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EFFECTS OF REDUCED CRUDE PROTEIN, AMINO ACID BALANCED DIETS ON PERFORMANCE, ECONOMICS, AND AMMONIA EMISSION IN A LARGE-SCALE COMMERCIAL LAYING HEN FLOCK

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ABSTRACT

Recent increases in feed prices for laying hens have renewed interest in reducing costly crude protein (CP) levels in diets and supplementing the lower protein diets with limiting amino acids (AAs) in order to reduce feed costs while maintaining performance. This feeding strategy has the additional benefit of potentially reducing ammonia (NH_3) emissions by lowering hen nitrogen (N) excretion. This dietary strategy was investigated under commercial conditions, based on the promising results of previous university scale studies. The objective was to establish if reduced CP, AA supplemented laying hen diets could maintain hen production performance, reduce feed costs, increase farm revenue, lower hen N excretion, and/or decrease NH_3 emissions, compared to hens fed typical levels of CP, on a commercial scale.

A total of 50,760 Lohmann LSL Lite laying hens were divided into three groups that were each fed diets containing different levels of CP and supplemented with limiting AAs to their required levels. Hens were housed in a high-rise facility with a deep-pit manure storage system. Each diet was fed to two out of the six total cage rows. Diets were corn-soybean meal based and least-cost formulated weekly based on current ingredient prices and nutrient concentrations. Hens were fed these diets ad libitum from 18 to 51 weeks of age (January 20, 2008-September 7, 2008). Diet C was a control diet formulated with the highest level of CP and was meant to represent a typical commercial laying hen diet. Diets B and A were formulated to contain intermediate and low levels of CP, respectively.

Weekly diet formulations were examined to establish if diets were formulated to be isocaloric and AA balanced while also achieving the designed CP differences between diets. Monthly replicated data was collected at the front (F), middle (M), and end (E) of the feed trough

for each cage row. Monthly samples and data included feed for CP, AA, and particle size analysis, hen body weight, egg weight, albumen height, Haugh units, yolk color, shell strength, shell thickness, and manure percent dry matter (DM), total N, ammonium (NH_4^+) N, organic N, phosphate (P_2O_5), potash (K_2O), and NH_3 flux. Weekly non-replicated production data and egg grade out results by dietary treatment were collected and reported by the producer. Weekly egg income, feed consumption, and feed prices were used to calculate weekly feed cost and egg income minus feed cost, e.g. farmer revenue. A 3 x 3 factorial analysis was performed using the PROC MIXED procedure of SAS© software version 9.1 to detect differences between parameters by diet (A, B, and C), location on the feed trough (F, M, and E), and their interaction. Mean comparisons were made using Tukey's procedure.

Examination of weekly diet formulations revealed that, overall, diets were isocaloric and AA balanced and that diets B and A were formulated at 0.85 and 1.40% less CP than diet C, respectively. Analyzed feed samples collected from within cage rows proved the diets to be AA balanced; however, overall, diets B and A were actually shown to contain 1.53% and 1.98% less CP than diet C, respectively; quite different than formulated CP differences between diets.

Feed particle size analysis showed that the high CP diet consistently contained a numerically smaller proportion of large particles (2380-3360 µm) and larger proportion of small particles (840-1190 µm and 420-590 µm) than the lower CP diets throughout the eight month trial, however, these results were not statistically significant. Additionally, feed collected at the M location along the feed trough was made up of a greater proportion of large particles (>2380 µm) than feed collected from the F and/or E locations ($P<0.05$).

Hen body weight and egg weight were numerically lower for the low CP diet versus the two higher CP diets throughout the study, though these differences were not statistically significant. Hen day egg production averaged 87.9, 87.4 and 87.1% for diets A, B, and C, respectively, and albumen height, Haugh units, yolk color, shell strength, and shell thickness did not differ by dietary treatment. Diets A, B, and C consistently had the highest to lowest percentage of large, grade A eggs and lowest to highest percentages of extra-large and jumbo, grade A eggs, respectively, throughout the study. Hen body weights and egg weights were consistently lower at the M location on the feed trough compared to the F and/or E, while shell strength was numerically higher at the M than at the F and/or E throughout the trial, though these findings were not statistically significant. Additionally, overall, albumen height and Haugh units were shown to be significantly higher at the M than at the F and/or E ($P<0.05$). Yolk color and shell thickness did not differ by location on the feed trough.

Mean weekly egg income per hen housed for diet A was \$0.0022 and \$0.0024 less than for diets B and C, respectively. However, mean weekly feed costs per hen housed for diets A and B were \$0.0083 and \$0.0070 less than for diet C, respectively. The resulting weekly egg income minus feed costs per hen housed for diets A and B were \$0.0059 and \$0.0068 higher than for diet C, respectively.

Manure percent DM, total N, (NH_4^+) N, organic N, and P_2O_5 did not differ by dietary treatment; however, manure K_2O was numerically lowest for the low CP diet and highest for the high CP diet consistently for seven out of the eight months of the trial. Additionally, manure percent DM, (NH_4^+) N, organic N, P_2O_5, and K_2O did not differ by location on the feed trough; however, manure total N tended to be highest at the M and lowest at the F location along the feed trough. Manure ammonia flux increased each sampling period until it peaked at 97.41 mg/cm^2/min in May and then decreased until rising again in September; however, ammonia flux did not differ by diet or location on along the feed trough. There were no significant interactions overall between diet and location along the feed trough for any manure components or NH_3 flux.

This study found that when reduced CP, AA balanced diets were fed to a large scale commercial laying hen flock, it was possible to maintain hen production performance while reducing dietary costs and increasing farm revenue. However, it was also shown that this dietary strategy did not significantly influence ammonia flux or manure composition. These results indicate that although this dietary strategy may not be effective for reducing hen N excretion or NH_3 emissions on a large scale basis, it would, however, be economically beneficial for producers to implement in commercial laying operations.

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ABBREVIATIONS

AA = Amino Acid

CP = Crude Protein

DM = Dry matter

E = End location along the feed trough

F = Front location along the feed trough

g = Grams

Ile = Isoleucine

K_2O = Potash

lbs = Pounds

Lys = Lysine

M = Middle location along the feed trough

M+C = Methionine + Cysteine

Met = Methionine

N = Nitrogen

NH_3 = Ammonia gas

(NH_4^+) N = Ammonium nitrogen

Ovr = Overall values for the entire trial

P_2O_5 = Phosphate

Thr = Threonine

Trp = Tryptophan

TSAA = Total Sulfur Amino Acids

Val = Valine

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CHAPTER 1

LITERATURE REVIEW

Reducing Dietary Crude Protein

Numerous investigations over the past several years have focused on the concept of formulating laying hen diets to contain an ideal quantity of crude protein (CP). Birds do not require specific quantities of CP in their diets, but instead are obligated to consume definitive amounts of specific essential amino acids (AAs). When AAs are consumed beyond their required levels, they are subsequently deaminated and excreted, mainly as uric acid, thus contributing N to the manure (Parsons, 1995; Nahm, 2007). Uric acid can then be converted to ammonia gas (NH_3) by a variety of microbial enzymes normally present in poultry manure (Goldstein and Skadhauge, 2000; Bregendahl and Roberts, 2006). Based on this information, the “ideal protein” concept seeks to formulate laying hen diets with essential AAs provided at ideal levels for all hen production parameters, without supplying AAs in excess of their requirements (Novak et al., 2006).

Implementation of this dietary strategy has become of particular interest in commercial livestock feeding operations in recent years due to the increasing availability and affordability of crystalline AAs for use as supplements in livestock diets (Meluzzi et al., 2001; Keshavarz and Austic, 2004). Use of reduced CP, AA supplemented diets has been studied in broilers, turkeys, swine, and laying hens (Boling and Firman, 1997; Firman and Boling, 1998; Figueroa et al., 2002; Shriver et al., 2003; Keshavarz and Austic, 2004; Roberts et al., 2007ab; Namroud et al., 2008). Since CP is a costly component of poultry diets, providing lower quantities of dietary CP could potentially lower production expenses for both farm owners and integrators. If egg production and quality can be maintained at the level of hens fed diets containing normal commercial levels of CP, then farmer revenue also has the potential to be increased. There is also the added benefit of low CP diets potentially leading to lower N excretion by hens and/or reduced NH_3 emissions from laying hen facilities, due to the decrease in dietary N intake (Meluzzi et al., 2001).

Hen Performance

Numerous studies have evaluated production performance of laying hens maintained on reduced CP, AA supplemented diets or feeding programs. Several of these investigations, both past and present, have shown reduced performance for hens fed reduced CP diets compared to those fed higher CP diets. Reduced egg weight and hen body weight have been observed even when lower CP diets were supplemented with AAs to their required levels (Penz and Jensen, 1991; Keshavarz and Jackson, 1992; Roberts et al., 2007a).

One early study reported a reduction in egg weight for a 13% versus a ≥16% CP diet, despite addition of supplemental Met, Lysine (Lys) and Tryptophan (Trp) or glycine and glutamic acid (Penz and Jensen, 1991). These authors also observed lower body weight gain for hens fed the lower CP diet, and determined lower egg weights resulted from decreased albumen content of the egg. Egg production in this study was shown not to differ by the level of dietary CP provided. Keshavarz and Jackson (1992) similarly found that when CP was reduced to 15% or below, with supplemental Met, Lys, Trp, and/or isoleucine (Ile), egg mass and hen body weight were reduced compared to hens fed higher levels of CP, though no differences were observed for egg production or egg weight. Another study evaluating a less dramatic CP reduction in laying hen diets, from ~20% to ~19% CP, demonstrated that, while egg weight, feed consumption, and body weight gain were maintained, egg production, egg mass, and feed utilization were reduced for the low CP diet, despite supplementation with required levels of essential AAs (Roberts et al., 2007a).

Some researchers suggest that one reason for poorer production performance for hens fed reduced CP, AA balanced diets could be that in the past some studies may have been performed without sufficient understanding of the essential AA requirements, ratios of essential AAs, or content, digestibility, and bioavailability of essential AAs in laying hen diets (Harms and Russell, 1993; Keshavarz, 1997; Keshavarz and Austic, 2004). A recent study by Sklan and Noy (2005), which evaluated the optimum AA levels for growth and maintenance of broiler chicks, found that most AAs were required at higher levels than those designated by the NRC (1994) requirements. One reason for the increased AA requirements since the NRC (1994) requirements were published is that poultry have been selected for faster growth with each successive generation, resulting in increasing nutritional requirements for their maintenance (Novak et al., 2004). Another example of why lower production could have been observed for reduced CP, AA balanced diets is discussed in a more recent study by Roberts et al. (2007a). The authors of this investigation state that levels of essential AAs may have been set too low, in formulation, for the specific dietary phases evaluated in their study, which could have led to the lower production performance observed for laying hens fed a low CP diet compared to a high CP diet.

Much work has been done, since the most recent edition of the NRC *Nutrient Requirements of Poultry* was published in 1994, to update the requirements of essential AAs for laying hen diets and our understanding of how they function and interact with one another. As these variables are elucidated, researchers are better able to evaluate whether low CP, AA balanced diets have the potential to maintain production parameters. As a result, several studies have shown little to no difference in production performance of laying hens fed typical commercial levels of CP versus those fed low CP diets supplemented with essential AAs (Harms and Russell, 1993; Meluzzi et al., 2001; Keshavarz and Austic, 2004).

Harms and Russell (1993) reported similar production performance, including egg weight, for heat stressed hens fed a 13% versus a 15.5% CP, AA supplemented diet or a 15% versus a 17.6% CP diet in two different studies, respectively. Another study by Kesharvarz and Austic (2004) found that production performance was comparable between hens fed an ~16% CP diet and those fed a 13% CP diet with supplemental Met, Lys, and Trp, with the exception of egg weight, which was greater for the low CP, AA supplemented diet. The authors stated that greater egg weight may have been observed for the low CP diet because the higher CP diet was deficient in one or more limiting AAs. Meluzzi et al. (2001) also demonstrated that performance could be maintained by laying hens fed low (13.6 and 15.3%) CP diets, compared to control diets of 17.1% CP, for a period of 8 weeks; however, egg production and egg mass were shown to decline after this time, despite ensuring that dietary levels of Lys, Met, Met + cysteine (M+C) or total sulfur AAs (TSAAs), Trp, and Thr were maintained at their required levels.

The recent work of Yakout et al. (2006a) demonstrated that, when CP was reduced to 15%, with supplemental Lys, Met, and Thr, egg production and egg mass could be maintained at a level equivalent to that of hens fed 19% CP during the first phase of production (24-36 weeks of age). Additional findings from this study during the second phase of production for the same birds (38-50 weeks of age) found that providing only 13% CP, with supplemental Lys, Met, Thr, and Trp, was not a feasible option, as this diet was far less cost effective than the higher CP diets, based on the lower levels of production observed (Yakout et al., 2006b).

Economic Value

Feed costs in the poultry industry have increased a great deal in recent years due to the dramatic rise in corn and soybean prices. Current corn prices are a result of increased corn usage for ethanol production as an alternative fuel to gasoline derived from crude oil. Corn prices were reported to have increased 157% from $2.69/bushel in September, 2006 to $6.92/bushel in July, 2008 (Cunningham, 2009). Additionally, ethanol production is projected to continue increasing to 14.8 billion gallons per year by 2011 given the current federal ethanol policy and the anticipated continuing rise in crude oil prices (Tokgoz et al., 2007). As a result of more acres planted for corn production, land available for growing soybeans has decreased, leading to an increase in the price of soybeans as well. Demand for soybeans for biodiesel production has also increased prices (Cunningham, 2009). Soybean prices have increased 122% from $182/ton in September, 2006 to $405/ton in July, 2008 (Cunningham, 2009).

Current price increases for corn and soybeans have led to a rise in feed and production costs for many species of livestock, including poultry, which, in turn has led to greater costs for animal products for the consumer as well. Feed costs for laying hens were reported to have increased 105% from $0.19/dozen eggs in September, 2006 to $0.39/dozen eggs in July, 2008 and production costs for laying hens were reported to have increased 68% from $0.34/dozen eggs in September, 2006 to $0.57/dozen eggs in July, 2008 (Cunningham, 2009). These production costs will only continue to increase over the next few years, so the industry must respond accordingly.

In order to relieve the economic burden of greater corn and soybean meal prices in poultry feed, dietary manipulation to reduce levels of CP in combination with supplementation of essential crystalline AAs has become an increasingly common practice in the poultry industry.

Crude protein is a costly component of poultry diets, especially taking current and projected increases in soybean prices into account. Therefore, reducing CP in hen diets may have the potential to lower production costs considerably, especially with the use of crystalline AAs in livestock diets becoming increasingly economical (Meluzzi et al., 2001).

To demonstrate the increased affordability of crystalline AAs today; it was shown back in 2004 by Kesharvarz and Austic, that a 16% CP control laying hen diet cost $129/ton and a 13% CP, AA supplemented diet cost $270/ton due to the high cost of crystalline AAs at that time. However, a more recent study, by Yakout et al. (2006a), showed that a 15% CP laying hen diet with supplemental Met, Lys, and Thr had the potential to save the poultry industry up to $11/ton of feed while maintaining production performance and egg quality at the level of hens fed typical commercial levels of CP.

Ammonia Emissions

Regulations. In 2004, the United States Environmental Protection Agency (EPA) published an article estimating that poultry (specifically chickens and turkeys) accounted for 27% of all NH_3 emissions produced in 2002 (Roberts et al., 2007b). This estimation identified poultry as the highest producers of NH_3 emissions of all domesticated species in the United States that were surveyed, including dairy, beef, sheep, goats, horses, and swine (EPA, 2004). Large quantities of NH_3 in the air can lead to odor problems in surrounding areas, increased fuel costs for ventilation of poultry facilities, decreased bird health and production, reduced employee health, less N available in poultry litter as a fertilizer, and a higher N load in aquatic environments (Blake and Hess, 2001; Roberts et al., 2007b).

Because large poultry facilities are capable of producing significant NH_3, a number of regulations and recommendations are currently in place to limit quantities of NH_3 released from poultry houses. The EPA published a final ruling in December, 2008, which exempted animal waste emissions from being reported under section 103 of the Comprehensive Environmental Response, Compensation Liability Act (CERCLA). However, the EPA maintained the requirement for NH_3 emissions from large concentrated animal feeding operations (CAFOs) exceeding 100 lbs in a 24 hour period to be reported under section 104 of the Emergency Planning and Community Right to Know Act (EPCRA) (EPA, 2008). Additionally, operations were required to comply with this policy by reporting NH_3 emissions beyond these levels by January 20, 2009. The United Egg Producers Animal Husbandry Guidelines for U.S. Egg Laying Flocks: 2006 Edition also recommends that birds should preferably be exposed to less than 10 ppm and never more than 25 ppm of NH_3 within hen houses (United Egg Producers, 2006). Health standards for employees working with poultry on a daily basis are covered by the Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH) and both have set limits for human NH_3 exposure averaged over an 8 hour work day, which are 50 and 25 ppm, respectively (Roberts et al., 2007b).

In order to comply with these policies and ensure the health and well being of the birds, their care takers, and the environment, researchers have been investigating both dietary and non-dietary strategies to reduce NH_3 emissions from poultry facilities. If alternative strategies are not identified, the number of birds per farm or facility would potentially have to be reduced, which could be detrimental to both farm productivity and profitability (Patterson and Lorenz, 1996).

Non-Dietary Strategies. There are a variety of non-dietary strategies currently being employed to reduce NH_3 emissions from hen houses. One such strategy involves the remodeling of older high-rise facilities, which use in-house deep-pit manure storage systems, to instead use belt systems to remove manure to a separate site where it can be composted. Modern laying hen facilities are often built to take advantage of belt manure removal systems. Through the drying of manure on the belts and regular removal to an external location via the belt system, this movement dramatically reduces the quantity of NH_3 released within hen houses compared to high-rise houses where manure in deep pits is only removed annually or bi-annually (Rotz, 2004). According to Rotz (2004), the typical percentage of total N excreted by hens and lost from the facilities (mainly in the form of NH_3) was 50% for high-rise houses using deep-pit manure storage versus only 10% for houses using a belt system.

However, renovation of high-rise, deep-pit hen houses to employ newer manure management systems is often not practical or economical for a farm owner or integrator. In these situations, there are a variety of other non-dietary strategies to reduce NH_3 emissions, mainly involving application of various additives to the manure within the pit. The five most common manure additives or amendments are urease inhibitors, digestive additives, adsorbents, acidifying additives, and saponins from the Mohave yucca (Yucca schidigera Roezl ex Ortgies) (McCrory and Hobbs, 2001).

Reducing Nitrogen Excretion. One major dietary strategy to reduce NH_3 emissions from poultry houses is to reduce N excretion with reduced CP diets simultaneously supplemented with crystalline essential amino acids. It has been reported in both laboratory and commercial scale studies performed with broilers, pullets, and laying hens that 25-75% of N consumed by these birds is either lost or excreted, mainly as a result of daily protein turnover in the body, including

AA catabolism (Parsons, 1995; Patterson and Lorenz 1996; Patterson and Lorenz, 1997; Patterson et al., 1998; Meluzzi et al., 2001; Nahm, 2007). When AAs are consumed beyond their required levels, they are subsequently deaminated and excreted, mainly as uric acid, thus contributing N to the manure (Parsons, 1995; Nahm, 2007). Therefore, large quantities of uric acid are inherently available in poultry manure for microbial hydrolysis to NH_3.

A direct relationship can be found between the level of dietary CP and N excreted in manure (Lopez and Leeson, 1995; Yakout et al., 2006b). Schutte et al. (1993) found a 10% reduction in N excreted for every percentage point decrease in dietary N fed to swine. Kerr (1995) similarly found an 8.5% decrease in N excreted from both poultry and swine for every percentage point decrease in dietary CP. As a result, dietary CP concentrations have been reduced in poultry diets over the past several years, while supplementing essential AAs, in order to balance requirements with dietary levels (Yakout et al., 2006b). However, standard commercial corn-soybean meal based laying hen diets, with supplemental TSAA and Lys, still contain several AAs in excess of their requirements (Roberts et al., 2007b). Therein lies the opportunity to reduce the catabolism of AAs provided in excess and reduce N excretion with modern feeding and formulating (Roberts et al., 2007b).

Investigations continue with the goal of formulating hen diets with practices that set no CP minimum, but provide required levels of essential AAs, in order to reduce dietary CP levels and subsequently reduce manure N (Roberts et al., 2007b). Meluzzi et al. (2001) reported a consistent linear decrease in manure N as dietary CP content was decreased and N excreted was approximately 50% of dietary N intake for all levels of CP. Roberts et al. (2007b) additionally reported a 10% reduction in N excretion from a ~20% CP diet to a ~19 CP, though they found no quantifiable effect on NH_3 emission.

Reducing Ammonia Emissions. Ammonia release from poultry manure results from the microbial break down of uric acid by the bacterial enzyme uricase, which is produced by several strains of bacteria, including Bacillus pasteurii, that are normally present in poultry waste (Blake and Hess, 2001; Bregendahl and Roberts, 2006). This reaction is favored at pH>7 (optimum enzyme activity is at pH 9) and since untreated poultry manure normally ranges from pH 9-10, it is understandable why poultry are the leading producers of NH_3 among most other domesticated species (Blake and Hess, 2001; EPA, 2004).

A limited amount of success reducing NH_3 emissions from poultry manure has been demonstrated using reduced CP, AA balanced diets. One successful study by Ferguson et al. (1998) reported both a 16.5% reduction in N excretion and a 31% reduction in equilibrium NH_3 gas concentration by reducing CP from 215 g/kg to 196 g/kg in broiler diets. Body weight and gain were not significantly different for birds fed normal levels versus low levels of dietary CP in this study; however, feed intake and feed conversion were increased for birds fed the low CP diet.

The results of these previous works indicate that this dietary strategy may be beneficial for producers and integrators to implement in large scale commercial laying operations both from an economical and managerial perspective.

CHAPTER 2

REDUCED CRUDE PROTEIN, AMINO ACID BALANCED DIETS MAINTAINED PERFORMANCE AND REDUCED PRODUCTION COSTS IN A LARGE-SCALE COMMERCIAL LAYING HEN FLOCK

SUMMARY

Recent increases in laying hen feed prices have renewed interest in reducing costly dietary crude protein (CP) levels, with supplementation of limiting amino acids (AAs), in order to reduce feed costs. A study was initiated to evaluate this dietary strategy under commercial conditions based on the promising results of numerous university-scale studies. The objective of this investigation was to determine if reduced CP, AA balanced laying hen diets could maintain hen performance and egg quality while reducing feed costs and/or increasing farm revenue.

A total of 50,760 Lohmann LSL Lite laying hens were divided into three groups that were each fed diets containing different levels of CP and supplemented with limiting AAs to their required levels. Hens were housed in a high-rise facility with a deep-pit manure storage system. Each diet was fed to two out of the six total cage rows. Diets were corn-soybean meal based and least-cost formulated weekly based on current ingredient prices and nutrient concentration. Hens were fed these diets ad libitum from 18 to 51 weeks of age (January 20, 2008-September 7, 2008). Diet C was a control diet formulated with a high level of CP and was meant to represent a typical commercial laying hen diet. Diets B and A were formulated to contain intermediate and low levels of CP, respectively.

Weekly diet formulations were examined to establish if diets were isocaloric and AA balanced while also achieving the goal CP differences between diets. Monthly, replicated data was collected at the front (F), middle (M), and end (E) of the feed trough from each cage row. Monthly data collection included obtaining feed samples for CP, AA, and particle size analysis, as well as measuring hen body weight, egg weight, albumen height, Haugh units, yolk color, shell strength, and shell thickness. Weekly non-replicated production and egg grade out data by dietary treatment was collected by the producer. Weekly egg income, feed consumption, and feed prices were used to calculate weekly feed cost and egg income minus feed cost (e.g. farmer revenue). A 3 x 3 factorial analysis was performed using the PROC MIXED procedure of SAS© software version 9.1 to detect differences between measured parameters by diet (A, B, and C), location along the feed trough (F, M, and E), and their interaction. Mean comparisons were made using Tukey's procedure.

Examination of weekly diet formulations revealed that, overall, diets were isocaloric and AA balanced and that diets B and A were formulated to contain 0.85 and 1.40% less CP than diet C, respectively. Analyzed feed samples collected from within cage rows proved the diets to be AA balanced; however, overall, diets B and A were actually shown to contain 1.53% and 1.98% less CP than diet C, respectively; quite different than formulated CP differences between diets.

Feed particle size analysis showed that the high CP diet consistently contained a numerically smaller proportion of large particles (2380-3360 µm) and larger proportion of small particles (840-1190 µm and 420-590 µm) than the lower CP diets throughout the eight month trial; however, these results were not statistically significant. Additionally, feed collected at the M location on the feed trough tended to be made up of a greater proportion of large particles (>2380 µm) than feed collected from the F and/or E locations ($P<0.05$).

Hen body weight and egg weight were numerically lower for the low CP diet versus the two higher CP diets throughout the study, though these differences were not statistically significant. Hen day egg production averaged 87.9, 87.4 and 87.1% for diets A, B, and C, respectively, and albumen height, Haugh units, yolk color, shell strength, and shell thickness did not differ by dietary treatment. Diets A, B, and C consistently had the highest to lowest percentages of large grade A eggs and lowest to highest percentages of extra-large and jumbo grade A eggs, respectively, throughout the study. Hen body weight and egg weight were consistently lower at the M location on the feed trough compared to the F and/or E, while shell strength was numerically higher at the M than at the F and/or E throughout the trial, though these findings were not statistically significant. Additionally, overall, albumen height and Haugh units were shown to be significantly higher at the M than at the F and/or E ($P<0.05$). Yolk color and shell thickness did not differ by location along the feed trough.

Mean weekly egg income per hen housed for diet A was \$0.0022 and \$0.0024 less than for diets B and C, respectively. However, mean weekly feed costs per hen housed for diets A and B were \$0.0083 and \$0.0070 less than for diet C, respectively, and mean weekly egg income minus feed costs per hen housed for diets A and B were \$0.0059 and \$0.0068 higher than for diet C, respectively.

These results demonstrate that when reduced CP, AA balanced diets were fed to a large scale commercial laying hen flock, it was possible to maintain hen production performance while reducing dietary costs and increasing farm revenue. Therefore, it would be economically beneficial for producers to implement this feeding strategy on a commercial scale.

DESCRIPTION OF THE PROBLEM

Recent increases in feed prices for laying hens have resulted from the rising costs of corn and soybean meal, which are a consequence of the increased demand for ethanol as an alternative fuel source (Tokgoz et al., 2007). In order to alleviate the burden of elevated feed prices, a large number of recent studies have focused on the development of dietary formulations aimed at reducing feed costs while maintaining hen production performance.

One such dietary strategy involves formulating diets on an “ideal protein” basis. The goal of this concept is to formulate diets that provide ideal levels of AAs for optimizing hen performance while minimizing excess AAs provided by dietary CP. This is accomplished by reducing CP in diets combined with supplementation of limiting AAs to their required levels. It is possible to formulate laying hen diets in this manner because poultry do not need a definitive amount of CP in their diets, but rather have requirements for specific quantities of individual AAs. With a variety of synthetic AAs becoming ever more available and affordable, this feeding strategy is becoming increasingly implemented in the poultry industry (Meluzzi et al., 2001).

Formulating diets on an “ideal protein” basis has been studied with broilers, turkeys, pigs, and laying hens (Boling and Firman, 1997; Firman and Boling, 1998; Figueroa et al., 2002; Shriver et al., 2003; Keshavarz and Austic, 2004; Roberts et al., 2007ab; Namroud et al., 2008). Many university-scale studies have shown that this approach to diet formulation has both the potential to increase producer revenue through the reduction of feed price and to reduce ammonia emissions by limiting hen nitrogen excretion that results from feeding AAs in excess of their requirements (Ferguson et al., 1998; Meluzzi et al., 2001; Nahm, 2007; Yakout et al., 2006ab).

Several studies in the past have found that many production parameters were not maintained when levels of CP were reduced in laying hen diets, despite supplementation with required levels of limiting AAs (Penz and Jensen, 1991; Keshavarz and Jackson, 1992; Roberts et al., 2007a). There are a number of potential explanations for the lower production performance observed in these studies, including providing insufficient quantities of limiting AAs required for specific phases of production, supplying inaccurate ratios of limiting AAs required for optimum production, and/or having inadequate knowledge of the content, digestibility, and bioavailability of limiting AAs provided by treatment diets (Harms and Russell, 1993; Keshavarz and Austic, 2004; Roberts et al., 2007a). In one study by Sklan and Noy, 2005, which evaluated the optimum AA levels for growth and maintenance of broiler chicks, it was specifically found that most AA requirements determined in their study were higher than those listed in the NRC (1994) recommendations, which are often used as the basis for treatment diet formulation in many present day investigations.

In contrast, other studies have found that when reduced CP diets, supplemented with limiting AAs, were fed to laying hens, most production parameters were maintained at or above the level of birds provided normal commercially fed quantities of CP for various lengths of time and phases of production (Meluzzi et al., 2001; Keshavarz and Austic, 2004; Yakout et al., 2006ab). One series of studies by Yakout et al. (2006a) specifically reported that, when 24-36 wk old laying hens were fed diets with CP levels reduced to 15% that were supplemented with required levels of lysine (Lys), methionine (Met), and threonine (Thr), these diets had the potential to save the poultry industry up to $11 per ton of feed while maintaining hen production performance at the level of diets containing 19% CP, supplemented with only Met.

The promising results of these recent university scale studies have led to the question of whether reduced CP laying hen diets, supplemented with limiting AAs, can provide a practical solution to reducing feed costs while maintaining production at the level of diets containing normal levels of CP on a commercial scale. The objective of this study was to determine if reduced CP, AA balanced diets could maintain hen performance while reducing feed costs and/or increasing farm revenue when fed to a commercial laying hen flock.

MATERIALS AND METHODS

Birds and Housing

A total of 50,760 Lohmann LSL Lite laying hens were obtained from a commercial pullet farm at 18 weeks of age (hatch date 09-12-07) and placed into an environmentally controlled, mechanically ventilated high-rise house that employed a deep-pit manure storage system. The birds were housed in six rows of 8,460 birds each. Two rows of birds were assigned randomly to each of the three dietary treatments used in this study. Hens were housed in 61 x 51 cm^2 cages with 7-8 hens per cage (density 389-444 cm^2/bird). Experimental diets were provided to each of the treatment groups from their date of arrival at the research facility at 18 wks of age until 51 wks of age.

All lighting and management practices aside from dietary alterations specified by dietary treatments were in accordance with current recommendations for the breed (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany) and guidelines from Wenger’s Feed Mill, Inc. (Rheems, PA). Photoperiod was progressively increased to 15 hr light: 9 hr dark at 25 weeks of age and maintained on this schedule for the remainder of the trial. The house temperature was kept at ~25˚C throughout the trial. The Pennsylvania State University Institutional Animal Care and Use Committee approved all techniques and procedures involved in animal care and handling (IACUC #27579).

Experimental Diets

Hens were divided into three treatment groups, which were fed low (diet A), intermediate (diet B), or high (diet C) CP diets, all isocaloric and supplemented with Lys, Met, and/or Thr to their required levels. The high CP diet represented a typical commercially fed laying hen diet. All diets were formulated by Wenger's Feed Mill, Inc. Diets were primarily corn and soybean meal based, but also included poultry by-product meal, dried distillers grains with solubles (DDGS), canola meal, blended fat, bakery by-product meal, and wheat middlings. A commercial type phase feeding program was used and diets were least-cost formulated weekly based on current ingredient prices and nutrient concentration. **Table 2.1** displays the high and low values for each of the major feed ingredients providing energy and protein in each of the dietary treatments throughout the study. Corn and soybean meal are shown to be the predominate feed ingredients, with corn averaging 52.37, 51.08, and 49.14% and soybean meal averaging 10.02, 11.59, and 17.39% for diets A, B, and C, respectively, over the eight months of the trial. Feed and water were provided ad libitum, with feed allocated daily into multiple feedings.

Table 2.1. High and low percentages of major energy and protein containing ingredients in dietary treatments

	Dietary treatment					
	A		B		C	
Ingredient (%)	High	Low	High	Low	High	Low
Corn	58.06	45.30	59.35	43.26	56.03	41.32
Soybean meal	14.83	7.25	18.15	8.70	26.31	13.09
Poultry by-product meal	8.00	5.75	8.00	5.80	8.00	1.10
DDGS[1]	8.01	5.00	8.00	5.00	8.00	1.20
Canola meal	5.00	2.94	5.00	4.55	5.00	0.00
Blended fat	2.00	0.00	2.25	0.00	2.70	0.15
Bakery by-product meal	6.00	0.00	6.20	0.00	6.00	0.00
Wheat middlings	6.00	0.00	6.00	0.00	5.32	0.00

[1] Dried distillers grains with solubles.

Monthly Production Data

Hen Body Weight. Hens from a single cage at the F, M, and E of the feed trough in each cage row were repeatedly weighed every four weeks (eight total sampling dates from February to September) resulting in a total of six cages of hen body weights for each treatment diet at each sampling date. Sampling locations are illustrated in **Figure 2.1**.

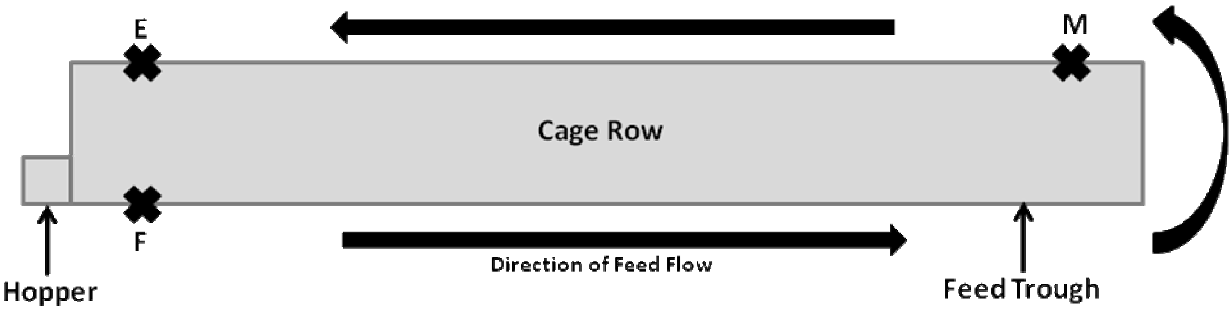

FIGURE 2.1. Direction of feed flow from the hopper to the front (F), middle (M), and end (E) sampling locations along the feed trough (feed was distributed by a chain mechanism).

Egg Sample Collection. Fifteen eggs were collected at the F, M, and E locations of the feed trough in each cage row, before the morning egg collection, once every four weeks (eight total sampling dates from February to September), resulting in a total of six replicate samples of fifteen eggs for each treatment diet at each sampling date. Eggs collected at each time period were then transported to The Pennsylvania State University for interior egg quality analysis and/or to Wenger's Feed Mill, Inc. for shell quality analysis.

Interior Egg Quality. Egg samples were transported to The Pennsylvania State University for interior egg quality analysis on the February, April, May, July, and September sampling dates. The Technical Services and Supplies QCM+ Egg Quality System was connected to a computer equipped with software to automatically record egg weight (g), measured with a digital balance (Ohaus Corporation, Florham Park, NJ), and albumen height (mm), measured with a tripod micrometer (TSS, York, England) and electronic height gauge (TSS, York, England). Haugh units (HU) were calculated from egg weight and albumen height with this software using the HU formula (Haugh, 1937). Yolk color was subjectively assigned using a Roche yolk color fan.

Egg Shell Quality. Egg samples were transported to Wenger's Feed Mill, Inc. for egg shell quality analysis after the March, April, June, and August sampling dates. Shell strength was determined using a QC-SPA shell strength and packaging analyzer (TSS, York, England). Shell thickness was determined by cracking open the sample egg, discarding the albumen and yolk, removing the egg shell membrane, and using a micrometer to measure shell thickness at a location near the equator of the shell.

Feed Sample Collection. Feed samples were collected at the F, M, and E of the feed trough from each cage row before the morning feeding, once every four weeks (eight total sampling dates from February to September), resulting in a total of six replicate feed samples for each treatment diet at each sampling date. One representative feed sample for each dietary treatment was collected directly from the feed mill and another was collected from the hoppers at the beginning of each cage row by pooling equal amounts of feed from the two hoppers for each diet. Feed trough samples were collected by vacuuming all feed from the trough beneath two adjacent cages at each sampling location. Feed samples were then transported back to The Pennsylvania State University and segregated into portions to be sent for AA analysis or used for feed particle size analysis.

Feed Sample Segregation. Feed samples were segregated in 100 g portions to be sent for AA analysis and 200 g quantities for feed particle size analysis using the procedure described by Tang (2004), in order to assure consistent and even distribution of feed particle sizes in all samples to be analyzed. Briefly, feed samples were poured onto a flat surface so that feed formed a cone shape with equal distribution of feed around the cone. The cone of feed was then flattened into a disk shape using a spatula. The disk of feed was then divided into four equal portions and two of these portions, diagonal from one another, were selected at random and discarded. This procedure was repeated until the desired sample sizes were acquired. Segregated feed samples were then double-bagged to prevent moisture loss and were stored at -20°C until they were either sent for AA analysis or used for feed particle size analysis.

***Feed Composition Analysis*.** The 100 g feed samples were sent to the Evonik-Degussa Corporation (Kennesaw, GA) for AA analysis to obtain accurate measurements of dry matter (DM), CP, Lys, Met, Met + Cysteine (M+C) or total sulfur AA (TSAA), and Thr for all treatment diets.

***Feed Particle Size Analysis*.** Feed samples were separated into ten fractions, based on particle size, using an AS 200 sieve shaker (Retsch, Inc., Newtown, PA) and a series of nine U.S.A. Standard Testing sieves. The 200 g feed samples stored at -20°C were poured onto a flat plate, covered with plastic wrap to prevent moisture loss, and allowed to defrost for 12 to 24 hours. A $2^{½}$ sieve series with openings between 3,360 and 210 µm was used for this analysis, meaning that the width of the openings of each successive sieve was 1.414 times larger than that of the previous sieve in the series (Tang, 2004). The diameter of each sieve was 203.2 mm. The nine sieves were stacked from top to bottom in the following order: sieve number 6 (3360 µm openings), 8 (2380 µm), 12 (1680 µm), 16 (1190 µm),20 (840 µm), 30 (590 µm), 40 (420 µm), 50 (297 µm), and 70 (210 µm). A pan of the same diameter as the sieves was placed beneath sieve number 70 to capture all fine particles less than 210 µm. Feed particles from the top two sieves (numbers 6 and 8) were collected throughout the sieving process and 5 g of these particles were added to each of sieve numbers 30, 40, 50, and 70 before each feed sample was shaken in order to reduce adhering of fine particles to these smaller opening sieves. A 200 g feed sample was then placed in the top sieve (number 6) and the stack of sieves was shaken for 10 minutes at an amplitude of 2.50 mm/”g”. Particles from each sieve and the bottom pan were then weighed. The quantity of feed in each sieve or pan was then divided by the total amount of feed remaining in all sieves and the pan and this number was multiplied by 100, resulting in the percentage of total feed made up by each particle size fraction. The particle size fractions of feed were divided into the following ten fractions sizes: >3360 µm, 3360-2380 µm, 2380-1680 µm, 1680-1190 µm, 1190-840 µm, 840-590 µm, 590-420 µm, 420-297 µm, 297-210 µm, and < 210 µm.

Statistical Analysis. A 3 x 3 two factor factorial statistical analysis was performed to detect any significant differences in hen production, egg quality, and feed parameters by three levels of dietary CP (low-A, intermediate-B, and high-C) and three sampling locations along the feed trough (F, M, and E). Significant differences in feed AA composition between diet formulations (formulation), feed samples collected at the feed mill (mill), feed samples collected from the hopper at the front of each cage row in the hen house (hopper), and feed samples collected at locations within each feed trough in the hen house (trough) were detected using a one-way ANOVA. Significant differences in feed particle size between mill, hopper, and location samples were also evaluated using a one-way ANOVA. Data analysis was done using the PROC MIXED procedure of SAS (SAS Institute, 2003, Version 9.1 ed., SAS Inst. Inc., Cary, NC). Mean comparisons were made using Tukey's procedure (Steel and Torrie, 1980).

Weekly Production Data

Diet Formulations. Formulated levels of CP ("as is" basis), metabolizable energy (ME), and digestible Lys, Met, M+C, and Thr for treatment diets were provided weekly by Wenger's Feed Mill, Inc.

Hen Body Weight. Three cages of hens per dietary treatment were weighed each week by personnel at the Jim Charles research facility (Lancaster, PA). These values were used to determine a mean hen body weight (lbs) for each dietary treatment using **Equation 2.1**:

Mean hen body weight (lbs) =
[Cumulative weight of hens weighed for a dietary treatment (lbs)]/
(Cumulative # of hens weighed for a dietary treatment) **(2.1)**

Cumulative Hen Mortality. The number of hen mortalities each week for each dietary treatment was recorded. Cumulative hen mortality for a given week was calculated using **Equation 2.2**:

Cumulative hen mortality =
(# Hen mortalities in a given week) +
(# Hen mortalities in all proceeding weeks) **(2.2)**

Feed and Water Consumption. Feed consumption (lbs/100 birds/d) and water consumption (gallons/100 birds/d) were determined weekly for each diet.

Percent Egg Production. Percent egg production was determined weekly for each diet.

Cumulative Eggs Per Hen Housed. Cumulative number of eggs per hen housed was determined weekly for each diet.

Egg Case Weight. Mean egg case weight (lbs) was determined weekly for each diet.

Egg Mass. Mean egg mass (g of egg/hen/d) was determined weekly for each diet.

Feed Conversion. The mean number of lbs of hen feed consumption to produce one lb of egg mass (lbs feed/lb egg mass) and the mean number of lbs of feed to produce one dozen eggs (lb feed/dozen eggs) was determined weekly for each diet.

Egg Grade Out. Egg processing and grade out was carried out by R.W. Sauder’s Eggs (Lititz, PA). Percent of eggs produced that were grade A, large grade A, extra-large grade A, jumbo grade A, grade B, cracked, dirty, and losses were all determined weekly for each diet.

Price of Diets. Price [dollars ($)/ton] of treatment diets were provided for all weekly diet formulations by Wenger's Feed Mill, Inc.

Economic Analysis. Weekly production data was used to determine weekly egg income per hen housed ($/wk), feed cost per hen housed ($/wk), and egg income minus feed cost per hen housed ($/wk) for each dietary treatment. Weekly feed consumption (lbs/wk) for each dietary treatment was first determined using **Equation 2.3**:

Weekly total treatment diet feed consumption (lbs/wk) =
{[Treatment diet feed consumption (lbs/100 birds/d) x 7]/100} x
(16,920 starting hens) **(2.3)**

Weekly treatment diet feed cost per hen housed ($/week) was then determined with **Equation 2.4**:

Weekly treatment diet feed cost per hen housed by treatment diet ($/wk) =
{[Price of treatment diet for that week ($/ton)/2000] x
[Weekly treatment diet feed consumption (lbs/wk)]}/16,920 starting hens **(2.4)**

Weekly treatment diet egg income per hen housed ($/wk) was then determined with **Equation 2.5:**

Weekly egg income per hen housed by treatment diet ($/wk) =
[Weekly treatment diet egg income ($/wk)]/16,920 starting hens **(2.5)**

Finally, weekly treatment diet egg income minus feed cost per hen housed ($/wk) was determined using **Equation 2.6**:

Weekly egg income minus feed cost per hen housed by treatment diet ($/wk) =
[Weekly treatment diet egg income per hen housed ($/wk)] –
[Weekly treatment diet feed cost per hen housed ($/wk)] **(2.6)**

RESULTS AND DISCUSSION

Feed Composition

Selected nutrients of each dietary treatment are shown in **Table 2.2** to summarize the average diet formulations provided to hens during each month of the trial. Overall, diets B and C were formulated to differ by a mean value of 0.85% CP and diets A and C by a mean value of 1.40% CP. A list of all weekly dietary formulations and selected critical nutrients are provided in **Appendix A.1**, **A.2**, and **A.3**.

Table 2.2. Mean monthly formulated concentrations of selected nutrients in treatment diets

		Month									
Diet	Nutr[1]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr[2]
A	CP	19.41	19.05	18.07	17.61	17.35	17.47	17.19	17.10	17.08	17.76
	ME	1300	1302	1305	1302	1297	1295	1295	1295	1295	1299
	Lys	0.91	0.90	0.84	0.85	0.88	0.90	0.85	0.85	0.82	0.87
	Met	0.48	0.47	0.43	0.41	0.40	0.40	0.39	0.38	0.38	0.41
	M+C	0.76	0.74	0.69	0.70	0.72	0.72	0.69	0.68	0.66	0.71
	Thr	0.62	0.61	0.57	0.59	0.62	0.63	0.59	0.59	0.57	0.60
B	CP	20.07	19.62	18.52	17.95	17.40	18.20	17.93	17.85	17.87	18.31
	ME	1300	1303	1305	1301	1296	1296	1296	1295	1295	1299
	Lys	0.91	0.90	0.84	0.82	0.88	0.90	0.85	0.85	0.82	0.86
	Met	0.47	0.46	0.42	0.40	0.39	0.40	0.38	0.37	0.36	0.40
	M+C	0.76	0.74	0.69	0.67	0.71	0.73	0.69	0.68	0.66	0.70
	Thr	0.62	0.61	0.57	0.56	0.62	0.65	0.61	0.62	0.59	0.61
C	CP	20.11	19.63	18.33	19.40	19.11	19.44	19.12	18.92	18.90	19.16
	ME	1300	1303	1306	1302	1296	1295	1295	1296	1296	1299
	Lys	0.91	0.90	0.84	0.82	0.89	0.91	0.86	0.85	0.82	0.87
	Met	0.48	0.46	0.43	0.38	0.38	0.38	0.36	0.36	0.35	0.40
	M+C	0.76	0.74	0.69	0.68	0.72	0.73	0.69	0.68	0.66	0.71
	Thr	0.63	0.63	0.59	0.62	0.70	0.70	0.67	0.66	0.63	0.65

[1] Nutr = Nutrient; CP = crude protein (%) (as is); ME = metabolizable energy (kcal/lb); Lys = digestible lysine (%); Met = digestible methionine (%); M+C = digestible methionine + cysteine (%); Thr = digestible threonine (%).
[2] Ovr = overall means of selected items over the entire eight months of the trial.

Treatment diets were formulated to be isocaloric, with nearly equal metabolizable energy (ME) in all three diets **(Table 2.2)**. Diets were also formulated to be AA balanced, as shown by the nearly equal concentrations for all diets, with the exception of Thr being higher for the high

CP diet (C) than for the other two diets. This was due to the higher CP content of diet C, which inherently contains a higher percentage of Thr.

In order to determine if the formulated nutrient values were achieved in the actual diets provided to the hens, feed composition was determined for samples taken from the feed mill directly (mill), from the hoppers at the front of each cage row (hopper), and from F, M, and E locations along the feed trough within each cage row (trough). Analyzed values of selected nutrients in feed samples from the mill and hoppers are presented in **Appendix B.1** and **Appendix B.2**, respectively. Nutrients from feed samples collected at the F, M, and E locations along the feed trough, which best represent nutrients being consumed by the birds, were pooled for all three locations along the feed trough and reported by dietary treatment in **Table 2.3**.

For feed sampled from the mill, hoppers, and trough, analyzed values of CP and AAs revealed that diets A, B, and C had the lowest to highest percent CP and AAs were nearly equal for all diets, again with the exception of higher values of Thr for diet C. DM values, not shown in the formulation means of **Table 2.2**, were also nearly equal for all diets from the mill, hoppers, and trough. **Table 2.3** demonstrates that diets B and C differed by a mean value of 1.53% CP and diets A and C by a mean value of 1.98% CP, much different than the 0.85 and 1.40% CP differences in formulation.

Nutrients (DM, Lys, Met, Cys, and M+C) delivered to the hens differed very little between dietary treatments **(Table 2.4)**. However, percent Thr was shown to be significantly greater in diet C than in diets A and B. The higher Thr levels in diet C are the result of the higher CP content of this diet. This trend is not of particular concern, however, as treatment diets in this study were formulated to meet the Thr requirements of the hen. As a result, dietary levels of Thr shown in **Table 2.4** satisfied both the NRC (1994) guideline of 0.47% dietary Thr and the current recommendation for the breed of 0.64% dietary Thr (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany) for hens consuming 100 g of feed per day.

Table 2.3. Mean monthly dietary treatment nutrients in feed collected from all locations along the feed trough (as is basis)

		Month								
Diet	Nutr (%) [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
A	DM	90.86	90.87	90.18	90.16	89.85	89.87	90.48	90.83	90.39
	CP	21.93	20.92	19.68	19.07	19.26	19.16	18.75	20.43	19.90
	Lys	1.18	1.12	1.03	1.04	1.02	0.99	0.96	1.07	1.05
	Met	0.56	0.50	0.45	0.40	0.41	0.40	0.41	0.44	0.45
	Cys	0.41	0.41	0.41	0.37	0.40	0.37	0.37	0.39	0.39
	M+C	0.97	0.91	0.86	0.78	0.81	0.78	0.77	0.83	0.84
	Thr	0.87	0.81	0.74	0.72	0.72	0.70	0.71	0.77	0.75
B	DM	91.03	90.93	90.11	90.32	90.12	89.92	90.35	91.13	90.49
	CP	22.12	21.24	19.68	19.83	19.47	20.20	19.55	20.69	20.35
	Lys	1.16	1.07	1.00	1.14	1.01	1.00	0.95	1.03	1.04
	Met	0.55	0.49	0.43	0.41	0.40	0.41	0.39	0.41	0.43
	Cys	0.42	0.42	0.41	0.37	0.40	0.38	0.38	0.40	0.40
	M+C	0.96	0.90	0.83	0.78	0.80	0.79	0.77	0.81	0.83
	Thr	0.86	0.80	0.73	0.76	0.73	0.73	0.73	0.77	0.76
C	DM	90.72	91.15	90.32	90.52	90.41	89.59	90.71	91.10	90.57
	CP	22.10	20.61	22.25	22.49	21.62	21.85	21.31	22.83	21.88
	Lys	1.14	1.07	1.03	1.16	1.02	1.02	0.96	1.08	1.06
	Met	0.55	0.51	0.44	0.40	0.41	0.40	0.39	0.42	0.44
	Cys	0.38	0.36	0.44	0.41	0.44	0.40	0.40	0.43	0.41
	M+C	0.93	0.86	0.89	0.81	0.85	0.81	0.79	0.85	0.85
	Thr	0.85	0.78	0.84	0.87	0.83	0.80	0.80	0.86	0.83

[1] Nutr = Nutrient; DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; Thr = threonine.
[2] Ovr = overall means of selected items over the entire eight months of the trial.

Mean percent CP in diet C was significantly greater than in diets A and B; however, though diet B was numerically greater than diet A, mean percent CP did not differ significantly between diets A and B for these samples taken from the feed troughs. The distinctly higher level of CP observed in diet C, compared to the other two diets, may have led to more dramatic differences in the production parameters measured in this study between diet C versus diets A and B. However, feed trough CP and AA levels, shown in **Table 2.4**, all exceeded both the NRC (1994) requirements and current recommendations for the breed (Lohmann LSL Lite Layer

Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany), in even the low CP diet. For example, the recommended percent CP for laying hens, consuming 100 g of feed per hen per day, is 15.0% according to NRC (1994) requirements and 18.80% according to current recommendations for the breed (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). In the present study, percent CP was analyzed to be 19.90% for the low CP diet from feed trough samples. With CP and AA levels well beyond the hens' nutrient requirements for growth and production, it is possible that treatment differences could have been masked.

Table 2.4. Mean nutrient concentrations for feed samples collected from all locations along the feed trough (as is basis)

	Dietary nutrient (%) [1]						
Diet [2]	DM	CP	Lys	Met	Cys	M + C	Thr
A	90.39	19.90^b	1.05	0.45	0.39	0.84	0.75^b
B	90.49	20.35^b	1.04	0.43	0.40	0.83	0.76^b
C	90.57	21.88^a	1.06	0.44	0.41	0.85	0.83^a
SEM	0.08	0.20	0.01	0.01	0.00	0.01	0.01
P-value	0.3864	0.0114	0.6670	0.6203	0.1093	0.5114	0.0107

[a-b] If numerical values do not have the same superscript letter within a column, then they are significantly different ($P<0.05$).
[1] DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Thr = threonine.
[2] A = Low CP level diet; B = Intermediate CP level diet; C = High CP level diet; SEM = Pooled standard error of the means; P = P-value ($P<0.05$ considered statistically significant).

Next, the change in dietary nutrients progressing from the formulated diets, to the mill, hopper, and feed trough was considered. Interestingly, **Table 2.5** demonstrates that all nutrients were significantly lower for the formulated diets and increased linearly at the feed mill, hopper, and feed trough locations, respectively. Lower AA values in formulation are due to their being presented on a digestible basis in formulation, rather than on an "as is" basis for feed samples analyzed from the feed mill, hopper, and feed trough. Only percent dry matter (DM) differed significantly between feed mill and hopper samples. Higher DM values from the feed mill to the hopper and feed trough may have been the result of increasing feed desiccation during transport and distribution to the birds.

Table 2.5. Mean nutrient concentrations across all diets at formulation, feed mill, hopper, and feed trough

	Dietary nutrient (%) [1, 2]						
Location	DM	CP	Lys	Met	Cys	M + C	Thr
Formulation	---	18.47^{c}	0.87^{c}	0.41^{b}	---	0.71^{c}	0.62^{c}
Feed mill	89.28^{c}	18.84^{bc}	0.94^{b}	0.42^{ab}	0.37^{b}	0.79^{b}	0.71^{b}
Hopper	89.68^{b}	19.50^{b}	0.97^{b}	0.42^{ab}	0.38^{b}	0.80^{b}	0.73^{b}
Feed trough	90.48^{a}	20.71^{a}	1.05^{a}	0.44^{a}	0.40^{a}	0.84^{a}	0.78^{a}
SEM [3]	0.08	0.18	0.01	0.01	0.00	0.01	0.01
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a-c}$ If numerical values do not have the same superscript letter within a column, then they are significantly different ($P<0.05$).

[1] DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; Thr = threonine.

[2] All nutrient values are reported on an "as is" basis, with the exception of formulated values of Lys, Met, M+C, and Thr, which are reported on a digestible basis.

[3] Pooled standard error of the means.

Lower values of CP and AAs in feed mill and hopper samples compared to those collected from the feed trough may have resulted from a combination of sampling technique and bird selection of certain particle sizes in feed. Tang et al. (2006) reported that when poultry mash feed is distributed by a drag chain, which was the system used in the current study, it has the tendency to segregate such that fine and dense feed particles percolate toward the bottom and center of the feed trough, and less dense particles rise to the top of the feed and accumulate towards the sides of the feed trough, thus encouraging hen selection of large-particle size ingredients, such as corn, which mainly contribute carbohydrates, gross energy, and nitrogen-free extract to the birds. As feed samples were collected from the feed trough one to two hours after feed had been distributed, this would have given the birds time to select certain particles sizes of feed over others. If the birds had already consumed these large-particle size ingredients when samples were collected, this would have led to a concentrating of smaller ingredients (such as soybean meal), crystalline AAs, vitamins and minerals, etc., which would have resulted in the higher values of CP and AAs for the feed trough samples compared to the feed mill and hopper.

Additionally, feed mill samples were collected by personnel at Wenger's Feed Mill, Inc., so collection method for these samples is not clear and samples from the hoppers were collected at the surface of the feed, as this was the only accessible location for feed collection. Conversely, feed trough samples were gathered very uniformly such that all feed at each sampling location was completely collected. Sample collection from the feed surface in the hoppers may have led to a higher proportion of large feed particles in these samples, as it is the nature of smaller, higher density particles to segregate towards the bottom of the feed. It is possible that samples from the feed mill were collected similarly, from the surface of a feed storage bin, which would have led to a similar accumulation of larger particles in these samples. These methods of sample collection could have led to a concentrating of high energy, low protein ingredients in feed mill and hopper samples compared to feed trough samples, adding to the discrepancy in nutrient values between these sampling locations.

Feed Particle Size

In order to determine if variation in nutrient composition between feed mill, hopper, and feed trough samples was occurring due to differences in feed particle size distribution, an analysis of feed particle size composition of diets was performed for samples collected from these locations. It can be seen in **Table 2.6** that feed particle size of diets was indeed significantly different between sampling locations for most fractions of particle sizes. Percentage of total feed in sieves 6, 8, and 12 (>1,680 µm) tended to be largest to smallest for feed mill, hopper, and cage row feed trough samples, respectively (P=<0.0001). Additionally, sieves 20, 30, 40, 50, and 70 (210-1190 µm) all contained smaller percentages of total feed for feed mill and hopper samples than for the feed trough samples, (P=<0.0001). The proportion of total feed in sieve 16 (1,190-1,680 µm) and the pan below the sieves (<210 µm) did not differ significantly by sampling location (P=0.0875 and P=0.0624, respectively). These results are similar to those reported by Tang et al. (2006), which found that percentage of total feed particles <1,180 µm were significantly higher ($P<0.05$) at the feed trough (61.7%) than at the hopper (45.8%) when a drag chain feed delivery system was employed, which they reported to be due to hen consumption of a large portion of feed particles >1,180 µm.

Table 2.6. Mean sieving fractions (% of total feed) for feed mill, hopper, and feed trough samples

	Sieve [1]									
Location	6	8	12	16	20	30	40	50	70	Pan
Feed mill	11.09^{a}	15.77^{a}	12.21^{a}	11.10	10.05^{b}	11.65^{b}	8.57^{b}	6.08^{b}	5.44^{b}	8.01
Hopper	8.92^{b}	13.87^{b}	12.26^{a}	11.68	10.74^{b}	12.30^{b}	9.65^{b}	6.78^{b}	6.17^{b}	7.66
Feed trough	5.59^{c}	9.22^{c}	9.89^{b}	11.05	11.68^{a}	14.10^{a}	13.00^{a}	8.77^{a}	8.01^{a}	8.69
SEM [2]	0.28	0.36	0.22	0.21	0.22	0.47	0.39	0.18	0.36	0.36
P-value	<.0001	<.0001	<.0001	0.0875	<.0001	<.0001	<.0001	<.0001	<.0001	0.0624

[a-c] If numerical values do not have the same superscript letter within a column, then they are significantly different ($P<0.05$).

[1] Particle size fractions: Sieve 6 (>3,360 µm), 8 (3,360-2,380 µm), 12 (2,380-1,680 µm), 16 (1,680-1,190 µm), 20 (1,190-840 µm), 30 (840-590 µm), 40 (590-420 µm), 50 (420-297µm), 70 (297-210 µm), and pan (< 210 µm).

[2] Pooled standard error of the means.

It was also brought into question whether treatment diet or location along the feed trough had any influence on feed particle size. In order to assess this, a 3 x 3 factorial statistical analysis was performed to look for effects of treatment diet, location on the feed trough, or an interaction of both. **Appendix C.1** summarizes several significant interactions between treatment diet and location, therefore, the effect of treatment and location cannot be considered individually for those feed fractions at those times. Although several interactions were significant during individual months, they were mainly the result of high or low values at a single location on the feed trough within a single treatment. Also, no significant or repeatable trends were observed overall for the entire trial.

Appendix C.2 shows that treatment diet appeared to have some influence on feed particle size in feed trough samples. The high CP diet was found to contain a numerically lesser proportion of large particles from 2,380-3,360 µm (sieve 8) and a numerically greater proportion of small particles from 840-1,190 µm (sieve 20) and 420-590 µm (sieve 40) than the two lower

CP diets for seven or eight months out of the eight month trial. There were no differences between dietary treatments for any other particle size fractions. This numerical pattern for the high CP diet containing a greater proportion of small particles and a lesser proportion of large particles compared to the two lower CP diets may be due to the higher soybean meal content of the high CP diet, as soybean meal averaged 10.02, 11.59, and 17.39% for the low, intermediate, and high CP diets, respectively.

Table 2.7 demonstrates that location on the feed trough within a cage row also had an effect on feed particle size. The M location on the feed trough tended to have a greater portion of large particles, >2,380 µm (sieves 6 and 8), than the F and/or E locations (P=0.0008 and P=0.0022 for sieves 6 and 8, respectively). There were no other differences by location on the feed trough for any other particle size fractions. Accumulation of large feed particles (>2,380 µm) at the M location along the feed trough could have resulted from a combination of smaller sized, high protein content ingredients segregating towards the bottom of the feed trough and their subsequent accumulation there as feed traveled along the trough and hen selection of feed particle sizes smaller than 2,380 µm at the F location on the feed trough leading to a concentrating of particles larger than this at the M location.

Feed nutrient composition by location along the feed trough was also investigated to determine if nutrient content was impacted by particle size distribution of the feed. The results of this analysis are given in **Table 2.8**. Percent DM was shown to decrease numerically from the F to E of the feed trough, potentially due to increasing desiccation of feed as it traveled along the feed trough. Also, percent CP and Lys both tended to be lowest at the M location along the feed trough. This finding corresponds with the tendency for large feed particles (>2,380 µm), mainly composed of high carbohydrate and gross energy, low protein content dietary ingredients, to be

Table 2.7. Mean sieving fractions (% of total feed) by location on the feed trough

		Month								
Sieve [2]	Loc [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [3]
6	F	4.88	4.55	5.63	5.52	6.10	7.23	5.93	4.00	5.48^{b}
	M	6.83	6.27	5.95	4.67	7.22	7.77	6.43	4.70	6.23^{a}
	E	4.23	4.95	5.13	3.73	5.95	6.32	5.77	4.28	5.05^{b}
	SEM	0.65	0.43	0.62	0.60	0.56	0.82	0.31	0.23	0.32
	P-value	0.0697	0.0659	0.6563	0.1448	0.1325	0.2016	0.3582	0.1261	0.0008
8	F	8.57	8.83^{b}	9.40	9.80	8.83	11.72	10.47	7.13	9.34^{ab}
	M	10.72	11.08^{a}	9.67	8.15	9.52	11.32	10.63	7.63	9.84^{a}
	E	7.55	9.05^{ab}	8.57	7.02	8.10	10.37	9.93	7.25	8.48^{b}
	SEM	1.00	0.5159	0.56	0.87	0.48	0.83	0.42	0.29	0.3761
	P-value	0.1423	0.0398	0.4086	0.1380	0.0803	0.2498	0.3374	0.4787	0.0022
12	F	10.05	9.93	10.23	10.88	9.78	11.02	11.47^{a}	8.07	10.18
	M	11.22	11.10	9.98	9.43	9.25	10.18	10.40^{b}	8.20	9.97
	E	9.72	9.78	9.47	8.43	8.98	10.80	10.92^{ab}	8.07	9.52
	SEM	0.70	0.46	0.42	0.54	0.43	0.33	0.20	0.21	0.23
	P-value[4]	0.2674	0.1463	0.2571	0.0506	0.4614	0.0195	0.0257	0.8786	0.0633
16	F	12.13	11.83	11.50	12.22^{a}	11.05	11.42	11.40	9.07	11.33
	M	12.53	12.23	11.47	11.15^{ab}	10.48	10.38	10.38	8.78	10.93
	E	12.23	11.52	11.10	10.67^{b}	10.68	11.37	10.98	8.73	10.91
	SEM	0.62	0.43	0.31	0.30	0.35	0.11	0.41	0.21	0.22
	P-value[4]	0.6874	0.5029	0.3564	0.0269	0.5466	0.0004	0.2908	0.2329	0.2129
20	F	13.28	12.62	12.00	12.23	12.10	11.73^{ab}	10.03	10.68^{a}	11.84
	M	12.63	12.23	12.05	12.03	11.60	11.13^{b}	9.18	10.37^{ab}	11.40
	E	13.82	12.40	11.80	12.02	11.98	12.17^{a}	9.98	10.33^{b}	11.81
	SEM	0.50	0.25	0.15	0.32	0.25	0.29	0.39	0.21	0.26
	P-value[4]	0.0418	0.3832	0.5108	0.8496	0.3760	0.0385	0.2042	0.0413	0.1769
30	F	19.40	17.78	15.13	12.85	13.20	12.20	12.08	13.73^{a}	14.55
	M	16.00	14.55	13.62	13.43	12.90	12.00	11.73	13.13^{b}	13.42
	E	19.10	16.33	14.03	13.82	13.37	12.43	12.15	13.30^{ab}	14.32
	SEM	1.28	0.76	0.31	0.38	0.33	0.48	0.24	0.18	0.40
	P-value[4]	0.1567	0.0635	0.0226	0.1543	0.2211	0.7180	0.4739	0.0205	0.1072
40	F	9.40	10.67	12.77	12.22	14.18	11.03^{b}	12.27	16.05	12.32
	M	9.75	10.42	13.42	14.13	14.18	13.00^{a}	14.73	16.00	13.20
	E	10.65	11.22	14.10	15.35	14.23	12.58^{ab}	13.33	16.42	13.49
	SEM	0.71	0.52	0.53	0.83	0.66	0.66	0.71	0.33	0.36
	P-value	0.3508	0.5093	0.1954	0.0930	0.9981	0.0390	0.1224	0.6481	0.0596
50	F	9.13	8.72	8.20	8.30	8.53	7.65	8.92	10.30	8.72
	M	7.63	8.03	8.38	9.00	8.70	8.00	8.70	10.08	8.57
	E	8.67	8.92	8.83	9.53	9.20	7.92	9.00	10.20	9.03
	SEM	0.93	0.37	0.33	0.53	0.26	0.37	0.36	0.19	0.23
	P-value	0.4903	0.2023	0.4249	0.3241	0.1657	0.5338	0.8345	0.4826	0.1501
70	F	9.60	9.20	7.05	7.18	6.37	6.48	8.05	10.00	7.99
	M	7.45	7.65	6.87	7.85	6.37	6.03	8.08	10.28	7.57
	E	8.82	9.07	7.97	9.85	7.20	5.88	8.55	10.33	8.46
	SEM	1.44	1.10	0.74	1.14	0.40	0.37	0.97	0.64	0.42
	P-value	0.4836	0.2922	0.3715	0.2986	0.3122	0.4769	0.9220	0.8586	0.1782
Pan	F	3.57	5.85	8.08	8.75	9.85^{b}	9.45^{b}	9.35	10.97	8.23
	M	5.18	6.45	8.67	10.15	9.77^{b}	10.20^{a}	9.67	10.87	8.87
	E	5.20	6.53	9.03	9.60	10.32^{a}	10.18^{a}	9.32	11.10	8.98
	SEM	0.58	0.28	0.41	0.71	0.23	0.16	0.62	0.38	0.34
	P-value	0.1539	0.2484	0.1947	0.4235	0.0080	0.0241	0.9090	0.7078	0.2504

[a-b] Numerical values without the same superscript letter within a specific sieve size column are significantly different ($P<0.05$).

[1] Loc = Location on the feed chain; F, M, E = Front, middle, or end of the feed chain; SEM = Pooled standard error of the means.

[2] Particle size fractions: Sieve 6 (>3,360 µm), 8 (3,360-2,380 µm), 12 (2,380-1,680 µm), 16 (1,680-1,190 µm), 20 (1,190-840 µm), 30 (840-590 µm), 40 (590-420 µm), 50 (420-297µm), 70 (297-210 µm), and pan (< 210 µm).

[3] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

[4] If $P<0.05$, but no subscripts are shown, then the interaction between treatment and location is significant and effect of treatment could not be analyzed individually.

higher at the M location, which would lead to the observed lower proportion of smaller, high protein ingredients (such as soybean meal) and crystalline AAs at the M of the feed trough. However, no other AAs were significantly different by location along the feed trough, despite numerical trends. There were no significant interactions between dietary treatment and location along the feed trough for any of these nutrients **(Appendix C.3)**.

Table 2.8. Mean nutrient values for feed samples collected from the feed trough by location along the feed trough (as is basis)

	Dietary nutrient (%) [1]						
Location	DM	CP	Lys	Met	Cys	M + C	Thr
F	90.36	20.71^{ab}	1.05^{ab}	0.44	0.40	0.84	0.78
M	90.47	20.43^{b}	1.03^{b}	0.43	0.39	0.83	0.77
E	90.61	20.99^{a}	1.07^{a}	0.45	0.40	0.85	0.79
SEM [2]	0.08	0.17	0.01	0.01	0.00	0.01	0.01
P-value	0.0777	0.0489	0.0497	0.6139	0.1597	0.2568	0.1022

[a- b] If numerical values do not have the same superscript letter within a column, then they are significantly different ($P<0.05$).

[1] DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; Thr = threonine.

[2] Pooled standard error of the means.

Monthly Hen Performance Data

Hen body weights and egg quality data were collected monthly in order to determine if differences in these parameters occurred due to either dietary treatment or location on the feed trough. **Appendix D.1** demonstrates several significant interactions between treatment diet and location during individual months and overall for hen body weight and yolk color; therefore, effect of treatment and location cannot be considered individually for these parameters at those times. Though these interactions were significant, they were mainly the result of high or low values at a single location on the feed trough within a single treatment, at various time periods.

Monthly results for body weight and egg parameters by dietary treatment can be seen in **Table 2.9**. No statistically significant differences were measured between dietary treatments for hen body weight or any egg quality parameters, indicating that lowering dietary CP to the levels used in this study, with supplementation of required levels of AAs, did not reduce hen performance. A number of studies have similarly found little to no difference in various production parameters for hens fed reduced CP, AA balanced diets compared to those fed higher levels of CP. Harms and Russell (1993) found similar production performance, including egg weight, for heat stressed hens fed 13% versus 15.5% CP or 15% versus 17.6% CP, AA supplemented diets for two different studies, respectively. Another study by Kesharvarz and

Table 2.9. Means of monthly production parameters by dietary treatment

		Month								
	Diet[1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr[2]
Body weight (Kg)	A	1.48	1.54	1.52	1.57	1.55	1.58	1.61	1.56	1.55
	B	1.50	1.55	1.55	1.59	1.58	1.63	1.63	1.58	1.58
	C	1.52	1.56	1.56	1.58	1.58	1.61	1.63	1.60	1.58
	SEM	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.01
	P-value	0.2755	0.8931	0.4099	0.7795	0.6252	0.3664	0.7684	0.4654	0.1441
Egg weight (g)	A	53.91	---	60.89	60.57	---	61.21	---	63.55	60.02
	B	53.94	---	60.77	61.63	---	61.77	---	64.07	60.48
	C	54.44	---	61.39	61.31	---	61.63	---	63.68	60.51
	SEM	0.39	---	0.51	0.40	---	0.51	---	0.65	0.30
	P-value	0.6077	---	0.6968	0.3101	---	0.7414	---	0.8472	0.5140
Albumen height (mm)	A	8.89	---	9.70	9.75	---	8.43	---	8.14	8.98
	B	8.78	---	9.89	9.72	---	8.57	---	8.37	9.09
	C	8.77	---	9.82	9.75	---	8.61	---	7.80	8.95
	SEM	0.10	---	0.13	0.13	---	0.31	---	0.14	0.07
	P-value	0.6794	---	0.6174	0.9784	---	0.9115	---	0.1437	0.4627
Haugh Unit	A	95.42	---	97.40	97.84	---	91.09	---	88.68	94.05
	B	94.83	---	98.20	96.83	---	91.21	---	89.82	94.30
	C	94.65	---	97.88	97.23	---	91.39	---	86.75	93.57
	SEM	0.50	---	0.61	0.91	---	1.40	---	0.84	0.39
	P-value	0.5862	---	0.6835	0.7527	---	0.9890	---	0.1699	0.4909
Yolk Color (Roche scale)	A	8.13	---	7.64	7.64	---	7.82	---	7.81	7.81
	B	8.06	---	7.58	7.91	---	7.92	---	7.79	7.85
	C	7.86	---	7.47	7.68	---	7.94	---	7.71	7.73
	SEM	0.12	---	0.12	0.10	---	0.11	---	0.06	0.06
	P-value	0.3822	---	0.6170	0.2371	---	0.7215	---	0.5028	0.4045
Shell Strength (g force at failure)	A	---	4805.94	4531.84	---	4148.98	---	3994.29	---	4370.26
	B	---	4832.63	4362.71	---	3948.77	---	4142.57	---	4322.78
	C	---	4842.64	4378.53	---	3925.12	---	3999.69	---	4302.00
	SEM	---	64.29	75.44	---	109.82	---	62.23	---	60.99
	P-value	---	0.9189	0.3478	---	0.3982	---	0.3028	---	0.7426
Shell Thickness (mm)	A	---	0.37	0.37	---	0.38	---	0.36	---	0.37
	B	---	0.37	0.36	---	0.38	---	0.37	---	0.37
	C	---	0.37	0.31	---	0.38	---	0.37	---	0.36
	SEM	---	0.00	0.04	---	0.00	---	0.00	---	0.01
	P-value	---	0.8409	0.4944	---	0.6796	---	0.0605	---	0.6982

[1] A = Low CP level diet; B = Intermediate CP level diet; C = High CP level diet; SEM = Pooled standard error of the means; P-value<0.05 considered statistically significant.

[2] OVR = Overall mean values and statistics for each parameter over the eight month data collection period.

Austic (2004) reported that body weight, hen-day egg production, egg weight, egg mass, feed intake, and feed conversion could all be maintained by hens fed a 13% CP diet supplemented with Met, Lys, and tryptophan (Trp) to their required levels compared to those fed ~16% CP from 36-48 wks of age. Meluzzi et al. (2001) also demonstrated that performance could be maintained by laying hens fed low (13.6 and 15.3%) CP diets supplemented with adequate levels of Lys, Met, M+C, Trp, and Thr, compared to control diets of 17.1% CP, for a period of 8 weeks; however, egg production and egg mass were shown to decline after this time. A recent study by Yakout et al. (2006a) demonstrated that, when CP in laying hen diets was reduced to 15%, with supplemental Lys, Met, and Thr, egg production and egg mass could be maintained compared to hens fed a 19% CP diet from 24-36 weeks of age. Additional findings from this study for the same birds from 38-50 weeks of age found that providing only 13% CP, with supplemental Lys, Met, Thr, and Trp, was not a feasible option, as this diet was far less cost effective than the higher CP diets in this study, based on the lower levels of production observed for this diet compared to the others (Yakout et al., 2006b).

Conversely, other investigations have shown lower performance of hens fed reduced CP diets compared to those fed higher levels of dietary CP. Calderon and Jenson (1990) reported that 32-64 wk old laying hens fed 13% CP diets had reduced body weight, egg production, egg weight, egg mass, feed intake, and feed efficiency compared to hens fed the 16 and 19% CP diets, despite adequate methionine supplementation in the lower CP diet. Another study by Penz and Jensen (1991), which assessed the effect of reduced CP diets on performance of growing pullets and laying hens, similarly found lower body weight gain, egg weight, and also decreased egg albumen content for hens fed a 13% CP diet versus those fed ≥16% CP diets despite adequate supplementation of Met, Lys, Trp or glycine and glutamic acid. These studies were formulated to meet the National Research Council (NRC) (1984) AA requirements, which have since been revised in 1994. However, a number of recent studies following the NRC (1994) requirements for essential AAs have also found reduced performance for hens fed reduced CP, AA supplemented diets. Ferguson et al. (1998) found that feed intake and feed:gain ratio were increased for broilers fed a 196 g/kg versus a 215 g/kg CP diet. Another study by Roberts et al. (2007) reported lower egg production, egg mass, and feed utilization when hens were fed ~19% versus an ~20% CP diets. These studies indicate that genetic improvements in poultry performance require greater nutrient density than outlined by NRC (1994) requirements.

The effect of hen cage location on the feed trough on hen body weight and egg quality was also investigated. This effect was considered due to the observation of a significantly greater proportion of large feed particles (>2,380 µm), consisting mainly of high carbohydrate and gross energy, low protein ingredients accumulating at the M location along the feed trough compared to the F and/or E (**Table 2.7**), which led to significantly lower analyzed values of percent CP and Lys at the M compared to the E of the feed trough (**Table 2.8**). Mean monthly hen body weight and egg quality by location on the feed trough can be seen in **Table 2.10**.

Hen body weight was numerically lower at the M location than at the F and/or E of the feed trough for six out of the eight months of the trial, though this observation was not found to be significant overall. Egg weight was similarly found to be numerically lower at the M of the feed trough than at the F and/or E for three out of five months that egg weight was measured; however, this finding was again not significant. Albumen height and Haugh units tended to be greater at the M location on the feed trough than at the F and/or E (P=0.0041 and P=0.0007, respectively) and shell strength was also numerically greater at the M location for all four months that shell strength was measured, though this occurrence was not found to be significant. Yolk color and shell thickness did not differ by location on the feed trough.

Table 2.10. Means of production parameters by location on the feed trough [3]

		Month								
	Location[1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
Body Weight (Kg)	F	1.51	1.58^{a}	1.55	1.59	1.57	1.60	1.62	1.58	1.57
	M	1.48	1.52^{b}	1.52	1.57	1.56	1.61	1.61	1.58	1.56
	E	1.51	1.56^{ab}	1.56	1.58	1.58	1.61	1.63	1.59	1.58
	SEM	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01
	P-value	0.2489	0.0320	0.2966	0.7794	0.6054	0.9495	0.7971	0.9331	0.0566
Egg Weight (g)	F	53.91	---	61.55	61.23	---	62.21	---	63.77^{ab}	60.60
	M	54.20	---	60.77	60.80	---	60.65	---	62.96^{b}	59.86
	E	54.17	---	60.73	61.50	---	61.76	---	64.56^{a}	60.56
	SEM	0.39	---	0.51	0.40	---	0.48	---	0.52	0.27
	P-value	0.8487	---	0.4471	0.4536	---	0.0564	---	0.0361	0.0658
Albumen Height (mm)	F	8.81	---	9.60	9.56^{b}	---	8.43	---	7.88	8.86^{b}
	M	8.76	---	9.94	10.00^{a}	---	8.74	---	8.34	9.17^{a}
	E	8.86	---	9.87	9.67^{ab}	---	8.43	---	8.10	8.99^{ab}
	SEM	0.10	---	0.13	0.13	---	0.21	---	0.14	0.07
	P-value	0.7655	---	0.1464	0.0496	---	0.1839	---	0.0883	0.0041
Haugh Unit	F	94.96	---	97.00	95.99	---	90.05	---	87.13^{b}	93.00^{b}
	M	94.73	---	98.36	98.93	---	92.77	---	90.03^{a}	95.06^{a}
	E	95.21	---	98.13	96.98	---	90.86	---	88.08^{ab}	93.85^{ab}
	SEM	0.50	---	0.61	0.91	---	1.07	---	0.82	0.38
	P-value	0.7821	---	0.2460	0.0715	---	0.0696	---	0.0430	0.0007
Yolk Color (Roche scale)	F	8.15	---	7.59^{ab}	7.67	---	7.88	---	7.79	7.81
	M	7.96	---	7.43^{b}	7.77	---	7.92	---	7.74	7.77
	E	7.93	---	7.67^{a}	7.79	---	7.89	---	7.78	7.81
	SEM	0.09	---	0.08	0.08	---	0.07	---	0.06	0.04
	P-value	0.0586	---	0.0183	0.4628	---	0.5878	---	0.8199	0.4386
Shell Strength (g force at failure)	F	---	4836.13	4381.64	---	4003.13	---	4050.36	---	4318.92
	M	---	4857.54	4547.09	---	4091.58	---	4064.60	---	4399.14
	E	---	4787.54	4344.36	---	3928.15	---	4021.59	---	4276.97
	SEM	---	64.29	70.20	---	87.53	---	62.23	---	46.89
	P-value	---	0.7328	0.0791	---	0.3065	---	0.8834	---	0.0714
Shell Thickness (mm)	F	---	0.37	0.36	---	0.38	---	0.37	---	0.37
	M	---	0.37	0.37	---	0.38	---	0.37	---	0.37
	E	---	0.37	0.31	---	0.38	---	0.37	---	0.36
	SEM	---	0.00	0.03	---	0.00	---	0.00	---	0.01
	P-value	---	0.4337	0.3568	---	0.9056	---	0.9303	---	0.3169

[a-b] Numerical values without the same superscript letter within a month and parameter are significantly different ($P<0.05$).

[1] F = Front of the feed chain; M = Middle of the feed chain; E = End of the feed chain; SEM = Pooled standard error of the means; P = P-value ($P<0.05$ considered statistically significant).

[2] OVR = Overall mean values and statistics for each parameter over the eight month data collection period.

A higher prevalence of larger feed particles than smaller feed particles was also observed at the M than at the F and E of the feed trough, as seen in **Table 2.7**. This led to a concentration of larger, high carbohydrate and gross energy, low protein feed ingredients and subsequent lower quantities of CP and Lys at the M compared to the E, as reported in **Table 2.8**. The lower proportion of CP and Lys detected at the M location on the feed trough could have led to the numerically lower hen body weights and egg weights observed at the M compared to the F and E of the feed trough. Significantly greater albumen height and Haugh units and numerically greater egg shell strength observed at the M location compared to the F and E could have resulted from a reduced rate of lay and/or the numerically lower egg weights observed at the M of the feed trough due to lower quantities dietary CP AAs at this location, as other studies have reported lower egg weight and production to be correlated with greater egg shell quality and Haugh units, respectively (Calderon and Jensen, 1990; Patterson et al., 1988).

Differences in production parameters were observed between the M and the F and/or E of the feed trough in this study. However, Tang et al. (2006) conversely found a linear reduction in performance parameters, including albumen height, Haugh units, and eggshell thickness, from the F to E of the feed trough, which the authors associated with a decrease in small particle size nutrients along the feed trough.

Production parameter differences detected at the M location along the feed trough could have resulted from differences in particle size distribution between these locations in the current study, or they may be explained by the influence of external environmental factors. The possibility of an environmental difference between the F and E versus the M location along the feed trough exists because both the F and E of the feed trough are located at the front of the hen house and the M of the feed trough is positioned at the back, as illustrated in **Figure 2.1**. These locations within the hen house could have experienced different temperatures, degrees of ventilation, and environmental disturbances, such as higher human traffic at the front of the house or the influence of the insect zapper at the back of the house.

Daily high/low temperature measurements were recorded at four locations within the house, as illustrated in **Figure 2.2**. Location 2 was nearest to where F and E hen body weight and egg quality data was gathered and location 4 was nearest to where M data was collected.

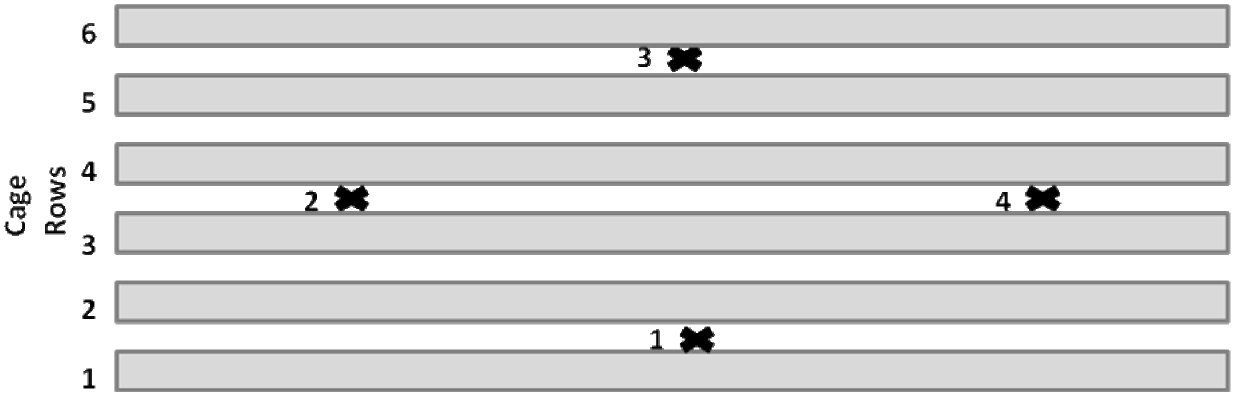

Figure 2.2. Four locations of daily high/low temperature measurements.

It can be seen in **Table 2.11** that the highest mean high/low temperatures for the entire trial were found at location 4, which could have led to the lower production performance observed at the M location along the feed trough.

Table 2.11. Mean daily high/low temperatures at four locations within the hen house

	Temperature (°F)	
Location [1]	High	Low
1	85.45	77.42
2	85.29	76.26
3	85.04	77.27
4	87.05	78.65

[1] Locations of temperature measurements illustrated in Figure 2.2.

Weekly Hen Performance Data

In addition to once per month replicated data collection, weekly production data was collected by personnel at the research facility for each dietary treatment. Though this data was non-replicated and thus could not be analyzed statistically, it was useful to examine to ensure that hens followed normal, commercial patterns and to document any trends associated with dietary treatments that may have occurred over time.

Two parameters of interest were weekly body weights and cumulative hen mortality, which are shown by dietary treatment in **Figure 2.3**. Mean hen body weight measurements taken at these weekly intervals were consistently lower for hens fed the low CP diet (A) compared to hens fed the other two treatment diets. Furthermore, this numerical trend was observed for the overall mean body weights for the entire trial at 3.39, 3.47, and 3.47 lbs for diets A, B, and C, respectively. Lower hen body weights were also observed among the replicated data for the low CP diet compared to the other two treatment diets consistently for all eight months of the trial, as seen in **Table 2.7**. It is likely that numerically lower body weights were the result of the reduced level of CP in the low CP diet, as differences in body weight were not observed until hens had been consuming the treatment diets for several weeks (about 21 weeks of age). Other studies have found similar reductions in body weight and body weight gain for both broilers and laying hens fed reduced CP, AA balanced diets (Calderon and Jenson, 1990; Penz and Jensen, 1991; Keshavarz and Jackson, 1992). Conversely, other work has shown that body weight and body

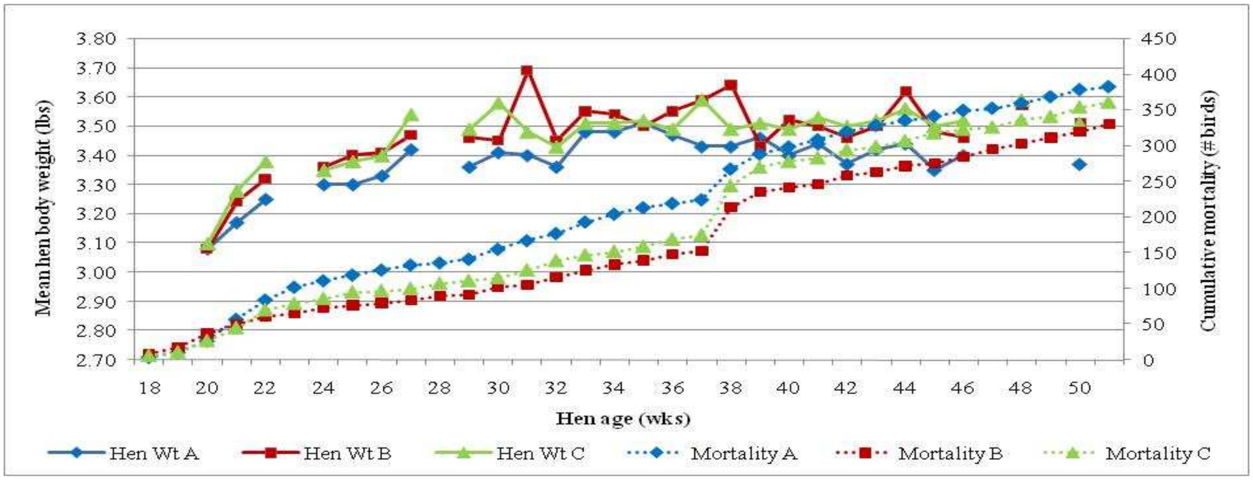

Figure 2.3. Weekly mean hen body weights and cumulative hen mortality by dietary treatment.

weight gain can be maintained when hens are fed reduced CP, AA supplemented diets (Meluzzi et al., 2001; Yakout et al., 2006a; Roberts et al., 2007a).

Weekly cumulative hen mortality was consistently higher for hens fed the low CP diet (A) and lowest for the intermediate CP diet (B). This observation is supported by the total number of mortalities over the length of the trial, which were 382, 361, and 330 birds for diets A, C, and B, respectively. As hen mortality did not vary in a linear fashion with dietary CP level, and since hen nutrient requirements were met in all treatment diets, the observed numerical pattern in hen mortality is most likely due to random chance.

Weekly hen water and feed consumption by dietary treatment are shown in **Figure 2.4**. Both water and feed consumption increased from their starting levels at 18 weeks of age and then leveled off in a manner similar to that of a normal commercial flock. Neither of these parameters differed by dietary treatment. This observation is reflected by the similarity of the overall means across all dietary treatments for the entire trial, which were 4.35, 4.37, and 4.42 gallons/100 birds/day for water consumption and 21.91, 21.94, and 22.25 lbs/100 birds/day for feed consumption for diets A, B, and C, respectively. These observations suggest that level of dietary

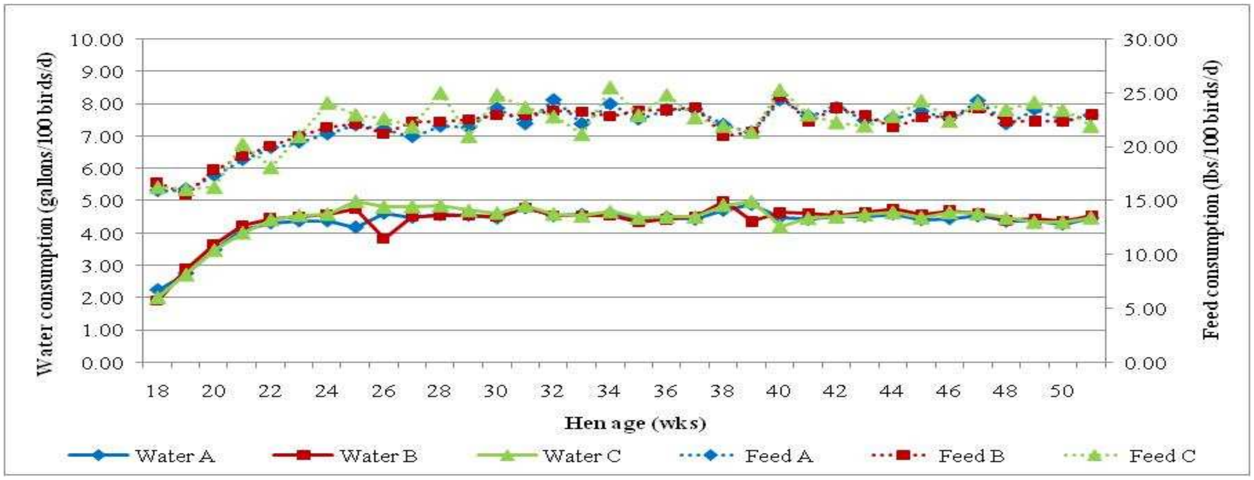

Figure 2.4. Weekly hen water and feed consumption by dietary treatment.

CP does not impact hen water or feed consumption. Several previous studies have similarly observed no change in feed consumption when CP was reduced in hen diets and required levels of AAs were supplemented (Kesharvarz and Austic, 2004; Roberts et al., 2007a). However, increased feed intake has been reported for broilers fed reduced CP, AA supplemented diets (Ferguson et al., 1998).

Weekly percent hen-day egg production and cumulative eggs per hen housed by dietary treatment are shown in **Figure 2.5**. Percent hen-day egg production increased and leveled off in a manner similar to that of a normal commercial flock. Peak hen-day egg production was exceptional for all dietary treatments and occurred at 27 weeks of age, with production at 96.94, 96.68, and 96.68% for diets A, B, and C, respectively, compared to a normal commercial peak egg production rate of 92-95% for this breed of hen (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). Cumulative eggs per hen housed increased over time with above average numbers for 51 week old hens, averaging 205 eggs across all dietary treatments for the study herein, compared with 195.1 cumulative eggs for this hen under commercial conditions (Lohmann LSL Lite Layer

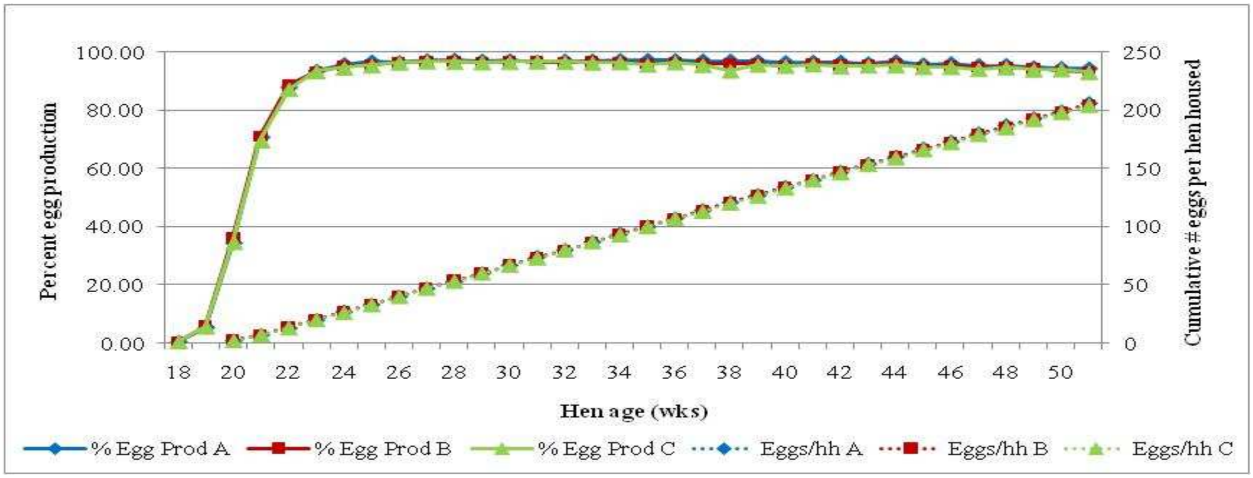

Figure 2.5. Weekly percentage hen-day egg production and cumulative number of eggs per hen housed by dietary treatment.

Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). These two parameters did not differ by dietary treatment, which is reflected by the similarity of the overall means for the entire trial. Egg production averaged 87.86, 87.39, and 87.08% and egg numbers per hen housed were 206, 205, and 204 for diets A, B, and C, respectively, at 51 wks of age. Egg production in several previous works was similarly maintained for hens fed reduced CP, AA balanced diets (Penz and Jensen, 1991; Keshavarz and Jackson, 1992; Meluzzi et al., 2001; Yakout et al., 2006a). However, egg production has also been reported to be lower when hens are fed reduced CP diets, despite adequate AA supplementation (Roberts et al., 2007).

Mean weekly egg case weight and egg mass by dietary treatment are shown in **Figure 2.6**. Case weight increased numerically in a linear fashion from the lowest to highest CP diets, with overall means of 46.11, 46.49, and 47.07 lbs for diets A, B, and C, respectively, indicating the weights for the high CP diet to be nearly a pound greater than the low CP diet on average. Hens fed the high CP diet reached the 45 lb (large egg size) case weight benchmark at 24 weeks of age, whereas one additional week was required for hens fed the lower CP diets to achieve this. Mean egg mass increased to an above average level compared to a normal commercial flock, averaging 58.0 g across all dietary treatments in this study, compared to 55.7 g for a commercial flock at 51 weeks of age (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). As with mean egg case weight, mean egg mass for the 33 week study also increased numerically in a linear manner from the lowest to highest CP diets, with overall means of 56.13, 56.28, and 56.84 g/hen/day for diets A, B, and C, respectively.

These non-replicated patterns in egg mass and case weight by dietary treatment are strikingly similar to the replicated egg weight data collected monthly, which showed the low CP diet (A) to have numerically lower egg weights for four out of the five months that egg weight was measured during the eight month trial. Though the observed pattern for the low CP diet was not found to be significant in the monthly replicated data, when this is considered with the numerical trends for weekly egg mass and case weight by dietary treatment, these results suggest that lower levels of CP may lead to lower egg weights on a commercial scale.

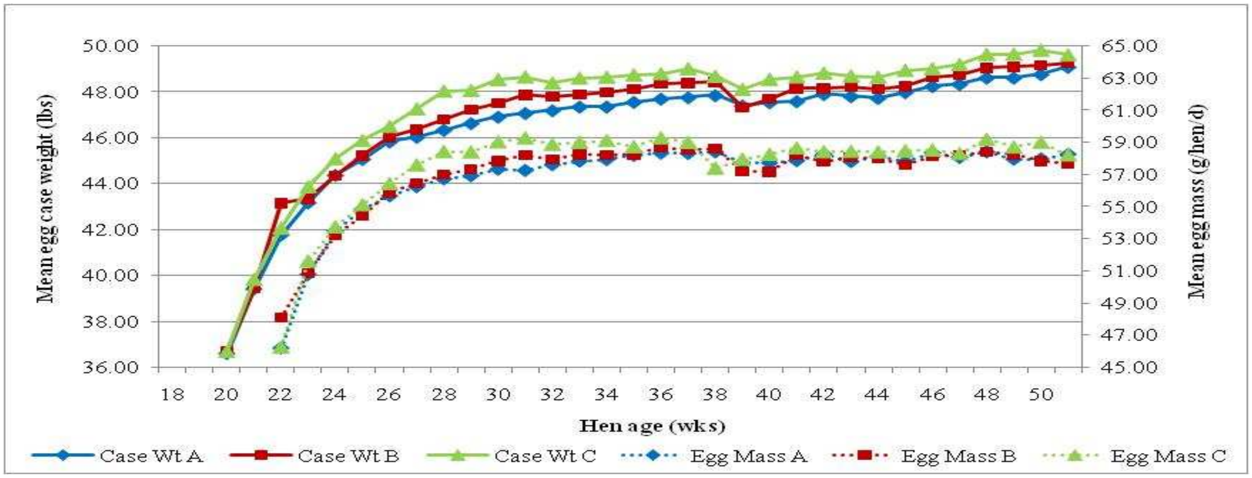

Figure 2.6. Mean weekly egg case weight and egg mass by dietary treatment.

These numerical trends for egg weight and/or mass with lower CP diets fed in this study are similar to the results of a number of past laboratory scale studies, which observed reduced egg weights when CP in hen diets was reduced by more than 0.5%, despite dietary supplementation with adequate levels of essential AAs (Keshavarz, 1984; Penz and Jensen, 1991; Keshavarz and Jackson, 1992; Keshavarz and Nakjima, 1995; Novak et al., 2006). However, maintenance of egg weight and/or mass has also been reported when hens were fed diets with CP levels reduced by one percent, with AA supplementation, on a small scale basis (Keshavarz and Jackson, 1992; Roberts et al., 2007).

Mean weekly pounds of feed per pound of egg mass and pounds of feed per dozen eggs by dietary treatment are presented in **Figure 2.7**. Both of these measures of feed conversion decreased early in lay then leveled off in a manner similar to normal commercial flocks. Neither of these parameters appeared to differ greatly as a result of dietary treatment. This observation is supported by the overall means, which were 1.92, 1.91, and 1.92 lb feed/lb egg and 2.94, 2.95, and 3.00 lb feed/dozen eggs for diets A, B, and C, respectively. This would indicate that level of dietary CP did not influence hen feed conversion. These findings are supported by previous studies, which similarly found no change in feed conversion when hens were fed a reduced level of dietary CP, with supplemental AAs (Keshavarz and Austic, 2004; Yakout et al., 2006b). However, increased rates of feed conversion have also been observed for hens fed lower CP diets, despite supplementation with required levels of essential AAs (Calderon and Jensen, 1990; Ferguson et al., 1998; Roberts et al. 2007a).

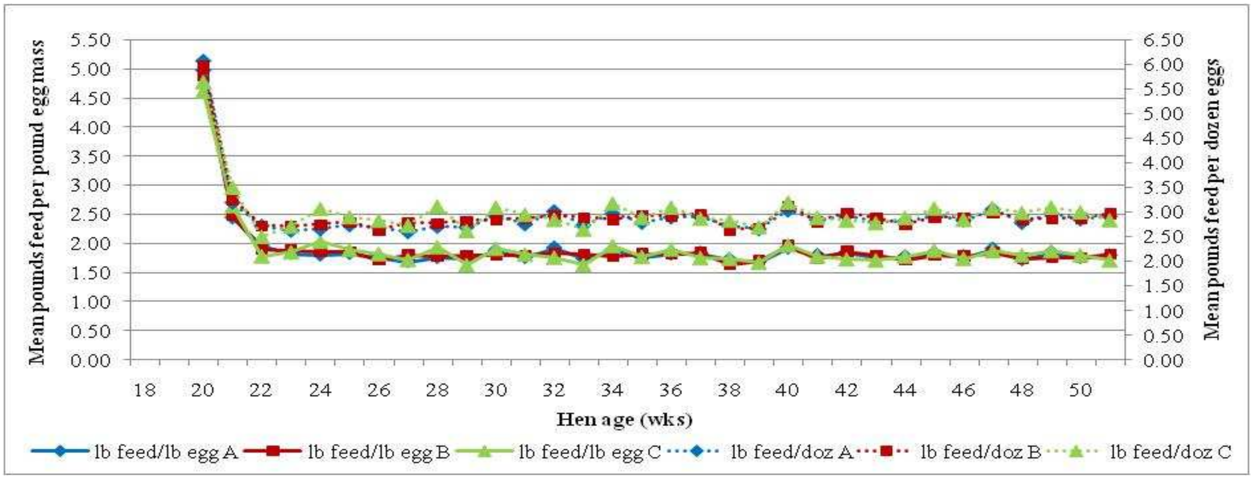

Figure 2.7. Mean weekly pounds of feed per pound of egg mass and mean pounds of feed per dozen eggs by dietary treatment.

Weekly, non-replicated data in this study indicated egg weight was lower for reduced CP diets. Though this numerical pattern was not found to be statistically significant, the marketability of lower weight eggs produced by hens fed the lower CP diets is still brought into question. In order to further assess this critical egg weight threshold, egg grade out reports, summarized weekly by R.W. Sauder's Eggs processing plant, were reviewed. Since the most numerous, marketable eggs produced by commercial flocks are large grade A eggs, weekly percentages were assessed and reported by dietary treatment in **Figure 2.8**.

Interestingly, numerically higher percentages of large grade A eggs were observed for the lowest to highest CP diets (A, B, and C, respectively). These observations are further verified by the overall means for the entire study at 51.58, 49.86, and 45.14% large grade A eggs from hens fed diets A, B, and C, respectively.

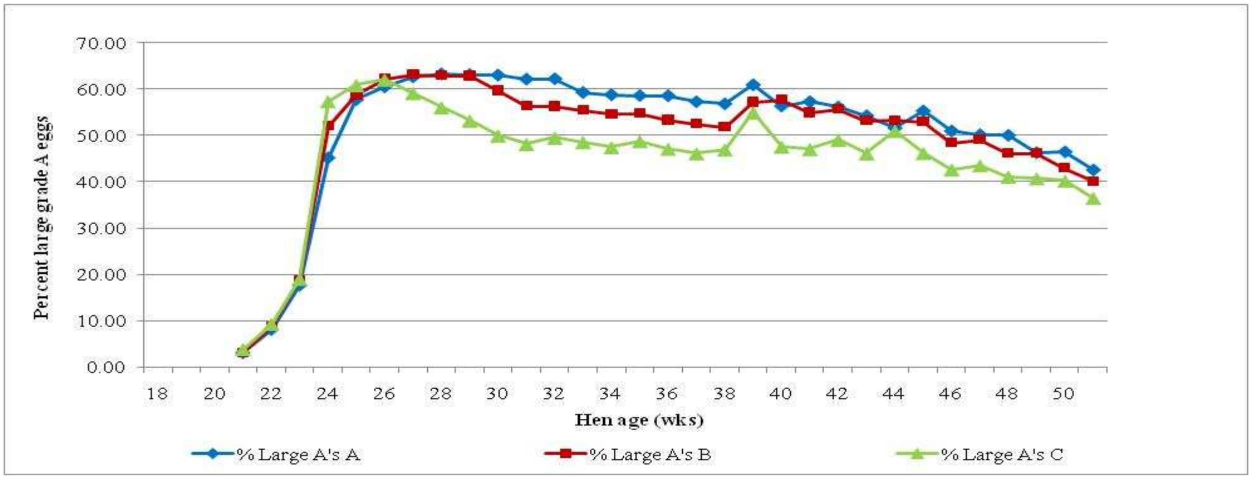

Figure 2.8. Weekly percentage large grade A eggs by dietary treatment.

Hens fed the higher CP diets produced a lesser percentage of large eggs since they were generating a greater proportion of extra large and jumbo eggs. This can explain why numerically lower egg weights were observed for hens fed the reduced CP diets. Marketable, large, grade A eggs produced by these birds were higher than normally observed for commercial flocks, with egg weight averaging 60.34 g in this study across all dietary treatments (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). It can be seen in **Appendix D.2**, that the weekly percentages of extra large and jumbo eggs did indeed increase numerically from the lowest to highest CP diets (A, B, and C, respectively). This observation is further supported by the overall means for the entire trial, which were 22.55, 25.77, and 31.93% for extra large eggs and 2.03, 2.31, and 3.48% for jumbo eggs for hens fed diets A, B, and C, respectively.

Additional egg grade out data is given in **Appendix D.3**, which shows weekly percent grade B and cracked eggs, and **Appendix D.4**, which illustrates percent dirty and loss eggs. Percentage of grade B eggs did not appear to differ by dietary treatment. However, the percentage of cracked, dirty, and loss eggs were all numerically greatest for the high CP diet. These findings correspond with the overall means for the entire trial: cracked eggs were 1.47, 1.51, and 1.68%; dirty eggs were 0.78, 0.76, and 0.87%; and loss eggs were 0.88, 0.85, and 0.92% for diets A, B, and C, respectively. These parameters combined are called “under-grades” and amount to 3.13, 3.12, and 3.47% for eggs produced by hens fed diets A, B, and C, respectively. These observations provide further evidence that hens fed higher CP diets produce larger eggs, since these eggs can potentially have thinner shells, which can break more easily, leading to higher percentages of cracked and loss eggs. Larger egg size also provides greater surface area for the collection of fecal material at oviposition, leading to a higher rate of contamination and corresponding larger percentage of dirty eggs from hens fed higher CP diets.

Economic Analysis

Finally, an economic analysis was performed to evaluate whether lowering CP in diets would be profitable. **Table 2.12** shows mean egg income, feed cost, and egg income minus feed cost for the entire trial.

Table 2.12. Mean weekly egg income, feed cost, and egg income minus feed cost per hen housed by dietary treatment

Diet[1]	Egg income ($/week)	Feed cost ($/week)	Egg income – feed cost ($/week)
A	0.4748	0.2064	0.2684
B	0.4770	0.2077	0.2693
C	0.4772	0.2147	0.2625

[1] A = Low CP level diet; B = Intermediate CP level diet; C = High CP level diet.

Weekly egg income per hen housed ($/week) was distinctly lower for the low CP diet (A), compared to the two higher CP diets, with mean weekly egg income per hen housed for diet A being $0.0022 and $0.0024 less than for diets B and C, respectively. This finding may reflect the lower egg weights observed from hens fed diet A in comparison to eggs produced by hens fed the other two diets. Weekly feed cost per hen housed ($/week) increased from the lowest to highest CP diets (A, B, and C, respectively), with average costs for diets A and B being $0.0083 and $0.0070 less than for diet C, respectively. This is most likely due to larger quantities of expensive protein ingredients in the higher CP diets. Interestingly, weekly egg income minus feed cost was higher for the low and intermediate CP diets (by $0.0059 and $0.0068, respectively) compared with the high CP diet (C). Kesharvarz and Austic (2004) conversely found that when CP was reduced from 16% to 13% in laying hen diets, feed costs increased $141/ton, which was associated with the cost of the five crystalline essential AAs used to supplement the lower CP diet at the time this study was conducted. As crystalline AAs have become increasingly available and affordable over the last several years, reducing dietary CP has been shown to be an economically beneficial feeding strategy. Yakout et al. (2006a) demonstrated that reducing CP in laying hen diets from 19% to 15% had the potential to save the poultry industry as much as $11/ton of feed.

Though weekly feed costs and egg income minus feed costs in the current study only differ between dietary treatments by fractions of a cent on a per hen basis, when these results are considered for a flock of 100,000 birds, weekly feed costs for diets A and B would be $830 and

$700 less than diet C, respectively. Weekly egg income minus feed cost for diets A and B would be $590 and $680 more than for diet C, respectively. This would mean a yearly farm revenue increase of $30,680 for diet A and $35,360 for diet B compared to the control, diet C, containing a commercial level of dietary CP. This indicates that lowering CP below levels that are currently fed in normal hen diets would be a profitable alternative feeding strategy for both producers and integrators on a commercial scale.

CHAPTER 3

EFFECT OF REDUCED CRUDE PROTEIN, AMINO ACID BALANCED DIETS ON MANURE NITROGEN AND AMMONIA EMISSION IN A LARGE-SCALE COMMERCIAL LAYING HEN FLOCK

SUMMARY

Laying hens were recently estimated to be the highest producers of ammonia gas (NH_3) emissions of all other domestic species surveyed by the United States Environmental Protection Agency (EPA, 2004). Consequently, a number of policies have been put in place in recent years regulating quantities of NH_3 both within and released from hen houses in order to protect bird, human, and environmental health. One potential dietary strategy for lowering NH_3 emissions is reducing costly crude protein (CP) levels in diets along with supplementation of limiting amino acids (AAs). This concept was investigated on a commercial scale in this study, based on the promising results of numerous university-scale studies. The objective of this investigation was to determine if isocaloric, reduced CP, AA balanced laying hen diets could reduce nitrogen (N) excretion and/or NH_3 emissions from a commercial laying hen flock.

A total of 50,760 Lohmann LSL Lite laying hens were divided into three groups that were each fed diets containing different levels of CP and supplemented with limiting AAs to their required levels. Hens were housed in a high-rise facility with a deep-pit manure storage system. Each diet was fed to two out of the six total cage rows. Diets were corn-soybean meal based and least-cost formulated weekly based on current ingredient prices and nutrient concentration. Hens were fed these diets ad libitum from 18 to 51 weeks of age (January 20,

2008-September 7, 2008). Diet C was a control diet formulated with a high level of CP and was meant to represent a typical commercial laying hen diet. Diets B and A were formulated to contain intermediate and low levels of CP, respectively.

Weekly diet formulations were examined to establish if diets were isocaloric and AA balanced while also achieving the CP differences between diets. All other data and samples were collected once every four weeks at the front (F), middle (M), and end (E) of the feed trough for each cage row. Feed samples were collected for CP, AA, and particle size analyses. A small-scale flux chamber was used to measure manure NH_3 flux. Manure samples were analyzed to determine manure percent dry matter (DM), total N, ammonium (NH_4^+) N, organic N, phosphate (P_2O_5), and potash (K_2O). A 3 x 3 factorial analysis was performed using the PROC MIXED procedure of SAS© software version 9.1 to detect differences between measured parameters by diet (A, B, and C), location on the feed trough (F, M, and E), and their interaction. Mean comparisons were made using Tukey’s procedure.

Examination of weekly diet formulations revealed that, overall, diets were isocaloric and AA balanced and that diets B and A were formulated to contain 0.85 and 1.40% less CP than diet C, respectively. Analyzed feed samples collected from within cage rows proved the diets to be AA balanced; however, overall, diets B and A averaged 1.53% and 1.98% less CP than diet C, respectively; quite different than formulated CP differences between diets.

Feed particle size analysis showed that the high CP diet consistently contained a numerically smaller proportion of large particles (2380-3360 µm) and larger proportion of small particles (840-1190 µm and 420-590 µm) than the low CP diets throughout the eight month trial; however, these results were not statistically significant. Additionally, feed collected at the M location along the feed trough was made up of a greater proportion of large particles (>2380 µm) than feed collected from the F and/or E locations ($P<0.05$).

Manure percent DM, total N, (NH_4^+) N, organic N, and P_2O_5 did not differ by dietary treatment; however, manure K_2O was numerically lowest for the low CP diet and highest for the high CP diet consistently for seven out of the eight months of the trial. Additionally, manure percent DM, (NH_4^+) N, organic N, P_2O_5, and K_2O did not differ by location along the feed trough; however, manure total N was significantly higher at the M than at the F location. Ammonia flux increased each sampling period until it peaked at 97.41 mg/cm^2/min in May and then decreased until rising again in September; however, ammonia flux did not differ by diet or location on along the feed trough. There were no significant interactions overall between diet and location on the feed trough for any manure components or NH_3 flux.

This investigation demonstrated that reducing CP in the diets of a commercial laying hen flock to the levels used in this study, with supplementation of limiting AAs to their required levels, did not significantly influence ammonia flux or manure composition. These results indicate that this dietary strategy may not be the most effective for the reduction of hen N excretion and NH_3 emissions on a large scale basis.

DESCRIPTION OF THE PROBLEM

It is known that poultry produce large quantities of NH_3 emissions that can lead to odor problems, increased fuel costs for ventilation, decreased bird health and production, negative impacts on human health, lower quantities of nitrogen (N) in manure, resulting in a lower quality fertilizer, higher quantities of N deposition in the adjacent environment, and increased eutrophication of surface water (Blake and Hess, 2001). Poultry, specifically chickens and turkeys, were estimated by the United States Environmental Protection Agency (EPA) to have produced 27% of all NH_3 emissions in 2002, indicating them to be the highest producers of NH_3 emissions of all domesticated species surveyed in the United States, including dairy, beef, sheep, goats, horses, and swine (EPA, 2004; Roberts et al., 2007b).

A number of polices have been put in place in recent years in attempts to reduce NH_3 emissions both within and released from hen houses in order to combat their negative impacts. The United Egg Producers Animal Husbandry Guidelines for U.S. Egg Laying Flocks: 2006 Edition recommends that, ideally, birds should be exposed to less than 10 ppm and never more than 25 ppm of NH_3 within hen houses (United Egg Producers, 2006). The Occupational Safety and Health Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH) have also both set limits of 50 ppm and 25 ppm, respectively, for human exposure to NH_3 over an eight hour day (Roberts et al., 2007b). The U.S. EPA also published a final ruling in December, 2008, which exempted animal waste emissions from being reported under section 103 of the Comprehensive Environmental Response, Compensation Liability Act (CERCLA), but maintained the requirement for NH_3 emissions from large concentrated animal feeding operations (CAFOs) exceeding 100 lbs in a 24 hour period to be reported under section 104 of the Emergency Planning and Community Right to Know Act (EPCRA) (EPA, 2008). Additionally, operations were required to comply with this policy by reporting NH_3 emissions beyond these levels by January 20, 2009. It has been reported in both laboratory and commercial scale studies performed with broilers, pullets, and laying hens that 25-75% of N consumed by these birds is either lost or excreted, mainly as a result of daily protein turnover in the body, including AA catabolism (Parsons, 1995; Patterson and Lorenz 1996; Patterson and Lorenz, 1997; Patterson et al., 1998; Meluzzi et al., 2001; Nahm, 2007). When AAs are consumed beyond their required levels, they are subsequently deaminated and excreted, thus contributing N to the manure (Parsons, 1995; Nahm, 2007). Therefore, large amounts of N are inherently available in poultry manure for microbial synthesis of NH_3. Patterson and Lorenz (1996) reported, in a mass balance study involving eight commercial flocks of 85,849 to 128,193 laying hens, that ~40% of total feed N was lost into the atmosphere. Because there is this direct relationship between feed N consumption, manure N content, and N lost into the atmosphere, dietary manipulations can be used to reduce N excretion and/or NH_3 emissions.

One dietary strategy for lowering poultry NH_3 emissions that has been of particular interest in recent years involves reducing dietary levels of CP with simultaneous supplementation of limiting AAs. Poultry diets can be formulated in this manner as they do not require a specific amount of CP in their diets, but instead require definitive quantities of specific AAs. Over the past several years, dietary CP levels have been gradually reduced in poultry diets, in combination with supplementation of crystalline limiting AAs, which have become increasingly available and affordable (Meluzzi et al., 2001; Yakout et al., 2006b). However, even current commercial corn-soybean meal based, AA balanced diets fed to poultry still contain many AAs in surplus of their requirements, which continue to contribute to N excretion into manure (Roberts et al., 2007b).

The eventual goal is to determine the ideal quantities of CP and limiting AAs to be supplied in poultry diets that will maintain bird performance while also reducing AAs provided in excess of their requirements.

Several university scale studies have been successful at using reduced CP, AA balanced diets to lower poultry N excretion and/or NH_3 emissions. Meluzzi et al. (2001) reported a consistent linear decrease in manure N as dietary CP content was decreased, where N excreted was approximately 50% of dietary N intake for all levels of CP. Roberts et al. (2007b) showed a 10% decrease in N excretion for laying hens fed diets containing 1% less CP than typically fed in commercial diets; however, reducing dietary CP in this study did not result in a decrease in NH_3 emissions and hens fed the reduced CP diets had lower egg production and egg mass. Ferguson et al. (1998) also found that, when CP in broiler diets was decreased from 215 g/kg to 196 g/kg, N excretion was reduced by 16.5% and, additionally, equilibrium NH_3 gas concentration was reduced by 31%; however, in this case, the lower CP diet resulted in increased feed intake and feed conversion.

Based on the promising results of these laboratory scale experiments, this study sought to determine if reduced CP, AA balanced diets could lower N excretion and/or NH_3 emissions in large-scale commercial facilities while maintaining hen production performance. The effects of dietary treatments on hen N excretion and/or NH_3 emissions are presented here, with hen performance data presented separately in the previous chapter.

MATERIALS AND METHODS

Birds and Housing

A total of 50,760 Lohmann LSL Lite laying hens were obtained from a commercial pullet farm at 18 weeks of age (hatch date 09-12-07) and placed into an environmentally controlled, mechanically ventilated high-rise house that employed a deep-pit manure storage system. The birds were housed in six rows of 8,460 birds each. Two rows of birds were assigned randomly to each of the three dietary treatments used in this study. Hens were housed in 61 x 51 cm^2 cages with 7-8 hens per cage (density 389-444 cm^2/bird). Experimental diets were provided to each of the treatment groups from their date of arrival at the research facility at 18 wks of age until 51 wks of age.

All lighting and management practices aside from dietary alterations specified by dietary treatments were in accordance with current recommendations for the breed (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany) and guidelines from Wenger's Feed Mill, Inc. (Rheems, PA). Photoperiod was progressively increased to 15 hr light: 9 hr dark at 25 weeks of age and maintained on this schedule throughout the rest of the trial. The house temperature was kept at ~25˚C throughout the trial. The Pennsylvania State University Institutional Animal Care and Use Committee approved all techniques and procedures involved in animal care and handling (IACUC #27579).

Experimental Diets

Hens were divided into three treatment groups, which were fed low (diet A), intermediate (diet B), or high (diet C) CP diets, all isocaloric and supplemented with Lys, Met, and/or Thr to their required levels. The high CP diet represented a typical commercially fed laying hen diet. All diets were formulated by Wenger's Feed Mill, Inc. Diets were primarily corn and soybean meal based and also included poultry by-product meal, dried distillers grains with solubles (DDGS), canola meal, a blended fat, bakery by-product meal, and wheat middlings. A commercial type phase feeding program was used and diets were least-cost formulated weekly based on current ingredient prices and nutrient concentration. **Table 2.1** displays the high and low values for each of the major feed ingredients providing energy and protein in each of the dietary treatments throughout the study. Corn and soybean meal are shown to be the predominate feed ingredients, with corn averaging 52.37, 51.08, and 49.14% and soybean meal averaging 10.02, 11.59, and 17.39% for diets A, B, and C, respectively, over the eight months of the trial. Feed and water were provided ad libitum, with feed allocated daily into multiple feedings.

Data Collection

Diet Formulations. Formulated levels of CP ("as is" basis), metabolizable energy (ME), and digestible lysine (Lys), methionine (Met), methionine + cysteine (M+C), and threonine (Thr) for treatment diets were provided weekly by Wenger's Feed Mill, Inc.

Feed Sample Collection. Feed samples were collected at the front (F), middle (M), and end (E) of the feed trough from each cage row before the morning feeding, once every four weeks (eight total sampling dates from February to September), resulting in a total of six replicate feed samples for each treatment diet at each sampling date. Sampling locations are illustrated in **Figure 2.1**. One representative feed sample for each dietary treatment was collected directly from the feed mill and another was collected from the hoppers at the beginning of each cage row by pooling equal amounts of feed from the two hoppers for each diet. Feed trough samples were collected by vacuuming all feed from the trough beneath two adjacent cages at each sampling location. Feed samples were then transported back to The Pennsylvania State University and segregated into portions to be sent for AA analysis or used for feed particle size analysis.

Feed Sample Segregation. Feed samples were segregated in 100 g portions to be sent for AA analysis and 200 g quantities for feed particle size analysis using the procedure described by Tang (2004), in order to assure consistent and even distribution of feed particle sizes in all samples to be analyzed. Briefly, feed samples were poured onto a flat surface so that feed formed a cone shape with equal distribution of feed around the cone. The cone of feed was then flattened into a disk shape using a spatula. The disk of feed was then divided into four equal portions and two of these portions, diagonal from one another, were selected at random and discarded. This procedure was repeated until the desired sample sizes were acquired. Segregated feed samples were then double-bagged to prevent moisture loss and were stored at -20°C until they were either sent for AA analysis or used for feed particle size analysis.

Feed Composition Analysis. The 100 g feed samples were sent to the Evonik-Degussa Corporation (Kennesaw, GA) for AA analysis to obtain accurate measurements of dry matter (DM), CP, Lys, Met, Met + Cysteine (M+C) or total sulfur AA (TSAA), and Thr for all treatment diets.

Feed Particle Size Analysis. Feed samples were separated into ten fractions, based on particle size, using an AS 200 sieve shaker (Retsch, Inc., Newtown, PA) and a series of nine U.S.A. Standard Testing sieves. The 200 g feed samples stored at -20°C were poured onto a flat plate, covered with plastic wrap to prevent moisture loss, and allowed to defrost for 12 to 24 hours. A $2^{½}$ sieve series with openings between 3,360 and 210 μm was used for this analysis, meaning that the width of the openings of each successive sieve was 1.414 times larger than that of the previous sieve in the series (Tang, 2004). The diameter of each sieve was 203.2 mm. The nine sieves were stacked from top to bottom in the following order: sieve number 6 (3360 µm openings), 8 (2380 µm), 12 (1680 µm), 16 (1190 µm),20 (840 µm), 30 (590 µm), 40 (420 µm), 50 (297 µm), and 70 (210 µm). A pan of the same diameter as the sieves was placed beneath sieve number 70 to capture all fine particles less than 210 µm. Feed particles from the top two sieves (numbers 6 and 8) were collected throughout the sieving process and 5 g of these particles were added to each of sieve numbers 30, 40, 50, and 70 before each feed sample was shaken in order to reduce adhering of fine particles to these smaller opening sieves. A 200 g feed sample was then placed in the top sieve (number 6) and the stack of sieves was shaken for 10 minutes at an amplitude of 2.50 mm/”g”. Particles from each sieve and the bottom pan were then weighed. The quantity of feed in each sieve or pan was then divided by the total amount of feed remaining in all sieves and the pan and this number was multiplied by 100, resulting in the percentage of total feed made up by each particle size fraction. The particle size fractions of feed were divided into the following ten fractions sizes >3360 µm, 3360-2380 µm, 2380-1680 µm, 1680-1190 µm, 1190-840 µm, 840-590 µm, 590-420 µm, 420-297 µm, 297-210 µm, and < 210 µm.

Manure Sample Collection. Approximately 200 g manure samples were collected in the pit of the hen house directly under the F, M, and E locations on the feed trough from each cage row, every four weeks (eight total sampling dates from February to September), resulting in a total of six replicate manure samples for each treatment diet at each sampling date. The manure was sampled from the surface of the pile, to a depth of no greater than 10 cm, at the same location the NH_3 flux measurements were made.

Manure Sample Analysis. The Agricultural Analytical Services Lab at the Pennsylvania State University analyzed all manure samples for manure percent DM, total N, (NH_4^+) N, organic N, P_2O_5, and K_2O. Extensive descriptions of the methods used by the Agricultural Analytical Services Lab are described by Peters et al. (2003). Brief descriptions are below.

- ***Manure Percent DM.*** Samples were dried in heat stabile containers at 110˚C for 6-24 hours, depending on sample size. Manure percent DM was calculated with **Equation 3.1**:

$$\text{Percent DM} = \frac{[(\text{dried sample} + \text{container weight}) - (\text{container weight})]}{[(\text{un-dried sample} + \text{container weight}) - (\text{container weight})]} \times 100 \qquad \textbf{(3.1)}$$

- ***Manure Total N.*** The Dumas method of flash combustion was used to analyze manure samples for total N content. Samples were ignited in an induction furnace at 950-1350˚C with oxygen (O_2) and helium (both 99.996% purity) used as carrier gases. Samples of the resultant combustion gases were passed through a Cu catalyst to remove O_2 and change nitrous oxides into nitrogen gas (N_2). Magnesium perchlorate and ascarite scrubbers acted to remove carbon dioxide (CO_2) and moisture. Manure total N content could then be detected using a thermal conductivity cell.
- ***Manure (NH_4^+) N.*** Manure (NH_4^+) N content was determined using an ion specific electrode. A strong base was added to both manure samples and standards to raise the pH above 11. This causes all dissolved ammonia (both $NH_{3\,(aq)}$ and NH_4^+) to be converted to solely $NH_{3\,(aq)}$. The $NH_{3\,(aq)}$ concentration could then be measured using an NH_3-specific electrode.
- ***Manure P_2O_5 and K_2O.*** P_2O_5 and K_2O in manure samples were determined by microwave-assisted acid digestion. Using microwave heating, up to 0.5 g of the dried manure samples were digested in 10 mL of concentrated nitric acid within a capped

fluorocarbon microwave vessel. After the acid-manure sample mixtures were cooled, they were allowed to settle, centrifuged or filtered, and then diluted. Samples were then analyzed for P_2O_5 and K_2O content using either inductively coupled plasma emission spectrophotometry (ICP-AES) or atomic absorption spectrophotometry (AAS). Wet weight concentrations of manure P_2O_5 and K_2O were calculated using **Equation 3.2** below:

$$\text{Wet weight concentration (ug/g)} = \frac{\text{ICP-AES or AAS result (ug/mL) x final volume (mL)}}{\text{Sample size (g)}} \qquad \textbf{(3.2)}$$

Ammonia Flux. An airflow recirculation portable flux chamber (chamber volume = 0.02707 m^3) was used to measure hen manure NH_3 flux. Readings were taken at a representative location on the surface of the manure piles corresponding with F, M, and E locations along the feed trough for each cage row, every four weeks (eight total sampling dates from February to September), resulting in a total of six replicate measurements for each treatment diet at each sampling date. Ammonia flux measurements were made at the same locations as subsequent manure sample collections. Flux measurements were made prior to manure sample collection so the manure surface was undisturbed for measurement of NH_3 flux, as disturbing the surface of the manure can encourage additional NH_3 release. Ammonia concentration (ppm) was measured using two varieties of Dräger tubes for short term NH_3 measurement, with measuring ranges of 2.5-100 ppm and 5-700 ppm, respectively (Dräger Safety AG & Co. KGaA, Luebeck, Germany). After the flux chamber was placed onto the surface of the manure (surface area covered by the chamber = 0.06701 m^2), three serial measurements of NH_3 concentration within the chamber were taken at time zero, two minutes, and four minutes. Ammonia flux was then calculated from these readings using a series of calculations outlined by Wheeler et al. (2008). First NH_3 concentration (ppm) was converted to mg/m^3 using **Equation 3.3**:

$$NH_3 \text{ concentration (mg/m}^3\text{)} = [NH_3 \text{ concentration (ppm)}] \times 0.7 \tag{3.3}$$

Ammonia emission or uptake was then calculated by examining the increase or decrease of measured NH_3 concentration over time, respectively. Ammonia concentration measured at time zero, two minutes, and four minutes will hereafter be represented as C_0, C_2 and C_4, respectively. If NH_3 concentration in the headspace of the chamber was shown to increase over time ($C_0 < C_2 < C_4$) non-linearly, such that $[(C_2 – C_0)/(C_4 – C_2)] > 1$, then NH_3 flux was a positive value (NH_3 was emitted from manure) and was calculated using **Equation 3.4** below, where V was the volume of the chamber (m^3), A was the surface area covered by the chamber (m^2), C was NH_3 concentration (mg/m^3), and t was the duration of time between C_0 and C_2 (Hutchinson and Mosier, 1981):

$$NH_3 \text{ flux (mg/m}^2\text{/min)} = (V/A) \times [(C_2 – C_0)^2]/\{[2(C_2) – C_4 – C_0]t\} \times \ln[(C_2 – C_0)/(C_4 – C_2)] \tag{3.4}$$

If NH_3 concentration in the headspace of the chamber was shown to decrease over time ($C_0 > C_2 > C_4$) non-linearly, again such that $[(C_2 – C_0)/(C_4 – C_2)] > 1$, then NH_3 flux was also calculated using **Equation 3.4**, however, in this case NH_3 flux was a negative value (NH_3 was taken up by manure). If NH_3 concentration either increased or decreased linearly over time, such that $[(C_2 – C_0)/(C_4 – C_2)] \leq 1$, then NH_3 flux was calculated with **Equation 3.5** below, where $\Delta C/\Delta t$ was the average rate of change of NH_3 concentration between $(C_2 – C_0)$ and $(C_4 – C_2)$:

$$NH_3 \text{ flux (mg/m}^2\text{/min)} = (V/A) \times (\Delta C/\Delta t) \tag{3.5}$$

Statistical Analysis

A 3 x 3 two factor factorial statistical analysis was performed to detect any significant differences in measured parameters by dietary treatment (A, B, and C), location on the feed trough (F, M, and E), or an interaction of both, for each monthly sampling date and overall. Study parameters that were evaluated included feed AA composition, feed particle size, manure percent DM, total N, (NH_4^+) N, P_2O_5, K_2O, and NH_3 flux. Significant differences in feed AA composition between diet formulations (formulation), feed samples collected at the feed mill (mill), feed samples collected from the hopper at the front of each cage row in the hen house (hopper), and feed samples collected at locations within each feed trough in the hen house (trough) were determined using a one-way ANOVA. Significant differences in feed particle size between mill, hopper, and location samples were also evaluated using a one-way ANOVA. Data analysis was done using the PROC MIXED procedure of SAS (SAS Institute, 2003, Version 9.1 ed., SAS Inst. Inc., Cary, NC). Mean comparisons were made using Tukey's procedure (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Feed Composition

Selected nutrients of each dietary treatment are shown in **Table 2.2** to summarize the average diet formulations provided to hens during each month of the trial. Overall, diets B and C were formulated to differ by a mean value of 0.85% CP and diets A and C by a mean value of 1.40% CP. A list of all weekly dietary formulations and selected critical nutrients are provided in **Appendix A.1**, **A.2**, and **A.3**.

Treatment diets were formulated to be isocaloric, with nearly equal metabolizable energy (ME) in all three diets **(Table 2.2)**. Diets were also formulated to be AA balanced, as shown by the nearly equal concentrations for all diets, with the exception of Thr being higher for the high CP diet (C) than for the other two diets. This was due to the higher CP content of diet C, which inherently contains a higher percentage of Thr.

In order to determine if the formulated nutrient values were achieved in the actual diets provided to the hens, feed composition was determined for samples taken from the feed mill directly (mill), from the hoppers at the front of each cage row (hopper), and from F, M, and E locations along the feed trough within each cage row (trough). Analyzed values of selected nutrients in feed samples from the mill and hoppers are presented in **Appendix B.1** and **Appendix B.2**, respectively. Nutrients from feed samples collected at the F, M, and E locations along the feed trough, which best represent nutrients being consumed by the birds, were pooled for all three locations along the feed trough and reported by dietary treatment in **Table 2.3**.

For feed sampled from the mill, hoppers, and trough, analyzed values of CP and AAs revealed that diets A, B, and C had the lowest to highest percent CP and AAs were nearly equal for all diets, again with the exception of higher values of Thr for diet C. DM values, not shown in the formulation means of **Table 2.2**, were also nearly equal for all diets from the mill, hoppers, and trough. **Table 2.3** demonstrates that diets B and C differed by a mean value of 1.53% CP and diets A and C by a mean value of 1.98% CP, much different than the 0.85 and 1.40% CP differences in formulation.

Nutrients (DM, Lys, Met, Cys, and M+C) delivered to the hens differed very little between dietary treatments **(Table 2.4)**. However, percent Thr was shown to be significantly greater in diet C than in diets A and B. The higher Thr levels in diet C are the result of the higher CP content of this diet. This trend is not of particular concern, however, as treatment diets in this study were formulated to meet the Thr requirements of the hen. As a result, dietary levels of Thr shown in **Table 2.4** satisfied both the NRC (1994) guideline of 0.47% dietary Thr and the current recommendation for the breed of 0.64% dietary Thr (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany) for hens consuming 100 g of feed per day.

Mean percent CP in diet C was significantly greater than in diets A and B; however, though diet B was numerically greater than diet A, mean percent CP did not differ significantly between diets A and B for these samples taken from the feed troughs. The distinctly higher level of CP observed in diet C, compared to the other two diets, may have led to more dramatic differences in the production parameters measured in this study between diet C versus diets A and B. However, feed trough CP and AA levels, shown in **Table 2.4**, all exceeded both the NRC (1994) requirements and current recommendations for the breed (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany), in even the low CP diet. For example, the recommended percent CP for laying hens, consuming 100 g of feed per hen per day, is 15.0% according to NRC (1994) requirements and 18.80% according to current recommendations for the breed (Lohmann LSL Lite Layer Management Guide 0501/E, 2005, Lohmann Tierzucht GmbH Veterinary Laboratory, Cuxhaven, Germany). In the present study, percent CP was analyzed to be 19.90% for the low CP diet from feed trough samples. With CP and AA levels well beyond the hens' nutrient requirements for growth and production, it is possible that treatment differences could have been masked.

Next, the change in dietary nutrients progressing from the formulated diets, to the mill, hopper, and feed trough was considered. Interestingly, **Table 2.5** demonstrates that all nutrients were significantly lower for the formulated diets and increased linearly at the feed mill, hopper, and feed trough locations, respectively. Lower AA values in formulation are due to their being presented on a digestible basis in formulation, rather than on an "as is" basis for feed samples analyzed from the feed mill, hopper, and feed trough. Only percent dry matter (DM) differed significantly between feed mill and hopper samples. Higher DM values from the feed mill to the hopper and feed trough may have been the result of increasing feed desiccation during transport and distribution to the birds.

Lower values of CP and AAs in feed mill and hopper samples compared to those collected from the feed trough may have resulted from a combination of sampling technique and bird selection of certain particle sizes in feed. Tang et al. (2006) reported that when poultry mash feed is distributed by a drag chain, which was the system used in the current study, it has the tendency to segregate such that fine and dense feed particles percolate toward the bottom and center of the feed trough, and less dense particles rise to the top of the feed and accumulate towards the sides of the feed trough, thus encouraging hen selection of large-particle size ingredients, such as corn, which mainly contribute carbohydrates, gross energy, and nitrogen-free extract to the birds. As feed samples were collected from the feed trough one to two hours after feed had been distributed, this would have given the birds time to select certain particles sizes of feed over others. If the birds had already consumed these large-particle size ingredients when samples were collected, this would have led to a concentrating of smaller ingredients (such as soybean meal), crystalline AAs, vitamins and minerals, etc., which would have resulted in the higher values of CP and AAs for the feed trough samples compared to the feed mill and hopper.

Additionally, feed mill samples were collected by personnel at Wenger's Feed Mill, Inc., so collection method for these samples is not clear and samples from the hoppers were collected at the surface of the feed, as this was the only accessible location for feed collection. Conversely, feed trough samples were gathered very uniformly such that all feed at each sampling location was completely collected. Sample collection from the feed surface in the hoppers may have led to a higher proportion of large feed particles in these samples, as it is the nature of smaller, higher density particles to segregate towards the bottom of the feed. It is possible that samples from the feed mill were collected similarly, from the surface of a feed storage bin, which would have led to a similar accumulation of larger particles in these samples. These methods of sample collection could have led to a concentrating of high energy, low protein ingredients in feed mill and hopper samples compared to feed trough samples, adding to the discrepancy in nutrient values between these sampling locations.

Feed Particle Size

In order to determine if variation in nutrient composition between feed mill, hopper, and feed trough samples was occurring due to differences in feed particle size distribution, an analysis of feed particle size composition of diets was performed for samples collected from these locations. It can be seen in **Table 2.6** that feed particle size of diets was indeed significantly different between sampling locations for most fractions of particle sizes. Percentage of total feed in sieves 6, 8, and 12 (>1,680 µm) tended to be largest to smallest for feed mill, hopper, and cage row feed trough samples, respectively (P=<0.0001). Additionally, sieves 20, 30, 40, 50, and 70 (210-1190 µm) all contained smaller percentages of total feed for feed mill and hopper samples than for the feed trough samples, (P=<0.0001). The proportion of total feed in sieve 16 (1,190-1,680 µm) and the pan below the sieves (<210 µm) did not differ significantly by sampling location (P=0.0875 and P=0.0624, respectively). These results are similar to those reported by Tang et al. (2006), which found that percentage of total feed particles <1,180 µm were significantly higher (P<0.05) at the feed trough (61.7%) than at the hopper (45.8%) when a drag chain feed delivery system was employed, which they reported to be due to hen consumption of a large portion of feed particles >1,180 µm.

It was also brought into question whether treatment diet or location along the feed trough had any influence on feed particle size. In order to assess this, a 3 x 3 factorial statistical analysis was performed to look for effects of treatment diet, location on the feed trough, or an interaction of both. **Appendix C.1** summarizes several significant interactions between treatment diet and location, therefore, the effect of treatment and location cannot be considered individually for those feed fractions at those times. Although several interactions were significant during individual months, they were mainly the result of high or low values at a single location on the feed trough within a single treatment. Also, no significant or repeatable trends were observed overall for the entire trial.

Appendix C.2 shows that treatment diet appeared to have some influence on feed particle size in feed trough samples. The high CP diet was found to contain a numerically lesser proportion of large particles from 2,380-3,360 µm (sieve 8) and a numerically greater proportion of small particles from 840-1,190 µm (sieve 20) and 420-590 µm (sieve 40) than the two lower CP diets for seven or eight months out of the eight month trial. There were no differences between dietary treatments for any other particle size fractions. This numerical pattern for the high CP diet containing a greater proportion of small particles and a lesser proportion of large particles compared to the two lower CP diets may be due to the higher soybean meal content of the high CP diet, as soybean meal averaged 10.02, 11.59, and 17.39% for the low, intermediate, and high CP diets, respectively.

Table 2.7 demonstrates that location on the feed trough within a cage row also had an effect on feed particle size. The M location on the feed trough tended to have a greater portion of large particles, >2380 µm (sieves 6 and 8), than the F and/or E locations (P=0.0008 and P=0.0022 for sieves 6 and 8, respectively). There were no other differences by location on the feed trough for any other particle size fractions. Accumulation of large feed particles (>2380 µm) at the M location along the feed trough could have resulted from a combination of smaller sized, high protein content ingredients segregating towards the bottom of the feed trough and their subsequent accumulation there as feed travels along the trough and hen selection of feed particle sizes smaller than 2380 µm at the F location on the feed trough leading to a concentrating of particles larger than this at the M location.

Feed nutrient composition by location along the feed trough was also investigated to determine if nutrient content was impacted by particle size distribution of the feed. The results of this analysis are given in **Table 2.8**. Percent DM was shown to decrease numerically from the F to E of the feed trough, potentially due to increasing desiccation of feed as it traveled along the feed trough. Also, percent CP and Lys both tended to be lowest at the M location along the feed trough. This finding corresponds with the tendency for large feed particles (>2380 µm), mainly composed of high carbohydrate and gross energy, low protein content dietary ingredients, to be higher at the M location, which would lead to the observed lower proportion of smaller, high protein ingredients (such as soybean meal) and crystalline AAs at the M of the feed trough. However, no other AAs were significantly different by location along the feed trough, despite numerical trends. There were no significant interactions between dietary treatment and location along the feed trough for any of these nutrients **(Appendix C.3)**.

Manure Composition

Results of monthly manure sample analysis by dietary treatment are shown in **Table 3.1**. Manure percent DM, total N, (NH_4^+) N, organic N, and P_2O_5 all did not differ by dietary treatment. Manure K_2O, however, was numerically higher for the high CP diet (C) for seven out of eight months of the trial; however, this inclination was not found to be significant. Keshavarz and Austic (2004) reported lower dietary potassium concentration when CP was reduced from 16% to 13%, due to the lower soybean meal content of their lower CP diet, as soybean meal is high in potassium. Therefore, the lower average potassium levels in the manure from hens fed the reduced CP diets in the current study may be due to the lower quantity of soybean meal in these lower CP diets, as soybean meal was the predominate dietary protein ingredient provided in this study **(Table 2.1)**.

The similarity of manure N composition observed in the current study across all dietary treatment containing various levels of CP is contrary to the findings of a numerous previous small scale investigations. Meluzzi et al. (2001) found that N excretion decreased linearly as CP was reduced in laying hen diets. Other studies have more specifically reported that N excretion in both poultry and swine can be lowered 8.5 to 10% for each percentage point that feed N is reduced (Schutte et al., 1993; Kerr, 1995; Roberts et al., 2007b).

Table 3.1. Mean manure nutrient concentrations by dietary treatment (wet weight basis)

		Month								
	Diet [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
% DM	A	33.40	34.73	47.25	48.55	51.77	51.37	54.43	57.45	47.37
	B	38.13	41.77	48.20	45.53	52.70	46.90	55.30	56.60	48.14
	C	29.77	35.58	45.17	44.12	54.63	53.87	56.27	59.58	47.37
	SEM	2.08	2.27	3.53	4.12	4.35	5.82	4.42	3.77	2.38
	P	0.1391	0.2010	0.8336	0.7592	0.8968	0.7200	0.9584	0.8543	0.9661
Total N (lb/ton)	A	43.61	41.44	56.51	54.08	48.34	41.89	59.13	51.12	49.52
	B	47.14	54.05	62.63	54.82	46.01	40.27	54.82	60.97	52.59
	C	39.97	40.81	59.89	58.91	54.53	52.03	55.49	57.25	52.36
	SEM	3.31	3.20	7.44	5.51	7.71	7.49	9.24	6.76	4.58
	P	0.4199	0.1004	0.8516	0.8125	0.7446	0.5541	0.9402	0.6298	0.8750
(NH_4^+) N (lb/ton)	A	19.24	14.85	13.34	12.79	9.69	9.15	8.88	9.16	12.14
	B	15.79	14.49	14.25	12.71	9.41	9.57	9.51	10.02	11.97
	C	19.32	12.76	13.25	12.96	8.61	8.36	8.83	9.08	11.65
	SEM	0.97	0.62	0.60	0.94	0.61	0.54	0.73	0.98	0.53
	P	0.1321	0.1801	0.5076	0.9809	0.5111	0.4001	0.7800	0.7691	0.8139
Org N [3] (lb/ton)	A	24.38	26.59	43.17	41.29	38.65	32.75	50.25	41.95	37.38
	B	31.35	39.57	48.38	42.11	36.60	30.70	45.31	50.95	40.62
	C	20.65	28.05	46.64	45.95	45.93	43.66	46.66	48.17	40.71
	SEM	4.23	3.55	7.22	5.08	8.09	7.57	8.52	5.81	4.37
	P	0.3283	0.1421	0.8792	0.8004	0.7202	0.5107	0.9166	0.5915	0.8372
P_2O_5 (lb/ton)	A	33.53	34.76	44.01	47.77	47.44	50.16	53.29	79.46	48.80
	B	33.96	39.33	41.21	45.09	46.32	50.16	48.29	73.06	47.18
	C	28.32	35.59	39.05	44.31	45.65	48.54	50.32	74.91	45.84
	SEM	2.74	2.43	2.63	3.62	4.94	3.14	1.79	4.86	2.09
	P	0.3894	0.4631	0.4966	0.7932	0.9672	0.9170	0.2833	0.6693	0.6471
K_2O (lb/ton)	A	17.49	19.96^{b}	22.61	24.21	25.94	26.04	27.40	29.08	24.09
	B	21.26	25.80^{a}	24.00	24.27	29.34	25.75	28.30	30.16	26.11
	C	19.51	25.90^{a}	24.83	25.43	30.92	32.13	29.56	33.36	27.70
	SEM	1.50	1.10	1.65	2.17	2.17	2.26	1.50	2.04	0.72
	P	0.3393	0.0496	0.6688	0.9082	0.3762	0.2267	0.6365	0.4156	0.0835

[a-b] Numerical values without the same superscript letter within a month and parameter are significantly different ($P<0.05$).

[1] A = Low CP level diet; B = Intermediate CP level diet; C = High CP level diet; SEM = Pooled standard error of the means; P = P-value ($P<0.05$ considered statistically significant).

[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

[3] Org N = Organic nitrogen content of the manure sample.

The results of monthly manure sample analysis by location on the feed trough are shown in **Table 3.2**. Manure percent DM, (NH_4^+) N, organic N, P_2O_5, and K_2O did not differ by location along the feed trough. Manure total N, however, was highest at the M and lowest at the F location overall for the entire trial (P=0.0288). Additionally, there were no significant interactions between dietary treatment and location on the feed trough overall for the entire trial **(Appendix F.1)**. The observation of higher manure total N at the M location along the feed

Table 3.2. Mean manure nutrient concentrations by location along the feed trough (wet weight basis)

		Month								
	Loc [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
% DM	F	37.12	36.72	42.75	49.17	50.40	49.37	54.07	54.63	46.78
	M	29.18	37.22	49.73	43.62	56.97	52.90	57.88	57.57	48.13
	E	35.00	38.15	48.13	45.42	51.73	49.87	54.05	61.43	47.97
	SEM	2.08	2.27	3.35	2.72	4.35	3.54	2.94	3.77	1.86
	P	0.0816	0.9036	0.3503	0.1231	0.5614	0.2130	0.2902	0.4852	0.7924
Total N	F	43.86	42.10	59.18	57.22	40.42	43.38	52.10	44.18^{b}	47.80^{b}
(lb/ton)	M	40.33	47.53	62.96	54.95	56.77	47.33	60.93	62.69^{a}	54.18^{a}
	E	46.54	46.67	56.89	55.64	51.70	43.49	56.40	62.47^{a}	52.47^{ab}
	SEM	3.28	3.20	6.23	4.02	6.69	4.82	5.84	4.53	3.00
	P	0.4523	0.4801	0.7467	0.8634	0.2332	0.5124	0.1792	0.0052	0.0288
(NH_4^+) N	F	16.07^{b}	14.64	13.35	12.04	8.75	8.56	8.53	7.92^{b}	11.23
(lb/ton)	M	20.78^{a}	13.50	13.90	12.98	9.25	9.66	8.54	10.85^{a}	12.43
	E	17.50^{b}	13.96	13.59	13.44	9.71	8.86	10.16	9.49^{ab}	12.09
	SEM	0.83	0.62	0.53	0.94	0.61	0.54	0.63	0.71	0.53
	P	0.0114	0.4771	0.7495	0.5875	0.5762	0.3940	0.1516	0.0237	0.2633
Org N [3]	F	27.79	27.46	45.83	45.18	31.67	34.81	43.58	36.25^{b}	36.57
(lb/ton)	M	19.55	34.03	49.06	41.97	47.52	37.67	52.39	51.84^{a}	41.75
	E	29.04	32.71	43.30	42.20	41.99	34.63	46.24	52.98^{a}	40.39
	SEM	3.83	3.55	6.11	3.91	7.12	4.83	5.55	3.93	2.96
	P	0.2108	0.4360	0.7656	0.7396	0.2970	0.6547	0.2064	0.0055	0.1379
P_2O_5	F	35.22	36.18	37.73	46.67	41.78	46.02	48.92	72.03	45.57
(lb/ton)	M	24.99	36.67	45.66	41.49	49.64	50.44	53.38	74.79	47.13
	E	35.60	36.83	40.88	49.01	47.99	52.40	49.60	80.62	49.11
	SEM	2.74	2.42	2.63	2.56	4.17	2.67	1.68	3.62	2.09
	P	0.0565	0.9804	0.1811	0.0641	0.3551	0.2362	0.1952	0.1679	0.4867
K_2O	F	21.73	23.33	22.50	25.57	26.89	26.15^{b}	27.91	30.02	25.51
(lb/ton)	M	16.47	23.96	25.17	23.32	30.32	29.56^{a}	29.32	30.21	26.04
	E	20.06	24.37	23.76	25.02	28.99	28.21^{ab}	28.04	32.36	26.35
	SEM	1.50	1.06	1.54	1.39	2.17	1.38	0.98	1.73	0.72
	P	0.1130	0.7833	0.4870	0.1489	0.5617	0.0142	0.2425	0.5358	0.7078

[a-b] Numerical values without the same superscript letter within a month and parameter are significantly different ($P<0.05$).

[1] Loc = Location on the feed chain; F, M, E = Front, middle, or end of the feed chain; SEM = Pooled standard error of the means; P = P-value ($P<0.05$ considered statistically significant).

[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

[3] Org N = Organic nitrogen content of the manure sample.

trough contradicts the finding that dietary CP was lower at the M location in this study (P=0.0489) **(Table 2.8)**. Therefore, the reason for the observed differences in total N by location on the feed trough is not entirely clear.

Ammonia Flux

Monthly measurements of NH_3 flux are shown by dietary treatment in **Table 3.3**. Ammonia flux increased each month, starting in February, until peaking at 97.41 $mg/cm^2/min$ in May. It then decreased in June, July, and August until rising again in September. Because the manure pit was cleaned out before the start of the trial, the buildup of manure over time likely resulted in increasing manure temperature and moisture, therefore leading to the observed rise in NH_3 flux in the first four months. Increasing NH_3 flux over this time period could have also been the consequence of rising hen house temperatures, which would have encouraged NH_3 release from manure. Increased facility ventilation in the warmer months may have stabilized manure (NH_4^+) N volatilization to NH_3 by preventing a continued rise in house temperature and promoting manure desiccation. The observed numerical increase in manure percent DM over time **(Table 3.1; Table 3.2)** provides evidence of manure desiccation in these later, warmer months of the trial. Ammonia flux may have risen again in September due lower house ventilation, as air inlets tend to be open more often for increased ventilation in warmer than in cooler months.

There was a high degree of variability in NH_3 flux observed for all months, as evidenced by the large monthly pooled standard error of the means (SEM), shown in **Table 3.3** and **Table 3.4**. As a result, no differences in NH_3 flux could be detected by dietary treatment **(Table 3.3)** or location along the feed trough **(Table 3.4)**. Additionally, it can be seen in **Appendix F.1** that no interactions between dietary treatment and location along the feed trough were found to be significant for NH_3 flux.

Table 3.3. Monthly mean manure ammonia flux ($mg/cm^2/min$) by dietary treatment

	Month								
Diet [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
A	14.20	33.79	66.26	112.54	108.21	69.97	22.43	48.34	59.47
B	22.52	34.37	80.12	82.89	77.44	48.70	25.05	37.93	51.13
C	23.80	37.19	80.87	96.80	101.19	92.31	30.44	60.58	65.40
SEM	6.85	7.17	24.71	12.65	10.57	33.34	4.63	12.51	6.60
P-value	0.6129	0.9393	0.8986	0.3768	0.2455	0.6864	0.5341	0.5195	0.4192

[1] A = Low CP level diet; B = Intermediate CP level diet; C = High CP level diet; SEM = Pooled standard error of the means; (P-value<0.05 considered statistically significant).
[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

Similar to the results found in the present study, Roberts et al. (2007b) were unable to discern any differences in NH_3 emissions when CP was reduced by one percentage point in laying hen diets. On the other hand, Liang et al. (2005) noted a 10% reduction in annual NH_3 emission rates when commercial laying hen flocks were fed diets reduced by 1% CP, although these results were not found to be significant (P=0.22), which the authors proposed was due to the low number of replications in their study. Additionally, Ferguson et al. (1998) was able to detect a significant ($P<0.10$) 31% reduction in equilibrium NH_3 concentration when CP was reduced from 215 g/kg to 196 g/kg (~9%) in broiler diets in a laboratory scale study.

Table 3.4. Mean manure ammonia flux ($mg/cm^2/min$) by location on the feed trough

	Month								
Loc [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
F	29.07	41.42	80.27	96.74	107.72^a	46.09	22.97	36.47	57.59
M	16.19	29.63	70.81	88.70	57.33^b	95.04	31.34	64.18	56.65
E	15.26	34.29	76.18	106.79	121.79^a	27.30	23.61	46.20	61.75
SEM	6.85	7.04	24.71	12.65	10.35	69.84	4.12	12.51	6.60
P-value	0.3470	0.5221	0.9641	0.6227	0.0099	0.4015	0.3011	0.3485	0.8452

[a-b] If numerical values do not have the same superscript letter, then they are significantly different ($P<0.05$).
[1] Loc = Location on the feed chain; F, M, E = Front, middle, or end of the feed chain; SEM = Pooled standard error of the means.
[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

Many challenges arise with any approach used to measure manure NH_3 flux. Variability between sampling locations could result from a variety of factors, including differences in manure surface temperature and manure moisture content. Human error in using the Dräger tubes to measure NH_3 flux could also have contributed to variability in measurements. Additionally, all readings were taken from the same manure pit (as all hens in this study were housed in the same facility), which most likely had a fairly uniform NH_3 levels in the air. All these factors could have masked differences in NH_3 flux between dietary treatments and location on the feed trough.

Ammonia flux and manure composition were not found to differ significantly by level of dietary CP in the present study. These results suggest that reducing dietary CP in commercial laying hen flocks may not be an effective strategy to reduce N excretion and/or NH_3 emissions on a large scale basis.

CHAPTER 4

CONCLUSIONS AND APPLICATIONS

Three dietary treatments were least-cost formulated to be isocaloric and AA balanced, while supplying a single commercial laying hen flock with three distinct levels of CP. The high CP diet was formulated to represent a normal commercially fed laying hen diet and the intermediate and low CP diets to contain 0.85% and 1.40% less CP than the high CP diet when averaged for the entire study. Treatment diets provided to the birds in the cage rows were analyzed and shown to indeed be AA balanced; however, mean values indicated that the intermediate and low CP diets actually contained 1.53% and 1.98% less CP than the high CP diet, quite different than in formulation. Feed and water consumption did not appear to differ by dietary treatment.

The high CP diet consistently contained a numerically smaller proportion of large feed particles and larger proportion of small particles than the lower CP diets throughout the eight month trial, though this finding was not statistically significant. Feed collected at the M location along the feed trough was made up of a significantly greater proportion of large particles than feed collected from the F and/or E locations.

Numerically lower body weight and egg weight was observed for hens fed the low CP diet compared to the higher CP diets consistently throughout the trial; although these patterns were not found to be significant overall in replicated data. Hen day egg production averaged 87.9, 87.4 and 87.1% for the low, intermediate, and high CP diets, respectively, and albumen height, Haugh units, yolk color, shell strength, and shell thickness did not differ by dietary treatment. Hens fed the lowest to highest CP diets numerically had the largest to smallest percentages of large, grade A eggs and the smallest to largest percentages of extra-large and jumbo, grade A eggs, respectively, consistently throughout the study. Numerically lower hen body weights and egg weights and greater shell strength were consistently observed at the M location along the feed trough compared to the F and/or E throughout the trial, though these findings were not statistically significant. Albumen height and Haugh units overall were shown to be significantly greater at the M than at the F and/or E locations. Egg yolk color and shell thickness did not differ by location on the feed trough.

Mean weekly egg income per hen housed was shown to be lowest for the low CP diet. Also, weekly feed costs were lowest for the low CP diet and progressively increased for the intermediate and high CP diets, respectively. Egg income minus feed cost (e.g. farmer revenue) was greater for the two lower CP diets compared with the high CP commercial diet.

Manure percent DM, total N, (NH_4^+) N, organic N, and P_2O_5 did not differ by dietary treatment; however, manure K_2O was numerically less concentrated for the low CP diet and more concentrated for the high CP diet consistently throughout the trial. Manure percent DM, (NH_4^+) N, organic N, P_2O_5, and K_2O did not differ by location on the feed trough; however, overall, manure total N was significantly higher at the M, and lower at the F location. Ammonia flux did not differ by dietary treatment or location along the feed trough.

This study determined that when reduced CP, AA balanced diets were fed to a large scale commercial laying hen flock, it was possible to maintain hen production performance while reducing dietary costs and increasing farm revenue. It was also determined that this dietary strategy did not significantly influence ammonia flux or manure composition. These results indicate that although this dietary strategy may not be effective for reducing hen N excretion or

NH_3 emissions on a large scale basis, it would be economically beneficial for producers to implement in commercial laying operations.

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APPENDIX A. DIET FORMULATIONS

Appendix A.1. Diet formulations January through March [2]

Date	Diet [1]	CP	ME	Lys	Met	M+C	Thr	Market $/Ton
1/7	A	19.24	1300	0.91	0.48	0.75	0.62	248.31
	B	19.90	1300	0.91	0.47	0.75	0.62	250.51
	C	19.95	1300	0.91	0.48	0.75	0.63	254.01
1/14	A	19.41	1300	0.91	0.48	0.76	0.62	281.47
	B	20.07	1300	0.91	0.47	0.76	0.62	283.64
	C	20.11	1300	0.91	0.48	0.76	0.63	287.20
1/21	A	19.41	1300	0.91	0.48	0.76	0.62	285.75
	B	20.07	1300	0.91	0.47	0.76	0.62	287.81
	C	20.11	1300	0.91	0.48	0.76	0.63	291.90
1/28	A	19.41	1300	0.91	0.48	0.76	0.62	280.23
	B	20.07	1300	0.91	0.47	0.76	0.62	281.93
	C	20.11	1300	0.91	0.48	0.76	0.63	284.97
2/4	A	19.24	1299	0.91	0.47	0.75	0.61	254.93
	B	19.92	1299	0.91	0.47	0.75	0.62	256.93
	C	19.96	1300	0.91	0.47	0.75	0.64	261.51
2/11	A	19.24	1300	0.91	0.48	0.75	0.61	260.27
	B	19.92	1300	0.91	0.47	0.75	0.62	262.31
	C	19.94	1300	0.91	0.47	0.75	0.63	266.49
2/18	A	19.24	1306	0.91	0.48	0.75	0.61	259.27
	B	19.65	1306	0.91	0.47	0.75	0.62	261.77
	C	19.64	1305	0.91	0.47	0.75	0.63	265.36
2/25	A	18.49	1304	0.86	0.44	0.71	0.59	258.72
	B	18.99	1305	0.86	0.43	0.71	0.59	259.96
	C	18.99	1305	0.86	0.44	0.71	0.61	264.69
3/3	A	18.54	1305	0.86	0.44	0.72	0.59	266.78
	B	19.14	1305	0.86	0.44	0.72	0.59	268.23
	C	18.79	1305	0.86	0.44	0.71	0.61	271.97
3/10	A	18.38	1305	0.86	0.44	0.71	0.59	292.04
	B	18.99	1305	0.86	0.43	0.71	0.59	293.49
	C	18.66	1305	0.86	0.44	0.71	0.60	298.25
3/17	A	17.59	1305	0.82	0.42	0.68	0.56	253.69
	B	18.03	1305	0.82	0.41	0.68	0.56	254.75
	C	17.87	1306	0.82	0.42	0.68	0.58	256.27
3/24	A	17.77	1305	0.82	0.42	0.69	0.56	254.83
	B	18.21	1305	0.82	0.42	0.69	0.56	255.67
	C	18.06	1306	0.82	0.42	0.69	0.58	262.96
3/31	A	18.06	1305	0.82	0.42	0.68	0.56	280.53
	B	18.25	1305	0.82	0.41	0.68	0.56	288.80
	C	18.25	1306	0.82	0.42	0.68	0.58	288.50

[1] A = Lowest CP level diet; B = Intermediate CP level diet; C = Highest CP level diet.

[2] Date = date of diet formulation; CP = crude protein (%) (as is); ME = metabolizable energy (kcal/lb); Lys = digestible lysine (%); Met = digestible methionine (%); M+C = digestible methionine + cysteine (%); Cys = digestible cysteine (%); Thr = digestible threonine (%); Market $/ton = market price per ton of feed.

Appendix A.2. Diet formulations April through June [2]

Date	Diet [1]	CP	ME	Lys	Met	M+C	Thr	Market $/Ton
4/7	A	18.07	1305	0.94	0.44	0.77	0.66	260.33
	B	18.65	1305	0.82	0.41	0.68	0.56	261.74
	C	19.94	1306	0.82	0.39	0.68	0.61	266.62
4/14	A	17.38	1300	0.78	0.39	0.64	0.53	288.91
	B	17.77	1300	0.78	0.38	0.64	0.53	289.90
	C	19.27	1300	0.78	0.36	0.64	0.59	295.18
4/21	A	17.40	1300	0.78	0.39	0.64	0.53	291.72
	B	17.82	1300	0.78	0.38	0.64	0.53	292.89
	C	19.21	1300	0.78	0.37	0.64	0.58	298.16
4/28	A	17.58	1301	0.90	0.42	0.74	0.63	256.15
	B	17.55	1300	0.90	0.42	0.74	0.63	256.69
	C	19.20	1301	0.91	0.40	0.74	0.70	260.52
5/5	A	17.60	1301	0.90	0.42	0.74	0.64	253.54
	B	17.87	1300	0.90	0.42	0.74	0.63	253.96
	C	19.25	1300	0.91	0.40	0.74	0.70	257.95
5/12	A	17.26	1296	0.88	0.39	0.71	0.62	252.32
	B	17.24	1295	0.87	0.39	0.70	0.62	253.00
	C	19.07	1295	0.89	0.37	0.71	0.70	257.96
5/19	A	17.26	1296	0.88	0.39	0.71	0.62	247.27
	B	17.24	1295	0.87	0.39	0.70	0.62	248.32
	C	19.07	1295	0.89	0.37	0.71	0.70	253.34
5/26	A	17.26	1296	0.88	0.39	0.71	0.62	247.27
	B	17.24	1295	0.87	0.39	0.70	0.62	248.32
	C	19.07	1295	0.89	0.37	0.71	0.70	253.34
6/2	A	17.24	1295	0.88	0.39	0.71	0.62	251.65
	B	17.98	1296	0.89	0.39	0.71	0.64	253.24
	C	19.24	1295	0.89	0.37	0.72	0.70	258.57
6/9	A	18.34	1295	0.97	0.44	0.77	0.68	283.23
	B	19.04	1295	0.97	0.44	0.78	0.68	284.12
	C	20.36	1295	0.98	0.42	0.78	0.74	291.21
6/13	A	17.24	1295	0.88	0.39	0.71	0.62	271.04
	B	17.98	1296	0.89	0.39	0.71	0.64	272.59
	C	19.24	1295	0.89	0.37	0.72	0.70	278.60
6/16	A	17.26	1296	0.88	0.40	0.71	0.62	283.71
	B	18.01	1295	0.88	0.40	0.71	0.64	285.17
	C	19.12	1295	0.88	0.38	0.71	0.69	291.57
6/23	A	17.24	1295	0.88	0.39	0.71	0.62	287.72
	B	17.98	1296	0.89	0.39	0.71	0.64	289.57
	C	19.24	1295	0.89	0.37	0.72	0.70	296.40
6/30	A	17.24	1295	0.88	0.39	0.71	0.62	295.09
	B	17.98	1296	0.89	0.39	0.71	0.64	297.05
	C	19.24	1295	0.89	0.37	0.72	0.70	304.65

[1] A = Lowest CP level diet; B = Intermediate CP level diet; C = Highest CP level diet.

[2] Date = date of diet formulation; CP = crude protein (%) (as is); ME = metabolizable energy (kcal/lb); Lys = digestible lysine (%); Met = digestible methionine (%); M+C = digestible methionine + cysteine (%); Cys = digestible cysteine (%); Thr = digestible threonine (%); Market $/ton = market price per ton of feed.

Appendix A.3. Diet formulations July through September [2]

Date	Diet [1]	CP	ME	Lys	Met	M+C	Thr	Market $/Ton
7/7	A	17.03	1295	0.76	0.37	0.61	0.51	328.95
	B	17.77	1295	0.76	0.36	0.61	0.53	330.99
	C	18.77	1295	0.76	0.35	0.61	0.57	338.13
7/14	A	17.24	1295	0.88	0.39	0.71	0.62	264.41
	B	17.98	1296	0.89	0.39	0.71	0.64	265.11
	C	19.24	1295	0.89	0.37	0.72	0.70	271.14
7/21	A	17.24	1295	0.88	0.39	0.71	0.62	264.41
	B	17.98	1296	0.89	0.39	0.71	0.64	265.11
	C	19.24	1295	0.89	0.37	0.72	0.70	271.14
7/28	A	17.24	1295	0.88	0.39	0.71	0.62	264.41
	B	17.98	1296	0.89	0.39	0.71	0.64	265.11
	C	19.24	1295	0.89	0.37	0.72	0.70	271.14
8/4	A	17.24	1295	0.88	0.39	0.71	0.62	250.71
	B	17.98	1296	0.89	0.39	0.71	0.64	250.97
	C	19.24	1295	0.89	0.37	0.72	0.70	256.04
8/11	A	16.99	1295	0.76	0.36	0.61	0.51	282.06
	B	17.73	1295	0.76	0.35	0.61	0.54	283.44
	C	18.73	1295	0.76	0.34	0.61	0.58	288.09
8/18	A	17.08	1295	0.87	0.39	0.70	0.62	250.63
	B	17.84	1295	0.88	0.38	0.71	0.64	251.44
	C	18.85	1296	0.88	0.37	0.71	0.69	257.22
8/25	A	17.08	1295	0.87	0.39	0.70	0.62	258.32
	B	17.84	1295	0.88	0.38	0.71	0.64	258.93
	C	18.85	1296	0.88	0.37	0.71	0.69	265.58
9/2	A	17.08	1295	0.87	0.39	0.70	0.62	252.02
	B	17.84	1295	0.88	0.38	0.71	0.64	252.69
	C	18.85	1296	0.88	0.37	0.71	0.69	259.13
9/4	A	17.15	1295	0.88	0.39	0.71	0.62	254.10
	B	17.98	1295	0.88	0.38	0.71	0.65	255.13
	C	19.06	1295	0.89	0.37	0.71	0.70	260.57
9/8	A	17.08	1295	0.76	0.37	0.62	0.52	289.16
	B	17.91	1295	0.76	0.35	0.62	0.54	290.27
	C	18.96	1295	0.76	0.34	0.62	0.58	295.60

[1] A = Lowest CP level diet; B = Intermediate CP level diet; C = Highest CP level diet.

[2] Date = date of diet formulation; CP = crude protein (%) (as is); ME = metabolizable energy (kcal/lb); Lys = digestible lysine (%); Met = digestible methionine (%); M+C = digestible methionine + cysteine (%); Cys = digestible cysteine (%); Thr = digestible threonine (%); Market $/ton = market price per ton of feed.

APPENDIX B. AMINO ACID ANALYSES

Appendix B.1. Monthly analyzed concentrations of crude protein and amino acids in feed mill samples ("as is" basis)

		Month									
Diet	Nutr (%) [1]	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
A	DM	89.91	89.20	89.33	89.02	88.76	89.47	88.88	89.41	88.94	89.21
	CP	21.49	19.96	18.17	18.23	17.02	17.34	16.56	17.08	17.31	18.13
	Lys	1.04	1.02	0.94	0.93	0.97	0.94	0.85	0.88	0.92	0.94
	Met	0.54	0.50	0.45	0.43	0.37	0.42	0.38	0.39	0.40	0.43
	Cys	0.42	0.40	0.36	0.39	0.34	0.37	0.35	0.33	0.34	0.37
	M+C	0.96	0.90	0.82	0.82	0.71	0.79	0.72	0.72	0.74	0.80
	Thr	0.83	0.78	0.70	0.69	0.65	0.66	0.62	0.65	0.67	0.69
B	DM	90.08	89.46	89.45	88.66	88.82	89.49	88.98	89.33	89.54	89.31
	CP	21.38	20.98	19.48	17.94	17.23	18.58	18.82	19.09	17.78	19.03
	Lys	1.10	1.05	0.98	0.88	0.96	0.94	0.90	0.90	0.90	0.96
	Met	0.53	0.53	0.46	0.39	0.36	0.38	0.38	0.38	0.39	0.42
	Cys	0.41	0.43	0.38	0.36	0.33	0.39	0.36	0.34	0.35	0.37
	M+C	0.93	0.96	0.83	0.76	0.69	0.77	0.75	0.72	0.74	0.79
	Thr	0.82	0.81	0.73	0.67	0.66	0.70	0.67	0.71	0.68	0.72
C	DM	89.65	89.20	89.63	88.81	89.25	89.74	89.08	88.99	89.38	89.30
	CP	20.76	19.53	19.00	19.48	19.18	20.05	19.74	16.89	19.64	19.36
	Lys	1.04	0.93	0.97	0.89	0.98	0.91	0.91	0.81	0.96	0.93
	Met	0.50	0.48	0.46	0.36	0.35	0.38	0.37	0.35	0.39	0.40
	Cys	0.38	0.37	0.32	0.38	0.35	0.43	0.38	0.31	0.37	0.37
	M+C	0.89	0.85	0.77	0.74	0.70	0.80	0.75	0.67	0.76	0.77
	Thr	0.80	0.73	0.71	0.72	0.73	0.76	0.73	0.61	0.77	0.73

[1] Nutr = Nutrient; DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; THR = threonine.

[2] Ovr = overall means of selected items over the entire nine months of the feed sample collection from the mill.

Appendix B.2. Monthly analyzed concentrations of crude protein and amino acids in hopper feed samples (“as is” basis)

		Month								
Diet	Nutr (%) [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
A	DM	89.24	90.46	89.60	89.38	89.84	89.26	89.60	89.65	89.63
	CP	19.74	20.67	18.71	18.3	18.37	18.65	18.01	17.64	18.76
	Lys	1.01	1.12	0.95	0.94	1.05	0.99	0.88	0.89	0.98
	Met	0.5	0.51	0.43	0.39	0.39	0.41	0.4	0.38	0.43
	Cys	0.37	0.4	0.39	0.36	0.38	0.37	0.35	0.35	0.37
	M+C	0.87	0.9	0.82	0.75	0.78	0.78	0.75	0.73	0.80
	Thr	0.78	0.8	0.71	0.67	0.72	0.69	0.68	0.66	0.71
B	DM	89.99	90.68	89.63	88.69	89.66	89.38	89.87	89.58	89.69
	CP	20.57	22.21	18.80	18.22	18.16	19.80	19.01	19.65	19.55
	Lys	1.02	1.13	0.95	0.94	0.97	0.92	0.9	0.95	0.97
	Met	0.49	0.53	0.41	0.39	0.37	0.41	0.39	0.4	0.42
	Cys	0.38	0.43	0.39	0.36	0.37	0.39	0.37	0.39	0.39
	M+C	0.88	0.96	0.8	0.75	0.74	0.8	0.75	0.78	0.81
	Thr	0.79	0.85	0.72	0.69	0.69	0.72	0.7	0.73	0.74
C	DM	90.19	90.01	88.98	89.59	89.7	89.46	90.12	89.76	89.73
	CP	21.69	20.12	19.72	20.39	18.74	20.97	20.19	19.76	20.20
	Lys	1.08	1.02	0.91	0.95	0.95	0.95	0.89	0.91	0.96
	Met	0.54	0.49	0.38	0.42	0.35	0.4	0.37	0.37	0.42
	Cys	0.37	0.35	0.39	0.39	0.37	0.4	0.38	0.37	0.38
	M+C	0.91	0.84	0.77	0.81	0.72	0.81	0.75	0.73	0.79
	Thr	0.84	0.76	0.74	0.77	0.72	0.78	0.75	0.74	0.76

[1] Nutr = Nutrient; DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; Thr = threonine.
[2] Ovr = overall means of selected items over the entire eight months of the trial.

APPENDIX C. PARTICLE SIZE ANALYSIS

Appendix C.1. P-values for interaction between treatment and location along the feed trough for sieve feed fractions [1]

	Month								
Sieve [2]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [3]
6	0.3135	0.2311	0.7259	0.4885	0.9179	0.1149	0.4203	0.1123	0.3149
8	0.4411	0.0911	0.5640	0.7445	0.5262	0.1041	0.2729	0.4246	0.2629
12	0.5947	0.2532	0.6972	0.3509	0.5367	0.0374	0.3464	0.5898	0.8602
16	0.8831	0.8094	0.4800	0.0904	0.6560	0.0277	0.6366	0.2578	0.9881
20	0.4317	0.2854	0.6711	0.5677	0.6922	0.0570	0.1859	0.0790	0.6538
30	0.1437	0.5573	0.0050	0.5491	0.4931	0.1876	0.1556	0.6048	0.3102
40	0.4810	0.2919	0.1627	0.2353	0.7899	0.1004	0.7081	0.6048	0.7781
50	0.6599	0.1758	0.4249	0.7332	0.2693	0.2802	0.5363	0.0329	0.5398
70	0.6606	0.1761	0.4041	0.6891	0.4597	0.8493	0.4092	0.2170	0.7075
Pan	0.4375	0.0942	0.3593	0.7724	0.3238	0.0553	0.2448	0.0094	0.7714

[1] P-value < 0.05 considered statistically significant.

[2] Particle size fractions: Sieve 6 (>3,360 µm), 8 (3,360-2,380 µm), 12 (2,380-1,680 µm), 16 (1,680-1,190 µm), 20 (1,190-840 µm), 30 (840-590 µm), 40 (590-420 µm), 50 (420-297µm), 70 (297-210 µm), and pan (< 210 µm).

[3] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

Appendix C.2. Mean sieving fraction values (% of total feed) by dietary treatment for feed trough samples

		Month								
Sieve [2]	Diet [1]	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [3]
6	A	5.83	5.93	6.83	5.90	6.55	8.27	6.78	5.03^{a}	6.39
	B	5.28	5.68	5.47	4.45	7.15	6.28	6.32	4.75^{ab}	5.67
	C	4.83	4.15	4.42	3.57	5.57	6.77	5.03	3.20^{b}	4.69
	SEM	0.65	0.43	0.63	0.69	0.78	1.23	0.31	0.27	0.45
	P-value	0.6091	0.1079	0.1573	0.1989	0.4534	0.5602	0.0594	0.0326	0.1606
8	A	9.87	10.52^{ab}	10.87^{a}	10.07	9.87	11.87	11.35	8.15^{a}	10.32
	B	8.77	10.72^{a}	9.50^{ab}	8.35	9.60	11.12	10.73	8.02^{a}	9.60
	C	8.20	7.73^{b}	7.27^{b}	6.55	6.98	10.42	8.95	5.85^{b}	7.74
	SEM	1.05	0.52	0.56	0.94	0.66	1.24	0.57	0.29	0.53
	P-value	0.5842	0.0445	0.0444	0.1629	0.0917	0.7345	0.1184	0.0180	0.0830
12	A	10.03	10.37	10.40	10.35	9.92	10.92	11.10	8.27	10.17
	B	10.12	10.98	10.13	9.73	9.83	11.03	11.25	8.50	10.20
	C	10.83	9.47	9.15	8.67	8.27	10.05	10.43	7.57	9.30
	SEM	0.85	0.51	0.58	0.54	0.43	0.54	0.20	0.21	0.27
	P-value	0.7797	0.2563	0.3984	0.2345	0.1221	0.4652	0.1174	0.1044	0.1635
16	A	11.47	11.33	11.07	10.97	10.55	10.53	10.62	8.48	10.63
	B	11.67	11.92	11.48	11.42	11.12	11.35	11.17	8.77	11.11
	C	13.77	12.33	11.52	11.65	10.55	11.28	10.98	9.33	11.43
	SEM	0.97	0.46	0.4577	0.30	0.35	0.13	0.41	0.32	0.28
	P-value	0.3186	0.4119	0.7606	0.3826	0.5044	0.0352	0.6701	0.3040	0.2786
20	A	12.18	11.60^{b}	11.32^{b}	11.20	11.07	10.73	9.53	9.60	10.90
	B	12.75	11.70^{b}	11.97^{ab}	12.27	12.00	12.02	9.40	10.25	11.54
	C	14.80	13.95^{a}	12.57^{a}	12.82	12.62	12.28	10.27	11.53	12.60
	SEM	0.80	0.34	0.16	0.36	0.27	0.41	0.49	0.35	0.36
	P-value	0.1945	0.0272	0.0258	0.1082	0.0584	0.1370	0.4944	0.0624	0.0961
30	A	17.75	16.75	12.78	12.45	12.33	11.62	11.77	12.67^{b}	13.51
	B	21.30	16.42	15.55	13.52	12.78	12.23	11.67	13.00^{ab}	14.56
	C	15.45	15.50	14.45	14.13	14.35	12.78	12.53	14.50^{a}	14.21
	SEM	1.46	0.76	0.35	0.49	0.52	0.65	0.24	0.28	0.40
	P-value[4]	0.1401	0.5547	0.0246	0.1906	0.1369	0.5242	0.1503	0.0362	0.3049
40	A	10.08	10.02	13.28	13.52	14.03	12.42	12.67	15.63^{ab}	12.71
	B	8.48	10.05	11.50	13.67	13.20	11.98	13.55	15.23^{b}	12.21
	C	11.23	12.23	15.50	14.52	15.37	12.22	14.12	17.60^{a}	14.10
	SEM	0.92	0.60	0.65	0.83	0.66	0.97	0.71	0.33	0.36
	P-value	0.2522	0.1253	0.0510	0.6894	0.2078	0.9522	0.4482	0.0288	0.0679
50	A	9.03	8.42	8.18	8.30	8.72	7.75	8.62	10.27	8.66
	B	8.35	8.30	8.67	9.00	8.55	7.90	8.63	10.42	8.73
	C	8.05	8.95	8.57	9.53	9.17	7.92	9.37	9.90	8.93
	SEM	1.05	0.44	0.34	0.53	0.32	0.56	0.36	0.28	0.31
	P-value	0.8070	0.5999	0.6241	0.3784	0.4645	0.9738	0.3670	0.4919	0.8244
70	A	8.02	8.45	6.42	7.07	6.98	5.80	7.70	10.08	7.56
	B	8.73	8.00	7.33	7.07	6.10	6.08	7.73	9.83	7.61
	C	9.12	9.47	8.13	10.75	6.85	6.52	9.25	10.70	8.85
	SEM	1.84	1.6405	1.02	1.14	0.40	0.43	0.97	0.90	0.56
	P-value	0.9143	0.8217	0.5601	0.1646	0.3733	0.5614	0.5181	0.7973	0.3243
Pan	A	5.70	6.38	8.82	10.18	10.02	10.10	9.87	11.80	9.17
	B	4.55	6.23	8.45	10.48	9.63	10.00	9.48	11.25	8.76
	C	3.70	6.22	8.52	7.83	10.28	9.73	8.98	9.88	8.14
	SEM	0.59	0.28	0.53	0.71	0.39	0.16	0.62	0.61	0.34
	P-value	0.1964	0.9086	0.8790	0.1346	0.5562	0.3630	0.6447	0.2182	0.2456

[a-b] Numerical values without the same superscript letter within a specific sieve size column are significantly different ($P<0.05$).

[1] A = Lowest CP level diet; B = Intermediate CP level diet; C = Highest CP level diet; SEM = Pooled standard error of the means

[2] Particle size fractions: Sieve 6 (>3360 µm), 8 (3360-2380 µm), 12 (2380-1680 µm), 16 (1680-1190 µm),20 (1190-840 µm), 30 (840-590 µm), 40 (590-420 µm), 50 (420-297 µm), 70(297-210 µm), and pan (< 210 µm).

[3] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

[4] If $P<0.05$, but no subscripts are shown, then the interaction between treatment and location is significant and effect of treatment could not be analyzed individually.

Appendix C.3. P-values for interactions between treatment and location along the feed trough for dietary nutrients

Dietary nutrient [1, 2]						
DM	CP	Lys	Met	Cys	M + C	Thr
0.7821	0.8198	0.8665	0.9893	0.7607	0.9313	0.8748

[1] DM = dry matter; CP = crude protein; Lys = lysine; Met = methionine; M+C = methionine + cysteine; Cys=cysteine; Thr = threonine.

[2] $P<0.05$ considered statistically significant.

APPENDIX D. PERFORMANCE DATA

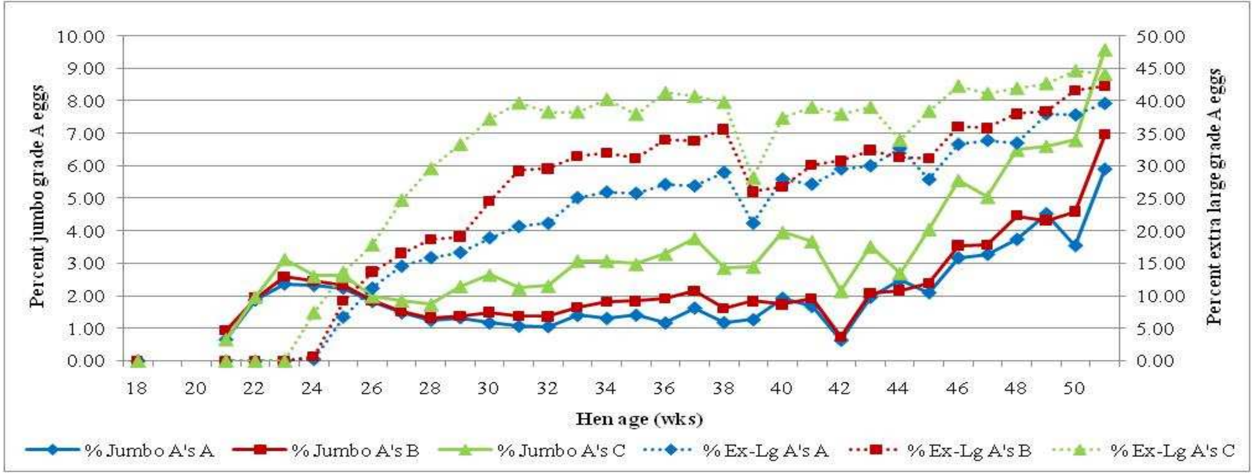
Appendix D.1. P-values for interactions between treatment and location along the feed trough for production parameters [1]

	Month								
Variable	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
Body weight (Kg)	0.5089	0.6546	0.4817	0.6618	0.1080	0.0823	0.0245	0.0587	<.0001
Egg weight (g)	0.0919	---	0.0099	0.8768	---	0.7922	---	0.0657	0.3404
Albumin height (mm)	0.7041	---	0.0012	0.5335	---	0.7838	---	0.1752	0.4522
Haugh unit	0.8051	---	0.0214	0.4889	---	0.8475	---	0.1860	0.7346
Yolk color (Roche scale)	0.0437	---	0.1963	0.0433	---	0.0006	---	0.3514	0.0073
Shell strength (g force at failure)	---	0.0768	0.3201	---	0.2655	---	0.2410	---	0.7714
Shell thickness (mm)	---	0.3366	0.4172	---	0.2325	---	0.0087	---	0.4259

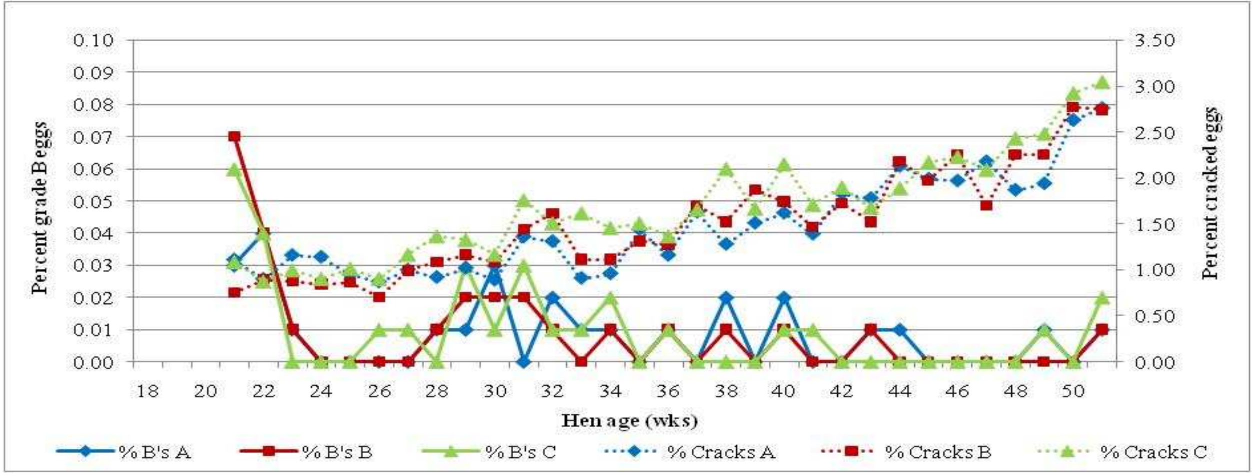
[1] P-value < 0.05 considered statistically significant.

[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

Appendix D.2. Weekly percentage jumbo and extra large grade A eggs



Appendix D.3. Weekly percentage grade B and cracked eggs



Appendix D.4. Weekly percentage dirty and loss eggs

APPENDIX E. MANURE ANALYSES

Appendix E.1. P-values for the interaction between dietary treatment and location along the feed trough for manure analysis [1]

	MONTH [1]								
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
% DM	0.6698	0.2845	0.4557	0.5842	0.5383	0.2771	0.1800	0.8643	0.2967
Total N (lb/ton)	0.6298	0.1320	0.5847	0.6379	0.7483	0.2748	0.1423	0.1055	0.2230
(NH_4^+) N (lb/ton)	0.1764	0.4357	0.9333	0.6193	0.2240	0.4980	0.6865	0.3511	0.9532
Organic N (lb/ton)	0.6153	0.1865	0.5709	0.8454	0.7601	0.2265	0.1423	0.0757	0.2376
P_2O_5 (lb/ton)	0.4473	0.2561	0.4322	0.9695	0.1279	0.6410	0.7225	0.7070	0.6615
K_2O (lb/ton)	0.5968	0.1466	0.2959	0.5850	0.5191	0.1989	0.0277	0.8162	0.2218

[1] $P<0.05$ considered statistically significant.

[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.

APPENDIX F. AMMONIA ANALYSIS

Appendix F.1. P-values for the interaction between dietary treatment and location along the feed trough for manure ammonia flux [a]

Month [1]								
Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ovr [2]
0.7124	0.9939	0.7044	0.1077	0.1724	0.4971	0.5473	0.7801	0.2175

[1] $P<0.05$ considered statistically significant.

[2] Ovr = Overall mean values and statistics for each parameter over the eight month data collection period.