Effects of dietary corn distiller's dried grains with solubles on ammonia emission, production performance, manure characteristics, and economic efficiency for laying hens

Stacey Ann Roberts

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Effects of dietary corn distiller's dried grains with solubles on ammonia emission, production performance, manure characteristics, and economic efficiency for laying hens

by

Stacey Ann Roberts

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

DOCTOR OF PHILOSOPHY

Major: Nutritional Sciences (Animal Nutrition)

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Iowa State University
Ames, Iowa
2009
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ABSTRACT

A series of experiments was designed to evaluate the mechanisms and effects of dietary corn distiller's grains with solubles on NH₃ emission from laying hens. A diet containing 15% DDGS was fed to laying hens in high-rise houses on a farm in central Iowa. Hens in three houses were fed the DDGS treatment diet while hens in the other three houses were fed a 0% control diet. Emission, egg production, and economic parameters as well as manure characteristics were measured over the course of a one-year period (between fall manure clean-out). The manure pH was lower for the DDGS than the control regimen (7.10 vs. 7.42 ± 0.08, respectively; \( P = 0.01 \)). Contrary to the hypothesis, the lower manure pH did not lead to a decrease in NH₃ emission (1.24 vs 1.32 ± 0.08 g/hen-d for the DDGS and control, respectively; \( P = 0.54 \)). However, higher N consumption by the DDGS hens did not lead to increased NH₃ emission. The DDGS diet did not cause any adverse effects on production performance. Economic analyses revealed lower diet cost for the DDGS regimen than the control (10.8 vs. 11.2 ± 0.1 c/hen-wk, respectively; \( P = 0.10 \) and 34.2 vs. 31.3 ± 0.5 c/kg egg, respectively; \( P = 0.06 \)). In a separate study, no change in pH or short-chain fatty acid contents of the laying-hen ceca could be detected between a 15% DDGS and control regimen. Because the DDGS treatment was expected to impact manure nutrient values, a study was conducted to determine which sampling strategies yielded the most precise nutrient values. The variation in dry-matter content was greater than the variation in nitrogen or phosphorus. Sampling strategies that accurately measure dry-matter content must be used, including collection of samples near the sidewall and in the center manure rows under high-rise houses. Diets containing 15% DDGS yielded lower feed cost while supporting egg production. However, no decrease in NH₃ emission was detected.
CHAPTER 1. GENERAL INTRODUCTION

Corn distiller’s dried grains with solubles (DDGS) has become an important feed ingredient in poultry diets in the United States. In addition to the contribution of nutrients, such as vitamins, minerals, and amino acids, to the diet, DDGS may have other beneficial effects including decreasing ammonia (NH$_3$) volatilization from poultry manure and lowering the populations of *Escherichia coli* and *Salmonella* in the digestive tract of the hen. This dissertation is the culmination of three research projects aimed to determine the effects of dietary corn DDGS in commercial laying-hen diets with respect to NH$_3$ emission and short-chain fatty acid (SCFA) contents. The two-part hypothesis driving this research was:

1) The fiber in corn DDGS would provide energy to the commensal bacteria in the ceca, thereby increasing total bacterial growth. The increased bacterial growth would incorporate nitrogen (N) into stable bacterial protein, thereby shifting N excretion from uric acid to bacterial protein. Uric acid is readily converted to ammonia after excretion; whereas bacterial protein is a relatively stable form of N in poultry manure and

2) Increased bacterial populations would produce increased quantities of SCFA as a normal by-product of their metabolism. If sufficient quantities of short-chain fatty acids were produced such that manure pH was decreased, the equilibrium of NH$_3$ and ammonium (NH$_4^+$) would be shifted towards NH$_4^+$, which is water soluble and less volatile compared to NH$_3$. 
The objectives of the dissertation research were to determine if dietary DDGS would cause a decrease in NH₃ emission from commercial high-rise laying-hen houses, to elucidate the mechanism of the effect, if any, on NH₃ emission, and evaluate production and economic parameters to determine if DDGS is a viable ingredient.

**Thesis Organization**

A literature review and three manuscripts are contained within this dissertation. Chapters 1 and 6 are the general introduction and general conclusion, respectively. Chapter 2 of the dissertation is an overall literature review that considers several aspects of potential environmental pollution and NH₃ emission from poultry housing. Chapter 3 is a manuscript, which describes the primary study of the dissertation research. A commercial-scale experiment was designed to determine the effect of 15% dietary corn DDGS on ammonia emission from high-rise laying-hen houses. Chapter 4 is the second manuscript included in the dissertation and reports the results of a study designed to evaluate the effects of 15% dietary DDGS on SCFA content and pH as well as *Escherichia coli* (*E. coli*) and *Salmonella* colonization in the ceca of laying hens. Chapter 5 is the third and final manuscript included in the dissertation and reports the findings of a study in which the spatial variation of manure nutrients in high-rise laying-hen houses was evaluated.

Combined, the three studies included in the dissertation provide a broad overview of the effects of dietary DDGS for laying hens in commercial production. The study presented in Chapter 3 followed previous research that showed decreased manure pH and lower NH₃ emission from laying hens fed 10% DDGS. In the present study, 15% DDGS diet was fed at a commercial production site to determine if similar results could be obtained in a production situation and with slightly higher
DDGS inclusion. The study presented in Chapter 4 was designed to elucidate the mechanism of manure pH reduction for the DDGS regimen and determine if manure pH changes could be related to pH and SCFA contents in the ceca. *E. coli* and *Salmonella* were evaluated as part of the study because SCFA have been reported to inhibit both species, which are detrimental to the laying-hen industry. Finally, the study presented in Chapter 5 evaluates the nutrient variation of manure under high-rise houses. Dietary manipulations may change the nutrient profile of manure, thereby making accurate sampling and analyses vital to proper manure management.
CHAPTER 2. REVIEW OF THE LITERATURE

Introduction

Nutrient pollution, including nitrogen (N) and phosphorus (P) to the environment is a major concern to poultry producers. Nitrogen is mainly lost to the atmosphere through the volatilization of ammonia (NH₃) and can seriously impair indoor air quality as well as area and regional ecosystems. Atmospheric NH₃ lead to N loading of sensitive waterways, acid rain, and fine particulate matter. As more attention is directed towards environmental management and regulations are enacted, more research is directed towards methods that lower NH₃ emission without causing decreasing production and without excessively increasing cost. A multitude of NH₃-lowering methods are available with varying efficiencies. These methods can be divided into two main categories: pre-excretion (dietary) and post-excretion (manure or air handling).

A description of the problem along with possible mitigation strategies is included herein. Additionally, considerations with regard to other effects of dietary manipulations are considered. Altering diets by including chemicals or non-traditional ingredients may have far-reaching effects on the animal and its environment.

Ammonia

Chemistry of Ammonia Emission

An understanding of the chemical origin of NH₃ found in poultry manure as well as the behavior of the compound will facilitate efforts to decrease NH₃ volatilization from poultry production facilities. Chickens do not have a functional urea cycle and, therefore, excrete nitrogenous waste products primarily as uric acid (Leeson and Summers, 2001). Because poultry excrete urine and feces together,
the uric acid in the urine immediately comes into contact with the bacteria in the feces. The uric acid is broken down to allantoin by microbial urate oxidase (i.e., uricase) and the allantoin is further broken down to urea and finally NH$_3$ by other microbial enzymes.

Schefferle (1965) evaluated the microbial populations in built-up poultry litter and found that an average of 25% of the bacteria in litter are capable of aerobically decomposing uric acid with most forming urea as the end product. Urease enzyme decomposes urea to NH$_3$ and carbamate (Mobley and Hausinger, 1989). Carbamate spontaneously decomposes to NH$_3$ and carbonic acid (H$_2$CO$_3$) yielding a total of 2 NH$_3$ molecules per molecule of urea and, therefore, 4 NH$_3$ molecules per molecule of uric acid (Figure 1). The urease-positive bacteria responsible for hydrolysis of urea to NH$_3$ were predominantly coryneform bacteria and micrococci (Schefferle, 1965). Bachrach (1957) found that pseudomonads isolated from poultry litter were capable of completely degrading uric acid to NH$_3$, CO$_2$, and H$_2$O (Figure 1). The NH$_3$ and CO$_2$ produced from the breakdown of uric acid equilibrate with their respective protonated and deprotonated forms, resulting in an increase in pH (Mobley and Hausinger, 1989). The rapid conversion of uric acid to NH$_3$ by microbial enzymes cannot be eliminated so efforts must be focused towards minimizing the conversion either by decreasing the uric acid content of the manure or by adjusting the chemical properties of the manure such that the conditions do not favor the enzyme activity.

Generation of NH$_3$ is dependent on temperature, pH, and air velocity (Monteny, 2000). Therefore, methods to impact any of the three aforementioned characteristics such that conversion of uric acid to NH$_3$ is minimized may be potential mitigation strategies to decrease NH$_3$ emission from livestock and poultry systems.
**Health Effects**

Aerial NH₃ is detrimental to the health of both poultry and the workers who care for them. Ammonia is water-soluble and, therefore, can be absorbed by mucus membranes, especially those of the eyes and respiratory tract. Extended exposure to NH₃ concentrations greater than 75 ppm can cause damage to the respiratory tract including loss of cilia, increase in goblet cells, and a decrease in the respiratory rate (Crespo and Shivaprasad, 2003). Exposure of broiler chickens to NH₃ concentrations greater than 25 ppm continually from 1 to 28 d of age increased the incidence of air sac lesions (Kling and Quarles, 1974). Atmospheric NH₃ can damage the eye, causing burns to the conjunctiva and making birds unwilling to open their eyes, move, or eat (Crespo and Shivaprasad, 2003).

The detrimental effects of NH₃ exposure on bird health and performance have been well documented in the literature. Miles et al. (2004) evaluated the performance of broilers reared in the presence of 0, 25, 50, or 75 ppm atmospheric NH₃ and found depressed weight gain and increased mortality in birds exposed to 50 or 75 ppm NH₃ compared to birds reared in a low-NH₃ environment. In a study by Beker et al. (2004), broiler chicks were grown to 21 days of age with 0, 30, or 60 ppm added NH₃. The 30 and 60 ppm NH₃ treatments resulted in higher incidence of conjunctival lesions while the 60 ppm NH₃ treatment caused a decrease in feed
utilization. Kling and Quarles (1974) evaluated the effects of atmospheric NH₃ when broiler chickens were challenged with infectious bronchitis vaccination. Results showed that 25 or 50 ppm NH₃ caused lower body weight, higher (i.e., poorer) feed efficiency, and greater lung weights compared to that of control chickens that were vaccinated but not exposed to high atmospheric NH₃ concentrations (Kling and Quarles, 1974). Broiler chickens raised to 28 d of age in the presence of 200 ppm NH₃ weighted half as much as control birds raised in low-NH₃ environment (Reece et al., 1980). When laying hens were exposed to 200 ppm NH₃ for 17 days, egg production and body weight were both depressed (Deaton et al., 1982). The lower body weight was attributed to numerically lower feed consumption.

Exposure to high atmospheric NH₃ concentrations is detrimental to bird health causing damage to the eyes, mucus membranes of the respiratory tract, and decreased growth rate. High levels of NH₃ also cause decreased production performance in laying hens and broiler chickens. Decreased production performance caused by ineffective management of NH₃ concentrations in poultry houses will be realized as decreases in economic returns. While NH₃ impairs the quality of indoor air in poultry houses, NH₃ emitted to the environment can cause damage to local and regional ecosystems including acidification, eutrophication, and damage to vegetation. Therefore, the incentive to decrease NH₃ emission from poultry production is two-fold: to improve indoor air quality and to decrease environmental pollution.

**Environmental Impact**

The environmental impact of intensive livestock and poultry production has come under increased scrutiny in the past decades. The consolidation and vertical integration of animal agriculture has improved the efficiency of livestock and poultry
production and has decreased the land area required for the production of animal products but, at the same time, has geographically concentrated manure nutrients and created a point source of aerial emissions.

In a ranking of aerial pollutants lost from livestock production facilities, the National Research Council Committee on Air Emissions from Animal Feeding Operations (NRC, 2002) ranked NH$_3$ as the most important pollutant with regards to global, national, and regional air pollution. Global anthropogenic NH$_3$ emission in 2004 was estimated at 47 million tons of N with livestock contributing 64% of the total NH$_3$-N emission, mainly from manure (Galloway et al., 2004; Steinfeld et al., 2006). The Environmental Protection Agency (EPA) estimated that poultry emitted 24% of the NH$_3$ lost from animal production facilities in the United States in 2002, accounting for more NH$_3$ than any other segment of the livestock industry (EPA, 2005). Approximately 22% of the NH$_3$ lost from poultry operations was from egg production, while the remainder was mostly from broiler and turkey production. A summary of reported NH$_3$ emission factors is shown in Table 1. Methods to decrease the NH$_3$ emission from egg-production facilities have great potential to decrease the overall environmental impact of livestock and poultry production, not only in the United States but also throughout the world.

Based on the findings of a study utilizing the Delphi technique (Dalkey and Helmer, 1963), Angus et al. (2003) reported that the environmental effects of NH$_3$ emission, ranked in order of importance, are N eutrophication, acidification from NH$_3$ deposition, and toxicity of NH$_3$ on vegetation. Eutrophication is the excessive input of nutrients into an ecosystem, which often leads to the overgrowth of plants and many undesirable side effects, such as oxygen depletion, fish kills, and offensive tastes and odors of drinking water (Smith and Schindler, 2009). The effects of NH$_3$ were mainly considered to be a local concern with lesser impacts at the national and
Table 1. Summary of ammonia (NH₃) emission rates (g NH₃/hen·d⁻¹) from commercial laying hen houses (adapted from Liang et al., 2005).

<table>
<thead>
<tr>
<th>Country</th>
<th>Housing</th>
<th>Season</th>
<th>NH₃ ER</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>England</td>
<td>Deep pit</td>
<td>Winter</td>
<td>0.58</td>
<td>Wathes et al. (1997)</td>
</tr>
<tr>
<td>England</td>
<td>Deep pit</td>
<td>Summer</td>
<td>0.87</td>
<td>Wathes et al. (1997)</td>
</tr>
<tr>
<td>England</td>
<td>Deep pit</td>
<td>N/A</td>
<td>0.72</td>
<td>Nicholson et al. (2004)</td>
</tr>
<tr>
<td>USA</td>
<td>High-rise</td>
<td>March</td>
<td>1.57</td>
<td>Keener et al. (2002)</td>
</tr>
<tr>
<td>USA</td>
<td>High-rise</td>
<td>July</td>
<td>1.25</td>
<td>Keener et al. (2002)</td>
</tr>
<tr>
<td>USA</td>
<td>High-rise</td>
<td>All year</td>
<td>0.90</td>
<td>Liang et al. (2005)</td>
</tr>
<tr>
<td>USA</td>
<td>High-rise</td>
<td>Dec–May</td>
<td>1.12</td>
<td>Li et al. (2009)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Manure belt</td>
<td>N/A</td>
<td>0.09</td>
<td>Kroodsma et al. (1988)</td>
</tr>
<tr>
<td>Denmark</td>
<td>Manure belt</td>
<td>All year</td>
<td>0.16</td>
<td>Groot Koerkamp et al. (1998)</td>
</tr>
<tr>
<td>Germany</td>
<td>Manure belt</td>
<td>All year</td>
<td>0.04</td>
<td>Groot Koerkamp et al. (1998)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Manure belt</td>
<td>All year</td>
<td>0.12</td>
<td>Groot Koerkamp et al. (1998)</td>
</tr>
<tr>
<td>England</td>
<td>Manure belt</td>
<td>All year</td>
<td>0.11</td>
<td>Nicholson et al. (2004)</td>
</tr>
<tr>
<td>USA</td>
<td>Manure Belt</td>
<td>All year</td>
<td>0.05</td>
<td>Liang et al. (2005)</td>
</tr>
</tbody>
</table>

¹Assuming, when necessary, hen body mass of 1.5 kg.

global scales (Angus et al., 2003).

Additionally, NH₃ contributes to the formation of secondary fine particulate matter (PM) with an aerodynamic diameter less than 2.5 µm. Atmospheric NH₃ can react with sulfate and nitric acid in the atmosphere to form ammonium sulfate and ammonium nitrate (McCubbin et al., 2002; Sharma et al., 2007). Fine PM has been implicated as a cause of respiratory disease including asthma, bronchitis, hospital admissions, and even premature mortality (Bates, 2000; McCubbin et al., 2002).
McCubbin et al. (2002) estimated that a 20% decrease in NH$_3$ emission from poultry operations would be associated with $1.212$ billion dollars related to health care and premature mortality in the United States. Lowering the NH$_3$ emissions from livestock and poultry facilities is a key step in the overall reduction of anthropogenic NH$_3$ and can help decrease pollutant loads to sensitive ecosystems and formation of secondary PM.

**Regulations**

While high concentrations of NH$_3$ are detrimental to worker and bird health and environmental NH$_3$ pollution damages sensitive ecosystems, federal and industry regulations are in place to provide incentive for producers to decrease NH$_3$ production. Federal regulations address NH$_3$ emission from animal production facilities into the environment. The federal Emergency Planning and Community Right-to-Know Act (EPCRA; 40 CFR part 355) and the Comprehensive Environmental Response, Compensation, and Liability Act of 1986 (CERCLA; 40 CFR part 302) require reporting of NH$_3$ releases greater than 45.4 kg (100 pounds) per day (CFR, 2009). On January 20, 2009, the EPA released an administrative exemption of CERCLA reporting for emissions of hazardous substances from farm animal manure and exemption of EPCRA reporting for emission of hazardous substances from animal waste on farms with limited numbers of animals (EPA, 2009). The exemption applies to egg production facilities with fewer than 82,000 hens and states that these facilities do not have to report NH$_3$ emissions greater than the 45.4 kg/d reportable quantity. However, farms with more than 82,000 hens must still report continuous releases of NH$_3$ greater than 45.4 kg/d. Table 2 outlines predictions of the number of hens necessary to emit 45.4 kg NH$_3$/d based on emission factors reported in Table 1. Per hen, the most NH$_3$ was
Table 2. Number of hens to emit 100 pounds (45.4 kg) NH₃/d calculated from emission factors reported in Table 1.

<table>
<thead>
<tr>
<th>Housing type</th>
<th>Emission Factor (g NH₃/hen-d)¹</th>
<th>Number of Hens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep pit</td>
<td>0.72</td>
<td>62,768</td>
</tr>
<tr>
<td>High rise</td>
<td>1.21</td>
<td>37,521</td>
</tr>
<tr>
<td>Manure belt</td>
<td>0.10</td>
<td>477,895</td>
</tr>
</tbody>
</table>

¹Mean of values reported in Table 1.

emitted from high-rise houses and, on average, it would take only 37,521 hens to emit 45.4 kg NH₃/d. Manure belt houses, with the lowest average emission factor, would require 477,895 hens to reach the reporting threshold. Many commercial production sites will maintain hen populations higher than 500,000 hens and would be expected to report NH₃ emissions.

Industry regulations address atmospheric NH₃ content in poultry facilities and federal regulations limit human exposure in the workplace. The United Egg Producers (UEP) specify that NH₃ concentrations in laying-hen houses should ideally be below 10 ppm and must be maintained below 25 ppm for egg producers to be certified under the Animal Husbandry Guidelines (UEP, 2008). The Occupational Safety and Health Administration (40 CFR 1910) requires worker exposure to remain below 50 ppm NH₃ time-weighted average for an 8-hr work day (CFR, 2009) and the National Institute of Occupational Safety and Health restricts NH₃ exposure to 25 ppm time-weighted average for an 8-hr work day (CDC, 2005). Personal protective equipment (e.g., a respirator appropriate to filter NH₃) should be worn when farm workers anticipate exposure to high atmospheric NH₃ concentrations.
Mitigation of Environmental Impacts

The excretion of excess N and P is a major concern for the poultry industry as both N and P are pollutants when deposited into sensitive ecosystems. Furthermore, volatile N, as NH₃, is detrimental to bird and worker health and can cause impairments of local ecosystems, as discussed previously. The nutrient content of the feed directly influences the nutrient content of the resulting manure. Additionally, dietary factors can directly influence characteristics of the manure (i.e., pH), which may decrease the pollution potential of that manure. Dietary manipulations are a valid method to lower the environmental impact of intensive livestock and poultry production (Sutton et al., 1999; Powers and Angel, 2008).

Manure management is extremely important, and when manure is land applied, care must be taken to minimize the run-off of N and P into area waterways. The 2008 proposed list of impaired waterways includes 445 water bodies in the state of Iowa (DNR, 2008), many of which are impaired by nutrient loading. Nutrient loading of waterways may lead to algal blooms, high nitrate concentrations, low dissolved-oxygen content, or fish kills.

Limitations of N and P naturally restrict phytoplankton and algal growth in freshwater lakes. However, excessive inputs of nutrients can favor rapid growth and decay cycles of plants, such that dissolved oxygen content is markedly decreased and other life forms are unable to survive. Schindler (1977) performed a series of studies on freshwater lakes and found that P is the most-limiting nutrient in lakes over long periods of time. Extreme C loads can cause overgrowth of plant material in a freshwater ecosystem, but is rarely a limiting factor because atmospheric CO₂ allows lakes to self-correct C deficiencies. Nitrogen may be limiting over the short term, and excessive inputs may cause plant overgrowth, but blue-green algae and some phytoplankton can fix N from the atmosphere. Therefore, in N-limited
ecosystems, N fixation can correct the deficiency when sufficient time is allowed for N-fixing organisms to establish adequate populations. However, there is no atmospheric form of P from which P deficiencies can be corrected. While C and N pollution to freshwater ecosystems should be considered, Schindler (1977) proposed that lakes can self-correct C and N deficiencies but the P available to the ecosystem will limit eutrophication until excessive pollution allows plant overgrowth.

**Dietary Manipulations (Pre-Excretion Techniques)**

*Phytase*

Phosphorus content of poultry manure is directly affected by P content of the diet and the extent that birds are able to digest and utilize the dietary P. Dietary phytase addition with concurrent decrease in total P content of the diet can lower the P content of poultry manure. Most plant-based ingredients (i.e., corn or soybean meal) contain P bound in phytate. Monogastric animals do not possess an endogenous enzyme to digest phytate, making that P unavailable to the animal. Phytase is a microbial enzyme that releases phytate-bound P from plant materials in poultry diets, making it available to the bird (Selle and Ravindran, 2007). Inclusion of phytase decreases the total P necessary in diets and lowers manure P, as the animal utilizes more of the dietary P.

Research results have shown that dietary phytase can cause a decrease in P excretion from chickens. Keshavarz and Austin (2004) reported a 48% decrease in P excretion for hens fed 0.2% dietary P and 300 units of phytase per kilogram of diet compared to hens fed 0.4% dietary P and no phytase. In an experiment evaluating the P requirements of broiler chickens, Yan et al. (2003) calculated up to a 52% reduction in P excretion when phytase was added to low-P diets. Similarly, Plumstead et al. (2007) reported a 42% decrease in manure P when broiler breeder
hens were fed diets containing phytase and decreased inorganic P and Applegate (2003) found a 42% decrease in litter P content when broiler chickens were fed phytase and low-phytate corn.

When including phytase in poultry diets, care should be taken to avoid over-formulating, which may result in an increase in soluble P and increase in the potential for P loss in runoff (Powers and Angel, 2008). Formulating diets with P contents that more closely match the P requirements of birds and including exogenous phytase enzyme will decrease the P contents of manure and decrease the potential of that manure to cause environmental deterioration when properly managed.

**Low crude protein**

Decreasing the crude-protein, and hence N contents, of poultry diets can decrease the uric-acid N excretion and, therefore, decrease NH₃ emission. Poultry diets in the United States are typically formulated with protein-supplying ingredients to meet the birds’ requirements for the second-limiting amino acid (i.e., lysine) with synthetic methionine included to meet the total sulfur amino acid (i.e., first-limiting amino acid) requirements. Amino acids supplied above the hen’s requirements are deaminated and the N is excreted as uric acid. If protein-supplying ingredients are included to meet the 3rd-limiting amino acid (i.e., threonine) and synthetic methionine and lysine are included, the crude protein contents of the diet can be further decreased with a concurrent decrease in the amount of excess amino acids supplied. Synthetic amino acids other than lysine and methionine are typically not economical to add to commercial poultry diets at this time and further decreases in dietary crude protein would not be possible. Diets should be formulated on a true-digestible amino acid basis to assure supply adequate amino acids to meet hen requirements.
The addition of crystalline amino acids to pig diets in order to decrease the crude protein (and hence, N contents) from 16.5% to 12.5% caused a decrease in total N excretion from 37.9 to 24.5 g/d (Canh et al., 1998). In laying-hen diets, a decrease in crude protein from 19 to 11% resulted in a decrease in N excretion from 2.0 to 1.1 g/d (Summers, 1993).

Low-protein diets must be carefully formulated to avoid losses in production. Roberts et al. (2007b) fed laying hens a 1 percentage unit lower protein diet and, while a decrease in N excretion was realized, egg production was also lower compared to that observed from the control-fed hens. The low-protein diet was likely deficient in one or more amino acids.

Research has shown that dramatically decreased crude protein diets do not support optimal production performance. When broiler chickens were fed diets with 4.2 percentage units lower crude protein and supplemental synthetic amino acids, nitrogen excretion was significantly decreased (Bregendahl et al., 2002). However, the low-protein diets did not support growth performance similar to that observed when birds were fed a high-protein control diet. Upon further investigation, Bregendahl et al. (2002) showed that inclusion of 1% L-Gln or 1% L-Asn, additional essential amino acids, or additional non-essential amino acids did not improve growth performance comparable to that of the control-fed birds. The addition of non-essential and essential amino acids seemed to improve growth performance but not enough to fully reach the growth observed for the control-fed birds (Bregendahl et al., 2002). In an experiment to evaluate the ideal amino acid ratio for laying hens, hens fed diets with 4.4 percentage units lower crude protein had lower egg production, egg weight (and consequently lower egg mass), lower feed utilization, and lower body weight change (Bregendahl et al., 2008). Bregendahl et al. (2008) considered non-essential and essential amino acid contents of the diets and
determined that the decreased production performance was not caused by an amino acid deficiency. Therefore, it is difficult to determine what is limiting the performance when dietary crude protein contents are decreased more than 4 percentage units by lowering the inclusion of intact protein and increasing the inclusion of synthetic amino acids.

While the NRC (1994) does not employ the ideal amino acid concept, several studies have determined ideal amino acid profiles for poultry. Formulating diets on a digestible amino acid basis according to the ideal amino acid profile will allow lower N consumption, and consequently lower N excretion, while maintaining production performance (Baker, 1994; Baker et al., 2002; Dari et al., 2005; Bregendahl et al., 2008). However, caution should be exercised when dietary protein contents are lowered more than 2 or 3 percentage units by decreasing the contents of intact protein (e.g., soybean meal).

While decreasing N consumption causes a decrease in total N excretion, research has shown that the lower N consumption is typically accompanied by lower NH$_3$-N loss (van der Peet-Schwering et al., 1996; Liang et al., 2005). Excess dietary N is primarily excreted as uric acid in poultry or urea in mammals (Leeson and Summers, 2001; Stipanuk and Watford, 2006) and both are readily converted to NH$_3$ after excretion.

**Dietary fiber**

Fiber is typically defined as carbohydrates that are not digestible by endogenous enzymes (Lupton and Turner, 2006). The fiber escapes digestion in the stomach and small intestine, arriving largely intact in the large intestine. In the large intestine (i.e., cecum, rectum, and colon), the fiber provides an energy source to commensal bacteria. With the increased supply of energy, bacterial metabolism
increases and populations grow. Bacteria produce short-chain fatty acids (e.g., acetate, butyrate, and propionate) during normal metabolism and increased populations of bacteria produce larger quantities of short-chain fatty acids. These acids may cause a decrease in the pH of digesta in the large intestine, which may be reflected as a decrease in the pH of manure. The equilibrium of NH$_3$ and NH$_4^+$ is highly dependent on pH, with an increased proportion of NH$_4^+$ at lower pH.

In addition to the decrease in pH, the increased microbial metabolism can shift the N excretion from urinary N (i.e., urea in mammals or uric acid in poultry) to fecal N. Urea and uric acid are readily degraded to NH$_3$ by microbial uricase and urease enzymes. However, fecal N is more stable and more likely to remain in the manure and not be volatilized to the atmosphere compared to urea N or uric acid N.

Dietary fiber has been shown to cause a decrease in manure pH and decrease uric acid-N excretion with a concurrent decrease in NH$_3$ emissions. Roberts et al. (2007a) fed laying hens diets that contained 10.0% corn distiller’s dried grains with solubles, 7.3% wheat middlings, or 4.8% soybean hulls. The NH$_3$ emission from the laying-hen manure was decreased 41%, 38%, and 27% for the corn distiller’s dried grains with solubles, wheat middlings, and soybean hulls, respectively (Roberts et al., 2007a). No repartitioning of N excretion could be detected in the study but manure pH was decreased from 7.08 for the control-fed hens to 6.77, 6.80, and 6.85, respectively, for the hens fed corn distiller’s dried grains with solubles, wheat middlings, and soybean hulls (Roberts et al., 2007a). The decreased pH was attributed to higher concentrations of SCFA in the manure from the fiber-fed hens.

Increased SCFA contents may have other implications besides causing a decrease in NH$_3$ volatilization. The SCFA have a bacteriostatic effect on certain groups of bacteria, namely the family *Enterobacteriaceae* of which *Escherichia coli*
*E. coli* and *Salmonella* are members (Barnes et al., 1979; Cherrington et al., 1990; Courrier et al., 1990; van der Wielen et al., 2000). *E. coli* species are a primary causative agent of peritonitis, which causes the laying-hen industry thousands of dollars annually from increased mortalities (Barnes et al., 2003; Trampel et al., 2007). While several *Salmonella* species can cause disease in poultry (e.g., Pullorum disease and fowl typhoid), *S. enteritidis* is a major concern for the egg industry (Gast, 2003). Therefore, dietary fiber may have wide-ranging effects if SCFA can inhibit bacteria that are harmful to either the hen or to human consumers.

While dietary fiber has been shown to lower NH$_3$ emission from laying-hen manure, much of the early work was done in swine. In pig experiments, repartitioning of N excretion from urinary N to fecal N is easier to evaluate as urine and feces can be collected separately, whereas extensive surgery would be required to collect urinary excretions and feces separately from birds. Shriver et al. (2003) fed pigs diets with 10% soybean hulls or 10% beet pulp. Although NH$_3$ emission was not measured directly, the additional dietary fiber led to a decrease in slurry NH$_4^+$, a decrease in urinary urea N, and an increase in short-chain fatty acid content of the slurry (Shriver et al., 2003). When feeding pigs increasing quantities of bacterially fermentable substrates, Kruezer and Machmuller (1993) observed 0.6% decrease in volatile N loss from manure for each percentage point increase in bacterially fermentable substrates in the diet. Urinary N was decreased and the ratio of NH$_4^+$-N to total N was decreased in the manure (Kreuzer and Machmuller, 1993). The inclusion of 30% dietary sugar beet pulp caused pigs to shift N excretion with 22% to 37% less urinary urea N compared to a control treatment (Canh et al., 1997). Therefore, the inclusion of high-fiber ingredients into poultry or pig diets has been shown by multiple researchers to cause a decrease in NH$_3$ emission.
**Acidifying additives**

Decreasing the pH of manure can have a profound impact on N volatilization. The pH of manure impacts the proportions of NH$_3$ and NH$_4^+$. The pKa of NH$_3$ is 9.23, which means at pH of 9.23, equal proportions of NH$_3$ and NH$_4^+$ exist (Brown et al., 2000). As pH decreases from 9.23, more of the NH$_3$ is in the protonated NH$_4^+$, which is more water-soluble and, therefore, less likely to volatilize and escape to the atmosphere. Machida and Nakanishi (1980) reported that the optimal pH for bacterial uricase, the enzyme that decomposes uric acid, is 9.5. At pH values below 9.5, the activity of the enzyme is decreased and less decomposition of uric acid would be expected in manure. Furthermore, low pH values may inhibit the growth and metabolism of uric acid-utilizing bacteria, which would decrease the rate of uric acid decomposition (Kim and Patterson, 2003). If manure can be altered such that pH is lower, a decrease in NH$_3$ emission would be anticipated.

Acidifying ingredients in the diet have been shown to decrease pH and cause lower NH$_3$ emission. One percent adipic acid added to pig diets caused a 2.2 point decrease in urinary pH and a 25% decrease in NH$_3$ emission (van Kempen, 2001). When a combination of calcium sulfate and phosphoric acid were included in pig diets, urine pH decreased 1.0 points and ileal digesta pH decreased 1.1 points (Kim et al., 2004). The decrease in pH was accompanied by a 30% decrease in NH$_3$ emission from pigs fed the calcium sulfate plus phosphoric acid diet.

**Other dietary treatments**

Extracts of *Yucca shidigera* have been used as a feed additive to lower NH$_3$ emission but inconsistent results have been reported (McCrory and Hobbs, 2001). The NH$_3$-lowering effect of the saponin fraction of *Y. shidigera* is primarily attributed to the inhibition of proteolytic microorganisms in the intestine and manure (Wallace
et al., 1994; Westendarp, 2005). A separate glyco-component was shown to bind NH₃ directly in aqueous solution when concentrations of NH₃ were low and the Y. shidigera extract was added in relatively large quantities (20%, vol/vol in vitro) (Wallace et al., 1994). A 40% decrease in aqueous NH₃ was reported when 300 mL glyco-component was added to artificial saliva (Ryan et al., 2003). Yeo and Kim (1997) fed broiler chicks a diet containing 0.2% yucca extract and found a decrease in urease activity of the small intestinal contents at three weeks of age. Because urease enzyme catalyzes the breakdown of uric acid to NH₃, a decrease in urease might lead to a decrease in NH₃ production from the manure. Indeed, Amon et al. (1997) showed a 50% decrease in NH₃ emissions from broiler-chicken housing when yucca extract was included in the diet.

Zeolites are hydrated alumino-silicate minerals that have been shown to have high affinity for NH₄⁺ (Mumpton and Fishman, 1977). Natural zeolites have a crystalline lattice-like structure and relatively high ion-exchange capacity, both properties that favor the binding of NH₄⁺. Zeolites have been included in poultry, swine, and cattle diets in Japan since 1965 (Mumpton and Fishman, 1977) and are still finding use in animal agriculture today.

A multitude of dietary strategies exist that can lower NH₃ emission from livestock and poultry production facilities. Combining dietary strategies may result in an even greater decrease in NH₃ emission compared to the emission observed when dietary strategies are applied separately. Wu-Haan, et al. (2007) fed laying-hens diets that contained calcium sulfate as an acidifier, zeolite as an NH₃ binder, and low crude protein and reported up to a 39% decrease in NH₃ emission. While NH₃ emission was decreased, an increase in H₂S emission was observed. The S in the calcium sulfate likely contributed to a 3-fold increase in H₂S emissions. The highest observed H₂S emission rate for hens fed diets containing calcium sulfate
was 7.1 mg/hen-d (Wu-Haan et al., 2007). At that emission rate, it would require 6.4 million hens at one production site to excrete 45.4 kg (100 pounds) H₂S per day, the EPCRA reportable quantity (CFR, 2009). Therefore, H₂S emission from laying-hen facilities when calcium sulfate is used as a dietary acidifier is not likely to approach the reporting threshold. However, if H₂S excretion does become problematic, the selection of an acidifying agent that does not contain S may be preferred.

In a commercial-scale study, Li et al. (2009) evaluated the effect of 3.5% dietary EcoCal, a proprietary mixture of calcium sulfate and zeolite, fed to laying hens in high-rise houses. A 23.2% decrease in NH₃ emission was reported compared to the emission from hens fed a control diet (Li et al., 2009). Similar to the H₂S emission increase observed by Wu-Haan et al. (2007), the S in the dietary calcium sulfate led to a 105% to 176% increase in H₂S emissions (Li et al., 2009).

A multitude of dietary strategies have been investigated with the goal of manipulating livestock and poultry waste to be less damaging to the environment. Dietary phytase improves the availability of plant-derived phosphorus in diets for monogastric animals (e.g., pigs and poultry), thereby decreasing P excretion. Low-crude protein, amino acid supplemented diets have been shown to decrease N excretion, which may be accompanied by a decrease in NH₃ emission. Dietary fiber lowers NH₃ emission by repartitioning N excretion from urinary N to bacterial protein and by causing an increase in the SCFA content of manure from pigs. Research indicates that this mechanism may also function in poultry. Dietary acidifiers shift N excretion from volatile NH₃ towards water-soluble NH₄⁺, which causes a decrease in NH₃ emission. Additionally, the more acidic environment does not favor the growth of ureolytic bacteria. The final dietary, pre-excretion manipulations considered in this review were the inclusion of yucca extract or a combination of zeolite and calcium sulfate. Most research with regards to yucca extract has been focused on ruminants.
Two components of yucca extract, namely a glyco-fraction and a saponin fraction, function separately to bind NH$_3$ and decrease bacterial urease, respectively. Zeolite is a mineral with a high binding affinity for NH$_3$ and, when combined with calcium sulfate has been shown to cause a marked decrease in NH$_3$ emission from poultry facilities.

**Post-Excretion Techniques**

Various manure treatments, manure management, and engineering practices have been developed and researched in an effort to lower NH$_3$ emission from livestock and poultry production facilities.

**Chemical treatments**

Decreasing manure pH through dietary alterations (e.g., diet acidifiers or high fiber) has been shown to cause a decrease in NH$_3$ emission from manure, and similarly, decreasing manure pH by the addition of chemical treatments after excretion has been shown to cause a reduction of NH$_3$ emission. The mechanisms of NH$_3$ reduction through pH decrease are the same whether caused by dietary manipulations or by post-excretion chemical application. Lower pH shifts the NH$_3$ and NH$_4^+$ equilibrium towards NH$_4^+$, decreases the activity of microbial uricase enzymes, and decreases the growth of bacteria that degrade uric acid. Liquid or dry alum (i.e., aluminum sulfate) decreases the NH$_3$ emission from stored manure or from litter in poultry houses.

Li et al. (2008) applied 1, 2, or 4 kg liquid alum/m$^2$ of laying-hen manure or 0.5, 1.0 or 1.5 kg dry granular alum/m$^2$ of manure and found NH$_3$ emission reductions between 63% and 94% compared to a control with no alum addition. Do et al (2005) applied 1.15 kg alum/m2 of floor space to fresh bedding in a commercial broiler house. The cumulative NH$_3$ concentration was 86% lower over a 42-d grow-
out period when the litter was treated with alum compared to NH₃ concentrations from non-treated controls (Do et al., 2005). Application of ferrous sulfate to fresh broiler litter resulted in a 91% decrease in cumulative NH₃ concentration during a 42-d grow-out period (Do et al., 2005).

However, there was no difference in pH observed in the litter from either the alum- or ferrous sulfate-treated litter after 42 d (Do et al., 2005). Over the duration of the trial, accumulation of manure may have influenced the pH of the litter to a greater extent than any residual pH decrease caused from the treatments to the fresh litter at the beginning of the study (Sommer and Husted, 1995). Kim and Patterson (2003) observed a decreased pH immediately after zinc sulfate was added to broiler manure but after a 21-d incubation period, with no additional manure accumulation, there was no difference in pH between treated and untreated manure samples.

Although the ferrous sulfate caused a decrease in NH₃ emission, an increase in mortality was observed, presumably from consumption of the litter material and subsequent iron toxicity (Do et al., 2005). Therefore, ferrous sulfate is a poor choice to be used as a chemical amendment to litter in a poultry house. It may be useful for application onto manure in storage buildings where birds would not have access to consume the litter material.

Kim and Patterson (2003) evaluated the effect of zinc sulfate on NH₃ loss from broiler manure in a laboratory-scale study. Manure treated with 1% or 2% zinc sulfate lost 15% and 26% less NH₃, respectively, over a 21-d incubation period compared to untreated manure (Kim and Patterson, 2003). Prior experiments had demonstrated that zinc sulfate was inhibitory to uricase enzyme activity and that zinc sulfate caused a decrease in the growth rate of uric acid-utilizing bacteria (Kim and Patterson, 2003).
Various other acidic compounds have been tested as possible treatments to lower NH₃ emission from poultry manure. Sulfuric, hydrochloric, nitric, phosphoric, and lactic acid have been considered as post-excretion treatments for poultry manure (McCrory and Hobbs, 2001). However, few have found practical use. For example, sulfuric, hydrochloric and nitric acid are inexpensive but quite corrosive and dangerous to handle. Stevens et al. (1989) used sulfuric acid to acidify pig and cow slurries and found a pH value of 6.0 was necessary to obtain an 80% decrease in NH₃ emission reduction from pig slurries and a further decrease to pH 5.5 was necessary for cow slurries. However, the authors acknowledged cost, safety, and possible side effects must be considered before sulfuric acid can be used as a manure treatment (Stevens et al., 1989). Phosphoric acid is relatively expensive and likely not economical for use in commercial production systems. The choice of a manure acidifier must be made based not only on efficacy of NH₃ reduction but also cost, safety, and practicality of use.

Zeolites have found efficacy as a feed additive but can also decrease NH₃ emission when applied directly to manure. Li et al. (2008) evaluated the topical application of zeolite to laying-hen manure at 2.5, 5.0, and 10% of manure weight and found that the 5.0 and 10% treatments caused a decrease in NH₃ emission over the 14-d monitoring period. The 2.5% application decreased NH₃ emission only during the first 7 d of the trial. To simulate addition of manure to a storage facility as would be the case for a manure-belt production system, Li et al (2008) evaluated NH₃ emission when fresh manure was added every other day and zeolite was applied at 5% of accumulated manure weight with each manure addition. The NH₃ emission was decreased 33% when zeolite was added along with fresh manure every other day (Li et al., 2008).
**Engineering strategies**

While dietary modifications and chemical treatments can lower the NH$_3$ emission from poultry manure, engineering methods such as manure handling and exhaust air treatment also cause a decrease in NH$_3$ emission to the atmosphere. Li et al. (2005) reported lower NH$_3$ emission from laying-hen manure with a lower surface-to-volume ratio. Ammonia is primarily lost from the top layer of a manure stack; therefore, minimizing the surface area of a manure stack is a valid method to decrease emissions. In the same study, Li et al. (2005) evaluated the effect of ventilation rate on NH$_3$ emission from stacks of laying-hen manure and found that higher ventilation rate caused increased NH$_3$ emission after four weeks of storage. Therefore, decreasing the ventilation rate of manure-storage structures can contribute to a lower overall NH$_3$ emission from egg-production facilities.

An electrostatic space charge system was originally evaluated for its effectiveness in decreasing dust and airborne bacterial counts in incubators and hatchers. Dust concentrations were decreased by an average of 93.6% in hatching cabinets from pipping (the onset of hatch) until the chicks were removed from the cabinets (Mitchell et al., 2002). Because NH$_3$ attaches to dust particles, it follows that a similar decrease in airborne dust in poultry houses could lead to a decrease in NH$_3$ concentrations and, hence, NH$_3$ emissions. Ritz et al. (2006) tested an electrostatic space charge system in a commercial broiler house during the grow-out of 7 flocks and found a 43% decrease in dust concentrations and 13% decrease in NH$_3$. The reduction of NH$_3$ concentration was generally higher when NH$_3$ concentrations were higher during cooler months of the year.

Biofilters have received much attention with regards to aerial emissions from livestock and poultry housing. A biofilter is a porous layer of organic material (typically wood chips) that is kept moist to support bacterial populations. Building
exhaust air is passed through the biofilter where the bacteria convert odorous compounds to carbon dioxide and water (Nicolai et al., 2008). The average odor removal of biofilters was 44% and NH₃ removal was 70% on pig farms in the Netherlands (Melse and Ogink, 2005). However, multi-stage air scrubbers can increase the efficiency of NH₃ removal from exhaust air compared to a single-stage biofilter. The first stage is a dust-removal system that includes a packing material over which water is recirculated. The second stage is an acid scrubber to remove NH₃ and the third stage is a biofilter to remove odor. Melse et al. (2008) reported that multi-stage scrubbers removed an average of 83% of NH₃ and 40% of odor. Multi-stage scrubbers have been developed mainly in Europe, where air emission regulations are more stringent compared to those in the US. However, as human and animal populations come into closer proximity, such technologies are likely to become more common on farms in the US.

**Corn Distiller’s Dried Grains with Solubles**

As the production of fuel alcohol has become more important in the United States, the supply of corn DDGS, a primary by-product of corn fermentation, has increased exponentially. Furthermore, high corn grain prices have made DDGS more favorable in least-cost diet formulations. Corn DDGS has become an important ingredient in livestock and poultry diets.

Various research studies have been designed to investigate the optimal inclusion of corn DDGS in monogastric animal (e.g., pig and poultry) diets. In the early days of computer diet formulation, studies were conducted to determine the nutritional value of DDGS and the appropriate inclusion rate in poultry diets. Runnels (1966) performed a series of three experiments in an effort to establish the biological value of DDGS compared to soybean meal. Although the study was unsuccessful in
determining the appropriate nutrient parameters to use for DDGS in computer diet formulations, results indicated that up to 20% dietary DDGS could support adequate production performance of broiler chickens. It is interesting to note that the unexpectedly good performance of the birds in Runnels’s trial led him to speculate that corn DDGS should be allowed a biological nutrient value equal to that of soybean meal or that unidentified growth factors must be present in the ingredient. An early study by Waldroup et al. (1980) concluded that 20% DDGS was the maximum inclusion that should be used in broiler chicken diets. Decreases in feed efficiency were observed when birds were fed 25% DDGS. The authors of the two aforementioned papers did not state the source of the DDGS.

Recent research has focused on the maximum inclusion rate of corn DDGS in modern high-nutrient dense diets. Lumpkins et al. (2005) fed laying hens commercial or low-density diets and determined 10–12% DDGS was the maximum inclusion that should be used. The 15% DDGS supported production in the commercial diet. However, the low-density diet provided a more-sensitive analysis and showed decreased production at the 15% inclusion rate. The diets used were formulated on a total amino-acid basis, which may have resulted in a lysine deficiency in the 15% DDGS diets (Lumpkins et al., 2005). Masa’deh and Scheideler (2008) reported decreased egg weight from hens fed greater than 15% DDGS and hens fed 20% or greater DDGS had lower weight gain. Feed intake, egg production, haugh units, and specific gravity were not affected up to 25% inclusion of DDGS. Again, diets were formulated in a total amino-acid basis, which may have led to a deficiency. Roberson et al. (2005) reported that 15% dietary DDGS was not detrimental to production of laying hens post-peak.

Contrary to research indicating 15 or 20% maximum DDGS inclusion in laying-hen diets, Pineda et al. (2008) fed diets containing up to 69% DDGS. Higher
inclusions of DDGS caused an increase in egg weight and a decrease in egg production. Treatment diets were fed for eight weeks and the authors speculated that metabolic imbalances, namely ketosis and fatty liver, might arise with long-term feeding of diets with extremely high contents of DDGS and low starch content. Future research should focus on the long-term effects of high DDGS inclusion rates.

Several factors limit the inclusion of DDGS in commercial poultry diets. The nutrient variability has long been implicated as the main limiting factor to higher DDGS use in pig and poultry diets. However, Spiehs et al. (2002) reported coefficients of variation between 5% and 10% for dry matter, calculated ME, crude protein, crude fat, and crude fiber among 118 samples collected across three years from ten ethanol plants in South Dakota and Minnesota. The variability was generally higher for total lysine and methionine content (coefficient of variation = 17.3 and 13.6, respectively). Among ten Dakota Gold DDGS samples obtained from ten different ethanol plants, lysine standardized ileal digestibility in pigs varied from 43.9% to 63.0% and the methionine digestibility varied from 73.9 to 84.7 (Stein et al., 2006). Among five samples sourced from five ethanol plants, another study reported total lysine variability from 0.48 to 0.76% with apparent lysine digestibility ranging from 38.6% to 69.5% (Fastinger et al., 2006). Methionine and lysine are typically the first- and second-limiting amino acids, respectively, in corn-soybean based poultry diets. Therefore, while many of the nutrients in DDGS may be consistent both within and between plants, variations in lysine and methionine can severely impact the value and inclusion rates of DDGS.

Mycotoxin content of DDGS may be a concern if the ethanol plant accepts contaminated corn (Shurson et al., 2003). Of the original corn, approximately 1/3 becomes ethanol, 1/3 becomes carbon dioxide, and 1/3 becomes DDGS. Because mycotoxins do not leave the system in the ethanol or the carbon dioxide and
because they are not inactivated by fermentation or heat, the DDGS will contain approximately three times the mycotoxin content as the corn that was used. Mycotoxins can be quite detrimental to bird health and performance with aflatoxin causing liver damage, tricotheces affecting protein metabolism, and ochratoxin impacting kidney function (Leeson and Summers, 2005).

Sodium content of DDGS should be considered when included in laying-hen diets. Batal and Dale (2003) evaluated the mineral content of 12 DDGS samples obtained from the upper Midwest United States and found the sodium content generally varied from 0.09 and 0.12%, much lower than the 0.48% reported by NRC (NRC, 1994). However, while most samples contained relatively low sodium, one plant consistently provided samples with 0.39–0.44% sodium (Batal and Dale, 2003). The source of the sodium was unclear and was attributed the inclusion of solubles from other manufacturing streams that may have contributed sodium to the final DDGS product. Dietary sodium content can influence water consumption and production. In a class demonstration, nearly total cessation of egg production was demonstrated when diets contained insufficient sodium (Batal and Dale, 2003). Leeson and Summers (2001) reported increased water consumption in birds fed 0.35% dietary sodium with toxicity at 0.50% total dietary sodium. However, Shaw et al. (2006) found that of nitrogen, calcium, phosphorus, sodium, potassium, or chloride, only daily intake of nitrogen and potassium were correlated with water intake in pigs and sodium did not directly impact the water consumption.

Considering the variation in nutrient content, possibility for mycotoxin contamination, and drastic differences in sodium content, laboratory analyses should be conducted for DDGS samples before diet formulation. With adequate knowledge of the characteristics of DDGS and a consistent supply, as with any feed ingredient,
nutritionists can successfully include it in livestock diets that support optimal production.

**Manure Sampling**

Accurate assessment of manure nutrients allows managers and agronomists to apply manure at the appropriate rates to crop fields, assuring that excess nutrients are not applied to a given parcel of land and that crops will have adequate resources for optimal yields. However, many poultry producers are not also crop farmers and do not own much if any land adjacent to the production site, on which to spread manure. Therefore, much of the manure produced in the United States is sold to local crop producers based on fertilizer value (i.e., nutrient contents) (Leibold and Olsen, 2007). Accurate sampling and analytical techniques are necessary to establish a fair price that reflects the value of manure for agronomic purposes and to avoid over-application of nutrients, which may lead to pollution of area ecosystems.

Although tables and reference values for manure nutrient contents are published (MWPS, 1993; Collins et al., 1999), the actual nutrient contents of manure varies depending on many factors. Season, characteristics of the animals' feed, housing scheme, use and type of bedding, and manure handling can all impact manure nutrients, which can be drastically different from published values (Rieck-Hinz et al., 1996; Lorimor and Xin, 1999; Dou et al., 2001; Derikx et al., 2007). For example, Peters (2000) reported that the N content of solid dairy manure varied between 1.5 and 16.5 g/kg for 388 samples submitted over a 6-year period. Lorimor and Xin (1999) examined the nutrient contents of laying-hen manure on four farms in Iowa and reported that the average observed N content was 42% lower, P was 60% higher, and K was 58% higher than published manure values for Iowa. In addition to inherent variability in the manure, sampling technique can have a significant impact
on the results of nutrient analyses (Derikx et al., 2007). Therefore, sampling with consistent methodology and subsequent laboratory analyses are necessary to establish the actual nutrient contents of manure before land application.

Manure samples can be collected from manure storage or during load-out and subsequent land application. Dou et al. (2001) reported that sample-to-sample variability was much lower when manure was agitated either in liquid or solid handling systems and the mixing that occurred during load-out of broiler manure was sufficient to decrease sample variability. Similarly, Peters (2000) recommended sampling stacked or bedded-pack manure (in this case, dairy manure) during load-out rather than directly from the stack in order to get a representative sample. However, while uniform samples may be obtained during the manure load-out, nutrient analyses performed at commercial laboratories cannot be available for determining land application rates. Sampling manure from the storage area, several days before load out, allows sufficient time for analyses such that the actual manure nutrient contents can be used to calculate application rates.

**Effects of DDGS on manure nutrient contents**

Livestock diets directly impact the characteristics and nutrient contents of the manure (Powers and Angel, 2008), and inclusion of DDGS in poultry diets is no exception. Increasing DDGS from 0% to 69% of laying-hen diets caused a linear increase in N excretion and a decrease in dry-matter digestibility (Pineda et al., 2008). Although DDGS contains more P compared to corn, reports have indicated the bioavailability of P in DDGS is higher than that for corn (Martinez Amezcua et al., 2004; Lumpkins and Batal, 2005). When dietary formulations consider the higher P availability in DDGS and especially if phytase is included in the diet, total P contents in the diet can be decreased and lower P excretion would be anticipated. However, if
the availability of P in the DDGS is not considered, an increase in P excretion may be realized.

Theoretically, DDGS may not significantly impact nutrient management plans. Regassa et al. (2008) developed a computer simulation to predict land and labor requirements to apply laying-hen manure to crop ground when 0%, 8%, or 16% DDGS were included in the diet. While the DDGS caused slightly higher manure application costs attributed to increased land requirements, other management decisions such as N-based, 1-year P-based, or 4-year P-based application rates impacted the manure management plan much more significantly. However, when land available for manure application is severely limited, increased manure nutrient contents might warrant more attention.

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CHAPTER 3. EFFECTS OF DIETARY 
CORN DISTILLER’S DRIED GRAINS WITH SOLUBLES 
ON AMMONIA EMISSION, MANURE NUTRIENTS, AND 
EGG PRODUCTION PARAMETERS 
FOR LAYING HENS IN HIGH-RISE HOUSES

A paper to be submitted to the Journal of Applied Poultry Research

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Summary

This one-year field study was conducted to evaluate the effect of including 
15% dietary corn distiller’s dried grains with solubles (DDGS) in Hy-Line W-36 
laying-hen diet on ammonia (NH₃) emission, nitrogen (N) mass balance, production 
performance, manure characteristics, and economic efficiency for commercial high-
rise laying-hen houses. We hypothesized that the 15% DDGS diet would cause a 
decrease in manure pH, which would lead to reduced NH₃ emission, as compared to 
the control diet (i.e., no DDGS added). Six high-rise houses, in three pairs, on a farm

¹ Mention of trade name, proprietary product, or specific equipment does not 
constitute a guarantee or endorsement by Iowa State University and does not 
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in Iowa were used. Although manure pH was lower for the DDGS regimen, there
were no differences in NH$_3$ emission, measured over a 1-year period, between the
control and DDGS regimens. The N consumption was higher for the DDGS regimen
and N deposition in eggs was higher, attributed to the higher egg mass. Economic
analyses revealed lower feed cost for the DDGS regimen than the control. The
DDGS diet had no adverse effects on production and was suitable for commercial
production. However, a decrease in NH$_3$ emission was not observed.

**Description of the Problem**

The emission of NH$_3$ is a serious concern for the livestock and poultry
industries [1]. Poultry production has been implicated as a major anthropogenic
source of NH$_3$ emission, which damages area and regional ecosystems [2-4].
Ammonia not only can be damaging to the environment, it is detrimental to indoor air
quality and the health of workers and birds [5-7]. Methods to decrease NH$_3$ emission
from egg-production facilities have great potential to decrease the overall
environmental impact of livestock and poultry production.

A previous *lab-scale* study [8] had revealed substantially lower (~ 40%) NH$_3$
emission from manure of laying hens fed diets containing 10% corn DDGS. We
hypothesized that dietary corn DDGS would cause lower NH$_3$ emission from
commercial egg-production facilities by causing a decrease in manure pH while
causing no negative impacts on egg production or economic return. Therefore, the
objective of this *field-scale* study was to evaluate the effects of feeding diets
containing 15% corn DDGS to hens in high-rise houses on the following
environmental and production parameters: NH$_3$ emission rate (ER), egg production,
manure characteristics, nitrogen (N) balance, and economic efficiency.
Materials and Methods

Hens and Housing

A total of six high-rise laying-hen houses located on a commercial farm in central Iowa were used for this study (Table 1). The Hy-Line W-36 laying hens were housed at 61 in²/hen (394 cm²/hen). Dietary regimens were assigned according to a randomized complete block design with three blocks and two treatments. Houses were blocked such that the two houses in each block (pair) contained a similar number of hens of similar age. Blocking avoided confounding effects on egg production, nutrient density of the diet, and manure production due to hen age and also avoided differences in NH₃ emission due to the number of hens in the house.

Hens in one house in each pair were randomly assigned a diet containing 15% corn DDGS (treatment). Hens in the other house in each pair were fed a diet that contained 0% corn DDGS (control). The study began in November after complete manure cleanout and continued for 12 months until manure cleanout the following October.

The cooperating producer formulated all diets. Example diets, fed from 85 to 80% egg production and 91 to 93 g/ hen-d feed consumption, are shown in Table 2. Diets fed throughout the study were chosen from a library, which included 21 control diets, 23 corn DDGS diets, and 4 molt diets. Formulations were adjusted throughout the study in consideration of hen age, feed consumption, egg size, and egg production. All corn DDGS treatment diets included 15% DDGS with the exception of the molting period when pre-molt diets contained 10% DDGS and post-molt diets contained 0% DDGS.

Corn DDGS was sourced from new-generation fuel ethanol plants in Iowa. Diets were corn- and soybean meal-based and fed in mash form. Each of the six
flocks was molted once during the study using a wheat middlings and fine limestone non feed-withdrawal molting regimen as prescribed by the United Egg Producers [9]. The cooperating producer made all management decisions with regards to animal care according to commercial practices and conditions.

**Ammonia Emission Measurement**

Portable monitoring units (PMU) [10, 11] were used to measure NH₃ and carbon dioxide (CO₂) concentrations of the exhaust and intake air in each house. One PMU was placed at each end of each house (12 PMU total, Figure 1). Each PMU contained 2 electro-chemical sensors (0–200 ± 3 ppm; PAC III, Draeger Safety, Inc., Pittsburg, PA) for NH₃ measurement and an infrared sensor (0–7,000 ± 20 ppm, model GMT222, Vaisala, Inc., Woburn, MA) for CO₂ measurement. The use of dual NH₃ sensors provided redundancy measurement to ensure data completeness and quality. A programmable on/off timer controlled a three-way solenoid valve, which regulated the air entering the unit as either incoming air or exhaust air from the laying-hen house. Incoming air was sampled from the attic space and exhaust air was sampled from two composite points approximately five meters (16.4 ft) from the exhaust fans. To avoid saturation of the NH₃ sensors, six minute sampling of exhaust air was followed by 24 min purging with incoming (fresh) air throughout the measurement periods.

The temperature was measured at two locations inside each house at approximately one-third and two-thirds the length of the house in an empty cage in the third of five tiers in the second of five rows from the north side of the house. The temperature was measured using portable temperature loggers (0°C–50°C ± 3%, HOBO Pro RH/Tmp, Onset Computer Corporation). Temperature was also monitored outdoors using the same type of loggers. Static pressure of each building
was measured with an electronic static pressure sensor (4–20 mA ± 1% for 1 to 125 Pa, Setra model 262, Stage, Inc., Pittsburgh, PA). All measuring devices were placed in the houses at the beginning of the study and remained until the end. Gas measurements were taken over two consecutive days (e.g., Tuesday and Wednesday) every other week throughout the 12-month experiment. Loggers were cleared and launched at the beginning of the measurement period and data were collected from the loggers at the end of each measurement period. The NH₃ sensors were calibrated before each measurement period and were verified after each measurement period. The CO₂ sensors were calibrated every three months throughout the study.

The ventilation rates were calculated using the CO₂ balance method, based on the principle of indirect animal calorimetry [12] and respiratory quotient values determined by Chepete et al. [13]. The NH₃ ER for each two-day measurement period was calculated according to the following equation, referred to as the concentration × flow integration (CFI) method:

\[ ER = Q \times (C_0 - C_i) \times 10^{-6} \times (W_m/V_m) \times (T_{std}/T_a) \times (P_a/P_{std}) \]  

[1]

where:

ER = emission rate (g NH₃ per day)
Q = ventilation rate (L/min)
Cᵢ = ammonia concentration at building inlet (ppm)
C₀ = ammonia concentration at building outlet (ppm)
Wₘ = molar weight of ammonia (17.031 g/mole)
Vₘ = molar volume of ammonia at standard temperature (0°C) and pressure (101.325 kPa) (22.4 L/mole)
T_{std} = standard temperature (273.15 K)
\( T_a \) = ambient temperature in K (°C + 273.15)

\( P_{\text{std}} \) = standard barometric pressure (101.325 kPa)

\( P_a \) = ambient barometric pressure (kPa)

The ER was considered representative of the week before and the week after the measurements were taken to determine ER throughout the year. The amount of NH\(_3\)-N lost from each house was calculated as the amount of NH\(_3\) multiplied by 0.8224 (weight of N in NH\(_3\)/total weight of NH\(_3\)).

Although feeding of treatment diets and data collection were initiated the beginning of November 2006, reliable NH\(_3\) emission data became available from the beginning of May 2007 through the end of the study (due to technical issues encountered with the NH\(_3\) sensors early in the trial). Commercial electro-chemical \( \text{NH}_3 \) sensors were used in the PMU. Electrochemical sensors contain an electrolyte solution and a series of electrodes [14]. The chemical of interest must first diffuse across a selectively permeable membrane to a working electrode, then into the electrolyte solution, and finally react with a counter electrode and a reference electrode. Changing the properties of the electrolyte solution directly affects the reaction of the target gas with the electrodes in an electrochemical sensor. The research group had used the PAC III electrochemical NH\(_3\) sensors in several previous experiments. However, the chemical formulation of the electrolyte in the sensors had been changed prior to the current study and the new sensors unexpectedly did not give reliable results. After several months of testing prototype sensors, reliable readings were obtained beginning in May 2007.

**Nitrogen (N) Mass Balance**

Nitrogen (N) mass balance is a process-based measurement of the flow of N into and out of an animal-production system. These measurements are important in
determining the fate of N required for egg production and in calculating the proportion of N entering the system that is converted to egg N and the proportion that is lost as NH$_3$. The calculated N loss was compared to the measured N loss from the NH$_3$-emission data obtained from the CFI measurements. The N mass balance was calculated for each house according to the following equation:

$$N_{\text{feed}} - N_{\text{egg}} - N_{\text{manure}} - N_{\text{mortality}} - N_{\text{tissue}} = \text{NH}_3\text{-N}$$  \[2\]

where $N_{\text{feed}}$ is the N consumed in the feed, $N_{\text{egg}}$ is the N deposited in eggs; $N_{\text{manure}}$ is the N retained in manure, $N_{\text{mortality}}$ is the N increment from weight gain in mortalities; $N_{\text{tissue}}$ is the incremental body N deposition from weight gain; and NH$_3$-N is the N volatilized into the atmosphere in the form of NH$_3$. Feed N values were calculated weekly as 16% of the crude protein contents of the diets as reported in the formulations provided by the cooperating producer. The $N_{\text{feed}}$ was calculated by multiplying each week’s feed consumption by the corresponding week’s feed N value. Eggs were considered to contain 1.70% N as reported by Roberts et al. [15]. The $N_{\text{egg}}$ was calculated by multiplying each week’s egg mass by 1.70%. Body weight gain of live or dead hens was considered to contain 4.08% N as reported by Haque et al. [16]. The $N_{\text{tissue}}$ was calculated by subtracting the initial body weight at the beginning of the study from the body weight measured the week of the mortality and multiplying that weight gain by 4.08%. Subtracting the initial body weight was done so that only N gained during the study, and hence N represented in the $N_{\text{feed}}$ term, was considered as lost due to mortality. All the N input and output terms were expressed as grams per hen per day.

The NH$_3$ emission, calculated according to the N-mass balance, was reported as grams of NH$_3$ per hen per day, grams of NH$_3$ per kilogram of feed N, and grams of NH$_3$ per kilogram of egg production. The use of multiple units to express the NH$_3$
emission considers differences in feed N consumption between the two dietary treatments as well as potential differences in egg production.

Production Performance

Hen performance data were obtained from farm records weekly throughout the 12-month study. Performance data included hen age, house population, mortality, egg production, feed consumption, feed cost, egg weight, water consumption, and body weight. House population was calculated as the difference between the initial number of hens housed and the number of mortalities removed through the end of each week. Mortality was counted daily when dead hens were removed from the house and the value was summed for each week. The number of eggs collected each day was counted using mechanical counters positioned along the egg elevator on each tier of each cage row. Egg production percentage was calculated by dividing the number of eggs produced daily by that day’s population and a weekly average was reported.

Feed consumption was measured as feed disappearance according to the weight of feed delivered by truck to each house during each week. Feed cost was calculated by the cooperating producer and reported as dollars per 2,000-pound ton of feed. Egg weight was measured weekly by collecting and weighing 180 eggs from each house. Egg mass (grams per hen) was subsequently calculated as egg weight (grams per egg) multiplied by egg production (percent).

Water consumption was measured using one water meter for each cage row (i.e., five water meters per house). Water meters were read daily and water consumption was calculated for each week as the sum of the water use for all cage rows in each house. Body weight was measured by weighing the same 100 hens in each house once per week. Performance data during the molt period, defined as the
time between the first week that a molt-specific diet was fed and the last week egg production was below 70% (i.e., typically nine weeks), were excluded from analyses.

**Manure Sampling**

Fresh manure samples, collected once per month, were used for pH measurement. Samples were collected from the scraperboard below the bottom tier of cages in the second and fourth of five cage rows. Approximately 200 g of manure was collected from each location. The pH of each manure sample was measured (Accumet AR-15, Fisher Scientific, PA) on the same day as collection by mixing one part manure (approximately 1 g) with 10 parts double-distilled water with a vortex mixer. Each sample was measured in duplicate.

Manure samples for N and phosphorus (P) analysis were collected from each house immediately before manure cleanout at the end of the study. Core samples of manure were collected using a probe (see Chapter 5 of this dissertation). Samples were collected at nine locations evenly spaced ¼, ½, and ¾ the length of the house and along the two outside and the middle rows of the five total manure rows. Two samples were collected side-by-side at each location giving a total of 18 samples per house.

The collected manure samples were stored at −20°C (−4°F) in plastic zip-top bags and subsequently freeze-dried. Manure samples were ground through a 1-mm screen and analyzed for N content using the micro-Kjeldahl method 988.05 with selenium catalyst [17] and a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA). The manure P content was analyzed using the photometric method 965.17 [17] adapted to a 96-well plate.
Economic Analyses

Economic analyses included calculation of feed cost, egg income, and manure values. Return over feed cost was calculated as the difference between the sum of egg and manure income and feed cost. The cooperating producer reported feed cost weekly in dollars per 2,000-pound ton (Figure 2), which was converted, using records of feed consumption, to feed cost per hen weekly. To calculate egg income, the egg mass was converted to dozens of eggs assuming an average case minimum net weight of 48 pounds per case (a case is 30 dozen eggs or 360 eggs). Central states breaking-stock prices [18] for each week throughout the study were used to calculate egg value (Figure 3). Although the actual case weight of eggs was known, the adjustment to 48-pound case equivalents was made because breaking-stock prices are reported only for 48-pound case weights.

Manure was valued based on N and P contents (personal communication, Angela Rieck-Hinz, Iowa State University Extension). Anhydrous ammonia (82.4% N) price of $523/ton and di-ammonium phosphate (21.2% N, 23.5% P) price of $442/ton, as reported for 2007 [19], were used to calculate manure N and P values. The value of manure per 1,000 hens per day was calculated for the situation where N and P both have value (e.g., for manure application onto land requiring both N and P fertilizer for crop production) and also for the situation where only the N was required (e.g., for application onto land requiring only N, in which case, the P would have no value to the crop producer). Actual manure values paid to the cooperating producer for nutrient application to local crop ground were reported at the end of the study.
Statistical Analyses

Statistical analyses were performed using JMP (version 7.0.2, SAS Institute Inc., Cary, NC). Data were analyzed according to a randomized complete-block design using analysis of variance (ANOVA) with house as the experimental unit. The model included the effect of diet and pair. Mortality data were not normally distributed so the percentages were arcsine-square root transformed prior to analysis [20]. A $P$-value less than or equal to 0.10 was considered significant.

Results and Discussion

Ammonia Emission

Ammonia ER was determined based on the CFI measurements as well as the N mass balance calculations (Equation 2). In either case of determination methods, NH$_3$ emission was not different between the two dietary regimens (Figure 4). In this study, CFI data were only available for the summer months, as described previously; whereas N mass balance was based on the entire (one-year) study period. The NH$_3$ ER calculated from N mass balance for the control regimen was 118% of the value measured by the CFI method. For the DDGS regimen, the mass-balance value was 106% of the value measured by the CFI method. The differences could have been attributed to incomplete coverage of the measurements by the CFI method and inherent uncertainties associated with both methods.

Liang et al. [21], using similar instrumentation, found from 1.1% to 5.6% disagreement between N mass balance and CFI measurements with the N mass balance resulting in higher NH$_3$ ER in three of four houses studied. Although some numerical differences in NH$_3$ emissions existed between the regimens in the current study, none of the differences were statistically significant.
Ammonia ER was also calculated as grams NH₃ per kilogram feed N consumed and grams NH₃ per kilogram egg production (Table 3). The trend towards lower NH₃ ER per kilogram of feed N consumption from the DDGS hens compared to the control indicates that the DDGS hens partitioned N consumption to a greater extent to egg production or non-volatile manure N rather than uric acid. The N partitioning observed in this study is discussed in more detail in the following section. The NH₃ ER per kilogram of egg production was somewhat lower from the DDGS hens compared to the control hens. Lower NH₃ emission per unit of production would allow egg producers to supply similar quantities of eggs at reduced NH₃ emission.

Ventilation rate might have been higher in the treatment houses compared to the control because NH₃ ER was not different between the two regimens and manure moisture content was lower (i.e., higher dry-matter content) in the presence of greater water consumption for the DDGS treatment. Higher ventilation might have increased NH₃ loss [22, 23] and dried the manure more in the DDGS houses. However, ventilation rate was not different between the control and DDGS regimens (3.62 and 3.58 ± 0.25 CFM/hen; P = 0.92).

**Nitrogen (N) Mass Balance**

Nitrogen (N) mass balance values are shown in Table 4. The DDGS hens consumed more N than the control hens. The higher N consumption was expected because amino acids in high-fiber ingredients (e.g., DDGS) are typically less digestible than those in low-fiber ingredients [24], requiring consumption of greater amounts of total amino acids (and, therefore, N) to satisfy the requirements for digestible amino acids. Furthermore, Stein et al. [25] found lysine apparent ileal digestibility for pigs in corn DDGS sourced from 10 different ethanol plants ranged
from 35% to 55%. When the amino acids digestibilities of corn DDGS vary so widely, excesses must be added to the diet to ensure hens consume adequate amounts of nutrients to support egg production.

While DDGS hens consumed more N than the control hens, they also deposited more N in the eggs. The greater egg mass observed from the corn DDGS treatment (Table 5; discussed in the next section) contributed to the higher egg N deposition. Additionally, the DDGS hens excreted a greater proportion of consumed N in the manure (27.3% vs. 20.1% for the control hens). The calculated NH₃-N emission was not different between the control and corn DDGS treatments.

Although the hens fed the DDGS diets consumed more N, NH₃ emission was not higher. Therefore, NH₃ emission is not directly proportional to N consumption for all dietary regimens. The DDGS diets fed in the current study led to greater N deposition in eggs and a trend towards higher manure N deposition, thereby directing feed N away from NH₃ emission.

**Production Performance**

There were no significant differences in egg production or egg weight (Table 5) between the control and corn DDGS dietary regimens. However, egg mass was greater for the DDGS treatment than the control. Numerically higher egg production and egg weights likely contributed to the increased egg mass because egg mass is the product of egg production and egg weight. The control and DDGS diets were both formulated by the cooperating producer and both were expected to support optimal hen performance.

Feed consumption was not different between the two dietary regimens. Feed conversion, calculated as grams of egg output per kilogram of feed consumption,
was better for the DDGS regimen than for the control. The higher egg mass observed for the DDGS regimen led to the improved feed conversion.

Water consumption was 9.5% higher for the DDGS hens than for the control hens. Corn DDGS may contain up to 0.50% sodium (Na), depending on the source [26]. In a survey of DDGS samples collected from 12 manufacturers, most samples contained between 0.09 and 0.12% Na, however, one plant consistently produced samples with 0.39–0.43% Na [27]. Dietary Na can have an impact on water intake. Leeson and Summers [28] reported increased water consumption in birds fed 0.35% dietary Na with toxicity at 0.50% total dietary Na. However, Shaw et al. [29] found that of N, Ca, P, Na, K, or Cl, only daily intake of N and K were correlated with water intake in pigs. The correlations between N and K consumption and water consumption were weak (r = 0.39 and 0.32, respectively) and Shaw et al. [29] concluded that other factors affect water intake to a greater extent than the effects of diet. Although water consumption was higher for the DDGS hens, the 4.7 gallons/100 hens observed in this study was well within the expected daily water consumption of 4.0 to 5.5 gallons/100 hens for Hy-Line W-36 hens [30].

Body weight and mortality rate of the hens were not different between the two dietary regimens. Hence, the production performance data showed that there were no differences in feed consumption, egg production, egg weight, hen body weight, or mortality between the 15% corn DDGS and the control regimens, as measured over one year, including performance data from the first and second cycles of lay.

**Manure Properties**

Manure properties are shown in Table 6. The manure N content (expressed on a dry-matter basis) was not different between the dietary regimens. The manure N content measured in this study was similar to the 3.0 ± 0.8% N in laying-hen
manure sampled from 4 high-rise houses in central Iowa and reported by Lorimor and Xin [31]. Patterson and Lorenz [32], however, found 4.8 ± 1.8% N in laying-hen manure from five flocks in Pennsylvania, which is relatively higher than the 3.28% and 3.24% manure N observed in the current study.

Manure from the control hens contained more P than that from the DDGS hens. The control and DDGS diets were formulated to contain similar amounts of total P. Although there is more P in corn DDGS than in corn grain [26, 33], the P in corn DDGS has been reported to be more bioavailable [34, 35]. Lumpkins and Batal [35] suggested the higher availability might be due to the formation of microbial phytases during the fermentation process. Martinez A mezcu a [34], however, suggested that higher drying temperatures might increase P availability because the highest phosphorus availability was observed in the sample with the lowest lysine digestibility. Low lysine digestibility is typically associated with the presence of Maillard-reaction compounds developed during excessive heating of a feedstuff. In a follow-up study, Martinez Amezcu a [34] observed higher P availability in corn DDGS samples that were autoclaved compared to samples that were not autoclaved (87% and 75%, respectively) The P content of manure from the control regimen was similar to the 3.1% and 3.0% reported by Lorimor and Xin [31] and Patterson and Lorenz [32], respectively. The P content of the DDGS manure was slightly lower than the average literature values.

Although the DDGS hens consumed more water, the manure of the DDGS regimen was drier than that for the control. The observed higher water consumption conflicts with the measured drier manure and it was difficult to explain. There was a trend towards higher excretion of dry-matter manure from the DDGS hens, however, feed consumption was not different between the two dietary regimens; thus any increase in dry-matter excretion could not be attributed to higher feed consumption.
The dry-matter digestibility of corn DDGS has been reported to be relatively low compared to that of corn. Pineda et al. [36] found that hens fed diets with increasing DDGS content excreted more manure on a dry-matter basis. Pineda et al. [36] observed that fecal dry-matter digestibility decreased linearly with increasing dietary DDGS content from 0 to 69% inclusion. Additionally, in a study with pigs, Stein et al. [25] found that the dry-matter digestibility of diets containing 67% corn DDGS (and 27% corn starch) was 76% compared to 88% dry-matter digestibility for a control diet containing 0% corn DDGS and 97% corn grain. Lower dry-matter digestibility indicates that more of the consumed dry matter from the diet will be excreted, as the hen does not digest it.

The dietary DDGS was anticipated to cause a lower manure pH. In slurry, pH is primarily influenced by short-chain fatty acid (SCFA; e.g., acetate) content [37]. Canh et al. [38] found that higher fiber content in the diets for growing pigs caused an increase in slurry SCFA content and the increased SCFA content was accompanied by a decreased slurry pH and lower NH₃ emission. Lower pH shifts the equilibrium of ammonium (NH₄⁺) and NH₃ towards NH₄⁺, which is more water soluble and less volatile compared to NH₃.

We hypothesized that the fiber in the DDGS would increase bacterial growth and metabolism in the hen’s intestine, which would increase SCFA concentration in the intestine and in the manure. Indeed, a lower manure pH was observed for the DDGS regimen compared to the control. The lower pH is in agreement with our previous research [8] where pH was decreased from 7.08 in the manure of control-fed hens to 6.77 in the manure of DDGS-fed hens. In the laboratory-scale study, the DDGS regimen led to a lower NH₃ emission, which was attributed to the lower manure pH. However, the lower pH in the present commercial-scale study was not accompanied by measurable decrease in NH₃ emission.
The manure pH decrease may not have been sufficient to affect NH₃ emission over the relatively long duration of the study. Factors other than the characteristics of the diet, such as manure moisture content, ventilation rate, and temperature affect NH₃ emission. These influences, external to the treatment assigned in the study, are difficult if not impossible to control on a commercial egg-production farm. Furthermore, external factors may have influenced NH₃ emission to a greater extent than any influences of the lower pH, thereby making the emission rates similar between the treatments.

**Economic Analyses**

Economic analyses were performed to evaluate the effects of the 15% DDGS diet on return over feed cost and the effects of the treatment diet on the manure values based on N and P content. The feed cost was lower for the DDGS regimen both when expressed per hen and per unit of egg production (Table 7). Egg income and return over feed cost were not different between the two dietary regimens. A relatively small number of experimental units (i.e., houses; n = 3) were used for this study, which makes it difficult to detect small differences in responses. If responses had been measured for more houses, the numerically higher egg income and return over feed cost for the corn DDGS treatment might have been statistically significant.

Manure values were calculated by valuing the N and P in manure on an equivalent basis to the N and P in commercial fertilizer (e.g., anhydrous ammonia and di-ammonium phosphate). A N value of 31.7¢/pound and a P value of 65.4¢/pound were calculated according to the commercial fertilizer values reported for 2007 [19]. Manure values were calculated when both N and P or when only N content were valued for their fertilizer content. If manure is to be land-applied to fields with adequate soil P contents, the P in the manure may have little to no value
to the crop producer. The value of manure produced per 1,000 hens per day was not
different between the two dietary regimens. The N content of the manure was not
different, thus the manure value for N-based fertilizer application was not anticipated
to be different. The manure P content for the control hens was greater than that for
the DDGS hens, although the difference was not enough to cause difference in
manure value. The DDGS hens excreted relatively more manure, which contributed
to the numerically higher manure values for the corn DDGS treatment.

When manure value was considered for N and P per 2,000 pounds, manure
from the corn DDGS treatment was $6.35 (19%) more valuable than that from the
control; however, the difference was not statistically significant. When only N value
was considered, manure was $4.06 (36%) more valuable; but again, no significant
difference was detected ($P = 0.11$). The numerically higher N content in the manure
from the corn DDGS treatment was enough to offset the significantly lower P content
when manure was valued according to both N and P contents. The actual manure
value paid to the farm by the local co-op was $1.57/ton (19%) more for manure from
the control regimen ($P = 0.07$). Locally, only the P content, and not the N content, of
manure was considered to have value to crop farmers for fall-season application.
Therefore, the actual prices obtained for the manure only considered P value on an
as-is basis. The P value was calculated based on locally reported di-ammonium
phosphate prices.

**Conclusions**

1. A diet containing 15% corn DDGS caused no adverse effects on hen
   performance and is suitable for commercial egg production.
2. Although manure pH was lower for the DDGS treatment, no concurrent
decrease in NH$_3$ emission could be detected in this study. Factors not directly
related to the dietary treatment, such as ventilation rate, temperature, or manure moisture might have affected the NH$_3$ emission, thereby masking any effects of the diet.

3. Manure P, expressed on a DM basis, was lower for the DDGS regimen than the control, while manure N content was not different. Manure from the DDGS regimen was drier than the control but there was no difference in the quantity of DM-manure excreted.

4. Economic analyses revealed lower feed cost both per hen and per egg mass produced for the DDGS regimen compared to the control. Return over feed cost was numerically but not statistically higher for the DDGS regimen. Because manure was valued on a P-basis when sold to the local co-op, manure from the DDGS regimen yielded less cash return per ton.

5. Additional research is on going and expected to help elucidate any effects of dietary DDGS on NH$_3$ emission from laying-hen manure.

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Acknowledgements

Funding for the study was provided by Dakota Gold Research Association, Sioux Falls, South Dakota; the Iowa Egg Council, Urbandale, Iowa; and the Iowa State University College of Agriculture Air Quality Initiative, Ames, Iowa. In-kind donations of feed ingredients, facilities, and services were provided by Sparboe Farms, Litchfield, Minnesota.
Figures and Tables

Table 1. House population at the onset of monitoring, age at the onset, and age at molting for each house.

<table>
<thead>
<tr>
<th>Treatment/pair</th>
<th>Population at onset</th>
<th>Age at onset (weeks)</th>
<th>Age at molting (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control/1</td>
<td>72,577</td>
<td>36</td>
<td>67</td>
</tr>
<tr>
<td>DDGS/1</td>
<td>72,038</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Control/2</td>
<td>69,495</td>
<td>60</td>
<td>66</td>
</tr>
<tr>
<td>DDGS/2</td>
<td>70,942</td>
<td>56</td>
<td>69</td>
</tr>
<tr>
<td>Control/3</td>
<td>66,814</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>DDGS/3</td>
<td>67,696</td>
<td>68</td>
<td>71</td>
</tr>
</tbody>
</table>
Table 2. Sample control and DDGS diets fed during the experiment\(^1\).

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>DDGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (% of the diet)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>65.24</td>
<td>54.52</td>
</tr>
<tr>
<td>Corn DDGS</td>
<td>—</td>
<td>15.00</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>19.45</td>
<td>14.00</td>
</tr>
<tr>
<td>Limestone(^2)</td>
<td>10.40</td>
<td>10.25</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>2.50</td>
<td>4.35</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.90</td>
<td>0.75</td>
</tr>
<tr>
<td>Animal-vegetable fat</td>
<td>0.75</td>
<td>0.35</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
<td>0.35</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>Vitamin and mineral pre-mix</td>
<td>0.10</td>
<td>0.13</td>
</tr>
<tr>
<td>BioLys</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td>Choline (70%)</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Aspergillus niger phytase</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>(\alpha)-galactosidase enzyme</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Calculated chemical composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>15.45</td>
<td>16.63</td>
</tr>
<tr>
<td>Metabolizable energy (kcal/kg)</td>
<td>2936</td>
<td>2932</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>3.59</td>
<td>4.81</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>2.09</td>
<td>2.64</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>4.49</td>
<td>4.50</td>
</tr>
<tr>
<td>Phosphorus (available, %)</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td>Phosphorus (total, %)</td>
<td>0.59</td>
<td>0.59</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>Lysine (total, %)</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td>Methionine (total, %)</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>Methionine + cystine (total, %)</td>
<td>0.69</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\(^1\)These diets were fed from 85 to 80% egg production and 91 to 93 g/hen-d feed consumption.

\(^2\)Supplied a 50:50 mixture of large (2.27 mm) and small (0.14 mm) diameter limestone.
Table 3. Ammonia emission rates calculated from N-mass balance.

<table>
<thead>
<tr>
<th>Unit of Ammonia Emission Rate</th>
<th>Control</th>
<th>DDGS</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grams per hen daily</td>
<td>1.32</td>
<td>1.24</td>
<td>0.08</td>
<td>0.54</td>
</tr>
<tr>
<td>Grams per kilogram N consumption</td>
<td>61.0</td>
<td>51.8</td>
<td>3.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Grams per kilogram egg production</td>
<td>28.2</td>
<td>25.0</td>
<td>1.8</td>
<td>0.35</td>
</tr>
</tbody>
</table>

\(^1\)Pooled standard error of the mean; n = 3.
Table 4. Nitrogen (N) mass balance of hens fed either a control diet or a diet containing 15% corn distiller’s dried grains with solubles (DDGS).\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Feed</th>
<th>Eggs</th>
<th>Manure</th>
<th>Mortality</th>
<th>Tissue</th>
<th>Ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (g/hen-d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet/pair</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control/1</td>
<td>2.24</td>
<td>0.70</td>
<td>0.42</td>
<td>0.0004</td>
<td>0.003</td>
<td>1.12</td>
</tr>
<tr>
<td>DDGS/1</td>
<td>2.48</td>
<td>0.74</td>
<td>0.58</td>
<td>0.0005</td>
<td>0.003</td>
<td>1.15</td>
</tr>
<tr>
<td>Control/2</td>
<td>2.17</td>
<td>0.67</td>
<td>0.37</td>
<td>0.0009</td>
<td>0.010</td>
<td>1.12</td>
</tr>
<tr>
<td>DDGS/2</td>
<td>2.37</td>
<td>0.71</td>
<td>0.80</td>
<td>0.0013</td>
<td>0.011</td>
<td>0.85</td>
</tr>
<tr>
<td>Control/3</td>
<td>2.10</td>
<td>0.61</td>
<td>0.47</td>
<td>0.0017</td>
<td>0.042</td>
<td>1.03</td>
</tr>
<tr>
<td>DDGS/3</td>
<td>2.31</td>
<td>0.69</td>
<td>0.57</td>
<td>0.0008</td>
<td>0.005</td>
<td>1.05</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.17</td>
<td>0.66</td>
<td>0.42</td>
<td>0.0010</td>
<td>0.018</td>
<td>1.07</td>
</tr>
<tr>
<td>DDGS</td>
<td>2.39</td>
<td>0.71</td>
<td>0.65</td>
<td>0.0009</td>
<td>0.007</td>
<td>1.02</td>
</tr>
<tr>
<td>SEM(^2)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.07</td>
<td>0.0003</td>
<td>0.009</td>
<td>0.08</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0031</td>
<td>0.06</td>
<td>0.15</td>
<td>0.77</td>
<td>0.44</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>30.4</td>
<td>20.1</td>
<td>0.05</td>
<td>0.9</td>
<td>49.3</td>
</tr>
<tr>
<td>DDGS</td>
<td>100</td>
<td>29.8</td>
<td>27.3</td>
<td>0.03</td>
<td>0.3</td>
<td>42.6</td>
</tr>
<tr>
<td>SEM(^2)</td>
<td>–</td>
<td>0.5</td>
<td>3.5</td>
<td>0.01</td>
<td>0.4</td>
<td>3.2</td>
</tr>
<tr>
<td>P-value</td>
<td>–</td>
<td>0.47</td>
<td>0.29</td>
<td>0.55</td>
<td>0.42</td>
<td>0.27</td>
</tr>
</tbody>
</table>


\(^2\)Pooled standard error of the mean; n = 3.
Table 5. Performance responses of laying hens fed either a control diet or a diet containing 15% corn distiller’s dried grains with solubles (DDGS).

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>DDGS</th>
<th>SEM(^1)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed consumption (pounds/100 hens)</td>
<td>22.6</td>
<td>22.5</td>
<td>0.1</td>
<td>0.57</td>
</tr>
<tr>
<td>Feed consumption (g/hen)</td>
<td>103</td>
<td>102</td>
<td>0.4</td>
<td>0.57</td>
</tr>
<tr>
<td>Egg production (%)</td>
<td>77.0</td>
<td>78.4</td>
<td>0.6</td>
<td>0.26</td>
</tr>
<tr>
<td>Egg weight (g/egg)</td>
<td>61.6</td>
<td>63.0</td>
<td>0.7</td>
<td>0.28</td>
</tr>
<tr>
<td>Egg weight (pounds/case)</td>
<td>48.9</td>
<td>50.0</td>
<td>0.5</td>
<td>0.28</td>
</tr>
<tr>
<td>Egg mass (g/hen)</td>
<td>47.0</td>
<td>49.3</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Feed conversion (g egg/kg feed)</td>
<td>458</td>
<td>482</td>
<td>5</td>
<td>0.07</td>
</tr>
<tr>
<td>Water consumption (gallons/100 hens)</td>
<td>4.32</td>
<td>4.73</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>Water consumption (L/100 hens)</td>
<td>19.6</td>
<td>21.5</td>
<td>0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Body weight (pounds/hen)</td>
<td>3.35</td>
<td>3.34</td>
<td>0.04</td>
<td>0.98</td>
</tr>
<tr>
<td>Body weight (kg/hen)</td>
<td>1.52</td>
<td>1.51</td>
<td>0.02</td>
<td>0.98</td>
</tr>
<tr>
<td>Mortality (%/week)</td>
<td>0.21</td>
<td>0.21</td>
<td>-(^2)</td>
<td>0.88</td>
</tr>
<tr>
<td>Cumulative mortality (%)</td>
<td>9.9</td>
<td>11.6</td>
<td>1.1</td>
<td>0.42</td>
</tr>
</tbody>
</table>

\(^1\)Pooled standard error of the mean; n = 3.

\(^2\)Data were arcsine square-root transformed prior to analysis.
Table 6. Properties of manure from laying hens fed either a control diet or a diet containing 15% corn distiller’s dried grains with solubles (DDGS).

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>DDGS</th>
<th>SEM&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (% DM basis)</td>
<td>3.24</td>
<td>3.28</td>
<td>0.24</td>
<td>0.91</td>
</tr>
<tr>
<td>Phosphorus (% DM basis)</td>
<td>2.96</td>
<td>2.66</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Dry Matter (%)</td>
<td>57.7</td>
<td>70.7</td>
<td>2.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Dry manure at clean-out (g/hen-d)</td>
<td>13.5</td>
<td>18.8</td>
<td>1.7</td>
<td>0.16</td>
</tr>
<tr>
<td>As-is manure (tons/1,000 hens-year)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>9.4</td>
<td>10.6</td>
<td>0.6</td>
<td>0.32</td>
</tr>
<tr>
<td>pH</td>
<td>7.42</td>
<td>7.10</td>
<td>0.08</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<sup>1</sup>Pooled standard error of the mean; n = 3.

<sup>2</sup>As measured at the time of annual manure clean out and calculated to 2,000-pound tons over a 365-d year.
Table 7. Economic analyses for flocks of laying hens fed either a control diet or a diet containing 15% corn distiller’s dried grains with solubles (DDGS).

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>DDGS</th>
<th>SEM¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed cost (¢/hen-wk)²</td>
<td>11.2</td>
<td>10.8</td>
<td>0.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Feed cost (¢/kg egg)³</td>
<td>34.2</td>
<td>31.3</td>
<td>0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Egg income (¢/hen-wk)⁴</td>
<td>26.9</td>
<td>28.4</td>
<td>0.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Manure N and P value ($/1,000 hen/d)⁵</td>
<td>0.58</td>
<td>0.72</td>
<td>0.07</td>
<td>0.31</td>
</tr>
<tr>
<td>Manure N value ($/1,000 hen/d)⁵</td>
<td>0.29</td>
<td>0.45</td>
<td>0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>Manure N and P value ($/2,000 pounds)⁵</td>
<td>33.66</td>
<td>40.01</td>
<td>2.17</td>
<td>0.18</td>
</tr>
<tr>
<td>Manure N value ($/2,000 pounds)⁵</td>
<td>11.33</td>
<td>15.39</td>
<td>1.03</td>
<td>0.11</td>
</tr>
<tr>
<td>Actual manure value ($/2,000 pounds)⁶</td>
<td>9.79</td>
<td>8.22</td>
<td>0.32</td>
<td>0.07</td>
</tr>
<tr>
<td>Return over feed cost (¢/hen-wk)⁷</td>
<td>15.9</td>
<td>17.7</td>
<td>0.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>

¹Pooled standard error of the mean; n = 3.
²Calculated from records of feed cost (dollars per ton) and feed consumption over a 1-year period.
³Calculated from the feed cost per hen and records of egg mass.
⁴Calculated from records of egg mass and breaking stock egg prices reported by the USDA [18] for central states markets for a blended minimum average case weight of 48 pounds per case of 30-dozen eggs over a 1-year period.
⁵Manure N and P contents were assigned values equal to N and P values in anhydrous ammonia and di-ammonium phosphate, respectively, for 2007 [19].
⁶Manure value paid to the farm by the local co-op.
⁷Calculated as egg income + actual manure value – feed cost.
Figure 1. Schematic layout of high-rise laying-hen house showing cross section and floor plan of the house and the sampling locations.
**Figure 2.** Cost of diets (dollars per 2,000-pound ton) used in the study. The DDGS diets contained 15% corn distiller’s dried grains with solubles.
Figure 3. Central states breaking stock egg prices as reported by the USDA [18] for a blended minimum average case weight of 48 pounds per case of 30-dozen eggs.
Figure 4. Ammonia emission calculated from N mass balance or measured by the CFI method. Means ± pooled standard error of the mean; n = 3. Mass balance was measured over the entire 12-month period from November to October of the following year. The CFI ER measurements were taken over 48 hr every 2 wk from May through October.
CHAPTER 4: EFFECTS OF DIETARY CORN DISTILLER’S DRIED GRAINS WITH SOLUBLES ON GENERIC E. COLI POPULATIONS, pH, AND SHORT-CHAIN FATTY ACID CONTENTS IN THE CECA OF LAYING HENS

A paper to be submitted to the Journal of Applied Poultry Research

S. Roberts, H. Xin, D. Trampel, H. Medina, and K. Bregendahl

Summary

Distiller’s dried grains with solubles (DDGS) from fuel ethanol production have become an important feed ingredient in livestock and poultry diets. While DDGS is often an economic source of nutrients in poultry diets, there may be additional desirable results. The study described here examined the effects of 15% dietary DDGS on SCFA and pH as well as Escherichia coli and Salmonella in the cecal

5 Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or endorsement by Iowa State University and does not imply the approval to the exclusion of other products that may be suitable.
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contents of laying hens. The hypothesis was that DDGS would cause an increase in metabolism and populations of commensal bacteria in the ceca, which would increase the SCFA contents and lead to a decrease in *E. coli* and *Salmonella* in the cecal contents. *E. coli* is a major cause of peritonitis in laying hens and *Salmonella* contamination of shell eggs is a major cause of food-borne illness in human consumers. Results showed that 15% DDGS diets did not cause an increase in SCFA content, decrease in pH, or change in *E. coli* populations in the laying-hen ceca. *Salmonella* were not detected in any hens, regardless of treatment. Therefore, dietary DDGS is not expected to cause a decrease in *E. coli* peritonitis through SCFA production.

**Description of the Problem**

Egg producers must continually strive to sustain or improve the production of high-quality eggs in both economically and environmentally sound ways. Formulating least-cost diets to optimize animal production is the key to success and, over the past few years, corn distiller’s dried grains with solubles (DDGS) has become an economical ingredient for laying-hen diets. Furthermore, with the growth of the ethanol industry in the United States, the supply of DDGS is plentiful. In addition to assuring that dietary DDGS will not depress egg production, it is of great academic and practical values to understand the broad range of effects and mechanisms concerning the dietary ingredients on the hen. The relatively high fiber content of the DDGS makes it a unique ingredient compared to corn and soybean meal. The fiber is not digested by the hen and, therefore, passes through the small intestine and arrives intact in the large intestine. Here, the fiber serves as an energy source for bacteria, thereby changing and potentially increasing the total bacterial
populations. This change in bacterial populations may have a broad range of effects in the intestine of the hen.

A DDGS-caused change in the bacterial populations and the intestinal environment in the hen could have an immense impact on the egg industry. Increased populations of anaerobic bacteria in the intestine are expected to cause an increase in short-chain fatty acid (SCFA) contents in the digesta because SCFA are a normal by-product of bacterial metabolism [1]. The SCFA have a bacteriostatic effect on certain groups of bacteria, namely the family Enterobacteriaceae of which Escherichia coli (E. coli) and Salmonella are members [1-4]. E. coli species are a primary causative agent of peritonitis, which costs the laying-hen industry thousands of dollars annually from increased mortalities [5, 6]. While several Salmonella species can cause disease in poultry (e.g., Pullorum disease and Fowl typhoid), S. enteritidis is a major concern for the egg industry [7]. S. enteritidis can be deposited in clean, intact eggs and cause severe gastroenteritis in human consumers. Therefore, if dietary corn DDGS can cause a decrease in E. coli and Salmonella populations in laying hens through an increase in SCFA, a decrease in E. coli peritonitis and Salmonella infection of shell eggs may result.

The objective of this study was to comparatively quantify the populations of generic E. coli, determine the presence of Salmonella, measure the pH, and measure the SCFA contents in the ceca, a portion of the large intestine, of laying hens fed diets with 0% corn DDGS (control) or 15% corn DDGS (treatment).

**Materials and Methods**

The Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals. Seventy-two Hy-Line CV-20 hens at 91 wk of age were obtained from a single high-rise laying-hen house on a commercial
farm in central Iowa. Prior to the study, all hens were fed the same diet, which did not contain corn DDGS. Hens were housed three per cage in 24 cages at the Iowa State University poultry research farm. Two commercial laying-hen diets were formulated with either 0% (control) or 15% corn DDGS (Table 1). Each of the 24 cages was assigned to one of the two dietary treatments \( n = 12 \) according to a completely randomized design.

After hens were fed the respective treatment diet for 6 wk, they were euthanized by CO$_2$ asphyxiation and the ceca were aseptically removed. The contents from one randomly chosen cecum of each hen were removed, mixed in a 1:10 dilution with buffered peptone water, serially diluted to $10^{-6}$, and plated onto appropriate media. For \textit{E. coli} enumeration, dilutions were plated once onto Petri film (3M Corporation, Minneapolis, MN) and in duplicate to Rapid \textit{E. coli} 2 Agar (BioRad, Hercules, CA) and MacConkey Agar (BBL, Cockeysville, MD). All media were incubated at 44°C for 24 h (agar plates) or 18 h (Petri films). For \textit{Salmonella} culture, a 1:10 suspension of cecal contents was prepared with buffered peptone water and incubated for 24 h at 37°C. A 100 \( \mu \)L aliquot was transferred to 9.9 mL of Rappaport Vassiliadis broth (BD Diagnostics, Sparks, MD) supplemented with novobiocin (20 \( \mu \)L/mL; Sigma, St. Louis, MO) and incubated for 24 h at 42°C after which 100 \( \mu \)L was transferred to Rappaport Vassiliadis broth without novobiocin and incubated for 24 h at 42°C. A loop of the final enrichment was plated, in duplicate, to XLT-4 agar (Remel, Lenexa, KS) and incubated at 37°C for 24 h.

The contents of the remaining cecum were removed and stored at –20°C until further analyses for pH and short-chain fatty acid contents. Prior to analyses, samples were thawed at 4°C for 12 hours. The pH was measured (Accumet AR-15, Fisher Scientific, Pittsburg, PA) after mixing 1 part cecal contents with 10 parts double-distilled water using a vortex mixer.
The short-chain fatty acid contents were measured after mixing 0.5 g cecal contents with 1 mL of 10% phosphoric acid plus 0.4 μL 4-methyl valeric acid per milliliter added as an internal standard [8]. The solution was mixed using a vortex mixer and centrifuged at 17,000 × g for 20 minutes. The SCFA content of the supernatant was measured using an HP-FFAP column 30 m long with a 0.25 mm internal diameter, HP 6890 series gas chromatograph, and HP 5973 mass selective detector. The parameters were as follows: 1 μL injection volume, 240°C injector temperature, 12.15 psi pressure, with 1.1 mL/min constant flow and helium carrier. The following oven program was used: 80°C initial temperature hold for 5 minutes, ramp 10°C/min to 240°C and 12 minute hold at 240°C.

The average number of *E. coli* colony forming units (CFU) per gram of cecal contents among the three hens in each cage was calculated and logarithmically transformed. Cage was the experimental unit so individual responses from each of the three hens in a cage were averaged to yield one observation per cage. Data were analyzed using JMP (Version 7.0.2, SAS Institute, Inc., Cary, NC) and analysis of variance (ANOVA) appropriate for a randomized complete block design with the model including the main effect of treatment; P ≤ 0.10 was considered significant.

**Results and Discussion**

The SCFA contents are shown in Table 2. There were no statistically significant differences in acetic acid, propionic acid, butyric acid, valeric acid or total SCFA contents in the ceca of hens fed 15% dietary corn DDGS compared to hens fed a 0% corn DDGS control diet. The hypothesis was that the fiber in the corn DDGS would provide an energy source for anaerobic bacteria in the intestine, which would lead to greater concentrations of short-chain fatty acids. However, observations from this study do not support the aforementioned hypothesis. Hens
were fed the 15% DDGS treatment diet for 6 weeks before short-chain fatty acid measurements were taken. Although this was expected to provide enough time for stabilization of the bacterial populations in the intestine, it is possible that six weeks was not sufficient time for bacterial populations to adjust to the treatment diet.

The concentrations of individual SCFA was calculated as a percentage of the total SCFA content in the ceca to determine if DDGS caused a shift in SCFA production from one acid to another. The percentage of propionic acid was higher in hens fed the 15% DDGS treatment diet compared to the control (23.7 vs. 20.4 ± 1.0 %, respectively; \( P = 0.03 \)). There were no significant differences observed for the percentages of acetic, butyric, or valeric acid, indicating that the increase in propionic acid was not merely a shift from another SCFA to propionic acid.

The \textit{E. coli} populations and the pH of the laying-hen ceca are shown in Figure 1. There were no statistically significant differences in either the numbers of \textit{E. coli} \( (P = 0.32) \) or the pH \( (P = 0.74) \) in the ceca of laying hens fed either 0% or 15% corn DDGS. The second part of the hypothesis driving this research was that short-chain fatty acids would cause a decrease in pH of the cecal contents, in turn inhibiting \textit{E. coli} populations. Because there was no measurable difference in short-chain fatty acid contents, a pH shift would not be expected. Without a pH response, the 15% corn DDGS treatment diet did not cause an effect on the \textit{E. coli} populations in the ceca. The lack of pH effect is contrary to laboratory-scale research that previously showed a decrease in manure pH when hens were fed 10% corn DDGS [9].

No \textit{Salmonella} colonies grew with the selective enrichment techniques employed and meaningful results could not be obtained from this trial. Without having specifically inoculated the hens, naturally occurring \textit{Salmonella} populations in the hens obtained from the commercial farm may have been prohibitively low to evaluate effects of the treatment diet on the presence of \textit{Salmonella}. Not all laying-
hen flocks are naturally contaminated with *Salmonella*. Poppe et al. [10] evaluated the prevalence of *Salmonella* in laying flocks across Canada and isolated *Salmonella* from 156 of 295 flocks studied. Furthermore, if *Salmonella* is present on a commercial egg-production farm, not all hens will test positive. When the prevalence of *Salmonella* was measured on a single farm with 12 houses, only 3 of 18 samples (16.7%) returned positive for hens older than 75 wk of age [11].

**Conclusions and Applications**

1. No differences in *E. coli* populations, pH, or short-chain fatty acid concentrations could be detected in the cecal contents of laying hens fed either a 15% corn DDGS treatment diet or a 0% DDGS control diet. Therefore, no effect of dietary DDGS on *E. coli* peritonitis, caused by SCFA, would be anticipated.

2. No meaningful results could be obtained from the *Salmonella* analyses. However, because there was no difference in SCFA contents or pH between the control and DDGS treatment diets, no difference in *Salmonella* would be expected.

3. Future research should evaluate the effects of dietary DDGS inclusion greater than the 15% considered here and fed over a period longer than 6 wk.

**References and Notes**


commercial high-rise houses and characterization of the *Salmonella* isolates by serotyping, antibiotic resistance analysis, and pulsed field gel electrophoresis. Poul. Sci. 86(3): 591–597.

**Acknowledgements**

The authors would like to thank the Iowa Egg Council, Urbandale, Iowa and Dakota Gold Research Association, Sioux Falls, South Dakota for financial support as well as Sparboe Farms, Litchfield, Minnesota for in-kind contributions.
Figure 1. Populations of *Escherichia coli* (*E. coli*) and the pH of the cecal contents from laying hens fed diets with either 0% (control) or 15% corn distiller’s dried grains with solubles (DDGS). Values are least-squares means ± standard error of the mean (n = 12).
Table 1. Ingredient composition of diets used in the study.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control (%)</th>
<th>DDGS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (% of the diet)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn grain</td>
<td>68.88</td>
<td>57.39</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>18.40</td>
<td>15.00</td>
</tr>
<tr>
<td>Corn DDGS</td>
<td>—</td>
<td>15.00</td>
</tr>
<tr>
<td>Limestone(^1)</td>
<td>10.95</td>
<td>11.05</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.15</td>
<td>0.90</td>
</tr>
<tr>
<td>Salt</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Vitamin and mineral pre-mix</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>BioLys</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Aspergillus niger phytase</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analyzed crude protein (%)</td>
<td>14.58</td>
<td>16.60</td>
</tr>
<tr>
<td>Calculated metabolizable energy (kcal/kg)</td>
<td>2871</td>
<td>2831</td>
</tr>
<tr>
<td>Calculated crude fat (%)</td>
<td>2.75</td>
<td>3.79</td>
</tr>
<tr>
<td>Calculated crude fiber (%)</td>
<td>2.07</td>
<td>2.61</td>
</tr>
<tr>
<td>Analyzed neutral detergent fiber</td>
<td>8.43</td>
<td>9.98</td>
</tr>
<tr>
<td>Calculated calcium (%)</td>
<td>4.51</td>
<td>4.50</td>
</tr>
<tr>
<td>Calculated available phosphorus (%)</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Calculated total phosphorus (%)</td>
<td>0.53</td>
<td>0.52</td>
</tr>
<tr>
<td>Calculated total lysine (%)</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>Calculated total methionine (%)</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Calculated total methionine + cystine (%)</td>
<td>0.54</td>
<td>0.59</td>
</tr>
</tbody>
</table>

\(^1\)Supplies a 50:50 mixture of fine (0.14 mm average diameter) and coarse (2.27 mm average diameter) particle sizes.
Table 2. Short-chain fatty acid (SCFA) contents in the ceca of laying hens fed either 0% (control) or 15% corn distiller’s dried grains with solubles (DDGS).

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>DDGS</th>
<th>SEM¹</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (µmol/g)</td>
<td>62.46</td>
<td>58.04</td>
<td>4.08</td>
<td>0.45</td>
</tr>
<tr>
<td>Propionic acid (µmol/g)</td>
<td>21.20</td>
<td>23.61</td>
<td>1.70</td>
<td>0.33</td>
</tr>
<tr>
<td>Butyric acid (µmol/g)</td>
<td>15.73</td>
<td>14.74</td>
<td>1.79</td>
<td>0.70</td>
</tr>
<tr>
<td>Valeric acid (µmol/g)</td>
<td>4.70</td>
<td>3.50</td>
<td>0.72</td>
<td>0.26</td>
</tr>
<tr>
<td>Total SCFA (µmol/g)</td>
<td>104.09</td>
<td>99.90</td>
<td>6.46</td>
<td>0.65</td>
</tr>
</tbody>
</table>

| Acetic acid (mol % of total) | 60.39 | 58.15 | 1.90 | 0.41 |
| Propionic acid (mol % of total) | 20.35 | 23.70 | 1.01 | 0.03 |
| Butyric acid (mol % of total) | 14.96 | 14.64 | 1.07 | 0.83 |
| Valeric acid (mol % of total) | 4.30  | 3.51  | 0.43 | 0.21 |

¹SEM = Pooled standard error of the mean; n = 12.
CHAPTER 5: SPATIAL VARIATION OF MANURE NUTRITENTS AND MANURE SAMPLING STRATEGY IN HIGH-RISE LAYING-HEN HOUSES

A paper to be submitted to the Journal of Applied Poultry Research

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Summary

Reliable knowledge of manure nutrient contents for intensive animal operations is imperative to the development of effective comprehensive nutrient management plans, which will minimize nutrient runoff and pollution of adjacent waterways. The objectives of this study were to evaluate the spatial variation of manure dry-matter (DM), phosphorus (P), and nitrogen (N) contents in commercial high-rise laying-hen houses; and to determine the sampling locations and number of samples that will lead to good assessment of the nutrient contents of the manure in

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the houses. Two side-by-side manure samples were collected from nine locations in each of six high-rise laying-hen houses (i.e., 18 samples per house) and analyzed for DM, N, and P contents. The nine sample locations were distributed as one-fourth, half, and three-fourths of the building length, with three sample locations (every other manure row) per cross-section of the five manure rows. The average of DM, N or P content from the 18 samples per house was used as the reference value for comparison of eight sampling scenarios. Results showed that duplicate sampling at a location added little to the precision of the data. Manure samples collected crossways across the middle of the house or diagonally across the house in either direction yielded results most similar to the reference value for that house. Hence, when collecting manure samples for nutrient assessment in high-rise laying-hen houses, a single sample collected from every other manure pile across the middle of the building should be sufficient to obtain representative samples of the house and is recommended.

**Description of the Problem**

Livestock and poultry manure from confined animal feeding operations is often used as a source of nutrients for agricultural crops. Of egg producers who spread manure onto crop fields, 73% applied that manure based on nutrient requirements for crop production [1]. However, an accurate assessment of the nutrient contents of manure must be made before sound management decisions with regards to land application of manure are possible. Prior to the analysis of manure nutrient contents, samples must be collected that are representative of and reflect the characteristics of the entire quantity of manure considered. Inaccurate sampling technique will lead to inaccurate nutrient analyses and impede sound management decisions with regards to manure application.
Improper management of manure nutrients can cause extensive pollution and damage to sensitive ecosystems. Over-application of manure may lead to runoff of nitrogen (N) and phosphorus (P) and subsequent nutrient loading into area waterways [2]. Eutrophication is the overproduction of organic matter caused by nutrient imbalances, typically in aquatic systems. Excesses of N or P in a sensitive ecosystem can lead to algal blooms and hypoxia (i.e., low dissolved-oxygen content) contributing to a decrease in biodiversity and fish kills [3, 4]. Agricultural and, to a lesser degree, industrial activities are the primary contributors of excess nutrients into ecosystems around the world [3, 5]. Therefore, proper management of agricultural and specifically manure nutrients is paramount to improving the quality of impaired waterways and to limit nutrient runoff into non-impaired systems.

In addition to the direct pollution of aquatic ecosystems, over application of P-containing fertilizer (i.e., manure) can lead to accumulation of P in the soil, as the corn and soybean crops typically grown in the Midwestern United States require a lower P:N ratio compared to that contained in poultry manure. Application rates based on N requirements for crop production lead to an increase in soil P over time [6]. When soil is over-enriched with P, erosion and sedimentation from fields carry P from agriculturally productive areas to natural environments [5, 7] where the excess P can be harmful, as described previously. Furthermore, P imbalances may persist for years after land-use changes have been implemented [8].

Accurate assessment of manure nutrients allows managers and agronomists to apply manure at the appropriate rates to crop fields, assuring that excess nutrients are not applied to a given parcel of land and that crops will have adequate resources for optimal yields. Because many poultry producers do not own much if any crop ground on which to spread manure, much of the manure produced in the United States is sold to crop producers based on fertilizer value (i.e., nutrient
content). Accurate sampling and analytical techniques are necessary to establish a fair price that reflects the value of the manure for agronomic purposes.

Although tables and reference values for manure nutrient contents are published [9, 10], the actual nutrient content of manure varies depending on many different factors. Season, characteristics of the animals’ feed, housing scheme, use and type of bedding, and manure handling can all impact manure nutrients which can be drastically different from published values [11-14]. For example, Peters [15] reported that the N content of solid dairy manure varied between 1.5 and 16.5 g/kg (3 to 33 pounds per ton) for 388 samples submitted to the Wisconsin Soil Testing Labs over a 6-year period. Lorimor and Xin [14] examined the nutrient contents of laying-hen manure on 4 farms in Iowa and reported that the average observed N content was 42% lower, P was 60% higher, and K was 58% higher than published manure values for Iowa. Therefore, sampling and laboratory analyses are necessary to establish the actual nutrient contents of manure before land application.

Manure samples can be collected from manure storage or during load-out and subsequent land application. Dou et al. [12] reported that sample-to-sample variability was much lower when manure was agitated either in liquid or solid handling systems and the mixing that occurred during load-out of broiler manure was sufficient to decrease sample variability. Similarly, Peters [15] recommended sampling stacked or bedded-pack manure (in this case, dairy manure) during load-out rather than directly from the stack in order to get a representative sample.

However, while uniform samples may be obtained during manure load-out, nutrient analyses performed at commercial laboratories cannot be available for determining land application rates. Sampling manure from the storage area, several days before load out, allows sufficient time for analyses such that the actual manure nutrient contents can be used to calculate application rates.
The objective of this study was to evaluate the spatial variation of nutrients (i.e., N and P) as well as dry matter (DM) contents in laying-hen manure in high-rise houses and to determine which sampling locations should be used and how many samples should be collected that would yield representative manure nutrients of the houses.

**Materials and Methods**

This experiment utilized six commercial high-rise laying-hen houses, designated 1 through 6, each containing between 66,800 and 72,600 Hy-Line W-36 hens housed at 61 in²/hen. Houses had five cage rows and, therefore, five piles of manure. Houses were 48 by 430 feet with 408-foot cage rows and were oriented east to west. Schematic diagrams of the high-rise laying-hen houses are shown in Figures 1 and 2. Hens in three of the houses were fed industry-standard diets while hens in the other three houses were fed diets that contained 15% corn distiller’s dried grains with solubles. Hens in each house were molted once during the 12-month manure-accumulation period and flocks were replaced in two of the six houses.

Manure samples were collected from the undisturbed manure piles in October immediately before the annual manure removal. Samples were collected at nine evenly spaced locations throughout each house at ¼, ½, and ¾ the length of the house (Figure 1). Crosswise samples were collected at every other manure row—the first, third, and fifth manure rows (i.e., the two outside rows and the middle row). Samples from the outside manure rows were collected from the side of the row nearest the sidewall. Samples from the middle row were collected from the south face of the row. Duplicate samples were collected side-by-side at each of the nine locations; hence 18 samples were collected per house.
Manure samples were collected using a manure probe that was designed and built by our research group (Figure 3). A 1-in. inside diameter coreless feed auger was placed inside a 1.5-in. diameter galvanized steel pipe. The pipe was 41 in. long and the auger was 43.5 in. long, i.e., 2.5 in. of the auger extended past the bottom end of the pipe. A 1-in. outside diameter, 18-in.-long steel water pipe was attached inside the top of the coreless auger. The water pipe had a 90° bend at the top with a spinning knob, designed for the steering wheel on a farm tractor, added to facilitate turning the auger. To collect a sample, the manure probe was placed mid-way between the peak and the valley of the manure row at an angle perpendicular to the face of the pile aiming at a floor-level center point of the manure row. The auger was manually turned clockwise while applying gentle pressure to encourage the probe into the manure row. The probe was advanced through the pile until it reached the concrete floor. The probe was not suitable for piles in which the manure was deeper at the sampling location than the 37 inches from the bottom of the probe to the support handle on the side. However, none of the six houses sampled contained manure piles that were too deep for sampling with the probe. After sample collection, the probe was withdrawn from the manure pile. Turning the auger counterclockwise extracted the sample from the probe into an appropriate collection vessel (e.g., 5-gallon bucket). The sample volume was approximately 1 quart (0.95 liters).

Samples were stored at −4°F (−20°C) in plastic zip-top bags until lyophilization (freeze-drying) and grinding through a 1 mm screen. The DM contents of the manure were determined as the sample weight remaining after lyophilization. The N [16] and P [17] contents of the manure samples were measured and were reported on a dry-matter basis.
Statistical Analyses

Statistical analyses were performed using JMP [18]. Least squares means for the nine locations were calculated for each response variable (i.e., DM, N, and P); when the main effect of location was significant, the locations were compared pairwise [19].

Variance components analysis was performed to assess the sources of sampling variability. Variability associated with location within house (termed “location”) and the variability associated with replication within location (termed “replication”) were estimated [20]. The standard error of the mean (SEM) for each variable was reported for different numbers of replications and locations to assess the effect of collecting different numbers of samples on the precision of the estimate.

The variance and SEM for any response variable Y (i.e., DM, N, and P) in a house was calculated according to Equations 1 and 2:

\[
Var(\bar{Y}) = \frac{\hat{\sigma}_L^2}{L} + \frac{\hat{\sigma}_R^2}{L \times R} \tag{Equation 1}
\]

\[
SE(\bar{Y}) = \sqrt{Var(\bar{Y})} \tag{Equation 2}
\]

where \( \bar{Y} \) indicates the mean response in a house, \( \hat{\sigma}_L^2 \) and \( \hat{\sigma}_R^2 \) represent the estimated variability between locations and replications, respectively, and \( L \) and \( R \) denote the number of locations and replications, respectively.

Eight sampling scenarios (designated a through h; Table 1) were examined to determine the locations from which manure should be sampled. A reference value was calculated for manure N, P, and DM contents for each house by calculating the average value obtained from all 18 samples collected from each house (Table 2). The average N, P, and DM contents based on the samples for each scenario,
assuming that only those specified samples were collected, was calculated for each house such that a N, P, and DM response was obtained for each scenario and each house. The response for each scenario was reported as the percentage deviation greater than or less than the reference value. Scenarios that yielded deviations nearer zero were considered more desirable than scenarios that yielded large deviations from the 18-sample reference values.

**Results and Discussion**

Least squares means for DM, P, and N for each of the nine locations sampled in six high-rise laying-hen houses are shown in Figures 4, 5, and 6, respectively. The DM content of samples ranged from 56.9% to 74.6% and higher DM contents were observed in the middle manure row compared to the manure along the perimeter walls in the north and south rows. Samples collected at the middle middle location contained the numerically highest DM content and were not statistically different from the other samples collected along the middle row. Samples collected along the perimeter walls in the north and south rows were not different from each other. During the winter months, moisture in the exhaust air may have condensed on the cooler sidewalls of the houses, resulting in an accumulation of water and lower DM content of the manure in the rows along the perimeter. Furthermore, the moisture gradient in the exhaust air may lead to greater drying ability near the center of the house and lower drying ability (due to more humid air) along the perimeter of the house near the exhaust fans.

The manure P content ranged from 2.65% to 2.96% on a DM basis; however, there were no differences ($P = 0.25$) among the nine locations. The N content of manure varied across sampling locations from 3.01 to 3.44% N on a DM basis. As
with the P content, there were no significant differences among the locations ($P = 0.29$)

Estimated variances from the variance components analysis are used to show how the precision of manure contents, expressed as SEM, changes with varying number of locations and replications (Table 3). For the DM measurements, decreasing the number of replications by half (1 rather than 2) and maintaining 9 locations increased the SEM from 2.85 to 3.03. However, decreasing the locations from 9 to 4 and sampling 2 replications per location increased the SEM from 2.85 to 4.28. Therefore, precision was relatively unaffected when half as many samples are collected by decreasing replications but not by decreasing locations. If twice as many samples are considered, the SEM decreases from 2.85 to 2.76 when the number of replications is doubled while keeping the number of locations the same. However, a greater decrease in the SEM can be realized with 36 samples collected at 18 locations with 2 replications.

A greater proportion of the variance in the P measurements was due to location rather than replication, as was observed for the DM measurements. The SEM increased from 0.085 to 0.102 when half as many replications were considered (i.e., 1 rather than 2). However, when 4 rather than 9 locations were sampled, the SEM increased from 0.085 to 0.128. Doubling the number of replications from 2 to 4 decreased the SEM from 0.085 to 0.076. However, doubling the number of locations from 9 to 18 decreased the SEM from 0.085 to 0.060. The N measurements revealed a similar trend with more variance associated with location compared to that from replication. Therefore, more total samples collected per house will always give a smaller SEM (more precise value) and collecting samples from more locations within a house will be more beneficial than collecting more samples at each location. When resources are limited, collecting replicate samples at each location may not be
necessary to achieve a precise estimate of the manure nutrient contents in a high-rise laying-hen house.

Scenarios were created to determine which configuration of sampling locations gave results closest to the 18-sample average for each barn. The percent deviations from the reference values for DM, P, and N are shown in Figures 7, 8, and 9, respectively. The DM contents of the manure showed the greatest variation across locations and between different sampling scenarios. Scenarios b, c, and d (middle north to south, diagonal from NW to SE, and diagonal from SW to NE, respectively) yielded manure DM results with the least deviation from the reference 18-sample average, all within 6.0 percent of the reference. Any of these three sampling scenarios would be appropriate to determine the DM contents of manure in high-rise laying-hen houses. These three scenarios all contain samples collected from both the perimeter and the central part of the house. Therefore, it seems that samples must be collected from manure rows that proportionally cover the variations across the width of the building, e.g., every other row, to adequately represent the nutrient profiles of the manure in high-rise houses.

The remaining five sampling scenarios yielded inferior results with greater deviations from the reference values. Scenarios a and f consistently overestimated the DM contents of the manure by up to 26.6 percent higher than the reference values. Scenario a involved three samples along the middle row of manure and Scenario f involved one sample collected directly in the middle of the house. These observations indicate that manure in the middle row had higher DM contents compared to the average, which agrees well with observations from Figure 4. Scenarios e, g, and h underestimated the DM contents of the manure by up to 16.8 percent. These scenarios involved four samples at the corners of the barn, the north outside row, and the south outside row, respectively; indicating that manure along
the perimeter of the houses tended to have less DM (i.e., more moisture) compared to the average.

The N and P contents of manure, when expressed on a DM basis, showed less variation among sampling scenarios compared to the DM analysis. Scenario f, one sample collected at the middle of the house, provided erratic results with up to a 20-percent overestimation of the manure N contents and a 20-percent underestimation of the P contents. Therefore, one sample is insufficient to reliably determine the nutrient contents of manure in a high-rise house. The N and P contents of manure were predicted within 12 percent of the reference for Scenarios g and h while Scenarios a through e yielded predictions within 10 percent of the reference values.

The present research was conducted in high-rise laying-hen houses with five manure rows each. Different housing schemes may require different sampling procedures. High-rise houses with more rows of manure likely will require the collection of additional samples to represent manure throughout the house. Furthermore, more intensive sampling of broiler or turkey litters in floor-rearing systems may be necessary compared to the 3-sample methods proposed for manure in high-rise houses. Dou et al. [12] sampled broiler litter with no mixing and reported that 75 samples were necessary to determine the manure nutrient contents with 10% accuracy compared to a reference value calculated as the average of all samples collected at each farm. However, the study did not evaluate manure sampling in high-rise houses.

The method used to collect manure samples can markedly impact the reliability of that sample [13]. The present study used a prototype manure probe to collect all samples, with the objective of determining spatial variation of manure samples within a laying-hen house. Because all samples were consistently collected
using the same protocol, the results of nutrient analyses among samples could be compared. However, further research is necessary to assess the quality of manure samples collected with this probe compared to samples collected by traditional means (e.g., grab samples collected during manure load-out or samples collected using a shovel).

**Conclusions and Applications**

1. Collecting manure samples from multiple locations in a high-rise house improved the precision of DM and nutrient measurements more so than did the collection of multiple samples from each location.
2. The following scenarios yielded results closest to the 18-sample reference values: a) three samples bisecting the middle of the house including both outside rows and the center row of manure, b) three samples diagonally from NW to SE, or c) three samples diagonally from SW to NE.
3. The manure piles nearer the sidewalls tended to have lower DM content than those near the center of the house, presumably due to moisture gradient in the ventilation air and thus drying ability.

**References and Notes**


16. The N contents were measured using AOAC method 984.13 on a Kjeltech 1028 distilling unit (U.S. Tecator Inc., Herndon, VA).

17. The P contents were determined using AOAC method 965.17 adapted to a 96-well plate.

18. JMP. Version 7.0.2, SAS Institute, Inc., Cary, NC.

19. Least squares means is the average of observations for each location adjusted for missing values. The least squares means were calculated using a model that included house, location, and house by location as the error term. The least squares means were compared pair-wise using Tukey's test to adjust for multiple comparisons.

20. The statistical model included house as a fixed effect and location nested within house as a random effect. Replication within location was the overall error term for the model.
Acknowledgements

The authors would like to acknowledge Dakota Gold Research Association, Sioux Falls, South Dakota and the Iowa Egg Council, Urbandale, Iowa for financial support as well as Sparboe Farms, Litchfield, Minnesota for in-kind contributions. Statistical guidance from Dr. Philip Dixon in the Department of Statistics at Iowa State University was greatly appreciated. Staff in the Monogastric Animal Nutrition Laboratory at Iowa State University analyzed all manure samples.
## Figures and Tables

### Table 1. Manure sampling scenarios in high-rise laying-hen houses.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Sample locations(^1)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>MW, MM, ME</td>
<td>Middle row east to west</td>
</tr>
<tr>
<td>b</td>
<td>NM, MM, SM</td>
<td>Middle north to south</td>
</tr>
<tr>
<td>c</td>
<td>NW, MM, SE</td>
<td>Diagonal northwest to southeast</td>
</tr>
<tr>
<td>d</td>
<td>SW, MM, NE</td>
<td>Diagonal southwest to northeast</td>
</tr>
<tr>
<td>e</td>
<td>NW, NE, SE, SW</td>
<td>Four corners</td>
</tr>
<tr>
<td>f</td>
<td>MM</td>
<td>One location at the middle</td>
</tr>
<tr>
<td>g</td>
<td>NW, NM, NE</td>
<td>North row</td>
</tr>
<tr>
<td>h</td>
<td>SW, SM, SE</td>
<td>South row</td>
</tr>
</tbody>
</table>

\(^1\)Abbreviations are as follows: NW = northwest; NM = north middle; NE = northeast; MW = middle west; MM = middle middle; ME = middle east; SW = southwest; SM = south middle; and SE = southeast. Refer to Figure 1 for sampling locations.
Table 2. Average and standard error of the means (in parenthesis) for dry matter (DM), phosphorus, and nitrogen contents of manure collected from each of six high-rise laying-hen houses.¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Dry Matter (%)</th>
<th>Phosphorus (% of DM)</th>
<th>Nitrogen (% of DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>House</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>59.9 (0.9)</td>
<td>2.98 (0.07)</td>
<td>2.87 (0.08)</td>
</tr>
<tr>
<td>2</td>
<td>65.9 (3.0)</td>
<td>2.62 (0.07)</td>
<td>3.20 (0.08)</td>
</tr>
<tr>
<td>3</td>
<td>80.3 (2.1)</td>
<td>2.69 (0.08)</td>
<td>3.58 (0.10)</td>
</tr>
<tr>
<td>4</td>
<td>65.9 (2.1)</td>
<td>2.67 (0.08)</td>
<td>3.40 (0.07)</td>
</tr>
<tr>
<td>5</td>
<td>59.8 (2.6)</td>
<td>2.88 (0.07)</td>
<td>3.08 (0.10)</td>
</tr>
<tr>
<td>6</td>
<td>53.2 (1.7)</td>
<td>3.03 (0.06)</td>
<td>3.45 (0.07)</td>
</tr>
<tr>
<td>Average</td>
<td>64.2 (1.2)</td>
<td>2.81 (0.03)</td>
<td>3.27 (0.04)</td>
</tr>
</tbody>
</table>

¹These house values are the reference values to which each scenario was compared (Figures 7, 8, and 9).
Table 3. Standard error of the mean (SEM) for different numbers of locations or replications (i.e., samples per location) per house.

<table>
<thead>
<tr>
<th>Locations</th>
<th>Replications</th>
<th>Total Samples</th>
<th>SEM$^{1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dry Matter</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>18$^{2}$</td>
<td>2.85</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>9</td>
<td>3.03</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>8</td>
<td>4.28</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>36</td>
<td>2.76</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>36</td>
<td>2.02</td>
</tr>
</tbody>
</table>

$^{1}$SEM was calculated according to Equations 1 and 2 using the estimated variance associated with location and replication. The variance estimates for location and replication ($\hat{\sigma}_L^2$ and $\hat{\sigma}_R^2$, respectively, from Equation 1) were as follows: dry matter 63.6 and 19.1; phosphorus 0.037 and 0.057; and nitrogen 0.071 and 0.061.

$^{2}$18 samples collected at 9 locations with 2 replications was the sampling scheme used to determine the variability associated with location or replication.
Figure 1. Diagram showing five manure piles in gray and nine sampling locations under high-rise laying-hen houses. Two samples were collected at each location to yield 18 samples in total per house. Abbreviations are as follows: NW = northwest; NM = north middle; NE = northeast; MW = middle west; MM = middle middle; ME = middle east; SW = southwest; SM = south middle; and SE = southeast. All measurements are in feet (1 foot = 0.3 meters); not drawn to scale.
Figure 2. Schematic cross-sectional view of the high-rise laying-hen houses where manure samples were collected.
**Figure 3.** a. Schematic diagram of manure-sampling probe developed by the research group. b. Cross-sectional view of the coreless auger (black ring) inside the 1.5-inch diameter pipe. The auger extends 2.5 inches below the pipe. All measurements are in inches (1 in. = 2.54 cm); not drawn to scale.
Figure 4. Gray-scale plot of the spatial variation of manure dry-matter content (%) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of six houses; SEM = 2.4. Values not connected by the same letter are different ($P \leq 0.05$).
Figure 5. Gray-scale plot of the spatial variation of manure phosphorus content (% of DM manure) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of six houses; SEM = 0.10; $P = 0.25$. 
Figure 6. Gray-scale plot of the spatial variation of manure nitrogen content (% of DM manure) under high-rise laying-hen houses; darker gray corresponds to higher values. Values are least-squares means of six houses; SEM = 0.13; $P = 0.29$. 

<table>
<thead>
<tr>
<th></th>
<th>West</th>
<th>Middle</th>
<th>East</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>3.29</td>
<td>3.12</td>
<td>3.38</td>
</tr>
<tr>
<td>Middle</td>
<td>3.41</td>
<td>3.44</td>
<td>3.26</td>
</tr>
<tr>
<td>South</td>
<td>3.01</td>
<td>3.30</td>
<td>3.17</td>
</tr>
</tbody>
</table>
Figure 7. Deviation of dry matter (DM) measurements from the reference values for each of the eight sampling scenarios considered in each of six high-rise laying-hen houses (refer to Table 1 for scenario configurations).
Figure 8. Deviation of phosphorus (P) measurements from the reference values for each of the eight sampling scenarios considered in each of six high-rise laying-hen houses (refer to Table 1 for scenario configurations).
Figure 9. Deviation of nitrogen (N) measurements from the reference values for each of the eight sampling scenarios considered in each of six high-rise laying-hen houses (refer to Table 1 for scenario configurations).
CHAPTER 6. GENERAL CONCLUSIONS

The objectives of the dissertation research were to evaluate the effects of corn distiller’s dried grains with solubles (DDGS) on ammonia (NH₃) emission, production parameters, and economic efficiencies for high-rise laying-hen houses and to elucidate the mechanism of NH₃-emission reduction.

The experimental diet, formulated with 15% DDGS, did not cause a decrease in NH₃ emission rate, when expressed per hen, per unit of nitrogen consumption, or per unit egg production. We hypothesized that lower manure pH would lead to decreased NH₃ emission; however, based on the findings of this research, the hypothesis must be rejected. The nitrogen-balance calculations revealed that DDGS-fed hens consumed more nitrogen compared to the control hens, but the increased consumption was not associated with increased nitrogen excretion. Rather, egg nitrogen deposition was higher, attributed to higher egg mass production. Feed consumption was not different between the two treatments, thus the higher egg mass production led to improved feed conversion for the DDGS regimen. The economic analyses revealed lower feed cost per hen and per kilogram of egg production for the DDGS treatment. The results of the study lead to the conclusions that 15% DDGS is an acceptable diet for commercial egg production, with no detrimental effects on production performance and lower feed costs. However, DDGS should be included in laying-hen diets for its nutrient contributions and not to cause lower NH₃ emissions.

While the DDGS diet caused a decrease in manure pH, no change in the short-chain fatty acid content or pH of cecal contents could be detected. Furthermore, E. coli populations in the ceca were not different between the DDGS and the control regimens. The DDGS diets were fed for six weeks prior to measurement of the cecal characteristics, which might not have been enough time
for the bacterial populations to adjust to the treatment diet. To test the length of time necessary for bacterial populations in the ceca to stabilize after a diet change, future studies should include weekly sampling until no further changes are observed.

The final part of the dissertation research considered the variation in manure nutrient contents in high-rise houses and which sampling scheme produced the most consistent results. Dietary manipulations, such as the inclusion of DDGS, directly impact manure nutrient contents, which makes proper sampling techniques paramount to responsible nutrient management. Sampling more locations increased the precision of measurements more so than collecting multiple samples at each location. The dry-matter content of manure varied more within a house than did the nitrogen or phosphorus content. Therefore, sampling schemes that properly assess dry-matter content of the entire quantity of manure should be used. Manure had lower dry-matter content near the sidewalls compared to the center manure row. Sampling scenarios that included perimeter as well as the center manure pile produced the best results.

In conclusion, DDGS remains an important ingredient in commercial laying-hen diets. However, results of laboratory-scale research, that showed significant decrease in NH₃ emission, were not validated by this research. Additional studies should be conducted to further evaluate the effects of dietary DDGS on NH₃ emission. Commercial-scale studies often include relatively low numbers of experimental units (n = 3 in this research), which limits the statistical power. Furthermore, uncontrollable factors, other than the treatment diet, on a working egg-production farm may influence NH₃ emission and mask any effects of the dietary treatment.