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An Analysis of Liquid Aluminum Sulfate (Alum) Use in Broiler Production Houses to Control In-House Ammonia (NH3) Concentrations and Naturally-Occurring Salmonella and Campylobacter; the Development of an NH3 Emission Factor for a Typical Tennessee Broiler House.

Kenneth A. Armstrong
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Kenneth A. Armstrong entitled "An Analysis of Liquid Aluminum Sulfate (Alum) Use in Broiler Production Houses to Control In-House Ammonia (NH3) Concentrations and Naturally-Occurring Salmonella and Campylobacter; the Development of an NH3 Emission Factor for a Typical Tennessee Broiler House.." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering Technology.

Dr. Robert T. Burns, Major Professor

We have read this thesis and recommend its acceptance:

Dr. Ann Draughon, Dr. Forbes Walker, Dr. Luther Wilhelm

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dr. Forbes Walker

Dr. Luther Wilhelm

Acceptance for the Council:

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(original signatures are on file with official student records)

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A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Kenneth A. Armstrong
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DEDICATION

To David R. Smith,

a University of Tennessee Research Associate in the Biosystems Engineering and Environmental Science Department, for his professional support but most of all for his friendship, loyalty, persistence, obedience to God and taking the time to lend a sympathetic ear to my tribulations during this program and research project.

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ABSTRACT

Dry granular alum (aluminum sulfate) has been used effectively as a broiler litter amendment to reduce ammonia (NH_3) volatilization in broiler production houses. Some broiler producers are currently using liquid alum, but no published information is available concerning its use in treating broiler litter. Likewise, information has not been published on the use of liquid alum to reduce *Salmonella* and *Campylobacter*. The three goals of this project were to enumerate and test the survival of *Salmonella* and *Campylobacter* in four commercial broiler production houses, compare in-house ammonia levels with four different liquid alum treatments, and compare two ammonia emissions estimate methods in a broiler house: a nitrogen mass-balance approach and a flow-integration approach.

This project investigated four treatment levels of liquid aluminum sulfate (Al^+Clear® Liquid Alum, General Chemical Corporation, Parsippany, New Jersey) in four adjacent broiler production houses of the same design. The houses were treated with the following rates of liquid alum: 0, 0.82, 1.64, and 2.46 L m^{-2}. These rates are equivalent to 0, 45, 90, and 135 kg of dry aluminum sulfate per 93 m^2 of production unit floor area on an aluminum sulfate basis. Each broiler house contained approximately 30,000 birds with a six-week grow-out period per flock. There were approximately two weeks between harvest of birds and introduction of the next flock when the houses were empty. The study was conducted over an 18-month period, and eight flocks of broilers were grown in each house.

Pathogen Component

Pathogen sampling events for each grow-out occurred at the beginning of the grow-out and 21 d after alum application. Four composite litter samples were collected for each sample event. Modified Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) methods were used to detect and enumerate Aerobic Plate Counts (APCs), coliforms, *Salmonella* and *Campylobacter* in the litter samples (AOAC, 1998). All isolates were confirmed biochemically and serologically. Data are reported for 15 sample events (2002-2003 production year).

In all four broiler houses, there was a decreasing trend over 12 months in *Salmonella* levels when compared to pre-treatment (baseline) bacteria levels. *Salmonella* levels increased within individual grow-outs in each of the houses during the study. There was a negative correlation between levels of *Salmonella* and litter pH when litter pH was reduced below 3.5. *Campylobacter* was detected for four months during the study in house 1, 2, and 4. The 1.64 L m^{-2} alum application rate reduced *Campylobacter* levels by log 0.94 CFU ml^{-1} and the 0.82 L m^{-2} rate reduced *Campylobacter* levels by log 3.5 CFU ml^{-1}. In the three alum-treated houses, coliform levels were reduced when compared to baseline levels. In the control house, coliform levels increased when compared to baseline levels. Liquid alum did not reduce APCs over the production period. These research findings suggest that the use of alum can reduce *Salmonella, Campylobacter*, and coliforms over a 12-month period in a poultry broiler production facility.

In-house NH_3 Concentrations Component

In the four units, in-house gaseous ammonia levels were measured every 5 s using Dräger Polytron I electrochemical gas sensors. Data are reported on four flocks of birds (October 2002 – May 2003). The 0.82 L m^{-2} application rate was effective at maintaining in-house NH_3 concentrations below 25 ppm for the first two weeks of the grow-out. Both the 1.64 L m^{-2} and 2.46 L m^{-2} rates were effective in keeping in-house NH_3 levels below 25 ppm for the first three weeks of the grow-out.

Ammonia Emissions Component

Total nitrogen inputs (bedding shavings, new birds, and feed) and outputs (broilers and litter) were used to arrive at the mass-balance ammonia emission estimate. The difference between nitrogen inputs and outputs was assumed to be volatilized nitrogen. In addition, a flow-integration emission estimate was determined by collecting house 4 NH_3 concentrations and exhaust fan flow-rate data every 5 s for 168 d (four flocks of birds).

The nitrogen mass-balance estimate was calculated as 9754 kg NH_3 $yr^{-1}house^{-1}$. The flow-integrated method yielded an NH_3 emission estimate of 9161 kg NH_3 $yr^{-1}house^{-1}$, within 6% of the mass-balance approach. Both methods resulted in an average daily NH_3 emission factor of 17 g hr^{-1} 500 kg bird $mass^{-1}$. Using the flow-integration method, the maximum NH_3 emission rate was 61 kg d^{-1} $house^{-1}$ and the average NH_3 emission rate was 28 kg d^{-1} $house^{-1}$. Mass-balance methods appear to be a useful technique for providing accurate long-term (e.g. year) NH_3 emission estimates from poultry broiler production units. The flow-

integrated approach with carefully designed ammonia measurement equipment can supply short-term (e.g. days, weeks) NH_3 emission factors.

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INTRODUCTION

BACKGROUND AND JUSTIFICATION

Between 1934 and 1987, the USDA reported that U.S. broiler production increased from 34 million to over 5 billion broilers per year (Austic et al., 1990). The total number of broilers produced in 2002 was 9 billion (USDA, 2003b). From 1960 to 2001, the U.S. per capita consumption of broilers rose from 11 to 35 kg (24 to 77 lbs), and by 2006 the National Chicken Council predicts the consumption will increase to 38 kg (83 lbs) (National Chicken Council, 2003).

The Center of Disease Control and Prevention (CDC) reported to the USDA that 76 million illnesses and 5,000 deaths in the U.S. were a result of foodborne diseases each year (USDA, 2003a). The major bacterial pathogens defined by the USDA are *Escherichia coli* O157, *Campylobacter*, *Listeria monocytogenes*, and *Salmonella*. These bacteria cost the U.S. an estimated $6.9 billion annually (USDA, 2003a). Two of the major bacterial pathogens are *Salmonella* and *Campylobacter* and the primary source of these bacteria is poultry (USDA, 2003a). In the U.S., *Campylobacter* is the number one cause of foodborne illnesses with approximately 2-4 million foodborne illness cases per year (Bad Bug Book, 2003). As of April 2003, the CDC reported over 1.4 million foodborne illnesses per year due to foodborne *Salmonella* and a cost of $3 billion annually (USDA, 2003a). Between the foodborne pathogens *Campylobacter* and *Salmonella*, over 600 deaths occur annually (USDA, 2003a). A literature review found no published research concerning the use of liquid alum in a broiler production setting to study the effects on foodborne pathogens. This work contributes one possible approach to reducing foodborne pathogens from

poultry. Liquid alum is an acidic chemical and was used as a litter amendment in this study to observe the effects on naturally occurring *Salmonella* and *Campylobacter* in broiler houses.

Ammonia (NH_3) gas concentrations can reach high levels (e.g. 80-100 ppm) during a broiler production grow-out due to ammonia volatilization from the litter in the broiler house. Various studies on the effects of ammonia on poultry have shown that high ammonia levels can cause rapid weight loss, reduced growth rate, and respiratory problems for birds (Charles and Payne, 1966; Bullis et al., 1950). Carlile (1984) reported that bird performance is hindered if in-house NH_3 concentrations surpass 25 ppm. The described disadvantages of high ammonia levels result in a reduction in the bird slaughter weight and a reduction in profits for the farmer. The Occupational Safety and Health Administration (OSHA) has set an 8-h human exposure limit of 25 ppm and a short-term (15 min) exposure limit of 35 ppm for ammonia in the workplace (OSHA, 2003). Ammonia concentrations in a broiler house can be effectively suppressed with the use of aluminum sulfate as a litter amendment. Moore et al. (1999, 2000) have conducted studies applying dry alum and have shown that alum-treated broiler litter has lower NH_3 volatilization, and thereby reduced NH_3 losses to the atmosphere. The lower in-house NH_3 levels were shown to improve feed conversion, decrease bird mortalities, and lower electricity and propane consumption in the houses. Less atmospheric NH_3 losses also improves in-house air quality for the workers. Dry alum was shown to maintain in-house NH_3 concentrations to less than 25 ppm for 3-4 wk into the grow-out (Moore et

al., 2000). No field analysis results have been published regarding the application of liquid alum to broiler litter. This study reports the effects on in-house ammonia concentrations using three levels of liquid alum.

On a global scale, gaseous emissions and other volatiles from animal feeding operations (AFOs) are a concern. In 2002, the National Research Council (NRC) reported their findings on air emissions from AFOs, and ranked NH_3 emissions as a major concern at global, national, and regional scales due to atmospheric deposition (NRC, 2002). Although endeavors to accurately quantify NH_3 emissions from poultry production facilities have been conducted (Asman, 1992, Demmers et al., 1999, Koerkamp, 1994, Koerkamp et al., 1998a, Patni, 1996, Sneath et al., 1996, Wathes et al., 1997, Worley et al., 2002), the NRC charged the USDA and the U.S. Environmental Protection Agency (USEPA) to focus on the control and measurement of NH_3 emissions. One specific finding of the NRC report was that the amount of credible scientific information was small (NRC, 2002), and so continued research is suggested in the U.S. Ammonia emission inventories provided to date in the United States have relied on poultry emission factors developed in Europe (Battye et al., 1994, Asman, 1992). Broiler production systems are different in the U.S. and Europe, and therefore the U.S. should have a distinct emission factor. This study estimated a broiler ammonia emission factor calculated via two methods: a mass-balance and flow-integration approach.

Farmers and poultry industry experts must continue to focus on food safety, bird and producer health concerns, and gaseous emissions related to

broiler production. The project and results described herein addressed these three main areas and consisted of three research components: foodborne pathogen control using liquid alum, in-house ammonia concentration control using liquid alum, and ammonia emissions exiting a typical Southeastern United States broiler production facility. The work is divided into the corresponding three parts, and the study for the three research components was conducted at the same broiler farm. The hypotheses below have been developed for the three research areas.

LIQUID ALUM-RELATED HYPOTHESES

Three null hypotheses were tested to look at the effects of liquid alum:

Hypothesis 1: Bacterial levels in the four broiler houses were not reduced by liquid alum application rates from 0 to 2.46 L m^{-2}.

Hypothesis 2: There are no significant differences in the in-house NH_3 concentration levels between the four alum treatments.

Hypothesis 3: Alum treatment does not significantly influence farm mortality rates, water use, or processing plant condemnations.

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PART 1: PATHOGEN CONTROL

ABSTRACT

The use of dry alum (aluminum sulfate) as a broiler litter amendment has been shown to reduce ammonia emissions, but little information is available on the application of liquid alum to poultry litter to reduce *Salmonella* and *Campylobacter*. Our objective was to test the survival of both pathogens and to enumerate them in four adjacent broiler facilities of the same design that were treated with the following rates of liquid alum: 0, 0.82 (45 kg), 1.64 (90 kg) and 2.46 (135 kg) L m^{-2}. Each broiler house contained approximately 30,000 birds with a six-week grow-out period and approximately two weeks between harvest of birds and introduction of the next flock. Four different composite samples were taken for each sample event; the sample events for each growout occurred at the beginning of the growout and 21 d after alum application. Modified FDA and USDA methods were used to detect each bacterium in the litter samples and to enumerate pathogens. All isolates were confirmed biochemically and serologically. In all four broiler houses, there was a decreasing trend over 12 months in *Salmonella* levels when compared to pre-treatment (baseline) bacteria levels. *Salmonella* levels increased within individual grow-outs in each of the houses during the study. There was a negative correlation between levels of *Salmonella* and litter pH when litter pH was reduced below 3.5. *Campylobacter* was detected for four months during the study in house 1, 2, and 4. The 1.64 L m^{-2} alum application rate reduced *Campylobacter* levels by log 0.94 CFU ml^{-1} and the 0.82 L m^{-2} rate reduced *Campylobacter* levels by log 3.5 CFU ml^{-1}. In the three alum-treated houses, coliform levels were reduced when compared to

baseline levels. In the control house, coliform levels increased when compared to baseline levels. Liquid alum did not reduce APCs over the production period. These research findings suggest that the use of alum can reduce *Salmonella, Campylobacter*, and coliforms over a 12-month period in a poultry broiler production facility.

LITERATURE REVIEW

The control of bacterial pathogens in poultry should include the control of pathogens in the litter (Draughon, personal communication, 2001). Chemical analysis of broiler manure varies due to moisture, temperature, amount and kind of bedding, amount of soil picked up while a house is cleaned, broiler feed ingredients, average bird weight, number of flocks consecutively reared, and conditions under which the manure was stored and handled prior to spreading. The majority of U.S. broiler producers grow the birds in deep litter systems. In deep litter systems, broilers are raised on the same litter for one year or longer before cleaning out the entire house. Therefore, only caked litter is taken out of the units at the end of the grow-out (Arogo et al., 2001). This litter management routine facilitates a growing environment for numerous pathogens related to poultry.

Previous Studies

Recent laboratory research by the USDA in Athens, GA found that acidified broiler litter resulted in higher reduction of *Campylobacter* population

and frequencies in comparison to *Salmonella* (Line, 2002). The research analyzed the effects on bacteria after high and low application rates of dry alum and sodium bisulfate. The broilers studied were kept in 5 m^2 (50 ft^2) pens and the application rates for alum were 37 and 74 kg m^{-2} (8 and 16 lb ft^{-2}); the sodium bisulfate rates were 12 and 19 kg m^{-2} (2.5 and 4 lb ft^{-2}).

One of the primary hypotheses of the Line study was that a change in pH to less than 4.5 due to an acidified amendment would cause death or halt growth in *Salmonella* or *Campylobacter* (Line, 2002). Line's results showed that *Salmonella* colonization was not affected by the litter acidification, but concluded that a broiler litter acidified amendment may aid poultry producers and the food industry in controlling *Campylobacter* and decrease pathogen transmission risks (Line, 2002). Line's research was one of the first to test dry alum and its application to broiler litter in controlling foodborne pathogens.

Many factors contribute to the survival and growth of foodborne pathogenic cells. This project directed attention to *Salmonella spp*. and *Campylobacter spp.* Additionally, assays were performed to test for an overall presence of Gram-negative bacteria using aerobic plate counts and coliform. *Salmonella's* growth rate can increase or decrease depending on variables such as temperature, pH, water activity, and salinity (Doyle, 1989). One of the major factors of this research was the role of broiler litter acidity. As discussed, alum reduces the pH of the broiler litter (Worley et al., 1999, Moore et al., 1994). Studies have proven that the manipulation of the pH homeostasis will determine whether pathogen bacteria survive, grow, or die (Hill et al., 1995; Doyle, 1989).

A lower pH will cause an imbalance in the pH homeostasis and a cell will eventually die. However, a cell can adapt and survive acidic conditions because of its ability to adjust its own cytoplasmic pH (Hill et al., 1995). After an alum application, the acidity of the broiler litter creates an environmental stress on any pathogenic cells in the litter. This dynamic state will trigger a cell to compensate for the change by controlling cation movement across the cell membrane (Hill et al., 1995). During these conditions, the main purpose of the cell's adjustments is survival (Hill et al., 1995).

Salmonella has an optimal growth pH range of 6.5 to 7.5, but research has shown that the bacterium will grow as low as 4.5 and as high as 9.0 (Doyle, 1995). Along with acidity, the chemical makeup of the acid may result in varied outcomes. For example, citric acid can inhibit *Salmonella typhimurium* whereas lactic or hydrochloric acid is less effective in equal incubation conditions. Temperature can also affect the minimum pH at which *Salmonella* will grow. Doyle referred to one study where growth occurred at a pH of 4.0 using citric acid and the incubation temperatures were 25°C and 32°C (Chung et al., 1970). The incubation temperature was lowered to 16°C, and *Salmonella anatum* (*S. anatum*) did not grow. During the same research, the incubation temperature was increased to 37°C then to 43°C, but *S. anatum* still did not grow (Chung et al., 1970).

Concerning *Salmonella*, especially *Salmonella typhimurium (S. typhimurium)*, the broiler litter pH is one of the more crucial factors researchers must consider to control whether the bacterium survives and whether the

bacterium is carried on to the processing plant. Hill et al. (1995) demonstrated that *S. typhimurium* can tolerate low pHs and the stress adaptation takes place in two stages. The research examined how *S. typhimurium* can manufacture acid-shock proteins (ASPs) that allow the cells to survive if the external pH is steady at approximately 5.8 for a period of time. A pause at pH 5.8 permits the organism to maintain pH homeostasis and produce the ASPs before a more acidic environment is reached. The authors refer to the first stage (pH=5.8) as pre-acid shock and the second stage (pH $\leq$ 4.0) as post-acid shock. If the broiler litter pH could be lowered to 3.3 without the hesitation at 5.8 then the ASPs could not take effect and the bacteria would die.

The Hill et al. (1995) research may explain why Line (2002) did not receive favorable results in controlling *Salmonella* colonization's. Line's material and methodology did call for monitoring litter pH. The lowest pH in Line's work was approximately 4.5 using a high alum rate (7.2 kg alum 4.6 $m^{2\ -1}$). Line (2002) states in his introduction that "significant reductions in litter pH and exposure to pH levels below 4.5 would be expected to be bacteriostatic or bacteriocidal for *Salmonella* and *Campylobacter.*" Hill et al. (1995) concluded that a pH $\leq$ 4.0 must be attained without any pause at 5.8 so *S. typhimurium* could be eliminated. Thus, one could conclude the one key in controlling *S. typhimurium* is the pH and the length of time at a given pH level. Alum must be able to decrease the pH lower than 4.0 without any delays to be effective in combating *S. typhimurium*.

Additionally, Hill et al. (1995) carried out studies with acid-induced *Salmonella* on cheddar cheese, and the *S. typhimurium* bacteria were able to

adapt and survive even after 74 d of storage. Hill et al. (1995) also discovered that if *S. typhimurium* was introduced to a pH of 4.4 for 30 min the ASPs will disappear and the cells cannot survive at an even lower pH of 3.3. Once more, the time period at any given pH is also critical in controlling the survival of foodborne pathogenic cells.

Like *S. typhimurium* and many bacteria, *Campylobacter spp.* is susceptible to environmental factors, which affect its survival. For example, *Campylobacter jejuni (C. jejuni)* grows between 42°C and 45°C, but will die at 48°C (Jay, 2000). A 2% sodium chloride (NaCl) level is bactericidal for *C. jejuni* and 1% NaCl will significantly decrease growth rates (Jay, 2000). In respect to acidity, *C. jejuni* is inactivated at pH 3.0 to 4.5, but this range is temperature-dependent. *Campylobacter* has an optimal pH range of 6.5 to 7.5 and the minimum pH for growth is approximately 4.9 (Doyle, 1989). The use of lactic and acetic acids can reduce *Campylobacter* colony forming units (CFU's) ml^{-1} in chicken broiler halves (Doyle, 1989). Line (2002) had positive results lowering *Campylobacter* populations using dry alum as a broiler litter amendment.

Pope et al. (2000) evaluated the effect on the presence of pathogens of Poultry Litter Treatment® (PLT)–treated broiler litter (Pope et al., 2000). The PLT product contains sodium bisulfate. This report addressed the exact pH levels obtained during the first weeks of the broiler grow-out. During week 0 of the university's research, a pH of 1.2 was recorded in the PLT treated production unit and after one week the pH returned to 6.2. In the end, the authors concluded that PLT® inhibits the survival of *E. coli* and *Salmonella,* and broiler growers and

companies could integrate PLT® into the poultry industry's already existing Hazard Analysis Critical Control Point (HACCP) guidelines. As mentioned, the majority of the field research using alum has focused on minimizing ammonia volatilization by holding the litter pH to less than 7.0. Moore et al. (1999) research documented pH but the lowest recorded pH was 5.5-6.0. In one study, Moore et al. (1995) analyzed fecal coliform counts and pH levels but only at the end of the broilers' grow-out when the pH had returned to an average of 7.5-9.0.

Theoretically, alum will have a difficult time overcoming the survivability of *Salmonella typhimurium* and *Campylobacter jejuni* if we can lower pH to only 5.5. As mentioned earlier, *C. jejuni* should die at pH 3.0-4.5 and *S. typhimurium* at pH 3.3. *Campylobacter* is an obligate microaerophilic organism, which will serve to be advantageous in limiting the organism's growth and/or survivability in the broiler litter (Stern et al., 1995). One solution is the alum application rate: the higher the alum concentration then a lower pH may be achieved.

The research by Pope and Cherry showed the effectiveness of PLT® to reduce in-house ammonia levels and bacterial counts (Pope et al., 2000). In general, the objective was to identify a scientific way to reduce bacteria at the farm level thereby decreasing the quantity of pathogens cells carried on to the processing plant. Poultry Litter Treatment® was successful at lowering *E. coli* populations during weeks one and two of the grow-out. Pope et al. (2000) also analyzed assays of *Campylobacter*, and they concluded PLT® was not effective in combating *Campylobacter*. Pope and Cherry took samples on the farm in

addition to locations in the processing plant. Overall, they concluded PLT is beneficial in lowering bacterial counts on the farm.

As indicated, broiler litter is one of the perfect environmental habitats to harbor foodborne pathogens, but is not the sole pathway for pathogens to reach the broilers. Internal production unit air contains a high concentration of dust particles and ammonia gas molecules. Although dust particles can range from 1 micron to 10 mm in diameter (Wark et al., 1998), bacteria can attach and ride the particles through the atmosphere because dust particles have an overall positive electrical charge (Mitchell et al., 2002). The USDA-Agricultural Research Service (ARS) in Athens, GA took advantage of this characteristic and designed an apparatus with a negative charge to attract the dust particles (Mitchell et al., 2002). The apparatus is an electrostatic space charge system (ESCS) and it was mounted above hatching cabinets with –20,000 V_{DC} supplied by electrodes for a time period of 4 d for the research. Although the Mitchell et al. research was conducted with a hatchery setup in mind, the results were positive from the point of controlling airborne transmitted bacteria. Mitchell et al. (2002) analyzed assays for total aerobic bacteria, enterobacteriaceae (ENT), and *Salmonella* in addition to dust concentration and particle size distribution. As far as bacteria reductions, the average reduction for total aerobic bacteria was 85% and *Salmonella* was reduced by 12% (Mitchell et al., 2002).

MATERIALS AND METHODS

Pathogen Testing Overview

Since the effects of liquid alum on pathogens have not been scientifically evaluated in commercial broiler houses, this study was undertaken to identify an alum application rate to eliminate or to minimize the most frequent foodborne pathogens associated with the broiler industry. Four adjacent commercial broiler production houses of the same design were selected to conduct the experiment; they were tunnel-ventilated, and each house measured 152 m (500 ft) x 12 m (40 ft) (1860 m^2). Nine 130-cm (51 in) and two 91-cm (36 in) constant velocity fans ventilated each house. Litter sample collection was during the 2002-2003-production year (eight grow-outs), when approximately 30,000 birds were produced for each 42 d grow-out period. During the first 14 d of the grow-out, birds were maintained in the brooder end of the house, ventilated by the 91-cm fans. After day 14, a divider curtain was raised, and the birds occupied the entire production house. Enumeration data from grow-outs 1-8 are presented in this study.

A commercial applicator applied liquid alum to the broiler houses approximately four to seven days prior to bird introduction. At each alum application event, the truck driver applied alum to house 1 first, and then house 2, and last house 3. No alum was applied to house 4. The application rates were 0.82, 1.64, 2.46, and 0 L m^{-2} to houses 1, 2, 3, and 4, respectively. For the remainder of this part of the work, the author will refer to house 1 rate as low, house 2 rate as medium, house 3 rate as high, and house 4 rate as control.

The litter in each house was sampled and assays were performed for Aerobic Plate Counts (APCs), Coliforms, S*almonella,* and *Campylobacter*. Litter condition was analyzed after the old litter had been removed and new litter was added to the houses but before any liquid alum or broilers (brooders) had been placed on the litter. Additionally, samples were taken 21 d after the first sampling event of the grow-out occurred. The above-described methodology was followed March 4, 2002 – October 24, 2002. Starting with the December 9, 2002 sampling event, samples were taken after alum application but before new broilers were introduced into the houses. The protocol alteration was done to observe bacterial level changes immediately following a liquid alum application. Again, samples were taken 21 d after the first sampling event of each grow-out. Throughout the research, samples were collected following the sampling protocol described below after each flock had been removed but before birds for a new grow-out were placed in the houses. Therefore, sampling events occurred 15 times per house over a one-year period with samples taken in quadruple for a total number of 60 samples per house per year. Each sample consisted of five smaller, randomly chosen sub-samples that were pooled together to form the large composite sample that was tested in the University of Tennessee's Food Safety Laboratory. Refer to the calendar of events for specific dates and tasks of the research (Appendix, Table A.2).

Litter Sampling

The sampling protocol for the pathogen analysis was as follows:

1) Sterile plastic shoe covers were used on both feet to enter the production houses (only if the sampler had visited other broiler production sites prior to sampling at this project site).
2) The sampler wore latex gloves on both hands during sampling.
3) A commercial ziploc bag was held open with one hand.
4) Using the other hand, the sampler grabbed a handful of litter from the house floor and aseptically placed the litter into the commercial Ziploc bag.
5) The sampler placed the bag into an insulated container that was at ambient temperature for storage and transportation.
6) The above steps were repeated for each of the five litter sub-samples at the four sampling locations in each house.

Litter samples were also analyzed for pH and moisture content and the protocol was as follows:

1) The sample was a composite sample consisting of five sub-samples at each of the locations and the composite sample was approximately 70 g.
2) The sample was a litter surface sample. No soil probe or auger was necessary.
3) The materials required for each composite sample were one commercial ziploc bag and a pair of latex gloves. The latex gloves were required for prevention of pathogen contamination.
4) The bag was held open with one hand and the sub-sample was taken with the other hand. The sub-samples were taken from within a 61 cm (2 ft) radius of the sample location noted on figure 1.2.
5) After each sample, each bag was sealed and placed in a storage container for transport.
6) After the sampler arrived on campus, all samples were delivered to the waste management laboratory for immediate analysis or refrigerated at 4°C.

Preliminary Litter Analysis

The objective of this step was to determine how the levels of *Salmonella* and *Campylobacter* varied across the transverse (Figure 1.1) of broiler

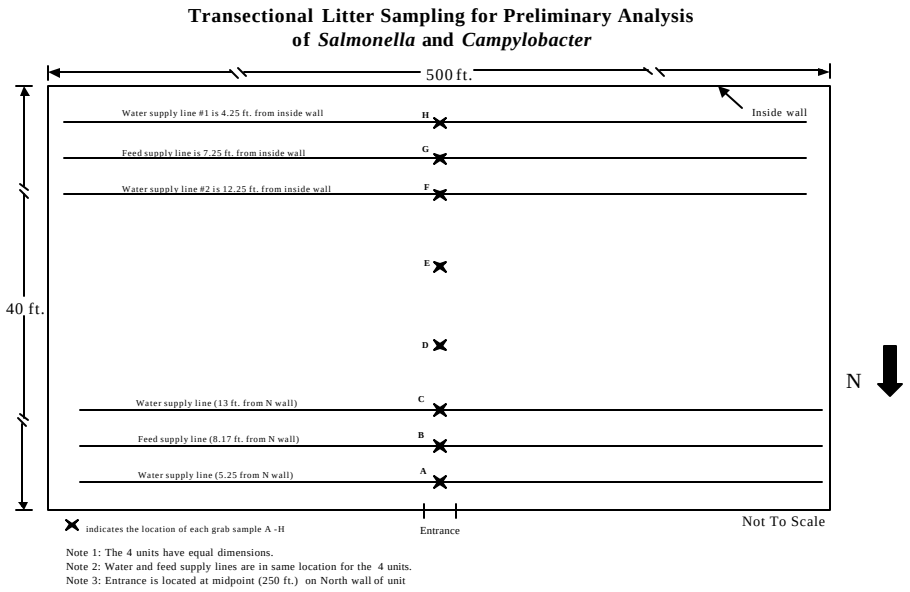

Figure 1.1 Preliminary Litter Analysis Sampling Pattern

production house 1 prior to alum application. The transverse sample was a representative sample for all four houses; because all four houses were identical in construction, all four houses had an equal quantity of chickens, and all four houses had zero alum amended to the litter at the beginning of the study. Eight surface samples were taken from house 1 to be analyzed for bacteria (Figure 1.1). The transverse litter samples were taken at the midpoint 76.2 m (250 ft.) of house 1. After the eight samples had been collected the samples were placed in an insulated container at ambient temperature. The insulated container was transported at ambient conditions to the University of Tennessee's Food Safety Laboratory. At the laboratory, the eight litter samples were analyzed for the presence of *Salmonella* and *Campylobacter* within 8 h. Only general analyses were performed for low, medium, or high levels of *Salmonella* and

Campylobacter. *Campylobacter* was not detected. The lowest levels of *Salmonella* were detected at location D, medium levels at location E, and the highest levels at location H.

Litter Analysis

Based on the preliminary litter analysis, four sampling locations were chosen to collect broiler litter for the study (Figure 1.2). Locations 3 and 4 were chosen because the middle of the house (approximately 6 m from north or south wall) had medium levels of *Salmonella*. Locations 1 and 2 (approximately 11 m from north wall) were chosen because high levels of *Salmonella* were detected. Therefore, four samples per bacterium were analyzed for the presence of *Salmonella* and *Campylobacter* within 8 h. The four sampling locations were used for the remainder of the research.

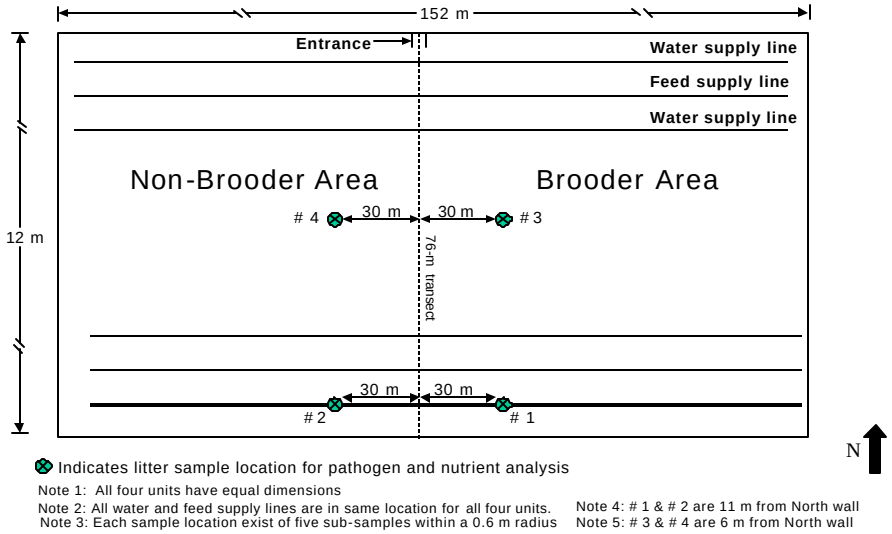

Figure 1.2 Final Litter Sampling Pattern.

Salmonella Isolation and Detection

Samples (25 g) were enriched in Tetrathionate Broth (225 ml) for enumeration and incubated for 18-24 h at 35°C. Each medium was streaked to XLT4 medium for isolation of *Salmonella* (Jackson, 2001). Presumptive *Salmonella* colonies were inoculated into Triple Sugar Iron (TSI) slants and Urease Broth and incubated at 35°C for 18-24 h. Positive reactions on TSI slants were confirmed by a serological polyvalent somatic (O) test. API biochemical test strips or Enterotube II were used for confirmation. Isolates were stored in the freezer culture collection for further study as needed (AOAC, 1998; Draughon, personal communication, 2001).

Campylobacter Isolation and Detection

The isolation and detection of *Campylobacter* took place in two stages. First, samples (25 g) were placed into *Campylobacter* Enrichment Broth (Bolton's Broth) and incubated under 85% N: 10% CO_2: 5% O_2 (NCO) atmosphere at 30°C for 3 h. In the second stage, antibiotics were added to the broths, and then the samples were incubated at 37°C for 48 h. Modified *Campylobacter* Blood-Free Selective Agar Base (CCDA) (Oxoid, UK; CM 689 plus SR 117) was prepared according to the procedures described in FDA/Bacteriological Analytical Manual (BAM, 8th ed.) with addition of CCDA Selective Supplement (Oxoid, SR 155) and *Campylobacter* Growth Supplement (FBP) (Oxoid, SR 84) to Abeyta-Hunt-Bark-Blood Agar (AHB-BA) (AOAC, 1998). Samples were direct plated for

enumeration onto AHB-BA and CCDA by preparing decimal dilutions of sample and incubation of plates under microaerobic atmosphere. Plates were examined for *Campylobacter*-like colonies, and if compatible with *Campylobacter spp*., presumptive positive colonies were inoculated into AHB-BA tubes. The inoculated AHB-BA plates were incubated for 48 h at 42°C under NCO atmosphere. Presumptive positive colonies were confirmed biochemically as *Campylobacter jejuni* with glucose fermentation, hippurate hydrolysis and oxidase reactions as described in the BAM, 8th ed., for each biochemical test. Serological confirmation was conducted using latex agglutination (AOAC, 1998; Draughon, personal communication, 2001).

Aerobic Plate Counts (APCs) and Coliform Testing

APCs and coliforms were isolated and enumerated using a selective plating procedure by placing 25-g samples into peptone water (0.1%) diluent and preparing serial dilutions. Samples were placed onto pre-poured agar plates of Standard Methods Agar (SMA) and Violet Red Bile (VRB) Agar and incubated for 18-24 h at 35°C. Colonies were randomly screened by Gram staining procedure (Speck, 1996, Draughon, 2001 personal communication).

Statistical Analysis

A randomized block design was used to test for alum application amount effects on the enumeration (CFU ml^{-1}) of the four bacteria. Analysis of variance (ANOVA) was conducted with mixed models (SAS, 1999), and least square

means (LSMs) were separated using Fisher's least significant difference test (α = 0.05). All pathogen data presented are log scale base 10 and statistical analysis was performed on the data set using this notation. All enumeration data are presented in the Appendix (Appendix, Pathogen Enumerations, pages 128-142)

ANOVA was used to test the effects of litter sampling locations: brooder vs. non-brooder areas and under water line vs. middle of house locations (figure 1.2). Also, ANOVA was used to test enumeration differences between 1st sampling event (beginning of a grow-out) and 2nd sampling event (21 d into a grow-out) during the 15 sample events. The LSMs of the sampling location analyses were calculated for each treatment.

RESULTS AND DISCUSSION

It is important to note the tasks described in the calendar of events (Appendix, Table A.2). In the calendar of events, alum applications, flock introduction and extraction, and sampling events are outlined. During the research period, the four broiler houses were completely cleaned out twice because litter build up made it difficult for the commercial alum applicator to apply alum. Analysis of variance showed that the litter moisture content increased from the first to second sampling event over all 15 sampling events ($P<0.0001$). At the beginning of grow-outs (odd numbered events, Figure 1.3), the moisture content least square mean was 26%. At the second sampling event (21 d afterwards; even numbered events, Figure 1.3), the moisture content least square mean was 34%. Analysis of variance showed that the litter moisture

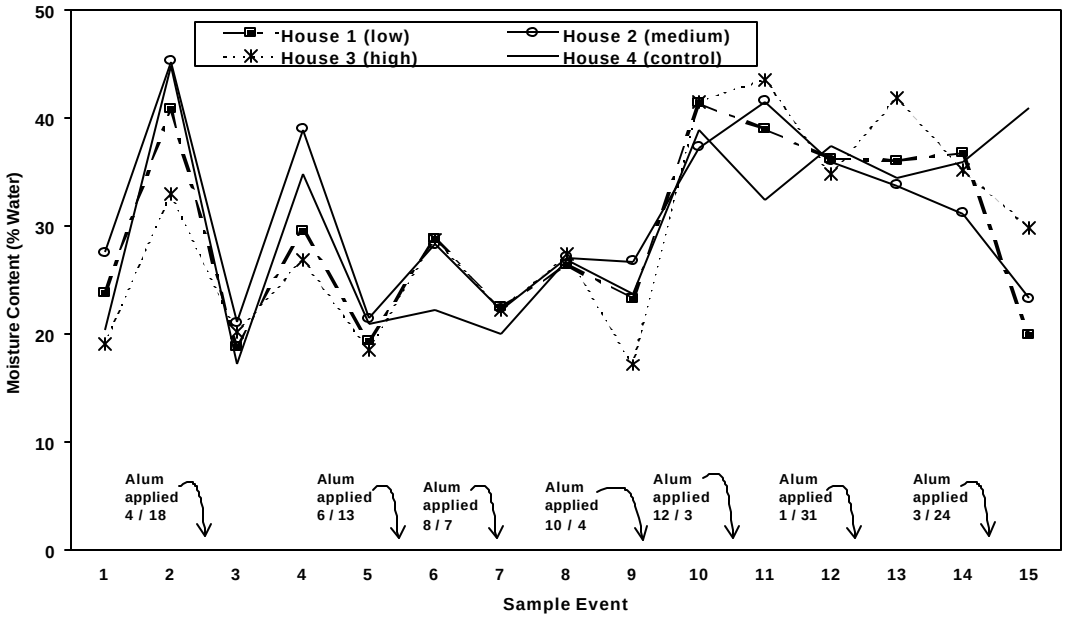

Figure 1.3 Litter Moisture Content By Alum Rate Over 15 Sampling Events.

content in the middle of the house (LSM=24%) was lower than under the water line (LSM=36%) over all 15 sampling events (P<0.0001). The interaction of sampling location (under water line vs. middle of house) and sampling event (first and second) within a grow-out proved significant for litter moisture content (P<0.0001). At the middle of the house location, the moisture content did not change from beginning to 21 d afterwards (LSM=24%). The moisture content of sampling locations under the water line 21 d afterwards was statistically higher (LSM=44%) than at the beginning of individual grow-outs (LSM=28%).

The liquid alum rate did have an effect on pH (P<0.0001). The low, medium, high, and control LSMs were pH=6, 5.9, 5.1, and 7.2, respectively. The low and high were statistically different; the control was statistically different from all alum rates; medium and high were not statistically different; low and medium

were not statistically different. The interaction of sampling location (under water line vs. middle of house) and sampling event (first and second) within a grow-out proved significant for litter pH ($P<0.001$). At the middle of the house location, the pH did change from beginning to 21 d afterwards (LSM=5.3 to 6.2). The pH of sampling locations under the water line 21 d afterwards was statistically higher (LSM=7.1) than at the beginning of individual grow-outs (LSM=5.3). The pH (Figure 1.4) and moisture content (Figure 1.3) trends indicate that both increase from the beginning to the second sampling event within the same grow-out.

The bacteria enumeration Least Square Mean for each house (low, medium, high, and control) shown in Table 1.1 are the results of using the enumerations from the four sampling locations in each house over the 15 sampling events: 15 sampling events x 4 samples house^{-1} x 4 houses = 240 sampling results bacterium^{-1}. Also, the effect (including all interaction combinations), on enumerations, litter pH, and litter moisture content from first and second sampling event within an individual grow-out, brooder vs. non-brooder sampling location, under water line vs. middle of house sampling location, and liquid alum rate was performed for APCs, coliforms, *Salmonella*, and *Campylobacter*.

Aerobic Plate Count assays showed a presence of microorganisms in the broiler litter in all four houses for each sample event (Figures 1.5-1.8). The baseline APC levels (enumerations at sample event 1) were log 6.5, 6.2, 4.8, and 5.5 CFU ml^{-1} in house 1, 2, 3, and 4, respectively. There was not a significant difference (Table 1.1) among the four treatment levels for APC assays ($P=0.35$).

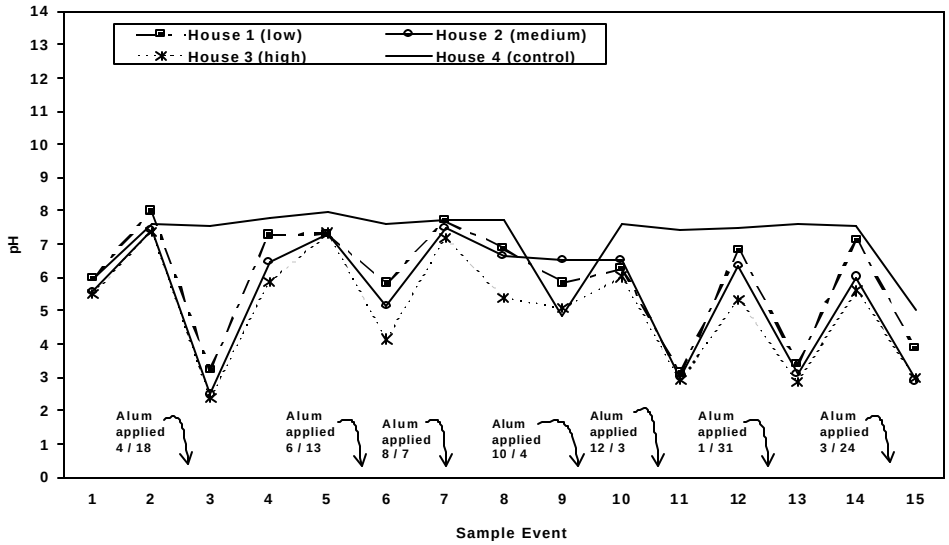

Figure 1.4 Litter pH By Alum Rate Over 15 Sampling Events.

Table 1.1 Bacterial Enumeration (log CFU ml^{-1}) Least Square Means and P values (calculation used all 15 sampling events).

Bacterium	Low	Medium	High	Control	P value	Result
APC	7.3	7.5	7.3	7.9	0.35	NS
Coliform	5.7	5.5	6.3	6.1	0.25	NS
Salmonella	2.8	2.3	2.4	1.6	0.02	S
Campylobacter	1.4	1.0	1.0	1.2	0.03	S

NS: Statistically Non-significant, S: Statistically Significant

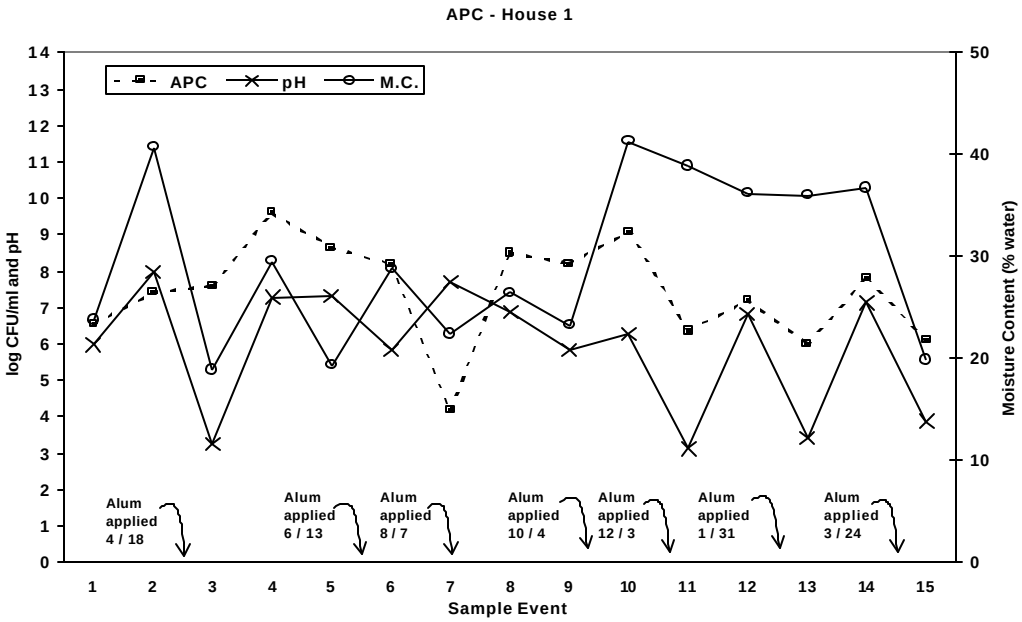

Figure 1.5 APC Enumerations, Litter pH, and Litter Moisture Content for Low Alum Rate.

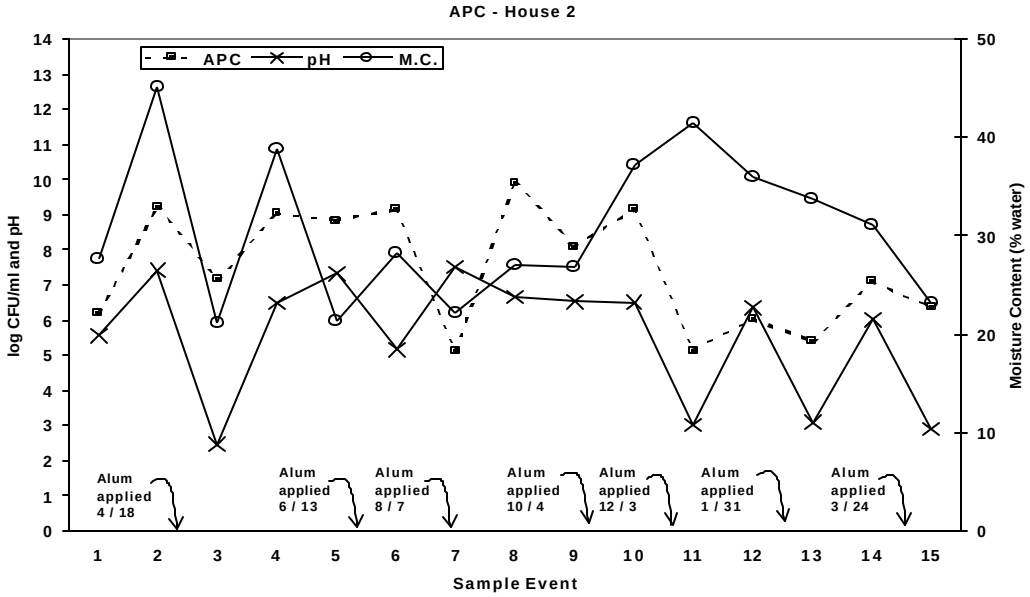

Figure 1.6. APC Enumerations, Litter pH, and Litter Moisture Content for Medium Alum Rate.

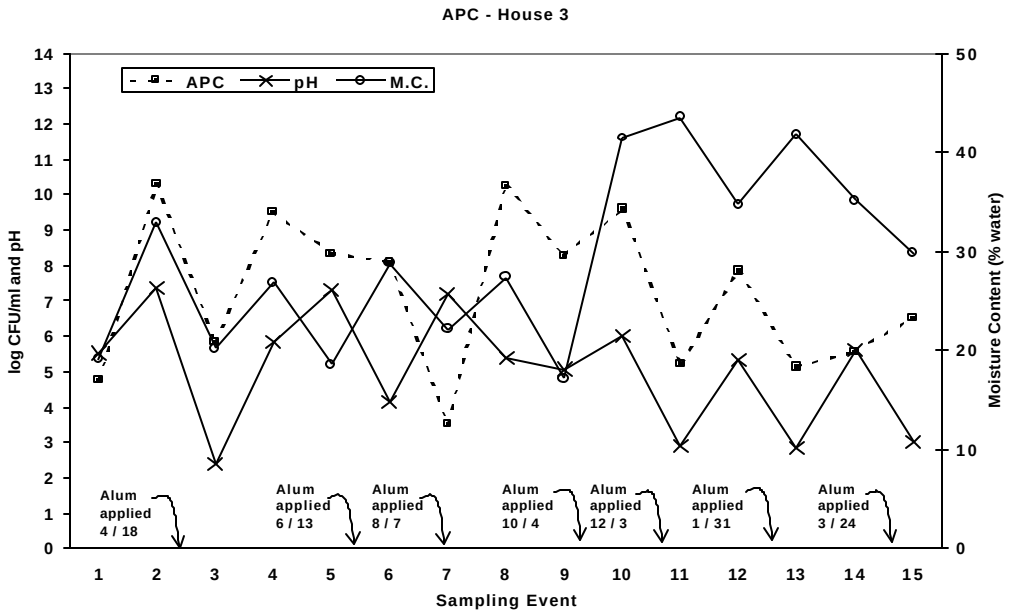

Figure 1.7 APC Enumerations, Litter pH, and Litter Moisture Content for High Alum Rate.

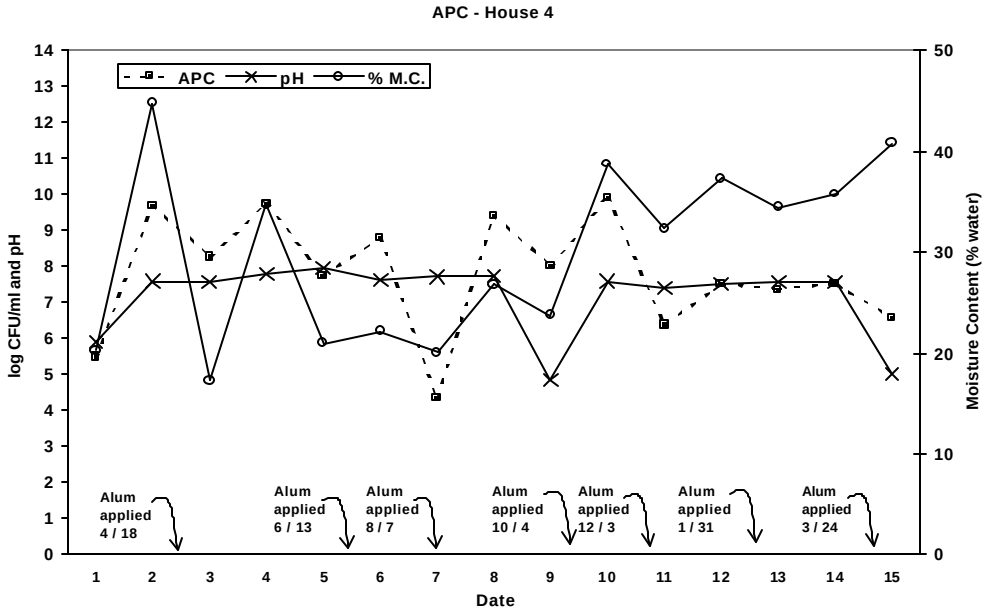

Figure 1.8 APC Enumerations, Litter pH, and Litter Moisture Content for Control Alum Rate.

Alum did not reduce APC levels between the first and second sampling event of an individual grow-out ($P<0.0001$). The APC LSM was log 6.4 CFU ml^{-1} at the beginning of the grow-outs, and after 21 d the APC LSM was log 8.6 CFU ml^{-1}. Aerobic Plate Counts were higher 21 d into the grow-outs in all houses. The effects of brooder/non-brooder location alone and under water line/middle of house sampling location proved non-significant in all houses for APCs.

Coliform assays and enumeration indicated the presence of Gram-negative, aerobic or facultative anaerobic, bacteria (Figures 1.9-1.12). The baseline coliform levels (baseline enumerations at sample event 1) were log 8.0, 7.2, 6.1, and 1.3 CFU ml^{-1} in house 1, 2, 3, and 4, respectively. Throughout the eight grow-outs, coliforms were present for the 15 sample events and showed similar trends to APCs. In house 1 and 2, five sampling events resulted in =log 4 CFU ml^{-1} (four sampling locations averaged). In house 3, four of the fifteen sampling events were <log 6 CFU ml^{-1} (the four sampling locations averaged). In house 4, 14 of the sampling events were >log 4 CFU ml^{-1} (the four sampling locations averaged). There was not a significant difference (Table 1.1) between the four treatment levels for coliform enumerations ($P=0.25$). The first sampling event of the grow-outs resulted in a coliform LSM of log 4.9 CFU ml^{-1}, and the second sampling event of the grow-outs resulted in a coliform LSM of log 6.9 CFU ml^{-1}. As with APC enumerations, coliforms were higher by the second sampling event of the grow-outs in all houses but there was a decreasing trend in coliform enumerations over the study period, except in house 4.

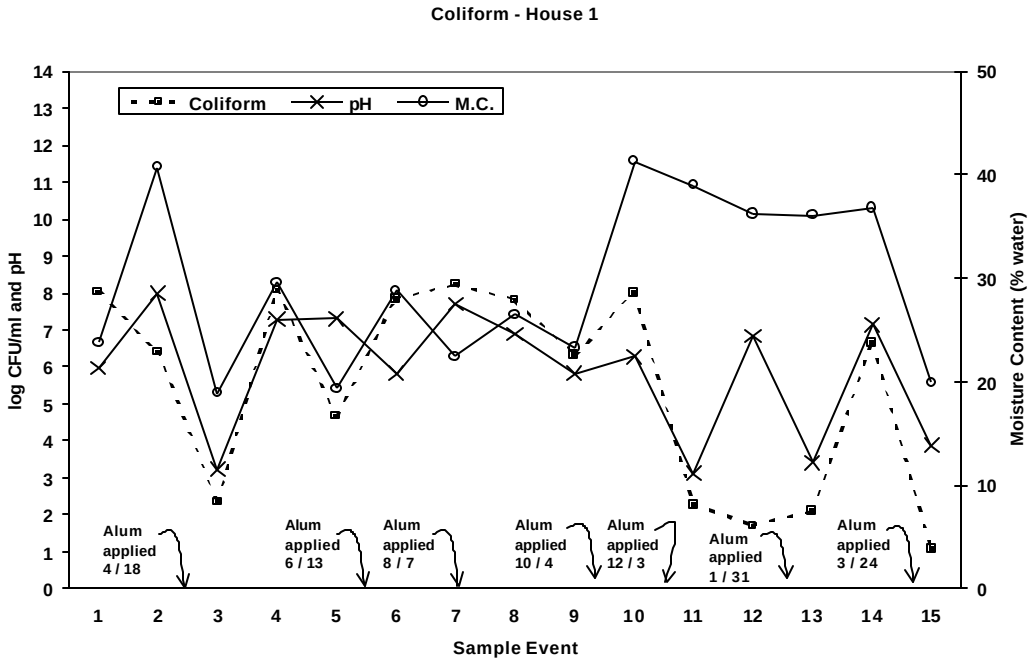

Figure 1.9 Coliform Enumerations, Litter pH, and Litter Moisture Content for Low Alum Rate.

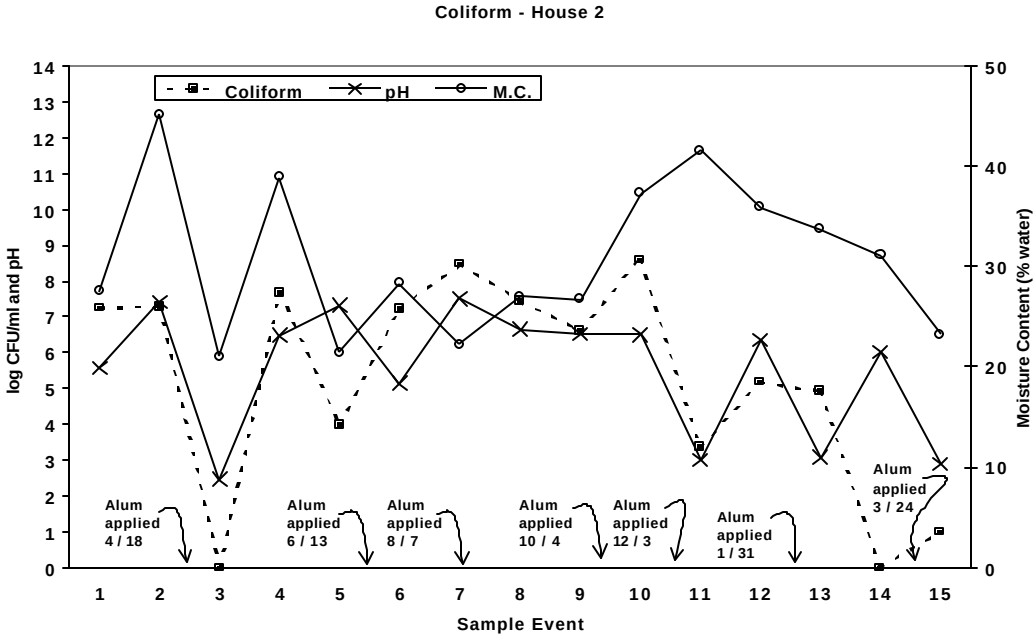

Figure 1.10 Coliform Enumerations, Litter pH, and Litter Moisture Content for Medium Alum Rate.

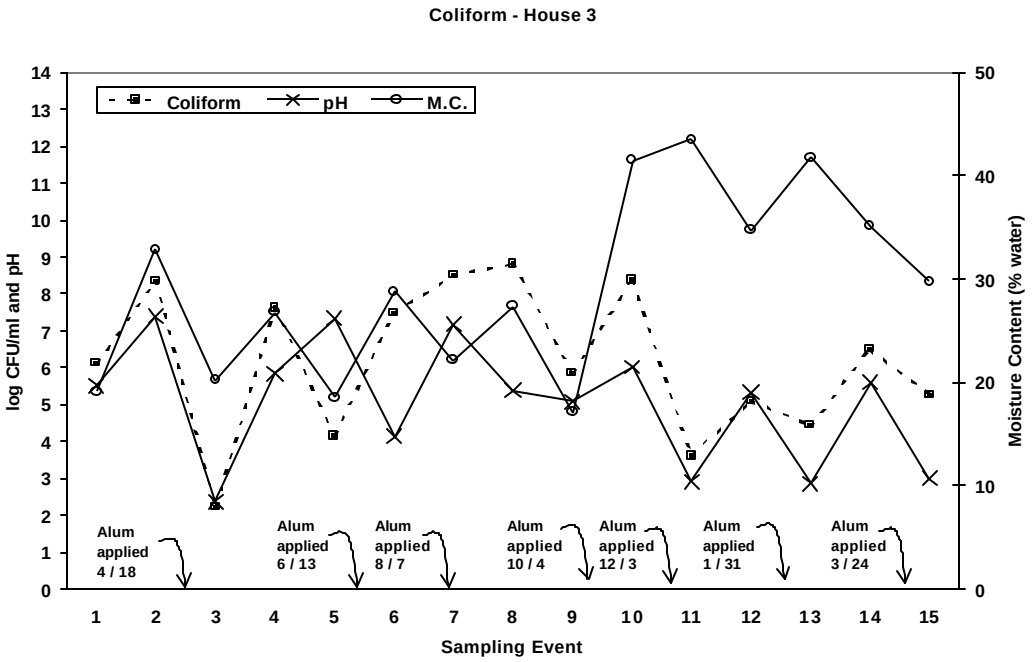

Figure 1.11 Coliform Enumerations, Litter pH, and Litter Moisture Content for High Alum Rate.

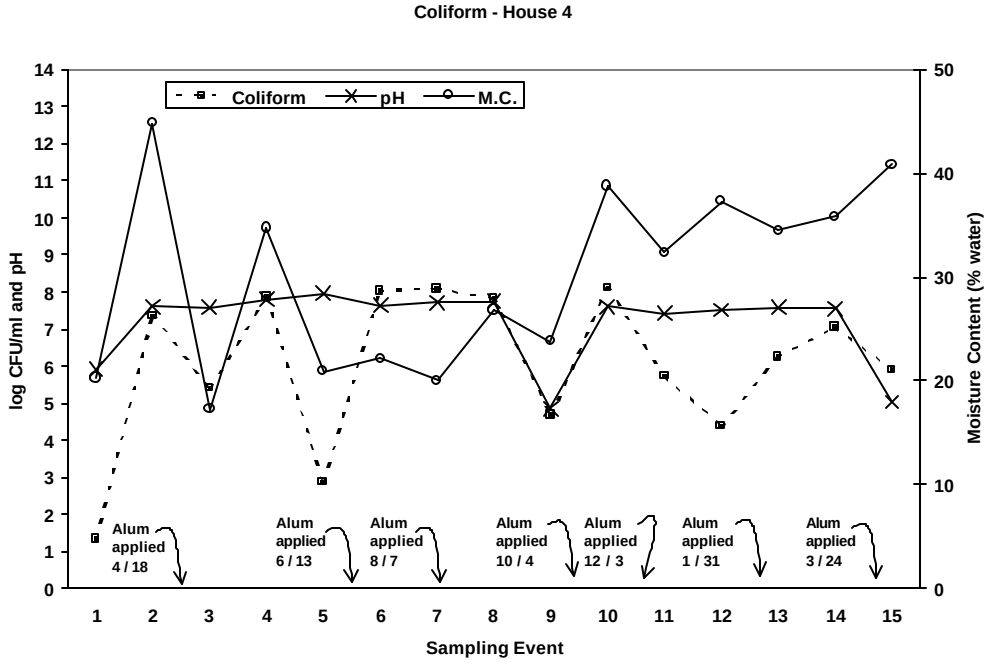

Figure 1.12 Coliform Enumerations, Litter pH, and Litter Moisture Content for Control Alum Rate.

The *Salmonella* enumeration results are shown in Figures 1.13-1.16. The baseline *Salmonella* levels (baseline enumerations at sample event 1) were log 5.5, 5.0, 3.4, and 5.0 CFU ml^{-1} in house 1, 2, 3, and 4, respectively. There was a significant difference in *Salmonella* levels between the four treatment levels ($P<0.05$). The three alum-treated houses were not significantly different, and house 4 (control) had the lowest LSM. *Salmonella* was not isolated for the last five sampling events in the four houses, which caused lower than expected LSMs. During the study, *Salmonella* was not detected for sampling events at the beginning of the grow-outs, but *Salmonella* did increase gradually over an individual grow-out in all houses. When pH was reduced to = 4, *Salmonella* was undetectable in house 1, 2, and 3. Although the control had the lowest LSM (Table 1.1), *Salmonella* levels declined from March to October in the three treated houses. At 21 d into the grow-outs, the *Salmonella* LSM was log 3.0 CFU ml^{-1}, and at the beginning the *Salmonella* LSM was log 1.6 CFU ml^{-1}. The interaction of first and second sampling event effect with liquid alum rate effect was significant ($P<0.02$). At the beginning of the grow-outs, the low (house 1), medium (house 2), high (house 3), and control *Salmonella* levels were log 1.7, 1.9, 1.4, and 1.5 CFU ml^{-1}, respectively. At the second sampling event, the low, medium, high, and control *Salmonella* levels were log 4, 2.8, 3.5, and 1.7 CFU ml^{-1}, respectively. The control rate *Salmonella* levels for this interaction analysis was not statistically different from beginning to 21 d afterwards; the low, medium, and high rate *Salmonella* levels from beginning to 21 d afterwards were statistically different. In the three alum-treated houses, *Salmonella* levels were

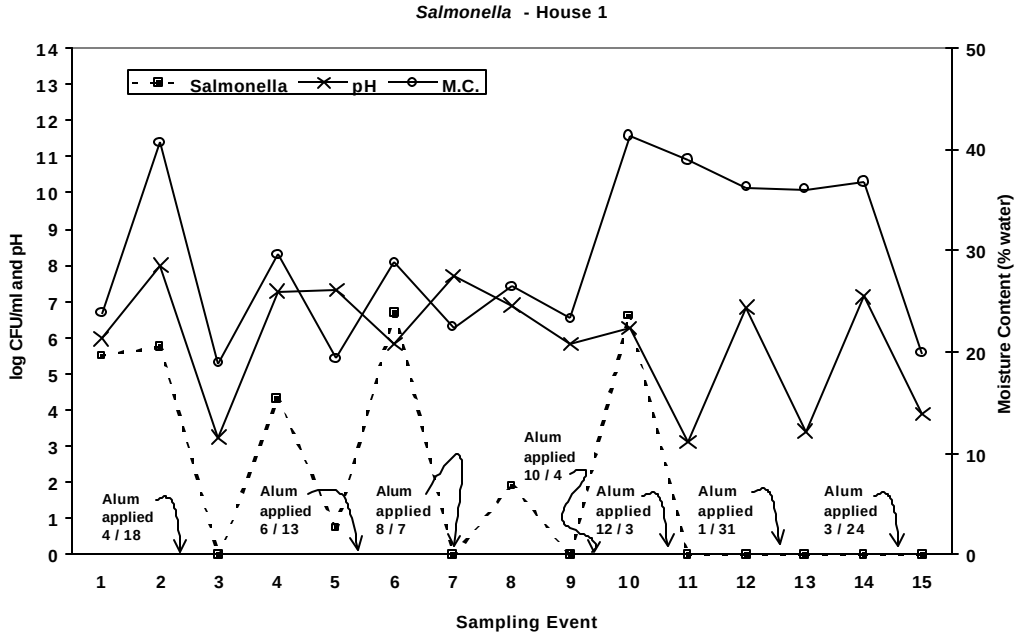

Figure 1.13 *Salmonella* Enumerations, Litter pH, and Litter Moisture Content for Low Alum Rate.

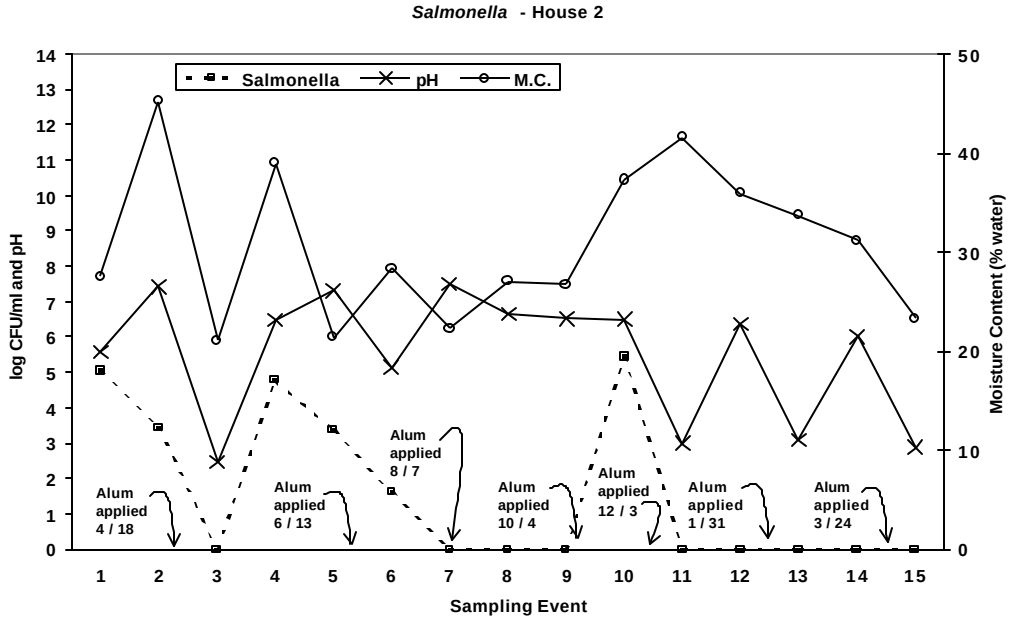

Figure 1.14 *Salmonella* Enumerations, Litter pH, and Litter Moisture Content for Medium Alum Rate.

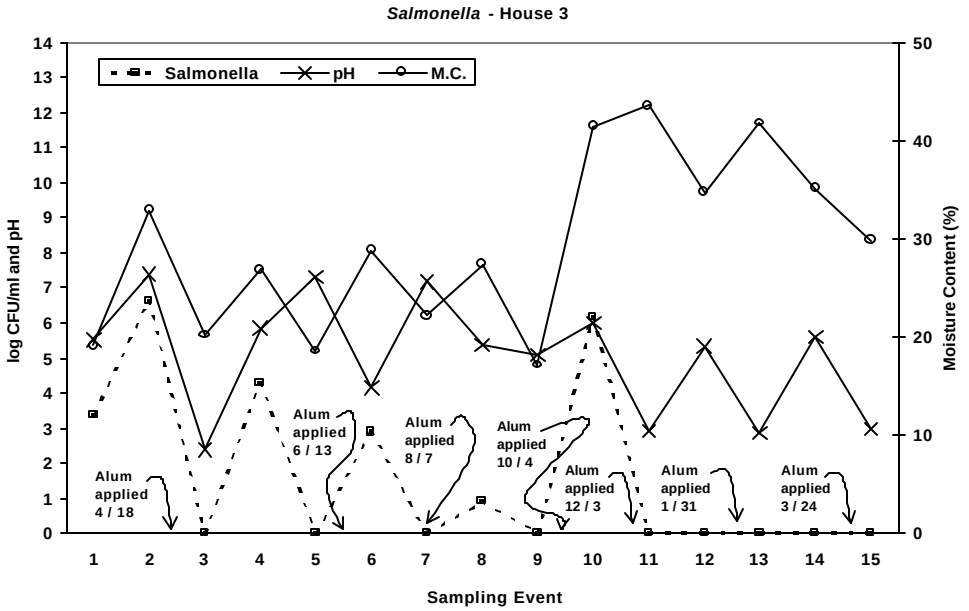

Figure 1.15 *Salmonella* Enumerations, Litter pH, and Litter Moisture Content for High Alum Rate.

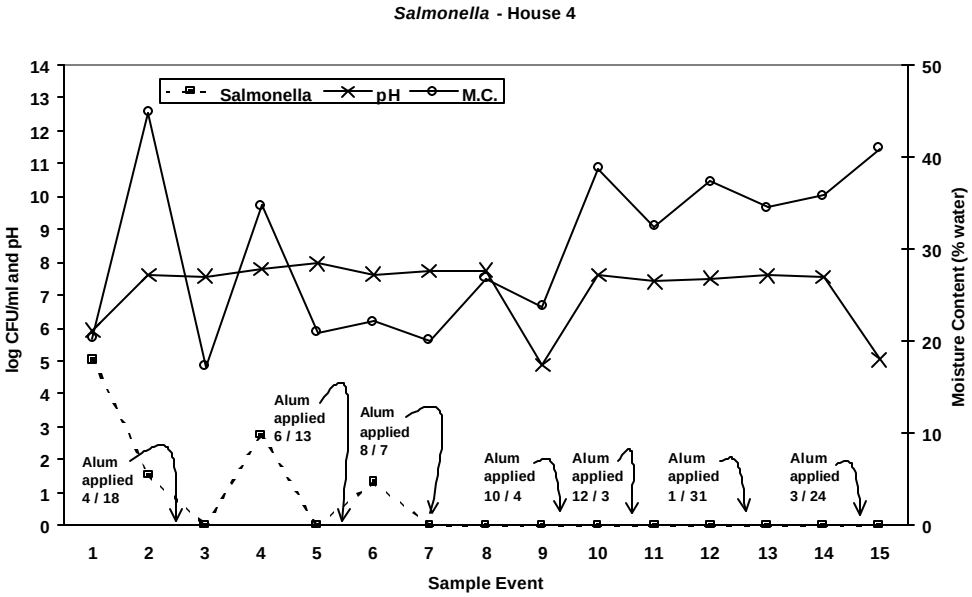

Figure 1.16 *Salmonella* Enumerations, Litter pH, and Litter Moisture Content for Control Alum Rate.

higher after 21 d. The effects of brooder/non-brooder location and under water line/middle of house sampling location proved non-significant for *Salmonella* enumerations. In house 4, *Salmonella* was isolated 10 times over the course of the study.

The *Campylobacter* enumeration results are shown in Figures 1.17-1.20. The baseline *Campylobacter* levels were log 3.5 (detected at sampling event 4), 0.94 (detected at sampling event 6), 0, and 1.2 (detected at sampling event 6) CFU ml^{-1} in house 1, 2, 3, and 4, respectively. There were significant differences in *Campylobacter* levels between the four treatment levels, and *Campylobacter* was detected for three months (Table 1.1). However, the ANOVA results showed the data not normally distributed (Shapiro-Wilk=0.43). The medium rate reduced pathogen levels by log 1 CFU ml^{-1} and the low rate reduced pathogen levels by log 3 CFU ml^{-1}. However, *Campylobacter* was only detected once in house 2 (medium). In house 4, *Campylobacter* was isolated twice, and the reduction was log 3 CFU ml^{-1}. In house 3 (high rate), *Campylobacter* was not detected. There was no first to second sampling event effect for *Campylobacter*. The effect of under water line/middle of house sampling location was significant ($P<0.03$). The *Campylobacter* LSM under the water line was log 1.3 CFU ml^{-1} and the LSM at the middle of the house was log 1.0 CFU ml^{-1}.

Some additional observations concerning house 4 (control) and the overall experiment are worth mentioning to help explain the confounding perception in the results. During numerous conversations with the grower, comments were

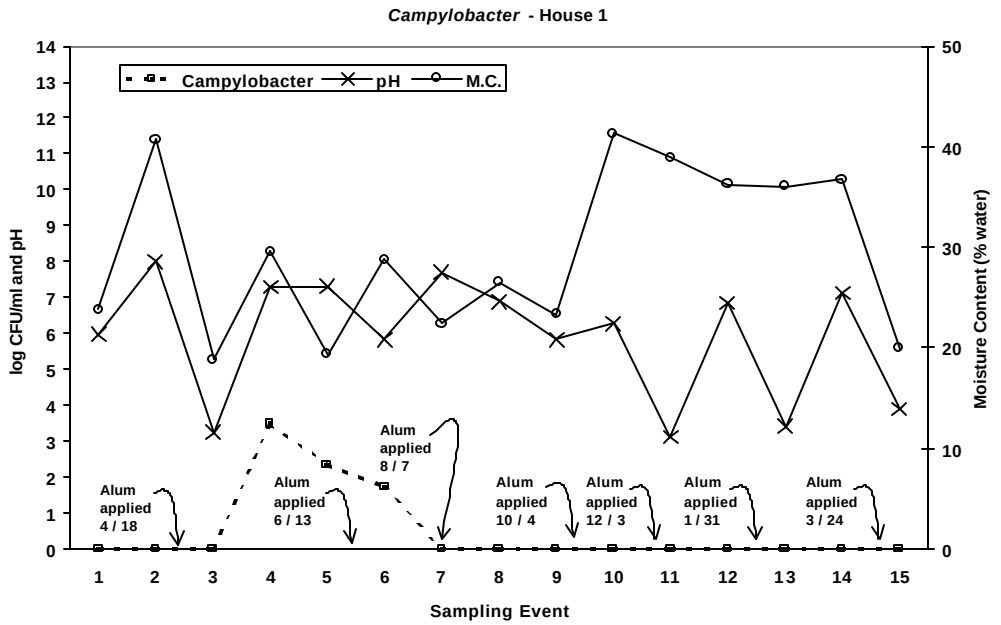

Figure 1.17 *Campylobacter* Enumerations, Litter pH, and Litter Moisture Content for Low Alum Rate.

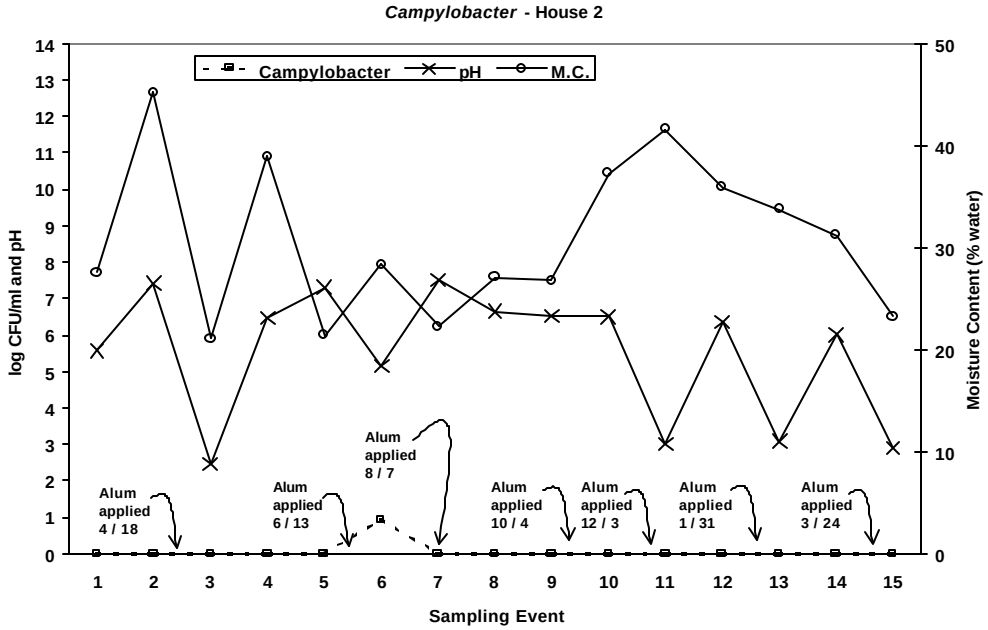

Figure 1.18 *Campylobacter* Enumerations, Litter pH, and Litter Moisture Content for Medium Alum Rate.

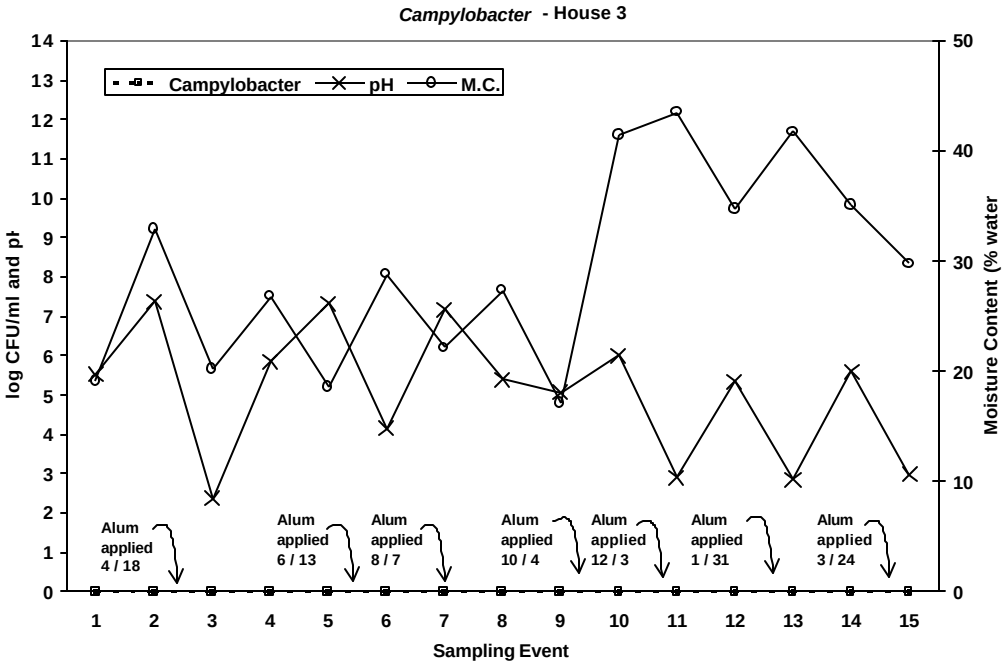

Figure 1.19 *Campylobacter* Enumerations, Litter pH, and Litter Moisture Content for High Alum Rate.

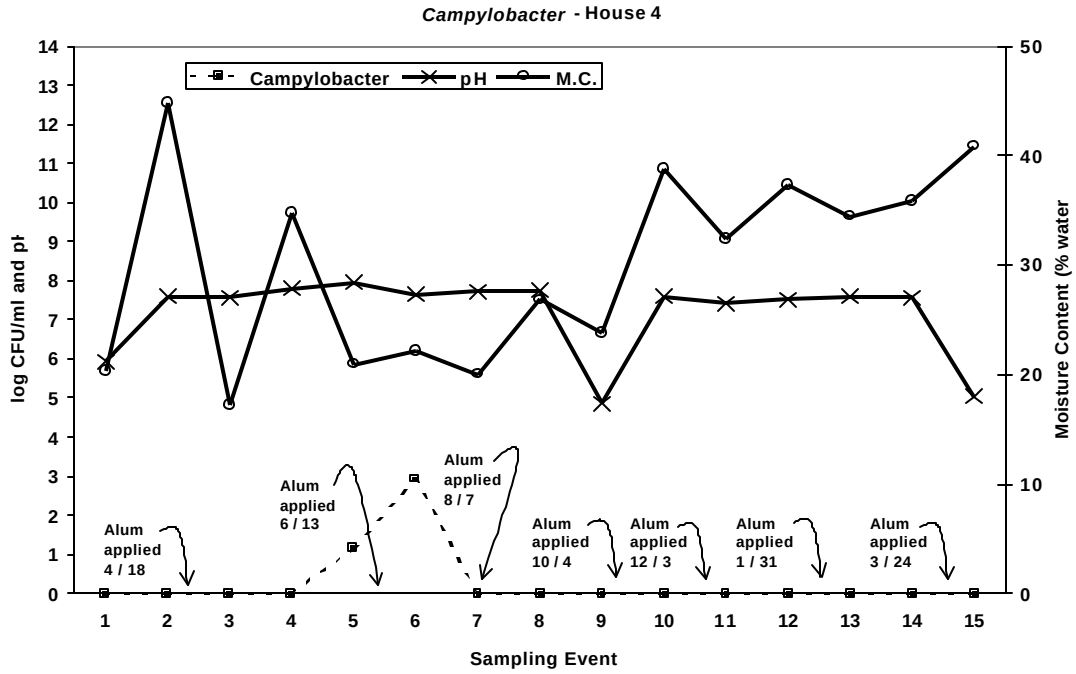

Figure 1.20 *Campylobacter* Enumerations, Litter pH, and Litter Moisture Content for Control Alum Rate.

made that house 4 repeatedly performed better than the other three broiler houses. The grower was referring to house 4 growing or producing a better bird. In house 4, the market broiler was historically heavier and healthier according to the grower. Therefore, the project members decided to make house 4 the control house to remove bias. In part 2 of this work, broiler production data is presented and house 4 did perform better on more than one occasion.

The second factor in the experiment design concerns the liquid alum application protocol. Upon arrival to the farm, the truck driver would apply in house 1, then move to house 2 and apply, and last house 3. The driver did not drive in house 4. The truck tires were not screened for pathogens and cross-contamination is a possibility. While the commercial applicator indicated the truck was disinfected between farms, no verification of disinfections was made prior to alum application in the three broiler houses.

Another issue is the source of newly hatched birds from the grower's integrator. Ideally, the grower will receive new birds for all the broiler houses on one day and begin a grow-out on the same day. On various instances, the houses were stocked with new birds on different days. However, all houses were stocked within 48 h of each other. The integrator indicated that the brooders used to stock the four houses may not have come from the same hatchery in these instances.

Data collection for this work was coordinated to begin when the grower performed a complete house cleanout of the four houses. After the complete house cleanout, litter samples were taken as outlined in the calendar of events

(Appendix, Table A.2). As noted, alum was not applied for the first grow-out. Sample event 1 enumerations serve as the baseline for the experiment. In sampling events 1 and 2, enumerations for APC, Coliform, and *Salmonella* resulted in approximately log 6-7 CFU ml^{-1}. *Campylobacter* was not detected in sample events 1 and 2. This work is suggesting more baseline data before starting with liquid alum could have contributed to better results.

CONCLUSIONS AND RECOMMENDATIONS

One reason the litter pH returned to a range of 7-8 within 21 d is because of manure accumulation. In the broiler manure, ammonium is in equilibrium with ammonia. During the first three weeks of a grow-out, the acidity effect of the alum diminishes due to ionic exchanges with the ammonia in the manure and returns to the more neutral range. Also, the feces accumulation and water from the broiler water delivery system are two reasons that contribute to the litter moisture increase. These described conditions of moisture content and pH offer an ideal survival and growth condition for microorganisms (Jay, 2000, Doyle, 1989).

There were no statistical treatment differences in APCs or coliform levels among the four houses over all 15 sampling events. These two assays are general indications of microorganism (pathogenic and non-pathogenic) activity in the litter. The litter conditions (with or without broilers) contain resources for microorganisms to live and grow throughout a production cycle. In house 1 (low), the coliform-baseline was log 8.0 CFU ml^{-1} and log 1.1 CFU ml^{-1} at sampling

event 15. In house 2 (medium), the coliform-baseline was log 7.2 CFU ml^{-1} and log 1.0 CFU ml^{-1} at sampling event 15. In house 3 (high), the coliform-baseline was log 6.1 CFU ml^{-1} and log 5.3 CFU ml^{-1} at sampling event 15. In house 4 (control), the coliform-baseline was log 1.3 CFU ml^{-1} and log 5.9 CFU ml^{-1} at sampling event 15. Although no significant difference in treatment level enumerations, the low, medium, and high liquid alum rates were found to be effective in reducing coliform levels below the initial baseline levels in broiler litter over a one-year period. The four liquid alum rates were not effective in reducing APC levels over a one-year period.

Salmonella was undetectable or at low levels at the beginning of each of the grow-outs because birds were not present, and, therefore no host environment for the pathogen (Jay, 2000, Jackson, 2001). The second sampling event was 21 d afterwards, and the pH had returned to a suitable level for *Salmonella* to survive and grow. As the birds grew, moisture content increased and feces accumulated providing conditions for the survival and growth of *Salmonella*. Statistically, there were differences in *Salmonella* levels between the treated houses when all 15 sampling events were considered in ANOVA analysis, but the control was the lowest; the low, medium, and high alum treatments were not different among each other. House 4 enumerations may have been lower because of the previously mentioned historical performance. In comparing all *Salmonella* enumerations at the beginning of a grow-out versus 21 d afterwards across the four houses, *Salmonella* levels increased (log 1.6 CFU ml^{-1} to log 3.0 CFU ml^{-1}) within a grow-out. In house 1 (low), the *Salmonella*-

baseline was log 5.5 CFU ml^{-1} and undetected at sampling event 15. In house 2 (medium), the *Salmonella*-baseline was log 5.0 CFU ml^{-1} and undetected at sampling event 15. In house 3 (high), the *Salmonella*-baseline was log 3.4 CFU ml^{-1} and undetected at sampling event 15. In house 4 (control), the *Salmonella*-baseline was log 5.0 CFU ml^{-1} and undetected at sampling event 15. The low, medium, high, and control liquid alum rates were effective in reducing *Salmonella* levels in broiler litter over a one-year period. This work suggests two reasons in explaining why *Salmonella* was not eliminated completely or reduced by the second sampling event within an individual grow-out. One reason is the litter pH was not reduced to = 3.3 without a delay at an intermediate pH level, and Hill et al. (1995) points out the importance of this step. This study sampled prior to alum application or within 48 h after application, and 21 d following bird introduction. No intermediate pH analyses were made between the beginning and 21 d into the growout. Secondly, Jay (2000) discusses that if broiler chicks are colonized with *Salmonella*, the bacteria may be transmitted to the feces. Once the feces is contaminated and excreted, *Salmonella* may be carried onto surrounding birds and colonize in the litter (Jay, 2000). It is noteworthy to recall that this study was conducted in four commercial broiler production facilities. Each broiler house contained an average of 30,000 birds per grow-out and achieved an average market weight of 2.2 kg during each of the eight grow-outs. Considering the mentioned factors, the *Salmonella* results were not abnormal.

The optimal growth temperature for *Campylobacter* is 42°C, and house temperature ranged 25-30°C (AOAC, 1998). Moreover, this bacterium is a

microaerophilic organism and the samples were surface grab samples. The litter surface is predominantly aerobic, and *Campylobacter* would not likely survive in this condition (Line, 2002, Stern et al., 1995). The combination of these two experiment circumstances made it difficult to isolate *Campylobacter*, and, therefore a challenge to attain sufficient data to statistically analyze thoroughly. This conclusion is supported by the Shapiro-Wilk statistic result, which is a normality test for data distribution by ANOVA and is recommended to be = 0.90 (Saxton, 2003). Like the other bacteria, the baseline enumerations are important to recall. In house 1 (low), the *Campylobacter*-baseline was log 3.5 CFU ml^{-1} and undetected at sampling event 15. In house 2 (medium), the *Campylobacter*-baseline was log 0.94 CFU ml^{-1} and undetected at sampling event 15. In house 3 (high), *Campylobacter* was undetected at all 15 sampling events. In house 4 (control), the *Campylobacter*-baseline was log 1.2 CFU ml^{-1} and undetected at sampling event 15.

While this work suggests that liquid alum was effective in reducing *Salmonella, Campylobacter,* and coliforms, the data is confounded by the fact that the control house experienced similar reductions from baseline levels. Within approximately 21 d, the acidity of alum will diminish and *Salmonella* may grow and multiply. While the low alum rate was effective in reducing *Salmonella*, several sampling events in the study demonstrated the medium and high were effective in reducing the litter pH below 3.3 for at least 21 d (events 3, 11, and 15). This pH could prove beneficial toward controlling *Salmonella* levels in the litter. While *Campylobacter* was not detected in house 3 (high), the medium rate

appears effective in reducing *Campylobacter* levels (= 1 log CFU ml^{-1}). Additionally, this rate will prove more cost effective to the grower. There was a *Campylobacter* reduction, but caution must be taken because the ANOVA results suggest the data is not normally distributed. Conversely, this work is not making suggestions or recommendations pertaining to colonized pathogens in the birds or further contamination once the birds have been removed from the houses at the end of a grow-out.

Hypothesis 1 states bacterial levels in the four broiler houses were not reduced by liquid alum application rates from 0 to 2.46 L m^{-2}. Therefore, hypothesis 1 is not supported, except in regard to APC, for the following reasons: salmonella and campylobacter levels were reduced over the 12-month period by the four treatment rates; coliforms were reduced over the 12-month period by the four treatment rates except in house 4 which had no alum applied during the study. Aerobic Plate Counts were not reduced over the 12-month study by the four treatment rates.

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PART 2: PRODUCTION HOUSE AMMONIA CONCENTRATIONS AND FARM PRODUCTION DATA

ABSTACT

High ammonia concentrations in commercial broiler production houses can result in poor bird performance, lower feed conversion ratios, and higher mortalities. Growers have traditionally controlled in-house ammonia levels by increasing ventilation. During cold weather, increased ventilation rates result in higher energy consumption to heat the incoming air. Granular alum (aluminum sulfate, $Al_2(SO_4)_3 \cdot 14H_2O$) has been successfully used as a litter amendment to reduce ammonia volatilization from litter inside the production house. In this study, liquid aluminum sulfate (Al^+Clear® Liquid Alum, General Chemical Corporation, Parsippany, New Jersey) was investigated as a litter amendment for ammonia suppression in four commercial poultry-broiler houses. This project investigates four treatment levels of liquid alum in four adjacent broiler facilities of the same design. The houses were treated with the following rates of liquid alum: 0, 0.82, 1.64 and 2.46 L m^{-2}. These rates are equivalent to 0, 45, 90, and 135 kilograms of dry aluminum sulfate per 93 m^2 of floor area on an aluminum sulfate basis. In-house gaseous ammonia levels, temperature, relative humidity, fan flow-rates and mortalities are reported over one grow-out cycle in this paper. The lowest rate of liquid alum application, 0.82 L m^{-2}, was effective at maintaining in-house ammonia levels below 25 ppm for the first two weeks of the grow-out. Both the 1.64 L m^{-2} and 2.46 L m^{-2} alum application rates were found to provide effective control of in-house ammonia concentrations for the first three weeks of the grow-out.

LITERATURE REVIEW

Broiler Litter Characteristics

Broiler litter is the largest source of nutrients for crop production generated by the poultry industry. According to the USDA's *National Engineering Handbook*, broiler litter is a specific form of poultry waste that results from floor production of birds after an initial layer of bedding material is spread on the floor (USDA, 1992). Bedding material, such as pine wood shavings or peanut hulls, is used to absorb liquids. Chicken litter contains water, nitrogen, phosphorus, potassium, wood shavings, and many other elements. In addition to the contents of chicken litter, ammonia gas is lost due to volatilization. Ammonia gas has been a known problem for many years in the poultry industry and researchers have used various chemical amendments to control ammonia gas as well as soluble phosphorus in chicken litter. As early as 1950, researchers have known that high levels of ammonia gas can cause respiratory problems for birds (Bullis et al., 1950). During the past decade, the University of Arkansas and the USDA have conducted field assessments, and they concluded that the application of aluminum sulfate (also referred to as alum) to chicken litter results in the best combination of environmental and economic benefits (Moore et al., 1999). Alum is a dry acid, but can also be applied in liquid form, with the chemical expression $Al_2 (SO_4)_3 \cdot 14H_2O$ and is sold commercially. Some key principles about alum concern its chemical reactions and benefits in a broiler production house.

The litter in a broiler production house consists of the chicken manure and the bedding material. Chicken manure contains uric acid, which has an ammonium ion. In addition, the broiler litter will have an average 25% moisture content according to the National Research Service (NRS) statistics (USDA, 1992). Ammonium and water are important in the control of ammonia volatilization. Ammonia volatilization is the passing off of ammonia in gas form to the atmosphere. Furthermore, the rate of ammonia volatilization from litter is dependent on pH, moisture content, wind speed, ammonium concentration, and temperature (USEPA, 2002, Moore et al., 1994, 1996).

One of the critical factors in controlling volatilization is the pH of the broiler litter because it attributes to the ratio of ammonia: ammonium. The chemical reaction that takes place evolves around the following equation: $NH_4^+ \Leftrightarrow NH_3 + H^+$. The left side of the equation is ammonium (aqueous) and the right side is ammonia (gaseous) and a hydrogen ion. The application of alum (acidic compound) to the litter can shift the above-mentioned equation to the left by changing the litter pH. In general, a pH < 7 shifts the equation towards ammonium, and therefore ammonia is less likely to volatilize. When alum is added to broiler litter, the litter pH decreases because more hydrogen ions are being added from the acid, which shifts the chemical balance to the left, and less ammonia gas is volatilized into the surrounding air of the chicken house. Also, temperature can shift the chemical balance between ammonium and ammonia. Assuming the amount of ammonium remains stable, an increase in temperature will result in an increased ammonia volatilization rate (Arogo et al., 2001). One

particular advantage liquid alum has over dry alum is liquid alum begins lowering the litter pH immediately when applied to the house floor. Dry alum's reaction is delayed because hydrolysis is necessary.

Alum Advantages

The above chemical characteristics of alum lead to direct benefits for the grower and can possibly improve the operations profit. High in-house ammonia concentrations have caused problems for a bird's performance in a typical broiler house setting. In general, the bird's immunity decreases allowing more susceptibility to various diseases, specifically, in the respiratory tract (Bullis et al., 1950). During the brooder stage (approximately the first 14 d of a grow-out), ammonia has the most negative effect on a bird's performance. As a result, alum can potentially reduce some notable disadvantages of ammonia in a broiler production house during the first crucial growing days. All of these attributes can lead to higher broiler productivity, lower mortalities, or lower operational costs and, therefore, higher profits for the producer (Moore et al., 2000). An additional cost benefit is the reduced use of propane and electricity. During the colder months, growers minimize fan operation to conserve propane. As a result, ammonia concentrations can build up in the house. Using alum can reduce litter ammonia losses thereby requiring less fan and heater operation time for production houses. Another benefit is a higher value for broiler litter because the reduction of ammonia volatilization results in an increased amount of nitrogen in the litter. Shreve et al. (1994) showed that there was a decrease in soluble

reactive phosphorus and total phosphorus concentrations in the agricultural runoff from soils where broiler litter amended with dry alum had been applied. The reduction of phosphorus concentrations in agricultural runoff could assist in preventing surface water eutrophication (Sharpley et al., 1999).

Previous Studies

Moore et al. (1996) showed an average of 14.4 g N kg^{-1} litter lost as a result of volatilization in the control houses. The rate of NH_3 volatilization is dependent on pH, moisture content, wind speed, ammonium concentration, and temperature. Moore et al. (1996) treated the litter of two experiment houses at rates of 65 g alum kg^{-1} litter and 130 g alum kg^{-1} litter. Moore reported ammonia losses of 4.07 and 0.70 g N kg^{-1} litter for the alum treatments, respectively. Moore tested 200 g alum kg^{-1} litter that resulted in only 0.2 g N kg^{-1} litter lost to volatilization. Since the higher application rates are not as economical as lower rates, growers will most likely take the frugal route and choose the lower rate. Moore showed that lower alum application rates result in more ammonia losses, and consequently, nitrogen emissions from broiler houses may continue as a result of NH_3 volatilization.

Several devices and methods have been used and tested in industry to measure ammonia (Demmers et al., 1997, Koerkamp et al., 1998b, Wilhelm and McKinney, 1999a, Muller et al., 2003, Worley et al., 2002). For example, the earlier acid traps, acid scrubbers, detector tubes, electrochemical cells, and passive diffusion devices are a few that engineers and scientists have used in

the past (Moore et al., 1994). When measuring atmospheric ammonia, there are two important factors to consider: we must realize NH_3 is present as a gas and is attached to particulate matter in the atmosphere; and we must know the NH_3 concentrations levels expected in the particular agricultural setting. Also, some detection devices are not practical because of NH_3 extraction precautions needed to detect low ambient atmospheric concentrations (Arogo et al., 2001). Arogo et al. (2001) mentions detector tubes but does not recommend them because they are typically used for higher NH_3 concentrations. Dräger manufactures a transmitter/sensor unit to measure lower concentrations. The unit functions on the electrochemical cell concept where the gaseous reaction in an electrolytic solution produces an electrical potential and, therefore, the output is measured in the form of current or voltage. One other commonly used NH_3 concentration-measuring devices is a chemiluminesence NO_x – analyzer (Wathes et al., 1997, Demmers et al., 1999). A few U.S. studies have used Dräger Pac III NH_3 Portable Monitoring Units (PMUs) (Xin et al., 2002, Liang et al., 2003).

One measurement method is a gas-measuring chamber and is built with a bucket, rubber septa, wire, and cardboard (Moore et al., 1997). Once the ammonia gas was captured in the chamber, a Sensidyne ammonia detection tube was used to detect an ammonia level at the sample location. This data acquisition technique is manual, and so an additional step must be taken to calculate the ammonia flux using the ideal gas law. The study that developed this gas chamber method pointed out that NH_3 flux was estimated and, thus,

independent of the ventilation rate (Moore et al., 1997). Point and case, ammonia flux exiting an animal production facility is not accurate without an exact air volume leaving the house. The correct ventilation rate is paramount because the majority of recently built broiler houses have up to eleven ventilation fans. The ammonia measurement design must account for the different ventilation rates and the differential pressure across the production house when a ventilation fan is on.

The measurement time interval must be addressed when measuring ammonia concentrations and even more so with ammonia emissions. One of the objectives of the gas chamber research was to "determine the optimum time needed to measure ammonia fluxes" (Moore et al., 1997). During one six-week experiment, Moore et al. (1997) measured the ammonia concentration every 5 min. As noted, the ammonia flux calculations were done using PV=nRT, but the volume of NH_3 gas was calculated over a one hour time interval. The same research concluded that ammonia levels are not static and several repetitive samples are needed for ammonia flux calculations leaving the production houses.

MATERIALS AND METHODS

Four modern commercial broiler production houses were instrumented to conduct the experiment; they were tunnel-ventilated, and each house measured 152 m (500 ft) x 12 m (40 ft) (1860 m^2). Nine 130-cm (51 in) and two 91-cm (36 in) constant velocity fans ventilated each house. Data collection was during the

2002-2003-production year (eight grow-outs), when approximately 30,000 birds were produced for each 42 d grow-out period. Data from grow-outs 5-8 are presented due to unacceptable performance of ammonia sensors during grow-outs 1-4 (explained in *Ammonia Gas Sensors* of this section). During the first 14 d of the grow-out, birds were maintained in the brooder end of the house, ventilated by the 91-cm fans. After day 14, a divider curtain was raised, and the birds occupied the entire production house; all eleven ventilation fans were used as needed for ventilation.

A commercial applicator applied liquid alum to the broiler houses approximately four to seven days prior to bird introduction. At each alum application event, the truck driver applied alum to house 1 first, then house 2, and last house 3. No alum was applied to house 4. The application rates were 0.82, 1.64, 2.46, and 0 L m^{-2} to houses 1, 2, 3, and 4, respectively. For the remainder of this part of the work, the author will refer to the house 1 rate as low, the house 2 rate as medium, the house 3 rate as high, and the house 4 rate as control.

Production House Instrumentation Overview

Four adjacent commercial broiler houses of the same design were instrumented to measure gaseous ammonia, fan status (on/off), differential pressure (house 4 only), temperature, relative humidity and feed motor run-time using a system similar to that developed by Wilhelm et al. (1999b). The basic data acquisition system design is shown in Figure 2.1. Wilhelm et al. (1999b)

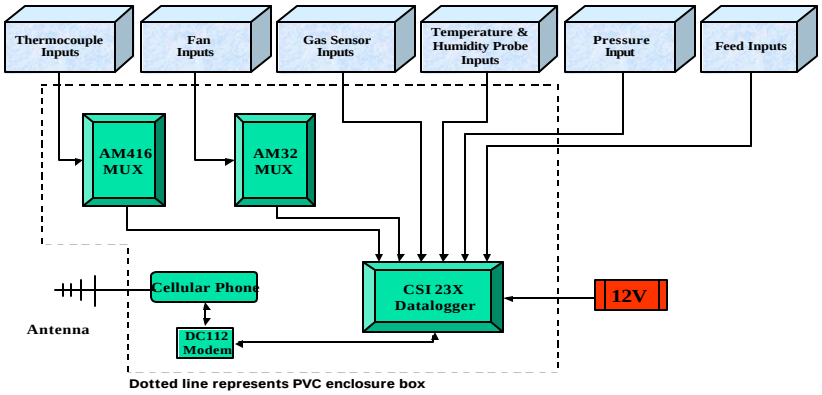

Figure 2.1 Data Acquisition System Configuration.

provides more detailed cost and other information on the data acquisition system design and gives a history of the system since its inception in 1994. The instrumentation system used in this project was updated with newer model components wherever we found a newer unit available that provided advantages over the component types originally used. Each of the four chicken houses had a control room as part of the house structure containing broiler production equipment. A set of data collection equipment was enclosed in a PVC manufactured enclosure located in control houses 2 and 4. Each PVC enclosure with the components noted in Figure 2.2 collected data from a pair of houses. House 1 and 2 inputs were connected to the 23X data logger and components in the house 2 equipment room. House 3 and 4 inputs were connected to the second 23X data logger in the house 4 equipment room. A brief description of the principal components used in this study is provided below.

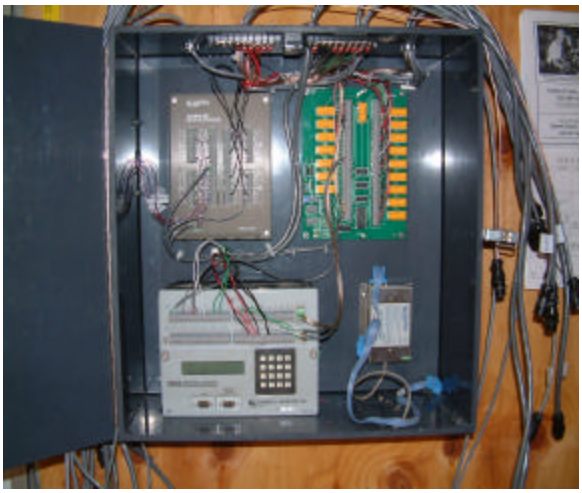

Figure 2.2 Data Acquisition Equipment in PVC Enclosure.

Data Acquisition

Two Campbell Scientific Inc. (CSI) 23X Microloggers (Campbell Scientific, Inc., Logan, UT) were used for data collection between the four houses. In conjunction with each micrologger, two CSI multiplexers (an AM416 and AM32) were programmed to scan the environmental input parameters every 5 s. A cellular phone and CSI DC112 modem was connected to each micrologger to enable remote data acquisition and viewing of real-time data. A CSI YAGI directional antenna was used with the units to improve signal strength.

Multiplexers

Four multiplexers were used to scan the environmental input parameters. A CSI AM416 and an AM32 were used in production houses 2 and 4. The CSI AM416 model permitted scanning of up to 32 differential inputs. It had two signal

channels, permitting thermocouple measurements and 0-5V (or other range) measurements via the same multiplexer. The AM32 was less flexible, having only one signal channel for all 32 differential inputs. Thus, two AM32 (one for each pair of houses) multiplexers were used to separately measure fan tilt switch voltage inputs from each production house.

Electrical/Electronic Equipment Enclosures

Two polyethylene (PVC) boxes (Figure 2.2) were used to house the key instrumentation components. The micrologger, the phone/modem unit, an AM416 and an AM32 multiplexer were mounted inside one PVC box located in production house 2. The second enclosure, located in production house 4, contained the same components.

Ammonia Gas Sensors

All ammonia sensors were Dräger Inc. (Dräger Safety, Inc., Pittsburgh, PA**)** electrochemical devices. One gas sensor unit was located in the center of each house (details in *Gas Sensor Housing*). Initially, Dräger Polytron II sensors were used for ammonia detection. These units consisted of a transmitter enclosure with the necessary electronics and a replaceable sensor. The 4-20 mA output from each transmitter was converted to a voltage signal through a 243-ohm precision resistor. This allowed the CSI 23X micrologger to use the 5 V full-scale measurement range to improve the resolution of recorded data. The

electrochemical sensors required periodic calibration to ensure accuracy. A warm-up period of at least 12 h was required before calibration. The zero calibration required 100% Ultra High Purity (UHP) nitrogen and the span calibration required a specific ammonia gas calibration concentration (50 ppm in N_2). The Polytron II sensor head accuracy is reported by the manufacturer to be ±5% for a range 21-100 ppm. The Polytron II units were provided by Dräger as direct replacements to the Polytron I units originally used by Wilhelm (Wilhelm et al., 1999b), but the Polytron II proved to be unsuitable for ammonia detection in broiler production houses. The Polytron II units were prone to provide decreasing and negative ammonia values; even while ammonia concentrations increased in the production houses. It is believed that the sensors used with the Polytron II units became saturated when exposed to continuous ammonia concentrations. Following the failure of the Polytron II units, Dräger determined that the Polytron I units were available in the European market. Between the fourth and fifth grow-outs, the Dräger Polytron II units were removed and replaced with Dräger Polytron I units. The manufacturer has been unable to provide information concerning the accuracy of the new Polytron I sensor heads at this writing. Documentation provided with the Polytron I units used by Wilhelm, was reported as ±2% in a range of 0-100 ppm. Ammonia sensors were scanned at 5-s intervals, averaged, and stored in the 23X micrologger for each 30-min period. Thus, the recorded ammonia value represents an average ammonia gas level concentration for a given 30-min period.

Sensors were calibrated between every grow-out, and gas-detection tube readings (LP-1200, RAE Systems Inc., Sunnyvale, CA) were periodically taken (ca. 20-d intervals) to verify the electrochemical sensor readings. A performance and calibration history is provided to compare and understand the performance of the Dräger Polytron I sensors against a commonly used gas-detection tube (Appendix, Table A.1).

Gas Sensor Performance

An important design feature for gas sensors is the sensor response time. The Dräger NH_3 Polytron I gas sensor time response was tested at the University of Tennessee Biosystems Engineering and Environmental Science Department using a 69-ppm test gas. The response of a Dräger NH_3 Polytron I gas sensor to a 69-ppm NH_3 test gas is shown (Figure 2.3). The time constant for the sensor is the time response for 70.7% of expected reading (.707 * 69 = 50 ppm). Based on the response to the test gas and the mentioned calculation, the sensor response time constant was 30 s. Also, T_{90} and T_{50} were 50 s and 19 s, respectively. The above sensor performance data are critical because a data-sampling interval must be chosen to accurately measure ammonia concentrations. Nyquist's Theorem states "the sampling rate must be equal to, or greater than, twice the highest frequency component in the analog signal" (Nyquist, 2003). The highest frequency component in the analog signal(s) was

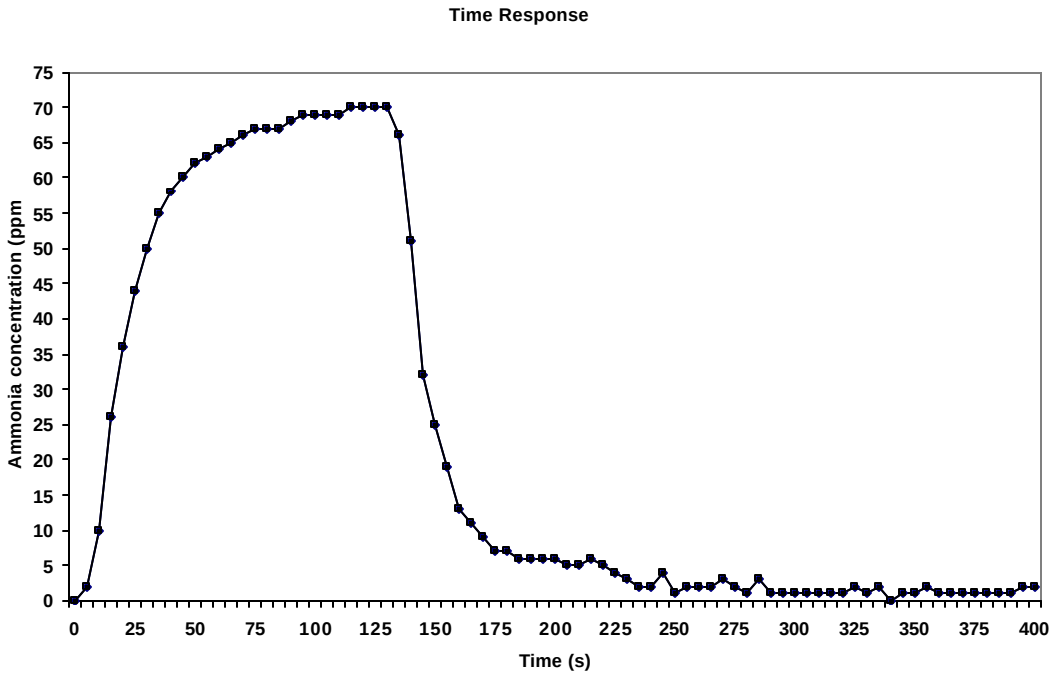

Figure 2.3 Dräger Polytron I NH_3 Sensor Time Response.

the ventilation fan signal (refer to *Fan Sensors* section below for details). Using Nyquist's Theorem and the sensor response results, a conservative 5-s data-sampling interval was chosen for the 23X micrologger program. The 5-s interval allowed us to detect the dynamic ammonia concentrations in the houses.

Gas Sensor Housing

The gas sensors in the four production houses were mounted inside a PVC housing approximately 0.5 m long x 0.22 m x 0.020 m deep, open at the bottom and at each end. This housing provided significant dust protection while

allowing air movement over the sensors. Also, the housing permitted all sensors to be handled as a single unit during installation and removal. Each sensor was bolted to the PVC housing to ensure the sensor head was parallel to the house floor. All gas sensor-housing assemblies were attached to nylon ropes and hung by a pulley arrangement from the production unit ceiling 46 cm (18 in) above the house floor. The University of Tennessee Biosystems Engineering and Environmental Science shop fabricated the gas sensor housings.

Fan Sensors

Mercury tilt switches were installed to sense fan operating status. A switch was riveted on a fan louver downstream of each ventilation fan such that the tilt switch contacts would open when the louver tilted during fan operation. This tilt switch was connected to a continuous analog output (CAO) output of the micrologger via a pull-up resistor. This system provided an output signal of 5V when the fan was operating and 0V when there was no airflow. These signals were scanned at 5-s intervals, averaged, and recorded for each 30-min period. Thus, the recorded value represented an average “on” time for each fan during that period. The Nyquist Theorem (Nyquist, 2003) approach was taken for sensing fan status as well because fans could cycle on and off as fast as every 60 s in the houses. Thus, the slowest recommended sampling rate was 30 s. The author chose a conservative 5-s sampling rate to coincide with the gas rate and to minimize programming difficulties.

Power

In houses 2 and 4, a 12-V battery provided power for the data logger, and a 'smart' charger maintained the battery voltage for long-term use 24 h day^{-1}. This approach assured uninterrupted power, which is necessary to avoid loss of gas sensor data. The six NH_3 Polytron I sensors were powered by a small $120V_{AC}$ to $24V_{DC}$ power supply.

Temperature and Humidity

In each production house, a combined temperature (T) and relative humidity (RH) sensor (HMP45C, Campbell Scientific, Inc., Logan, UT) was mounted adjacent to the central gas sensor. Rated sensor accuracies were ± 0.4 °C and ± 2% RH from 0 to 90% RH.

Wiring and Connections

To allow speed and flexibility in connecting the various components, connectors were installed for almost all wiring entering the equipment enclosures. Individual connectors were also used for cables to each gas sensor. All signal connections, with the exception of thermocouples, used AMP Series 2 circular plastic shell and plug connectors. Thermocouple connections were

made using quick disconnect thermocouple plugs and jacks. Approximately 2.7 km of cable was installed for the instrumentation of the four production facilities.

Farm Production Data Methodology

In the poultry industry, broiler integrators mandate growers to record specific production data. During the research period, farm production data from the eight grow-outs were collected from the grower. The data included on-farm mortalities, on-farm water use, USDA condemnations, and feed consumption. The production data was collected to determine if there were differences in the above-mentioned parameters for the four alum application rates. The feed consumption data were collected but was not reliable because there were instances the feed motor sensor would detect the motor running but the feed auger was empty. At various times, the grower's feed supply pipes would stop up because of feed moisture problems and cause the feed lines to operate without feed in them. The grower's integrator provided condemnation data and they represent carcasses that plant USDA inspectors condemn on the plant processing line. There are several reasons an inspector can condemn a broiler carcass. The processing equipment at the integrator's facility causes some reasons for condemnation. Contrarily, some birds arrive from the farm with physical and/or health conditions that do not meet USDA standards, and, thus are rejected. Hence, a carcass condemnation is production plant or farm level inflicted. The data reported are farm level inflicted condemnations.

The grower inspected each broiler house daily to remove the dead birds and recorded this quantity as mortalities. The grower had on-site utility water, and the grower recorded the water flow meter reading daily. Carcass condemnations are for the following reasons: tuberculosis, leucosis, septicaemia and toxemia, synovitis, tumors, bruises, cadavers, contamination, overscald, and airsacculitis. According to the integrator, all but cadavers, contamination, and overscald are farm-level inflicted (Wilds, personal communication, 2002). The integrator categorized the condemnations, and farm-level inflicted data were provided for interpretation. When broilers were taken out on the last day of the grow-out, paint was sprayed on the chicken cage that was the first cage for each chicken house. At the plant, the integrator was able to tally the condemnations on a house-by-house basis to allow alum treatment comparisons. The grower provided propane receipt photocopies for the propane data. The propane tanks were not filled at exactly the beginning and end of each grow-out, therefore propane data is analyzed for the entire production period (January 2002 – May 2003).

Statistical Analysis

A randomized block design was used to test for alum treatment level effects on farm mortalities, on-farm water use, condemnations, fan operation time, and in-house ammonia concentrations across the four houses. Analysis of variance (ANOVA) was conducted with mixed models (SAS, 1999), and least

square means (LSMs) were separated using Fisher's least significant difference test (a = 0.05).

IN-HOUSE AMMONIA CONCENTRATION RESULTS AND DISCUSSION

Prior to the arrival of brooders (broiler chicks) at the beginning of a grow-out, a grower will ignite the propane heaters to raise the temperature and establish a suitable environment for the birds. The rise in temperature causes NH_3 volatilization to increase and NH_3 concentrations increase as well (Moore et al., 1994, USEPA, 2002). Poultry producers refer to this practice as a 'cookoff' period, which lasts approximately 24 h. The 'cookoff' period for grow-out 7 is provided to illustrate NH_3 concentration trends for the alum treated houses (Figure 2.4). Alum was applied January 31, 2003 in houses 1, 2, and 3; bird introduction was 2/3/03 in all houses. After igniting the heaters on 2/3/03, the temperature rose to 13°C, and ammonia concentrations in house 4 (control) jumped to 60 ppm. In the treated houses, ammonia concentrations remained below 20 ppm, and even more importantly below 25 ppm, for 12 d. House 3 (high) had the lowest in-house NH_3 concentration with exception of one day. These results show that liquid alum can aid to suppress in-house NH_3 concentrations below the OSHA 8-h human exposure limit (25 ppm) during the 'cookoff' period and for at least 12 d after bird introduction.

Ammonia data is available for grow-outs 5, 6, 7, and 8 (October 2002 to March 2003). Liquid alum was applied for all the grow-outs presented in part 2 of

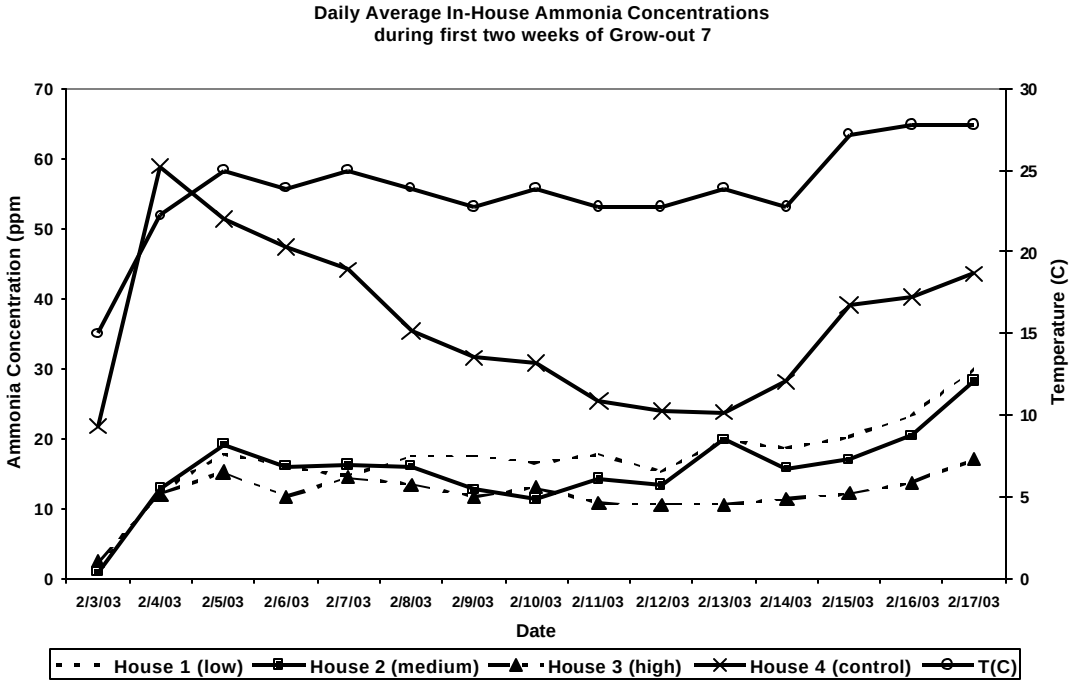

Figure 2.4 In-house Ammonia Levels by Alum Rate and Average In-house Temperature During Grow-out 7 'Cookoff' Period.

this work. The application occurred 47 d prior to bird introduction (Appendix, Table A.2). As noted, liquid alum begins lowering litter pH immediately. Figures 2.5-2.8 summarize the half-hour averages of ammonia gas concentrations taken from the beginning to the end of the grow-out. All three rates of liquid alum application were equally effective at maintaining ammonia concentrations below 25 ppm for the first 13 d of the grow-out. The ammonia concentration in the control consistently exceeded 25 ppm by day 6 of the grow-out. By day 17, the

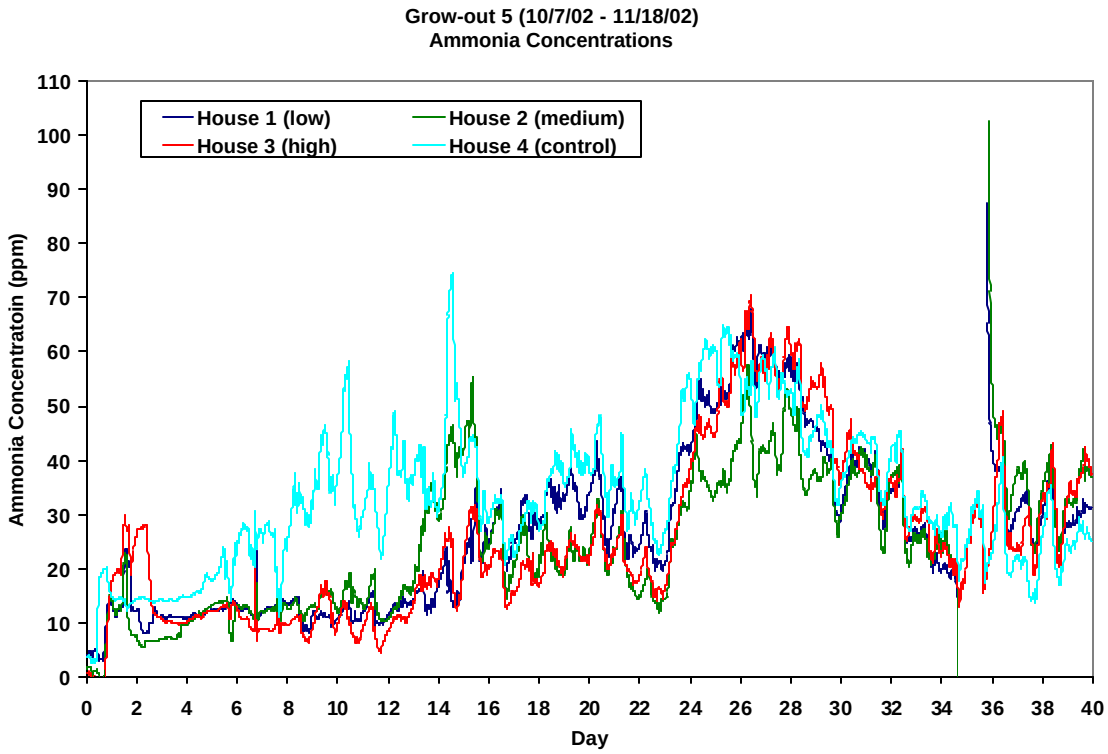

Figure 2.5 In-House NH_3 Concentrations During Grow-out 5 for all Treatments (plotted data represent the 30-min averages recorded by the CSI 23X micrologger).

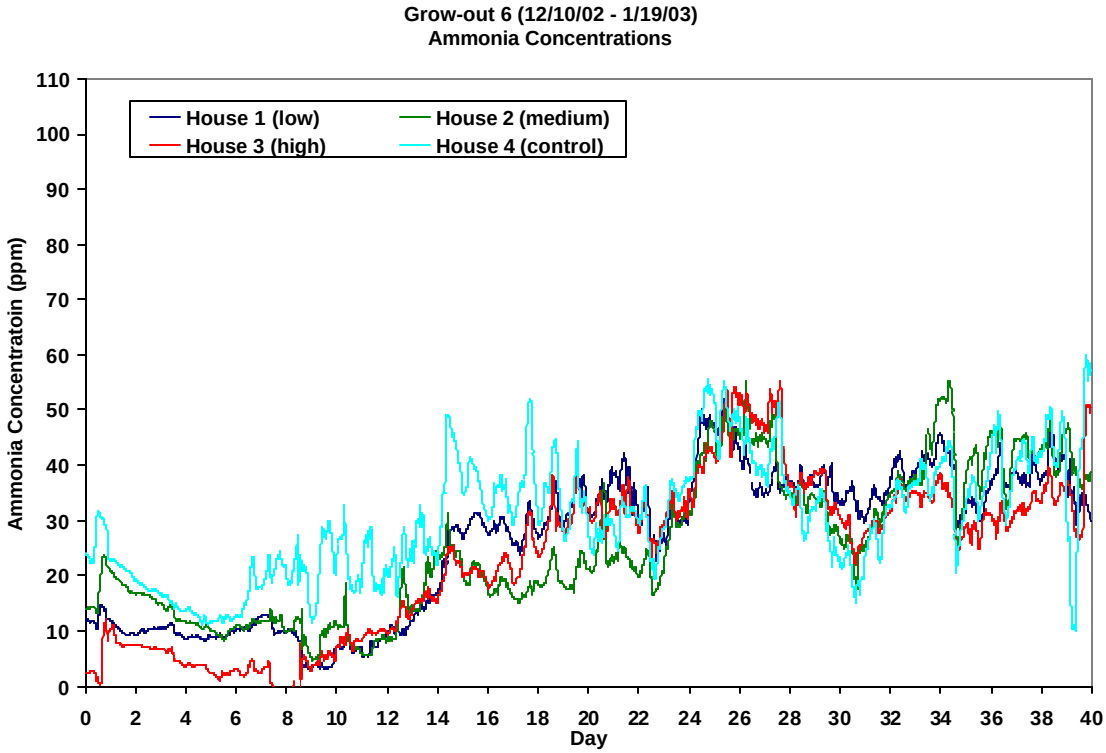

Figure 2.6 In-House NH_3 Concentrations During Grow-out 6 for all Treatments (plotted data represent the 30-min averages recorded by the CSI 23X micrologger).

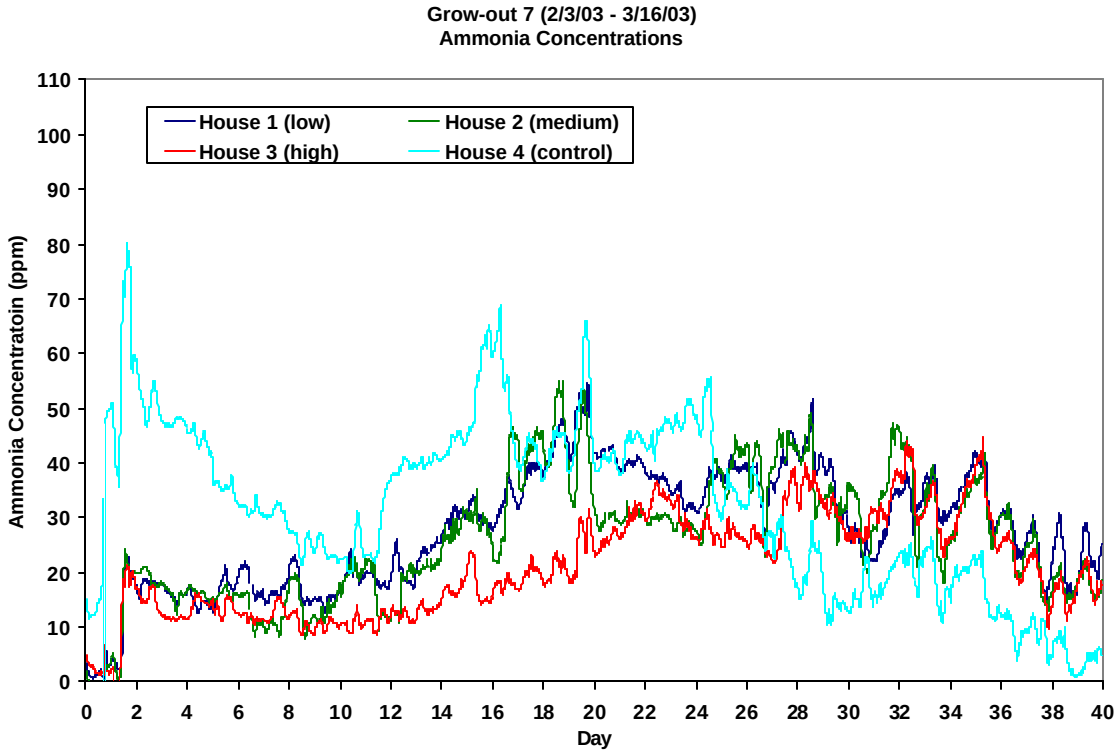

Figure 2.7 In-House NH_3 Concentrations During Grow-out 7 for all Treatments (plotted data represent the 30-min averages recorded by the CSI 23X micrologger).

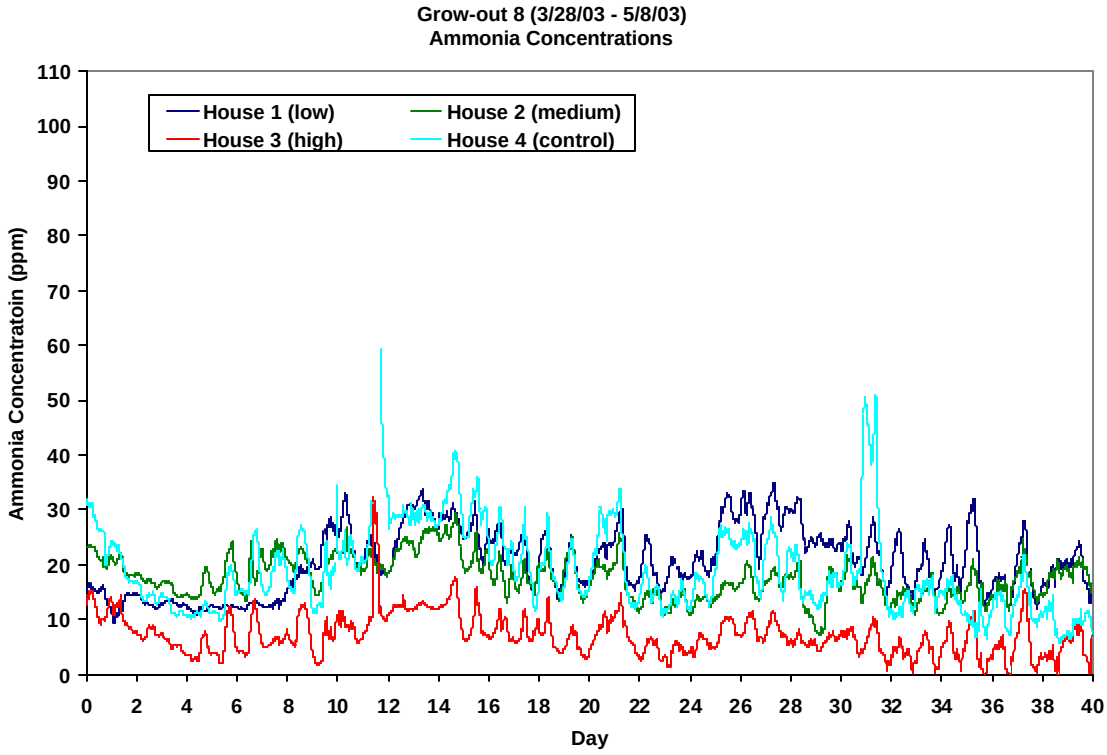

Figure 2.8 In-House NH_3 Concentrations During Grow-out 8 for all Treatments (plotted data represent the 30-min averages recorded by the CSI 23X micrologger).

ammonia levels begin to differ among treatments. House 1, the low rate, follows the control, more closely. The house 2 and house 3 gas concentrations essentially track each other until the end of the grow-out and are lower than house 1 and house 4. At day 23, the ammonia levels in all four houses consistently surpass 25 ppm.

This suggests that liquid alum applied at the low rate can suppress in-house ammonia levels to below 25 ppm for approximately the first two weeks of the grow-out. Liquid alum applications of medium and high suppressed in-house

ammonia levels to below 25 ppm for approximately the first three weeks of the grow-out. As pointed out in Part 1, the litter pH returns to a range 7-8 (Figure 1.3, Part 1) after 22-23 d. After 21 d, the litter moisture continues to increase in addition to the accumulation of chicken feces in the litter bedding. These two factors contribute to shifting of the chemical balance toward NH_3^+ and the build up of in-house ammonia concentrations. Additional ammonia results are provided in Table 2.1 as well as the LSMs for ammonia concentrations, 130-cm fan run-time, and 91-cm fan run-time. The least square means were calculated using ammonia concentrations from all four grow-outs. The NH_3 concentration LSM in house 3 was the lowest (20 ppm) and significantly different (P=0.02) from the other concentrations (Table 2.2). The above LSM results were calculated using NH_3 concentrations from the four grow-outs and ammonia levels from the beginning to the end of each grow-out. As mentioned, the medium and high are both effective in suppressing in-house NH_3 concentrations below 25 ppm for three weeks. As well, the LSM suggest that the medium and high are both effective in suppressing in-house NH_3 concentrations below 25 ppm for an

Table 2.1 Ammonia level information for grow-out 5.

Treatment (L/m^3)	Maximum ppm	Maximum ppm, 1st two weeks	Average ppm
Low	68	24	17
Medium	58	47	17
High	71	30	15
Control	74	74	29

Table 2.2 Least Square Means by House for NH_3 levels and Fan Run-Time (all 4 grow-outs used for means).

	Low	Medium	High	Control	P-value	Result
91-cm Fan Run-Time (minutes)	233	219	798	252	0.09	NS
130-cm Fan Run-Time (minutes)	2,902	2,992	4,270	2,523	0.01	S
Ammonia Concentration (ppm)	26	24	20	29	0.02	S

entire broiler grow-out. House 4 (no alum) had the highest NH_3 concentration LSM (29 ppm).

The LSMs for the 91-cm and 130-cm were calculated over the four grow-outs by house, but house 3 fan run-times are not accurate. During grow-outs 7 and 8, two ventilation fan voltage readings in house 3 were showing a continuous 'on' status. After recent conversation with the grower, it was found that the integrator's production manager recommended continually operating the particular fan in house 3 due to high litter moisture observations in one corner of the house. However, houses 1, 2, and 4 did show similar 91-cm and 130-cm fan run-times.

Figure 2.6 shows ammonia concentrations during a winter grow-out. During the first eight days, house 3 (high) has lower ammonia levels than the low and medium houses. This is important because the first week is important for bird health and performance as pointed out in the literature review. Newly hatched birds are more susceptible to diseases (Charles and Payne, 1966). Also, in grow-out 8 (figure 2.8), house 3 (high) maintains the lowest ammonia levels for the first crucial growing days. In grow-out 8, house 3 (high) consistently shows ammonia levels below 25 ppm. The statistics do show

differences among the treatments over the entire study, however, figures 2.5-2.8 also illustrate there are differences among the treatments within an individual grow-out.

Figure 2.9 illustrates the fan ventilation rate for production house 4 when one or more ventilation fans were operating. The measured differential pressure was applied to the appropriate manufacturer fan curve (91 cm or 130 cm) and used to calculate the proper airflow rate from each ventilation fan. Each of the 11 individual fan flow-rates was summed to attain the overall airflow for house 4. The ventilation rates recorded for houses 1, 2 and 3 were very similar to house 4. All four of the production houses were identical, chicks were introduced to all four houses within a 48 h period, and the grower was asked to continue normal operation, therefore the ventilation patterns in houses 1,2, and 3 were expected to behave similarly. For the first 14 d of the grow-out the birds remain in the

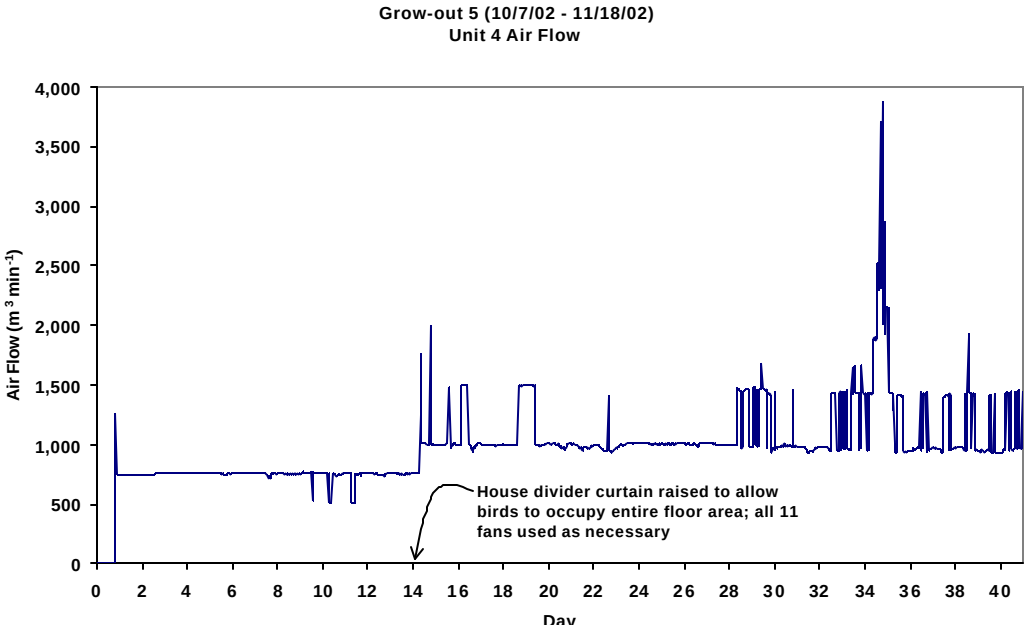

Figure 2.9 Ventilation Rates in House 4 for Grow-out 5.

brooder end of the house and only two 91-cm fans are used to provide ventilation. At day 14 of the grow-out, the grower raises the divider curtain allowing the birds to occupy the entire production house. At this time all eleven fans can be used as needed. If temperature increases enough the house will be placed into full tunnel ventilation using all nine 130-cm fans. Figure 2.10 shows temperature and relative humidity as recorded at the center of production house 4. House 4 had an average inside temperature of 25-30°C. As ventilation increases relative humidity decreases as drier air from the outside enters the production house.

In the poultry industry, broiler integrators provide guidelines that growers follow to maintain broiler house temperature. The recommendations vary according to broiler breed and to the type of house ventilation system (Vest, 1996).

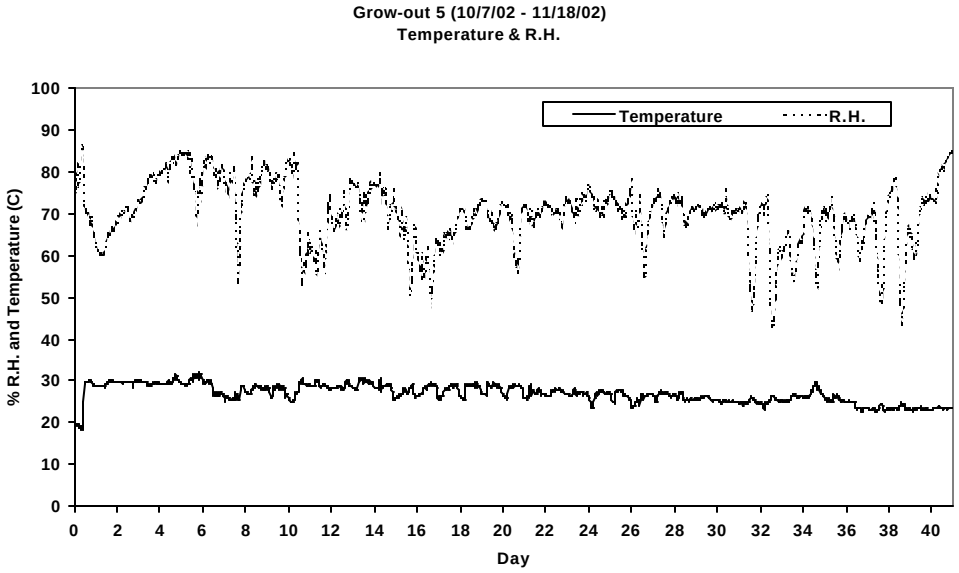

Figure 2.10 Temperature and Relative Humidity in House 4 for Grow-out 5.

For example, this grower's integrator suggests the following in-house temperatures during a grow-out: week 1: 31°C, week 2: 27°C, week 3: 26°C, week 4: 23°C, week 5: 21°C, and week 6: 18°C. Equally, the broiler house temperature is a factor that will affect NH_3 volatilization, and therefore, the in-house NH_3 concentrations (USEPA, 2002). As temperature increases, NH_3 volatilization increases. In evaluating in-house ammonia concentrations, it is fundamental to make note of how the NH_3 concentrations alter from hour to hour and how NH_3 levels and other parameters change throughout the grow-out.

In the following figures (Figures 2.11-2.16), three individual days from grow-out 6 and 8 were selected to illustrate various in-house environmental trends in house 4 (control). In the beginning of grow-out 6 (e.g. day 4) in-house ammonia concentrations remained relatively constant during the 24-h period because the birds are small and less manure is excreted on the floor. As the grow-out continues, the birds gain mass and manure quantities increase on the litter bedding. Although fan operation is thermostatically controlled, ammonia concentrations will increase or decrease according to in-house temperature fluctuations. As the last 7 d of a grow-out is approached, in-house ammonia concentrations will vary from season to season. In Figure 2.15, the 24-h period is January 18, 2003, which is a winter day in Tennessee. According to National Climatic Data Center (NCDC) data inventories, the average temperature on January 18, 2003 was –11°C (NCDC, 2003). The NCDC weather station is within 7 km of broiler farm.

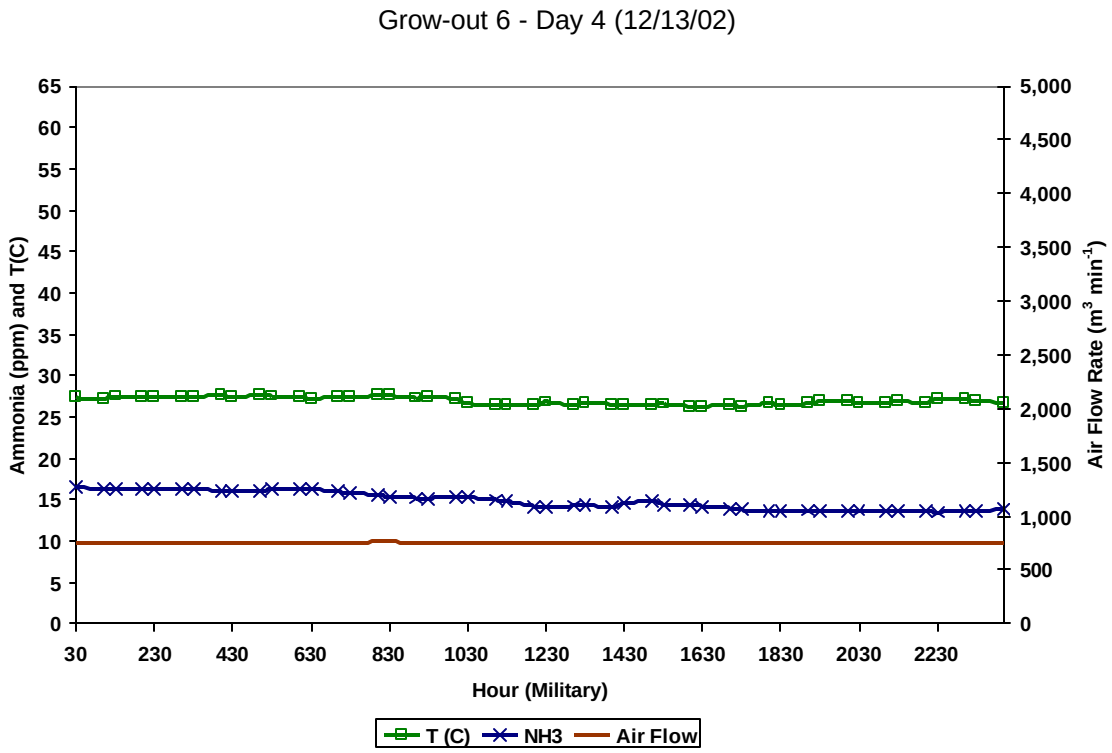

Figure 2.11 Grow-out 6 (day 4): Temperature, NH_3 levels, and Airflow in house 4.

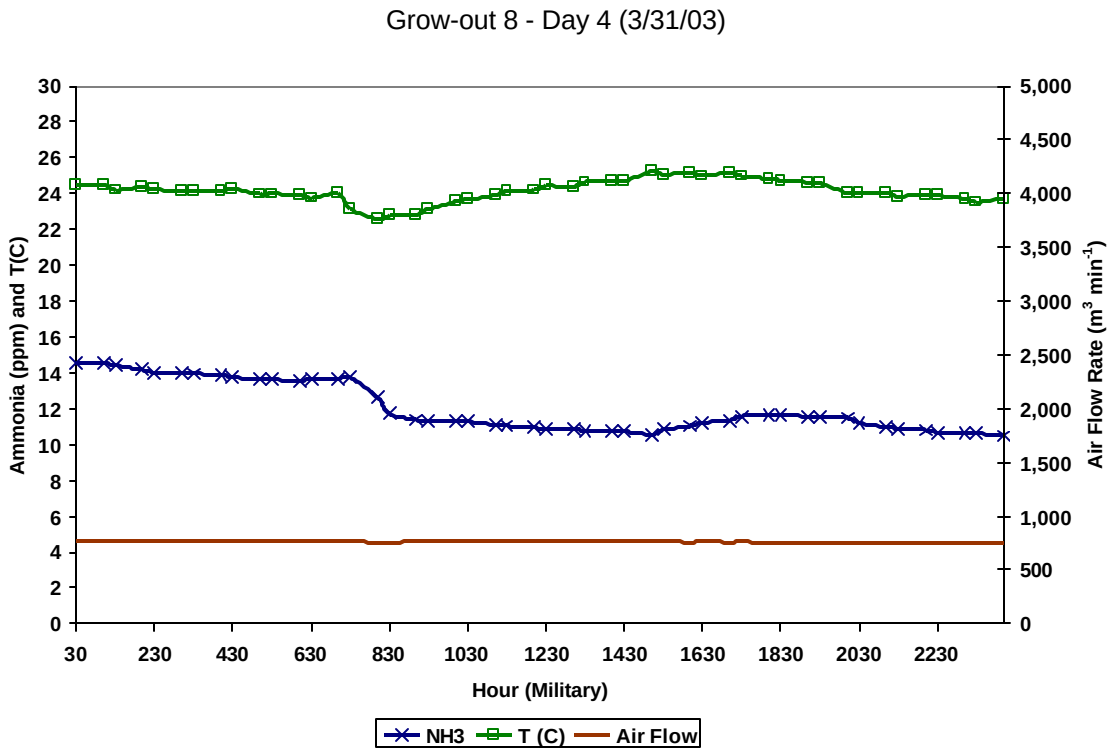

Figure 2.12 Grow-out 8 (day 4): Temperature, NH_3 levels, and Airflow in house 4.

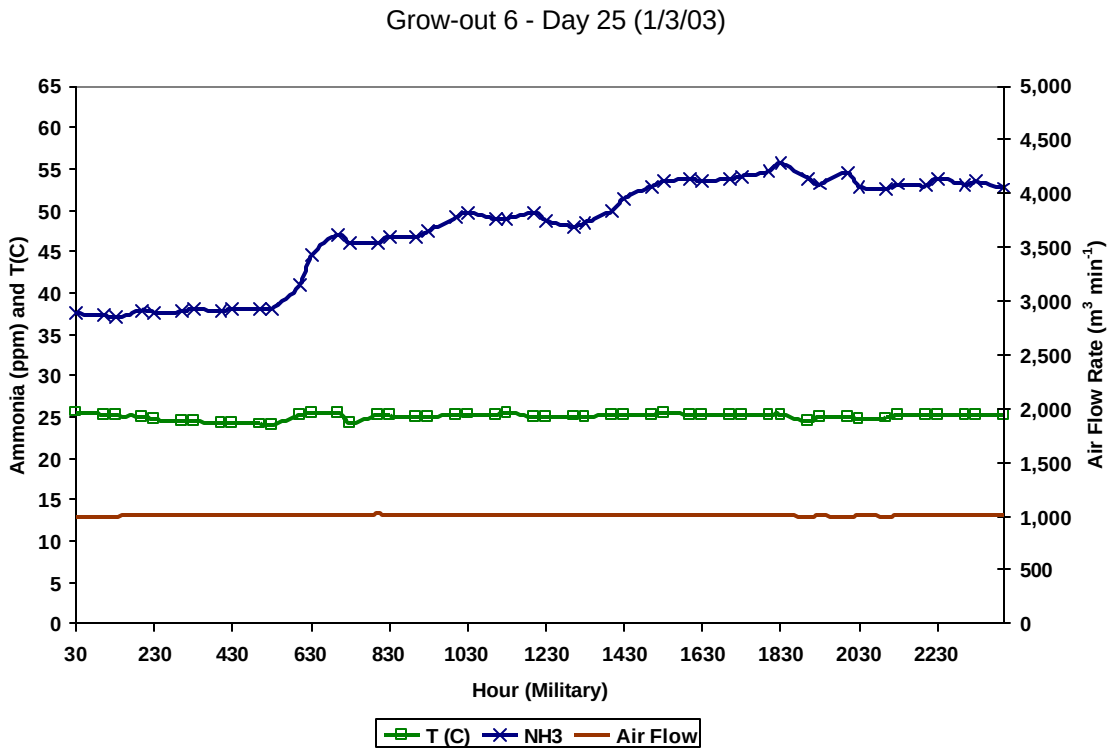

Figure 2.13 Grow-out 6 (day 25): Temperature, NH_3 levels, and Airflow in house 4.

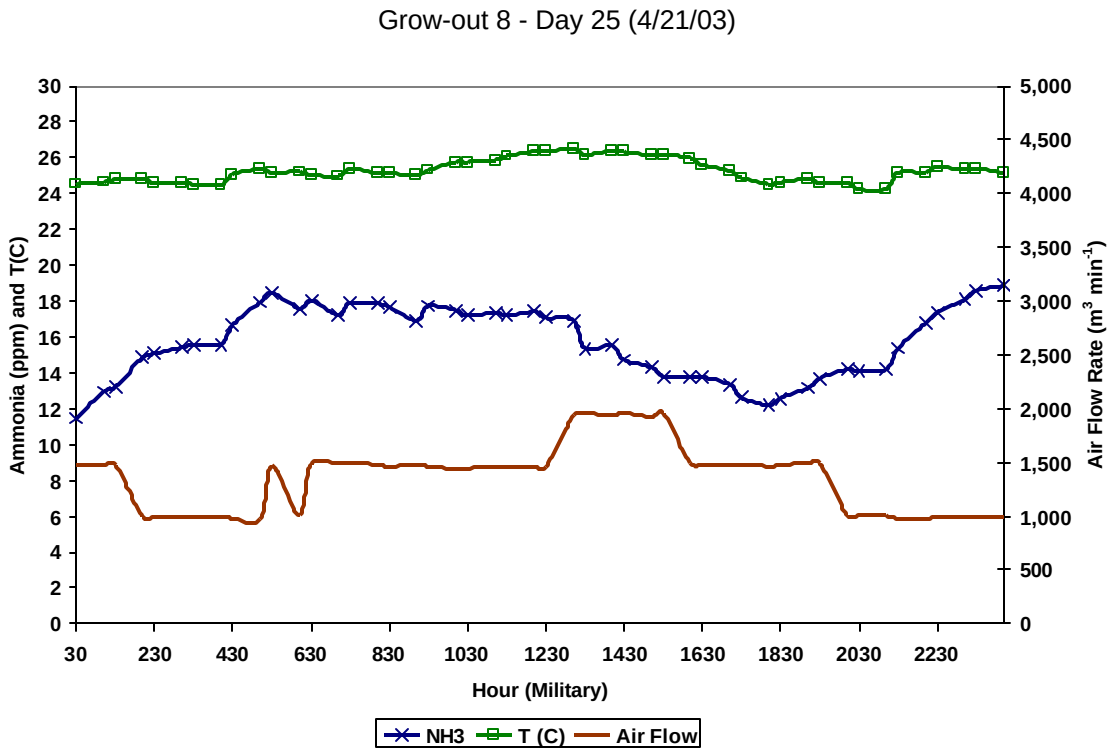

Figure 2.14 Grow-out 8 (day 25): Temperature, NH_3 levels, and Airflow in house 4.

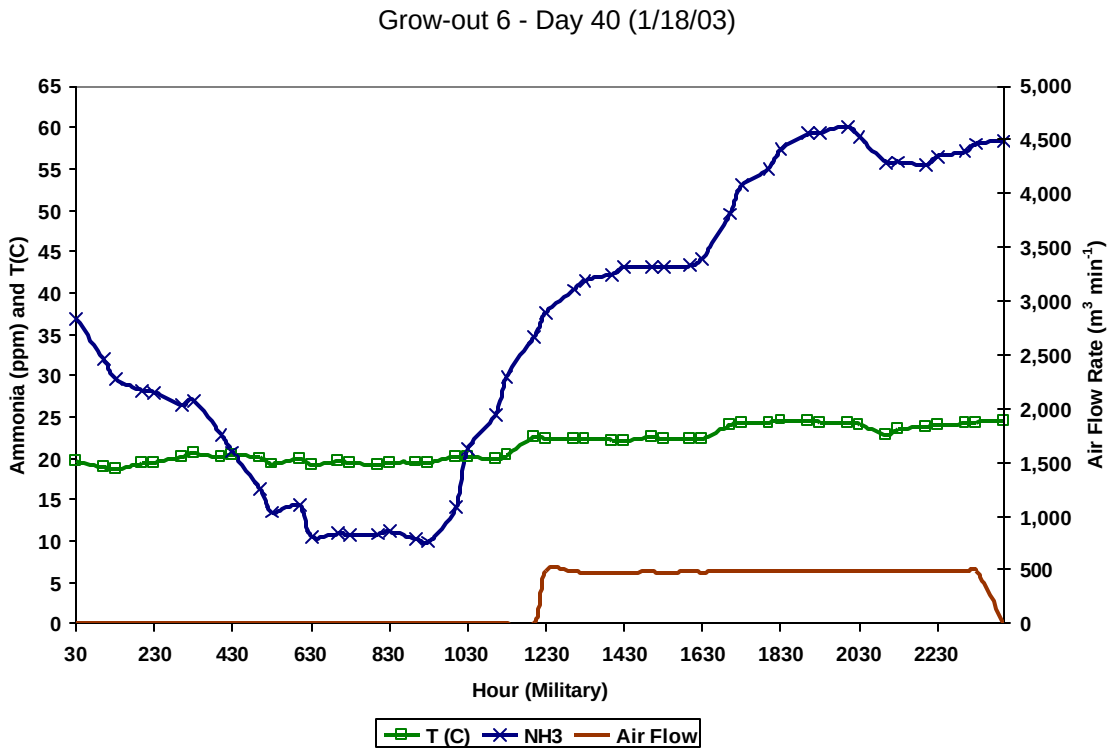

Figure 2.15 Grow-out 6 (day 40): Temperature, NH_3 levels, and Airflow in house 4.

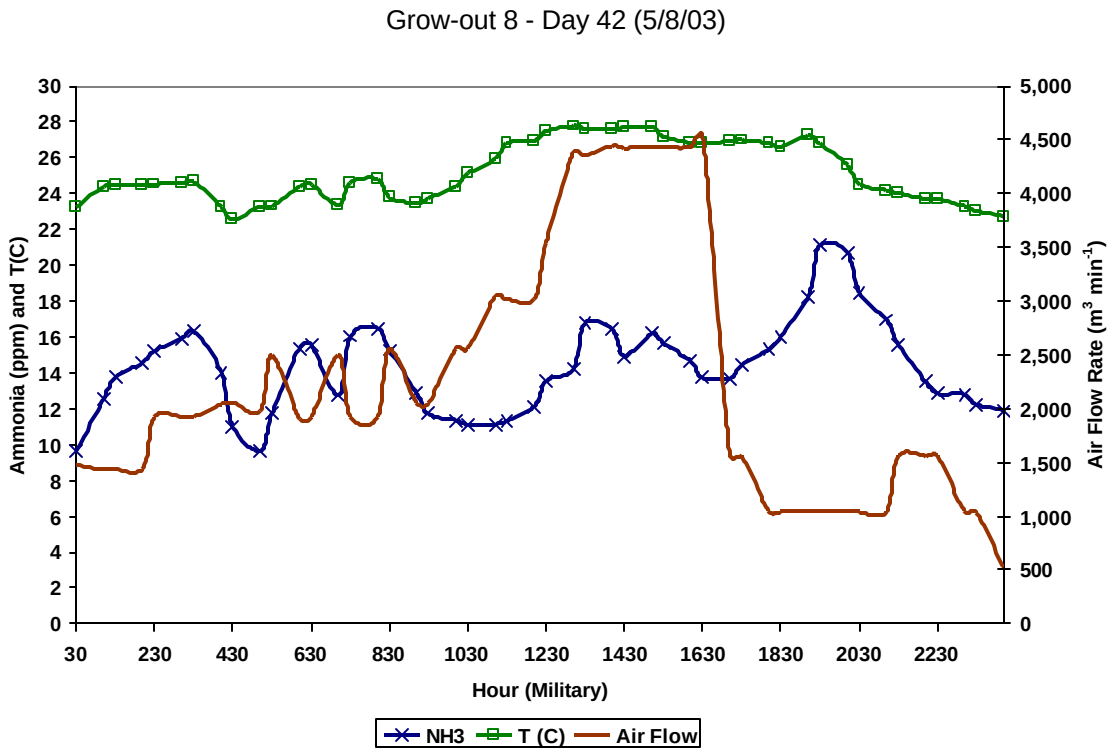

Figure 2.16 Grow-out 8 (day 42): Temperature, NH_3 levels, and Airflow in house 4.

The airflow rate on this day is lower because the grower adjusted the thermostatic controls to operate the ventilation fans less time. In winter months, growers prefer to operate the fans less time because the fans will bring colder air into the houses requiring the propane heaters to heat the inside air for optimum broiler growing conditions. For the last 12 h, the airflow rate was approximately 500 m^3 min^{-1}, and the ammonia concentration steadily increased to approximately 60 ppm because in-house temperature increased from 20°C to 23°C at 1200 hours and again to 24°C at 1630 hours.

Grow-out 8 started March 28, 2003, which is spring climate in Tennessee (Figure 2.12). Again, airflow was constant on day 4 (3/31/03), and ammonia concentration followed in-house temperature as it decreased at 0700 hours. On 3/31/03, the NCDC inventory reported average temperature was 3°C. On day 25 (4/21/03), ammonia concentrations react to both in-house temperature and airflow. If the airflow is constant for a few hours, ammonia will follow in-house temperature accordingly. However, as springtime air temperatures enter the house and additional 130-cm fans turn on to adjust in-house temperature, in-house ammonia concentrations will lower. The NCDC inventory reported 18°C on 4/21/03 (NCDC, 2003). When in-house temperature has stabilized, the ammonia level will once again track the 1-4°C fluctuations of in-house temperature. On the last day of grow-out 8 (Figure 2.16), parameters are even more variable. The warmer outside air brought in by the ventilation system causes in-house temperature to increase, thus airflow rate will increase to control in-house temperature. As the day progressed toward 1330, there were

significant variations in ammonia levels and airflow. In-house temperature reached a maximum of 28°C and the airflow rate settled at approximately 4500 m^3 min^{-1}. Hotter temperatures lasted approximately four hours and ammonia concentrations slowly lowered once cooler night temperatures entered the house to lower the in-house temperature.

FARM PRODUCTION DATA RESULTS AND DISCUSSION

The recorded data for condemnations, mortalities, and water use are shown in Table 2.3, Table 2.4, and Table 2.5, respectively. Propane data is shown in Table 2.6. Over the 2002-2003 production year, the propane use between the four houses was essentially equal. Mortalities, water use, and condemnation trends are shown in Figures 2.17-2.20. The ANOVA results for the three categories are shown in Table 2.7. Mortalities, condemnations, or water use on the broiler were not significantly different among the four houses. However, house 3 (high) had the lowest mortality LSM, 1093 birds, while house 4 had the highest number of mortalities (Table 2.7). House 3 had the lowest water consumption, 245,651 L (Table 2.5). House 4 (control) had the lowest condemnation LSM and house 2 (medium) had the highest (Table 2.3). At the highest alum application rate (high) the lowest number of mortalities was observed. It is important to note that all houses showed a decreasing trend in mortalities over the first four grow-outs (Figure 2.20). Between grow-out 4 and 5, all houses were completely cleaned out. Alum was applied prior to bird

Table 2.3 Processing Plant Condemnations By Alum Rate.

Alum Rate ?	Low	Medium	High	Control
Grow-out 1	84	96	87	105
Grow-out 2	75	64	59	53
Grow-out 3	97	89	109	56
Grow-out 4	64	71	45	56
Grow-out 5	97	163	140	82

Table 2.4 Broiler Mortalities (x1000) By Alum Rate.

Alum Rate ?	Low	Medium	High	Control
Grow-out 1	1.66	1.81	1.58	2.32
Grow-out 2	2.06	1.50	1.48	1.19
Grow-out 3	0.95	1.07	1.15	0.99
Grow-out 4	0.86	0.91	0.74	0.83
Grow-out 5	1.08	0.99	0.94	1.10
Grow-out 6	1.20	2.36	1.66	1.36
Grow-out 7	0.74	0.66	0.65	1.78
Grow-out 8	0.56	0.54	0.54	0.55

Table 2.5 Water Use (liters x1000) By Alum Rate.

Alum Rate ?	Low	Medium	High	Control
Grow-out 1	232	208	389	220
Grow-out 2	229	240	179	235
Grow-out 3	292	312	206	267
Grow-out 4	216	289	308	285
Grow-out 5	262	295	215	292
Grow-out 6	266	284	216	266
Grow-out 7	266	296	222	250
Grow-out 8	294	294	231	293

Table 2.6 Propane Use (liters x100) By Alum Rate.

Alum Rate ? Date Delivered ?	Low	Medium	High	Control
1/31/2002	16	21	23	21
3/4/2002	23	20	23	24
3/25/2002	20	23	19	19
5/23/2002	19	19	19	19
8/15/2002	15	12	16	15
11/21/2002	15	20	16	15
12/27/2002	22	23	21	18
1/31/2003	14	25	15	11
3/27/2003	20	16	11	16
4/9/2003	23	15	15	19
5/23/2003	16	19	22	19
Total Delivered	204	213	202	197

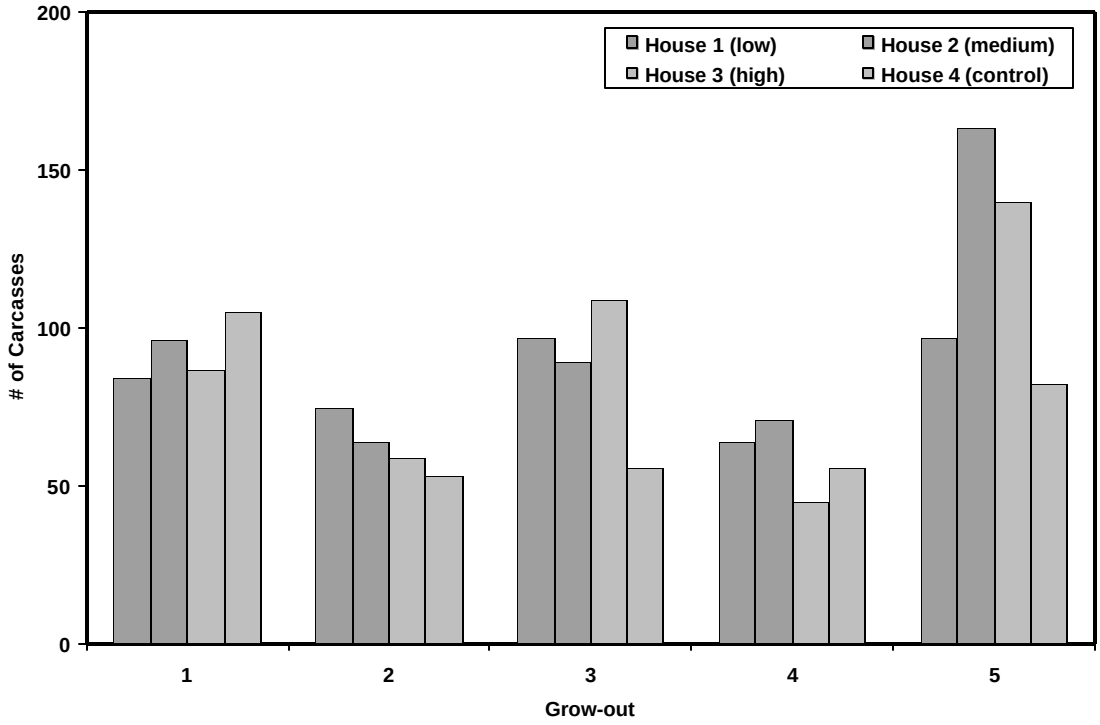

Figure 2.17 Processing Plant Condemnations By Alum Rate.

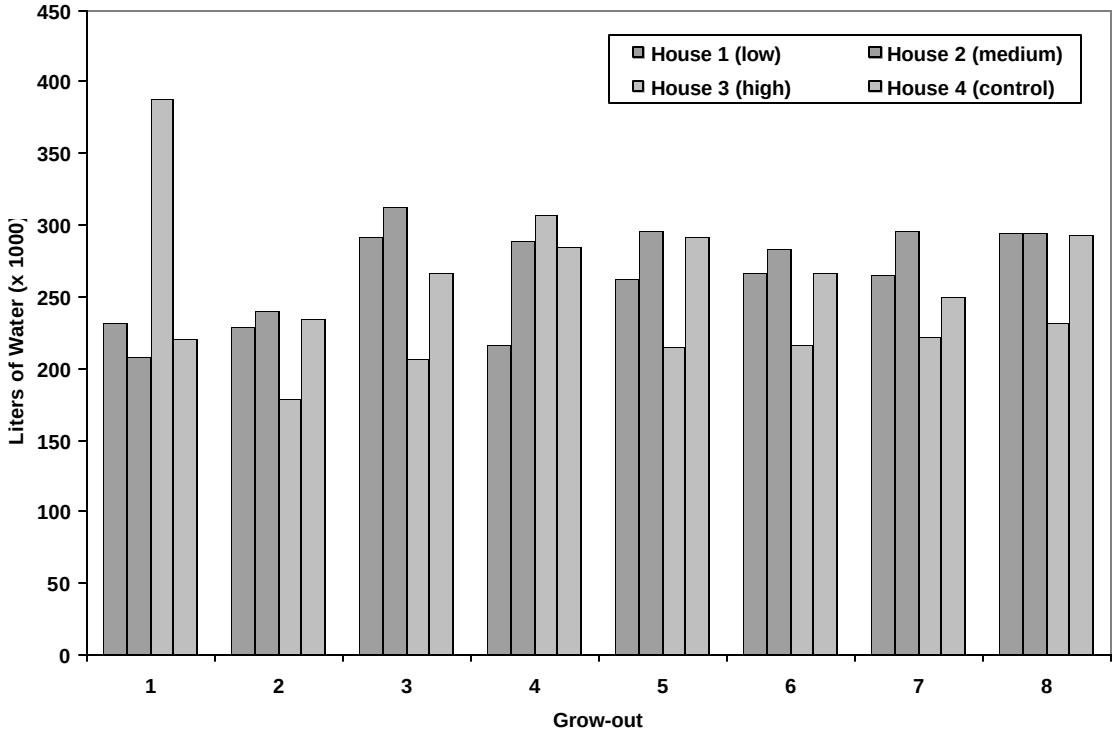

Figure 2.18 Water Use By Alum Rate.

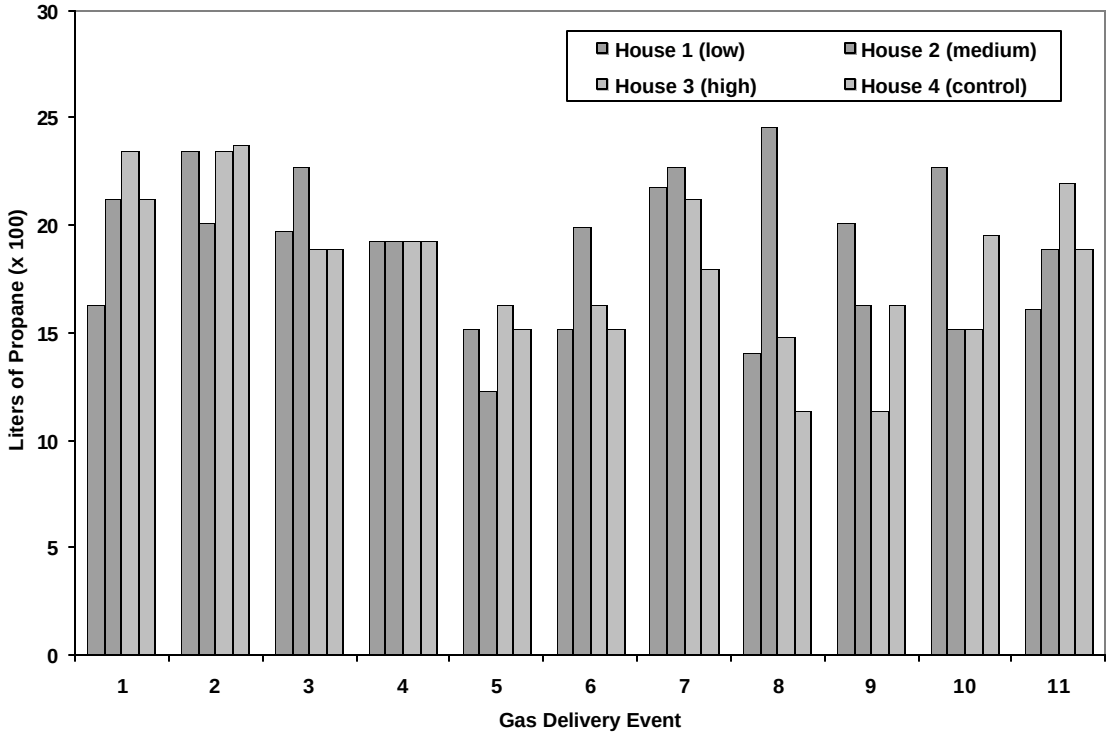

Figure 2.19 Propane Use By Alum Rate.

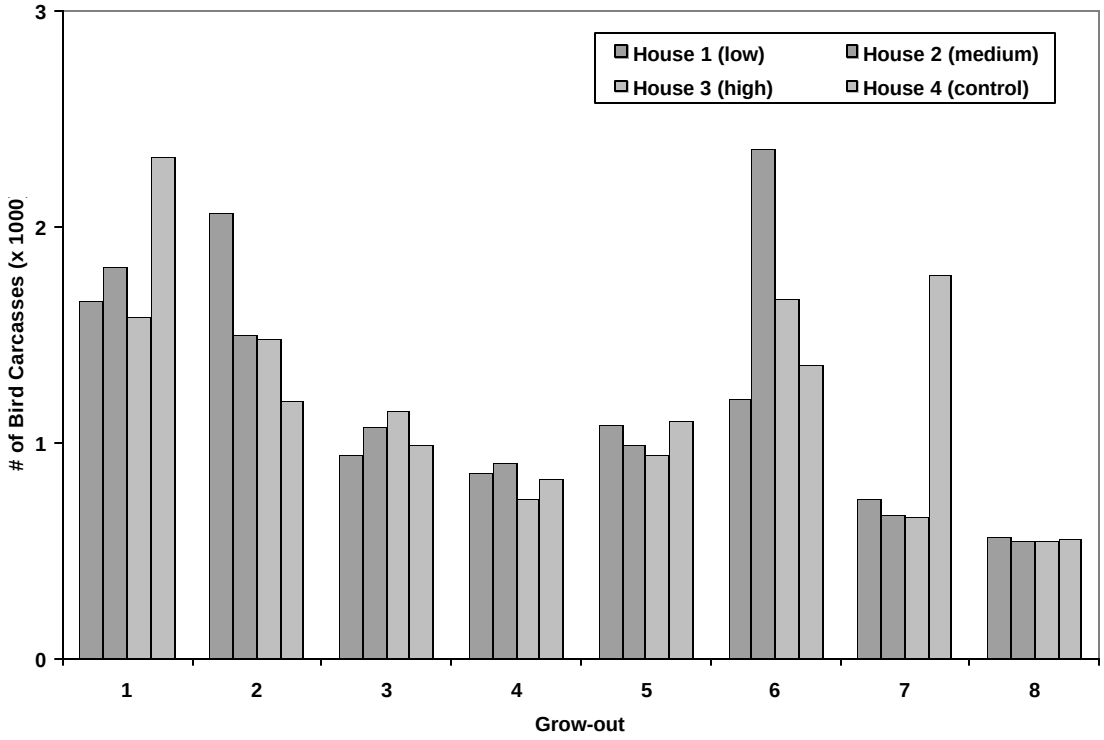

Figure 2.20 Broiler Mortalities By Alum Rate.

Table 2.7 Farm Production Data Least Square Means and P values.

	Low	Medium	High	Control	P value	Result
Mortalities	1,138	1,230	1,093	1,265	0.71	NS
Condemnations	83	97	88	70	0.27	NS
Water Use (L)	257,256	277,452	245,651	263,571	0.53	NS

introduction for grow-outs 5, 6, 7, and 8. After the cleanout, house 4 had an increasing trend for grow-outs 5-7, but the treated houses were lower by grow-out 7. By grow-out 8, the four houses had similar mortalities. Although ANOVA results (Table 2.7) showed a non-significant difference (P=0.71), the data suggest that the use of liquid alum has the potential to reduce mortalities in broiler production cycles.

CONCLUSIONS AND RECOMMENDATIONS

Ammonia concentration data from four grow-outs suggests that liquid alum is effective at suppressing in-house ammonia levels for up to three weeks in modern tunnel ventilated broiler production houses. The low rate of liquid alum was effective at maintaining in-house ammonia levels below 25 ppm for the first two weeks of the grow-out. Both the medium and high rates provided effective control of in-house ammonia concentrations for the first three weeks of the grow-out. The application of liquid alum at the three treatment levels was found to be very effective at reducing in-house ammonia levels as compared to no alum application. By the third week following application the ammonia reduction in the

treated houses ceased. Moreover, liquid alum is effective at maintaining ammonia levels below 25 ppm during the 'cookoff' period of a typical broiler grow-out. While no statistical difference was found in the reduction of mortalities from one production year among the treatment levels, the data suggests that within an individual grow-out the effect of liquid alum applications on broiler mortalities provides some advantage.

In conclusion, this work recommends the medium liquid alum rate (1.64 L m^{-2}) as a method to manage in-house ammonia levels based on the reasons discussed above as well as the following economic advantages. A cost analysis is provided in Table 2.8. On a per house basis, the medium rate cost less than the equivalent house 2 dry alum applications by approximately $117.00 per treatment. The high rate is more expensive for the grower and comments from the grower indicate that the high rate may cause eye irritation and occasional equipment corrosion. Also, this work concludes that liquid alum is as effective as dry alum in suppressing in-house NH_3 levels. As a result of the variation in location of liquid alum distributors, transportation cost will be a variable that influences the total application cost. The distributor used for this study charged 0.16 cents $liter^{-1}$ (0.60 cents $gallon^{-1}$) and was located approximately 370 km from the farm. Although the cost analysis is only applicable to this study, further research is warranted to investigate the economic implications and benefits of liquid alum in Tennessee. Hypothesis 2 states there are no significant differences in the in-house NH_3 concentration levels between the four alum treatments, thus hypothesis 2 was disproved. Hypothesis 3 states

Table 2.8 Dry and Liquid Alum Cost Comparison.

Product	Application amount	Cost per unit kg or L (cents)	Application Cost per house	Total Cost per house ($)
Dry Alum (45 kg per L)	907 kg	0.33	not considered	299
Dry Alum (90 kg per L)	1814 kg	0.33	not considered	599
Dry Alum (135 kg per L)	2721 kg	0.33	not considered	898
Liquid Alum (low)	1514 L	0.16	40	242
Liquid Alum (medium)	3028 L	0.16	40	484
Liquid Alum (high)	4543 L	0.16	40	727

alum treatment does not significantly influence farm mortality rates, water use, or processing plant condemnations, thus hypothesis 3 is supported.

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PART 3: PRODUCTION HOUSE AMMONIA EMISSIONS

ABSTRACT

A recent report by the National Research Council (NRC, 2002) ranked ammonia (NH_3) emissions from animal feeding operations as a major concern at global, national, and regional scales due to atmospheric deposition that results from these emissions, and recommended strengthening the scientific basis for estimating air emissions from animal feeding operations. This project compares two ammonia emissions estimate methods for a modern broiler production house: a nitrogen mass-balance approach and a flow-integration approach. The mass-balance estimate was derived by quantifying total nitrogen inputs (bedding shavings, chicks, and feed), and outputs (broilers and litter). The difference between inputs and outputs was assumed to be volatilized nitrogen, and was calculated as 7150 kg NH_3 yr^{-1} from a broiler production house growing 6 flocks of birds for a total of 252 d during the year. The flow-integrated emission estimate was determined by collecting NH_3 concentration and exhaust fan flow rate data every 5 s for 85 d (two flocks of birds). This method yielded an NH_3 emission estimate of 6950 kg yr^{-1}, within 3% of the mass-balance method. Both methods yielded an average daily NH_3 emission factor of 17 g hr^{-1} 500 kg bird $mass^{-1}$; the maximum daily emission estimated by flow-integration was 37 g hr^{-1} 500 kg bird $mass^{-1}$.

LITERATURE REVIEW

Previous Studies

A study by Asman (1992) entitled *Ammonia Emission in Europe: Updated emission and emission variations,* provides an updated ammonia inventory for Europe and includes the three general NH_3 sources: emission from livestock, emission from application of fertilizers, and other sources. For livestock emissions Asman defined an overall emission factor as the animal category's total emission divided by the total number of animals of that category (Asman, 1992). The study used the United Nations Food and Agricultural Organization livestock statistics. A mass-balance approach was used to arrive at ammonia emissions for each animal category. Asman points out that agricultural scientist evaluated nutritional research from the 1980's to calculate the nitrogen flows of the livestock. Also, the document states, " the emission factors used for Europe were derived from The Netherlands emissions." This statement is important because the study goes on to clarify that the nitrogen content of animal's food in The Netherlands is possibly higher than other European areas. Nitrogen in the animal's food is a source of NH_3 emissions (Asman, 1992). As a result, the study concluded that there was reason to believe an over-estimation of NH_3 emissions could have occurred in their mass-balance approach. Another observation of Asman's study was the differences in agricultural practices between the inventoried European countries. Participating countries were questioned, and the responses are detailed in the document, but Asman decided to apply The Netherlands emission factors for all European countries due to the survey's large

array of responses and the unavailability of information in some of the European countries. One of the studies broiler-specific conclusions was that the NH_3 emission rate was higher at higher temperatures and higher ventilation rates. Additionally, the research concluded that the largest diurnal variation in the ventilation rate would occur in the spring and autumn and varies according to local climatology (Asman, 1992). Asman reported a 0.167 kg NH_3 $animal^{-1}$ yr^{-1} for the broiler emission factor.

In 1994, the U.S. EPA completed a report on the selection of NH_3 emission factors and found that 80% of ammonia emissions in the U.S. came from livestock waste (Battye, 1994). This report was similar to the Asman study but went further to rank the existing animal NH_3 emission factors. The rankings were A to D with A being the best. The author refers readers to Battye et al. (1994) for the rating system and criteria of the emission factors. Battye et al. ranks the factor developed by the Asman study for poultry emission as a 'B' in part because of questionable distinctions between hens, pullets, breeders, and laying hens. While the European emission factor received a 'B', the U.S. EPA agreed to use the 0.167 kg NH_3 $animal^{-1}$ yr^{-1} (Asman, 1992) for the broiler emission factor in its U.S. NH_3 emission inventory.

In 1996, work was done to gather data and knowledge about in-house ammonia concentrations and livestock ammonia emissions in The Netherlands, Germany, Denmark, and England (Koerkamp, 1998b). In this multi-country project, the method to attain an emission rate was to take the product of the ammonia concentration and the ventilation rate, referred to as flow-integration

(FI) in this study (Koerkamp, 1998b). The mass of ammonia emitted from a broiler house is the sum of the net ammonia flows through all ventilation exits. The NH_3 concentration and ventilation parameters must be measured at the same time. Koerkamp et al. (1998b) noted mass-balance disadvantages such as litter sampling and nitrogen content errors, so chose a FI method to measure NH_3 in-house concentrations. The NH_3 sensor system used was a combination of a chemiluminescence NO_x analyzer and a thermal NH_3 converter. The ventilation rates for the broiler houses were calculated using "STALKL" program (van Ouwerkerk et al., 1994). Data sampling took place continuously for 24 h under summer and winter conditions and at seven sampling points in the broiler house. The broiler emission factors for England, The Netherlands, Denmark, and Germany were 20, 11, 9, and 19 mg h^{-1} $animal^{-1}$, respectively. The work also reported broiler emission factors in mg h^{1} 500 kg live $weight^{-1}$, and they were 8294, 4179, 2208, and 7499 for England, The Netherlands, Denmark, and Germany, respectively. Emission factors can be presented with various units. However, the Koerkamp et al. (1998b) study emphasizes emission rates per 500 kg live weight causes errors due to the animal weight assumptions necessary to convert to this unit (Koerkamp et al., 1998b). Koerkamp et al. (1998b) adds that the simplest way is NH_3 emissions per broiler and the number of broilers should be clearly stated. Other important conclusions of the study were that variations between countries existed, spatial variation between sampling points at the same moment existed, and diurnal variation due to, e.g. broiler activity was observed.

Two years later, work was done in England to measure NH_3 emissions in a mechanically ventilated broiler house (Demmers et al., 1999). Demmers et al. (1999) incorporated the same NH_3 emission rate technique, and specifically the same ammonia concentration measurement unit. The study used a tracer gas method and measuring fans to calculate ventilation rates (Demmers et al., 1999). The tracer gas was carbon dioxide and a known mass was introduced into the broiler house. The ventilation rate was calculated based on the conservation of mass equation that includes a correction for the background carbon dioxide levels. A measuring fan was mounted at each air exit duct of the house to attain the air flow-rate. Parameters were measured continuously in the broiler house for approximately 60 d. The broiler house contained 11000 birds, and the broiler litter was removed between each production cycle. Like previous studies, Demmers et al. (1999) did show a diurnal pattern in the ammonia emission rate. The annual emission rate was 17 kg lu^{-1} a^{-1}. The 'a' stands for animal, which is a broiler in the Demmers study. The LU is livestock unit and is equivalent to 500 kg live weight; emission rate based on 290 d y^{-1} broiler occupancy and a final live weight of 2 kg. The study concluded that the broiler NH_3 emission rate was higher than a previous experiment and that the dry and warm weather, thereby high ventilation rates, could have contributed to a higher emission rate (Demmers et al., 1999). The tracer gas method for ventilation rate calculation showed a 16% larger deviation compared to the measuring fan method. The NH_3 emissions measurement using the measuring fan method was able to

compensate for individual emissions whereas the tracer gas method used an average NH_3 concentration and average ventilation rate.

In August 2001, research was conducted to observe ammonia emissions from a commercial broiler house at the University of Georgia (Worley et al., 2002). The experiment was conducted in modern mechanically ventilated broiler houses (12 m x 152 m) and ammonia was measured with Dräger Polytron II electrochemical sensors. The researchers in this study tested three different ammonia sensors (Dräger Polytron II, Industrial Scientific, and Matheson MAT 4-20) prior to the study and found the Dräger Polytron II the most accurate. The fan status (on/off) was monitored with a programmable controller. Along with the fan status, static pressure, and the published manufacturer's flow rates, the airflow rate was calculated (Worley et al., 2002). Worley sampled data every two minutes and monitored the last three weeks of a growout. For comparison and accuracy verification, Worley spot-checked ammonia levels with RAE Gas detection tubes. At higher in-house ammonia concentrations (> 50 ppm), Worley et al. (2002) discovered the Dräger Polytron II's accuracy declined rapidly over time. Worley reported 15 g NH_3 h^{-1} 500 kg bird $mass^{-1}$ at bird age 33 d. At bird age 49 d, he reported 3 g NH_3 h^{-1} 500 kg bird $mass^{-1}$. During summer and fall conditions, the maximum emission rate was 16 and 23 kg d^{-1} $house^{-1}$, respectively.

In April 2002, the U.S. EPA and the State of North Carolina Department of Environment and Natural Resources (NCDENR) published another report on the review of NH_3 emission factors and methodologies to estimate NH_3 emissions

(USEPA, 2002). The principal purpose of this investigation was to model ammonia emissions from swine facilities in North Carolina. While an attempt was made to compare U.S. and European information on ammonia emissions, the USEPA and NCDENR noted that complete U.S. NH_3 emissions methodology was not found (USEPA, 2002). Thus, the authors provided a comprehensive analysis of typical European methods, which were mass balance approaches according to this report (USEPA, 2002). The report gives recommendations how European findings can be applied in developing U.S. emission factors for U.S. emissions from livestock operations.

MATERIALS AND METHODS

House 4, the control house in part 2 of this work, was used to conduct this component of the research; it was tunnel-ventilated, and measured 152 m (500 ft) x 12 m (40 ft) (1860 m^2). Nine 130-cm (51 in) and two 91-cm (36 in) constant velocity fans ventilated the house. Data collection was during the 2002-2003-production year (eight grow-outs), when approximately 30,000 birds were produced for each 42-d grow-out period. Data from grow-outs 5-8 are presented due to unacceptable performance of ammonia sensors during grow-outs 1-4 (explained in *Ammonia Gas Sensors* of this section). During the first 14 d of the grow-out, birds were maintained in the brooder end of the house, ventilated by the 91-cm fans. After day 14, a divider curtain was raised, and the birds occupied the entire production house; all eleven ventilation fans were used as needed for ventilation.

Flow-Integrated Emission Estimation

Overview: House 4 was instrumented to measure gaseous ammonia, fan status (on/off), differential pressure, temperature, relative humidity and feed motor run-time using a system similar to that developed by Wilhelm et al. (1999b). The instrumentation layout is shown in figure 3.1. The NH_3 mass emission per 30-min interval was calculated by multiplying average NH_3 concentration by the total air volume exhausted during the interval. The formula for concentration (C) is: C ($\mu g\ m^{-3}$) = ((ppm x molecular weight) / 24.5) x 10^3 (Wark, et al., 1998). The 24.5 represent a volume constant and must be adjusted for pressure and temperature accordingly.

Pressure Senor: The differential pressure between the house interior and exterior was sensed with an electronic pressure transducer (Model PX2670, Omega Engineering Inc., Stamford, CT). The pressures were similarly collected at 5-s

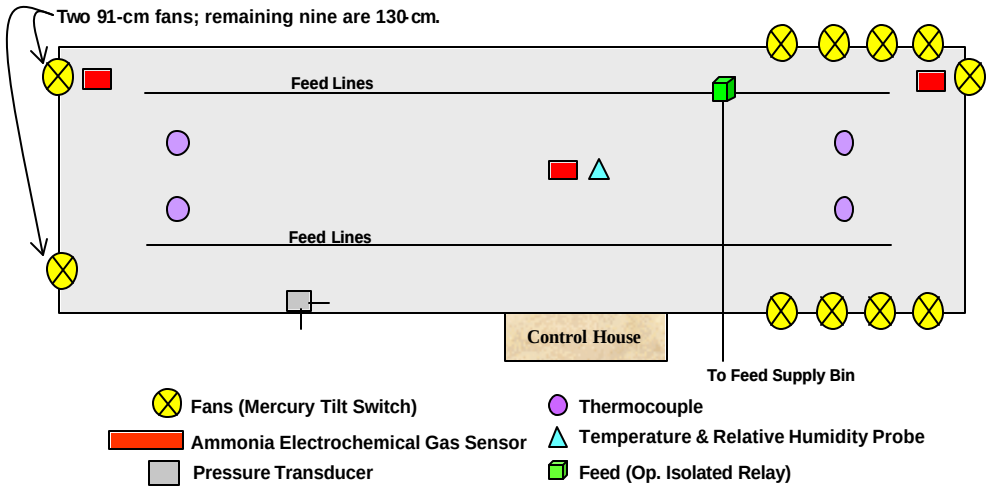

Figure 3.1 House 4 Instrumentation Layout.

intervals, averaged, and recorded for 30-min periods. During post-processing, pressure-drop was entered into manufacturer-provided fan performance equations (Figures 3.2 and 3.3) to determine the flow rate through each of the eleven fans.

Data Acquisition: One Campbell Scientific Inc. (CSI) 23X Microloggers (Campbell Scientific, Inc., Logan, UT) was used for data collection in house 4 (Figure 3.4). In conjunction with the micrologger, two CSI multiplexers (an AM416 and AM32) were programmed to scan the environmental input parameters every 5 s. A cellular phone and CSI DC112 modem was connected to each micrologger to enable remote data acquisition and viewing of real-time data. A CSI YAGI directional antenna was used with the units to improve signal strength.

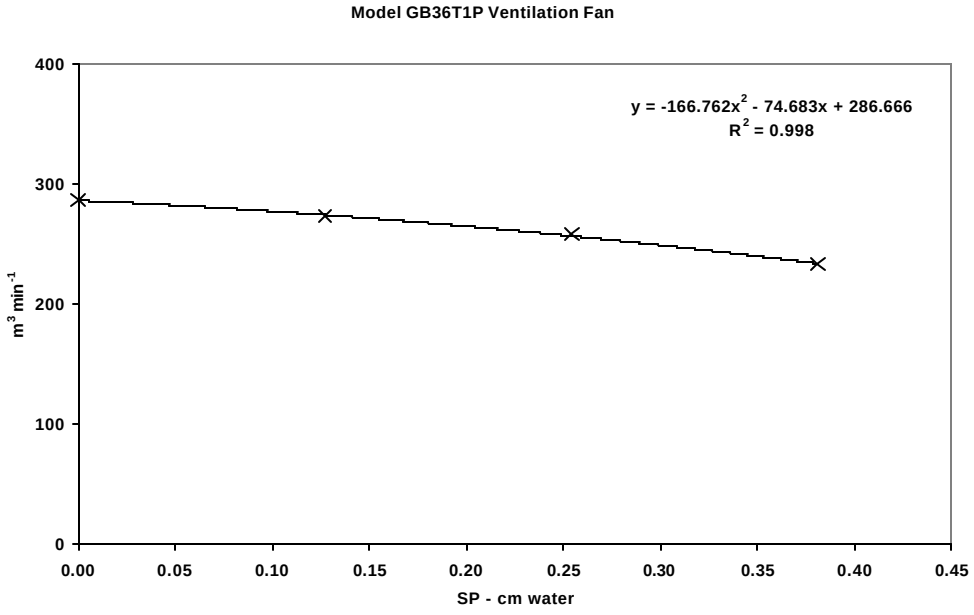

Figure 3.2 Fan Curve, 91-cm Ventilation Fan.

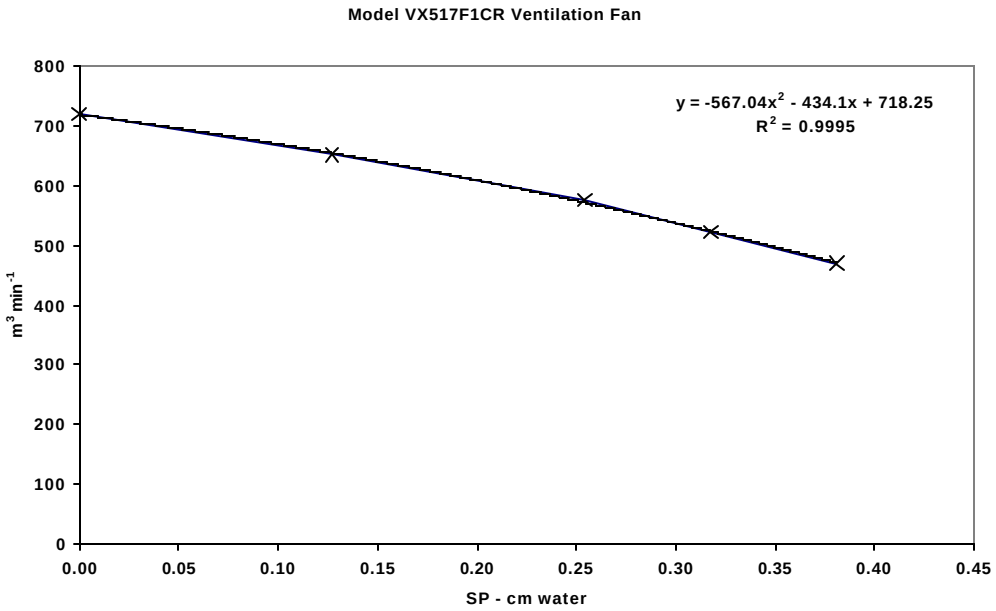

Figure 3.3 Fan Curve, 130-cm Ventilation Fan.

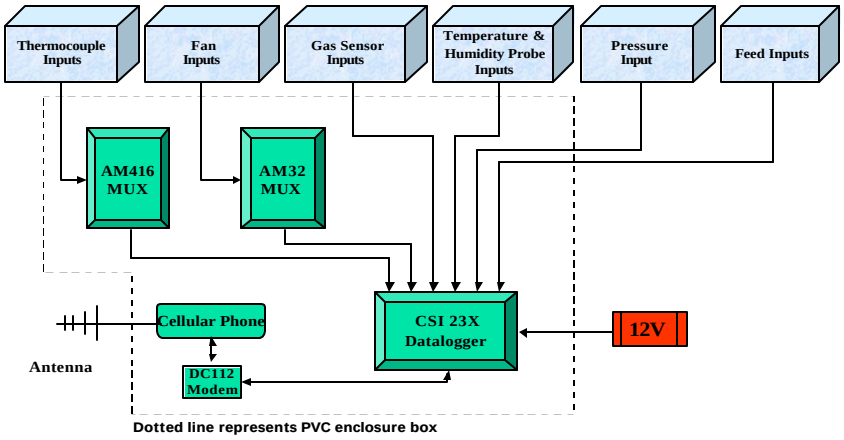

Figure 3.4 Data Acquisition System Configuration.

Multiplexers: Two multiplexers were used to scan the environmental input parameters. A CSI AM416 and an AM32 were used in production house 4. The CSI AM416 model permitted scanning of up to 32 differential inputs. It had two signal channels, permitting thermocouple measurements and 0-5V (or other range) measurements via the same multiplexer. The AM32 was less flexible, having only one signal channel for all 32 differential inputs. Thus, an AM32 multiplexer was used to separately measure fan tilt switch voltage inputs from production house 4.

Electrical/Electronic Equipment Enclosures: Two polyethylene (PVC) boxes (Figure 3.5) were used to house the key instrumentation components. The micrologger, the phone/modem unit, an AM416 and an AM32 multiplexer were mounted inside one PVC box located in production house 4.

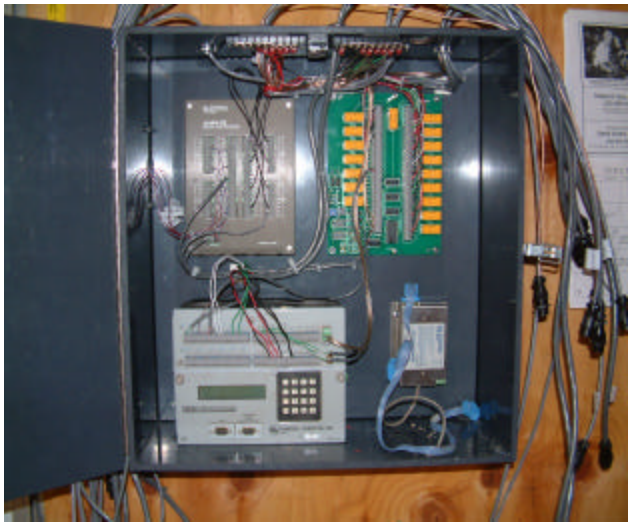

Figure 3.5 Data Acquisition Equipment in PVC Enclosure.

Ammonia Gas Sensors: The three ammonia sensors in house 4 were Dräger Inc. (Dräger Safety, Inc., Pittsburgh, PA**)** electrochemical devices. One gas sensor unit was located in the center of each house (details in *Gas Sensor Housing*). Initially, Dräger Polytron II sensors were used for ammonia detection. These units consisted of a transmitter enclosure with the necessary electronics and a replaceable sensor. The 4-20 mA output from each transmitter was converted to a voltage signal through a 243-ohm precision resistor. This allowed the CSI 23X micrologger to use the 5 V full-scale measurement range to improve the resolution of recorded data. The electrochemical sensors required periodic calibration to ensure accuracy. A warm-up period of at least 12 h was required before calibration. The zero calibration required 100% Ultra High Purity (UHP) nitrogen and the span calibration required a specific ammonia gas calibration concentration (50 ppm in N_2). The Polytron II sensor head accuracy is reported by the manufacturer to be ±5% for a range 21-100 ppm.

The Polytron II units were provided by Dräger as direct replacements to the Polytron I units originally used by Wilhelm (Wilhelm et al., 1999b), but the Polytron II proved to be unsuitable for ammonia detection in broiler production houses. The Polytron II units were prone to provide decreasing and negative ammonia values; even while ammonia concentrations increased in the production houses. It is believed that the sensors used with the Polytron II units became saturated when exposed to continuous ammonia concentrations. Following the failure of the Polytron II units, Dräger determined that the Polytron I

units were available in the European market. Between the fourth and fifth grow-outs, the Dräger Polytron II units were removed and replaced with Dräger Polytron I units. The manufacturer has been unable to provide information concerning the accuracy of the new Polytron I sensor heads at this writing. Documentation provided with the Polytron I units used by Wilhelm, was reported as ±2% in a range of 0-100 ppm. Ammonia sensors were scanned at 5-s intervals, averaged, and stored in the 23X micrologger for each 30-min period. Thus, the recorded ammonia value represents an average ammonia gas level concentration for a given 30-min period.

Sensors were calibrated between every grow-out, and gas-detection tube readings (LP-1200, RAE Systems Inc., Sunnyvale, CA) were periodically taken (ca. 20-d intervals) to verify the electrochemical sensor readings. A performance and calibration history is provided to compare and understand the performance of the Dräger Polytron I sensors against a commonly used gas-detection tube (Appendix, Table A.1).

Gas Sensor Housing: The gas sensors in the four production houses were mounted inside a PVC housing approximately 0.5 m long x 0.22m x 0.020m deep, open at the bottom and at each end. This housing provided significant dust protection while allowing air movement over the sensors. Also, the housing permitted all sensors to be handled as a single unit during installation and removal. Each sensor was bolted to the PVC housing to ensure the sensor head was parallel to the house floor. All gas sensor-housing assemblies were attached to nylon ropes and hung by a pulley arrangement from the production

unit ceiling 46 cm (18 in) above the house floor. The sensors located at each end of house 4 were hung from the ceiling approximately 152 cm (60 in) above the house floor. One was located in front of a 91-cm fan and the other near a 130-cm fan. The University of Tennessee Biosystems Engineering and Environmental Science shop fabricated the gas sensor housings.

Fan Sensors: Mercury tilt switches were installed to sense fan operating status. A switch was riveted on a fan louver downstream of each ventilation fan (11 fans in house 4) such that the tilt switch contacts would open when the louver tilted during fan operation. This tilt switch was connected to a continuous analog output (CAO) output of the micrologger via a pull-up resistor. This system provided an output signal of 5V when the fan was operating and 0V when there was no airflow. These signals were scanned at 5-s intervals, averaged, and recorded for each 30-min period. Thus, the recorded value represented an average "on" time for each fan during that period. The Nyquist Theorem (Nyquist, 2003) approach was taken for sensing fan status as well because fans could cycle on and off as fast as every 60 s in the houses. Thus, the slowest recommended sampling rate was 30 s. The author chose a conservative 5-s sampling rate to coincide with the gas rate and to minimize programming difficulties.

Power: In house 4, a 12-V battery provided power for the data logger, and a 'smart' charger maintained the battery voltage for long-term use 24 h day^{-1}. This approach assured uninterrupted power, which is necessary to avoid loss of gas

sensor data. The six NH_3 Polytron I sensors were powered by a small $120V_{AC}$ to $24V_{DC}$ power supply.

Temperature and Humidity: In house 4, a combined temperature (T) and relative humidity (RH) sensor (HMP45C, Campbell Scientific, Inc., Logan, UT) was mounted adjacent to the central gas sensor. Rated sensor accuracies were ± 0.4 °C and ± 2% RH from 0 to 90% RH.

Wiring and Connections: To allow speed and flexibility in connecting the various components, connectors were installed for almost all wiring entering the equipment enclosures. Individual connectors were also used for cables to each gas sensor. All signal connections, with the exception of thermocouples, used AMP Series 2 circular plastic shell and plug connectors. Thermocouple connections were made using quick disconnect thermocouple plugs and jacks.

Mass-Balance Emission Estimation

Overview: Mass flow measurements commenced when the producer had completely cleaned out the house just prior to the beginning of the first grow-out. The difference between nitrogen inputs and outputs were assumed to reflect gaseous emissions from the house as NH_3. Losses due to nitrate leaching and denitrification were assumed negligible. The N content of each mass balance component was obtained by analyzing a representative sample in a combustion-based nitrogen analyzer (FP-2000, LECO Corporation, St. Joseph, MI).

High-moisture samples were dried by lyophilizing (freeze drying) in a Virtis machine (The Virtis Co., Gardiner, NY). When grinding of samples was required,

a Wiley Mill (Wiley Mill Model 2, Arthur H. Thomas Co., Philadelphia, PA) was used. Dry matter (total solids) was determined for all samples by drying duplicate samples at 100°C; dry matter was used to adjust all N analysis results to a dry-basis (d.b.). Details regarding each component of the N balance follow:

Pine shavings for litter bedding: 250 g of clean pine shavings from the operation were ground to pass a 1-mm screen. Duplicate samples were then tested for N content.

Brooders (newly hatched birds): Four carcasses were frozen, lyophilized for 5 d, and ground to pass a 1-mm screen. Duplicate 0.5-g samples of ground material were analyzed for N.

Feed: Three feed formulations were used: starter, finisher, and withdrawal. Each formulation was sampled as follows: Four 150-g samples were oven dried at 60°C for 1 h and ground to pass a 1-mm screen. Duplicate 0.5-g samples of ground material were analyzed for N.

Poultry Broilers: Three market size broiler carcasses were frozen, chopped into smaller pieces, lyophilized for 34 d, and ground to pass a 4-mm screen. Five 0.5-g samples from each bird were analyzed for N.

Litter: Litter bedding depth was measured in 18 locations equally spaced throughout the house just prior to cleanout. Additionally, two bedding profiles were taken to estimate an average litter density. Three samples were lyophilized for 14 d, ground to pass a 1-mm screen, and then analyzed for N.

RESULTS AND DISCUSSION

Nitrogen Emission Estimation by Flow-Integration

Ammonia emissions estimated by the flow-integration method for grow-out 5 are illustrated in Figures 3.6-3.9; the significant temporal variability in emission rate is notable, and has implications for the design of emission experiments. Valid NH_3 concentration data was collected during grow-outs 5, 6, 7, and 8 and were used to compute flow-integration NH_3 emission estimates of 1477, 1044, 1099, and 961 kg NH_3 per flock, respectively.

The four grow-outs did not demonstrate equal in-house ammonia concentration or airflow trends. Therefore, the house 4 ammonia emission estimates differed in each grow-out because ammonia emissions are a product of air volume and in-house ammonia concentration. Grow-out 5 flow-integration measurements are used to explain ammonia emission behavior over a typical 42 d grow-out. The rationale for NH_3, temperature, and airflow rate responses are identical to that explained in Part 2 (grow-out 6 and 8) of this work, thus providing explanation for NH_3 flux from a broiler house. Figure 3.6 shows lower emissions for the first five days because NH_3 levels are low and airflow is relatively constant. Within the 5-day span NH_3 tracks in-house temperature. As NH_3 concentrations build again emissions gradually increase to a high of 51 kg on day 15. Within the 15 d, ammonia follows in-house temperature. In particular, on day 10, the temperature increases from 19°C to 29°C causing an ammonia concentration spike of 60 ppm in the house. At day 15, NH_3 levels begin a

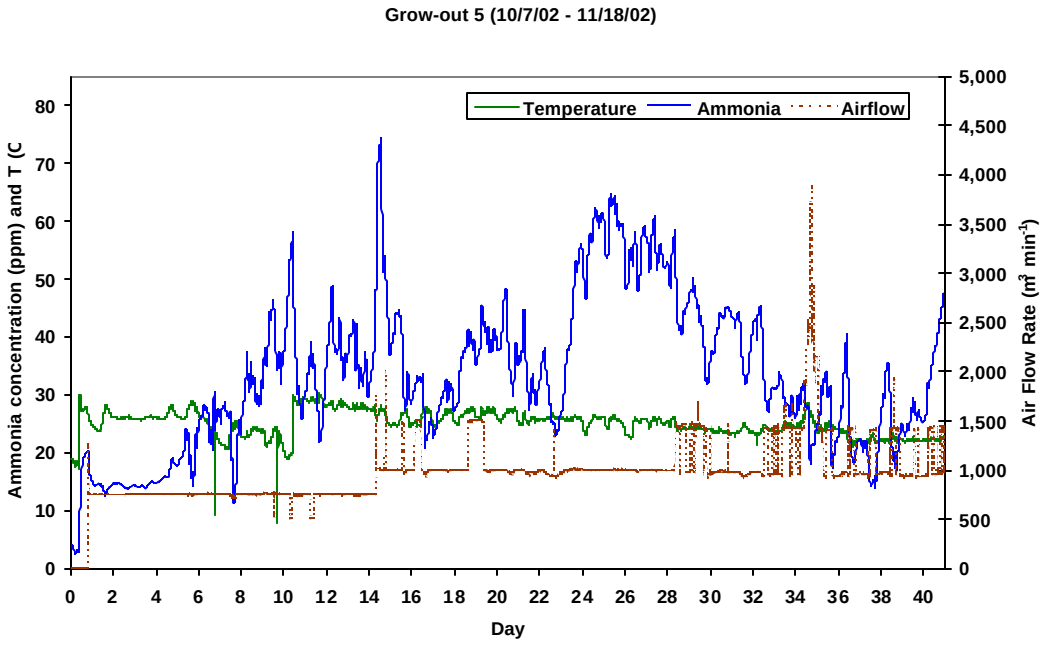

Figure 3.6 Grow-out 5 Temperature, In-house NH_3 concentrations, and Air flow (House 4).

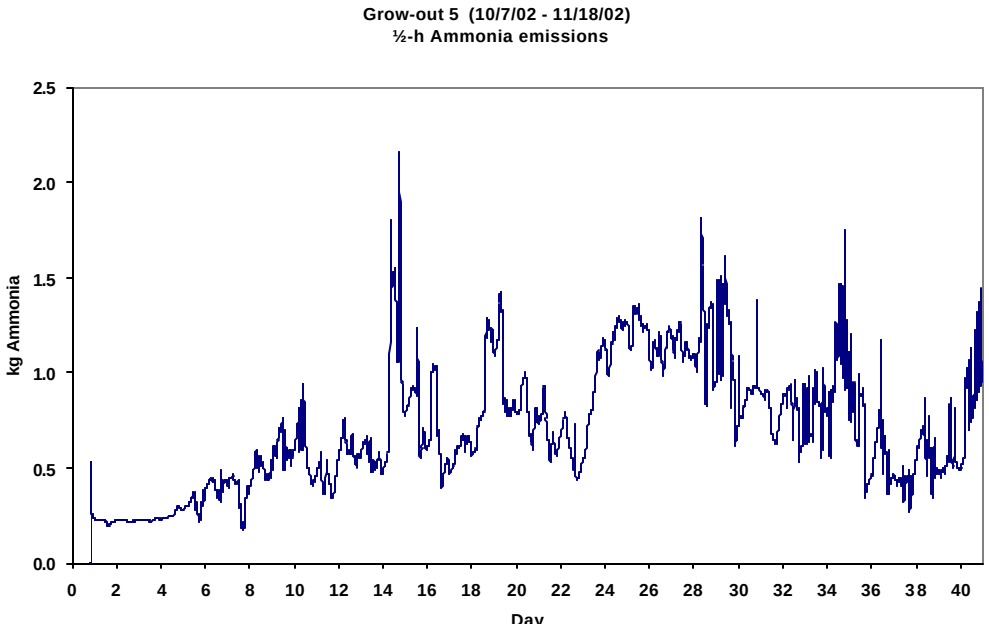

Figure 3.7 Grow-out 5 ½ -h NH_3 emissions (House 4).

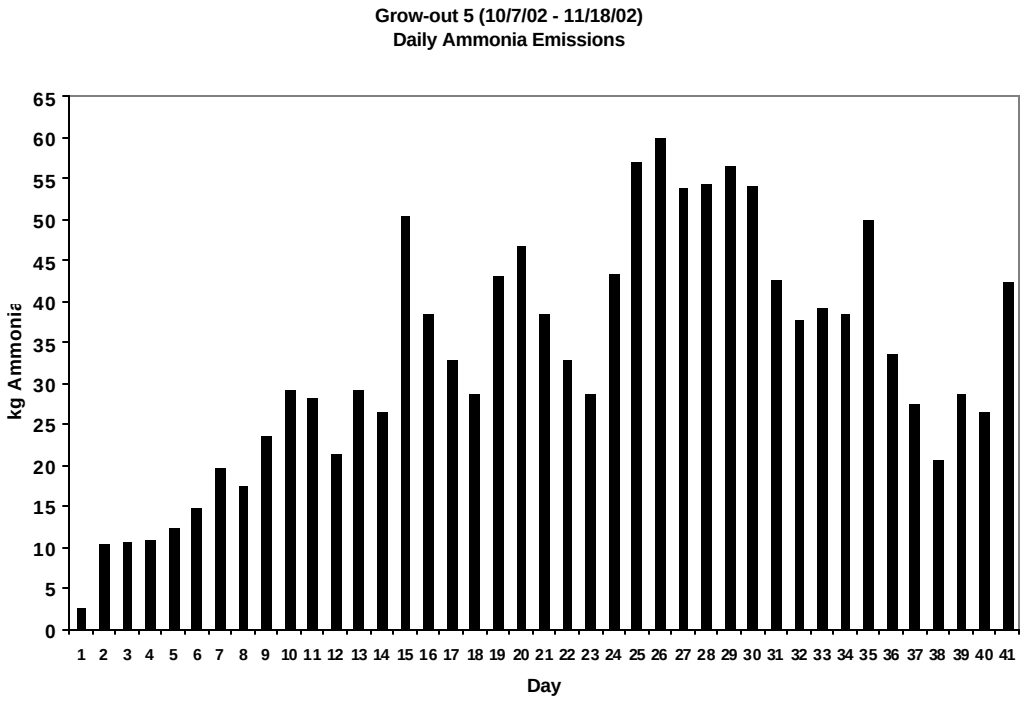

Figure 3.8 Grow-out 5 Daily NH_3 emissions (House 4).

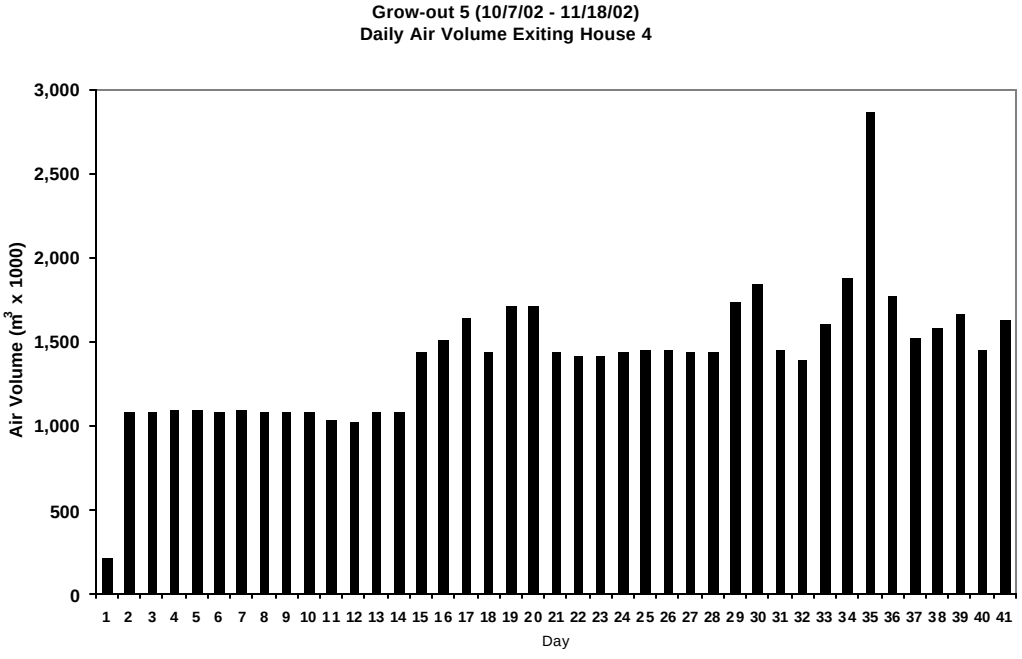

Figure 3.9 Grow-out 5 Daily Air Volume Exiting House (House 4).

downward trend because the divider curtain has been raised and an increase in airflow has occurred. Until day 28, airflow is an average of 1,000 m^3 min^{-1}, so ammonia levels track temperature. Specifically, there are two ammonia emission troughs before day 28, the first due to lowering temperature and the second due to a short increase in airflow. On day 28, fans begin to cycle on and off more frequently so ammonia concentrations gradually decrease to a low of 12 ppm on day 37. On day 35, there is a sharp increase in temperature and airflow rate immediately responds, thus causing an increase in ammonia emissions. During the last few days of the grow-out, ammonia concentrations rise and emissions follow as expected. The average temperature was 22°C for the last 4-5 d of grow-out 5. The grower was most likely attempting to maintain temperature constant just before broiler extraction by cycling the fans more frequently. This caused an increase in ammonia volatilization thereby increasing ammonia concentrations at the end of the grow-out.

Nitrogen Emission Estimation by Mass-Balance

The average N content of pine shavings was 0.0738% (d.b.) and the density was 160 kg m^{-3} (USDA, 1992). Based on the measured shavings additions, shavings input was assumed to remain constant at 15 kg N per flock over the course of the experiment. Brooder carcass samples averaged 9.69% N (d.b.), their average mass was 0.016 kg (d.b.), and 30000 brooders were introduced at the beginning of each grow-out. Therefore, a 48 kg N input was associated with brooders for each flock. Starter feed, finisher feed, and

withdrawal feed averaged 3.768% N (d.b.), 3.626% N (d.b.), and 3.322% N (d.b.), respectively. All feed was 12.5% moisture content, wet-basis (w.b.). Feed properties were assumed constant for all flocks, while the feed masses changed slightly each time causing changes in the N input associated with feed (Table 3.1). Broiler carcasses averaged 8.203% N (d.b.); N mass removal in broilers are listed in Table 3.1. Based on the observed litter density and removal volume, the average litter mass removed per flock was 25200 kg (w.b.); average litter moisture content was 30% (w.b.), and average N content was 2.714% (d.b.), yielding an average N removal in litter of 478 kg flock^{-1}. Table 3.1 summarizes the mass balance results.

Comparison of Methods

The mentioned sensor problems compromised the flow-integrated emission estimates for flocks 1 – 4. To enable comparison of flow-integrated and

Table 3.1 Nitrogen Loss Estimated Using Nitrogen Mass Balance.

	Grow-out 1	Grow-out 2	Grow-out 3	Grow-out 4	Grow-out 5	Grow-out 6	Grow-out 7	Grow-out 8	Total N (kg)
N Inputs (kg)									
Shavings	15	15	15	15	15	15	15	15	123
Chicks	48	48	48	48	48	48	48	48	381
Starter Feed	528	356	360	488	445	534	528	494	3,732
Finisher Feed	1,022	1,097	1,208	1,152	1,051	822	1,000	1,055	8,407
Withdrawal Feed	1,612	1,663	1,643	1,450	1,670	1,740	1,533	1,593	12,904
Total N Inputs	3,224	3,178	3,274	3,153	3,229	3,159	3,125	3,206	25,548
N Outputs (kg)									
Poultry Broilers	1,650	1,676	1,770	1,681	1,761	1,925	1,588	1,637	13,689
Litter	478	478	478	478	478	478	478	478	3,824
Total N Outputs	2,128	2,154	2,248	2,159	2,239	2,403	2,066	2,115	17,513
Input - Output (lbs N)	1,097	1,024	1,025	994	989	756	1,058	1,090	8,034
As lbs of NH3	1,331	1,244	1,245	1,207	1,201	917	1,285	1,324	9,754
Comparison of Measured Ammonia Emissions (FI) vs. Mass Balance (MB)									
Measured NH3	1,145	1,145	1,145	1,145	1,477	1,044	1,099	961	9,161
Mass Balance NH3	1,331	1,244	1,245	1,207	1,201	917	1,285	1,324	9,754
% of Measured NH3	86%	92%	92%	95%	123%	114%	86%	73%	94%

mass-balance results, the average flow-integrated emission determined for flocks 5, 6, 7, and 8 were applied to flocks 1 – 4. Table 3.2 summarizes the results of mass-balance and flow-integration methods for estimating NH_3 emission from a full-scale production broiler house. The close overall match between the two methods – better than 10% – is remarkable. The close match between mass-balance and flow-integration methods reported may reflect fortuitous cancellation of errors in the NH_3 measurements due to the long-term nature of the study. Because of the vagaries of electrochemical measurements, this work believes the mass-balance approach to be a more reliable emission estimation technique.

Table 3.2. Ammonia emission estimates by mass-balance (MB) and flow-integration (FI) approaches, in units of kg NH_3 $flock^{-1}$; % difference = 100 * (FI – MB)/MB.

Grow-out ? Method ↓	1	2	3	4	5	6	7	8	Total
Flow-Integration	1,145	1,145	1,145	1,145	1,477	1,044	1,099	961	9,161
Mass Balance	1,331	1,244	1,245	1,207	1,201	917	1,285	1,324	9,754
% Difference	-14	-8.0	-8.0	-5.0	+23	+14	-14	-27	-6.0

CONCLUSIONS AND RECOMMENDATIONS

The results suggest that short duration (less than one complete grow-out) measurements of NH_3 emissions are inadequate to accurately estimate long-term emission factors. Other investigators using a mass-balance approach have arrived at emission factors similar to those reported herein (Asman, 1992), whereas investigators using flow-integration methods arrived at lower emission estimates (Table 3.3). Given the cost and complexity of flow-integration methods, mass-balance methods appear to be a promising method of providing accurate long-term NH_3 emission estimates from poultry broiler production houses. If short-term (days or weeks) emission factors are desired, a carefully designed flow-integration system can provide such data, as shown by the excellent agreement between flow-integration and mass-balance results herein.

Table 3.3 U.S. and European NH_3 Emission Factors (originally reported values are shown in bold).

Reference (method)	kg / yr / broiler	g / hr / LU	kg / d / 30000-broiler house	g / d / broiler
Asman, 1992 (MB)	***0.167***	12.2	13.7	0.663
Burns et al., 2003 (MB + FI)	0.341	**17.1**	***28.0***	1.35
Casey et al., 2003 (FI)	0.030 - 0.250	1.80 - 18.0	2.07 - 20.3	***0.100 - 0.980***
Demmers et al., 1999 (FI)	0.026	***1.90***	2.14	0.104
Hutchings et al., 2001 (FI)	***0.200***	10.1	16.4	0.794
Muller et al., 2003 (FI)	0.043	***3.10***	3.50	0.169
Wathes et al., 1997 (FI)	0.124	***9.00***	10.2	0.490
Wheeler et al., 2003 (FI)	0.154	11.2	12.6	***0.610***

* Average final bird mass assumed to be 2.27 kg; emission estimate based on ½ this value to reflect average bird mass over 42-d grow-out.

SUMMARY

A summary is provided of published European and U.S. ammonia emission factors from studies conducted on broiler houses (Table 3.3). The original published emission factor units for each emission factor is noted in bold for each particular reference. The author has converted the published emission factor into the remaining three units. Burns et al. (2003), Casey et al. (2003), and Wheeler et al. (2003) are U.S. studies and the remainder are European. The table illustrates several points about broiler ammonia emission factors. As shown in the table, broiler emission factors are reported in various units. One key assumption that must be made in converting between these four units is the final bird mass for the grow-out studied. The final bird mass can range 2–4 kg in the U.S. and 1-2 kg in Europe. The other issue with the assumption of a bird mass is if an average bird mass for the entire grow-out or a daily bird mass was used in the emission factor calculations. This information is critical because broiler production systems are different between Europe and the U.S., and different between the production systems used for different bird types (e.g. Roaster broiler, White Leghorn broiler, or Cobb-Cobb broiler). Another important broiler industry difference between the two regions is litter management. Usually in Europe, the broiler houses are completely cleaned out at the end of each grow-out. Also, a grow-out is shorter in Europe (e.g. 32 d). In the U.S., growers use a deep-litter system to manage broiler houses and six or more flocks are

grown on the same litter. Typically, the deep-litter systems will have higher in-house ammonia concentrations.

Ammonia measurement methodology is crucial, specifically the data collection time period. Published research shows that experiment designs have measured ammonia concentrations continuously for 41 d (Demmers et al., 1999), every 2 min for approximately 3 weeks (Worley et al., 2002), every 5 s for 42 d (Burns et al., 2003), once an hour for 24 h (Wathes et al., 1997), and continuously for 24 h on selected days of a grow-out (Casey et al., 2003 and Wheeler et al., 2003). As discussed in Part 2 of this work, ammonia concentrations will vary from hour to hour in a broiler house. As shown in Part 3 of this work, ammonia emissions will vary from day to day. The studies using a short-term (e.g. minutes, days) (Koerkamp et al., 1998, Wheeler et al., 2003, Zhu et al., 1999) sampling time may not attain a representative sample of NH_3 concentrations from a broiler grow-out. Müller et al. (2003) emphasizes that short-term ammonia measurements are not recommended because the broiler mass will vary during the growout, and therefore, in-house concentrations will vary. The studies using a long-term (e.g. weeks, months) (Demmers et al., 1999, Worley et al., 2002, Burns et al., 2003) sampling time are expected to attain a more representative sample of NH_3 concentrations from a broiler grow-out. Although short-term NH_3 concentration measurements shorten the research duration and possibly lower equipment and labor cost, long-term is advisable for ammonia emissions inventory purposes.

As mentioned in previous parts of this work, a mass-balance approach was used to estimate NH_3 emissions. But, mass-balance calculations will differ as well because of the production system, litter management, and assumed bird mass. Koerkamp et al. (1998b) points out that caution should be taken in using the MB approach because the measured in-house ammonia losses can be as much as 20% less than the total nitrogen in the manure. Also, Koerkamp et al. (1998b) adds that litter sampling protocol and nitrogen content determination can lead to errors using the MB method. Burns et al. (2003) showed that the MB approach was successful in determining NH_3 emissions over a longer time period.

In comparing some of the emission factors expressed in g hr^{-1} LU^{-1}, Asman (1992), Burns et al. (2003), Casey et al. (2003), and Wheeler et al. (2003) are similar. However, if a comparison is done on the same authors in kg yr^{-1} $broiler^{-1}$ units, larger differences are present. One is reason is Casey et al. (2003) used broiler growth curves to calculate the daily broiler mass. Additionally, the broilers in the Casey et al. (2003) study were grown for 47-56 d. As mentioned, the author assumed 2.27 kg in converting the reported emission factors to other units. Another example of differences is among Demmers et al. (1999) and some U.S. authors. Demmers et al. (1999) measured ammonia long term using chemiluminesence detection. Casey et al. (2003) and Wheeler et al. (2003) measured short term using Dräger portable measuring unit sensors described in Xin et al., 2002. Burns et al. (2003) measured long term using Dräger Polytron I sensors. The variety of sensor technology could explain some

of the differences. A review of published literature did not find any comparison of these mentioned sensors. Last, broiler dietary practices are different in Europe which could explain the variation in U.S. and European broiler emission factors.

Ammonia emission inventories are necessary to provide information in establishing air quality standards. Although substantial advancement has been made in ammonia emissions, this work demonstrates that continued emissions research is warranted in order to accurately provide an ammonia emission inventory in the U.S.

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APPENDIX

Table A.1 History of Dräger Polytron NH3 Sensor Performance (Sensor 6808700 with Polytron Head 8311655)

Sensor I.D.	8/21/2002 Sensors calibrated in lab air was used to zero, cal gas to span	8/22/2002 Sensors Installed (except U1)
U1	0 and 50	Installed 9/1
U2	0 and 50	X
U3	0 and 50	X
U4NH3_1 (middle of unit)	0 and 50	X
U4NH3_2 (non-brooder)	0 and 50	X
U4NH3_3 (brooder)	0 and 50	X

Sensor I.D.	10/2/2002 Sensor Output ppm	w/ 50 ppm cal. gas	calibrated w/ N2 as 0 and using 50 ppm cal gas	10/8/2002 Sensor Output ppm
U1	-2	48	0 and 50	21
U2	-12	38	0 and 50	22
U3	-3	47	0 and 50	Sensor was unplugged
U4NH3_1 (middle of unit)	-12	36	0 and 50	1.5
U4NH3_2 (non-brooder)	-10	40	0 and 50	11
U4NH3_3 (brooder)	2	50	0 and 50	12

Sensor I.D.	10/13/2002 Sensor Output ppm	Sensor output with 50 ppm cal. gas	12/18/2002 Sensor output before calibration	Rae Tube before calibration	Sensor output after calibration	Rae Tube after calibration
U1	11	56	9	5	5	5
U2	11	58	13	9	9	9
U3	8	57	0	9	5	9
U4NH3_1 (middle of unit)	22	58	26	30	20	30
U4NH3_2 (non-brooder)	-2	48	-2	6	1	6
U4NH3_3 (brooder)	40	62	28	25	18	26

Sensor I.D.	2/3/2003 Sensor output before calibration	Rae Tube before calibration	Sensor output with 50 ppm cal. gas	Sensor output after calibration	Rae Tube after calibration
U1	1	1.5	38	0	1.5
U2	1	2	40	2	2
U3	1	2	38	2	2
U4NH3_1 (middle of unit)	17	40	30	0 (no span, sensor OOO)	40
U4NH3_2 (non-brooder)	32	38	32	20	38
U4NH3_3 (brooder)	9	25	30	40	25

Sensor I.D.	2/27/2003 Sensor output before calibration	Rae Tube before calibration	Sensor output with N2	Sensor output with 50 ppm cal. gas	Sensor output after calibration	Rae Tube after calibration
U1	38	18	not calibrated	not calibrated	not calibrated	not calibrated
U2	38	22	not calibrated	not calibrated	not calibrated	not calibrated
U3	29	18	not calibrated	not calibrated	not calibrated	not calibrated
U4NH3_1 (middle of unit)	57	18	22	45	not recorded	18
U4NH3_2 (non-brooder)	17	30	11	60	not recorded	30
U4NH3_3 (brooder)	OOS	OOS	OOS	OOS	OOS	OOS

Sensor I.D.	3/26/2003 Sensor output before calibration	Rae Tube before calibration	Sensor output with N2	Sensor output with 50 ppm cal. gas	Sensor output after calibration	Rae Tube after calibration
U1	not recorded	not recorded	4	57	not recorded	not taken
U2	not recorded	not recorded	4	53	not recorded	not taken
U3	not recorded	0	4	68	not recorded	0
U4NH3_1 (middle of unit)	not recorded	0	12	50	not recorded	0
U4NH3_2 (non-brooder)	not recorded	0	1	68	not recorded	0
U4NH3_3 (brooder)	OOS	OOS	OOS	OOS	OOS	OOS

Table A.1 (continued)

	5/16/2003					
Sensor I.D.	Sensor output before calibration	Rae Tube before calibration	Sensor output with N2	Sensor output with 50 ppm cal. gas	Sensor output after calibration	Rae Tube after calibration
U1	20	1	2	49	6	1
U2	21	1	2	49	5	1
U3	9	0	8	40	5	1
U4NH3_1 (middle of unit)	30	10	9	43	16	10
U4NH3_2 (non-brooder)	11	10	2	52	23	10
U4NH3_3 (brooder)	OOS	OOS	OOS	OOS	OOS	OOS

Pathogen Enumerations

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **04-Mar-02**
Growout Status: 1st growout after litter had been changed (Feb. 16, 2002); no alum ; 7th day of growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		7.23	8.26	4.46	6.18	4.69	8.26	6.76	5.11
Coliform		8.00	8.18	8.91	7.00	8.26	7.48	5.49	7.72
Salmonella		6.71	7.32	0	7.98	5.30	7.48	7.36	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	3.75	5.08	3.11 EST.	7.04	1.32	6.49	6.20	7.95
	7.30	4.71	5.20	7.30	0	0	0	5.32
	0	7.28	0	6.30 EST.	6.28	6.69	7.15	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **25-Mar-02**
Growout Status: 3 weeks after 1st sampling event of 1st growout ; no alum

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		2.89	8.74	9.41	8.62	7.79	9.36	9.87	9.89
Coliform		5.41	6.91	6.00 EST.	7.26	6.26	7.76	7.59	7.53
Salmonella		4.11 EST.	5.79	6.85	6.23	0	7.08	6.70	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	10.23	10.11	10.38	10.41 EST.	9.95	9.72	9.79	9.38
	10.36	8.40	7.18 EST.	7.41	7.59	6.63	7.32	7.92
	6.64	5.85	6.94	7.11	0	0	0	6.15
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **24-Apr-02**

Growout Status: 2nd growout ; alum applied Apr. 18, 2002 ; Chicks introduced 4/25/02 into all units

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		8.32	5.58	8.18	8.34	8.30	5.32	7.45	7.54
Coliform		4.00	0	0	5.43	0	0	0	0
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	5.43	4.95	6.36	6.67	8.32	8.15	8.20	8.41
	0	0	4.00	4.90	5.60	5.30	5.26	5.49
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **15-May-02**
Growout Status: 3 weeks after 1st sampling event of 2nd growout

Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
Alt. #	1	2	3	4	5	6	7	8
APC	9.91	9.45	9.38	9.69	8.40	9.41	9.52	8.78
Coliform	9.43	7.68	7.08	8.11	8.28	7.45	7.64	7.32
Salmonella	4.78 EST.	5.95 EST.	0	6.49	5.00 EST.	4.00 EST.	5.18 EST.	4.90 EST.
Campylobacter	0	7.41	6.48	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	9.74	10.30	8.98	8.99	10.51	9.72	9.34	9.41
	8.36	7.00	6.93	8.15	7.11	7.79	9.00	7.51
	5.48 EST.	6.43 EST.	0	5.23 EST.	0	0	6.00 EST.	5.00 EST.
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **12-Jun-02**
Growout Status: Beginning of 3rd growout, alum applied 6/13/02

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		8.04	8.62	8.94	9.00	8.76	8.32	9.20	9.11
Coliform		6.11	5.04	3.70 EST.	3.85 EST.	3.30 EST.	4.99	3.70 EST.	3.95 EST.
Salmonella		0	3.00 EST.	0	0	0	0	6.85 EST.	6.60 EST.
Campylobacter		5.18 EST.	4.18 EST.	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	8.48	7.91	7.87	9.15	7.64	7.58	7.20	8.56
	4.53	3.30 EST.	3.85 EST.	4.96	0.00	4.00 EST.	4.00 EST.	3.48 EST.
	0	0	0	0	0	0	0	0
	0	0	0	0	0	4.68	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **06-Jul-02**
Growout Status: 3 weeks into 3rd growout

	Sample #	U1-1		U1-2	U1-3	U1-4		U2-1	U2-2	U2-3	U2-4	
	Alt. #	1		2	3	4		5	6	7	8	
APC		7.60		8.72	7.96	8.45		9.76	9.68	8.76	8.40	
Coliform		7.28	EST.	7.56	8.18	8.36	EST.	7.66	7.85	7.08	EST. 6.32	EST.
Salmonella		6.79		6.91	7.20	5.78	EST.	6.45	0	0	0	
Campylobacter		0		6.91	0.00	0.00		3.78	0	0	0	

U3-1		U3-2	U3-3	U3-4		U4-1	U4-2	U4-3	U4-4
9		10	11	12		13	14	15	16
8.38	EST.	8.00	7.40	8.58		9.11	8.81	8.93	8.36
8.49		7.18	7.68	6.53		8.23	8.08	8.28	7.53
0		6.65	0	4.90	EST.	0	0	0	5.30
0		0	0	0		6.38	5.30	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **07-Aug-02**
Growout Status: Beginning of 4th growout; alum applied 8/7/02 but after sampling

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		0	0	0	4.18	5.79	5.34	5.58	3.78
Coliform		7.79	8.48	8.40	8.28	8.28	8.26	8.59	8.78
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	0.00	4.08	3.48	3.00	4.73	4.26	4.75	3.70
	8.41	8.72	8.83	8.04	7.58	7.92	8.45	8.40
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **02-Sep-02**
Growout Status: 3 weeks into 4th growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		9.04	7.79	8.43	8.75	9.97	9.97	9.96	9.81
Coliform		7.58	8.12	7.95	7.61	5.67	8.03	8.04	8.01
Salmonella		3.48	0	0	4.18	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	10.30	10.32	10.20	10.23	9.54	9.60	9.04	9.53
	9.30	9.08	7.28	9.59	7.05	7.72	8.52	7.98
	3.60	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **03-Oct-02**
Growout Status: Beginning of growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		8.20	8.15	7.76	8.71	8.30	8.08	7.76	8.23
Coliform		7.08	4.92	6.76	6.56	5.04	6.63	7.63	7.15
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	7.79	8.43	8.75	8.15	7.69	8.00	7.97	8.41
	4.89	4.11	7.08	7.34	4.52	4.84	4.83	4.49
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **24-Oct-02**
Growout Status: 3 weeks into 5th growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		9.30	9.45	8.64	9.00	9.26	9.36	9.26	8.77
Coliform		9.28	7.65	7.00	8.11	7.45	9.34	9.04	8.59
Salmonella		6.26	7.15	7.23	5.74	3.00	6.32	6.00	6.40
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	9.60	9.98	9.45	9.36	9.99	10.04	10.00	9.70
	10.04	7.82	6.56	9.04	7.11	8.48	7.81	9.04
	6.54	5.79	6.15	6.15	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **09-Dec-02**
Growout Status: Beginning of Growout 6

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2		U2-3	U2-4
	Alt. #	1	2	3	4	5	6		7	8
APC		7.00	7.76	4.04	6.62	5.46	3.00	EST.	5.65	6.45
Coliform		3.70	0.00	0	5.41	4.40	0		4.41	4.69
Salmonella		0	0	0	0	0	0		0	0
Campylobacter		0	0	0	0	0	0		0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	6.40	6.45	8.08	0	5.41	7.18	6.79	6.15
	3.00	3.00	4.45	4.00	4.58	7.11	6.49	4.76
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **30-Dec-02**
Growout Status: 3 weeks into 6th growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		7.71	6.57	7.36	7.20	6.67	6.95	5.92	4.60
Coliform		6.86	0	0	0	4.00	7.08	5.18	4.40
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
9	10	11	12	13	14	15	16
8.59	8.26	6.48	8.04	7.70	7.40	7.83	7.18
6.85	4.38	4.78	4.41	6.78	0	5.00	5.78
0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **03-Feb-03**
Growout Status: Beginning of Growout 7

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		4.45	5.61	6.08	7.86	4.70	5.04	4.48	7.34 EST.
Coliform		0.00	4.00 EST.	0	4.41	3.30 EST.	4.00 EST.	5.08	7.30
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	5.20	4.40	5.93	5.00	7.48	8.61	7.11	6.30
	4.46	3.00 EST.	5.93	4.36 EST.	6.20	6.56	6.18	6.04
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **27-Feb-03**
Growout Status: 3 weeks into 7th Growout

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		8.04	7.59	7.94	7.59	6.49	7.00	7.04	7.81
Coliform		7.32	7.04	6.04	6.20	0	0	0	0
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	7.91	0	7.04	7.34	6.69	7.58	7.32	8.56
	6.62	6.40	6.41	6.60	7.30	7.15	7.49	6.30
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Pathogen Enumerations (continued)

Sampling Notations:

U1 is Unit 1 U2 is Unit 2
U3 is Unit 3 U4 is Unit 4

The number after the unit designation is the sampling location within the house and are as follows:
1: Non brooder area under water feeder
2: Brooder area under water feeder
3: Non brooder area at center
4: Brooder area at center

Data Notations:

APC = Aerobic Plate Count
EST. = Estimated
All numerical data is represented in log scale base 10 CFU / ml.

Date: **26-Mar-03**
Growout Status: Beginning of Growout 8

	Sample #	U1-1	U1-2	U1-3	U1-4	U2-1	U2-2	U2-3	U2-4
	Alt. #	1	2	3	4	5	6	7	8
APC		7.41	5.43	5.91	5.71	5.58	5.00 EST.	9.00	5.86
Coliform		0	0	4.30	0.0	0	4.00	0	0
Salmonella		0	0	0	0	0	0	0	0
Campylobacter		0	0	0	0	0	0	0	0

	U3-1	U3-2	U3-3	U3-4	U4-1	U4-2	U4-3	U4-4
	9	10	11	12	13	14	15	16
	6.98	5.81	6.98	6.32	7.28	6.26	6.54	6.23
	5.18	4.78	6.04	5.04	6.52	5.77	5.68	5.65
	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0

Table A.2 Calendar of Events

Date	Event
February 16, 2002:	Houses cleaned out
February 26, 2002:	Flock 1 introduction (no alum applied in houses)
March 4, 2002:	***Sample Event 1***
March 25, 2002:	***Sample Event 2***
April 10, 2002:	Flock 1 taken out
April 18, 2002:	Alum applied
April 24, 2002:	***Sample Event 3***
April 25, 2002:	Flock 2 introduction
May 15, 2002:	***Sample Event 4***
June 5, 2002:	Flock 2 taken out
June 12, 2002:	***Sample Event 5***
June 13, 2002:	Alum applied
June 20, 2002:	Flock 3 introduction
July 6, 2002:	***Sample Event 6***
August 1, 2002:	Flock 3 taken out
August 7, 2002:	***Sample Event 7 (before alum application)***
August 7, 2002:	Alum applied
August 13, 2002:	Flock 4 introduction
September 2, 2002:	***Sample Event 8***
September 23, 2002:	Flock 4 taken out
September 24, 2002:	Houses cleaned out
October 3, 2002:	***Sample Event 9***
October 4, 2002:	Alum applied
October 8, 2002:	Flock 5 introduction
October 24, 2002:	***Sample Event 10***
November 18, 2002:	Flock 5 taken out
December 3, 2002:	Alum applied
December 9, 2002:	***Sample Event 11***
	Protocol change: sampled after alum application
December 10, 2002:	Flock 6 introduction
December 30, 2002:	***Sample Event 12***
January 19, 2003:	Flock 6 taken out
January 31, 2003:	Alum applied
February 3, 2003:	***Sample Event 13 (before bird introduction)***
February 3, 2003:	Flock 7 introduction
February 27, 2003:	***Sample Event 14***
March 16, 2003:	Flock 7 taken out

Table A.2 (continued)

Date	Event
March 24, 2003:	Alum applied
March 26, 2003:	***Sample Event 15***
March 28, 2003:	Flock 8 introduction
May 8, 2003:	Flock 8 taken out

VITA

Ken Armstrong was born on June 24, 1968, and is the son of E.M. and Phyllis Armstrong. He received his elementary and secondary education in Etowah, Tennessee, graduating from McMinn Central High School in May of 1986. After military service in the U.S. Navy, he entered DeVry Institute of Technology majoring in Business Operations with a minor in Accounting. Ken graduated from DeVry Institute of Technology with a B.S. in Business Operations in June 1994. After working in the private sector and serving the U.S. Peace Corps, he accepted a research assistantship at the University of Tennessee, Knoxville in spring of 2001 and began study toward a Master of Science degree in Biosystems Engineering Technology.