkness in color and degradation of lysine could have been due to overheating of the DDGS during processing [8, 16].

Spiehs et al. [17] also studied the variability in DDGS using samples from ten different ethanol plants over a three year span. The DM values averaged 88.9% with a range of 88.4 to 90.2%. The values reported for CP (30.2%) and crude fat (10.9%) [17], were higher than the values reported by others [10, 18]. Crude fiber was reported to be 8.8% [17] and was similar to the listed NRC value [10]. Authors found that of the amino acids analyzed, lysine was the most variable ranging from 0.72 to 1.02% with an average lysine concentration of 0.85%. They also stated that the second most variable amino acid was methionine as it ranged from 0.49 to 0.69% with an average of 0.55% [17]. They concluded that the variation in samples of DDGS was likely due to the differences between plants and differences between the years of sampling [17].

Lysine is one of the most important and variable nutrients in DDGS [19]. The variability in lysine availability in DDGS is attributed to the drying process as the exposure of the DDGS to high temperatures that can range from 315°C to 621°C [11, 15]. Several studies have reported values for lysine. The lysine concentration of 0.85% reported by Spiehs et al. [17] is higher than the lysine values that were reported by the NRC (0.75%) [10] and Cromwell et al. (0.70%) [18]. To evaluate the effect of heat treatments on the bioavailability of lysine, Martinez-Amezcua and Parsons [11] performed a study comparing four different heat processing methods with the following treatments: original DDGS, autoclaved DDGS, oven-dried plus autoclaved DDGS, and oven-dried DDGS. The heat treatments decreased the availability of all amino acids and the autoclaved treatments resulted in the lowest amino acid availabilities. Lysine availability values dropped from 0.9% in the original DDGS to 0.4%, 0.3%, and 0.6% in the autoclaved DDGS, oven-dried plus autoclaved DDGS, and oven-dried DDGS, respectively [11]. Therefore, lysine availability in DDGS samples can be extremely variable based on the heating treatments used in different ethanol processing plants.

Phosphorus is another costly ingredient in laying hen diets. There is the potential to more cost effectively provide phosphorus using by-product DDGS instead of supplemental phosphorus. In addition to the price, the availability of phosphorus is important because extra phosphorus excreted in manure can pose environmental concerns [15]. The previous study mentioned by Martinez-Amezcua and Parsons [11] also examined different processing methods on the bioavailability of phosphorus in different DDGS samples. They found that although increased heating of DDGS reduced lysine availability, heating treatments improved the bioavailability of phosphorus. They also concluded that there was no effect of particle size on phosphorus availability [11].

Differences in the availability of phosphorus are mostly due to the amount of phytate-bound phosphorus. The 1994 NRC lists that only 0.39% of the 0.72% total phosphorus is non-phytate phosphorus; therefore, only 54% of the total phosphorus is available [10] which is consistent with the range (54-68%) reported by Lumpkins and Batal [15]. However, other factors can greatly affect the phosphorus content of DDGS such as the amount of grains versus solubles added back to the mash during processing the differences in processing methods [16]. Martinez-Amezcua et al. [16] found phosphorus content of the grains and the solubles to be 0.39% and 1.24%, respectively, with an average concentration of 0.72%. The average phosphorus concentration is consistent with the total phosphorus concentrations reported by other research studies

[10, 15, 20], but lower than the 0.89% reported by Spiehs et al. [17], suggesting that the concentration of phosphorus is vastly variably between processing plants.

Although DDGS have reduced energy availability compared to the original grains due to the extraction of starch, they an increased concentration of important amino acids (lysine, methionine, etc.), phosphorus, and other vitamins. Variation in the amount of protein and fat is attributed to the processing methods used during fermentation and affects the market value of DDGS [13]. Other nutrients that affect the use of DDGS include minerals, lysine, and methionine [19]. Despite the variability of nutrients, the use of DDGS in laying hen diets could be efficacious as a substitute for costly nutrients like phosphorus and lysine. Distillers dried grains with solubles has the potential to be a partial substitute for corn, soybean meal or other cereals in laying hen diets [19]. This also gives the potential for the use of exogenous enzymes to increase the availability of phytate-bound substrates.

1.3 Dietary Inclusion of DDGS

Many studies have investigated the use of DDGS in laying hen diets as a cost effective feed alternative to corn, soybean meal, and other cereal grains. Upon review of the literature, Swiakiewicz and Koreleski [19] suggested that it is safe to feed up to 15% DDGS in the diet of laying hens. Other studies have investigated the use of higher levels of DDGS in laying hen diets; however, the results are inconsistent. Currently the commercial industry is using DDGS within the 5 to 15% range suggested by Swiakiewicz and Koreleski [19]. New enzyme technologies may enhance the digestibility of DDGS allowing the use of higher levels in poultry diets.

Prior to the development of ethanol for fuel, most DDGS came from the beverage industry (referred to as brewers grains). In 1966, Matterson et al. [21] performed two 40-week studies evaluating the effects of 10 and 20% brewers grains in the diets of laying hens. The authors concluded that brewers grains could compromise up to one-third of the protein in the ration without negative effects on production [21]. Previous research supports the use of 10% brewers grains [22, 23] and 20% brewers grains in diets [24]. However, Lilburn and Jensen [24] observed a reduction in hen body weight when 20% brewers grains was fed over the course of the study. However, the authors concluded that the inclusion of up to 20% DDGS from brewers industry was acceptable in laying hen diets.

1.3.1 Use of DDGS in white egg-laying hen diets

The increased use of DDGS in livestock diets was stimulated by the increase in the production of ethanol for fuel. However, increasing the number of ethanol plants and the variety of processing methods used made the new DDGS from ethanol production a more variable and less consistent product. Most of the published research has been focused on white egg-laying hens though the first phase of a laying cycle with limited data from later production cycles or second-cycle hens. Few studies have researched the use of DDGS over full length production cycles.

Lumpkins et al. [25] studied the inclusion of 15% DDGS in the early stages of a production cycle. The study investigated differences between a commercial grade diet and a low-density diet with and without the addition of DDGS. When the commercial grade diets plus 15% DDGS were fed Hy-Line® W36 laying hens between 25-43 weeks of age, no differences were observed in hen-day egg production. However, the hens fed the commercial diets plus 15% DDGS had numerically lower egg production. A decrease in egg production was observed when the low density diets were fed in combination with 15% DDGS. No differences in feed intake and feed conversion rates were noted throughout the 18 week trial [25]. Roberts et al. [26] also found no differences in production when feeding 10% DDGS in a well formulated diet to Hy-Line® W36 hens from 23-58 weeks of age.

Wu-Haan et al. [27] recently published results similar to those of Lumpkins and colleagues [25] in a study that fed Hy-Line® W36 hens commercial diets with the addition of 20% dietary DDGS from 21-26 weeks of age [27]. Similar research was also replicated by in a comparable study. Romero et al. [28] reported no significant differences in feed intake or egg production when feeding diets containing 20% DDGS to Hy-line W36 hens from 26 to 34 weeks of age [28]. These studies indicate that laying hen diets can include between 10 to 20% DDGS during the first phase of the production cycle.

Several other studies have focused on the effects of dietary DDGS inclusion during the first phase of production on laying hens. Egg production increased when 5% DDGS were fed to Ishans hens from 30 to 42 weeks of age [29]. However, the authors also reported a decrease in hen day production and feed conversion when 15 or 20% DDGS was added to the diet [29]. Deniz et al. [30] fed 5, 10, 15 or 20% DDGS to Super Nick white laying hens from 28 to 36 weeks of age. They did not observe the same increase in egg production with inclusion of 5% DDGS or the decrease in egg production with 15% inclusion level of DDGS [30]. The authors did note a decrease in egg production and feed intake, and a poorer feed conversion when the hens were fed diets with 20% DDGS [30]. In contrast to other findings that found negative effects of up to 20% DDGS, Masa'deh et al [31] reported no negative effects on production parameters when feeding up to 25% DDGS to Bovans White hens from 24 to 46 weeks of age.

Upon review of the literature from the ethanol industry, it appears to be acceptable to include up to 15% DDGS in white egg-laying hen diets without negative consequences on performance parameters in the first phase of production [25, 27, 28, 30-32]. However, certain genetic strains appear to be able to consume diets with up to 20% DDGS without negative effects on egg production during the first phase [27, 28]. This suggests the potential for different feeding strategies for the different genetic strains of white laying hens in the first phase of production.

Roberson et al. [32] evaluated the use of DDGS in later stages in production. They fed up to 15% DDGS to Hy-Line® W36 hens from 48-56 and 58-67 weeks of age without negatively affecting egg production. The authors suggested a gradual increase of inclusion when feeding higher levels of DDGS [32]. Masa'deh et al. [31] also found no negative differences in production in the second phase when DDGS was included up 25% (Bovans White hens from 47 to 76 weeks of age) [31].

Sun et al. [33] fed Single Comb White Leghorns levels as high as 0, 17, 35 and 50% DDGS, after an adjustment period on the diets from 60 to 84 weeks of age. They observed reduced egg production, feed intake, and feed efficiency when DDGS composed half of the diet during weeks 60 to 72 of age. Negative effects on egg production, feed intake, and feed efficiency were alleviated once the diets were reformulated to contain adequate levels of lysine and methionine, suggesting that up to

50% DDGS could be included in the diet if they were formulated accordingly [33]. Loar et al. [34] found no negative effects on production parameters measured in second-cycle Bovan White hens from 72 to 87 weeks of age when fed up to 32% DDGS [34].

The available publications on white egg laying hens after the first phase of production, suggest that inclusion of higher levels of DDGS (20-25%) can be added to the diets. Inclusion of DDGS between 25 and 50% of the diet is possible if the diets are well formulated with a consistent supply of quality DDGS [31, 33, 34].

1.3.2 Use of DDGS in brown egg-laying hen diets

There is less research on the effects of DDGS in brown egg laying hen diets when compared to white egg-laying hens and the genetic strains of hens used are more variable. Swiatkiewicz and Koreleski [9] fed experimental diets containing 0, 5, 10, 15, or 20% DDGS to Lohmann Brown hens from 26 to 68 weeks of age. During the first phase of production (26-43 weeks of age) there were no effects of DDGS on laying rate, feed intake, or feed efficiency. Koksal et al. [35] also found no negative effects of adding up to 15% DDGS to the diets of Lohmann Brown hen productivity. The results from the previous studies in Lohmann Brown hens are consistent with the publications of Cheon et al. [36] who evaluated the effects of up to 20% DDGS fed to Hy-Line® Brown hens from 24 to 34 weeks of age without effects on egg production. These studies suggest that feeding between 15 and 20% DDGS in brown egg-laying hens may be efficacious during the first phase of production (24-40 weeks of age) and agree with the research in white egg-laying hens in early stages of production [25, 27, 28].

During the second phase of production (44-68 weeks of age) Swiatkiewicz and Koreleski [9] reported a negative impact of 20% DDGS on egg laying rate. Niemiec et al. [37] also reported comparable results in ISA Brown laying hens during the first phase of production. They found decreased laying hen performance and feed conversion ratios when 20% DDGS was included in the diet between 31 to 48 weeks of age [37]. In brown egg laying hens from 40 to 52 weeks of age also suggest an appropriate inclusion rate of 15% DDGS during the second phase of production [35]. Ghazalah et al. [38] examined the use of 0, 25, 50, and 75% DDGS in the diets of Bovans Brown laying hens from 40-56 weeks of age. The authors reported decreased hen day egg production and poorer feed conversion as the inclusion of DDGS increased. The hen body weights were reduced with the 75% inclusion level of DDGS in comparison to the other treatments [38].

From the research available, it can be included that inclusion of up to 20% DDGS may be damaging to brown laying hen production between weeks 30 to 52 of age. The conclusions reported with the brown laying hen research advocates an inclusion level of DDGS at or below 15%, which is consistent with the white egg-laying hen literature [25, 27, 28, 30, 32]. However, future research should focus on how to include higher levels of DDGS (25 and 50%) in brown egg laying hen diets.

1.3.3 Effect of DDGS on egg quality parameters

Many of the studies previously discussed have investigated the effects of dietary DDGS on egg quality parameters in both brown and white laying hens. Lumpkins et al. [25] found no effect of up to 15% DDGS on overall egg weight when feeding Hy-Line® W36 hens and are consistent with results of Roberson et al. [32] and Deniz et al [30].

Other research in Lohmann Brown hens supports the use of up to 15% DDGS without a negative effect on egg weight [9, 35]. Select studies demonstrated that containing a 20% DDGS reduced egg weight [9, 29, 30]. Deniz et al. [30] reported a depression in egg weight in the early stages of lay when 20% DDGS was included in the diets of Super Nick white egg-laying hens. Other studies have found decreased egg mass during the second phase of production [9, 29].

In contrast, other published research found no effect of up to 20% DDGS on overall egg weight in both brown [36, 37] and white [22, 24, 27, 31, 34] hens over various stages in production. However, Masa'deh et al. [31] did note a trend toward reduced egg weight when 20 and 25% DDGS was included in the early stages of production. The numerical decrease found by Masa'deh et al. [31] disagrees with other findings that suggest egg weight does not decrease until DDGS is in included in the diet at levels above 35% [33, 34, 38]. Overall, the literature is inconsistent as to how much DDGS can be included in the diet without a depression in egg weight or egg mass. However, most results support the inclusion of between 15 to 25% DDGS to the diet without significant negative effects.

Numerous studies have evaluated the use of DDGS inclusion on the shell quality of eggs using measurements such as shell thickness, shell weight, specific gravity, percent shell, and egg breaking strength. Most of the literature published is consistent suggesting that the addition of DDGS does not affect the quality of egg shells from either white or brown egg laying hens [9, 22, 25, 30, 31, 34-36]. However, some depressions in eggshell quality have been noted [32, 37].

Roberson et al. [32] found a negative effect of 15% DDGS on the specific gravity parameter during 51 weeks of age. Niemiec et al. [37] found a decrease in percent shell and shell thickness at 48 weeks of age in diets containing 20% DDGS. In contrast to both studies that found a reduction in shell quality, Sun et al. [33] found a significant increase (10.1 vs. 9.8%) in shell percentage when 50% DDGS was included in the diet. Therefore, given the lack of studies published with negative impacts on shell quality, it can be concluded that the inclusion of dietary DDGS has little to no effect on shell quality parameters if the diets are well formulated.

Albumen height is also of importance to the egg industry and is normally expressed in Haugh units. The Haugh unit (HU) uses the height of albumen (h, millimeters) and the weight of the egg (w, grams) to determine the interior quality of the egg. Haugh units are calculated using this equation:

$$HU = 100 * \log(h - 1.7\, w^{0.37} + 7.6)$$

Several studies have looked at the effect of various levels of DDGS on the interior quality of the egg.

Jensen et al. [22] found an improvement in the interior quality of the egg when 10% DDGS was added to the diet. They concluded that the improvements in Haugh unit values were due to trace elements in the DDGS. In a second experiment, the authors confirmed their results using 0, 5, 10, 15, and 20% DDGS treatments along with two additional treatments: trace elements and vitamins. They reported increased Haugh unit values when DDGS or trace elements were added to the diet. Further Haugh unit improvements were also noted in the 10 and 20% DDGS treatments when compared to the control treatment (81.3 and 82.5 versus 71.6) [22].

Other studies have confirmed these positive improvements in interior egg quality with the addition of at least 20% DDGS [24, 33, 34]. However, Lumpkins et al. [25] only found a numerical improvement in Haugh units with the inclusion of 15% DDGS when comparing it to the control treatment (85.4 vs. 87.2). The authors reported that they might have observed significant results if the trial was carried out longer [25]. Other studies have seen similar responses to Lumpkins et al. [25]. Other experiments failed to observe significant improvements in Haugh units but saw numerical improvements in Haugh unit values with the addition of dietary DDGS at various levels [9, 25, 30, 31, 36, 38]. Therefore, given the research, the addition of dietary DDGS should not have a negative impact on Haugh unit values and feeding levels of 20% DDGS or more may improve the interior quality of the egg.

Yolk color can vary greatly with the diet of the hens. It is well known that the addition of carotenoids to the diets of laying hens will increase the pigmentation of both the birds' skin and yolk. The color of yolks can be measured using many different techniques. Measurements from colorimeters are a good indication of yolk color and give three color values; L*, a*, and b*. These values determine lightness (L*) versus darkness, redness (a*) versus greenness, and yellowness (b*) versus blueness in appearance [39].

Several publications have shown increased yolk color when various levels between 10 and 25% DDGS were added to the diet using color fans for the determination of yolk color [9, 30-33, 35-38]. However, using a colorimeter Lumpkins et al. [25] reported that the addition of 15% DDGS only improved a* values making yolks redder when 15% DDGS was added to the diet for 18 weeks. The authors stated that the a* value differences, although significant, would be hard to detect with the naked eye [25]. This disagrees with the findings of Roberson et al. [32] who suggested that yolk color is visually changed in one month with the addition of 10% DDGS and Loar et al. [34] who found increases in both L* and a* values when DDGS was included at 16%.

Additionally, two studies that used up to 75% DDGS found significant improvements in yolk color using Roche color fans with each increased level of DDGS. Ghazalah et al. [38] fed 0, 25, 50, and 75% DDGS to brown laying hens and reported significant increases in yolk color 4.54, 5.15, 5.34, and 6.46 respectively. These results are consistent with Sun et al. [33] who fed white laying hens 0, 17, 35, and 50% DDGS and saw improvements in yolk color (5.5 vs. 7.0 vs. 7.9 vs. 8.7 respectively). The colorimeter findings indicate that the inclusion of dietary DDGS improves yolk color making yolks darker and redder in appearance. These improvements in color have also been noted to have consumer preference and have been implicated in marketing techniques [34].

1.4 Additives Used to Improve Bird Health, Productive Performance, and Egg Quality

1.4.1 Exogenous enzymes utilized in the poultry industry

An enzyme is a protein that catalyzes a reaction (i.e. lowers the activation energy of the reaction) without being altered by the reaction itself [40]. Endogenous enzymes are produced inside the chicken and exogenous enzymes are supplemented in the feed [41]. Exogenous enzymes are important in poultry diets because poultry cannot digest 15-25% of the feed they consume due to indigestible factors and a limited ability to produce certain enzymes [40]. Available feedstuffs such as barley, wheat, rye, DDGS, and plant feedstuffs are high in non-starch polysaccharides (NSP) or other indigestible factors. High concentrations of NSP in the diet increase gut viscosity, reduce weight gain, and impair feed efficiency, the rate of digestion, and nutrient absorption [41]

Carbohydrases (i.e. enzymes that breakdown NSP) are not endogenously produced by chickens. Exogenous supplementation of common carbohydrases (cellulase, xylanase, and beta-glucanase) has been demonstrated to improve digestibility of high NSP feedstuffs [42]. Cellulose, a NSP located in plant cell walls, comprises approximately 35-50% of plant cell walls and can be broken down with exogenous cellulase supplementation [42]. Xylanase, another carbohydrase, breaks down the beta-(1, 4) linkages in arabinoxylans into dimers that can be further reduced into fructose molecules. Beta-glucanase also degrades NSP, thus releasing nutrients from the grain endosperm and aleurone layers within plant cell walls [41]. Therefore, inclusion of endogenous carbohydrases into laying hen diets could help increase the breakdown of NSP in high DDGS diets.

Chickens have a limited ability to hydrolyze phytate in the diet because of low microbial fermentation in the foregut and low endogenous phytase secretions [41]. Phytase supplementation releases phytate bound minerals, protein, and starches in the diet which may reduce the necessary inclusion of supplemental minerals, protein, and starch [43]. Phytase is one of the most extensively studied enzymes in high DDGS diets as there are many commercial phytase products available for use in poultry diets. Many phytase products have shown to be efficacious in breaking down DDGS.

Alpha-amylase and protease are both endogenously secreted by the pancreases to breakdown starches and polypeptide chains, respectively [41]. However, exogenous supplementation of these enzymes has demonstrated further improvements in starch and protein digestion. Both α-amylase and protease may reduce feed costs by improving digestibility of lower protein feedstuffs [44, 45].

1.4.2 Effect of the addition of exogenous enzymes in DDGS diets fed to laying hens

The use of exogenous enzymes in poultry diets has been studied intensely over the past decade. However, the results are not conclusive mainly due to the high number of commercial enzyme products available and the variety of DDGS supplies. Many commercial enzyme products have multiple enzyme activities including phytase, cellulase, protease, amylase, and xylanase. The major objective of dietary addition of enzymes was to help animals to further break down the NSP in feed stuffs allowing more flexibility in feed formulation (i.e. reduced energy, less digestible feed stuffs, limiting supplemental minerals/vitamins) [46].

Studies have evaluated the use of enzymes in laying hen diets containing DDGS and observed results have been with and without improvements in performance and egg quality. Koksal et al. [35] found no effect of up to 15% DDGS with or without enzyme (phytase) supplementation, suggesting that the DDGS inclusion levels were not high enough for the enzyme to work. Shalash et al. [29] reported decreased egg production, egg weight and a poorer feed conversion rate when 15 and 20% DDGS were included in the diet; egg production, egg weight and feed conversion rate were partially improved with the addition of an exogenous enzyme complex (α-amylase, beta-glucanase, cellulase, protease, and xylanase activity). However, the second enzyme complex (xylanase, α-amylase, beta-glucanase, and protease activity) did not exhibit the same improvements and was not effective at either 15 or 20% [29]. Similarly, Deniz et al. [30]

did not observe improvements in laying rate, egg weight or feed conversion with the addition of an exogenous enzyme complex (protease, pentosanase, pectinase, cellulase, beta-glucanase amylase, and phytase activity) when DDGS was included in the diet at 20% [30].

Despite this, positive improvements on performance parameters were noted in two studies. Swiatkiewicz and Koreleski [9] found improvements in laying rate when an enzyme complex (endo-1,4-beta-xylanase, endo-1,3(4)-beta-glucanase, pentozanase, hemicellulase, and pectinase activity) was added to the 20% DDGS diet (84.7 versus 87.9%). Ghazalah et al. [38] reported improvements in egg production, feed conversion, and egg weight when an enzyme complex (glucanase, xylanase, amylase, polygalacturonase, and protease activity) was included with the addition of 25, 50, and 75% DDGS. The authors reported that the addition of the enzyme complex reduced the negative effects of 25% DDGS on egg production. Egg production was 80.8% in the non-enzyme supplemented 25% DDGS, whereas, the enzyme supplemented birds produced at a rate of 83.4% [38].

The literature is inconsistent as to whether or not the supplementation of enzyme complexes will help maintain normal production in laying hens when higher levels of DDGS are included. The effect of enzyme supplementation needs to be evaluated with higher levels (25 and 35%) of DDGS; and through a full-length production cycle.

1.4.3 Antioxidants utilized in the poultry industry

The development of various diseases and declines in animal production and product quality have been attributed to the production of free radicals and lipid peroxidation [47]. Use of antioxidants in the poultry industry have shown improved bird health, reproductive health, oxidative stability of both meat and eggs, and are currently being increased in bird diets for the enrichment of products due to consumer preferences [48-51]. Two primary antioxidants are used to target these poultry industry needs: Vitamin E and selenium.

Because it is an important component of lipid soluble membranes vitamin E is said to work as an antioxidant by helping reduce free radicals responsible for oxidative damage [52]. Vitamin E scavenges peroxyl radicals in the following equation (adapted):

peroxyl radicals (ROO*) + Vitamin E (Toc-OH) → ROOH + Toc-O*

and forms a tocopheroxyl radical (Toc-O*) that can be recycled to a useable tocopherol (vitamin E) [47].

In the previous reaction the remaining hydroperoxide (ROOH) is damaging to membrane structures and functions and needs selenium (selenium dependent glutathione peroxidase; GSH-PX) to convert hydroperoxide to its non-destructive components [47, 53]. Selenium is a trace element that can be naturally found in feedstuffs, however, the levels in feed are related to the levels of selenium in the soil and can vary greatly [54]. The following reaction demonstrates the need for selenium dependent glutathione peroxidases (Se-GSH-PX; adapted):

ROOH + 2GSH → Se-GSH-PX → ROH + GSSG + ROH to convert hydroperoxide (ROOH) into a non-destructive from (ROH) [47]. Because of these reactions, vitamin E and selenium together play an important role in the maintenance of membrane structures and an important role in antioxidant defense.

1.4.4 Effect of antioxidant supplementation in laying hen diets

Without sufficient amounts of selenium in the diet, birds can incur impaired growth and immunocompetence increasing their susceptibility to disease and other health problems [55]. In laying hens selenium can also be an important factor in maintaining high performance and reproductive characteristics [56]. Vitamin E is also used similarly to selenium; added to diets to prevent disease and optimize growth production in chickens [57, 58].

For the laying industry, one of the bigger movements has been increased demand for "healthy" and "enriched" foods. Enriched or modified eggs can have many different meanings; they can be supplemented with different vitamins, omega-3 fatty acids, minerals, or combinations of the above [59]. Consumers are looking for foods that have the potential to decrease heart disease and improve their own antioxidant levels (vitamins) [50, 59]. However, changing the fatty acid profile of eggs with enriched omega-3 fatty acids can cause storage and taste issues [50]. Therefore, research has focused on adding antioxidants to laying hen diets to improve taste, storage and even add more nutritional value to eggs

Due to vitamin E's ability to reduce free radicals and its benefits to the consumer, it was thought that higher levels of vitamin E in the yolk might help improve the storage life and marketability of eggs. In order to determine the anti-oxidative principles of vitamin E with storage of eggs most researchers use a thiobarbituric acid reactive substances (TBARS) assay to measure TBA a by-product of lipid peroxidation. Egg yolks contain a high percentage of the total lipids in the whole egg, most of which are unsaturated. This makes them sensitive to oxidation and therefore, off-flavors, taste differences, changes in color and or texture, and potential loss of nutrients [60].

Scheideler et al. [52] showed reduced TBARS in storage eggs with added vitamin E, indicating benefit of vitamin E supplementation on the reduction of oxidative damage. Addition of 0.3 ppm of selenium (the legal limit) showed improvements in egg production, egg mass, and vitelline membrane strength of table eggs. Authors also reported improvements in egg yolk nutrients when vitamin E and selenium were fed in combination [52]. This study and others previously mentioned suggest increased interest in the addition of antioxidants in laying hen diets to improve both bird health and egg quality.

1.4.5 Effect of the addition of antioxidants in DDGS diets fed to laying hens

The use of antioxidants in DDGS diets fed to laying hens has not been extensively researched. However, given previous studies in non-DDGS diets, it appears that there might be some implication for antioxidants like vitamin E and selenium to be increased in diets high in DDGS for both bird health and egg quality. One study looked at varying levels of DDGS (0, 10 and 20%) and vitamin E (0 and 200 mg/kg) on both egg quality and egg production [61]. Jiang et al. [61] found that the 20% DDGS treatment had the worst feed conversion rate when compared to the 0% DDGS treatment and vitamin E supplementation at 200mg/kg improved egg production in all diets. Authors reported that no interaction between vitamin E and up to 20% DDGS was found in the study [61]. However, egg production improvements were noted with vitamin E and suggest further research as egg production in high DDGS diets is often reduced.

1.4.6 Proprietary premix

A proprietary premix containing enzyme and antioxidant technologies developed by Alltech, Inc. (Nicholasville, KY) was anticipated to improve the overall health and production of the laying hens fed diets with high levels of DDGS. It was expected that the enzyme and antioxidant technologies would reduce any negative effects of the inclusion of high levels of DDGS on mortality, hen body weight, feed intake, hen-day egg production, egg weight, and egg quality (Haugh units, egg shell breaking strength, egg shell percentage, yolk weight, and yolk color).

CHAPTER 2. Effect of a proprietary premix on productive performance and egg quality of white egg laying hens fed diets high in distillers dried grains with solubles through 59 weeks of production

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Summary

High demand for ethanol production has decreased the availability of grains for poultry consumption, making distiller's dried grains with solubles (DDGS) an attractive ingredient for higher inclusion levels in poultry diets. This study evaluated the effects of including 25 and 35% DDGS into corn-soybean meal based diet with and without a proprietary premix (enzyme and antioxidant; Alltech, Inc.) on egg quality and production parameters. At 16 weeks of age, 288 Hy-Line W-36® hens were allocated to five dietary treatment groups: 1) corn-soybean meal (control), 2) 25% DDGS, 3) 25% DDGS plus premix, 4) 35% DDGS, and 5) 35% DDGS plus premix. At 43 weeks of age, diets were adjusted from 18 to 16% CP and during both phases calcium and available phosphorus were reduced in the DDGS diets. Overall, hens fed 35% DDGS treatments were lighter ($P<0.05$) and had lowest body weight gains over the study ($P=0.013$). In the second phase of production (43 to 75 weeks of age) feed intake was decreased ($P=0.026$) with the addition of DDGS, however, the 25% DDGS plus premix treatment was not different from the control. Overall, no differences in either phase of production were noted with the addition of up to 35% DDGS on egg production. During times of potential metabolic stress (peak production and diet changes), addition of up to 35% DDGS decreased ($P<0.05$) egg weight, yolk weight, shell percentage and shell breaking strength. However, addition of the premix alleviated the negative effects on shell quality. Inclusion of DDGS improved yolk color value compared to the control treatment ($P<0.05$). Overall, no effect of dietary treatment was noted on egg weight, Haugh unit values, shell percentage, egg shell breaking strength and yolk weight, taste of the eggs, and bone quality. Therefore, addition of up to 35% DDGS may be included in the diet without substantial negative effects and the addition of the premix may help alleviate negative effects on egg quality.

KEYWORDS: enzyme and antioxidant, distillers dried grains with solubles, white egg laying hens, production, egg quality

2.1 Description of Problem

Distillers dried grains with solubles (DDGS) have been used as a feed ingredient in poultry diets for numerous years. However, since the Energy Policy Act of 2005 and the Energy Independence and Security Act of 2007, greater amounts of corn and other high quality grains are being used for ethanol production, thus increasing the cost of feed for animal consumption. Approximately one bushel (56 pounds) of corn is needed to produce 2.8 gallons of ethanol, resulting in 17.5 pounds of by-product DDGS for animal feed [3]. Therefore, researchers and producers have focused on feeding higher levels of this lower cost by-product to livestock in order to lower costs of production. Many companies have developed new technologies to extract more nutrients out of the DDGS.

It is estimated that poultry use approximately eight percent of the total DDGS produced for the livestock industry [1]. The DDGS by-product is considered a good source of energy, protein, water-soluble vitamins, and minerals [8]. There is great variability in DDGS as a feedstuff due to the different processing methods used at numerous ethanol plants. Higher temperatures used during the drying of DDGS seem to be of most concern when using DDGS as a feed ingredient in animal diets. This is because higher temperatures have been shown to decrease the amino acid availability in DDGS [12, 17]. Nonetheless, DDGS are a good source of energy, protein, and phosphorus and can provide cost savings when included in the diet in place some of the corn, soybean meal, and supplemental phosphorus [19].

The literature is fairly consistent when adding lower levels of DDGS to the diets of laying hens. Most studies agree that inclusion of up to 15% DDGS has no negative effects on egg production or egg quality [15, 26, 27, 31-33]. However, some authors reported decreased egg production or egg weight with inclusion of 20% DDGS [29, 30].

Several studies have evaluated the effects of DDGS inclusion above 20% in laying hen rations at various stages of production. Masa'deh et al. [31] formulated laying hen rations with up to 25% DDGS in two phases of production. They reported a decrease in egg weight during 24-46 weeks of age: however, during 47-76 weeks of age there was no effect of DDGS on egg production. It was also concluded that there was no effect of up to 32% DDGS on production parameters in second cycle hens [34]. Two other studies evaluated the effects of including DDGS at or above 50% of the diet [33, 62]. Both studies found a decrease in egg production; however Pineda et al. [62] reported increased egg weight and feed intake, which was opposite the findings of Sun et al. [33] who noted a decrease in feed intake and egg weight. Overall, Pineda et al. [62] found no differences in feed utilization (egg mass/feed) which was consistent with Sun et al. [33] who found no effect of DDGS on feed conversion when feeding 50% DDGS in diets balanced for amino acids.

The objective of this study was to evaluate higher levels of DDGS on the egg production and egg quality of Hy-Line W36® laying hens through a full production cycle with and without the addition of a proprietary premix (enzyme and antioxidant). The high inclusion levels (25 and 35% DDGS) were based on the previous work in our laboratory [63]. It was hypothesized that 35% DDGS would reduce egg production parameters and that the proprietary premix would help alleviate negative effects given the enzyme and antioxidant technology.

2.2 Materials and Methods

All procedures were carried out in compliance with the University of Kentucky Institutional Animal Care and Use Committee (IACUC) from March 7, 2012 to April 2, 2013.

2.2.1 Animal Care and Use

This study utilized 288 Hy Line W-36® hens that were arranged in a completely randomized design. At 16 weeks of age, hens were placed in battery cages (2 birds per 516 cm^2 cage) and adjusted to a 16-hour light: 8-hour dark lighting regimen. Body weights were adjusted; abnormally light or heavy birds were eliminated. For this experiment a replicate group consisted of 3 top and 3 bottom cages within a battery row. Each replicate group constituted an experimental unit. Due to space limitations, the control treatment was reduced to 4 replications, leaving enough room for each of the 4 DDGS treatments to have 5 replications. Birds were housed in a fan ventilated climate-controlled room and were allowed ad *libitum* access to feed and water throughout the experiment.

2.2.2 Dietary Treatments

Dietary treatments consisted of a basal corn-soybean meal diet and two levels of DDGS with and without a proprietary premix (enzyme and antioxidant; [4]). The five treatments were 1) corn-soybean meal (control), 2) 25% DDGS, 3) 25% DDGS plus premix, 4) 35% DDGS, and 5) 35% DDGS plus premix. Diets were formulated to meet or exceed the National Research Council (1994) recommendations for metabolizable energy, crude protein, and vitamins (Table 2.1). In the first phase of production (16-42 weeks of age), diets were formulated to be isocaloric, with approximately 18% crude protein and reduced available calcium (4.1 vs. 4.2%) and phosphorus (0.19 vs. 0.29%) in the DDGS treatments. At 43 weeks of age, hen diets were changed to the phase two diets that were also formulated to be isocaloric (Table 2.1). During the second phase crude protein levels were reduced to approximately 16% and again diets containing DDGS were formulated with reduced calcium (3.7 vs. 3.8%) and available phosphorus (0.22 vs. 0.30%) compared to the control.

2.2.3 Data Collection and Methods

Body weights were recorded monthly throughout the 59-week study and reported as the average of the replicate group weight. Mortality and egg production were recorded daily to calculate hen day production percentages. Once a month (in correspondence with body weight) the total egg production per replicate group was weighed and averaged for the determination of egg weight; from that collection a random sample of 6 eggs was selected for egg quality analysis.

Shell breaking strength was determined with a Shimadzu EZ-S texture analyzer [64] by steadying the egg horizontally and applying enough pressure (N) to crack the shell without puncturing the membrane. Eggs were broken out and albumen height was measured approximately one centimeter away from the yolk using the TSS Quantum Chromodynamics Super System [65]. Individual egg weights and albumin heights were used to calculate the Haugh Unit. Albumen and yolk were separated and yolks were placed in the Hunterlab Colorflex EZ colorimeter [39] to measure the transmittance and reflectance of the yolks. The colorimeter computed L*, a*, and b* values to determine lightness, redness, and yellowness respectively (Appendix 1). Yolks were weighed as a replicate group and averages were reported for the yolk weight.

2.2.4 Bone Sample Collection

At the end of the trial, three birds per pen were euthanized for bone quality analysis. The birds' right tibia and humerus were removed, cleaned, and broken using an Instron 4301 machine [66]. Bones were centered over a platform and enough pressure force (kg force) was applied to break the bone. Tibia and humerus samples were also collected from the birds left side for mineral analysis following the procedure outlined in Appendix 2.

2.2.5 Taste Panel

A taste panel was convened at the end of the 59-week study. Treatments were assigned a random three-digit number and samples were randomized using a random number generator. A total of 10 eggs from each treatment were scrambled for 1m with a wire whisk. Samples were prepared in a non-stick skillet that was heated to medium high heat (191-204° C). For each treatment 243 grams of eggs were cooked for 1 m and 30 s and immediately distributed into approximately 20 g aliquots to panelists. The taste panel was run under red lighting to avoid color bias. Untrained panelists were provided water to cleanse their pallet and asked to rate the eggs on a scale of 1-8 for the following categories: flavor, off-flavor, buttery flavor, metallic flavor, fishy flavor, painty flavor, rancid flavor, grassy flavor, and purchase intent (Appendix 3).

2.2.6 Statistical Analysis

Data was analyzed using the statistical program SAS [67]. The Proc GLM function was utilized to determine the main effects of dietary DDGS with and without the proprietary premix. Means were separated using Fisher's least significant difference test, with significance set at $P \leq 0.05$. Tendencies were considered between $P > 0.05$ and $P \leq 0.10$.

2.3 Results and Discussion

2.3.1 Production Parameters

Mortality was not different over the 59 week study (less than 5%; P=0.954). In the first phase of production (16-42 weeks of age), body weights were consistent (Table 2.2). Hens reached mature body weight around 34 weeks of age with no differences among treatments. At 42 weeks of age, the control treatment birds were heavier (P=0.012) than both premix treatments and the 35% DDGS treatment. Control treatment birds were also heavier (P<0.01) at 46 and 50 weeks of age. At weeks 54, 58, 66, and 75, the 35% DDGS plus premix treatment had hen body weights that were not different (P≤0.03) from the control treatment. Overall (16-75 weeks of age), the 35% DDGS treatment had the lowest change in body weight with a gain with an average of 468 grams per bird. Hen body weight gain values after reaching mature body weight (34-75 weeks of age) were minimal (less than 153 grams per bird) with the control and 25% DDGS treatments gaining more (P=0.005) then the other treatments.

Over the first phase of production (16-42 weeks of age) there was no effect of dietary treatment on feed intake (Table 2.3). These results are similar to Masa'deh et al.

[31] who fed up to 25% DDGS with no adverse effects on overall feed intake (24-46 weeks of age). In our study, inconsistent differences in feed intake between weeks were noted in the first phase of production and are consistent with Loar et al. [34] who also reported treatment by week interactions on feed intake. During 27-30 weeks of age, the treatments containing DDGS had increased feed intake (*P*=0.042) when compared to the control treatment (97.3 versus 92.3 g/day respectively). These increases in feed intake (27-30 weeks of age) could have been due to the physiological status of the hens (trying to eat to meet their needs) during peak egg production. From 39-42 weeks of age the premix treatments had reduced feed intake compared to the control treatment (*P*=0.023; 86.8 versus 92.2 g/bird/day). During this same period, the DDGS treatments (89.6 g/bird/day) were not different from either the control premix treatments, suggesting that the premix may have been releasing more energy or protein allowing the birds to eat less.

In the second phase of production, feed intake varied between weeks. Weekly variation in feed intake carried into phase two overall feed intake calculations (43-75 weeks of age) as the 25%, 35% and 35% plus premix treatments had increased (*P*=0.026) feed intake when compared to the control (104.2, 105.4 and 103.8 versus 99.7g/hen/day respectively). The 25% DDGS plus premix treatment (101.7g/hen/day) was not different from the control, 25%, or 35% plus premix treatments. However, the addition of the premix reduced feed intake equivalent to the control during the second phase of production. Other publications did not note a decline in feed intake when up to 35% DDGS was included in the diet, but did observe similar results when DDGS compromised over 50% of the ration [33, 62]. Overall, the average feed intake (16-75 weeks of age) was not different among dietary treatments (Table 2.4). A tendency

(*P*=0.088) was noted for DDGS treatments (except 25% DDGS plus premix) to increase feed intake when compared to the control treatment.

During the first phase of production, there was no effect of dietary treatment on hen day egg production from 16-42 weeks of age (Table 2.4). These results are consistent with the published literature at lower inclusion levels of DDGS (below 25%) [25-28]. However, two significant differences in hen day egg production were noted within 31 and 39 weeks of age as the 35% DDGS treatment (39 weeks of age) and 35% DDGS plus premix treatment reduced egg laying ($P \leq 0.002$) rate when compared to the other three treatments. This trend carried into the second phase of production until the hens were about 51 weeks of age (Table 2.5). Interestingly, after 56 weeks of age, when the control and 25% DDGS treatments started declining in egg production, the hens receiving the 35% DDGS treatments with and without the premix maintained approximately 80% hen day egg production and carried this through end of the second phase of production. Hen-day egg production improvements ($P \leq 0.016$) were noted in the 35% DDGS treatments during 62, 66, and 67 weeks of age when compared to the control and 25% DDGS treatments. Consistent with previous results, no differences were noted in hen-day egg production with inclusion of up to 35% DDGS from 16-75 weeks of age [31-33].

2.3.2 Egg Quality and Taste

Research on the effects of up to 35% inclusion of DDGS is inconsistent for egg weight measurements as some authors have reported no effect [33, 34], and others have observed a reduction in egg weight [29-31]. During the first dietary phase, egg weights ranged from approximately 50-60 grams and in the second dietary phase, 60-65 grams

(Table 2.6). We observed egg weight reductions (*P*<0.05) in the 25% DDGS plus premix treatment compared to the control during two weeks; once when the hens were reaching peak production (22 weeks of age) and again after the change in dietary phases (50 weeks of age). Overall, egg weight was not altered with the inclusion of up to 35% DDGS.

There was no effect of dietary treatment overall on egg shell quality parameters; egg shell percent and egg shell breaking strength. However, weekly differences were noted during potential times of metabolic stress (early stages of production and dietary changes) for the birds and differences were in connection with the egg weight changes previously mentioned. Egg shell percent decreased in the 35% DDGS treatments when hens were 26 and 46 weeks of age (Table 2.7). At 26 weeks of age, there was an association between egg shell percentage and egg shell breaking strength (Table 2.8); the 35% DDGS treatment decreased (*P*=0.036) shell percentage and translated into decreased shell breaking strength (*P*=0.040). When the premix was added to the 35% DDGS treatment shell quality was not different from the control. At 46 weeks of age, the 35% DDGS plus premix treatment had reduced egg shell percent (*P*=0.030), yet did not translate into poorer egg shell breaking strength. Overall, changes in egg shell quality are likely due to the birds' metabolic state in early production as most of the literature suggests that there is no effect of DDGS on egg shell quality [25, 30, 34, 35].

Overall, there was no effect of dietary treatment on Haugh unit values (Table 2.9). A trend was noted for increased Haugh unit values in the DDGS treatment eggs (*P*=0.077), similar to the numerical improvements reported by Lumpkins et al. [25]. When the hens were 30 weeks of age, an unexpected reduction in Haugh unit values were observed in the 25% DDGS plus premix and 35% DDGS treatments (*P*<0.001). Our results are not consistent with increased Haugh unit values found in previous studies [22, 24, 33, 34].

Yolk weight values were not affected by dietary treatment when averaged over the entire study (Table 2.10). Yolk weights were only affected at the beginning of the second dietary phase, when hens were 46 and 50 weeks of age. At 46 weeks of age, the 35% DDGS treatments had reduced yolk weight values compared to the control treatment and 25% DDGS treatments and the 35% DDG plus premix treatment was not different from either (P=0.043). At 50 weeks of age, yolk weights were reduced in the 35% DDGS treatments when compared to the other three treatments with no improvement of the premix (P=0.002). Previous work in our laboratory also reported decreases in yolk weight with the addition of up to 23% DDGS, however, improvements were noted with the addition of a phytase enzyme to the diet [68].

Yolk color parameters were also monitored throughout the study and varied in lightness, redness, and darkness. The lightness (L*) values were darker (P<0.017) with the addition of dietary DDGS from 26-42 and 50-58 weeks of age when compared to the control (Table 2.11). During 61-74 weeks of age, the 35% DDGS treatments were not different than the control treatment (P<0.020) and the 25% DDGS treatments were increased compared to the other treatments. Further increases in the 25% DDGS treatments cannot be explained by dietary DDGS color as the same DDGS was used in all DDGS treatments. The yolk color redness values (a*) in the first dietary phase were darkest (P<0.0001) for the 35% DDGS treatments, followed by the 25% DDGS treatments, and control treatment (Table 2.12). After the dietary phase change from 46-54 weeks of age, the 25% DDGS treatments had hens that laid redder yolks compared to the other treatments and continued to do so throughout the second phase (*P*<0.0001). From 66-70 weeks of age, the hens on the 35% DDGS treatments laid eggs with yolks less red then then both the control and 25% DDGS treatments (*P*<0.0001) suggesting that some of the pigmentation could have come from the corn. Yellowness (b*) values followed a pattern similar to that of the red (a*) values and can be viewed in Table 2.13.

Results from the taste panel showed no effect of dietary treatment on egg taste parameters measured (Table 2.14). The Pearson correlation (Appendix 4) showed that as the flavor parameter increased metallic, fishy, and grassy flavors decreased (-0.4), as well as painty (-0.2) and rancid (-0.6) flavors while purchase intent increased by almost one whole point (0.9), thus validating the panel. Thus, the addition of up to 35% dietary DDGS did not have an effect on the taste of the eggs.

2.3.3 Bone Quality

Breaking strength of the bones was unaffected by dietary treatment in both the tibia and the humerus (Table 2.15). However, ash content decreased (*P*=0.015) in the humerus bones of the control and premix treatments when compared to the 35% DDGS treatment. Calcium content also decreased (*P*=0.038) in the humerus bones in the control treatment compared to the 25% DDGS plus premix treatment and 35% DDGS treatment; this inconsistency among treatments was not expected. No differences were noted in the phosphorus content of the bones. The analyzed dietary calcium and phosphorus values can be observed in Appendix 6. Future studies should consider analyzing mineral and breaking strength of bones in correlation with diet changes and during peak production given the weekly differences we observed in egg shell quality at these potential times of metabolic stress for the birds.

2.4 Conclusions and Applications

1. The inclusion of up to 35% DDGS reduced hen body weights but had no effect on overall hen day egg production or feed intake.

2. The premix helped alleviate some of the weekly negative effects of up to 35% DDGS on feed intake, egg shell percentage, egg shell breaking strength and yolk weight parameters, but was not consistent among weeks.

3. Inclusion of up to 35% DDGS can be included in the diet through a full production cycle without negative effects on egg quality (egg weight, Haugh unit values, shell percentage, egg shell breaking strength and yolk weight, and taste of scrambled eggs) and bone strength.

4. Yolk color was increased with inclusion of up to 35% DDGS making yolks significantly darker, redder and more yellow in appearance and may have marketing benefits.

5. Addition of up to 35% DDGS may be included in the diet without substantial negative effects and the addition of the premix may help alleviate slight negative weekly effects on egg quality and feed intake when the birds are under stress.

2.5 Tables and Figures

Table 2.1 Diet composition and calculated analysis of the dietary treatments for phase one and phase two of production

	Percent of Diet (%)					
	Phase One (16-42 weeks of age)			Phase Two (43-76 weeks of age)		
Diet Composition	Control	25% DDGS	35% DDGS	Control	25% DDGS	35% DDGS
Corn	56.51	44.90	37.33	63.90	50.54	40.20
Soybean meal, 48% CP	28.20	14.70	11.10	21.50	10.00	6.30
DDGS[1]	0.00	25.00	35.00	0.00	25.00	35.00
Corn oil	3.05	3.95	4.90	2.50	3.00	4.90
Oyster Shell	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	7.30	7.50	7.56	6.20	6.60	6.70
Dicalcium phosphate	1.00	0.11	0.00	1.10	0.00	0.00
Salt	0.42	0.15	0.13	0.44	0.26	0.19
Vitamin-mineral mix*	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.17	0.13	0.10	0.11	0.09	0.09
L-Lysine	0.00	0.31	0.38	0.00	0.23	0.33
Tryptophan	0.00	0.00	0.00	0.00	0.03	0.04
Celite	0.10	0.00	0.25	1.00	1.00	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME, kcal/kg	2,891	2,892	2,894	2,893	2,895	2,903
CP, %	18.58	18.16	18.56	15.92	15.94	15.97
Ca, %	4.22	4.11	4.11	3.81	3.69	3.72
P, avail., %	0.29	0.19	0.19	0.30	0.20	0.24
Lys, %	0.98	0.98	0.98	0.84	0.78	0.78
Met, %	0.46	0.46	0.45	0.38	0.37	0.37

[1]DDGS Nutrient composition: 3,035 kcal/kg ME; 89.4% DM; 25.8% CP; 10.9% Crude fat; 5.6% Crude fiber; 4.9% Ash.

*Vitamin mineral mix provided (per kg diet): 11025 IU vitamin A, 3528 IU vitamin D, 33.075 IU vitamin E, 0.9096 mg vitamin K, 2.205 mg Thiamin, 7.7175 mg Riboflavin, 55.125 mg Niacin, 17.64 Pantothenate, 4.9613 mg vitamin B-6, 0.2205 mg d-biotin, 1.1025 mg Folic acid, 478.485 mg Choline, 0.0276 mg vitamin B-12, 75 mg Zinc, 40 mg Fe, 64 mg Mn, 10 mg Cu, 1.85 mg I, 0.3 mg Se.

Table 2.2 Effects of dietary treatments on hen body weight* (g/bird)

	Weeks of Age	TREATMENTS Control	25% DDGS	25%DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16	1163	1166	1154	1166	1161	9.2	0.869
	22	1442	1451	1430	1446	1473	13.7	0.285
	26	1494	1507	1477	1493	1531	14.3	0.127
	30	1504	1526	1502	1550	1537	33.5	0.817
	34	1567	1588	1544	1541	1587	21.3	0.355
	38	1610^{x}	1601^{x}	1552^{xy}	1524^{y}	1581^{xy}	20.9	0.052
	42	1615^{a}	1585^{ab}	1546^{bc}	1496^{c}	1530^{bc}	0.02	0.012
Phase 2	46	1617^{ab}	1620^{a}	1544^{bc}	1492^{c}	1537^{c}	25.0	0.007
	50	1637^{a}	1634^{a}	1537^{b}	1495^{b}	1543^{b}	24.8	0.002
	54	1594^{a}	1590^{a}	1505^{b}	1488^{b}	1542^{ab}	23.1	0.013
	58	1623^{ab}	1637^{a}	1569^{bc}	1554^{c}	1621^{ab}	21.0	0.037
	61	1649^{xy}	1677^{x}	1590^{y}	1603^{y}	1651^{xy}	21.9	0.053
	66	1688^{ab}	1699^{a}	1620^{bc}	1608^{c}	1672^{ab}	20.7	0.024
	70	1600	1697	1624	1605	1675	29.9	0.122
	75	1719^{ab}	1736^{a}	1645^{bc}	1634^{c}	1693^{abc}	24.5	0.030
Gain	22-75[1]	$+277^{a}$	$+284^{a}$	$+215^{b}$	$+188^{b}$	$+220^{b}$	15.4	0.001
	34-75[2]	$+153^{a}$	$+147^{a}$	$+102^{b}$	$+92^{b}$	$+107^{b}$	11.9	0.005
	16-75[3]	**$+557^{a}$**	**$+569^{a}$**	**$+492^{bc}$**	**$+468^{c}$**	**$+533^{ab}$**	**21.1**	**0.013**

[abc] Means without a common letter differ significantly ($P<0.05$).

[xyz] Trends considered for means without a common letter (P<0.10).

*Hen body weight values are reported as pen averages (n=24).

[1]Represents body weight gain from dietary adjustment period to end of study.

[2]Represents body weight gain from mature body weight to end of study.

[3]Represents the overall body weight gain from the beginning to end of study.

Table 2.3 Effects of dietary treatments on feed intake* (g/bird/day)

	Weeks of Age	TREATMENTS Control	25% DDGS	25%DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16-22	73.2	74.8	75.5	74.7	77.4	1.09	0.164
	23-26	94.3	94.4	95.4	95.0	95.5	1.30	0.950
	27-30	92.3^{b}	97.0^{a}	96.5^{a}	97.7^{a}	98.1^{a}	1.26	0.042
	31-34	95.6	95.5	93.8	93.3	92.7	1.26	0.578
	35-38	96.0	95.4	92.2	92.4	94.8	1.22	0.124
	39-42	92.2^{a}	90.1^{ab}	86.8^{b}	89.1^{ab}	86.8^{b}	1.16	0.023
Phase 2	43-46	95.1	97.2	95.2	97.4	94.0	1.47	0.416
	47-50	95.7^{a}	97.4^{a}	93.3^{ab}	95.9^{a}	91.2^{b}	1.43	0.045
	51-54	94.2^{c}	97.7^{ab}	96.6^{b}	99.7^{a}	95.2^{bc}	1.05	0.015
	55-58	97.4^{b}	102.1^{a}	101.7^{a}	105.2^{a}	103.5^{a}	1.29	0.009
	59-61	104.3^{b}	113.1^{a}	106.5^{a}	112.8^{a}	114.2^{a}	1.61	0.001
	62-66	103.9^{c}	110.0^{ab}	106.5^{bc}	110.7^{ab}	112.1^{a}	1.63	0.016
	67-70	102.3^{y}	105.1^{xy}	105.8^{xy}	110.7^{x}	109.0^{x}	2.13	0.095
	71-75	104.0	110.1	107.1	110.4	110.5	2.21	0.231
Overall	16-42[1]	88.3	89.8	89.2	88.9	90.3	0.94	0.604
	43-75[2]	99.7^{c}	104.2^{ab}	101.7^{bc}	105.4^{a}	103.8^{ab}	1.16	0.026
	16-75[3]	**95.1^{y}**	**98.3^{x}**	**96.6^{xy}**	**98.7^{x}**	**98.3^{y}**	**0.96**	**0.088**

abc Means without a common letter differ significantly (P<0.05).
xyz Trends considered for means without a common letter (P<0.10).
*****Feed intake values are reported as pen averages (n=24).
[1]Represents phase one average feed intake.
[2]Represents phase two average feed intake.
[3]Represents average feed intake from the beginning to end of study.

Table 2.4 Effects of dietary treatments on phase one hen-day egg production* (%)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16	1.8	0.0	0.2	0.0	0.0	0.66	0.335
	17	4.5	1.4	3.8	1.4	1.7	1.36	0.385
	18	25.6	18.8	24.1	19.5	21.4	4.20	0.763
	19	51.8	59.3	59.5	50.0	61.2	4.35	0.281
	20	79.5	79.3	80.2	76.7	76.4	3.13	0.861
	21	95.2	94.1	93.6	89.8	93.1	2.31	0.551
	22	96.1	94.5	95.0	94.6	93.3	1.11	0.562
	23	97.3^{x}	93.1^{y}	92.6^{y}	94.3^{xy}	92.9^{y}	1.18	0.086
	24	97.0	95.0	98.1	97.2	97.6	1.31	0.507
	25	96.4	95.9	97.4	96.0	95.7	1.41	0.918
	26	95.8	94.3	93.1	96.6	95.5	1.46	0.482
	27	97.0	96.2	95.5	95.1	95.5	1.18	0.819
	28	97.6	96.2	94.7	97.3	95.9	1.47	0.671
	29	96.1	94.0	93.7	96.6	93.1	1.68	0.501
	30	95.5	94.3	94.3	96.0	94.5	1.71	0.930
	31	93.2^{a}	94.7^{a}	91.7^{a}	91.2^{a}	84.8^{b}	1.53	0.002
	32	92.9	94.5	90.7	94.4	87.9	2.04	0.140
	33	92.0	92.5	89.7	89.2	86.7	1.96	0.259
	34	93.1	95.3	95.1	96.1	94.3	1.80	0.834
	35	90.2	90.6	91.7	91.5	92.6	1.74	0.878
	36	92.6	91.8	91.5	93.0	90.7	1.69	0.888
	37	86.3	89.6	90.2	89.1	87.1	1.87	0.565
	38	88.4	88.5	89.9	91.0	88.3	1.94	0.824
	39	88.1^{ab}	91.0^{a}	88.1^{ab}	84.5^{b}	78.8^{c}	1.52	<0.001
	40	90.2	90.1	90.5	90.5	86.9	1.70	0.501
	41	88.1^{x}	84.1^{xy}	82.3^{xy}	80.2^{y}	80.2^{y}	2.01	0.079
	42	87.5	87.4	85.2	84.8	84.4	2.56	0.854
	16-42[1]	81.6	81.0	80.9	80.1	79.5	0.75	0.336

abc Means without a common letter differ significantly (*P*<0.05).

xyz Trends considered for means without a common letter (P<0.10).

*****Hen day egg production percent values are reported as pen averages and adjusted for mortality (n=24).

[1]Represents phase one overall hen day egg production percentages.

Table 2.5 Effects of dietary treatments on phase two hen-day egg production* (%)

	Weeks of Age	Control	TREATMENTS 25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 2	43	87.2	84.1	85.7	80.7	83.3	2.12	0.304
	44	85.4^{ab}	86.0^{a}	81.1^{ab}	80.3^{b}	74.4^{c}	1.76	0.001
	45	83.6	75.8	84.9	82.2	75.5	3.23	0.147
	46	88.7^{a}	83.1^{a}	85.5^{a}	82.5^{a}	75.2^{b}	2.36	0.011
	47	81.0^{ab}	84.4^{a}	84.7^{a}	75.1^{b}	73.0^{b}	2.60	0.011
	48	83.1^{a}	79.0^{ab}	78.6^{ab}	75.3^{bc}	70.7^{c}	2.32	0.020
	49	83.3	84.1	85.4	80.1	75.5	2.92	0.149
	50	87.2^{x}	86.8^{x}	80.9^{xy}	80.1^{xy}	76.8^{y}	2.75	0.064
	51	81.6^{ab}	82.8^{ab}	86.4^{a}	78.4^{bc}	73.2^{c}	1.84	0.001
	52	82.2	77.2	79.7	74.0	71.6	3.00	0.147
	53	69.1	66.5	64.1	67.6	60.5	3.00	0.327
	54	72.9	73.8	68.2	73.2	69.8	3.60	0.751
	55	78.9	76.5	76.9	79.2	77.0	2.61	0.917
	56	76.5	76.0	77.0	72.5	79.3	2.80	0.546
	57	80.0	77.9	76.1	80.5	80.4	2.09	0.492
	58	80.0	79.7	77.1	79.3	80.7	2.71	0.898
	59	84.3	83.0	80.4	85.5	83.6	2.61	0.701
	60	76.9	78.5	76.0	82.0	75.8	2.35	0.326
	61	78.5^{xy}	80.4^{x}	74.2^{y}	81.7^{x}	81.8^{x}	2.10	0.092
	62	73.2^{b}	78.1^{ab}	75.7^{b}	82.0^{a}	82.4^{a}	1.64	0.004
	63	79.9	77.7	78.4	79.0	80.1	2.54	0.957
	64	81.0	77.8	81.1	83.2	83.3	2.94	0.668
	65	76.2	79.7	78.1	80.8	82.4	2.67	0.563
	66	74.2^{c}	78.2^{bc}	76.2^{c}	80.2^{ab}	83.3^{a}	1.38	0.002
	67	73.9^{c}	74.9^{bc}	76.7^{bc}	80.2^{ab}	83.3^{a}	1.95	0.016
	68	76.2	78.1	78.2	78.7	81.0	2.54	0.780
	69	76.9	74.9	75.9	80.2	77.4	2.08	0.435
	70	71.8	76.0	75.2	75.6	81.3	2.73	0.239
	71	71.7	75.4	72.9	75.6	77.1	2.77	0.668
	72	71.5	73.8	76.4	75.4	78.8	2.11	0.215
	73	72.9	78.3	72.4	76.3	80.2	3.39	0.435
	74	73.4	77.3	76.4	76.3	80.9	3.26	0.628
	75	72.7	75.6	75.0	79.2	75.2	3.16	0.708
	43-75[1]	78.7	78.8	78.2	78.8	77.9	1.53	0.990
Overall	**16-75[2]**	**80.0**	**79.8**	**79.3**	**79.4**	**78.6**	**4.76**	**0.881**

abc Means without a common letter differ significantly ($P<0.05$).

xyz Trends considered for means without a common letter ($P<0.10$).

*****Hen day egg production percent values are reported as pen averages and adjusted for mortality (n=24).

[1]Represents phase two overall hen day egg production percentages.

[2]Represents average overall hen day egg production from the beginning to end of study.

Table 2.6 Effects of dietary treatments on egg weight* (g)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	22	51.6[a]	50.8[ab]	49.8[b]	52.0[a]	50.9[ab]	0.50	0.047
	26	55.2	55.7	54.5	56.1	55.7	0.45	0.149
	30	57.7	57.7	57.0	57.2	57.8	0.54	0.797
	34	59.0	59.3	58.0	58.5	59.9	0.61	0.241
	38	60.2	59.1	58.2	59.3	59.8	1.01	0.716
	42	61.1	59.6	59.2	59.6	60.3	0.70	0.434
Phase 2	46	60.9	59.5	59.2	59.6	58.7	1.00	0.647
	50	62.7[a]	61.1[a]	57.1[b]	59.4[ab]	59.8[ab]	1.11	0.030
	54	61.4	62.3	60.8	59.9	61.0	0.65	0.154
	58	63.0	61.0	60.5	60.8	61.7	0.66	0.121
	61	63.0	62.1	62.9	61.7	61.9	1.05	0.880
	66	64.0	62.9	63.3	62.9	63.5	1.03	0.946
	70	64.3	64.4	63.3	63.9	64.2	0.90	0.922
	74	66.5	64.9	65.0	63.2	63.6	0.93	0.176
Overall	**22-74**[1]	**60.3**	**59.6**	**58.7**	**59.2**	**59.6**	**0.49**	**0.266**

[abc] Means without a common letter differ significantly (*P*<0.05).

[xyz] Trends considered for means without a common letter (P<0.10).

*****Egg weights are reported as pen averages (n=24).

[1]Represents average overall egg weight from beginning to end of study.

Table 2.7 Effects of dietary treatments on egg shell percent* (%)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	22	10.0	9.8	11.1	10.2	10.2	0.36	0.117
	26	9.9[a]	9.6[ab]	9.8[a]	9.4[b]	9.7[ab]	0.09	0.036
	30	10.4	10.8	10.8	10.2	10.5	0.40	0.736
	34	9.7	9.4	9.5	9.5	9.7	0.20	0.786
	38	9.2	9.2	9.1	9.0	8.9	0.09	0.269
	42	9.3	9.1	9.3	9.3	9.3	0.13	0.659
Phase 2	46	9.3[a]	9.0[ab]	9.1[a]	9.1[a]	8.8[b]	0.10	0.030
	50	9.2	9.2	9.4	9.3	9.3	0.15	0.946
	54	9.6	9.5	9.2	9.5	9.4	0.15	0.481
	58	9.4	9.2	9.4	8.8	9.8	0.27	0.153
	61	9.0	9.1	9.2	9.2	9.1	0.13	0.650
	66	9.2	9.1	9.2	9.2	9.2	0.14	0.996
	70	9.1	9.0	8.9	9.0	9.0	0.12	0.908
	74	8.8	8.7	9.0	9.2	8.8	0.15	0.125
Overall	**22-74[1]**	**9.4**	**9.3**	**9.5**	**9.3**	**9.4**	**0.08**	**0.663**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
***Egg shell percent values are reported as pen averages (n=24).
[1]Represents average overall egg shell percent from beginning to end of study.

Table 2.8 Effects of dietary treatments on egg shell breaking strength* (N)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	22	28	27	28	26	27	0.9	0.504
	26	32^{a}	30^{bc}	31^{ab}	29^{c}	30^{abc}	0.7	0.040
	30	28	28	29	26	28	0.7	0.394
	34	28	28	26	27	26	0.8	0.364
	38	25	26	24	24	24	0.7	0.382
	42	25	27	27	27	26	0.9	0.405
Phase 2	46	28	26	26	26	26	0.8	0.254
	50	28	29	28	28	30	0.8	0.677
	54	31	29	27	29	29	1.2	0.385
	58	30	29	29	28	30	1.2	0.920
	61	27	28	29	28	28	1.0	0.673
	66	29	28	30	28	29	1.1	0.733
	70	27	27	25	26	26	0.9	0.438
	74	25	25	26	27	25	0.7	0.605

^{abc} Means without a common letter differ significantly (P<0.05).

^{xyz} Trends considered for means without a common letter (P<0.10).

***Egg shell breaking strength values are reported as pen averages (n=24).

Table 2.9 Effects of dietary treatments on Haugh unit values*

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	22	87.9	91.3	92.3	91.7	91.3	1.72	0.475
	26	77.5	79.2	77.5	79.8	79.7	0.98	0.295
	30	96.1[a]	96.4[a]	93.0[b]	93.1[b]	96.8[a]	0.56	<.001
	34	88.1	88.9	87.7	88.7	88.5	0.87	0.882
	38	85.2	87.0	85.3	85.7	87.5	1.09	0.475
	42	92.2	95.2	93.5	93.1	94.7	1.29	0.527
Phase 2	46	90.9	93.9	92.3	92.6	93.5	0.79	0.138
	50	80.7	84.9	83.1	85.4	84.3	1.30	0.163
	54	81.1	83.6	82.1	81.0	82.7	1.42	0.680
	58	83.4	86.9	83.2	82.4	85.7	1.58	0.248
	61	78.4	80.6	78.0	79.3	78.9	1.15	0.540
	66	70.6	72.8	74.6	75.0	73.7	1.72	0.463
	70	71.7	77.3	76.2	73.5	73.9	1.77	0.243
	74	84.0[y]	90.6[x]	87.0[xy]	89.2[x]	90.1[x]	1.57	0.061
Overall	**22-74[1]**	**83.6[b]**	**86.4[a]**	**84.7[ab]**	**85.2[ab]**	**86.0[a]**	**0.68**	**0.077**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
***Haugh unit values are reported as pen averages (n=24).
[1]Represents average overall Haugh unit from beginning to end of study.

Table 2.10 Effects of dietary treatments on yolk weight* (g)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	22	12.3	12.3	11.9	12.8	12.3	0.27	0.226
	26	13.8[y]	13.9[xy]	14.2[x]	14.6[x]	14.0[xy]	0.21	0.134
	30	13.7	14.1	14.4	14.5	14.2	0.17	0.065
	34	15.0	15.7	15.5	15.5	15.6	0.19	0.161
	38	15.9	16.8	16.4	16.6	16.7	0.28	0.257
	42	16.1	16.3	16.1	15.8	15.8	0.16	0.156
Phase 2	46	16.3[ab]	16.9[a]	16.8[a]	16.0[b]	16.3[ab]	0.23	0.043
	50	17.5[a]	17.5[a]	17.4[a]	16.6[b]	16.5[b]	0.20	0.002
	54	17.4	17.2	17.4	17.2	17.2	0.25	0.911
	58	18.0	17.7	17.9	17.4	18.0	0.34	0.754
	61	18.3	18.7	18.1	18.4	18.3	0.30	0.656
	66	18.9	19.0	18.7	18.8	18.7	0.32	0.954
	70	19.6	19.3	18.5	18.7	19.0	0.30	0.147
	74	18.6	18.5	18.4	18.4	18.0	0.30	0.647
Overall	**22-74[1]**	**16.5**	**16.7**	**16.4**	**16.5**	**16.4**	**0.13**	**0.649**

[abc] Means without a common letter differ significantly ($P<0.05$).

[xyz] Trends considered for means without a common letter (P<0.10).

***Yolk weight values are reported as pen averages (n=24).

[1]Represents average overall yolk weight from beginning to end of study.

Table 2.11 Effects of dietary treatments on yolk color lightness[1] (L*)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	22	56.4	55.0	53.8	54.3	54.0	0.89	0.301
	26	61.6[a]	60.2[ab]	59.9[b]	58.8[bc]	58.1[c]	0.54	0.003
	30	59.7[a]	57.3[bc]	58.2[b]	57.8[b]	56.2[c]	0.46	0.001
	34	61.6[a]	59.6[b]	59.5[b]	59.6[b]	59.2[b]	0.37	0.002
	38	64.3[a]	62.2[b]	62.5[b]	61.9[b]	62.0[b]	0.37	0.002
	42	63.2[a]	60.6[b]	60.2[b]	59.9[b]	60.7[b]	0.29	<.0001
Phase 2	46	62.6	61.1	61.5	61.9	61.3	0.38	0.120
	50	64.3[a]	62.6[bc]	63.2[b]	63.3[b]	62.3[c]	0.27	0.001
	54	64.6[a]	62.7[b]	62.5[b]	62.6[b]	62.3[b]	0.36	0.003
	58	64.0[a]	63.0[b]	63.1[b]	63.1[b]	63.1[b]	0.19	0.017
	61	64.3[a]	63.3[b]	63.0[b]	64.3[a]	64.2[a]	0.14	<.0001
	66	63.5[a]	62.1[b]	62.9[ab]	63.3[a]	63.5[a]	0.31	0.020
	70	63.8[a]	62.8[b]	63.1[b]	63.7[a]	63.7[a]	0.17	0.001
	74	63.2[a]	62.1[c]	62.1[c]	62.6[bc]	62.7[ab]	0.19	0.003
Overall	**22-74[2]**	**62.6[a]**	**61.0[b]**	**61.1[b]**	**61.2[b]**	**60.9[b]**	**0.12**	**<.0001**

[abc] Means without a common letter differ significantly ($P<0.05$).
[xyz] Trends considered for means without a common letter ($P<0.10$).
[1]Yolk color lightness (L*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color lightness (L*) from beginning to end of study.

Table 2.12 Effects of dietary treatments on yolk color redness[1] (a*)

	Weeks of Age	TREATMENTS					SEM	*P*-value
		Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix		
Phase 1	22	9.6^{c}	14.0^{ab}	13.5^{b}	14.3^{ab}	14.5^{a}	0.33	<.0001
	26	9.8^{d}	14.5^{b}	13.9^{c}	15.3^{a}	15.0^{ab}	0.21	<.0001
	30	11.6^{c}	16.3^{b}	16.7^{b}	17.2^{a}	17.2^{a}	0.14	<.0001
	34	11.0^{c}	15.3^{b}	15.3^{b}	16.1^{a}	16.3^{a}	0.19	<.0001
	38	10.3^{c}	15.4^{b}	15.3^{b}	16.6^{a}	16.2^{a}	0.23	<.0001
	42	9.3^{c}	15.9^{ab}	15.7^{b}	16.3^{a}	15.7^{b}	0.17	<.0001
Phase 2	46	12.9^{d}	16.5^{a}	15.7^{b}	15.0^{c}	14.4^{c}	0.22	<.0001
	50	12.8^{c}	16.3^{a}	15.7^{a}	14.1^{b}	14.4^{b}	0.21	<.0001
	54	11.4^{d}	14.8^{a}	14.3^{ab}	14.1^{bc}	13.5^{c}	0.21	<.0001
	58	13.8^{b}	14.6^{a}	14.7^{a}	13.3^{b}	13.3^{b}	0.17	<.0001
	61	13.5^{b}	15.2^{a}	15.4^{a}	13.7^{b}	12.7^{c}	0.17	<.0001
	66	14.9^{c}	16.2^{a}	15.6^{b}	13.9^{d}	13.2^{e}	0.17	<.0001
	70	14.5^{b}	15.5^{a}	15.1^{a}	13.2^{c}	13.3^{c}	0.18	<.0001
	74	13.8^{b}	15.2^{a}	15.3^{a}	13.6^{b}	13.6^{b}	0.19	<.0001
Overall	**22-74**[2]	**12.1^{e}**	**15.4^{a}**	**15.1^{b}**	**14.8^{c}**	**14.6^{d}**	**0.07**	**<.0001**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
[1]Yolk color redness (a*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color redness (a*) from beginning to end of study.

Table 2.13 Effects of dietary treatments on yolk color yellowness[1] (b*)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	22	51.0	55.0	54.6	54.4	53.7	1.46	0.388
	26	53.0[b]	59.7[a]	58.4[a]	59.8[a]	59.1[a]	0.78	<.0001
	30	60.6[b]	65.1[a]	65.4[a]	64.4[a]	63.4[a]	0.73	0.002
	34	55.2[b]	60.2[a]	59.9[a]	60.5[a]	60.3[a]	0.75	0.001
	38	55.9[c]	64.2[ab]	62.8[b]	67.6[a]	62.5[b]	1.61	0.002
	42	55.6[c]	64.1[a]	64.9[a]	63.9[a]	61.4[b]	0.59	<.0001
Phase 2	46	59.9[ab]	63.8[a]	61.7[a]	62.9[a]	57.6[b]	1.29	0.019
	50	63.8[c]	67.7[a]	66.8[ab]	64.9[bc]	64.7[bc]	0.87	0.035
	54	57.7[bc]	60.6[a]	60.8[a]	59.4[ab]	57.4[c]	0.56	0.001
	58	63.7[a]	63.0[ab]	63.0[ab]	60.9[bc]	60.0[c]	0.88	0.036
	61	68.2[a]	67.5[a]	68.6[a]	67.3[a]	64.6[b]	0.62	0.001
	66	69.4[ab]	72.0[a]	71.4[a]	67.1[b]	67.2[b]	0.88	0.001
	70	70.5[a]	70.5[a]	70.2[a]	66.8[b]	68.0[ab]	0.87	0.019
	74	65.5[a]	66.2[a]	66.5[a]	65.6[a]	62.1[b]	0.60	<0.001
Overall	**22-74**[2]	**60.7[b]**	**64.2[a]**	**63.9[a]**	**63.2[a]**	**61.5[b]**	**0.37**	**<.0001**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
[1]Yolk color yellowness (b*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color yellowness (b*) from beginning to end of study.

Table 2.14 Effects of dietary treatments on scrambled egg taste and purchase intent at 74 weeks of age[1]

	TREATMENTS						
	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Flavor	5.4	6.4	5.6	5.8	5.6	0.4	0.587
Off Flavor	6.4	6.4	6.3	6.7	5.8	0.5	0.877
Buttery Flavor	3.9	3.9	3.9	3.8	3.8	0.5	0.990
Metallic Flavor	1.4	1.5	1.8	1.8	1.6	0.3	0.540
Fishy Flavor	1.6	1.4	1.7	1.3	1.6	0.2	0.633
Painty Flavor	1.3	1.2	1.3	1.6	1.6	0.2	0.173
Rancid Flavor	1.3	1.1	1.8	1.2	1.8	0.3	0.120
Grassy Flavor	1.4	1.8	1.5	2.1	1.6	0.3	0.181
Purchase Intent	5.1	6.3	5.6	6.0	5.3	0.5	0.359

[abc] Means without a common letter differ significantly ($P<0.05$).
[xyz] Trends considered for means without a common letter (P<0.10).
[1]Taste parameter values are reported as averages (n=16).

Table 2.15 Effects of dietary treatments on bone quality and mineralization* (75 weeks of age)

	Bone	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Breaking Strength (kg force)	Tibia	2.7	2.8	2.8	3.0	3.0	0.1	0.488
	Humerus	1.9	1.9	1.9	1.9	2.0	0.1	0.989
Ash (%)	Tibia	59.7	60.7	60.4	61.5	58.8	1.0	0.447
	Humerus	58.1^{bc}	60.7^{ab}	57.0^{c}	62.9^{a}	58.8^{bc}	1.2	0.015
Ca (%)	Tibia	31.8	31.8	31.5	33.8	31.0	0.85	0.213
	Humerus	31.1^{b}	32.0^{ab}	32.8^{a}	32.6^{a}	32.0^{ab}	0.36	0.038
P (%)	Tibia	18.6	18.6	18.6	19.5	18.4	0.33	0.237
	Humerus	18.4	18.8	18.8	18.8	18.6	0.16	0.348

^{abc} Means without a common letter differ significantly (*P*<0.05).
^{xyz} Trends considered for means without a common letter (P<0.10).
*****Bone measurements are reported as pen averages (n=24).

CHAPTER 3. Effect of a proprietary premix on productive performance and egg quality of brown egg laying hens fed diets high in distillers dried grains with solubles through 60 weeks of production

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Summary

Ethanol production has decreased the availability of grains for poultry consumption and increased the price of high quality grains, making distiller's dried grains with solubles (DDGS) a more attractive ingredient for increased inclusion in poultry diets. The objective of this study was to evaluate the effects of including up to 35% DDGS in corn-soybean meal based diets with and without a proprietary premix (enzyme and antioxidant; Alltech, Inc.) on egg quality and production parameters. Hy-Line® brown hens (288) were allocated in a completely randomized design at 16 weeks of age. The five dietary treatment groups consisted of the following: 1) corn-soybean meal (control), 2) 25% DDGS, 3) 25% DDGS plus premix, 4) 35% DDGS, and 5) 35% DDGS plus premix. At 46 weeks of age, crude protein levels were reduced from 18 to 16% and during both dietary phase's the DDGS diets contained reduced levels of calcium and available phosphorus compared to the control treatment. The addition of up to 35% DDGS had no effect on overall mortality or overall feed intake. Addition of the premix maintained body weights and sustained gain differences comparable to the control in the 25% DDGS plus premix treatment (P=0.016). Hens on the premix came into lay faster than the control and DDGS treatments (P=0.041); however, over the 60 week production cycle addition of 25 and 35% DDGS reduced hen-day egg production (P=0.019). Overall egg quality, shell quality, and bone quality were not affected by the addition of up to 35% DDGS. Improvements in overall Haugh unit values (P=0.033), yolk color scores (P<0.0001), and a tendency for increased buttery flavor of scrambled eggs (P=0.058) may explain the increased purchase intent score in the 35% DDGS plus premix diet (P=0.018). Therefore, addition of up to 35% DDGS reduced overall hen-day egg production and hen body weights. Addition of the premix may help alleviate the negative effects on body weight and increase some egg quality characteristics.

KEYWORDS: enzyme and antioxidant, distillers dried grains with solubles, brown egg laying hens, production, egg quality

3.1 Description of Problem

Distillers dried grains with solubles are a by-product of the ethanol industry and have been commercially accepted as a partial replacement for costly ingredients like corn and soybean meal. Ethanol production uses roughly one bushel of corn (56 pounds) to produce 2.8 gallons of ethanol, yet, it produces 17.5 pounds of by-product DDGS for animal consumption [3]. Due to the increased demand of corn for ethanol production and limited availability of high quality grains, research is looking into adding higher levels of DDGS to laying hen diets. Currently, the poultry industry is only using approximately eight percent of the total available DDGS supply for livestock [1]. Increased levels in DDGS to laying hen rations could help cut costs for producers.

In laying hen diets, DDGS are typically added in place of costly ingredients like corn, soybean meal, and supplemental phosphorus. Distillers dried grains with solubles are considered a good source of energy, protein, water-soluble vitamins and minerals [8, 19]. The literature suggests it is safe to include between 15-20% DDGS without adverse effects on production parameters; however, the availability of nutrients in DDGS are variable. This variability has potentially caused some negative effects on production parameters when DDGS was added to laying hen diets. Most of this variation in DDGS as a feedstuff is caused by the different processing methods used in a number of ethanol plants. One of the biggest sources of variation in DDGS is the drying temperature at the end of processing, as higher temperatures can decreased the amino acid availability in DDGS [12, 17].

Limited studies have published results about the effects of higher levels of DDGS in brown laying hen rations. It has been reported that including between 15 and 25%

DDGS in well-formulated diets to brown egg laying hen does not have an effect on production parameters in the early stages of production [9, 35, 36]. However, when the studies were carried into the middle of a production cycle negative effects on laying rate and egg weight were noted with 20% or higher inclusion level of DDGS [9, 37, 38]. The inclusion of up to 20% DDGS has been reported to reduce eggshell percentage [37] and improve yolk pigmentation [38]. However, inclusion of 25 to 35% DDGS to the diets of white egg-laying hens has shown no depression in egg production and egg weight when included in well formulated diets [33, 34].

Therefore the objectives of this study were to evaluate the inclusion of 25 and 35% DDGS with and without the addition of THE premix on the productive performance and egg quality of brown egg-laying hens. The premix contains a proprietary nutrition strategy containing enzyme technology along with other solutions to help release more nutrients out of the less digestible by-product DDGS. It was hypothesized that the inclusion of both 25% and 35% DDGS would negatively impact the egg production and egg weight based on previously published research in brown egg laying hens. It was also suspected that the addition of the premix would reduce negative effects of the 25% DDGS level and the 35% DDGS inclusion level.

3.2 Materials and Methods

All procedures were performed in accordance with the University of Kentucky's Institutional Animal Care and Use Committee (IACUC) March 7, 2012 until April 5, 2013.

3.2.1 Animal Care and Use

A total of 244 Hy-Line® Brown hens were used for this experiment that lasted 60 weeks from March 7, 2012 to April 2, 2013. At 16 weeks of age, hens were weighed and placed in elevated battery cages adjusting for extremely high or low weight hens. A replicate group consisted of three adjacent cages on the top and bottom tiers of the batteries. Two hens were placed per 516 cm^2 cage making a total of 12 birds per replicate group. Due to space limitations within the room, the control treatment was reduced by one replication in order to increase the chance of observing differences among the DDGS treatments. Hens were adjusted to a 16 light: 8 dark lighting schedule after placement, and provided *ad libitum* access to feed and water throughout the study. Birds were housed in a fan-ventilated, climate controlled room within the research facility.

3.2.2 Dietary Treatments

The basal was a corn-soybean meal diet. The DDGS diets contained a corn-soybean meal base and two adjusted levels of DDGS with and without a proprietary premix (enzyme and antioxidant; [4]). The five dietary treatments consisted of: 1) corn-soybean meal (control), 2) 25% DDGS, 3) 25% DDGS plus premix, 4) 35% DDGS, and 5) 35% DDGS plus premix. Diets were formulated using the NRC requirements with the two dietary phases based on crude protein levels (Table 3.1). Phase one diets were fed from 16-42 weeks of age and were calculated to be isocaloric, contain approximately 18% crude protein, and each of the DDGS diets were formulated with reduced calcium (4.1 vs. 4.2%) and available phosphorus (0.19 vs. 0.29%) compared to the control. During the second phase (starting at 43 weeks of age) the crude protein was reduced from

18% to 16% and the diets were formulated to be isocaloric, with reduced calcium (3.7 vs. 3.8%) and available phosphorus (0.22 vs. 0.30%) in the diets containing DDGS compared to the control.

3.2.3 Data Collection and Methods

Birds were weighed in their replicate groups once a month to monitor the average body weight of the hens. Feed intake was recorded for the replicate groups on an as-fed basis and reported in correspondence with body weight. Egg production was recorded daily for each replicate group in order to calculate the hen day production. Once a month, during the same week that the birds were weighed, the daily egg production was also weighed. From the weighed eggs that day, a representative sample of six eggs was randomly selected for egg quality analysis.

Shell breaking strength was analyzed using a Shimadzu EZ-S texture analyzer [64]. The machine was used to apply enough pressure (N) to crack the eggshell without puncturing the shell membrane. Eggs were placed horizontally under the load cells for the eggshell breaking procedure. Eggs were analyzed for albumin height with the TSS Quantum Chromodynamics Super System [65]. The super system probe was used to pierce the albumen approximately one cm away from the yolk. Albumen heights and individual egg weights were recorded and used to calculate Haugh Units for a good indication of internal egg quality. Yolks were separated from the albumen for color determination using a Hunterlab Colorflex EZ colorimeter [39] to measure the transmittance and reflectance. The colorimeter measured L*, a*, and b* values to analyze lightness, redness, and yellowness of the yolks respectively (Appendix 1). Yolks were also weighted in their respective replicate groups for the yolk weight parameter.

3.2.4 Bone Sample Collection

Bone samples were collected at the end of the trial for breaking strength and mineral analysis. During 76 weeks of age, three birds per replicate group were euthanized and tibia and humerus were removed. The birds' right tibia and humerus were collected for breaking strength and broken using the Instron 4301 machine [66]. The machine was used to apply enough pressure (kg force) in the middle of the bone to break the bone. Two tibia and humerus bones from the left side of the bird were boiled, cleaned, defatted, dried, and ashed (Appendix 2).

3.2.5 Taste Panel

A sensory panel was performed at the end of the 60-week study to analyze the taste of the eggs. Treatments were assigned random three-digit numbers using a random number generator and samples were also randomized to deter panelists from number preferences (bias). Ten eggs from each treatment were randomly selected from their respective replicate groups and scrambled for 60 s using a wire whisk. Samples were prepared in a non-stick skillet under medium high heat (191-204° C) using 243 g of prepared egg sample per treatment. Samples were cooked for 90 seconds and immediately removed and distributed to untrained panelists under red lights (to avoid color bias) in approximately 20-gram aliquots. The skillet was cleaned in between each treatment with water and the temperature was checked to ensure consistency between samples. Panelists were provided water to cleanse their pallet and asked to rate the eggs on a scale of 1-8 for the following categories: flavor, off-flavor, buttery flavor, metallic flavor, fishy flavor, painty flavor, rancid flavor, grassy flavor, and purchase intent (Appendix 3).

3.2.6 Statistical Analysis

Data was analyzed using the Proc GLM procedure in SAS [67] to determine the main effects of the five treatments. Means were separated using a Fisher's least significant difference test. Significance was accepted at $P \leq 0.05$, with tendency's considered between $P \geq 0.05$ and $P \leq 0.10$.

3.3 Results and Discussion

3.3.1 Production Parameters

No effect of dietary treatment was observed on mortality (less than 13%) throughout the 60 week study. Although body weights were adjusted during bird placement (16 weeks of age) the hens on the 25% DDGS plus premix, 35% DDGS, and 35% DDGS plus premix treatments were on average 51 grams heavier (P=0.028) than the control and 25% DDGS treatment (1581 vs. 1530 g/bird, Table 3.2). These effects were not observed in weeks following placement but did carry over into the overall gain calculations from both weeks 20 to 76 weeks of age and 16 to 76 weeks of age. A positive effect of the premix was noted in the 25% DDGS on hen body weight as the birds were not different than the control for the overall gain calculations previously mentioned. Positive effects of the premix on hen body weight were also observed during

52, 72, and 76 weeks of age in both the 25 and 35% DDGS diets ($P<0.029$). No differences were observed on overall gain (28 to 76 weeks of age) after hens reached mature body weight at 28 weeks of age.

Feed intake followed a similar pattern to body weight around placement (16 to 20 weeks of age) with the premix treatments having the same feed intake levels as the control treatment (Table 3.3). These improvements were not noted during 29 to 32 weeks of age when inclusion of dietary DDGS reduced feed intake in comparison to the control treatment ($P=0.014$). Overall no differences in feed intake were noted during dietary phase one and phase two. During 73 to 76 weeks of age, dietary DDGS increased feed intake when compared to the control ($P<0.001$), suggesting a need for hens to eat more feed to gain nutrients during the final stages of production. On average the birds ate approximately 111 grams of feed per bird per day, and was not different in overall feed intake (16-76 weeks of age) which is consistent with the some of the published literature in brown egg laying hens [9, 35, 36].

Birds on the premix came into production faster than the control and DDGS without premix treatments at 16 and 17 weeks of age ($P<0.041$; Table 3.4) and could be related to increased body weight and feed intake data at this time period. However, this suggests that future studies should look into the age at first egg with the addition of the premix. During 27 weeks of age, inclusion of the premix in the 25% diet reduced some of the negative effects of DDGS on egg production maintaining levels similar to the control treatment ($P=0.038$). Although there was no significant overall difference in egg production during dietary phase one and phase two, negative effects of DDGS inclusion started to show around 39 weeks of age and carried through 51 weeks of age (Table 3.5) and support the results of Niemiec et al. [37]. Hen day egg production varied through the middle of production cycle implying that some weekly differences could be due to an artifact of collection. However, the total overall production from 16 to 75 weeks of age was reduced in DDGS treatments in comparison to the control treatment (*P*=0.019). Production results are comparable to the results of Ghazalah et al. [38] who reported reduced laying rate with the addition of 25, 50 and 75% DDGS.

3.3.2 Egg Quality and Taste

Egg weight was unaffected by dietary treatment over the 60 week trial (Table 3.6) similar to previous findings [9, 29, 31, 36, 37]. Egg shell percentage was significantly reduced with the addition of DDGS during two potential stages of metabolic stress (Table 3.7); when the birds were placed on high DDGS diets (*P*=0.027; 20 weeks of age) and when hens were reaching peak egg production (*P*=0.049; 28 weeks of age). These reductions in percent shell were not of concern at the beginning of production as no differences in shell breaking strength were observed (Table 3.8). No other effects of dietary treatment were observed on shell quality during either dietary phase and are consistent with other studies [9, 31, 35].

Haugh unit improvements were noted with the addition of dietary DDGS during 20, 44, and 68 weeks of age, however, the noted improvements were not consistent among treatments/weeks (Table 3.9). Despite this the overall average Haugh unit values were increased with the addition of 35% DDGS (*P*=0.033). Other authors that have noted increased Haugh unit values with the addition of DDGS have suggested the improvements might be due to increased trace elements in the DDGS [22].

Overall yolk weight was unaffected by dietary treatment (Table 3.10). During 32 and 48 weeks of age depressions in yolk weight were noted in some of the DDGS treatments when compared to the control treatment. However, the reductions are not seen throughout the 60 week study, are less than approximately one gram in difference, and could have potentially been due to an artifact of collection. Therefore, it is conclude that addition of up to 35% DDGS did not reduce yolk weight.

Yolk color improved with the addition of DDGS similar to previous publications [9, 31, 34-37]. Lightness values (L*) were decreased with the addition of DDGS after 7 weeks on the experimental diets (P=0.007; Table 3.11). Hens on the 35% DDGS treatments had further decreases in yolk color L* values starting at 28 weeks of age (P=0.003) and carried into the end of the first dietary phase (P<0.0001). After the change to phase two diets no further reductions in L* values were noted with the 35% DDGS treatments. From 60 to 76 weeks of age, no differences were noted between the dietary treatments. Yolk color a* values (redness) were also improved with the addition of DDGS until 60 weeks of age when compared to the control treatment (P<0.0001; Table 3.12). The 25% DDGS treatments had additional improved red color when compared to the 35% DDGS treatments after the dietary phase change from 44 to 76 weeks of age (P<0.0001). From 60 to 68 weeks of age, the control and 35% DDGS treatments were not different. This is not explained by the data collected or pigmentation of the feed samples. In addition, yolk color b* values for yellowness follow the same pattern as a* values throughout both dietary phases and are shown in Table 3.13.

Results from the taste panel show no effect of dietary treatment on the flavor parameters measured in the scrambled egg taste panel (Table 3.14). However, a tendency

(*P*=0.058) for increased buttery flavor was noted in the 35% DDGS plus premix treatment when compared to the control and could have translated into the increased purchase intent (*P*=0.0182). The 35% DDGS plus premix dietary treatment had eggs with the highest purchase intent score (6.0) and was different from the eggs on the control treatment (4.3). The purchase intent values for the other DDGS treatments averaged 5.3 and were not different from the previous treatments mentioned above. The Pearson correlation (Appendix 5) showed that as the flavor parameter increased buttery flavor increased (0.4) and metallic (-0.3), painty (-0.4), and rancid (-0.4) flavor scores decreased while purchase intent increased (0.8), thus validating the panel. Thus, the addition of up to 35% DDGS did not have a negative effect on the taste of the eggs and may have a beneficial impact on taste scores and purchase intent.

3.3.3 Bone Quality

No differences in bone quality were noted at 76 weeks of age (Table 3.15). A trend (*P*=0.083) towards improved bone strength was noted in the tibia with the addition of 35% DDGS and could potentially be due to the trend (*P*=0.080) observed for increased calcium content. Overall no differences in bone quality parameters (breaking strength, ash, calcium, or phosphorus) were noted in the collected humerus bones. Analyzed dietary values referenced in Appendix 6.

3.4 Conclusions and Applications

1. Inclusion of up to 35% with reduced phosphorus DDGS in the diets of brown laying hens has no effect on mortality, feed intake, or bone quality.

2. The proprietary premix (enzyme and antioxidant) induced lay faster when included in DDGS diets however; over a long production cycle reduced hen-day egg production.

3. The proprietary premix showed additional improvements on hen body weight maintaining heavier birds then the high DDGS diets.

4. Inclusion of up to 35% DDGS has no negative effect on egg shell quality, egg weight, or yolk weight and improves Haugh unit values and yolk color scores.

5. The proprietary premix may increase the purchase intent of brown scrambled eggs when included in high DDGS diets.

3.5 Tables and Figures

Table 3.1 Diet composition and calculated analysis for phase one and phase two of production

	Percent of Diet (%)					
	Phase One (16-40 weeks of age)			Phase Two (41-76 weeks of age)		
Diet Composition	Control	25% DDGS	35% DDGS	Control	25% DDGS	35% DDGS
Corn	56.51	44.90	37.33	63.90	50.54	40.20
Soybean meal, 48% CP	28.20	14.70	11.10	21.50	10.00	6.30
DDGS[1]	0.00	25.00	35.00	0.00	25.00	35.00
Corn oil	3.05	3.95	4.90	2.50	3.00	4.90
Oyster Shell	3.00	3.00	3.00	3.00	3.00	3.00
Limestone	7.30	7.50	7.56	6.20	6.60	6.70
Dicalcium phosphate	1.00	0.11	0.00	1.10	0.00	0.00
Salt	0.42	0.15	0.13	0.44	0.26	0.19
Vitamin-mineral mix*	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.17	0.13	0.10	0.11	0.09	0.09
L-Lysine	0.00	0.31	0.38	0.00	0.23	0.33
Tryptophan	0.00	0.00	0.00	0.00	0.03	0.04
Celite	0.10	0.00	0.25	1.00	1.00	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME, kcal/kg	2,891	2,892	2,894	2,893	2,895	2,903
CP, %	18.58	18.16	18.56	15.92	15.94	15.97
Ca, %	4.22	4.11	4.11	3.81	3.69	3.72
P, avail., %	0.29	0.19	0.19	0.30	0.20	0.24
Lys, %	0.98	0.98	0.98	0.84	0.78	0.78
Met, %	0.46	0.46	0.45	0.38	0.37	0.37

[1]DDGS Nutrient composition: 3,035 kcal/kg ME; 89.4% DM; 25.8% CP; 10.9% Crude fat; 5.6% Crude fiber; 4.9% Ash.

*Vitamin mineral mix provided (per kg diet): 11025 IU vitamin A, 3528 IU vitamin D, 33.075 IU vitamin E, 0.9096 mg vitamin K, 2.205 mg Thiamin, 7.7175 mg Riboflavin, 55.125 mg Niacin, 17.64 Pantothenate, 4.9613 mg vitamin B-6, 0.2205 mg d-biotin, 1.1025 mg Folic acid, 478.485 mg Choline, 0.0276 mg vitamin B-12, 75 mg Zinc, 40 mg Fe, 64 mg Mn, 10 mg Cu, 1.85 mg I, 0.3 mg Se.

Table 3.2 Effects of dietary treatments on brown egg laying hen body weight* (g/bird)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16	1526^{b}	1533^{b}	1597^{a}	1561^{ab}	1584^{a}	16.6	0.028
	20	1824	1822	1860	1847	1866	17.8	0.314
	24	1875	1846	1886	1861	1891	17.2	0.324
	28	1933	1895	1935	1897	1922	17.2	0.564
	32	1927	1847	1894	1874	1865	17.2	0.325
	36	1942	1905	1962	1931	1939	17.2	0.541
	40	1912	1870	1896	1873	1868	17.3	0.771
Phase 2	44	1915	1886	1900	1824	1860	17.2	0.137
	48	1943	1892	1954	1882	1906	17.2	0.148
	52	2001^{a}	1947^{ab}	2004^{a}	1909^{b}	1950^{ab}	17.2	0.029
	56	1986^{x}	1898^{y}	1948^{xy}	1872^{y}	1913^{xy}	17.2	0.089
	60	1992	1889	1950	1859	1889	17.2	0.156
	64	2020^{x}	1946^{y}	1970^{xy}	1919^{y}	1934^{y}	17.2	0.065
	68	2023	1950	2001	1931	1948	26.6	0.121
	72	2013^{a}	1925^{bc}	1991^{ab}	1913^{c}	1973^{abc}	19.1	0.028
	76	1987^{a}	1917^{b}	1999^{a}	1899^{b}	1939^{ab}	19.5	0.013
Gain	20-76[1]	$+163^{a}$	$+95^{bc}$	$+139^{ab}$	$+52^{c}$	$+73^{c}$	17.6	0.002
	28-76[2]	+54	+22	+65	+2	+18	24.2	0.349
	16-76[3]	**$+461^{a}$**	**$+384^{b}$**	**$+402^{ab}$**	**$+338^{b}$**	**$+355^{b}$**	**19.9**	**0.016**

abc Means without a common letter differ significantly (*P*<0.05).

xyz Trends considered for means without a common letter (P<0.10).

*Hen body weight values are reported as pen averages (n=24).

[1]Represents body weight gain from dietary adjustment period to end of study.

[2]Represents body weight gain from mature body weight to end of study.

[3]Represents the overall body weight gain from the beginning to end of study.

Table 3.3 Effects of dietary treatments on brown egg laying hen feed intake* (g/bird/day)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16-20	90.2[ab]	88.6[b]	92.5[ab]	88.6[b]	93.6[a]	1.36	0.048
	21-24	102.7	101.4	105.0	100.6	102.4	1.38	0.234
	24-28	111.7	111.4	110.9	108.7	110.1	1.46	0.609
	29-32	105.4[a]	101.4[b]	101.9[b]	100.4[b]	99.7[b]	1.04	0.014
	33-36	110.9	107.6	108.4	106.3	108.8	1.53	0.373
	37-40	97.5	97.0	94.3	93.7	92.9	1.52	0.179
Phase 2	41-44	102.9	102.9	102.5	100.2	100.8	1.59	0.637
	45-48	105.6	103.2	106.6	103.7	104.7	1.42	0.440
	49-52	113.0	114.4	114.8	116.4	116.2	2.22	0.829
	53-56	155.0	151.7	154.3	151.1	153.1	2.71	0.835
	57-60	112.2	112.4	113.7	115.6	115.4	1.81	0.566
	61-64	116.9	117.4	117.5	120.0	118.0	1.99	0.820
	65-68	116.4	118.0	119.9	120.0	121.3	2.59	0.726
	69-72	116.2	119.2	120.5	124.6	122.0	2.76	0.333
	73-76	109.8[b]	118.7[a]	118.6[a]	123.8[a]	122.3[a]	1.90	<0.001
Overall	16-40[1]	103.0	101.2	102.1	99.6	101.2	1.00	0.235
	41-76[2]	116.3	117.4	118.7	119.1	119.1	1.64	0.710
	16-76[3]	**110.8**	**110.7**	**111.9**	**111.0**	**111.7**	**1.19**	**0.920**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
*****Feed intake values are reported as pen averages (n=24).
[1]Represents phase one average feed intake.
[2]Represents phase two average feed intake.
[3]Represents average feed intake from the beginning to end of study.

Table 3.4 Effects of dietary treatments on phase one brown hen day egg production* (%)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	16	0.0^{b}	0.5^{b}	3.8^{a}	1.2^{b}	2.6^{ab}	0.89	0.041
	17	15.8^{abc}	10.2^{c}	23.3^{a}	13.1^{bc}	18.8^{ab}	2.95	0.040
	18	64.3	55.0	63.6	52.2	52.4	4.81	0.239
	19	87.8	88.6	87.4	86.7	86.2	2.47	0.961
	20	94.4	93.3	94.5	93.6	91.4	1.53	0.623
	21	97.0	96.2	96.4	91.7	94.5	1.64	0.196
	22	98.8^{x}	98.8^{x}	98.8^{x}	95.7^{y}	98.1^{xy}	0.90	0.095
	23	98.2	98.6	98.1	95.4	98.8	1.17	0.272
	24	100.3	100.2	100.2	97.1	98.3	1.27	0.299
	25	98.2	98.3	98.3	97.6	96.2	1.40	0.778
	26	98.2	98.1	97.4	96.7	98.6	1.23	0.812
	27	100.0^{a}	97.1^{b}	98.8^{ab}	96.4^{b}	96.4^{b}	0.88	0.038
	28	96.4	97.1	96.4	95.4	97.6	1.24	0.755
	29	97.0	96.9	91.9	93.8	95.9	1.71	0.197
	30	96.4^{xy}	99.0^{x}	96.2^{xy}	93.7^{y}	95.5^{xy}	1.26	0.084
	31	96.4	96.9	91.2	93.5	95.0	1.64	0.132
	32	97.3	97.4	95.9	95.0	91.9	1.74	0.195
	33	97.6	96.6	95.7	95.5	94.5	1.41	0.635
	34	98.5	97.6	97.4	96.6	96.7	1.14	0.787
	35	96.4	95.3	93.8	94.2	96.2	0.85	0.170
	36	96.7	97.7	95.2	93.3	95.3	1.97	0.583
	37	98.5	93.3	93.8	91.6	93.4	2.03	0.253
	38	91.7	92.6	87.3	89.2	87.3	1.70	0.119
	39	90.5^{a}	90.0^{a}	83.0^{b}	83.4^{b}	81.0^{b}	2.09	0.013
	40	94.7^{a}	94.1^{a}	89.5^{ab}	89.3^{ab}	85.1^{b}	1.85	0.010
	16-40[1]	87.8^{x}	86.8^{x}	86.6^{xy}	84.6^{y}	85.6^{xy}	0.72	0.056

[abc] Means without a common letter differ significantly (*P*<0.05).

[xyz] Trends considered for means without a common letter (P<0.10).

*****Hen day egg production percent values are reported as pen averages and adjusted for mortality (n=24).

[1]Represents phase one overall hen day egg production percentages.

Table 3.4 Effects of dietary treatments on phase two brown hen day egg production* (%)

	Weeks of Age	TREATMENTS					SEM	*P*-value
		Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix		
Phase 2	41	95.5	95.6	91.9	92.0	89.5	1.88	0.152
	42	92.6^{a}	93.2^{a}	90.3^{a}	89.9^{a}	83.7^{b}	1.42	0.001
	43	93.4^{a}	92.1^{a}	89.8^{a}	88.7^{ab}	84.6^{b}	1.73	0.018
	44	95.8^{a}	92.7^{ab}	88.0^{bc}	83.3^{c}	85.7^{c}	2.25	0.007
	45	93.8	93.2	86.6	87.7	87.1	2.46	0.137
	46	93.5^{a}	82.7^{b}	85.0^{b}	79.5^{b}	83.2^{b}	2.52	0.018
	47	91.7^{x}	90.5^{xy}	85.8^{xy}	90.0^{xy}	84.6^{y}	2.04	0.096
	48	94.9^{a}	89.5^{ab}	82.2^{c}	83.9^{bc}	85.4^{bc}	1.97	0.002
	49	92.9	90.1	83.7	89.5	87.7	2.92	0.306
	50	94.6	92.1	84.6	84.7	88.2	3.17	0.154
	51	94.7^{a}	90.7^{ab}	85.6^{b}	86.9^{b}	86.3^{b}	2.02	0.034
	52	93.8^{x}	90.3^{xy}	85.7^{y}	86.0^{y}	86.3^{y}	2.17	0.082
	53	81.6	79.1	74.6	74.5	79.1	2.33	0.182
	54	85.1	80.6	79.9	82.3	82.9	3.26	0.830
	55	90.4	87.4	82.8	87.3	86.8	2.17	0.238
	56	91.1	87.8	85.3	87.3	81.6	3.34	0.409
	57	93.8	87.7	85.6	91.3	88.3	2.09	0.101
	58	92.8	90.6	91.3	93.0	90.8	2.24	0.905
	59	89.2	89.7	87.3	90.6	88.2	2.67	0.911
	60	91.5	85.0	86.1	88.7	87.5	2.42	0.441
	61	89.4	88.8	87.3	88.2	85.6	2.32	0.800
	62	89.3	88.7	85.1	88.1	86.7	2.18	0.670
	63	91.0	92.0	83.4	87.3	89.4	3.00	0.299
	64	92.7	92.9	87.6	89.3	87.5	2.87	0.513
	65	92.2	87.7	85.8	89.1	87.8	2.86	0.640
	66	91.4	89.2	83.5	89.1	85.8	3.02	0.411
	67	92.3^{a}	89.5^{ab}	81.6^{bc}	79.1^{c}	87.9^{ab}	2.81	0.020
	68	90.7	89.1	86.0	86.9	86.6	2.45	0.675
	69	91.0	87.6	87.6	85.2	82.1	2.75	0.280
	70	84.4	88.7	82.7	89.6	85.2	2.55	0.278
	71	82.4	84.5	79.8	87.8	83.9	3.25	0.518
	72	84.2	85.9	82.3	84.4	80.1	2.78	0.621
	73	84.6^{abc}	86.0^{ab}	77.4^{c}	89.1^{a}	80.5^{bc}	2.49	0.025
	74	81.3	86.3	81.6	86.5	85.7	2.80	0.510
	75	73.4	85.2	80.8	85.8	84.1	3.32	0.117
	43-75[1]	90.3^{x}	88.8^{xy}	84.8^{z}	86.9^{xyz}	85.6^{yz}	1.36	0.064
Overall	**16-75**[2]	**89.2^{a}**	**88.0^{ab}**	**85.6^{c}**	**85.9^{bc}**	**85.6^{c}**	**0.82**	**0.019**

^{abc} Means without a common letter differ significantly (P<0.05).

^{xyz} Trends considered for means without a common letter (P<0.10).

***Hen day egg production percent values are reported as pen averages and adjusted for mortality (n=24).

[1]Represents phase two overall hen day egg production percentages.

[2]Represents average overall hen day egg production from the beginning to end of study.

Table 3.6 Effects of dietary treatments on brown shell egg weight* (g)

	Weeks of Age	TREATMENTS: Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	20	49.1	48.5	50.0	48.9	49.0	0.76	0.712
	24	56.9	55.4	56.1	56.5	56.3	0.64	0.603
	28	59.2	58.5	58.9	58.3	58.8	0.58	0.843
	32	58.9	57.9	59.7	58.3	57.4	0.93	0.431
	36	60.4	60.0	59.1	59.2	59.3	0.57	0.428
	41	58.0	58.9	58.4	58.4	58.3	0.66	0.919
Phase 2	44	59.6	59.8	59.2	57.9	58.8	0.86	0.510
	48	61.3^{x}	60.9^{x}	60.1^{xy}	59.5^{xy}	58.4^{y}	0.67	0.057
	52	61.1	59.9	60.5	60.2	59.1	0.77	0.506
	56	62.3	60.0	60.9	58.5	59.4	1.04	0.166
	60	61.6	60.8	60.2	61.5	60.5	0.90	0.764
	64	63.3	61.7	63.1	62.0	61.4	1.00	0.597
	68	62.6	62.5	63.5	62.5	62.4	1.10	0.946
	72	63.1	63.3	63.9	63.8	62.9	1.06	0.947
	76	64.2	64.3	64.0	63.4	62.8	0.85	0.693
Overall	**20-76**[1]	**59.9**	**58.2**	**59.7**	**59.1**	**58.9**	**0.79**	**0.605**

[abc] Means without a common letter differ significantly (*P*<0.05).

[xyz] Trends considered for means without a common letter (P<0.10).

*****Egg weights are reported as pen averages (n=24).

[1]Represents average overall egg weight from beginning to end of study.

Table 3.7 Effects of dietary treatments on brown egg shell percent* (%)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	20	12.1[a]	10.9[ab]	9.9[b]	10.6[b]	10.7[b]	0.42	0.027
	24	9.8	10.6	9.5	10.1	9.1	0.38	0.137
	28	9.8[a]	9.3[abc]	9.2[bc]	9.4[ab]	8.8[c]	0.20	0.049
	32	9.9	10.1	10.1	10.1	9.8	0.16	0.609
	36	9.5	9.8	9.5	9.5	9.5	0.13	0.348
	41	8.7	9.3	9.1	8.6	8.6	0.25	0.218
Phase 2	44	9.5	9.5	9.4	9.3	9.4	0.26	0.975
	48	9.8	9.0	8.9	9.4	8.8	0.48	0.623
	52	9.3	9.6	9.3	9.7	9.8	0.27	0.618
	56	9.5	9.1	8.6	9.1	9.2	0.30	0.418
	60	9.7	9.1	9.2	9.4	9.0	0.30	0.644
	64	9.8	9.4	9.1	9.2	9.3	0.34	0.610
	68	10.4	9.4	9.2	9.3	9.5	0.34	0.155
	72	9.4	9.1	9.7	9.6	9.6	0.21	0.309
	76	9.2	9.0	8.6	9.2	8.8	0.23	0.296
Overall	**20-76[1]**	**9.7**	**9.4**	**9.3**	**9.5**	**9.3**	**0.15**	**0.263**

[abc] Means without a common letter differ significantly ($P<0.05$).

[xyz] Trends considered for means without a common letter ($P<0.10$).

***Egg shell percent values are reported as pen averages (n=24).

[1]Represents average overall egg shell percent from beginning to end of study.

Table 3.8 Effects of dietary treatments on brown egg shell breaking strength* (N)

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	20	36	31	35	34	33	1.5	0.298
	24	34	32	29	32	29	1.6	0.313
	28	32	30	29	30	28	1.3	0.266
	32	33	35	36	35	34	1.0	0.380
	36	34	32	30	32	32	1.2	0.328
	41	25	27	28	24	25	1.2	0.147
Phase 2	44	29	28	28	29	29	1.3	0.989
	48	29	28	26	29	28	1.1	0.328
	52	28	29	30	30	30	1.3	0.793
	56	32	29	28	28	31	1.5	0.189
	60	29	28	29	28	26	1.2	0.322
	64	31	32	28	28	30	1.3	0.254
	68	32	30	31	28	31	1.3	0.237
	72	29	28	32	30	32	1.2	0.191
	76	30	27	26	30	26	1.3	0.161

[abc] Means without a common letter differ significantly (P<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
*****Egg shell breaking strength values are reported as pen averages (n=24).

Table 3.9 Effects of dietary treatments on brown shell egg Haugh unit values*

	Weeks of Age	TREATMENTS Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	20	65.4[b]	66.5[b]	69.0[b]	69.1[b]	74.4[a]	1.27	0.001
	24	84.7	87.0	85.3	84.9	86.4	1.44	0.765
	28	81.9	83.9	84.6	83.4	83.6	1.27	0.708
	32	78.6	79.6	78.5	80.6	81.5	0.94	0.158
	36	82.2	83.0	80.6	81.3	82.9	0.97	0.351
	41	82.0	82.3	81.4	84.1	82.5	1.53	0.783
Phase 2	44	86.4[ab]	84.6[b]	86.7[ab]	88.4[a]	87.3[a]	0.81	0.038
	48	84.5	85.4	84.4	85.9	86.0	1.34	0.859
	52	77.0	75.7	78.3	78.2	77.2	1.51	0.724
	56	71.2	75.3	74.4	77.4	79.0	2.10	0.156
	60	73.3	76.0	74.4	75.6	75.6	1.46	0.700
	64	68.4	67.8	67.0	69.2	70.9	1.35	0.305
	68	60.3[b]	67.4[a]	61.0[b]	65.7[ab]	67.1[a]	1.90	0.041
	72	58.9	61.1	54.6	59.1	62.0	3.46	0.597
	76	79.6	79.1	79.7	80.7	79.5	1.75	0.973
Overall	**20-76[1]**	**75.8[c]**	**77.4[abc]**	**76.6[bc]**	**77.8[ab]**	**78.5[a]**	**0.566**	**0.033**

[abc] Means without a common letter differ significantly (*P*<0.05).

[xyz] Trends considered for means without a common letter (P<0.10).

***Haugh unit values are reported as pen averages (n=24).

[1]Represents average overall Haugh unit from beginning to end of study.

Table 3.10 Effects of dietary treatment on brown shell egg yolk weight* (g)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	20	8.5	11.2	12.3	11.6	11.0	0.98	0.143
	24	13.5	13.2	13.5	13.5	13.5	0.29	0.892
	28	14.7	14.9	14.6	14.9	14.8	0.23	0.943
	32	14.9^{a}	14.2^{b}	14.8^{a}	14.5^{ab}	13.9^{b}	0.22	0.023
	36	15.9	15.6	15.5	15.4	15.4	0.18	0.331
	41	15.3	15.0	15.4	15.4	15.6	0.23	0.555
Phase 2	44	15.8	15.8	15.6	15.5	15.5	0.31	0.906
	48	16.5^{a}	15.8^{b}	15.9^{ab}	15.8^{bc}	15.2^{c}	0.20	0.006
	52	16.1^{xy}	16.1^{xy}	16.6^{x}	15.9^{y}	15.7^{y}	0.21	0.067
	56	16.6	16.1	16.4	16.1	16.0	0.28	0.485
	60	16.7	16.7	16.6	16.8	16.2	0.40	0.821
	64	17.5	17.9	17.7	18.0	17.4	0.34	0.687
	68	18.1	17.9	17.6	18.3	17.6	0.41	0.610
	72	17.7	18.3	17.7	18.5	17.2	0.52	0.434
	76	16.8	17.2	16.7	16.8	16.8	0.37	0.895
Overall	**20-76[1]**	**15.3**	**15.6**	**15.8**	**15.7**	**15.5**	**0.17**	**0.415**

[abc] Means without a common letter differ significantly ($P<0.05$).
[xyz] Trends considered for means without a common letter ($P<0.10$).
***Yolk weight values are reported as pen averages (n=24).
[1]Represents average overall yolk weight from beginning to end of study.

Table 3.11 Effects of dietary treatments on brown shell egg yolk color lightness[1] (L*)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	20	56.2	54.7	54.6	53.1	53.0	0.95	0.180
	24	58.5^{a}	54.1^{b}	55.5^{b}	55.6^{b}	55.1^{b}	0.70	0.007
	28	59.6^{a}	57.8^{ab}	56.8^{bc}	55.9^{c}	56.0^{c}	0.61	0.003
	32	59.8^{a}	58.0^{ab}	58.2^{a}	56.4^{bc}	56.2^{c}	0.59	0.003
	36	61.2^{a}	58.2^{bc}	58.8^{b}	57.8^{bc}	57.5^{c}	0.40	<.0001
	41	63.6^{a}	60.3^{b}	59.8^{bc}	59.6^{bc}	58.9^{c}	0.32	<.0001
Phase 2	44	60.2^{a}	58.9^{ab}	58.6^{ab}	59.2^{a}	57.5^{b}	0.54	0.039
	48	63.2^{a}	60.9^{b}	60.6^{b}	60.9^{b}	60.2^{b}	0.27	<.0001
	52	63.3^{a}	61.3^{bc}	60.9^{c}	61.9^{b}	60.8^{c}	0.31	<.001
	56	62.6^{a}	61.6^{b}	61.3^{b}	61.0^{b}	61.3^{b}	0.29	0.016
	60	62.2	61.5	61.3	61.9	60.8	0.41	0.202
	64	61.0	61.8	61.0	61.6	60.2	0.48	0.187
	68	63.8^{a}	63.4^{a}	62.2^{b}	63.5^{a}	63.3^{a}	0.27	0.006
	72	64.2	62.7	63.1	64.1	63.5	0.49	0.195
	76	62.6^{x}	61.2^{y}	61.0^{y}	61.8^{xy}	61.2^{y}	0.39	0.081
Overall	**20-76[2]**	**61.4^{a}**	**59.7^{b}**	**59.5^{bc}**	**59.6^{b}**	**59.1^{c}**	**0.17**	**<.0001**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
[1]Yolk color lightness (L*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color lightness (L*) from beginning to end of study.

Table 3.12 Effects of dietary treatments on brown shell egg yolk color redness[1] (a*)

	Weeks of Age	TREATMENTS: Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Phase 1	20	10.6^{b}	14.4^{a}	15.0^{a}	15.4^{a}	14.9^{a}	0.42	<.0001
	24	10.0^{d}	14.3^{c}	14.7^{bc}	15.4^{a}	15.2^{ab}	0.21	<.0001
	28	9.8^{c}	15.1^{b}	15.6^{ab}	16.0^{a}	16.0^{a}	0.23	<.0001
	32	10.3^{b}	15.4^{a}	15.4^{a}	15.9^{a}	16.2^{a}	0.33	<.0001
	36	10.4^{d}	14.7^{c}	15.1^{bc}	15.5^{b}	16.1^{a}	0.18	<.0001
	41	8.8^{c}	15.2^{ab}	14.8^{b}	15.7^{a}	15.3^{ab}	0.19	<.0001
Phase 2	44	12.1^{d}	15.5^{ab}	15.9^{a}	14.6^{c}	14.9^{bc}	0.20	<.0001
	48	11.9^{d}	15.8^{a}	15.8^{a}	14.1^{c}	14.9^{b}	0.22	<.0001
	52	11.3^{c}	15.7^{a}	15.4^{a}	13.4^{b}	13.6^{b}	0.18	<.0001
	56	12.4^{c}	14.3^{a}	14.5^{a}	13.0^{bc}	13.2^{b}	0.21	<.0001
	60	12.9^{b}	14.1^{a}	14.5^{a}	12.8^{b}	12.4^{b}	0.17	<.0001
	64	13.6^{b}	15.3^{a}	15.8^{a}	14.2^{b}	13.8^{b}	0.28	<.0001
	68	14.1^{b}	15.6^{a}	15.7^{a}	13.6^{bc}	13.4^{c}	0.21	<.0001
	72	13.1^{b}	14.7^{a}	14.8^{a}	12.5^{c}	12.5^{c}	0.17	<.0001
	76	13.5^{c}	15.9^{a}	16.2^{a}	14.3^{b}	14.4^{b}	0.25	<.0001
Overall	**20-76**[2]	**11.6^{c}**	**15.1^{a}**	**15.3^{a}**	**14.4^{b}**	**14.5^{b}**	**0.90**	**<.0001**

[abc] Means without a common letter differ significantly (*P*<0.05).
[xyz] Trends considered for means without a common letter (P<0.10).
[1]Yolk color redness (a*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color redness (a*) from beginning to end of study.

Table 3.13 Effects of dietary treatments on brown shell egg yolk color yellowness[1] (b*)

	Weeks of Age	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
		TREATMENTS						
Phase 1	20	53.7[c]	57.5[ab]	59.4[a]	58.1[ab]	55.4[bc]	1.11	0.016
	24	53.1[b]	57.4[a]	59.4[a]	58.8[a]	59.0[a]	0.82	<0.001
	28	53.4[b]	61.7[a]	62.4[a]	62.3[a]	61.8[a]	0.82	<.0001
	32	54.1[b]	62.6[a]	62.0[a]	60.9[a]	61.1[a]	1.18	0.001
	36	56.6[b]	61.5[a]	62.2[a]	60.8[a]	62.1[a]	0.81	0.001
	41	53.9[c]	64.1[a]	62.5[b]	63.5[ab]	62.8[ab]	0.46	<.0001
Phase 2	44	55.6[c]	61.1[a]	61.6[a]	60.9[ab]	58.9[b]	0.71	<0.001
	48	60.0[c]	65.2[a]	64.9[a]	62.1[b]	64.0[a]	0.44	<.0001
	52	57.8[d]	65.3[a]	62.7[b]	60.8[bc]	60.3[c]	0.67	<.0001
	56	59.3[bc]	61.7[a]	61.8[a]	58.7[c]	59.4[bc]	0.78	0.026
	60	59.1[b]	61.9[a]	62.5[a]	59.3[b]	59.3[b]	0.79	0.011
	64	63.6[b]	68.5[a]	67.9[a]	67.0[a]	64.0[b]	0.96	0.004
	68	71.1[a]	71.8[a]	71.0[a]	68.5[b]	66.8[c]	0.56	<.0001
	72	67.5[ab]	67.7[ab]	68.6[a]	65.9[b]	63.8[c]	0.64	<0.001
	76	63.1[c]	65.8[a]	66.9[a]	63.6[b]	63.4[b]	0.60	0.001
Overall	**20-76[2]**	**58.7[c]**	**63.4[a]**	**63.6[a]**	**62.1[b]**	**61.5[b]**	**0.21**	**<0.001**

[abc] Means without a common letter differ significantly ($P<0.05$).
[xyz] Trends considered for means without a common letter ($P<0.10$).
[1]Yolk color yellowness (b*) values are reported as pen averages (n=24).
[2]Represents average overall yolk color yellowness (b*) from beginning to end of study.

Table 3.14 Effects of dietary treatments on scrambled brown shell egg taste and purchase intent at 74 weeks of age[1]

	TREATMENTS						
	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix	SEM	*P*-value
Flavor	5.1	5.3	5.4	5.7	5.9	0.4	0.467
Off Flavor	5.6	6.6	5.6	6.1	5.5	0.6	0.326
Buttery Flavor	2.8^{y}	3.7^{xy}	3.6^{xy}	3.1^{xy}	3.8^{x}	0.4	0.058
Metallic Flavor	1.9	1.6	1.9	1.3	1.3	0.3	0.578
Fishy Flavor	1.8	1.4	1.4	1.4	1.3	0.3	0.657
Painty Flavor	1.6	1.6	1.2	1.6	1.1	0.2	0.326
Rancid Flavor	1.6	1.4	1.2	1.5	1.1	0.2	0.685
Grassy Flavor	1.5	1.3	1.6	1.6	1.7	0.2	0.300
Purchase Intent	4.8^{b}	5.6^{ab}	4.9^{ab}	5.3^{ab}	6.0^{a}	0.4	0.018

[abc] Means without a common letter differ significantly ($P<0.05$).
[xyz] Trends considered for means without a common letter ($P<0.10$).
[1]Taste parameter values are reported as averages (n=16).

Table 3.15 Effects of dietary treatments on brown egg laying hen bone quality and mineralization* (76 weeks of age)

	Bone	TREATMENTS					SEM	*P*-value
		Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix		
Breaking Strength (kg force)	Tibia	2.1	2.4	2.3	2.7	2.7	0.15	0.083
	Humerus	2.0	2.2	2.1	2.3	2.2	0.08	0.150
Ash (%)	Tibia	61.1	61.4	60.2	59.4	60.0	1.21	0.775
	Humerus	59.9	59.4	57.8	57.6	59.6	1.15	0.515
Ca (%)	Tibia	31.7	30.4	30.7	31.1	32.3	0.48	0.080
	Humerus	31.0	30.6	30.5	31.8	31.3	0.47	0.282
P (%)	Tibia	18.8	18.9	18.9	18.8	18.8	0.14	0.985
	Humerus	18.1	18.6	18.4	18.5	18.6	0.23	0.588

^{abc} Means without a common letter differ significantly ($P<0.05$).

^{xyz} Trends considered for means without a common letter ($P<0.10$).

*****Bone measurements are reported as pen averages (n=24).

CHAPTER 4. Summary and Conclusions

4.1 Summary

Addition of up to 35% DDGS affected white and brown egg laying hens differently when considering certain production and egg quality parameters. Overall mortality was not significantly affected in either of the Hy®-Line strains used. In both strains, hen body weight and body weight gain values were decreased with the addition of 25 and 35% DDGS when compared to the control treatment. The premix helped hens maintain body weight and body weight gain values in the brown egg laying hens comparable to the control; however, this was not observed in the white egg laying hens. On average the brown hens were 200 to 300 grams heavier at the end of the 60 week production cycle and could have been a factor in the effectiveness of the premix. We feel the premix was helping to digest up to 35% DDGS better because of the inclusion of the enzyme technology.

The addition of up to 35% DDGS did not affect overall feed intake of both the white and brown egg laying hens with average feed intakes of 97.4 and 111.2 grams per bird per day; respectively. However, in the second phase of production, addition of DDGS increased overall feed intake of white laying hens which was comparable to control levels with the addition of the premix. No differences in overall hen day egg production were noted with the addition of dietary DDGS in the white egg laying hens and averaged approximately 79.4%. This was not the same for the brown egg laying hens. Brown hens on the premix came into lay faster than the control and DDGS treatments and over the 60 week production cycle DDGS had reduced overall hen day egg production (86.3%) when compared to the control treatment (89.2%).

Overall egg quality and shell quality were not affected by the addition of up to 35% DDGS in brown egg laying hens. Brown hens displayed improvements in Haugh unit values, yolk color scores, and purchase intent scores with the addition of DDGS. In the white egg laying hens, improvements were only noted in yolk color scores. The premix helped reduced the negative effects of DDGS on egg shell percentage, egg shell breaking strength and yolk weight parameters in the white egg laying hens that were noted during times of potential metabolic stressors. It is thought that differences in the brown egg laying were not noted due to the depression in egg production when compared to the white egg laying hens. Bone quality was not affected by the addition of up to 35% DDGS even with reduced calcium and available phosphorus levels.

4.2 Future Implications

Addition of up to 35% DDGS may be included in the diet without substantial negative effects on white egg laying hens. The addition of the premix may help alleviate negative effects on egg quality when the birds are under stress. Future research should look into the beginning of the production cycle and times of metabolic stress on calcium and phosphorus levels to evaluate the increased production noted with the addition of the premix.

APPENDICES

Appendix 1. Colorimeter Procedure (L*, a*, b*)

Procedure
Egg yolk color is analyzed using a Hunterlab Colorflex Colorimeter. The Colorimeter is standardized with a white and black pane prior to sample analysis. Once standardized, yolks are placed one at a time into a glass cup specifically designed for the machine. A black cover is placed over the glass cup and the sample is then analyzed as the machine transmits a light signal through the glass cup. The colorimeter measures the sample via reflectance and absorbance and renders L* (lightness), a* (redness), and b* (yellowness) values [39].

A reference diagram can be viewed at www.hunterlab.com.

Appendix 2. Bone Ash and Mineral Analysis Procedure

Procedures for determination of bone ash and mineral analysis were outlined by Ao *et al.* [69] with modifications.

Bone Collection

Bones were collected and boiled in deionized water for 15 minutes. An extra 5 minutes of boiling time was added due to the age of the birds for ease of cleaning the soft tissue from the bones.

Bone Ash

Samples were then placed in a drying oven at 60°C for 72 hours. Once dried the bones were placed in petroleum ether for 72 hours, changing out the ether after the first 24 hours. Bones were set out to air dry overnight at room temperature and dried in a 105°C oven for 12 hours. Dry weights were recorded and bones were placed in a 600°C muffle furnace overnight for determination of percent ash.

Mineral Analysis

Bone ash was digested with HNO_3 [70] and placed under the ICP-OES for determination of Ca and P.

Appendix 3. Taste Panel Scoring Sheet

Egg Purchase Intent and Flavor Description

Sample Number____________________ Date____________________

1. Flavor

1	2	3	4	5	6	7	8
Dislike Extremely							*Like Extremely*

2. Off Flavor

1	2	3	4	5	6	7	8
Intense Off-Flavor							*No Off-Flavor*

3. Buttery Flavor

1	2	3	4	5	6	7	8
No Buttery Flavor							*Intense Buttery Flavor*

4. Metallic Flavor

1	2	3	4	5	6	7	8
No Metallic Flavor							*Intense Metallic Flavor*

5. Fishy Flavor

1	2	3	4	5	6	7	8
No Fishy Flavor							*Intense Fishy Flavor*

6. Painty Flavor

1	2	3	4	5	6	7	8
No Painty Flavor							*Intense Painty Flavor*

7. Rancid Flavor

1	2	3	4	5	6	7	8
No Rancid Flavor							*Intense Rancid Flavor*

8. Grassy Flavor

1	2	3	4	5	6	7	8
No Grassy Flavor							*Intense Grassy Flavor*

9. Purchase Intent

1	2	3	4	5	6	7	8
Would Not Buy							*Would Buy*

Appendix 4. White Hen Taste Panel Results

	Panelist	Sample	Diet	Flavor	OffFlavor	Buttery	Metallic	Fishy	Painty	Rancid	Grassy	PurchaseIntent
Panelist	1.00000	0.27142	0.00000	-0.04553	-0.00859	0.07716	0.18946	0.06054	0.22248	-0.21317	0.23418	-0.13689
		0.0149	1.0000	0.6903	0.9398	0.4963	0.0923	0.5937	0.0473	0.0576	0.0365	0.2259
	80	80	80	79	80	80	80	80	80	80	80	80
Sample	0.27142	1.00000	0.47016	0.08236	0.01119	0.01074	0.13483	-0.18364	0.24809	-0.18984	0.19122	0.08997
	0.0149		<.0001	0.4705	0.9215	0.9247	0.2331	0.1030	0.0265	0.0917	0.0893	0.4274
	80	80	80	79	80	80	80	80	80	80	80	80
Diet	0.00000	0.47016	1.00000	-0.02063	-0.07750	-0.02769	0.07080	-0.04385	0.18822	0.13199	0.07119	0.01325
	1.0000	<.0001		0.8568	0.4944	0.8074	0.5326	0.6993	0.0945	0.2432	0.5303	0.9071
	80	80	80	79	80	80	80	80	80	80	80	80
Flavor	-0.04553	0.08236	-0.02063	1.00000	0.52180	0.19308	-0.39925	-0.41340	-0.26076	-0.60318	-0.35083	0.89685
	0.6903	0.4705	0.8568		<.0001	0.0882	0.0003	0.0002	0.0203	<.0001	0.0015	<.0001
	79	79	79	79	79	79	79	79	79	79	79	79
OffFlavor	-0.00859	0.01119	-0.07750	0.52180	1.00000	0.28113	-0.36478	-0.16237	-0.15778	-0.45426	-0.21012	0.42531
	0.9398	0.9215	0.4944	<.0001		0.0115	0.0009	0.1502	0.1622	<.0001	0.0614	<.0001
	80	80	80	79	80	80	80	80	80	80	80	80
Buttery	0.07716	0.01074	-0.02769	0.19308	0.28113	1.00000	0.02171	0.21451	0.10842	-0.17878	0.06592	0.13750
	0.4963	0.9247	0.8074	0.0882	0.0115		0.8484	0.0560	0.3384	0.1126	0.5612	0.2239
	80	80	80	79	80	80	80	80	80	80	80	80
Metallic	0.18946	0.13483	0.07080	-0.39925	-0.36478	0.02171	1.00000	0.39329	0.57155	0.27913	0.64257	-0.47741
	0.0923	0.2331	0.5326	0.0003	0.0009	0.8484		0.0003	<.0001	0.0122	<.0001	<.0001
	80	80	80	79	80	80	80	80	80	80	80	80
Fishy	0.06054	-0.18364	-0.04385	-0.41340	-0.16237	0.21451	0.39329	1.00000	0.26218	0.49708	0.36770	-0.39524
	0.5937	0.1030	0.6993	0.0002	0.1502	0.0560	0.0003		0.0188	<.0001	0.0008	0.0003
	80	80	80	79	80	80	80	80	80	80	80	80
Painty	0.22248	0.24809	0.18822	-0.26076	-0.15778	0.10842	0.57155	0.26218	1.00000	-0.09026	0.66506	-0.25944
	0.0473	0.0265	0.0945	0.0203	0.1622	0.3384	<.0001	0.0188		0.4259	<.0001	0.0201
	80	80	80	79	80	80	80	80	80	80	80	80
Rancid	-0.21317	-0.18984	0.13199	-0.60318	-0.45426	-0.17878	0.27913	0.49708	-0.09026	1.00000	0.05711	-0.56940
	0.0576	0.0917	0.2432	<.0001	<.0001	0.1126	0.0122	<.0001	0.4259		0.6148	<.0001
	80	80	80	79	80	80	80	80	80	80	80	80
Grassy	0.23418	0.19122	0.07119	-0.35083	-0.21012	0.06592	0.64257	0.36770	0.66506	0.05711	1.00000	-0.32683
	0.0365	0.0893	0.5303	0.0015	0.0614	0.5612	<.0001	0.0008	<.0001	0.6148		0.0031
	80	80	80	79	80	80	80	80	80	80	80	80
PurchaseIntent	-0.13689	0.08997	0.01325	0.89685	0.42531	0.13750	-0.47741	-0.39524	-0.25944	-0.56940	-0.32683	1.00000
	0.2259	0.4274	0.9071	<.0001	<.0001	0.2239	<.0001	0.0003	0.0201	<.0001	0.0031	
	80	80	80	79	80	80	80	80	80	80	80	80

Appendix 5. Brown Hen Taste Panel Results

	Panelist	Sample	Diet	Flavor	OffFlavor	Buttery	Metallic	Fishy	Painty	Rancid	Grassy	PurchaseIntent
Panelist	1.00000	-0.08327	0.00000	0.14083	-0.19912	-0.27981	-0.02908	-0.13233	-0.17788	-0.01914	-0.24769	0.31862
		0.4627	1.0000	0.2128	0.0766	0.0119	0.7979	0.2419	0.1168	0.8670	0.0268	0.0040
	80	80	80	80	80	80	80	80	79	79	80	80
Sample	-0.08327	1.00000	0.15637	0.04179	0.05899	-0.05334	-0.15338	0.00322	0.15122	0.03081	-0.04380	0.08722
	0.4627		0.1660	0.7129	0.6032	0.6384	0.1744	0.9774	0.1834	0.7875	0.6997	0.4417
	80	80	80	80	80	80	80	80	79	79	80	80
Diet	0.00000	0.15637	1.00000	0.19438	-0.04250	0.10730	-0.17500	-0.12781	-0.13008	-0.13041	0.09071	0.16696
	1.0000	0.1660		0.0840	0.7082	0.3434	0.1205	0.2586	0.2532	0.2520	0.4236	0.1388
	80	80	80	80	80	80	80	80	79	79	80	80
Flavor	0.14083	0.04179	0.19438	1.00000	0.33204	0.35752	-0.34287	-0.21623	-0.42743	-0.39157	-0.10601	0.79871
	0.2128	0.7129	0.0840		0.0026	0.0011	0.0018	0.0540	<.0001	0.0004	0.3493	<.0001
	80	80	80	80	80	80	80	80	79	79	80	80
OffFlavor	-0.19912	0.05899	-0.04250	0.33204	1.00000	0.44889	-0.20846	-0.09011	-0.08165	-0.30162	-0.17803	0.36766
	0.0766	0.6032	0.7082	0.0026		<.0001	0.0635	0.4267	0.4744	0.0069	0.1141	0.0008
	80	80	80	80	80	80	80	80	79	79	80	80
Buttery	-0.27981	-0.05334	0.10730	0.35752	0.44889	1.00000	-0.14292	-0.06318	-0.21297	-0.21521	-0.04691	0.45240
	0.0119	0.6384	0.3434	0.0011	<.0001		0.2060	0.5777	0.0595	0.0568	0.6795	<.0001
	80	80	80	80	80	80	80	80	79	79	80	80
Metallic	-0.02908	-0.15338	-0.17500	-0.34287	-0.20846	-0.14292	1.00000	0.37417	0.38929	0.48887	0.18891	-0.46620
	0.7979	0.1744	0.1205	0.0018	0.0635	0.2060		0.0006	0.0004	<.0001	0.0933	<.0001
	80	80	80	80	80	80	80	80	79	79	80	80
Fishy	-0.13233	0.00322	-0.12781	-0.21623	-0.09011	-0.06318	0.37417	1.00000	0.54912	0.68365	0.10493	-0.31302
	0.2419	0.9774	0.2586	0.0540	0.4267	0.5777	0.0006		<.0001	<.0001	0.3543	0.0047
	80	80	80	80	80	80	80	80	79	79	80	80
Painty	-0.17788	0.15122	-0.13008	-0.42743	-0.08165	-0.21297	0.38929	0.54912	1.00000	0.68034	0.03750	-0.54887
	0.1168	0.1834	0.2532	<.0001	0.4744	0.0595	0.0004	<.0001		<.0001	0.7428	<.0001
	79	79	79	79	79	79	79	79	79	79	79	79
Rancid	-0.01914	0.03081	-0.13041	-0.39157	-0.30162	-0.21521	0.48887	0.68365	0.68034	1.00000	0.09804	-0.42498
	0.8670	0.7875	0.2520	0.0004	0.0069	0.0568	<.0001	<.0001	<.0001		0.3900	<.0001
	79	79	79	79	79	79	79	79	79	79	79	79
Grassy	-0.24769	-0.04380	0.09071	-0.10601	-0.17803	-0.04691	0.18891	0.10493	0.03750	0.09804	1.00000	-0.11493
	0.0268	0.6997	0.4236	0.3493	0.1141	0.6795	0.0933	0.3543	0.7428	0.3900		0.3100
	80	80	80	80	80	80	80	80	79	79	80	80
PurchaseIntent	0.31862	0.08722	0.16696	0.79871	0.36766	0.45240	-0.46620	-0.31302	-0.54887	-0.42498	-0.11493	1.00000
	0.0040	0.4417	0.1388	<.0001	0.0008	<.0001	<.0001	0.0047	<.0001	<.0001	0.3100	
	80	80	80	80	80	80	80	80	79	79	80	80

Appendix 6. Analysis of Dietary Treatments

Item (% DM basis)	TREATMENTS					SEM	*P*-value
	Control	25% DDGS	25% DDGS + premix	35% DDGS	35% DDGS + premix		
Crude Protein	18.79	18.64	18.53	18.91	18.92	0.62	0.988
DM	90.08	90.58	90.59	90.96	90.92	0.23	0.102
Crude Fat	4.7[c]	8.41[b]	8.27[b]	10.61[a]	10.71[a]	0.34	<.001
Crude Fiber	3.22	3.82	3.72	3.96	3.91	0.37	0.676
Ash	16.85	16.00	16.54	17.70	15.40	1.01	0.561
Calcium	5.64	5.11	5.12	4.85	5.05	0.26	0.353
Phosphorus	0.59[a]	0.49[b]	0.51[b]	0.52[b]	0.52[b]	0.01	<.001

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VITA

Megan van Benschoten was born in Saginaw, Michigan. Megan grew up in Linwood, Michigan with the loving support of Mark and Debra van Benschoten. Megan graduated from Pinconning High School in May 2007. She then moved to Lansing, MI were she attended Michigan State University. During her time at Michigan State University she was active on the MSU Horse and MSU Livestock Judging Teams where she met her best friend and one true love Ethan Bosserd. Megan graduated from Michigan State University with a bachelor's of science degree in Animal Science in the summer of 2011.

Megan then moved to Lexington, Kentucky were she started a post baccalaureate program at the University of Kentucky. Megan chose to pursue her master's degree in poultry nutrition under the supervision of Dr. Anthony J. Pescatore. During her time as a M.S. student, Ethan Bosserd proposed to Megan and the two will be happily married on November 30th 2013.