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Ammonia emissions, feeding and defecation dynamics of W36 pullets and laying hens as affected by stocking density and manure accumulation time

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Ammonia emissions, feeding and defecation dynamics of W36 pullets and laying hens as affected by stocking density and manure accumulation time

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TABLE OF CONTENTS

ABSTRACT

A great deal of effort has been given through intensive research toward studying the sources of gaseous emissions from animal feeding operations (AFOs) and their influencing factors. Ammonia (NH_3) is the most predominant noxious gas released from poultry production facilities and it is mainly affected by diet composition, manure temperature, moisture content and stacking configuration and manure surface area exposed to ambient air. However, current literature lacks information on bird age effects on NH_3 emissions, even though changes in diet composition with bird age are expected to affect the emissions. Also, some producers have been using different bird stocking densities (SD) as an attempt to improve bird welfare. Nevertheless the effects of different bird SD regimens on NH_3 emissions remain unknown. Moreover, it has been shown that different housing styles can have significant impacts on the magnitude of NH_3 emissions from laying-hen facilities in that the high rise (HR) systems (typical of US egg production) emit 61 to 71 % more ammonia than the manure-belt (MB) systems (gaining more popularity in the US). The impact of manure accumulation time on the belts in MB systems on NH_3 emissions needs to be quantified. Hence, a research study was conducted in a laboratory setting that resembled a commercial MB system for laying hens. The results of the study are presented in this thesis.

Chapter 2 describes the effect of different SD regimens (155 to 619 cm^2 $bird^{-1}$ or 24 to 96 in^2 $bird^{-1}$) and manure accumulation time (MAT, 1 to 6 d) of pullets (hens < 18 weeks of age) and laying hens on NH_3emissions. Results showed that daily NH_3 emission rate (ER) for pullets and laying hens increased exponentially with bird age and MAT, while SD effect on NH_3 ER was more pronounced for MAT ≥ 3d ($P<0.0001$). In general,

higher SD led to higher ER. Specifically, for the laying hens, NH_3 emissions from the 3^{rd} to 6^{th} d MAT ranged from 41 to 251 mg/hen-d for the high density (HD) and from 29 to 160 mg/hen-d for the low density (LD). This outcome supports the current egg industry practice of removing manure at 1- to 3-d MAT for the MB house systems.

Chapter 3 assesses the dynamics of feeding, defecation and NH_3 emissions of pullets and laying hens under different SDs (as used in the trials described in Chapter 2), MAT (1 to 6 d) during light and dark periods of the day. Results indicate that SD did not adversely affect feed disappearance or fresh manure production ($P = 0.17 – 0.81$) at any of the tested ages. For each gram of feed use, the fresh manure produced varied from 0.58 to 1.15 g $bird^{-1}$ ($P < 0.0001$) varying according to bird age. The light and dark partitioning of feed disappearance was 92% to 8%, respectively, while the partitioning for fresh manure production was 80% to 20%. Results also revealed that 37% of the total daily NH_3 emission occurred during the dark period vs. 63% during the light hours.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Among all constituents emitted from poultry production facilities, ammonia (NH_3) is the predominant noxious gas due to the nature of the manure (Liang et al., 2005). Although NH_3 emission regulations have been in place for some time, state and federal regulatory agencies have not enforced these regulations for animal operations for various reasons including the limited information on emission of gaseous pollutants emitted from animal facilities (Jacobson et al., 2005).

Ammonia emissions from agricultural sources has been widely regarded as an important factor contributing to acid rain and excessive nitrogen input to natural ecosystems, which can lead to unwanted modification of such ecosystems (van Breemen et al., 1982; Demmers et al. 1998), such as eutrophication and soil acidification related environmental stress (Heij & Schneider, 1991: Heij & Erisman, 1997, Monteny & Erisman, 1998).

Several methods have been developed to reduce gas emissions from AFOs. These methods address four major areas: (1) manure, (2) exhaust air, (3) animal diet, and (4) housing design.

Manure additives are products generally intended to reduce ammonia volatilization from manure. Some of these are digestive (e.g., select microorganisms, enzymes) or NH3 absorbing additives (McCrory and Hobbs, 2001; Li et al. 2008). Another treatment is manure acidification through the use of sodium bisulfate or aluminum sulfate (Herber et al. 1999). At a pH of approximate 4.5 or lower, virtually all nitrogen present exists as nonvolatile ammonium (NH_4^+). Li et al. (2009) reported that topical application of certain low pH chemicals (e.g., aluminum sulfate) onto poultry manure may reduce NH_3 emission by up to 90%.

Another strategy to reduce emissions is biofiltration, where a filter bed is established at the exhaust with a diverse population of aerobic microorganisms, which subsequently oxidize the reduced compounds generated by indoor confinement to carbon dioxide, water, salts, and biomass (Leson and Winer 1991). Gas absorption is another potential strategy to reduce emissions at the exhaust. Air is collected and passed through an enclosed tower with the absorption media (typically water) flowing counter-current to the air stream. Gases are diffused into and absorbed by the media. Yet, another strategy to treat exhaust air is bio-scrubbing, which has a similar concept to that of biofiltration with the exception that the microorganisms are housed in an enclosed packed tower instead of in a filter bed (Lais, et al., 1997). One additional potential mitigation technology under investigation is wet or acid scrubber. The principle of the wet/acid scrubber is to subject contaminated (exhaust) air to an acidic solution that will dissolve NH_3 in the acidic solution. Melse and Ogink (2005) concluded that gaseous removal efficiency of acidic scrubbers could reach 91 to 99%.

There have been several recognized studies that have quantified NH_3 emission reductions from diet manipulation, as reported by Jacob et al. (2000) and Ndegwa et al. (2008). For instance, nutritionally balanced laying-hen diets with 1% lower crude protein would lead to approximately 10% reduction in ammonia emission (Liang et al., 2005). Inclusion of acidifiers or fiber in laying-hen diets has been shown to reduce manure pH and ammonia emissions from laying-hen manure (Wu-Haan et al., 2007; Roberts et al., 2007; Xin et al, 2010 – Personal Communication, Iowa State University).

A ‘long-term’ strategy to reduce gaseous emissions from AFOs is the use of alternative housing designs that lead to a shorter residence time of manure inside the building. The two primary housing styles for laying hens in the US are high-rise (HR) and manure-belt (MB).

The HR housing system is described by Fabbri et al. (2007) as being a cage system with aerated open manure storage (fig. 1). Manure is stored on the ground floor and the hens are housed on the first floor. In the HR house, the droppings are collected on baffles under the cages and scraped and removed to the under floor storage every day, where they remained in heaps for the complete laying cycle (typically one year). The HR system for layers described shown in figure 1 is more representative of the models used in Europe, the designs being current used in the US have the batteries of cages disposed in a pyramid or an “A” shape.

The MB housing system was also described by Fabbri et al. (2007) as being a one pavement construction only (fig. 2), with the manure collected on the manure belts. Manure is usually ventilated via air ducts to enhance faster drying. The manure is discharged every 1-7 d to a sheltered external storage.

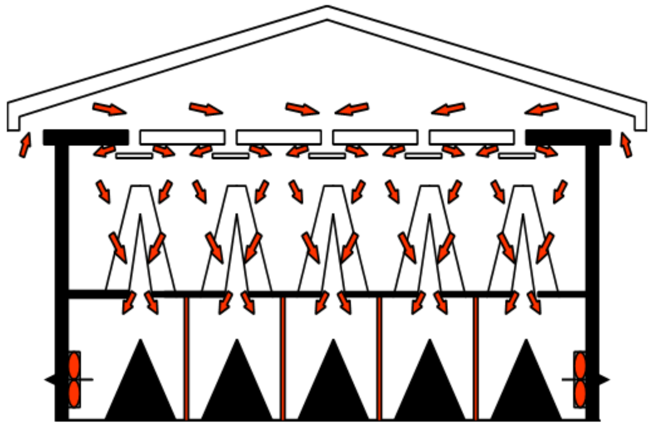

Figure 1. Cross section (a) of a high rise (HR) layer house. The ground floor is for manure storage and the hens are housed on the first floor. Source: Xin (2010, Personal Communication).

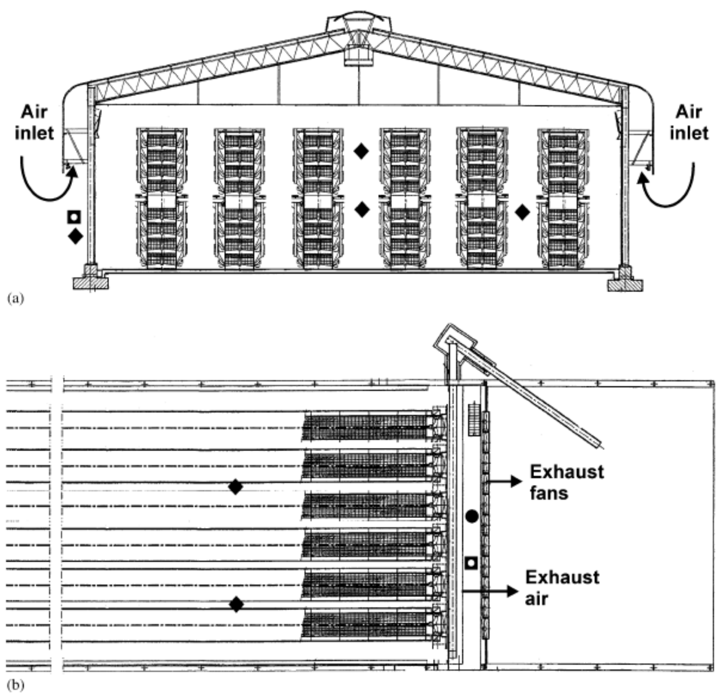

Figure 2. Cross-section (a) and top view (b) of a house with vertical tiered cages with manure belts (MB) and forced air drying (ventilated belt). Source: Fabbri et al. (2007).

Fabri et al. (2007) stated that the NH_3 reduction of a ventilated MB system (1-d manure accumulation time) vs. HR system was 61% (combining the emissions of both the barn and the manure storage area). Emissions also seem to increase dramatically with the increase in manure accumulation time (MAT) for MB houses. Liang et al. (2005) reported an increase in NH_3 rates of 71% for daily removal vs. semi-weekly removal from non-ventilated MB systems. Laboratory experiments designed to mimic a MB house system performed by Ning (2008) also showed that NH_3 emissions from laying hens (Hy-Line W-36) depend on duration of manure accumulation.

However, the documented or ongoing studies on NH_3 emissions from MB or other housing types do not include pullets (i.e., hens younger than 18 weeks of age, pre-laying), even though pullets are a major integral part of the egg operation. Moreover, pullets or hens may be housed at different stocking densities (SD) as producers respond to certain industry production management guidelines such as those by the United Egg Producers and/or fast food-chain restaurants (e.g., McDonald's). Hence there is also a need to quantify the impact of bird stocking density on aerial emission rate.

Thus, a study was carried out in a laboratory setting that mimics a MB system with the following objectives:

1. To quantify NH_3 emission rate (ER) of W36 pullets and laying hens vs. bird age and as affected by manure accumulation time (MAT) and stocking density (SD).

2. To delineate feeding and defecation dynamic behaviors and NH_3 emissions of pullets and laying hens housed under different SDs and different MAT.

Thesis organization

This thesis has been prepared in journal manuscript format, with two manuscripts, corresponding to the respective study objectives. The thesis includes four chapters – a General Introduction, one manuscript entitled "Ammonia Emissions of Pullets and Laying Hens as Affected By Stocking Density and Manure Accumulation Time", to be submitted to the *Transactions of the ASABE*; the other manuscript entitled "Dynamics of Feeding, Defecation and NH_3 Emissions of Pullets and Laying Hens under Different Stocking Densities and Manure Accumulation Time" to be submitted to the *Journal of Environmental Quality*. Figures are embedded in the text but tables relevant to each paper are included in the end of each paper. System calibrations, data acquisition program and statistical analysis programs essential to the experiment are included as appendices.

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CHAPTER 2. AMMONIA EMISSIONS OF PULLETS AND LAYING HENS AS AFFECTED BY STOCKING DENSITY AND MANURE ACCUMULATION TIME

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Abstract

Data on ammonia (NH_3) emissions from pullets (hens <18 weeks of age) are non-existent, despite the large differences in nutritional and environmental conditions between raising pullets and laying hens. Different stocking densities (SD) in housing the birds may be used in response to certain industry guidelines on production; however, information concerning the impact of SD on accumulated manure properties (e.g., moisture content) and thus NH_3 emissions is limited in the literature. It is hypothesized that bird SD affects the amount of manure per unit of storage or surface area as manure accumulates, and the exposed manure surface area would affect ammonia emission from the accumulated manure. A lab-scale study was carried out that resembled the conditions of manure-belt laying-hen houses with the objectives of (a) determining the magnitude of NH_3 emission rate (ER) of pullets as a function of age, and (b) assessing the effect of SD on NH_3 ER of pullets and laying hens during a 6-d manure accumulation time (MAT). Two different SD's at a given bird age were evaluated, being that the higher density (HD) had 33% lower per-hen floor area allocation than the lower density (LD). Stocking densities ranged from 155 to 619 cm^2 (24 to 96 in^2) per bird. Tests were conducted for W-36 pullets at 4 to 37 weeks of age. NH_3 ER was expressed in the units of amount of NH_3 emission per bird, per kg of feed nitrogen (N) disappearance, and per kg of ‘as-is’ and dry manure weight and g/AU (animal unit, 500 kg BW). Results showed that daily NH_3 ER for pullets and laying hens increased exponentially with bird age and MAT ($P<0.0001$). Stocking density effect on NH_3 ER was more pronounced for MAT ≥ 3d, where the treatment HD led to higher ER. Specifically, for the laying hens, NH_3 emissions from the 3^{rd} to 6^{th} d MAT ranged from 41 to 251 mg/hen-d for HD and from 29 to 160 mg/hen-d for LD. This outcome supports the current egg industry practice of removing manure at 1- to 3-d MAT for the manure-belt house systems. The results of this study will help set a foundation for further field-scale emissions measurement, and ultimately development of best management practices to effectively reduce aerial emissions from egg production operations.

Key Words: bird welfare, emission rates, manure accumulation, pullets’ age

Introduction

Animal feeding operations (AFOs) are associated with aerial emissions, primarily ammonia (NH_3), nitrous oxide (N_2O), nitric oxide (NO), methane (CH_4), hydrogen sulfide (H_2S), volatile organic compounds (VOCs), and particulate matters (National Academy of Science, 2003). In agriculture, several sources of air emissions have been studied as well as the substances being emitted from them. Aerial emission rate (ER) is the product of source concentration of the substance and the air exchange rate through the source.

Among all constituents emitted from poultry production facilities, ammonia (NH_3) is the predominant noxious gas due to the nature of the manure (Liang et al., 2005). Livestock is often fed high protein diet, which contains surplus nitrogen, to ensure that the animals' nutritional requirements are met. Nitrogen that is not metabolized into animal protein or product is excreted as uric acid where further microbial action releases NH_3 into the air during manure decomposition (Gay et al., 2009). The NH_3 volatilization rate from solid poultry manure is affected by nitrogen content, moisture content, stacking configuration of the manure pile, pH, temperature and oxygen availability, all of which contribute to the microbial activities and NH_3 release from the manure pile (Li, 2006). Research has shown that prolonged exposure to high levels of NH_3 can cause reduced body weight gain and egg production in laying hens, and also can have a negative impact on farm workers (Carlile, 1984; Ning, 2008).

The most recent studies on NH_3 emissions from commercial U.S. poultry operations include those reported by Liang et al. (2005) for laying hens, Wheeler et al. (2006) and Burns et al. (2007) for broilers, and Li et al. (2008) for turkeys. Currently a national study through an air compliance agreement (ACA) between the U.S. EPA and certain sectors of the livestock and poultry industry is ongoing that aims to collect more baseline data on AFO air emissions. Laboratory experiments performed by Ning (2008) showed that NH_3 ER from laying hens (Hy-Line W-36) depends on duration of manure accumulation. Ning (2008) also found that the emissions had an inverse relation to defecation events.

However, the documented or ongoing studies do not include pullets (i.e., hens younger than 18 weeks of age, pre-laying), even though pullets are a major integral part of the egg operation. Moreover, pullets or hens may be housed at different stocking densities as producers respond to certain industry production management guidelines, such as those by the United Egg Producers and/or fast food-chain restaurants (e.g., McDonald's). Hence there is a need to quantify the impact of bird stocking density on aerial emission rate.

The objectives of this study were a) to quantify NH_3 ER of pullets and laying hens vs. bird age and MAT, and b) to assess SD effects on NH_3 ER of pullets and layers. Results from this research will help filling a literature gap on pullet NH_3 emission, and provide insight on the impact of production management (SD) practices on aerial emissions.

Methodology

Dynamic gas emission chambers system

The study was conducted using four dynamic gas emission chambers (fig. 1) at the Iowa State University Livestock Environment and Animal Physiology Laboratory II (LEAP Lab II). The chambers each had a dimension of 86 cm L x 45 cm W x 66 cm H and were located inside an environmentally controlled room. The chamber walls were constructed with transparent plexiglass panels (5 mm thick). Inside each chamber was an iron-framed wire-mesh cage (44 cm L x 34 cm W x 58 cm H). Fresh air to each chamber was supplied through an air distribution plenum to improve spatial uniformity, and the air supply was powered with a diaphragm air pump (100 L min^{-1} capacity, DDL 120-101, GAST Manufacturing INC, Benton Harbor, MI, USA) placed on the inlet side of the chamber, thereby creating a positive-pressure ventilation system. Airflow rate through each chamber was measured with a thermoelectric air mass flow meter (capacity of 110

L/min, GFM57, Aalborg Instruments & Controls Inc., Orangeburg, NY, USA) placed in the supply air stream. Prior to onset of the experiment, calibration equations were developed to correlate output readings of the air mass flow meters with the actual flow rates (equations are presented in Appendix 1). Air flow through each chamber was adjustable via a by-pass, so that the concentration of target gases (NH_3, CO_2) inside the chamber could be controlled. One air temperature and relative humidity (RH) sensor (HMP45A/D, Vaisala, Woburn, MA, USA) was placed in each cage to measure the dry-bulb temperature. A plastic cup with tubing was placed underneath each nipple drinker to catch and divert any water leakage out of the manure pan or chamber.

To capture feeding and defecation events of the birds, two electronic balances (2200.0 ± 0.1 g, model GX2000, A&D Company Limited, Tokyo, Japan) with a 0-2.2 VDC analog output (sampling rate of 0.1 s, the data acquisition system averages data at every 10 s) were used in each chamber. One balance was used for measurement of the feeder weight or feeding activities and the other for measurement of the manure pan weight or defecation activities. Feed disappearance and 'as-is' manure production were calculated as being the difference between the weight on the scale in the beginning and the end of the day.

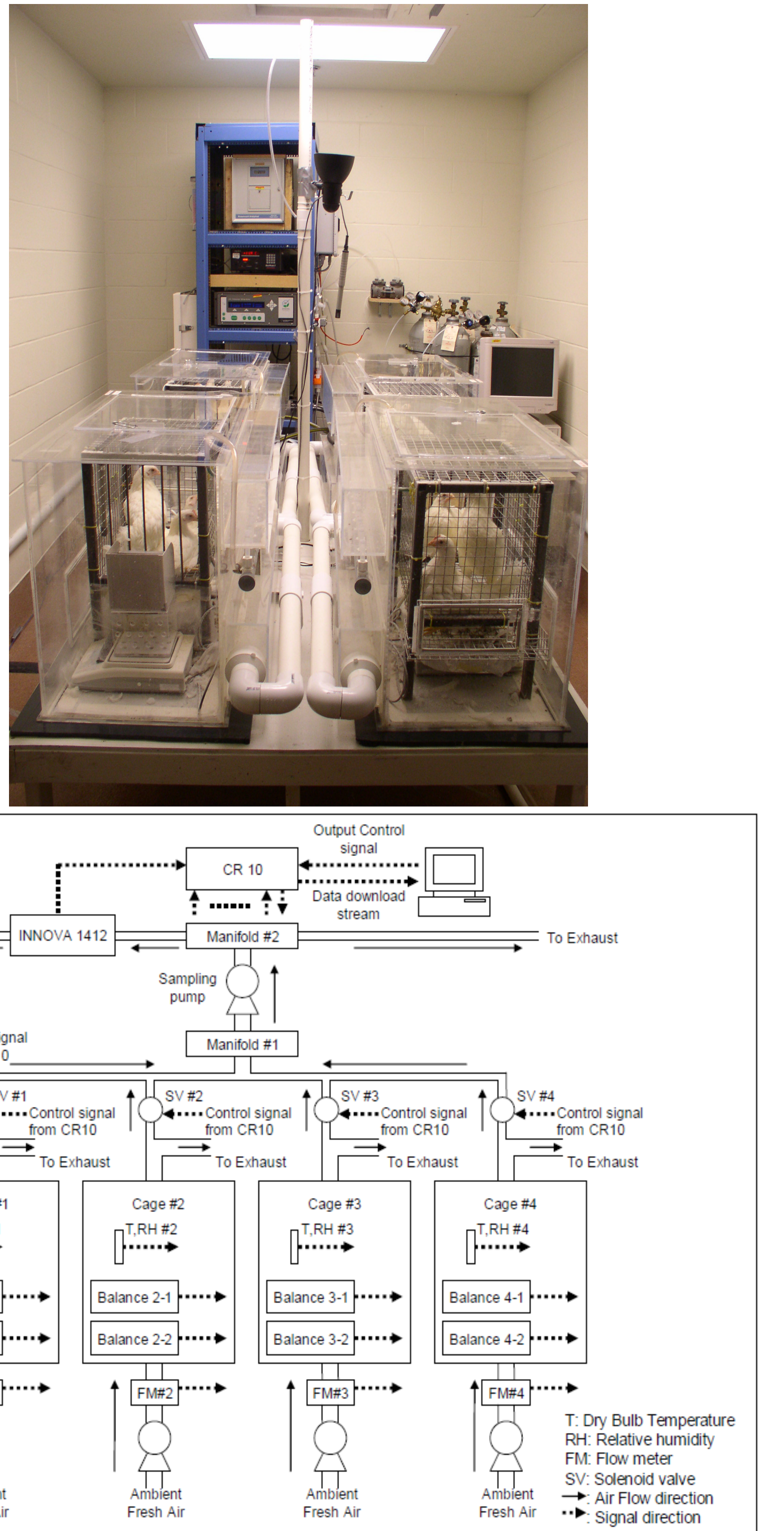

Figure 1. ISU multi-chamber system for feeding, defecation and air emissions measurement (top). Schematic representation of the system by Ning 2008 (bottom).

Samples of the exhaust air from each chamber were successively taken by a sampling pump (capacity of 20 L/min, Teflon wetted parts, Model No. 2107CA20B, Gardner Denver INC., Sheboygan, WI, USA) at 5 min intervals, with the first 3 min for stabilization and last 2 min for measurement. This sampling sequence yielded a measurement cycle of 25 min for the entire system (including 5 min for the ambient air). The successive sampling was accomplished through controlled operation of five solenoid valves (PKV-2R-D1/4NF, Takasago Electric Inc., Midori-ku, Nagoya, Japan). A Teflon filter (4.7 cm diameter, 5 μm pore diameter) connected to a Teflon tubing (0.32 cm ID x 0.64 cm OD) was placed in front of each solenoid valve. A photoacustic multi-gas analyzer (model 1412, INNOVA AirTech Instruments A/S, Ballerup, Denmark) was used to measure NH_3 and CO_2 concentrations. The multi-gas analyzer was challenged weekly and calibrated, as needed, with zero, 25 ppm NH_3 (balanced with air) span calibration and 2500 ppm CO_2 (N_2 balance) calibration gases (information on calibration of INNOVA is in Appendix 3). Dew-point temperature was measured with a dew-point hygrometer (model 2001, EG&G Moisture and humidity Systems, Burlinton, MA).

Analog outputs from the temperature, INNOVA gas analyzer, dew-point hygrometer, electronic balances, and the mass flow meters were logged at 10 s intervals into a measurement and control module (CR10, Campbell Scientific, Inc., Logan, UT). A sample of the CR10 program used in the study is in Appendix 1.All measurements were recorded as the average of output over the 10 s intervals.

To assess and ensure the integrity of the dynamic emission measurement system, CO_2 recovery tests with 100% ethanol (C_2H_5OH) lamps were conducted prior to the beginning of the experiment and repeated every other week, as performed by Ning (2008).

In the test, an alcohol lamp containing 100% ethanol was placed on the electronic balance in each chamber, so that the dynamic as well as cumulative alcohol consumption could be obtained from the weight changes. Detailed algorithm for the recovery test was described by Scott and Hillman (1983) and can be found in Appendix 4.

The system was set and handled in a way that would mimic a manure-belt house system with a 6-d manure accumulation time period, so that the results of the study can be extrapolated to that housing type.

Hen handling and experimental design

The experimental Hy-Line W-36 pullets/hens were procured from a commercial farm in Iowa. Two batches of 32 randomly assigned pullets (two weeks apart in age) at initial age of 2 weeks were acquired in the beginning of the experiment. Twenty-eight of the 32 pullets in each batch were randomly allotted to the four cages inside each respective chamber, two cages or chambers with 8 birds in each and the other two cages or chambers with 6 birds in each, thereby yielding two stocking densities. The four remaining birds were housed separately in a holding cage for replacement of the experimental birds in case of mortality. After 12-d measurement, the pullets inside the emission chambers were returned to the holding cages at similar SD to that used during the measurement. The following 2 days were used to check, calibrate, and perform maintenance on the instruments and prepare the system for the next trial. Then, 28 pullets from the second batch (now having reached the same measurement age as those in the first batch) were allotted to the emission chambers to repeat the measurement, as done with the first batch. After two more weeks, birds from the first batch were measured again. This procedure was repeated until birds from the second batch reached 18 weeks of age. For the section of the experiment regarding the laying hens, the two previous batches were used when the hens aged 23 wk, but in order to improve the statistical power of the results, another batch of 10 hens from the same precedence of the previously mentioned was acquired, with initial age of 34 wk. All the birds were kept at comfortable environmental conditions, as suggested by the Hy-Line Commercial Management Guide (i.e., 21.1-23.3°C, 40-50% relative humidity). Birds were weighed once a week. Detailed information about the pullets and the dietary N contents is shown in Table 1.

During the test period, fresh feed was added daily to the feeder, usually between 10:00 AM and 12:00 PM. Fluorescent lighting was provided at an illumination intensity of 10 lux with a lighting program specified for Hy-Line pullets and laying hens (Hy-Line, 2007). Nipple drinkers were used to supply drinking water. Manure pans were replaced after 6-d manure accumulation.

The treatment for this experiment was SD at various ages of the bird. Two SD's were tested: high SD (HD) and low SD (LD). For birds at 4 to 6 weeks of age, HD and LD were, respectively, 155 and 206 cm^2 $bird^{-1}$ (24 and 32 in^2 $bird^{-1}$), i.e., HD bird having 33% more floor space; for birds at 6 to 18 weeks of age, HD = 310 cm^2 $bird^{-1}$ (48 in^2 $bird^{-1}$) and LD = 413 cm^2 $bird^{-1}$ (64 in^2 $bird^{-1}$ - 33% more space). For birds at 23 weeks and older HD = 413 cm^2 hen^{-1} (64 in^2 hen^{-1}) and LD = 620 cm^2 hen^{-1} (90 in^2 hen^{-1}). To achieve the respective SD levels, the number of birds per chamber or cage was 8 for the HD and 6 for the LD for pullets at 4-6 wk of age; but 4 birds for the HD and 3 birds for LD for pullets from 6 to 18 wk of age. For layers at 23 to 37 wk of age, treatments consisted of 3 and 2 hens per cage for HD and LD, respectively.

To complete the randomization process and avoid chamber effect on measurements, groups under the same treatment switched chambers on a weekly basis, so that by the end of the trial, all SD would have been run in all four chambers and all ages.

Because the hens used in this research study ranged from 23 to 37 weeks of age, data sets for that respective section were first analyzed for age effect. However, no age effect was detected on feed disappearance, manure production rates or NH_3 ER. Consequently, the age factor was disregarded among the layers and the data were pooled over the age span.

Calculation of NH_3 ER and evaluation of SD effect on the NH_3 emissions

Daily ammonia emission rate (NH_3 ER) was calculated for 1 to 6 d of MAT with the following equation.

$$NH_3\ ER = \frac{Q_{STPD} x (C_{NH_3,e} - C_{NH_3,i}) x\, W_m\ x\ 10^3}{10^6\ x\, V_m\ x\ N} \qquad [1]$$

Where:

NH_3 ER – ammonia emission rate, mg·h^{-1}·bird^{-1}

Q_{STPD} – air flow rate, corrected for standard temperature (21^{o} C), pressure (1 ATM) and dry basis, L·h^{-1}·chamber^{-1}

$C_{NH3,e}$, $C_{NH3,i}$ –exhaust and inlet ammonia concentrations, respectively, ppm

W_m – molecular weight of ammonia (17.031 g·mol^{-1})

V_m – molar volume of ammonia, corrected for standard temperature (21^{o} C), pressure (1 ATM), 24.14 L·mol^{-1}

N – number of hens per cage or chamber.

NH_3 ER was calculated in several units, including g/kg N disappearance, g/kg 'as is' and 'dry basis' manure, and g/AU (animal unit, 500 kg BW). The feed N disappearance was calculated based on the feed disappearance and crude-protein (CP, Table 1) content of the diet. Crude protein was divided by 6.25 to yield the feed N content. Effects of SD were tested on a daily basis using *proc glm* in SAS. In addition, the percent of the difference in the mean ER values between the SDs was calculated using equations 2 and 3. Standard error values for the percent of the difference were calculated using the Delta Method, as described by Casella & Berger (2002).

$$\hat{\lambda} = \left(\frac{\hat{\mu}_{LD} - \hat{\mu}_{HD}}{\hat{\mu}_{HD}} \right) x100 \quad [2]$$

Where:

$\hat{\lambda}$ - estimated mean value of the percent of the difference (%)

$\hat{\mu}_{LD}$ - estimated mean value for the variable μ under the LD treatment

$\hat{\mu}_{HD}$ - estimated mean value for the variable μ under the HD treatment

$$SE^2\left(\hat{\lambda}\right) = \begin{bmatrix} \frac{\partial \lambda}{\partial \mu_{HD}} & \frac{\partial \lambda}{\partial \mu_{LD}} \end{bmatrix}_{(\mu_{HD},\mu_{LD})=(\hat{\mu}_{HD},\hat{\mu}_{LD})} \begin{bmatrix} \hat{\text{v}}\text{ar}(\hat{\mu}_{HD}) & \hat{\text{c}}\text{ov}(\hat{\mu}_{HD}, \hat{\mu}_{LD}) \\ \hat{\text{c}}\text{ov}(\hat{\mu}_{HD}, \hat{\mu}_{LD}) & \hat{\text{v}}\text{ar}(\hat{\mu}_{LD}) \end{bmatrix} \begin{bmatrix} \frac{\partial \lambda}{\partial \mu_{HD}} \\ \frac{\partial \lambda}{\partial \mu_{LD}} \end{bmatrix}_{(\mu_{HD},\mu_{LD})=(\hat{\mu}_{HD},\hat{\mu}_{LD})} \quad [3]$$

Where:

$SE\left(\hat{\lambda}\right)$- Standard error of the estimated percent of the difference;

$\hat{\text{v}}\text{ar}(\hat{\mu}_{HD})$- Estimated variance of the estimated mean under the HD treatment, obtained from SAS output;

$\hat{\text{v}}\text{ar}\left(\hat{\mu}_{LD}\right)$- Estimated variance of the estimated mean under the LD treatment, obtained from SAS output;

$\hat{\text{c}}\text{ov}\left(\hat{\mu}_{HD}, \hat{\mu}_{LD}\right)$ - Estimated covariance for the estimated means under the effects of both treatments (equal to zero, since the number of replicates per SD treatment is always the same).

Statistical Analyses

Statistical analysis was performed using the program SAS (version 6.2). Data modeling was performed in two different levels. The first, considered the overall effects of all possible factors and interactions (SD, MAT and age) on the analyzed variables, called the 'full' model; the second model, named 'reduced' model, only considered the effect of stocking density. As for the full model, statistical analysis was performed using *proc mixed* and *proc glimix* in SAS.

Considering that we wanted to take a closer look at SD effect on the variables, 'reduced' model compared only the means for different stocking densities regardless age and day MAT, the analysis of the plot of residuals indicated that for a specific age and MAT the variance is approximately constant, what allowed the datasets to be analyzed in the original scale. This was done using *proc glm* in SAS. A difference with p-value equal to or less than 0.05 was considered significant. The codes for the SAS programs used in the analysis are presented in Appendix 5.

Results and Discussion

Full Model Analysis: Effects of Age, MAT and SD on NH_3 Emissions

Prior to running the ANOVA test to look for overall effects of age, MAT and SD on the analyzed variables, the residual plots of the data sets were generated in SAS using *proc univariate*; e.g., Figure 2 (top) shows that for daily NH_3 ER, the distribution of the residuals (the difference between each observation and the overall mean) follows what looks like a *funnel shaped* pattern, indicating that there is an increase in the spread from the left to the right. According to Ramsey & Schafer (2002) the unequal spread revealed by the plot of residuals is an indication that the ANOVA test results might be unreliable because its assumption of 'equal distribution' is being violated. In such cases, Ramsey & Schafer (2002) suggested that a log transformation of the data set might be appropriate. Figure 2 (bottom) shows the residual plot for the log transformed daily NH_3 ER; the approximately equal spread indicates that the 'equal distribution' assumption of the ANOVA test is no longer being violated. All data sets were checked through residual plots before being tested with ANOVA, log-transformation was applied when needed.

P-values from Table 2 indicated that age had a significant impact in feed disappearance, manure weight in 'as-is' and dry basis and NH_3 emissions in all units ($P < 0.0023$). This outcome was expected since the birds will tend to eat more as they grow old, impacting manure production, which is the primary source of NH_3 emissions.

Data in Table 2 also evidenced that 'as-is' and dry manure weights were both significantly affected by MAT; consequently, NH_3 emissions in all units were significantly affected by MAT ($P < 0.0001$).

A regression analysis, using *proc reg* in SAS, was performed on the daily NH_3 ER data, as a function of age (wk) and MAT (d), the data sets were analyzed by SD treatments. Results from the statistical analysis indicated that NH_3 ER could be explained as a function of AGE and MAT following an exponential fashion ($P<0.0001$). From equations 4 and 5 one can see that daily NH_3 ER was positively correlated to bird age and MAT.

For the treatment HD:

$$NH_3ER = e^{\left[(-36\pm14) + (5.2\pm0.6)AGE + (2.8\pm0.8)MAT\right]} \qquad (R^2 = 0.51) \quad [4]$$

For the treatment LD:

$$NH_3ER = e^{\left[(-30\pm11) + (3.7\pm0.5)AGE + (4.2\pm1.2)MAT\right]} \qquad (R^2 = 0.56) \quad [5]$$

Where:

NH_3 ER – NH_3 emission rate (mg bird^{-1} d^{-1})

AGE – bird age (wk)

MAT – manure accumulation time (d)

There is evidence that ‘as-is’ manure weight was impacted by SD ($P<0.0001$), but no effect of SD was detected on dry manure weight, the effect on ‘as-is’ manure weight presumably arose from different manure moisture contents between the treatments HD and LD. According to Table 2, SD also had a significant impact on daily NH_3 ER ($P<0.0001$), and in NH_3 emissions in all other units. Detailed analysis of SD effect on NH_3 emissions is discussed later in this paper.

Data in table 2 also reveals that there was no evidence of an interaction between age and SD ($P \geq 0.44$) on any of the analyzed variables, neither there was evidence of an interaction among age, MAT and SD ($P \geq 0.98$).

An interaction between age and MAT on feed disappearance was non-existent ($P=0.59$), indicating that birds will ingest more feed as they get older but the amount of feed doesn't seem to change considerably over the course of a 6-d period. This result presumably arose from the fact that daily feed disappearance rate data was averaged over the total number of cycles that were run for each age tested. However, an interaction between age and MAT clearly existed on 'as-is' and dry manure production; consequently, the effect of the interaction affected NH_3 emissions in all analyzed units ($P \leq 0.05$).

Reduced Model Analysis: Effects of SD on NH_3 Emissions

The downside of analyzing the data sets through the full model is that if the analysis was performed in the log-transformed scale, converting SEs back to the original scale is not simple. Thus, a more simplified model was developed: the 'reduced' model, in which age and MAT were set constant, and the data was tested only for SD effects. The 'unequal distribution' problem is eliminated here because the analysis is performed on sub-sections of the entire dataset, which are small enough to have equal distribution.

The results of data analysis using the reduced model are shown in Tables 3 to 7. There was no significant difference ($P = 0.61 - 0.92$) in either feed or feed N disappearance between the two SD regimens for all bird ages or different MATs. This outcome indicates that the reduced floor space allocation did not seem to adversely affect feed disappearance. Figure 3 (top) illustrates how feed disappearance increased according to the bird age, but remained fairly constant within the 6-d cycles.

‘As-is’ manure weight data presented in Tables 3 to 7 show some significant effect of SD (P = 0.03), indicating that HD led to higher manure weight. Data also indicates that even with lack of significance, mean values for manure weight under the treatment HD were consistently higher than those under the treatment LD. The difference presumably arose from greater moisture evaporation for the LD manure because of larger exposed surface area per unit weight of manure. The larger number of birds under HD was also associated with a higher indoor RH, e.g., averaging 44% as compared to 37% for LD for the 8-wk trials. The lower RH and greater vapor pressure gradient between the manure surface and the ambient air for LD would be more conducive to moisture loss of the manure. Manure samples were collected at the end of the 6-d MAT and were analyzed for MC to confirm these speculations. Moisture content (MC) for the last day of MAT was lower for the LD manure (P = 0.009 – 0.04). The overall MC averaged 74 % for HD manure and 70% for LD manure (Figure 4). Mean ‘as-is’ manure weight is illustrated in Figure 3 (bottom) as a function of age and MAT.

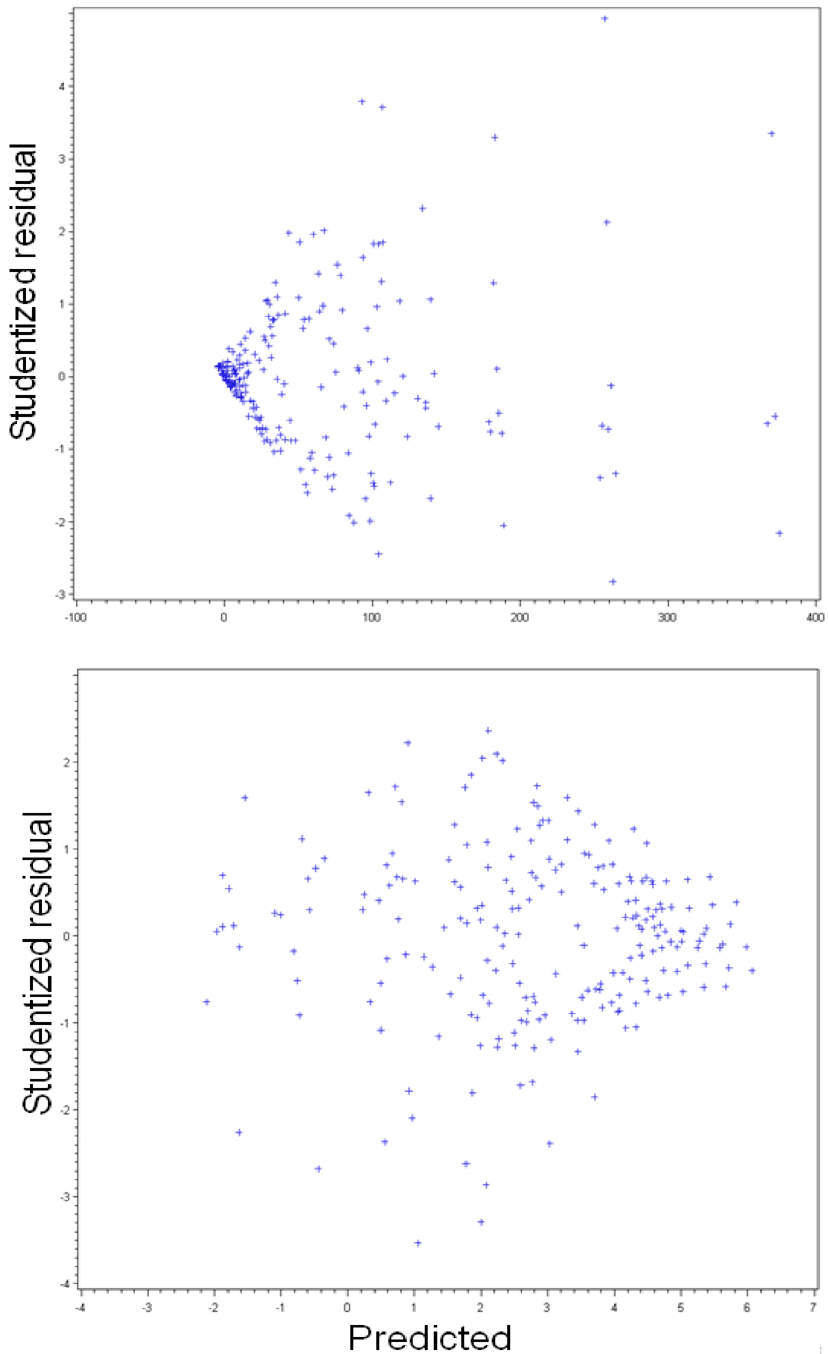

Figure 2. Residual plots obtained from SAS output for daily NH_3 ER (mg/hen-d) in the original scale (top) and in the log-transformed scale (bottom).

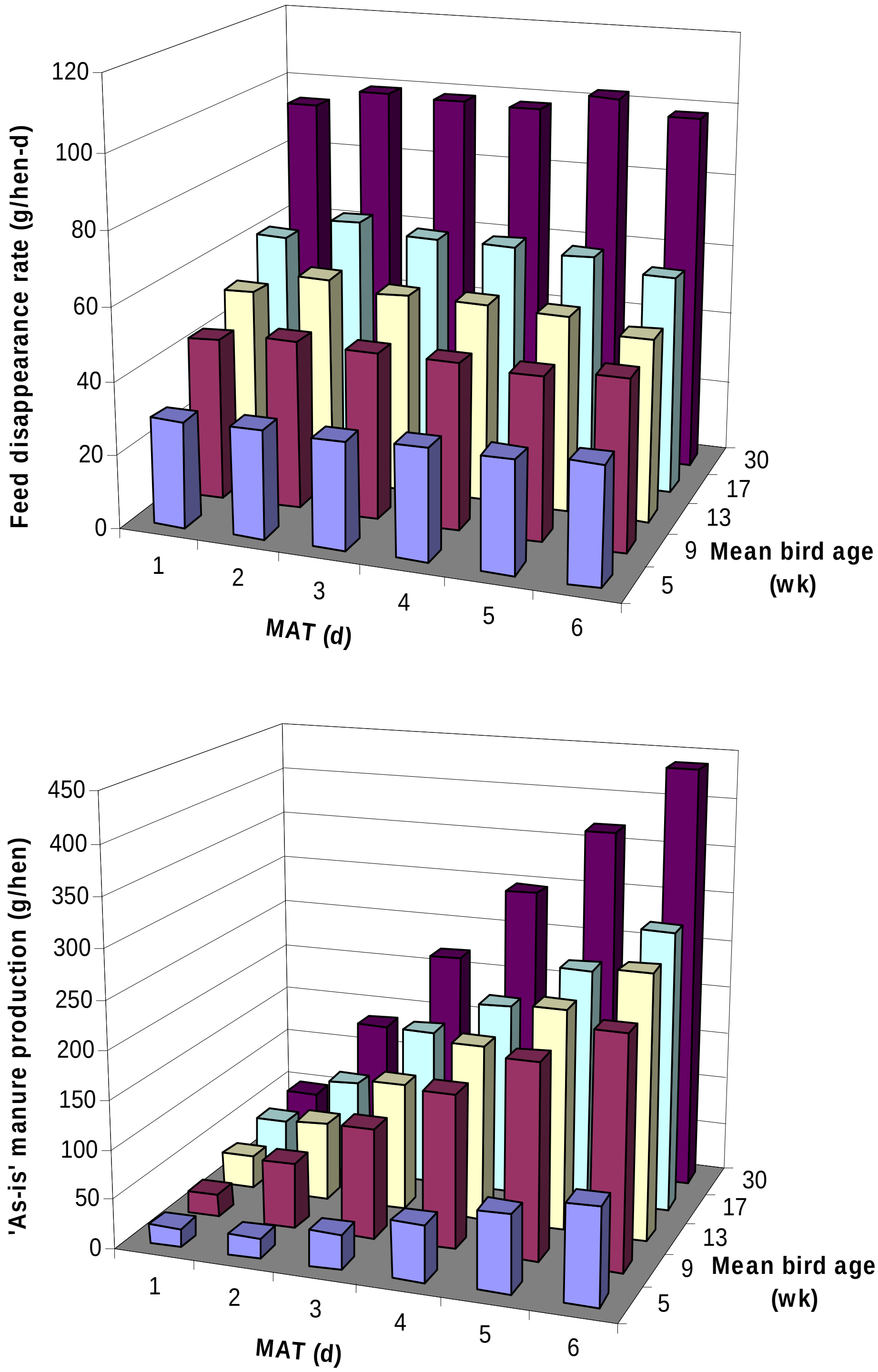

Figure 3. Daily feed disappearance (top) and 'as-is' manure production (bottom) of W-36 pullets/hens as a function of bird age (5 to 30 wks) and manure accumulation time (MAT, 1 to 6 d).

Daily NH_3 ER data is presented in Tables 3 to 7. For data analysis purposes, a distinction was made between 'clean' vs. 'non-clean' system and NH_3 ER in several units were derived from the daily NH_3 ER obtained from 'clean' system. It can be seen from the tables that data for 'clean' and 'non-clean' systems look different from each other for the first 2-d MAT; however after the 3rd day the 95% confidence intervals for both conditions overlap each other. Mean values for daily NH_3 ER for 'clean' and 'non-clean' system are illustrated in Figure 5 as a function of age and MAT.

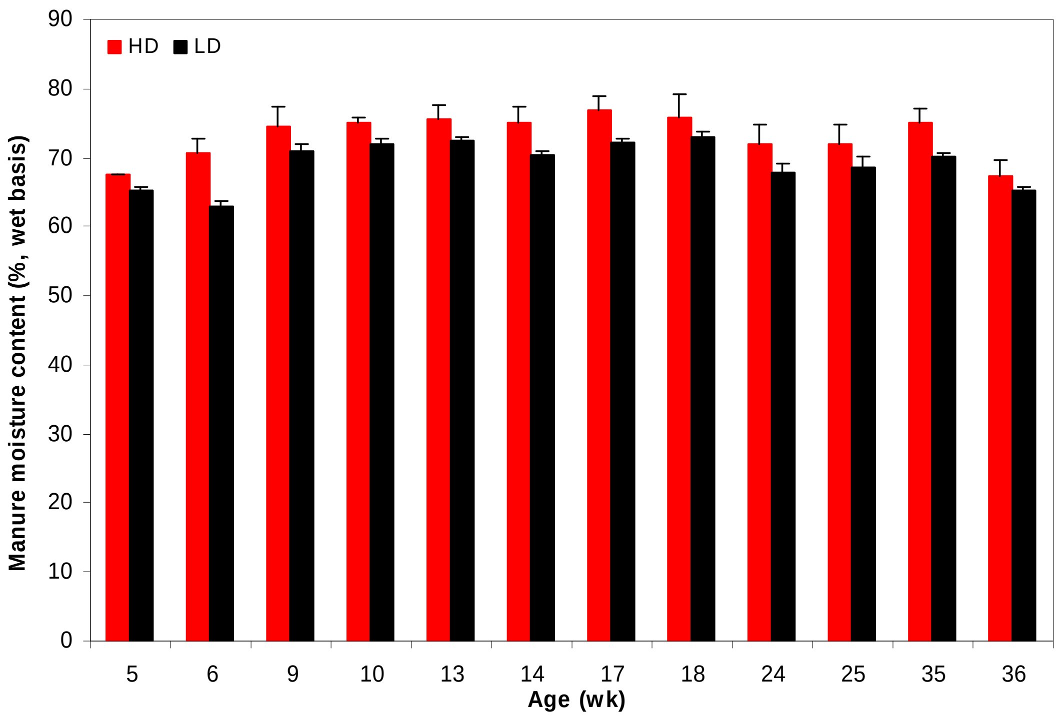

Figure 4. Manure moisture content on the 6-d manure accumulation time (MAT).

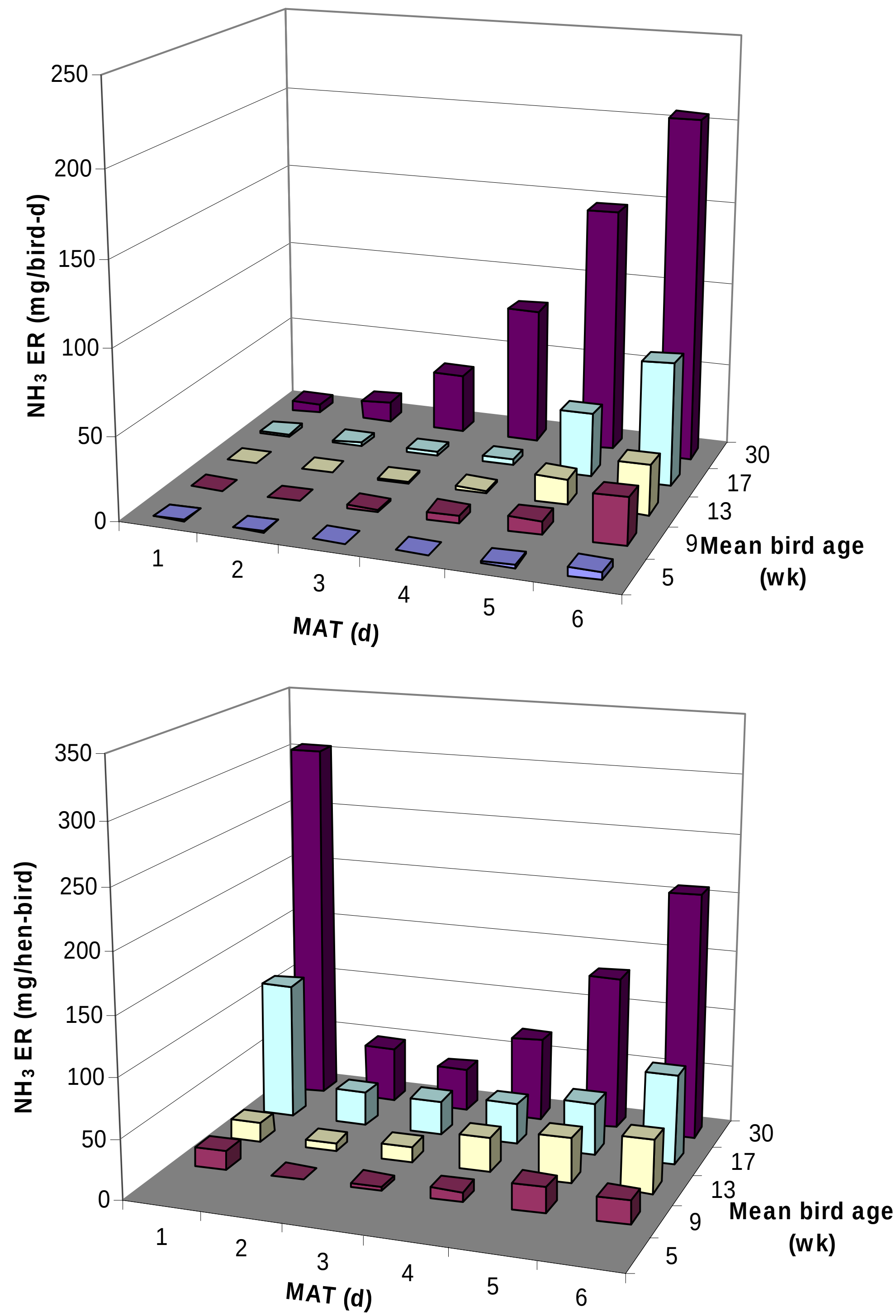

Figure 5. Daily NH_3 ER of W-36 pullets/hens as a function of bird age (5 to 30 wks) and manure accumulation time (MAT, 1 to 6 d) for the 'clean' (top) and the 'non-clean' (bottom) systems.

When looking at SD effect on NH_3 ER shown in tables 3 to 7, one can observe that percent of the difference values are consistently negative and relatively constant for MAT≥3 d, evidencing that the treatment HD led to higher NH_3 ERs, and with an overall difference that ranged from -41 to -27% and from -61 to -24% for 'clean' and 'non-clean' systems respectively.

This outcome indicates that the SD will likely impact the emission after 3-d MAT. In particular, significant effect of SD was detected at the 3rd d of MAT for pullets at 12 wk of age, for the 'clean' system, 1.0±0.1 mg/hen-d for HD vs. 0.6±0.1 mg/hen-d for LD; and for the laying hens at days 3 and 4 of MAT for the 'non-clean' system, with estimated means being 45±3 mg/hen-d for HD vs. 25±3 mg/hen-d for LD at 3-d MAT and 83±8 mg/hen-d for HD vs. 56±8 mg/hen-d for LD at 4-d MAT. The increase in the uncertainty of the emission as the estimated mean became larger, was most seemingly what caused the lack of significance of the treatment SD on the data, thus a greater number of replicates would presumably allow one to see more significant effects of SD on daily NH_3 ER for both 'clean' and 'non-clean' systems after the 3^{rd} d of MAT.

This outcome on daily NH_3 ER supports current management practices used in manure-belt housing systems, where manure is usually removed every 1 to 3 d MAT to avoid overload of the belts (Xin, 2010 – Personal communication). Results indicate that regardless of the stocking density, daily NH_3 ER will increase considerably for MAT>3 d.

Liang et al. (2005) measured NH_3 ER from manure-belt laying hen houses with MAT = 1-d (Iowa) or 3- to 4-d (Pennsylvania) and reported that the overall NH_3 ER was 54±5 mg/hen-d for MAT = 1 d and 94±2 g/hen-d for MAT = 4 d. These ER values parallel those in the current study, for the data presented in Table 7 (layers) and MAT=2 d of the non-clean HD system (57±6 mg/hen-d) and 3-4 d of the clean or non-clean HD system (83-94 mg/hen-d). The 2-d MAT of the non-clean system in the current study likely better resembles the commercial production situation in that some residual manure exists in the barn from the daily removal of the manure.

NH_3 ER in other units followed similar trends to that of ER in g/hen-d (Tables 3 to 7). The overall percent of difference in grams of NH_3 emissions per N disappearance varied from -41% to -23% (values averaged from 3- to 6-d MAT).

Stocking density effects on NH_3 emissions in g/kg manure were similar for manure expressed in 'as-is' and dry basis for most days of MAT. NH_3 emissions under the treatment LD was 26% to 44 % lower than that for the treatment HD in g/kg 'as-is' manure, and 12 % to 52% in g/kg dry manure.

Conclusions

Effects of bird age, manure accumulation time (MAT) and stocking density (SD) on ammonia emission were examined. The following conclusions were drawn:

1. Ammonia (NH_3) emission of pullets and laying hens increases with bird age and manure accumulation time, following an exponential pattern.
2. SD effect on NH_3 emission became more pronounced for MAT $\geq$ 3 d.
3. Daily NH_3 emission increases with age and manure accumulation time, but tends to decrease with increasing floor space allocation to the hens.

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Table 1. Bird body weight (mean ± SE) and feed crude protein (CP) content .

Bird Age (wk)	Bird body weight (kg)		Feed CP content (%)
	HD	LD	
4	0.22 ± 0.017	0.22 ± 0.017	19.0
5	0.30 ± 0.014	0.30 ± 0.018	19.0
6	0.35 ± 0.006	0.35 ± 0.009	19.0
8	0.58 ± 0.015	0.59 ± 0.012	19.0
9	0.67 ± 0.025	0.70 ± 0.022	17.5
10	0.76 ± 0.016	0.78 ± 0.013	17.5
12	1.00 ± 0.024	1.01 ± 0.022	17.5
13	1.03 ± 0.033	1.05 ± 0.025	17.5
14	1.02 ± 0.018	1.06 ± 0.017	15.5
16	1.15 ± 0.01	1.17 ± 0.02	15.5
17	1.18 ± 0.07	1.19 ± 0.02	17.0
18	1.25 ± 0.06	1.23 ± 0.01	17.5
23	1.46 ± 0.02	1.35 ± 0.04	17.5
24	1.48 ± 0.01	1.48 ± 0.06	17.5
25	1.48 ± 0.04	1.50 ± 0.10	17.5
34	1.49 ± 0.02	1.53 ± 0.11	17.5
35	1.51 ± 0.08	1.53± 0.11	17.5
36	1.54 ±0.01	1.56 ± 0.08	17.5

Table 2. P-values from the ANOVA for cumulative feed use, feed N use, cumulative manure weight (as is and dry matter) and NH_3 emissions in several units under two stocking density (SD), different manure accumulation time (MAT), block (batch) effect, and interaction among them the factors. Hen age = 4 - 37 wk.

Variables	**Factor/Interaction**							
	Block	AGE	MAT	AGE*MAT	SD	AGE*SD	MAT*SD	AGE*MAT*SD
Daily feed disappearance g/bird-d	**< 0.0001**	**< 0.0001**	0.5933	0.9427	0.7703	0.9276	0.9930	1.0000
Daily feed N disappearance g/bird-d	**< 0.0001**	**0.0002**	0.6004	0.9953	0.1373	0.1962	0.9762	1.0000
Manure weight (as is), g/bird	0.5857	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.1930	0.4409	0.9810
Manure weight (dry basis), g/bird	**0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.4759	0.8794	0.9853	1.000
Daily NH_3 emission, mg/bird-d*	**0.0469**	**0.0001**	**< 0.0001**	**0.0002**	**< 0.0001**	0.5102	0.8701	0.9996
NH_3 emission, g/kg N disappearance *	0.9394	**0.0003**	**< 0.0001**	0.0543	**< 0.0001**	0.6643	0.8841	1.0000
NH_3 emission, g/kg manure (as-is) *	**0.0446**	**0.0002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.8805	0.8462	0.9999
NH_3 emission, g/kg manure (dry basis) *	**0.0378**	**0.0005**	**< 0.0001**	**< 0.0001**	**< 0.0001**	0.6179	0.7582	0.9999
NH_3 emission, kg/AU-d	**0.0038**	**0.0023**	**< 0.0001**	**0.0050**	**< 0.0001**	0.3913	0.9956	1.0000

*Variables were analyzed in the log-transformed scale; ** bold P values are less than 0.05.

Table 3. Feed disappearance, feed nitrogen (N) disappearance, manure weight and ammonia emission of W-36 pullets over 6-day period at two cage stocking densities (SD): pullet age = 4 - 5 wk; pullet body weight = 220 - 329 g; HD = 155 cm^2/bird; LD = 206 cm^2/bird (mean ± SE); the percentage of difference (%dif) uses HD as basis. NH_3 emissions in g/kg N disappearance, g/kg 'as-is' and dry manure, g/AU-d were calculated using 'clean' system NH_3 ER data.

Variables		Manure Accumulation Time (MAT, d)						
		1	2	3	4	5	6	Overall
Daily feed disappearance, g/bird-d	HD	30±1	30±1	30±1	30±1	30±1	30±1	30±1
	LD	28±1	29±1	28±1	30±1	31±1	33±1	30±1
Daily feed N disappearance, g/bird	HD	0.91±0.02	0.91±0.02	0.91±0.02	0.94±0.03	0.91±0.02	0.94±0.03	0.92±0.02
	LD	0.85±0.02	0.88±0.02	0.85±0.02	0.91±0.03	0.94±0.02	1.00±0.03	0.91±0.02
Manure weight (as-is), g/bird	HD	20±4	23±3	38±2	62±4	88±6	108±6	-
	LD	10±4	18±3	34±2	52±4	70±6	88±6	-
Manure weight (dry basis), g/bird	HD	7±1	6±1a	11.2±0.4	19±1	25±1	31±1	-
	LD	7±1	9±1b	12.0±4	18±1	25±1	32±1	-
Daily NH_3 emission, mg/bird-d (clean system)	HD	0.05±0.07	0.10±0.18	0.3±0.1	0.6±0.2	2±1	6±2	-
	LD	0.02±0.07	0.05±0.18	0.1±0.1	0.3±0.2	1±1	3±2	-
Daily NH_3 emission, mg/bird-d (non-clean system)	HD	-	-	-	-	-	-	-
	LD	-	-	-	-	-	-	-
NH_3 emission, g/kg N disappearance	HD	0.05±0.07	0.10±0.05	0.3±0.1	0.6±0.2	2±1	6±2	-
	LD	0.02±0.07	0.06±0.05	0.2±0.1	0.3±0.2	1±1	3±2	-
NH_3 emission, g/kg manure (as-is)	HD	0.003±0.002	0.004±0.003	0.007±0.003	0.010±0.004	0.020±0.007	0.06±0.02	-
	LD	0.002±0.002	0.003±0.003	0.004±0.003	0.006±0.004	0.014±0.007	0.03±0.02	-
NH_3 emission, g/kg manure (dry basis)	HD	0.007±0.011	0.02±0.20	0.02±0.01	0.03±0.02	0.07±0.03	0.2±0.1	-
	LD	0.003±0.011	0.01±0.20	0.011±0.01	0.02±0.02	0.04±0.03	0.1±0.1	-
NH_3 emission, kg/AU-d	HD	0.09±0.10	0.2±0.40	0.5±0.5	1.0±0.6	3±1	10±4	-
	LD	0.03±0.10	0.09±0.40	0.2±0.5	0.5±0.6	2±1	5±4	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for 0.01< P ≤ 0.05; AU = animal unit = 500 kg live body weight. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 4. Feed disappearance, feed nitrogen (N) disappearance, manure weight and ammonia emission of W-36 pullets over 6-day period at two cage stocking densities (SD): pullet age = 8-9 wk; pullet body weight = 580 - 670 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird (mean ± SE); the percentage of difference (%dif) has LD as basis. NH_3 emissions in g/kg N disappearance, g/kg 'as-is' and dry manure, g/AU-d were calculated using 'clean' system NH_3 ER data.

Variables		Manure Accumulation Time (MAT, d)						
		1	2	3	4	5	6	Overall
Daily feed disappearance, g/bird-d	HD	44±1	45±1	44±1	44±1	43±1	46±1	44±1
	LD	44±1	47±1	47±1	47±1	46±1	47±1	46±1
Daily feed N disappearance, g/bird	HD	1.27±0.03	1.30±0.06	1.27±0.05	1.27±0.02a	1.24±0.04	1.32±0.02	1.28±0.03
	LD	1.27±0.03	1.35±0.06	1.35±0.05	1.35±0.02b	1.32±0.04	1.35±0.02	1.33±0.03
Manure weight (as-is), g/bird	HD	23±6	72±8	124±13	174±14	218±14	254±16	-
	LD	24±6	61±8	101±13	142±14	181±14	218±16	-
Manure weight (dry basis), g/bird	HD	5±2	19±3	32±4	45±4	56±5	66±5	-
	LD	5±2	19±3	31±4	43±4	55±5	66±5	-
Daily NH_3 emission, mg/bird-d (clean system)	HD	0.1±0.4	0.2±0.4	2±1	5±3	11±5	30±6	-
	LD	0.3±0.4	0.6±0.4	1±1	4±3	5±5	26±6	-
Daily NH_3 emission, mg/bird-d (non-clean system)	HD	19±4	1.6±0.5	5±1	12±3	27±5	24±10	-
	LD	13±4	0.4±0.5	2±1	5±3	16±5	15±10	-
NH_3 emission, g/kg N disappearance	HD	0.08±0.40	0.2±0.3	2±1	4±4	9±8	23±8	-
	LD	0.24±0.40	0.4±0.3	1±1	3±4	4±8	19±8	-
NH_3 emission, g/kg manure (as-is)	HD	0.004±0.002 b	0.003±0.008	0.016±0.005	0.03±0.01	0.05±0.02	0.12±0.02	-
	LD	0.013±0.002 a	0.010±0.008	0.010±0.005	0.03±0.01	0.03±0.02	0.12±0.02	-
NH_3 emission, g/kg manure (dry matter)	HD	0.02±0.34	0.01±0.03	0.06±0.02	0.11±0.05	0.2±0.1	0.5±0.1	-
	LD	0.06±0.34	0.03±0.03	0.03±0.02	0.09±0.05	0.1±0.1	0.4±0.1	-
NH_3 emission, kg/AU-d	HD	0.07±0.30	0.2±0.3	1.5±0.8	4±2	8±4	22±5	-
	LD	0.22±0.30	0.4±0.3	0.7±0.8	3±2	4±4	19±5	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$; AU = animal unit = 500 kg live body weight. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 5. Feed disappearance, feed nitrogen (N) disappearance, manure weight and ammonia emission of W-36 pullets over 6-day period at two cage stocking densities (SD): pullet age = 12-13 wk; pullet body weight = 1000 - 1030 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird (mean ± SE); the percentage of difference (%dif) has LD as basis. NH_3 emissions in g/kg N disappearance, g/kg 'as-is' and dry manure, g/AU-d were calculated using 'clean' system NH_3 ER data.

Variables		**Manure Accumulation Time (MAT, d)**						
		1	2	3	4	5	6	Overall
Daily feed disappearance, g/bird-d	HD	54±2	57±1	57±2	53±1	54±5	48±2	54±2
	LD	48±2	57±1	52±2	56±1	53±5	52±2	53±2
Daily feed N disappearance, g/bird-d	HD	1.45±0.06	1.53±0.03	1.53±0.06	1.42±0.03	1.4±0.5	1.29±0.05	1.45±0.02
	LD	1.29±0.06	1.53±0.03	1.40±0.06	1.51±0.03	1.4±0.5	1.40±0.05	1.42±0.02
Manure weight (as-is), g/bird	HD	36±3	83±5	141±6	198±10	252±15	299±16	-
	LD	32±3	76±5	121±6	164±10	201±15	243±16	-
Manure weight (dry basis), g/bird	HD	12±0.4	24±0.7	36±1b	47±2	58±3	70±4	-
	LD	11±0.4	25±0.7	47±1a	54±2	66±3	79±4	-
Daily NH_3 emission, mg/bird-d (clean system)	HD	0.3±0.2	0.3±0.1	1.0±0.1a	3±1	16±4	36±8	-
	LD	0.1±0.2	0.1±0.1	0.6±0.1b	2±1	14±4	23±8	-
	%dif	-	-	-45±31	-40±24	-43±29	-36±38	-41±31
Daily NH_3 emission, mg/bird-d (non-clean system)	HD	18±4	10±2	18±5	44±9	53±11	61±18	-
	LD	15±4	4±2	6±5	15±9	22±11	34±18	-
	%dif	-	-	-67±25	-66±23	-58±41	-54±40	-61±28
NH_3 emission, g/kg N disappearance	HD	0.2±0.2	0.2±0.2	0.6±0.2	2±2	11±4	28±9	-
	LD	0.1±0.2	0.1±0.2	0.4±0.2	1±2	10±4	16±9	-
	%dif	-	-	39±18	-81±42	-11±11	-41±51	-23±13
NH_3 emission, g/kg manure (as-is)	HD	0.008±0.010	0.004±0.010	0.007±0.010	0.015±0.020	0.06±0.02	0.12±0.03	-
	LD	0.003±0.010	0.001±0.010	0.005±0.010	0.012±0.020	0.07±0.02	0.010±0.03	-
	%dif	-	-	-36±12	-76±33	10±5	-21±9	-31±7
NH_3 emission, g/kg manure (dry basis)	HD	0.025±0.03	0.013±0.03	0.028±0.04	0.064±0.03	0.28±0.06	0.5±0.1	-
	LD	0.009±0.03	0.004±0.03	0.012±0.04	0.037±0.03	0.21±0.06	0.3±0.1	-
	%dif	-	-	-58±21	-83±38	-23±8	-43±28	-52±16
NH_3 emission, kg/AU-d	HD	0.15±0.04	0.15±0.04	0.49±0.05 a	1.47±0.05 a	7.82±0.06a	17.6±0.1a	-
	LD	0.05±0.04	0.05±0.04	0.27±0.05 b	0.29±0.05 b	6.84±0.06b	11.2±0.1 a	-
	%dif	-	-	-45±16	-80±33	-13±10	-36±18	-44±22

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$; AU = animal unit = 500 kg live body weight; %diff ($\hat{\lambda}$) values were calculated from: $\hat{\lambda} = [(\hat{\mu}_{LD} - \hat{\mu}_{HD}) / \hat{\mu}_{HD}].100\%$, where $\hat{\mu}_{LD}$ and $\hat{\mu}_{HD}$ are the estimated means for the considered variable under low and high density, respectively. The standard error for $\hat{\lambda}$ was estimated with the Delta Method. However, when the %diff was small, the Delta Method tended to output extremely high SEs, in other words, the function used to estimate SE got closer to its limit range ($\lim_{\%diff \to 0} SE = \infty$), thus %diff values with extremely magnified estimates for SE were deleted. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 6. Feed disappearance, feed nitrogen (N) disappearance, manure weight and ammonia emission of W-36 pullets over 6-day period at two cage stocking densities (SD): pullet age = 16-17 wk; pullet body weight = 1015 - 1018 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird (mean ± SE); the percentage of difference (%dif) has LD as basis. NH_3 emissions in g/kg N disappearance, g/kg 'as-is' and dry manure, g/AU-d were calculated using 'clean' system NH_3 ER data.

Variables		**Manure Accumulation Time (MAT, d)**						
		1	2	3	4	5	6	Overall
Daily feed disappearance, g/bird-d	HD	64±2	67±2	67±2	63±1	64±2	58±2	64±1
	LD	58±2	67±2	62±2	66±1	63±2	62±2	63±1
Daily feed N disappearance, g/bird-d	HD	1.77±0.05	1.85±0.07	1.85±0.06	1.7±0.3	1.77±0.04	1.60±0.04	1.76±0.08
	LD	1.6±0.05	1.85±0.07	1.71±0.06	1.8±0.3	1.74±0.04	1.71±0.04	1.74±008
Manure weight (as-is), g/bird	HD	45±1	102±4	149±5 b	197±3	244±4	292±6	-
	LD	46±1	91±4	173±5 a	199±3	243±4	288±6	-
Manure weight (dry basis), g/bird	HD	23±1 a	38±4	69±4	88±4	136±4 a	148±6	-
	LD	15±1 b	25±4	56±4	77±4	111±4 b	129±6	-
Daily NH_3 emission, mg/bird-d (clean system)	HD	2.00±0.03	2.4±0.1 a	2.4±0.2	5±2	49±13	90±21	-
	LD	2.03±0.03	1.8±0.1 b	1.9±0.2	2±2	26±13	57±21	-
	%dif	-	-	-21±11	-60±46	-47±24	-37±13	-41±21
Daily NH_3 emission, mg/bird-d (non-clean system)	HD	128±25	30±5	32±6	41±10	49±10	88±14	-
	LD	102±25	28±5	27±6	29±10	38±10	62±14	-
	%dif	-	-	-16±18	-28±12	-22±11	-30±22	-24±16
NH_3 emission, g/kg N disappearance	HD	1.13±0.03 a	1.28±0.07 a	1.3±0.1	2.9±1	28±9	56±16	-
	LD	1.27±0.03 b	0.98±0.07 b	1.1±0.1	1.1±1	15±9	33±16	-
	%dif	-	-	-14±2	-62±28	-46±523	-41±12	-41±14
NH_3 emission, g/kg manure (as-is)	HD	0.044±0.001	0.023±0.001	0.016±0.001a	0.025±0.008	0.20±0.05	0.30±0.08	-
	LD	0.044±0.001	0.020±0.001	0.011±0.001b	0.010±0.008	0.11±0.05	0.20±0.08	-
	%dif	-	-	-32±18	-60±1	-47±19	-36±29	-44±16
NH_3 emission, g/kg manure (dry basis)	HD	0.09±0.05 a	0.06±0.01	0.035±0.02	0.06±0.03	0.3±0.2	0.6±0.3	-
	LD	0.14±0.05 b	0.07±0.01	0.034±0.02	0.03±0.03	0.2±0.2	0.4±0.3	-
	%dif	-	-	15±10	-37±20	-12±10	-14±8	-12±10
NH_3 emission, kg/AU-d	HD	0.87±0.02	1.03±0.08	1.0±0.1	2.2±0.5	21±9	39±16	-
	LD	0.88±0.02	0.79±0.08	0.8±0.1	0.9±0.5	11±9	25±16	-
	%dif	-	-	-21±8	-60±24	-47±52	-37±42	-41±18

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$; AU = animal unit = 500 kg live body weight; %diff ($\hat{\lambda}$) values were calculated from: $\hat{\lambda} = [(\hat{\mu}_{LD} - \hat{\mu}_{HD}) / \hat{\mu}_{HD}].100\%$, where $\hat{\mu}_{LD}$ and $\hat{\mu}_{HD}$ are the estimated means for the considered variable under low and high density, respectively. The standard error for $\hat{\lambda}$ was estimated with the Delta Method. However, when the %diff was small, the Delta Method tended to output extremely high SEs, in other words, the function used to estimate SE got closer to its limit range ($\lim_{\%diff \to 0} SE = \infty$), thus %diff values with extremely magnified estimates for SE were deleted. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 7. Feed disappearance, feed nitrogen (N) disappearance, manure weight and ammonia emission of W-36 hens over 6-day period at two cage stocking densities (SD): hen age = 23-36 wk; hen body weight = 1351 - 1564 g; HD = 413 cm^2/bird; LD = 620 cm^2/bird (mean ± SE); the percentage of difference (%dif) has LD as basis. NH_3 emissions in g/kg N disappearance, g/kg 'as-is' and dry manure, g/AU-d were calculated using 'clean' system NH_3 ER data.

Variables		Manure Accumulation Time (MAT, d)						
		1	2	3	4	5	6	Overall
Daily feed disappearance, g/bird-d	HD	94±2	98±3	99±3	96±8	103±4	98±2	98±3
	LD	95±2	100±3	98±3	100±8	102±4	99±2	99±3
Daily feed N disappearance, g/bird-d	HD	2.60±0.07	2.72±0.08	2.73±0.08	2.63±0.08	2.83±0.09	2.70±0.05	2.70±0.08
	LD	2.62±0.07	2.75±0.08	2.73±0.08	2.77±0.08	2.80±0.09	2.72±0.05	2.73±0.08
Manure weight (as-is), g/bird	HD	45±4	137±5	225±9	307±12	375±18	455±18	-
	LD	46±4	134±5	214±9	288±12	360±18	419±18	-
Manure weight (dry basis), g/bird	HD	26±2	72±6	130±5	177±7	216±10	262±10	-
	LD	26±2	80±6	127±5	171±7	214±10	248±10	-
Daily NH_3 emission, mg/bird-d (clean system)	HD	5±3	12±4	41±9	98±13	179±26	251±33	-
	LD	7±3	12±4	29±9	64±13	114±26	160±33	-
	%dif	-	1±50	-31±33	-34±22	-36±24	-36±22	-27±28
Daily NH_3 emission, mg/bird-d (non-clean system)	HD	349±29	57±6	45±3 a	83±8a	154±19	245±27	-
	LD	260±29	38±6	25±3 b	56±8b	107±19	171±27	-
	%dif	-25±13	-33±17	-43±14	-32±16	-30±19	-30±17	-32±17
NH_3 emission, g/kg N disappearance	HD	2±1	4±1	16±3	40±4 a	61±11	105±11 a	-
	LD	2±1	4±1	11±3	24±4 b	42±11	62±11 b	-
	%dif	-	-	-35±30	-38±18	-32±29	-40±18	-36±22
NH_3 emission, g/kg manure (as-is)	HD	0.07±0.05	0.1±0.02	0.15±0.02	0.30±0.03	0.42±0.04	0.52±0.06	-
	LD	0.07±0.05	0.1±0.02	0.12±0.02	0.22±0.03	0.30±0.04	0.35±0.06	-
	%dif	-	-	-17±17	-25±15	-29±16	-33±19	-26±15
NH_3 emission, g/kg manure (dry basis)	HD	0.10±0.07	0.12±0.04	0.27±0.04	0.52±0.05	0.8±0.1	0.9±0.1	-
	LD	0.15±0.07	0.10±0.04	0.20±0.04	0.35±0.05	0.5±0.1	0.6±0.1	-
	%dif	-	-	-27±24	-33±14	-30±21	-32±17	-30±21
NH_3 emission, kg/AU-d	HD	2±1	4±1	14±3	32±4	58±8	82±10	-
	LD	2±1	4±1	9±3	20±4	36±8	51±10	-
	%dif	-	-	-34±32	-37±20	-38±23	-37±7	-36±18

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$; AU = animal unit = 500 kg live body weight; %diff ($\hat{\lambda}$) values were calculated from: $\hat{\lambda} = [(\hat{\mu}_{LD} - \hat{\mu}_{HD}) / \hat{\mu}_{HD}].100\%$, where $\hat{\mu}_{LD}$ and $\hat{\mu}_{HD}$ are the estimated means for the considered variable under low and high density, respectively. The standard error for $\hat{\lambda}$ was estimated with the Delta Method. However, when the %diff was small, the Delta Method tended to output extremely high SEs, in other words, the function used to estimate SE got closer to its limit range ($\lim_{\%diff \to 0} SE = \infty$), thus %diff values with extremely magnified estimates for SE were deleted. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

CHAPTER 3. DYNAMICS OF FEEDING, DEFECATION AND NH_3 EMISSIONS OF PULLETS AND LAYING HENS UNDER DIFFERENT STOCKING DENSITIES AND MANURE ACCUMULATION TIME

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Abstract

This study examines the dynamics of feeding and defecation behavior and ammonia (NH_3) emissions of W36 pullets (4 to 18 wk of age) and laying hens (23 to 36 wk of age) housed under different stocking densities (SD) over a 6-d manure accumulation time (MAT). The lab-scale experiment was carried out with a system that resembled the conditions of a manure-belt (MB) laying-hen house. Continuous measurements of feed disappearance (g/bird-h), 'as-is' manure production (g/bird-h) and NH_3 (mg/hen-h) emissions were taken. An algorithm was developed and validated to calculate the fresh manure production (g/hen-h) from 'as-is' manure production by accounting for the moisture loss of manure through evaporation. Two SDs at a given bird age were evaluated, ranging from 155 to 619 cm^2 (24 to 96 in^2) per bird. Results indicate that the SDs did not affect feed disappearance or fresh manure production ($P = 0.17 – 0.81$) at any of the tested bird ages. Each gram of feed use corresponded to a 1.15 g of fresh manure production (P <

0.0001) for laying hens. The light (16 hr) and dark (8 hr) partitioning of daily feed disappearance was 92% to 8%, respectively, while the concomitant partitioning of fresh manure production was 80% and 20%, respectively. The results also revealed that 37% of the daily NH_3 emission took place the dark period that accounts for 63% of the daily hours.

Key words: Emission dynamics, laying hens, manure accumulation, defecation behavior, stocking density

Introduction

Livestock is often fed high protein diet, which contains surplus nitrogen to ensure that the animals' nutritional requirements are met (Gay et al., 2009), and most of the unused feed nitrogen is lost in the manure, which is the primary source of ammonia (NH_3) emissions. Ammonia is an irritant, colorless gas with a characteristic pungent odor, which can be harmful to both animals and humans. Research has shown that prolonged exposure to high concentrations of NH_3 can cause significant lower body weight gain and reduced egg production in laying hens (Deaton et al., 1982; Ning, 2008).

Ammonia is the by-product of a 5-step enzymatic degradation of uric acid (Singh et al., 2009) and has been regarded as contributing to acid rain and excessive nitrogen input to natural N-sensitive ecosystems. The resultant consequence could be modification of ecosystems (van Breemen et al., 1982; Demmers et al. 1998) such as eutrophication and soil acidification related environmental stress (Heij & Schneider, 1991: Heij & Erisman, 1997, Monteny & Erisman, 1998).

Hence, there is an urgent need to develop and evaluate new technologies and practices that will cost-effectively mitigate NH_3 emissions from laying-hen operations. Several methods have been reported in the literature. One strategy to reduce gaseous emissions from inside animal feeding operations (AFOs) is the use of alternative housing designs that lead to a shorter residence time of manure inside the building. High-rise (HR) layer houses are the most popular housing type used in the US for laying hens. Manure in HR house is generally stored inside the barns for about one year and land-applied after crop harvest. Although HR system has been typical in the U.S., the trend in new laying-hen houses has been shifted to manure-belt (MB) style, where manure is frequently (daily to weekly) removed from the houses. Recent monitoring of NH_3 emissions from commercial laying-houses showed that MB houses with daily or semi-weekly manure removal emit less than 10% of the NH_3 as compared with HR houses (Liang et al., 2005; Li, et al. 2009). Fabbri (et al., 2007) reported a 61% NH_3 emission reduction for a ventilated MB system as compared to the HR system.

Laboratory experiments designed to mimic a MB house system performed by Ning (2008) showed that NH_3 ER from laying hens (Hy-Line W-36) depends on duration of manure accumulation. Ning (2008) also found that the emissions had an inverse relation to defecation events.

However, the documented or ongoing studies on NH_3 emissions from MB or other housing types do not include pullets (i.e., hens younger than 18 weeks of age, pre-laying), even though pullets are a major integral part of the egg operation. Moreover, pullets or hens may be housed at different stocking densities as producers respond to certain industry production management guidelines.

The objective of the research study described in this paper was to characterize the dynamics of feeding and defecation behaviors and NH_3 emissions of pullets and laying hens housed under different stocking densities (SDs) and different manure accumulation time (MAT) in a laboratory setup that resembles a MB house. The results obtained from this study will advance the scientific knowledge and provide researched-based information that enables the poultry industry to better make management decisions.

Materials and Methods

Dynamic gas emission chambers system, experimental hens and relation of this study to the one described in Chapter 2

The study was conducted using the same four dynamic gas emission chambers containing birds at different SD, ages and MAT as described in Chapter 2. In fact, the data sets analyzed for this chapter were the same collected for the experiment described in Chapter 2. However, feed disappearance, 'as-is' manure production and NH_3 ER were processed using a different methodology.

Description of the algorithm developed to analyze the dynamics of emission rates, feeding and defecation behaviors

Hourly ammonia emission rate (NH_3 ER) was calculated for 1 to 6 d MAT with equation 1, as described in Chapter 2 of this thesis.

Hourly feed disappearance and fresh manure production rates were calculated from the continuously measured feeder and manure pan weights. The total values of feed disappearance, manure production and NH_3 emission were broken down into light and dark periods, for each of the 6 d MAT.

To estimate the amount of fresh manure produced by the hens (accounting for moisture evaporation), an algorithm was written using Macros in Excel. The steps of the algorithm were described in figure 1. Manure weight data, originally recorded at 10 s intervals were averaged into 1-min intervals to reduce the noise. Then the change (delta) in manure weight after every minute was calculated, with negative deltas considered as moisture loss and positive delta indicating occurrence of a defecation event. Adding up the positive and negative deltas provided 'as-is' manure production. All variables were initially calculated in g/hen-h.

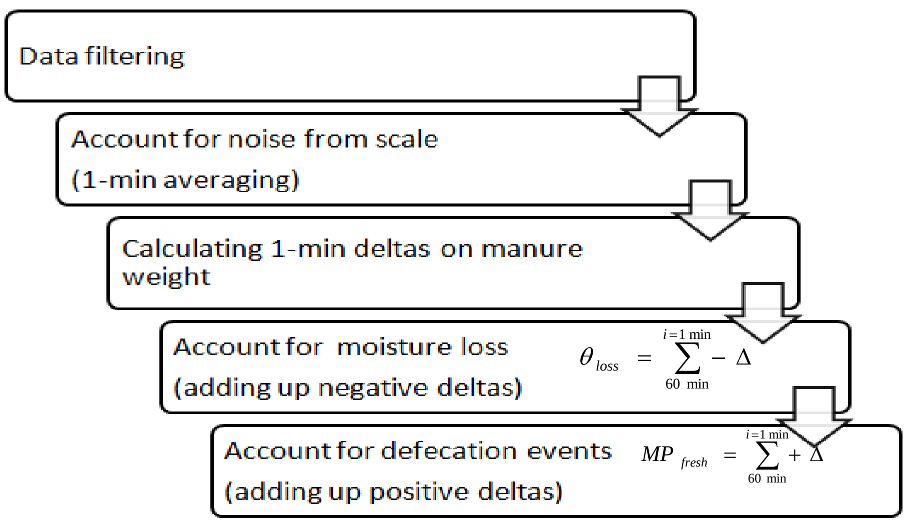

Figure 1. Flow chart illustrating the steps of the algorithm for feed disappearance and fresh manure production data processing.

Hourly feed disappearance data was obtained from the continuously monitored feeder weight data. Data sets were first processed for removal of the points corresponding to the birds pecking activity on the scale (figures 2 and 3), and then broken down into hourly rates.

All variables presented were tested for SD effect using *proc glm* in SAS through the ANOVA for a block design. Significant difference between means under different SD was tested through the Tukey Test and p-values less or equal to 0.05 were considered significant.

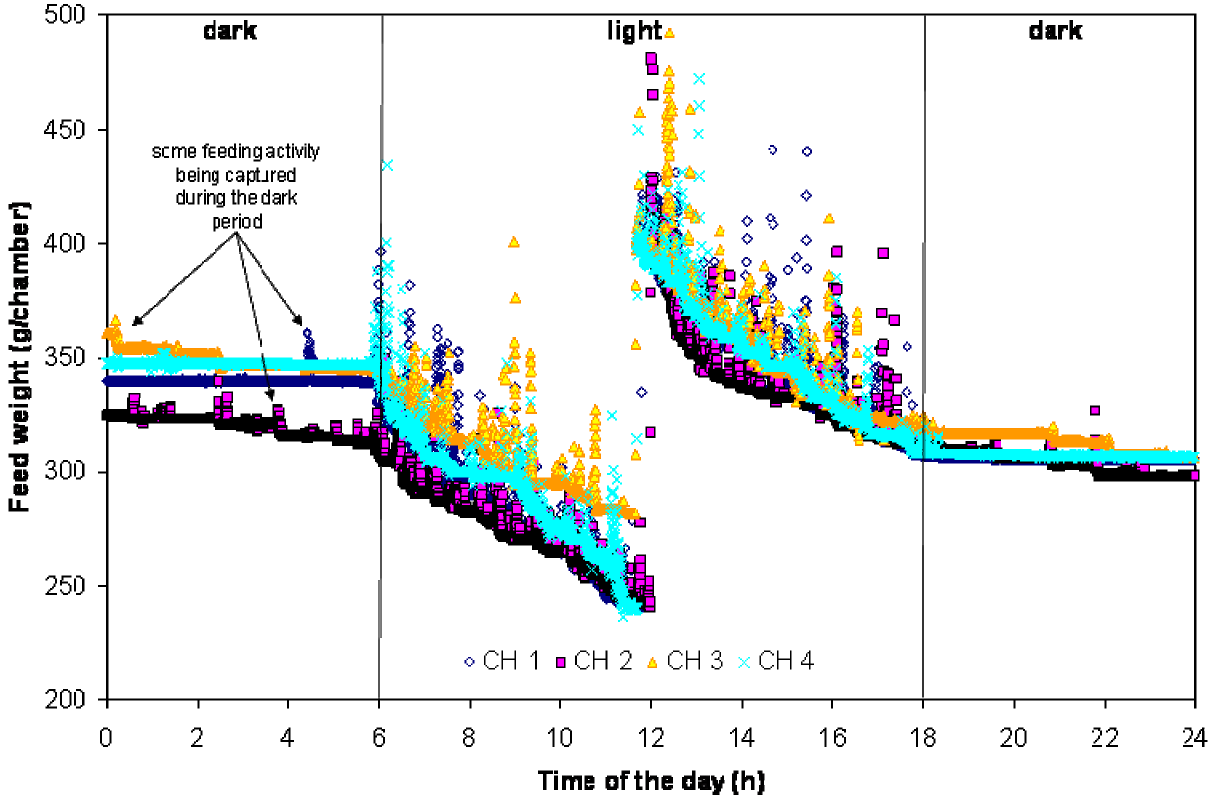

Figure 2. Sample of the continuously monitored feeder weight data of chambers (CH) 1 to 4 throughout dark and light periods. Bird age: 14 wk; 4 birds/chamber for CH 1 and CH 4 and 3 birds/chamber for CH 2 and CH 3; lighting program: 12 L: 12 D.

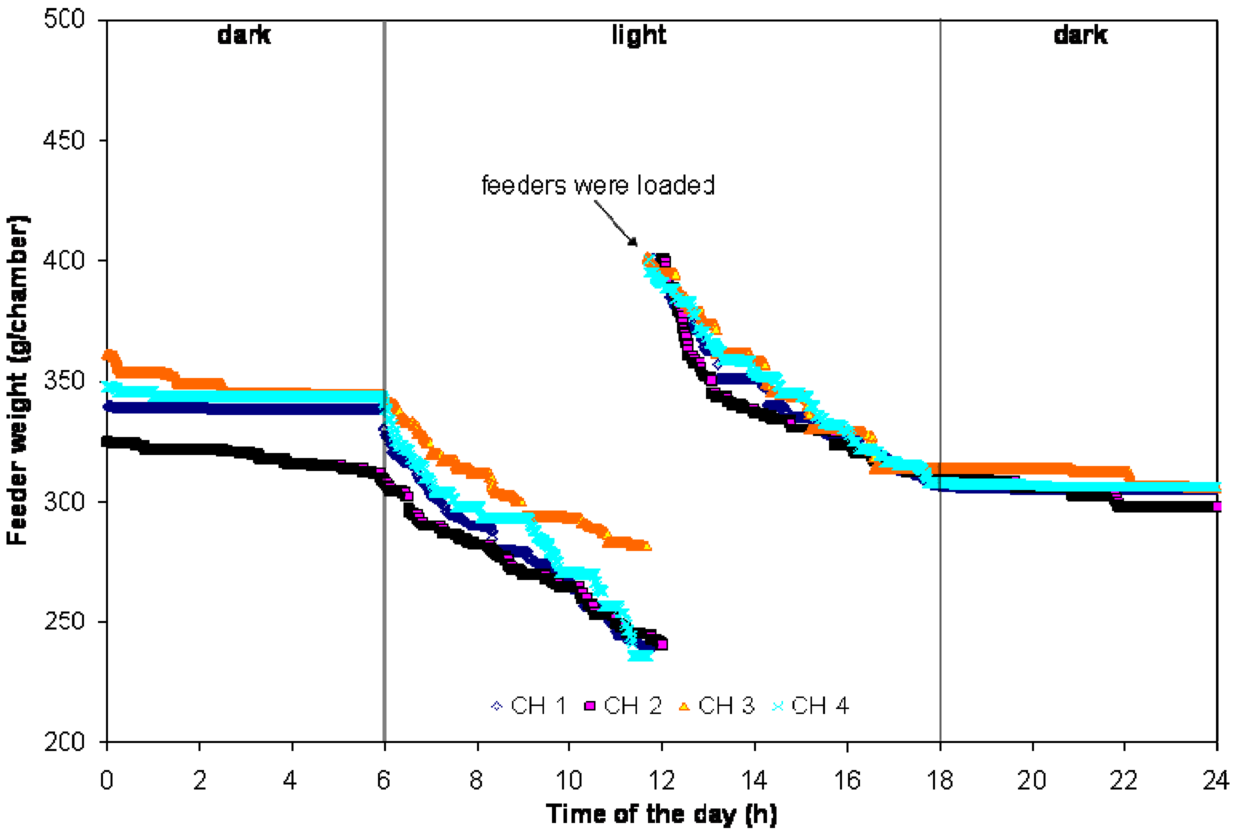

Figure 3. Sample of the processed, feeder weight data of chambers (CH) 1 to 4 throughout dark and light periods. Bird age: 14 wk; 4 birds/chamber for CH 1 and CH 4 and 3 birds/chamber for CH 2 and CH 3; current lighting program: 12 L: 12 D.

Results and Discussion

Validation of the algorithm for the estimation of fresh manure production

One can see from figure 4 how hourly 'as-is', fresh manure production rates and hourly moisture loss rates are related for laying hens aging 23-36 wk. The results confirm the mass balance of equation 2. The same held true for birds at the other tested ages.

$$\theta_{loss} = MP_{fresh} - MP_{'as-is'} \qquad [2]$$

Where:

θ_{loss} – moisture loss from manure (g/hen-h)

MP_{fresh} – fresh manure production (g/hen-h)

$MP_{'as-is'}$ – 'as-is' manure production (g/hen-h)

The algorithm was validated by correlating the daily 'as-is' manure production obtained from the difference between beginning and end of the day (called raw data method) with the daily 'as-is' manure production obtained from the algorithm by adding up the hourly rates for the whole 24-h period. The degree of correlation is quite good, as presented in figure 5 (top) (R^2=0.96, $P < 0.0001$).

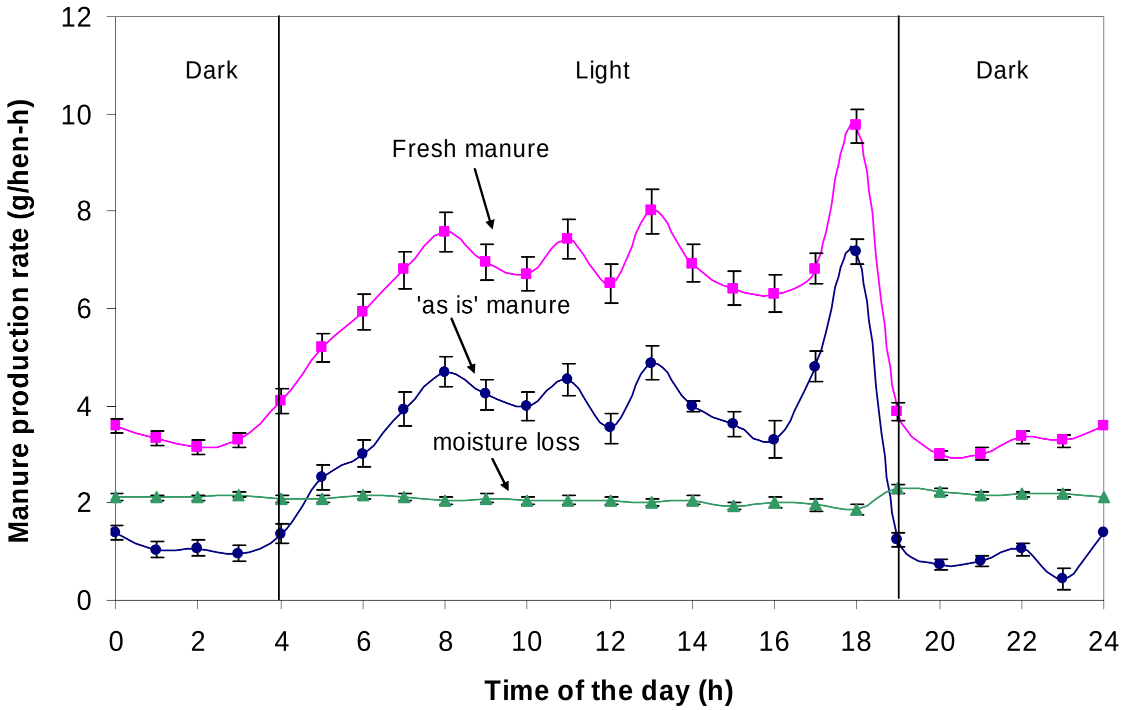

Figure 4. Dynamics of fresh, 'as is' manure production rates and moisture loss from manure throughout dark and light periods. Bird average age: 30 wk; photoperiod of 16L:8D.

A similar validation procedure was performed, which correlated the moisture loss data obtained from the algorithm with those calculated from the difference between fresh manure production (determined with the algorithm) and the 'as-is' manure production (determined from the scale readings). The graphical representation is shown in figure 5 (bottom), and the relationship was well represented by a linear model (R^2=0.98, P<0.0001).

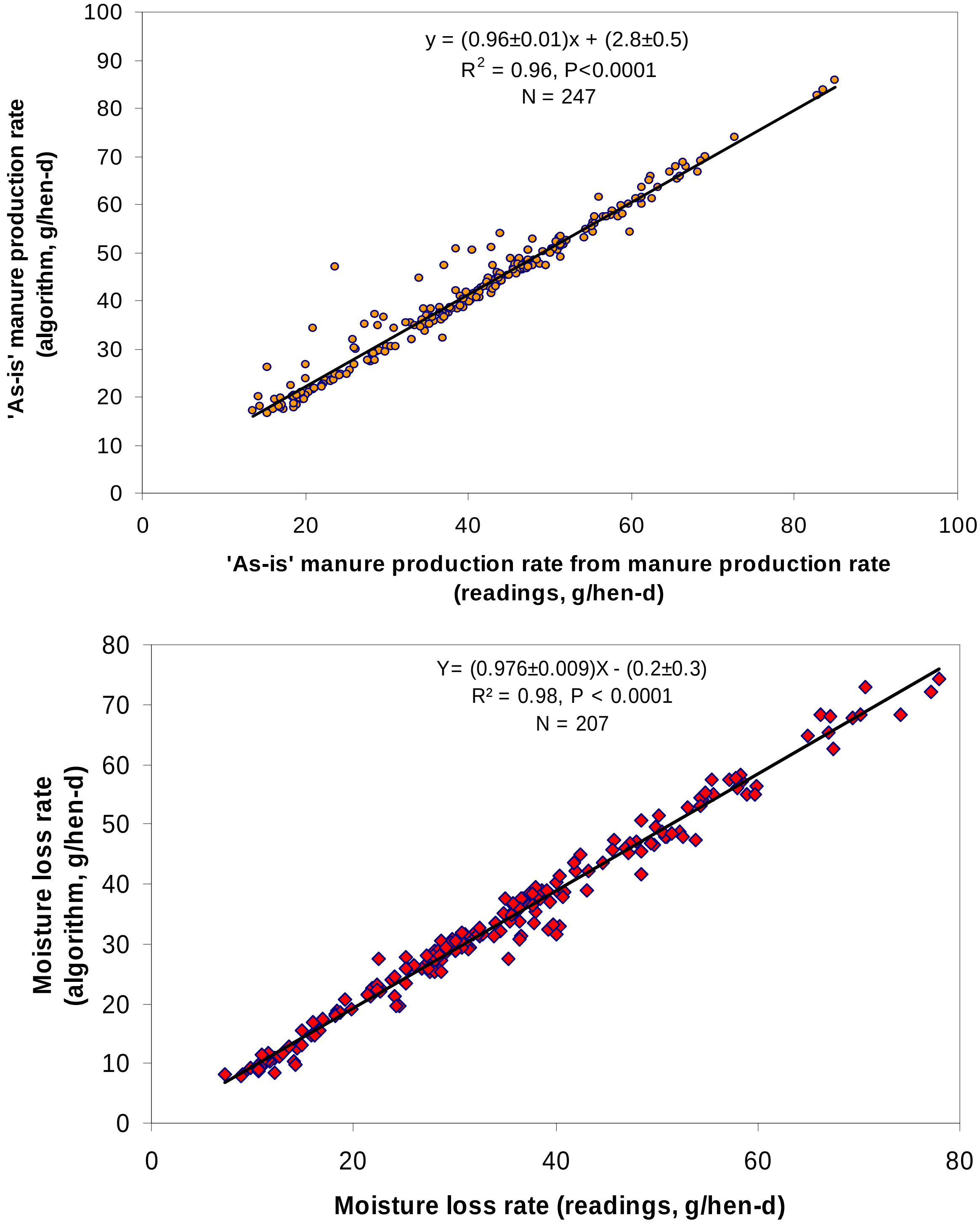

Figure 5. Correlation between 'as is' manure production obtained from the algorithm and calculated from manure weight readings (top) and correlation between moisture loss obtained from the algorithm and calculated from the difference between fresh manure production obtained from the algorithm and 'as-is' manure weight from readings and readings (bottom).

Light vs. dark period dynamics of bird feed disappearance, fresh manure production and NH_3 ER

The correlation between feed disappearance and fresh manure production for laying hens is shown in figure 6, regression analysis suggests that a linear model can be used to represent the relationship. Results of the regression analysis for fresh manure production vs. feed disappearance for birds at different ages are shown in table 2. The coefficient of determination (R^2) indicated that the proportion of change in fresh manure production was explained by the change in feed disappearance according to a linear model by 35 – 57 %, but the P-value of the regression ($P<0.0001$) indicated that the relationship is very likely to be linear, such as described by equation 3. Ning (2008) reported that the relationship (feed disappearance vs. fresh manure production) was well described by a linear model only when feed disappearance was lower than 8 g/bird-h.

$$MP_{fresh} = A * FD + B \quad [3]$$

Where:

MP_{fresh} – fresh manure production (g/bird-h);

FD – feed disappearance (g/bird-h);

A and B – parameters of the linear model, obtained empirically

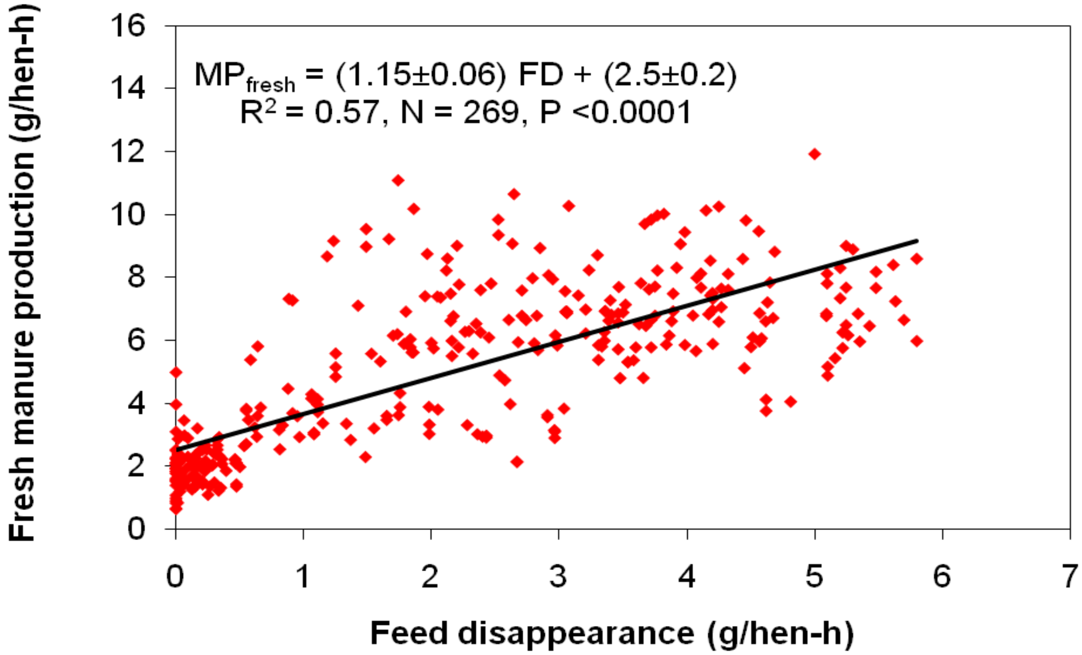

Figure 6. Linear relationship between feed disappearance and fresh manure production for laying hens. (laying hen age: 23-34 wk)

Results in table 2 also show that both coefficients (A and B) of the linear model in eq. 3 were significantly different from zero ($P<0.0001$) for all the tested ages. The coefficient A (multiplier or slope) indicated that the increase in manure production per each gram of increase in feed disappearance was 0.58 – 1.15 g. The coefficient B (offset or intersept) indicated that for periods of time when feed disappearance was zero, fresh manure production was 0.83 – 2.5 g/bird-h. This outcome suggests that during the dark periods (when feed disappearance was zero or minimal) manure was still being produced.

Bird fresh manure production and feed disappearance for each of the 6-d MAT and both SD treatments during light, dark and daily periods are shown in tables 3 to 7 for birds at different ages. Results indicated no significant difference in feed disappearance (P = 0.46 – 0.81) between the two SD regimens, suggesting that the reduced floor area allocation did not adversely affect feed use. Consequently, fresh manure production was not affected by the SDs either (P = 0.17 – 0.72).

About 8% of the total feed use took place during the dark period, while about 20% of the total fresh manure was produced during the same period. This outcome supports the above discussion that some manure is still produced during dark hours even when birds are not eating. These results are consistent with what Ning (2008) reported that laying hens used about 4% of the total feed use while producing 17% of the total fresh manure during dark hours.

Ammonia ER for dark, light and daily periods under the different SD regimens at 1-6 d MAT are also shown in tables 3 to 7 for birds at different ages.

For data analysis purposes, a distinction was made between 'clean' vs. 'non-clean' system and NH_3 ER in several units were derived from the daily NH_3 ER obtained from 'clean' system. NH_3 ER data in tables 3 to 7 are presented for both 'clean' and 'non-clean' systems. One can see that for the 'non-clean' system, most of the residual ammonia is flushed before the first 48-h of MAT, when 95% confidence intervals for both conditions started to overlap.

One can observe from daily NH_3 ER data presented in tables 3 to 7 (for both clean and non-clean systems), that considering the fact that the light period was always bigger than dark period, the emissions for HD and LD in the dark period would be relatively equal to the respective emissions for the light period if light and dark hours had the same number or hours. This fact is an indication that emissions tend to be more intense during the dark period. In fact, Ning (2008) observed a similar result and speculated that during the light period the newly defecated manure covers the old manure surface which is more responsible for NH_3 emission because new manure needs time to decompose and generate NH_3. The new manure covers the relatively old manure, reducing the effective surface area for emission. It is estimated that about 37% of the daily NH_3 was emitted during the dark hours. This outcome indicates that if an NH_3-supressing agent is applied to the hen manure, it would be more effective to apply the agent during the dark period.

Conclusions

Feed disappearance, fresh manure production and NH_3 ER were partitioned into dark and light periods for pullets and laying hens under different SD regimens from 1- to 6-d MAT. The analysis revealed the following:

1. Stocking density did not impact feed disappearance (P = 0.46 -0.81) or fresh manure production (P = 0.17 – 0.72). Each gram of feed use led to 0.53 to 1.15 g of fresh manure production.
2. Feed use during the dark period was minimal, while some fresh manure was still being produced;, ranging from 0.91 to 3.4 g/bird-h. The partitioning of feed disappearance between light and dark hours was 92 % and 8 %, respectively; and the partitioning of fresh manure production during dark and light hours was 80 % and 20 % respectively;
3. The partitioning for daily NH_3 ER between light and dark hours was 63% and 37% of the total daily emissions, respectively.

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Table 1. Bird body weight (mean and SE) and feed crude protein (CP) content .

Bird Age (wk)	Bird body weight (kg), mean (SE)		Feed CP content (%)
	HD	LD	
4	0.22 ± 0.017	0.22 ± 0.017	19.0
5	0.30 ± 0.014	0.30 ± 0.018	19.0
6	0.35 ± 0.006	0.35 ± 0.009	19.0
8	0.58 ± 0.015	0.59 ± 0.012	19.0
9	0.67 ± 0.025	0.70 ± 0.022	17.5
10	0.76 ± 0.016	0.78 ± 0.013	17.5
12	1.00 ± 0.024	1.01 ± 0.022	17.5
13	1.03 ± 0.033	1.05 ± 0.025	17.5
14	1.02 ± 0.018	1.06 ± 0.017	15.5
16	1.15 ± 0.01	1.17 ± 0.02	15.5
17	1.18 ± 0.07	1.19 ± 0.02	17.0
18	1.25 ± 0.06	1.23 ± 0.01	17.5
23	1.46 ± 0.02	1.35 ± 0.04	17.5
24	1.48 ± 0.01	1.48 ± 0.06	17.5
25	1.48 ± 0.04	1.50 ± 0.10	17.5
34	1.49 ± 0.02	1.53 ± 0.11	17.5
35	1.51 ± 0.08	1.53± 0.11	17.5
36	1.54 ±0.01	1.56 ± 0.08	17.5

Table 2. Summary of the regression analysis performed for feed disappearance (FD, g/bird-h) vs. fresh manure production (MP_{fresh}, g/bird-h), of the form MP_{fresh} = A*FD + B.

Bird age (wk)	Parameter estimate				N	R^2
	A ± SE (g_{manure}/g_{feed})	P	B ± SE (g_{manure}/bird-h)	P		
4-5	0.58±0.05	<0.0001	0.83±0.08	<0.0001	192	0.35
8-9	0.76±0.05	<0.0001	1.6±0.2	<0.0001	192	0.48
12-13	0.84±0.06	<0.0001	1.7±0.2	<0.0001	184	0.42
16-17	1.02±0.08	<0.0001	2.0±0.2	<0.0001	184	0.46
23-36	1.15±0.06	<0.0001	2.5±0.2	<0.0001	288	0.57

Table 3. Estimated means and SEs from the two-way ANOVA test for a block design of: fresh manure production, feed disappearance and NH_3 ER for 'clean' and 'non-clean' system during the light, dark and daily periods (17 L vs. 7 D) along the six days of MAT of W-36 pullets at two cage stocking densities (SD): hen age = 4 - 5 wk; hen body weight = 220 - 329 g; HD = 155 cm^2/bird; LD = 206 cm^2/bird.

Variable	**Period**	**SD**	**Manure accumulation time (MAT-d)**						
			1	2	3	4	5	6	Overall
Fresh manure weight (g/hen-period)	Light	HD	36±8	38±21	29±8	32±10	44±7	39±6	36±18
		LD	44±8	75±21	51±8	55±10	57±7	48±6	55±18
	Dark	HD	4±1	5±1	6.8±0.5	4±1	6±1	5.5±0.5	5±1
		LD	5±1	4±1	6.7±0.5	5±1	6±1	6.8±0.5	6±1
	Daily	HD	41±9	42±21	36±9	36±11	50±8	44±7	42±19
		LD	49±9	79±21	58±9	60±11	62±8	55±7	61±19
Feed disappearance (g/hen-period)	Light	HD	29±2	30±2	29±3	30±3	30±4	27±2	29±3
		LD	27±2	29±2	27±3	30±3	30±4	30±2	29±3
	Dark	HD	0.7±0.4	0.3±0.1	1±1	0.18±0.04	0.2±0.1	3.3±0.2	0.9±0.4
		LD	1.1±0.4	0.4±0.1	1±1	0.20±0.04	0.3±0.1	3.1±0.2	1.0±0.4
	Daily	HD	30±3	30±2	30±3	30±4	30±4	30±2	30±3
		LD	28±3	29±2	28±3	30±4	31±4	33±2	30±3
Daily NH_3 ER (mg/hen-period) (clean system)	Light	HD	0.03±0.04	0.06±0.12	0.20±0.07	0.4±0.2	1.2±0.6	4±1	-
		LD	0.01±0.04	0.03±0.12	0.07±0.07	0.2±0.2	0.7±0.6	2±1	-
	Dark	HD	0.02±0.02	0.04±0.30	0.10±0.02	0.20±0.04	0.8±0.3	2±1	-
		LD	0.01±0.02	0.02±0.30	0.03±0.02	0.10±0.04	0.3±0.3	1±1	-
	Daily	HD	0.05±0.07	0.10±0.18	0.3±0.1	0.6±0.2	2±1	6±2	-
		LD	0.02±0.07	0.05±0.18	0.1±0.1	0.3±0.2	1±1	3±2	-
Daily NH_3 ER (mg/hen-period) (non-clean system)	Light	HD	-	-	-	-	-	-	-
		LD	-	-	-	-	-	-	-
	Dark	HD	-	-	-	-	-	-	-
		LD	-	-	-	-	-	-	-
	Daily	HD	-	-	-	-	-	-	-
		LD	-	-	-	-	-	-	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$, and **x** and **y** for $P \leq 0.01$. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 4. Estimated means and SEs from the two-way ANOVA test for a block design of: fresh manure production, feed disappearance and NH_3 ER for 'clean' and 'non-clean' system during the light, dark and daily periods (14 L vs. 10 D) along the six days of MAT of W-36 pullets at two cage stocking densities (SD): hen age = 8 - 9 wk; hen body weight = 580 - 670 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird.

Variable	Period	SD	Manure accumulation time (MAT-d)						
			1	2	3	4	5	6	Overall
Fresh manure weight (g/hen-period)	Light	HD	35±9	48±7	52±7	44±14	46±12	39±12	44±10
		LD	42±9	52±7	51±7	47±14	51±12	48±12	49±10
	Dark	HD	15±4	23±2	27±5	12±4	11±4	12±4	17±4
		LD	23±4	22±2	17±5	20±4	15±4	25±4	20±4
	Daily	HD	50±13	71±8	79±10	56±17	57±8	51±15	61±14
		LD	65±13	74±8	68±10	67±17	66±8	73±15	69±14
Feed disappearance (g/hen-period)	Light	HD	38±10	45±5	35±8	38±6	35±8	38±3	38±7
		LD	41±10	38±5	43±8	43±6	45±8	44±3	42±7
	Dark	HD	6±2	7±3	9±4	6±2	8±6	8±8	7.3±5
		LD	3±2	2±3	4±4	4±2	1±6	3±8	2.8±5
	Daily	HD	44±11	45±7	44±7	44±6	43±8	46±7	46±7
		LD	44±11	47±7	47±7	47±6	46±8	47±7	45±7
Daily NH_3 ER (mg/hen-period) (clean system)	Light	HD	0.07±0.30	0.1±0.2	0.6±0.7	2±2	8±3	17±4	-
		LD	0.20±0.30	0.4±0.2	0.6±0.7	2±2	3±3	15±4	-
	Dark	HD	0.03±0.05	0.1±0.1	1.4±0.4	2.7±0.5	3±1	13±1	-
		LD	0.10±0.05	0.2±0.1	0.4±0.4	1.6±0.5	2±1	11±1	-
	Daily	HD	0.1±0.4	0.2±0.4	2±1	5±3	11±5	30±5	-
		LD	0.3±0.4	0.6±0.4	1±1	4±3	5±5	26±5	-
Daily NH_3 ER (mg/hen-period) (non-clean system)	Light	HD	13±2	1.2±0.3	3±1	8±2	17±2	16±	-
		LD	8±2	0.3±0.3	1±1	3±2	9±2	10±4	-
	Dark	HD	6±2	0.4±0.2	1.9±0.7	4±1	10±1	8±1	-
		LD	5±2	0.1±0.2	0.8±0.7	2±1	7±1	5±1	-
	Daily	HD	19±4	1.6±0.5	5±1	12±3	27±3	24±5	-
		LD	13±4	0.4±0.5	2±1	5±3	16±3	15±5	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$, and **x** and **y** for $P \leq 0.01$. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 5. Estimated means and SEs from the two-way ANOVA test for a block design of: fresh manure production, feed disappearance and NH_3 ER for 'clean' and 'non-clean' system during the light, dark and daily periods (13 L vs. 11 D) along the six days of MAT of W-36 pullets at two cage stocking densities (SD): hen age = 12 - 13 wk; hen body weight = 1000 – 1030 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird.

Variable	Period	SD	Manure accumulation time (MAT-d)						
			1	2	3	4	5	6	Overall
Fresh manure weight (g/hen-period)	Light	HD	63±6	64±6	65±5	62±6	67±6	62±4	60±5
		LD	61±6	65±6	80±5	72±6	62±6	65±4	67±5
	Dark	HD	15±1	16±1	15±1	16±1	12±3	13±1	14±2
		LD	13±1	14±1	16±1	13±1	12±3	14±1	16±2
	Daily	HD	79±6	80±7	80±5	78±6	79±8	75±5	74±6
		LD	74±6	79±7	96±5	85±6	74±8	79±5	83±6
Feed disappearance (g/hen-period)	Light	HD	48±10	47±5	48±8	49±6	46±8	37±3	46±7
		LD	45±10	49±5	45±8	50±6	41±8	38±3	45±7
	Dark	HD	6±2	10±3	9±4	4±2	8±6	11±8	8.0±5
		LD	3±2	8±3	7±4	6±2	6±6	8±8	6.3±5
	Daily	HD	54±11	57±7	57±7	53±6	54±8	48±7	54±8
		LD	48±11	57±7	52±7	56±6	47±8	46±7	51±8
Daily NH_3 ER (mg/hen-period) (clean system)	Light	HD	0.2±0.1	0.21±0.10	0.7±0.1	2±1	11±3	25±6	-
		LD	0.1±0.1	0.07±0.10	0.4±0.1	1±1	10±3	15±6	-
	Dark	HD	0.10±0.05	0.09±0.03	0.31±0.04	0.9±0.3	5.0±0.5	11±1	-
		LD	0.04±0.05	0.03±0.03	0.20±0.04	0.6±0.3	4.0±0.5	8±1	-
	Daily	HD	0.3±0.2	0.3±0.1	1.0±0.1a	3±1	16±4	36±8	-
		LD	0.1±0.2	0.1±0.1	0.6±0.1b	2±1	14±4	23±8	-
Daily NH_3 ER (mg/hen-period) (non-clean system)	Light	HD	13±3	8±2	13±4	40±6 a	48±9 a	40±13	-
		LD	11±3	3±2	5±4	11±6 b	15±9 b	22±13	-
	Dark	HD	5±1	2±1	5±2	4±3	5±3	21±6	-
		LD	4±1	1±1	1±2	4±3	7±3	12±6	-
	Daily	HD	18±4	10±2	18±5	44±9	53±11	61±18	-
		LD	15±4	4±2	6±5	15±9	22±11	34±18	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$, and **x** and **y** for $P \leq 0.01$. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 6. Estimated means and SEs from the two-way ANOVA test for a block design of: fresh manure production, feed disappearance and NH_3 ER for 'clean' and 'non-clean' system during the light, dark and daily periods (13 L vs. 11 D) along the six days of MAT of W-36 pullets at two cage stocking densities (SD): hen age = 16 - 17 wk; hen body weight = 1015 - 1018 g; HD = 310 cm^2/bird; LD = 413 cm^2/bird.

Variable	Period	SD	Manure accumulation time (MAT-d)						
			1	2	3	4	5	6	Overall
Fresh manure weight (g/hen-period)	Light	HD	74±6	79±8	73±6	74±4	74±6	83±5	76±6
		LD	70±6	80±8	78±6	80±4	89±6	77±5	79±6
	Dark	HD	17±1	19±5	19±2	17±2	15±3	18±2	18±3
		LD	14±1	22±5	21±2	22±2	15±3	17±2	19±3
	Daily	HD	91±7	98±12	92±7	91±7	89±6	101±7	94±8
		LD	84±7	102±12	99±7	102±7	104±6	94±7	98±8
Feed disappearance (g/hen-period)	Light	HD	59±10	61±5	58±8	54±6	58±8	55±3	58±7
		LD	52±10	64±5	58±8	63±6	53±8	58±3	58±7
	Dark	HD	6±2	6±3	9±4	9±2	6±6	3±8	6.5±5
		LD	3±2	3±3	4±4	3±2	10±6	6±8	4.8±5
	Daily	HD	65±11	67±7	67±7	63±6	64±8	58±7	64±7
		LD	55±11	67±7	62±7	66±6	63±8	64±7	63±7
Daily NH_3 ER (mg/hen-period) (clean system)	Light	HD	1.3±0.03 b	1.5±0.1	1.5±0.2	3±2	31±7	57±14	-
		LD	1.43±0.03 a	1.2±0.1	1.1±0.2	1±2	16±7	38±14	-
	Dark	HD	0.71±0.02 a	0.9±0.1 a	0.9±0.1	2.0±0.4	18±4	33±6	-
		LD	0.63±0.02 b	0.6±0.1 b	0.7±0.1	0.9±0.4	10±4	19±6	-
	Daily	HD	2.00±0.03	2.4±0.1 a	2.4±0.2	5±2	49±13	90±21	-
		LD	2.03±0.03	1.8±0.1 b	1.8±0.2	2±2	26±13	57±21	-
Daily NH_3 ER (mg/hen-period) (non-clean system)	Light	HD	79±19	21±3	21±5	26±7	30±8	51±11	-
		LD	59±19	21±3	18±5	18±7	25±8	39±11	-
	Dark	HD	49±4	9±1	11±1	15±2	19±3	37±4	-
		LD	43±4	7±1	9±1	11±2	13±3	23±4	-
	Daily	HD	128±25	30±5	32±6	41±10	49±10	88±14	-
		LD	102±25	28±5	27±6	29±10	38±10	62±14	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01 < P \leq 0.05$, and **x** and **y** for $P \leq 0.01$. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

Table 7. Estimated means and SEs from the two-way ANOVA test for a block design of: fresh manure production, feed disappearance and NH_3 ER for 'clean' and 'non-clean' system during the light, dark and daily periods (16 L vs. 8 D) along the six days of MAT of W-36 hens at two cage stocking densities (SD): hen age = 23-34 wk; hen body weight = 1351 - 1564 g; HD = 413 cm^2/bird; LD = 620 cm^2/bird.

Variable	Period	SD	Manure accumulation time (MAT-d)						
			1	2	3	4	5	6	Overall
Fresh manure weight (g/hen-period)	Light	HD	100±8	127±17	131±16	120±14	97±5	92±3	111±10
		LD	107±8	127±17	98±16	108±14	103±5	95±3	106±10
	Dark	HD	29±2	29±1 a	31±2	29±1 a	28±1 a	26±1	29±1
		LD	24±2	24±1 b	24±2	25±1 b	24±1 b	25±1	24±1
	Daily	HD	129±8	156±17	162±18	149±15	125±5	118±3	140±11
		LD	131±8	150±17	122±18	132±15	127±5	120±3	131±11
Feed disappearance (g/hen-period)	Light	HD	87±7	85±5	96±5	77±7	91±5	90±6	88±6
		LD	106±7	101±5	104±5	76±7	89±5	91±6	95±6
	Dark	HD	1±1	1±1	1.1±0.3	0.9±0.3	3.1±0.4 a	1±1	1.4±1
		LD	2±1	3±1	1.1±0.3	1.2±0.3	1.1±0.4 b	3±1	1.9±1
	Daily	HD	88±7	86±5 a	97±5	78±6	94±5	91±6	89±7
		LD	108±7	104±5 b	105±5	77±6	90±5	94±6	96±7
Daily NH_3 ER (mg/hen-period) (clean system)	Light	HD	3±2	8±2	25±7	61±10	108±17	168±24	-
		LD	4±2	7±2	18±7	39±10	72±17	102±24	-
	Dark	HD	2±1	4±1	16±2	37±4	71±9	83±8	-
		LD	3±1	5±1	11±2	25±4	42±9	58±8	-
	Daily	HD	5±3	12±4	41±9	98±13	179±26	251±33	-
		LD	7±3	12±4	29±9	64±13	114±26	160±33	-
Daily NH_3 ER (mg/hen-period) (non-clean system)	Light	HD	21215±	36±4	28±2	51±5	88±13	158±16	-
		LD	165±15	22±4	14±2	33±5	63±13	99±16	-
	Dark	HD	137±14	21±3	17±1	32±4	67±5	87±8	-
		LD	95±14	95±3	95±1	95±4	95±5	95±8	-
	Daily	HD	349±29	57±6	45±3	83±8	245±19	245±27	-
		LD	260±29	38±6	25±3	56±8	171±19	171±27	-

Values for the two stocking densities of each variable followed by different letters are significantly different (**a** and **b** for $0.01< P \leq 0.05$, and **x** and **y** for $P \leq 0.01$. Overall values for variables that have a cumulative nature wouldn't make sense and thus they were not shown in the table above.

CHAPTER 4. GENERAL CONCLUSIONS

This thesis research was conducted to fulfill two main objectives. The first objective was to delineate the magnitude of NH_3 emission rate (ER) of pullets and laying hens as affected by bird age, manure accumulation time (MAT) and stocking density (SD). The second objective was to investigate the dynamics of feeding, defecation and NH_3 emissions of birds housed under different SD regimens and different MAT. Tests were conducted in a laboratory setup that resembled the conditions of manure-belt (MB) laying-hen housing systems.

The first objective was accomplished by monitoring the emissions from pullets (4 to 18 weeks of age) and laying hens (23 to 37 weeks of age) at different SDs (155 to 619 cm^2 $bird^{-1}$) from 1- to 6-d MAT. NH_3 ER was expressed in several units, including the amount of NH_3 emission per bird, per kg of feed nitrogen (N) disappearance, and per kg of 'as-is' and dry manure weight and g/AU (animal unit). The study revealed the following:

- Ammonia (NH_3) emission of pullets and laying hens increases with bird age and manure accumulation time, following an exponential pattern.
- SD effect on NH_3 emission became more pronounced for MAT $\geq$ 3 d.
- Daily NH_3 emission increases with age and manure accumulation time, but tends to decrease with increasing floor space allocation to the hens.

The second objective was accomplished by continuously measuring feed disappearance and 'as-is' manure production. An algorithm was developed and validated to calculate the fresh manure production (g/hen-h) from 'as-is' manure weight readings by accounting for the moisture loss from manure through evaporation. Feed disappearance, fresh manure production and hourly NH_3 ER of the pullets and layers were partitioned according to dark and light periods for the same SD regimens as in the first objective and for 1- to 6-d MAT. The following conclusions were drawn:

- SD did not impact feed disappearance (P = 0.46 – 0.81) or fresh manure production (P = 0.17 – 0.72). Each gram of feed use yields 0.53 to 1.15 g of fresh manure.
- Feed use during the dark period was minimal, while some fresh manure was still being produced, ranging 0.91 to 3.4 g/bird-h. The partitioning of feed use into light and dark periods was 92 % and 8 %, respectively; whereas the concomitant partitioning of fresh manure production was 80 % and 20 % respectively.
- The partitioning for daily NH_3 ER between light and dark hours was 63 % and 37 % of the total daily emissions, respectively.

APPENDIX 1.

CR10 PROGRAM USED IN THE STUDY

```
*Table 1 Program
  01: 1          Execution Interval (seconds)

1:  Batt Voltage (P10)
 1: 1        Loc [ Batt_Volt ]

2:  If time is (P92)
 1: 01       Minutes (Seconds --) into a
 2: 1440     Interval (same units as above)
 3: 30       Then Do

3:  Signature (P19)
 1: 2        Loc [ Prog_Sig  ]

4:  End (P95)


5:  Do (P86)
 1: 1        Call Subroutine 1


6:  Do (P86)
 1: 45       Set Port 5 High

7:  Beginning of Loop (P87)
 1: 0        Delay
 2: 4        Loop Count

8:  Do (P86)
 1: 74       Pulse Port 4

9:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

10:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 3     -- Loc [ Flow_1    ]
 5: 0.044    Mult
 6: 0.1112   Offset

11:  End (P95)

12:  Do (P86)
 1: 74       Pulse Port 4

13:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
```

```
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

14:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 7        Loc [ NH3       ]
 5: 0.032    Mult
 6: -12.633  Offset

15:  Do (P86)
 1: 74       Pulse Port 4

16:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

17:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 8        Loc [ CO2       ]
 5: 5.0667   Mult
 6: -2021.6  Offset

18:  Do (P86)
 1: 74       Pulse Port 4

19:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

20:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 9        Loc [ dP        ]
 5: 0.076    Mult
 6: -89.227  Offset

21:  Do (P86)
 1: 74       Pulse Port 4

22:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

23:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 10       Loc [ FB_1      ]
 5: 1.911    Mult
 6: 26.355   Offset
```

```
24:  Do (P86)
 1: 74       Pulse Port 4

25:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

26:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 11    -- Loc [ FB_2      ]
 5: 2.3544   Mult
 6: -465.18  Offset

27:  Do (P86)
 1: 74       Pulse Port 4

28:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

29:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 12       Loc [ FB_3      ]
 5: 2.4734   Mult
 6: -508.08  Offset

30:  Do (P86)
 1: 74       Pulse Port 4

31:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

32:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 13    -- Loc [ FB_4      ]
 5: 2.5066   Mult
 6: -507.81  Offset

33:  Do (P86)
 1: 74       Pulse Port 4

34:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation
```

```
35:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 14       Loc [ MB_1      ]
 5: 1.9945   Mult
 6: 34.354   Offset

36:  Do (P86)
 1: 74       Pulse Port 4

37:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

38:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 15    -- Loc [ MB_2      ]
 5: 2.3489   Mult
 6: -467.39  Offset

39:  Do (P86)
 1: 74       Pulse Port 4

40:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

41:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 16    -- Loc [ MB_3      ]
 5: 2.4708   Mult
 6: -509.82  Offset

42:  Do (P86)
 1: 74       Pulse Port 4

43:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

44:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 17    -- Loc [ MB_4      ]
 5: 1.9072   Mult
 6: 24.022   Offset

45:  Do (P86)
```

```
 1: 74       Pulse Port 4

46:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

47:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 39       Loc [ Dewpoint  ]
 5: 0.0218   Mult
 6: -9.9804  Offset

48:  Do (P86)
 1: 74       Pulse Port 4

49:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 0        Delay W/Ex (units = 0.01 sec)
 3: 1        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

50:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 1        DIFF Channel
 4: 40       Loc [ O2        ]
 5: 1        Mult
 6: 20.091   Offset


51:  Do (P86)
 1: 55       Set Port 5 Low

52:  Temp (107) (P11)
 1: 1        Reps
 2: 3        SE Channel
 3: 1        Excite all reps w/E1
 4: 18       Loc [ Temp_0    ]
 5: 1.0      Mult
 6: 0.0      Offset

53:  Do (P86)
 1: 47       Set Port 7 High

54:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 15       Delay W/Ex (units = 0.01 sec)
 3: 0        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

55:  Volt (SE) (P1)
 1: 1        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 4        SE Channel
 4: 19       Loc [ RH_0      ]
 5: 1        Mult
 6: 0        Offset
```

```
56:  Do (P86)
 1: 57       Set Port 7 Low


57:  Temp (107) (P11)
 1: 4        Reps
 2: 5        SE Channel
 3: 1        Excite all reps w/E1
 4: 20       Loc [ Temp_1    ]
 5: 1.0      Mult
 6: 0.0      Offset

58:  Do (P86)
 1: 48       Set Port 8 High

59:  Excitation with Delay (P22)
 1: 1        Ex Channel
 2: 15       Delay W/Ex (units = 0.01 sec)
 3: 0        Delay After Ex (units = 0.01 sec)
 4: 0        mV Excitation

60:  Volt (SE) (P1)
 1: 4        Reps
 2: 25       2500 mV 60 Hz Rejection Range
 3: 9        SE Channel
 4: 24       Loc [ RH_1      ]
 5: 1        Mult
 6: 0        Offset

61:  Do (P86)
 1: 58       Set Port 8 Low

62:  If time is (P92)
 1: 0     -- Minutes (Seconds --) into a
 2: 5        Interval (same units as above)
 3: 10       Set Output Flag High (Flag 0)

63:  Set Active Storage Area (P80)
 1: 1        Final Storage Area 1
 2: 101      Array ID

64:  Real Time (P77)
 1: 1111     Year,Day,Hour/Minute,Seconds (midnight = 0000)

65:  Average (P71)
 1: 4        Reps
 2: 3        Loc [ Flow_1    ]

66:  Average (P71)
 1: 1        Reps
 2: 7        Loc [ NH3       ]

67:  Average (P71)
 1: 1        Reps
 2: 8        Loc [ CO2       ]

68:  Average (P71)
 1: 1        Reps
 2: 9        Loc [ dP        ]
```

```
69:  Average (P71)
 1: 8        Reps
 2: 10       Loc [ FB_1      ]

70:  Average (P71)
 1: 10       Reps
 2: 18       Loc [ Temp_0    ]

71:  Sample (P70)
 1: 5        Reps
 2: 34       Loc [ Valve_0   ]


*Table 2 Program
  01: 1         Execution Interval (seconds)

1:  Serial Out (P96)
 1: 71       Storage Module

*Table 3 Subroutines

1:  Beginning of Subroutine (P85)
 1: 1        Subroutine 1

2:  If time is (P92)
 1: 0        Minutes (Seconds --) into a
 2: 39       Interval (same units as above)
 3: 30       Then Do

3:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 34       Z Loc [ Valve_0   ]

4:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 35       Z Loc [ Valve_1   ]

5:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 36       Z Loc [ Valve_2   ]


6:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 37       Z Loc [ Valve_3   ]


7:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 38       Z Loc [ Valve_4   ]


8:  End (P95)

9:  If time is (P92)
 1: 10       Minutes (Seconds --) into a
```

```
 2: 49       Interval (same units as above)
 3: 30       Then Do

10:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 34       Z Loc [ Valve_0   ]

11:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 35       Z Loc [ Valve_1   ]


12:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 36       Z Loc [ Valve_2   ]

13:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 37       Z Loc [ Valve_3   ]


14:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 38       Z Loc [ Valve_4   ]

15:  End (P95)

16:  If time is (P92)
 1: 20       Minutes (Seconds --) into a
 2: 49       Interval (same units as above)
 3: 30       Then Do

17:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 34       Z Loc [ Valve_0   ]

18:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 35       Z Loc [ Valve_1   ]


19:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 36       Z Loc [ Valve_2   ]

20:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 37       Z Loc [ Valve_3   ]


21:  Z=F (P30)
 1: 0        F
```

```
 2: 0        Exponent of 10
 3: 38       Z Loc [ Valve_4   ]
22:  End (P95)

23:  If time is (P92)
 1: 30       Minutes (Seconds --) into a
 2: 49       Interval (same units as above)
 3: 30       Then Do

24:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 34       Z Loc [ Valve_0   ]

25:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 35       Z Loc [ Valve_1   ]


26:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 36       Z Loc [ Valve_2   ]

27:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 37       Z Loc [ Valve_3   ]


28:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 38       Z Loc [ Valve_4   ]

29:  End (P95)

30:  If time is (P92)
 1: 40       Minutes (Seconds --) into a
 2: 49       Interval (same units as above)
 3: 30       Then Do

31:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 34       Z Loc [ Valve_0   ]

32:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 35       Z Loc [ Valve_1   ]


33:  Z=F (P30)
 1: 0        F
 2: 0        Exponent of 10
 3: 36       Z Loc [ Valve_2   ]

34:  Z=F (P30)
 1: 0        F
```

```
 2: 0        Exponent of 10
 3: 37       Z Loc [ Valve_3   ]


35:  Z=F (P30)
 1: 1        F
 2: 0        Exponent of 10
 3: 38       Z Loc [ Valve_4   ]

36:  End (P95)


37:  SDM-CD16 / SDM-CD16AC (P104)
 1: 1        Reps
 2: 02       SDM Address
 3: 34       Loc [ Valve_0   ]



38:  End (P95)

End Program

-Input Locations-
1 Batt_Volt 1 0 1
2 Prog_Sig  1 0 1
3 Flow_1    1 1 1
4 Flow_2    0 0 0
5 Flow_3    0 0 0
6 Flow_4    0 0 0
7 NH3       1 1 1
8 CO2       1 1 1
9 dP        1 1 1
10 FB_1      1 1 1
11 FB_2      1 0 1
12 FB_3      1 0 1
13 FB_4      1 0 1
14 MB_1      1 0 2
15 MB_2      1 0 0
16 MB_3      1 0 2
17 MB_4      1 0 2
18 Temp_0    1 1 1
19 RH_0      1 0 1
20 Temp_1    5 0 1
21 Temp_2    9 0 1
22 Temp_3    9 0 1
23 Temp_4    17 0 1
24 RH_1      5 0 1
25 RH_2      1 0 1
26 RH_3      9 0 1
27 RH_4      17 0 1
28 _________ 1 1 0
29 _________ 1 1 1
30 _________ 1 2 3
31 _________ 1 5 1
32 _________ 1 1 2
34 Valve_0   5 2 7
35 Valve_1   9 2 7
36 Valve_2   1 3 7
37 Valve_3   1 4 7
38 Valve_4   1 5 8
```

```
39 Dewpoint  1 0 1
40 O2        1 0 1
45 _________ 1 0 0
46 _________ 1 0 0
47 _________ 1 0 0
48 _________ 1 0 0
49 _________ 1 0 0
50 _________ 1 0 0
51 _________ 1 0 0
52 _________ 1 0 0
-Program Security-
0000
0000
0000
-Mode 4-
-Final Storage Area 2-
```

APPENDIX 2.

LABORATORY CALIBRATIONS OF THE MASS FLOW METERS (MFMs) BASED ON THE FACTORY-CALIBRATED REFERENCE MFM

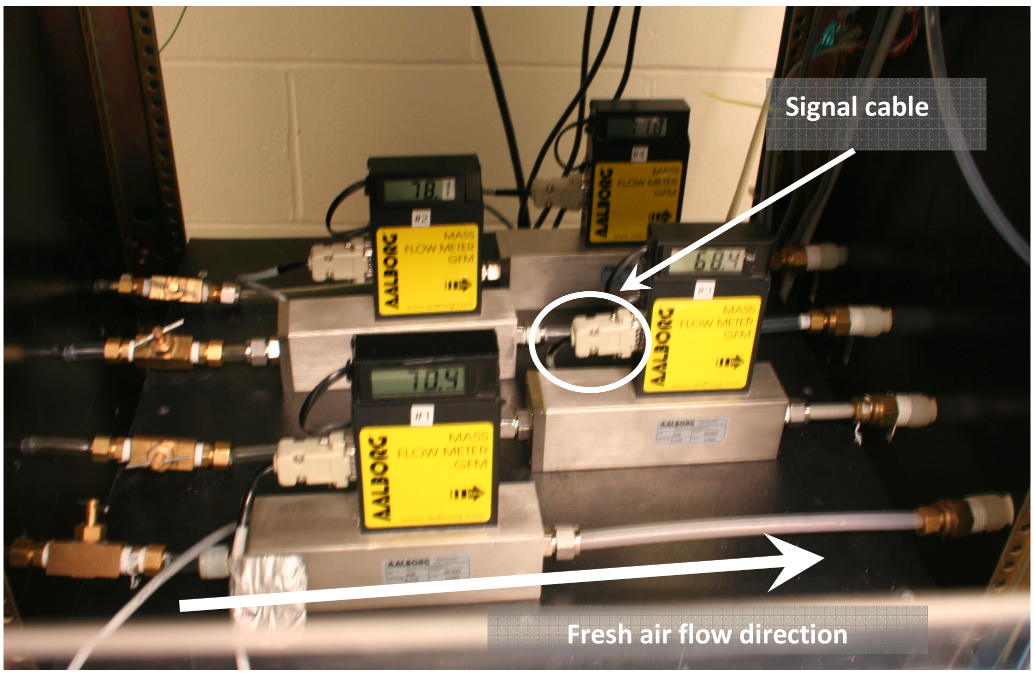

Figure 1. Pictorial representation of the four mass flow meters (MFM) for control and measurement of mass flow rates of fresh air into the chambers of the ISU multi-chamber system for air emissions measurement.

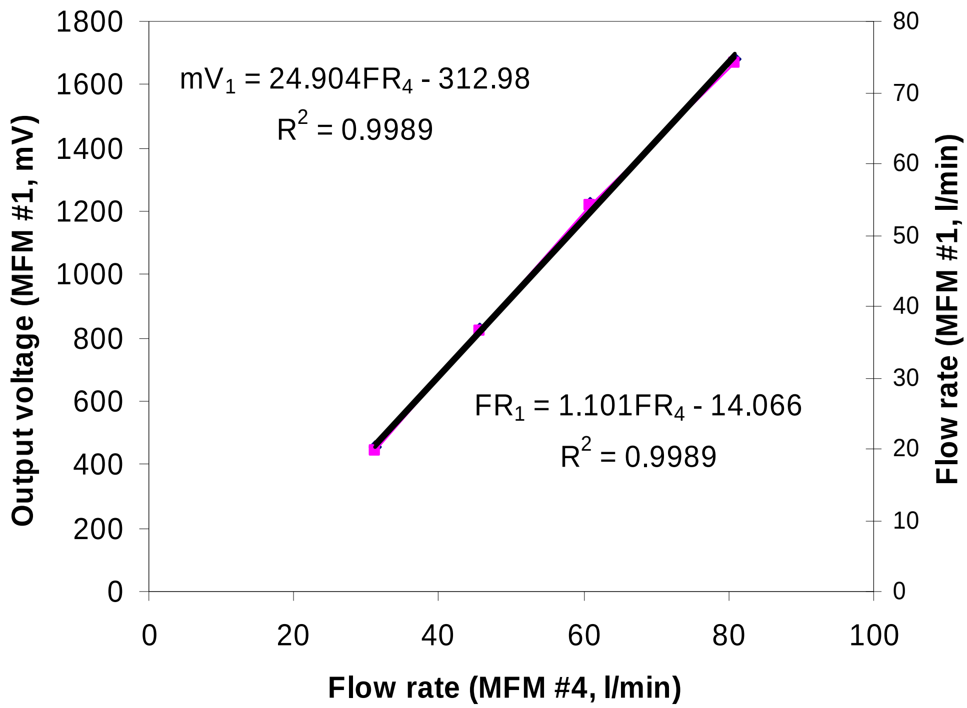

Figure 2. Calibration equations and correlation coefficients of the output voltage and display readings of mass flow meter #1 as a function of the display reading of mass flow meter #4 (calibrated by factory).

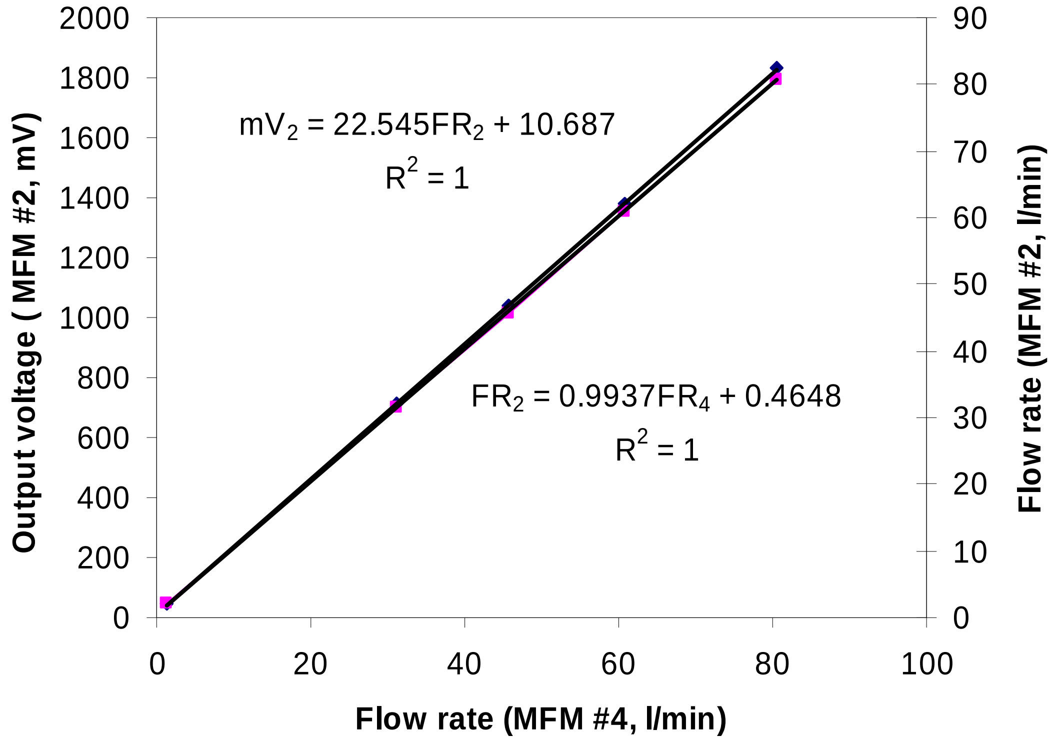

Figure 3. Calibration equations and correlation coefficients of the output voltage and display readings of mass flow meter #2 as a function of the display reading of mass flow meter #4 (calibrated by factory).

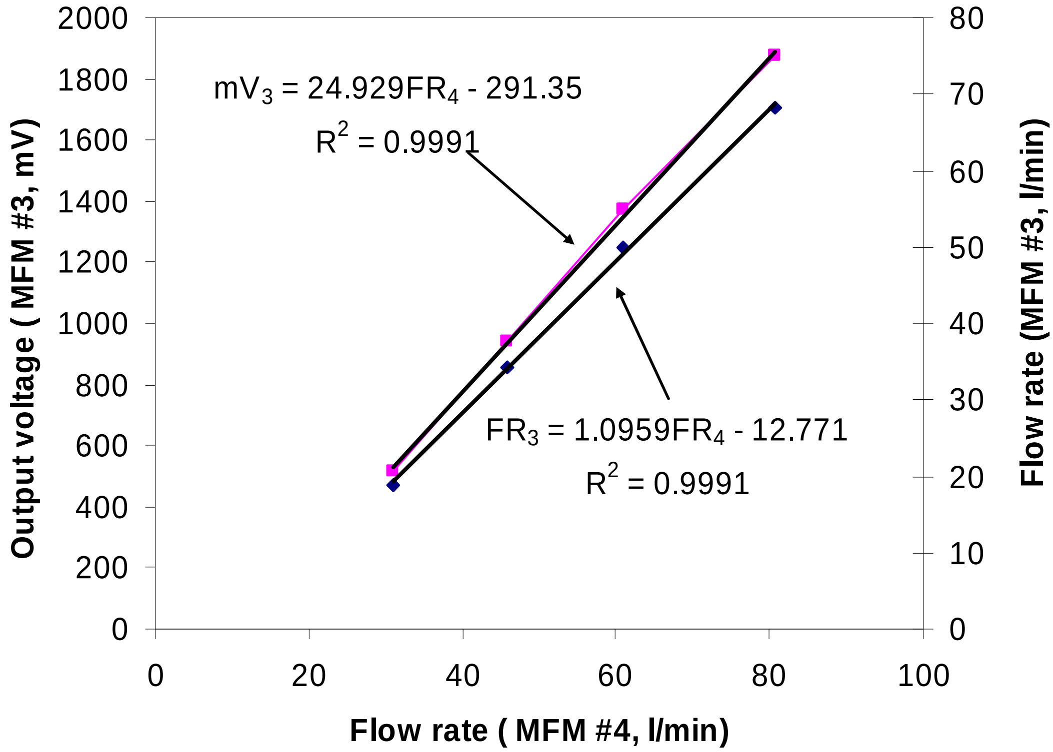

Figure 4. Calibration equations and correlation coefficients of the output voltage and display readings of mass flow meter #3 as a function of the display reading of mass flow meter #4 (calibrated by factory).

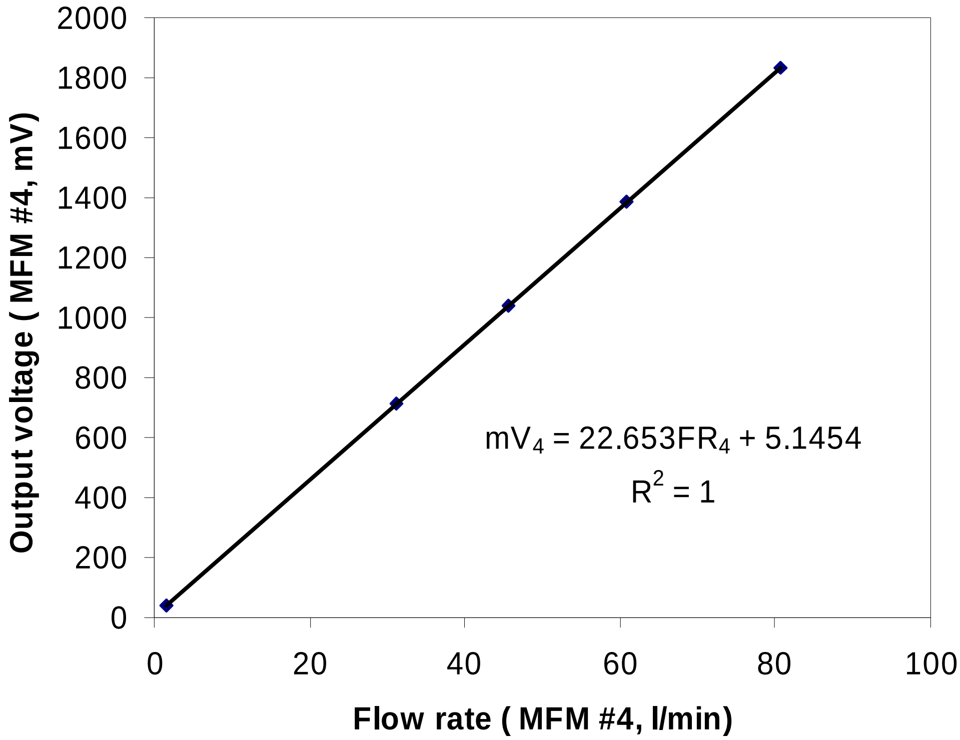

Figure 5. Calibration equation and coefficient of the correlation between the output voltage and display readings of mass flow meter #4 (calibrated by factory).

APPENDIX 3.

DYNAMIC BEHAVIOR OF THE SEMI-WEEKLY CALIBRATION OF THE INNOVA 1412 DURING THE COURSE OF SOME TRIALS

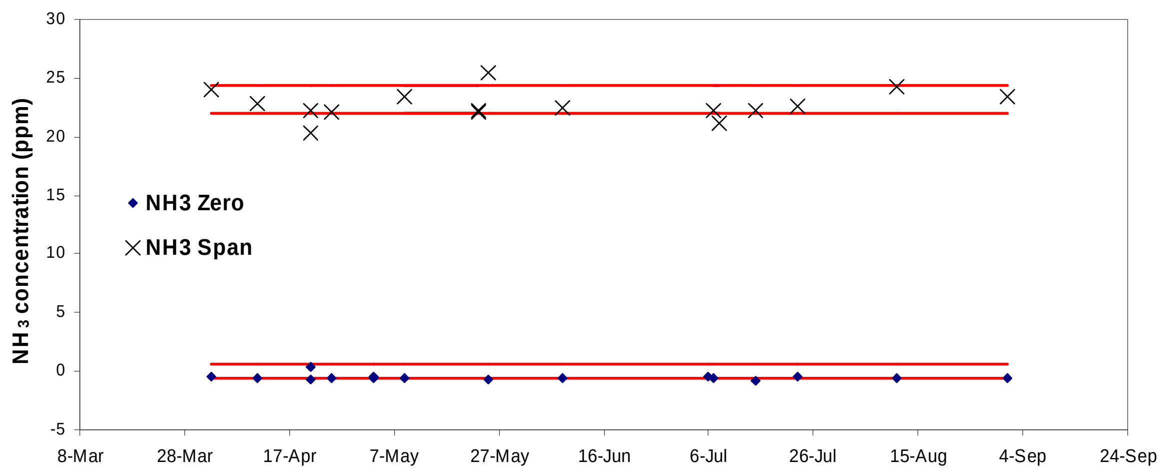

Figure 1. Behavior of the INNOVA 1412 when challenged and/or calibrated for NH_3 with zero (99.999% nitrogen) and span (23.2 ppm NH_3 balanced with nitrogen) during the course of some trials of the experiment. The lines represent a 0 ± 0.5 ppm NH_3 tolerance range for the zero gas and 23.2 ± 1.2 ppm NH_3 tolerance range for the span gas.

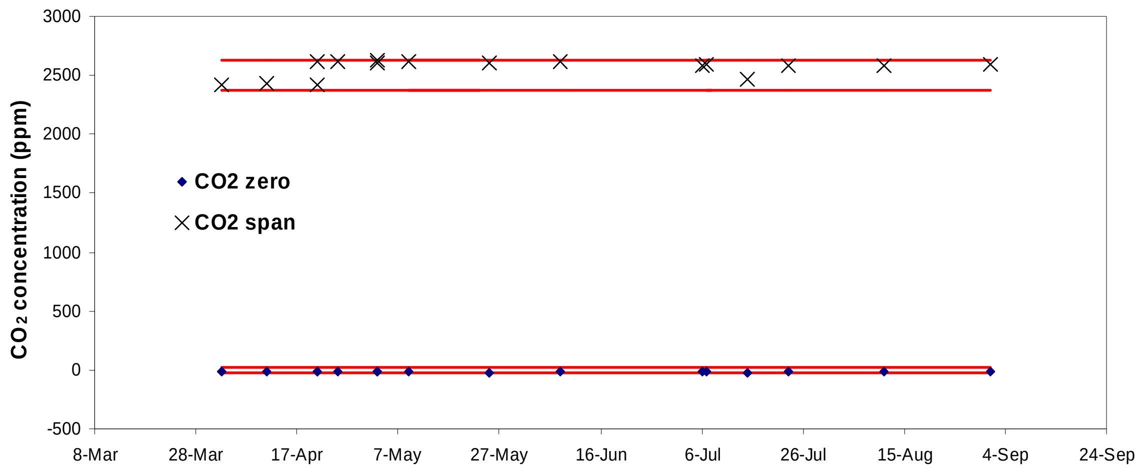

Figure 2. Behavior of the INNOVA 1412 when challenged and/or calibrated for CO_2 with zero (99.999% nitrogen) and span gas (2500 ppm CO_2 balanced with nitrogen) calibrations during the course of some trials of the experiment. The lines represent a 0 ± 25 ppm CO_2 tolerance range for the zero gas and 2500 ± 125 ppm CO_2 tolerance range for the span gas.

APPENDIX 4.

ASSESSMENT OF THE INTEGRITY OF THE ISU MULTI-CHAMBER AIR EMISSIONS MEASUREMENT SYSTEM THROUGH CO_2 RECOVERY TESTS

a) Experimental set up and data collection

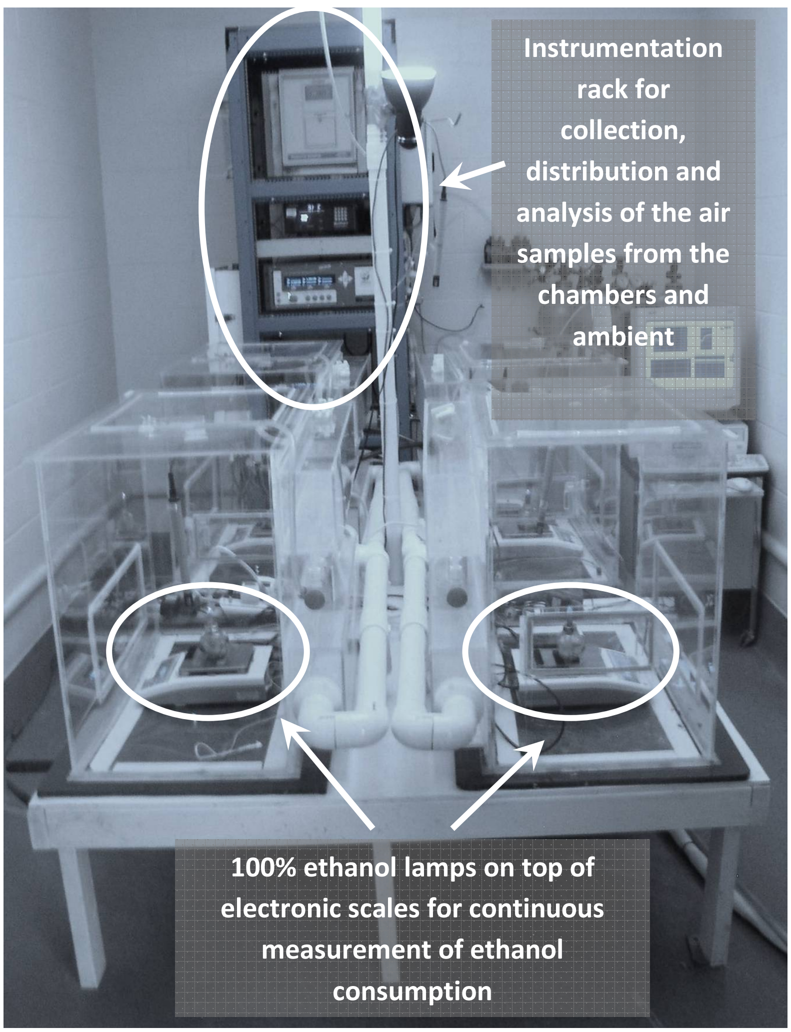

Figure 1. Illustration of the ISU Multi-chamber Air Emissions Measurement System set up for the CO_2 recovery test.

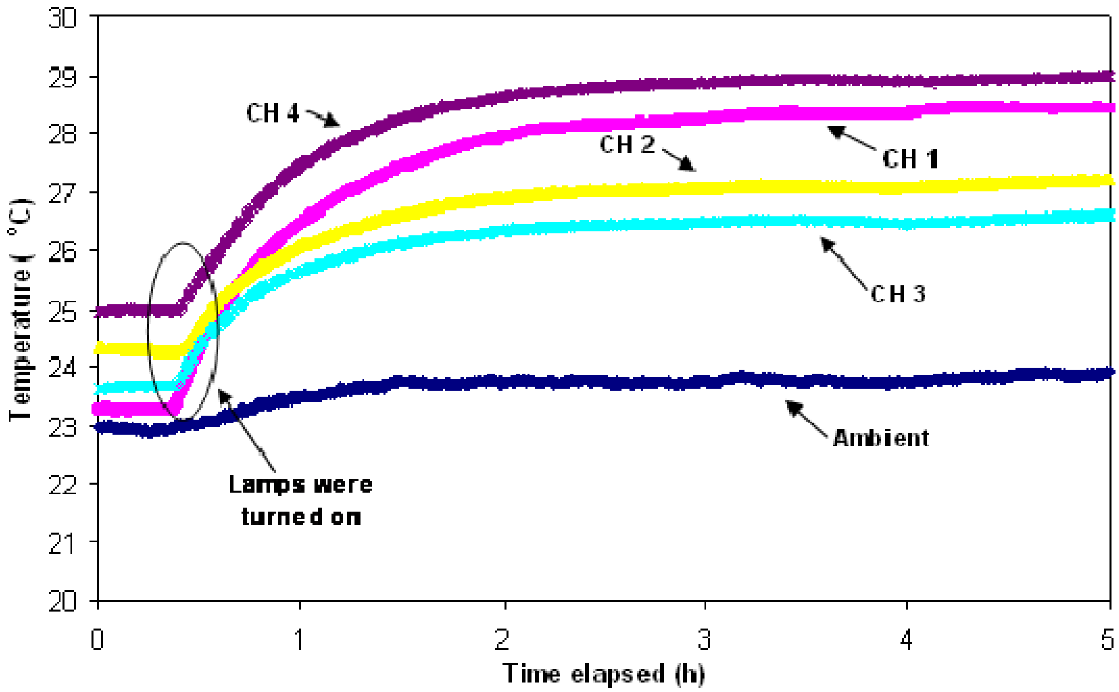

Figure 2. Sample of the dynamics of change in temperature in the ambient and inside chambers (CH) 1, 2, 3 and 4 after the ethanol were turned on for a CO_2 recovery trial. Air flow rate was constant and approximately equal to 75 l/min inside all four chambers.

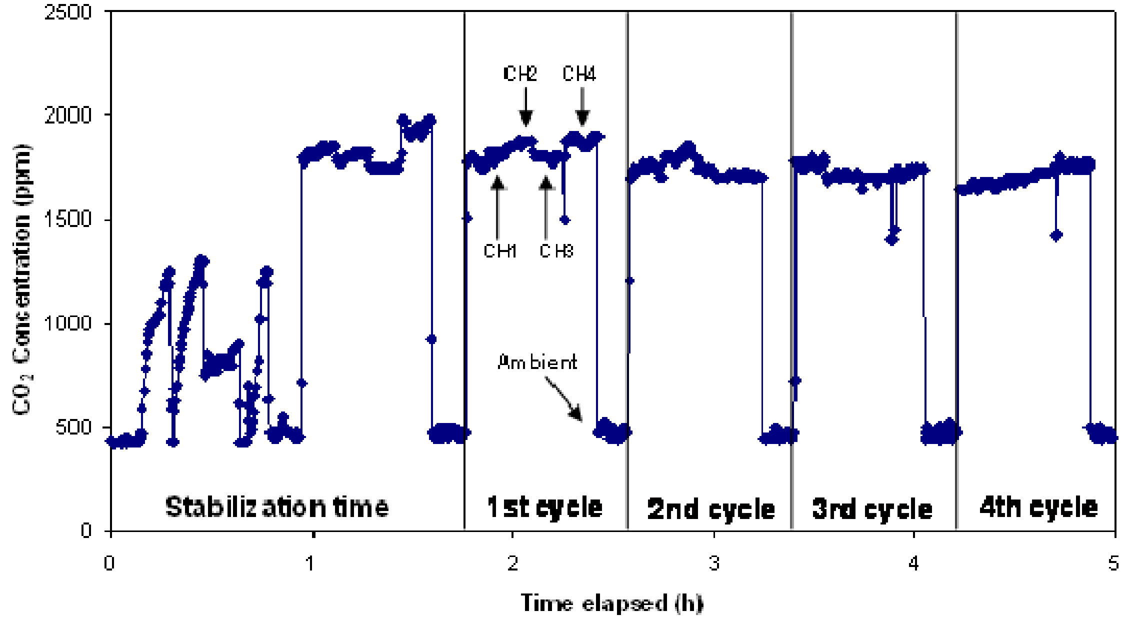

Figure 3. Sample of the dynamics of the carbon dioxide (CO_2) concentration in the ambient air and inside chambers (CH) 1, 2, 3 and 4 during several cycles of 50 min of a CO_2 recovery trial.

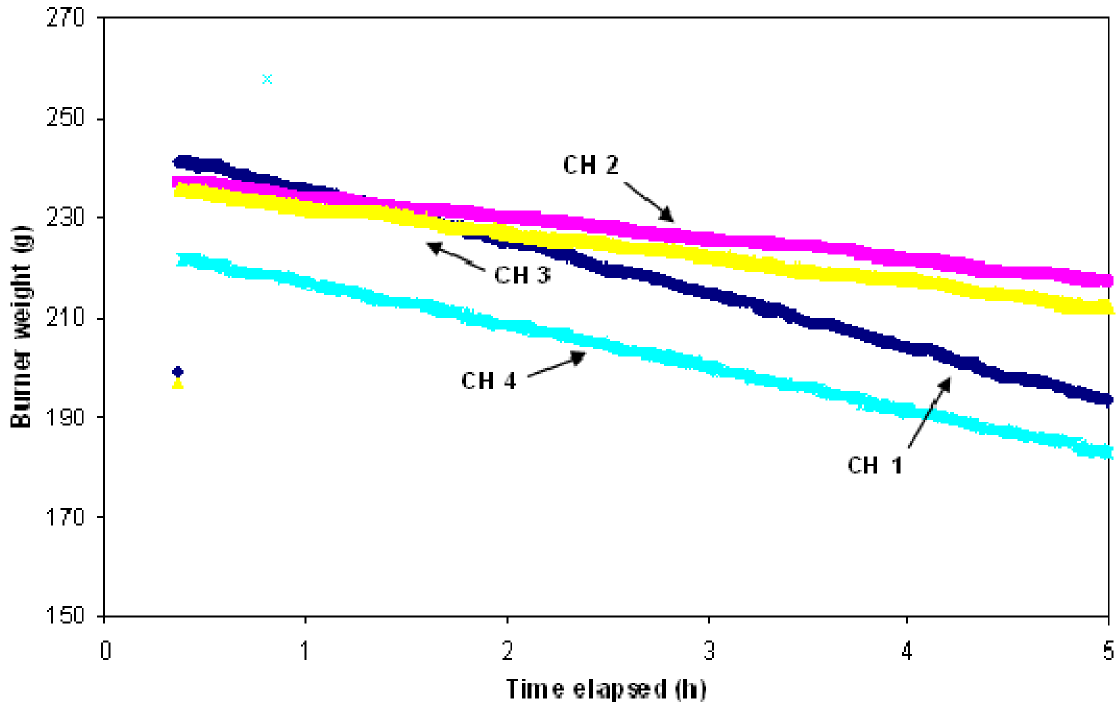

Figure 4. Dynamics of the change in the weight of the burner inside chambers (CH)1, 2, 3 and 4 indicating that the consumption of 100% ethanol occurred during a CO_2 recovery trial.

b) Algorithm used to estimate the CO_2 recovery:

(Equations presented by Scott & Hillman, 1983)

1. Given:

IO_2 (%) – O_2 concentration of the entering air (ambient);

EO_2 (%) – O_2 concentration inside the chamber after ethanol lamps burned at least 90 min;

ICO_2 (%) – CO_2 concentration of the entering air (ambient);

ECO_2 (%) - CO_2 concentration inside the chamber after ethanol lamps burned at least 90 min;

EFR (l/min) – Flow-rate;

EDB (^{o} C) – Dry-bulb temperature inside chamber;

CDB (^{o} C) – Chamber dry-bulb temperature;

CDP (^{o} C) – Chamber dew-point temperature;

BP (mmHg) – chamber barometric pressure;

ERH (%) – Chamber air relative humidity (calculated using EDB and DCP, assuming no condensation between chamber and flow meter;

Ees (mmHg) – saturated vapors pressure of exhaust air (determined at EDB);

COH (g) - amount of alcohol consumed

T (min) – time required for COH

2. General Equations

$$EFR_{STPD} = EFR.(BP - ERH.Ee_s)\frac{.273.16^o C}{760 mmHg} \quad \text{(Equation 1)}$$

$$IN_2 = 100 - IO_2 - ICO_2 \quad \text{(Equation 2)}$$

$$EN_2 = 100 - EO_2 - ECO_2 \quad \text{(Equation 3)}$$

$$IFR_{STPD} = \frac{EFR_{STPD}.EN_2}{IN_2} \quad \text{(Equation 4)}$$

$$VCO_2 = \frac{EFR_{STPD}.ECO_2}{100} - \frac{IFR_{STPD}.IO_2}{100} \quad \text{(Equation 5)}$$

$$PVO_2 = \frac{COH.(3\ mole)}{(46.0694\ g).(22.414 l\ mole^{-1})} \quad \text{(Equation 6)}$$

$$RCO_2 = \frac{VCO_2.T}{PVO_2}100 \quad \text{(Equation 7)}$$

Where:

EFR_{STPD} (l/min) – exhaust flow rate at standard temperature and pressure;

IN_2 (%) – incoming nitrogen;

EN_2 (5) – exhaust nitrogen;

IFR_{STPD} (l/min) – incoming flow rate; assumes temperature, barometric pressure and vapor pressure same as exhaust air;

VO_2 (l/min) – rate of oxygen consumption;

VCO2 (l/min) – rate of carbon dioxide production;

RQ (non dimensional) – respiratory quotient

PVO_2 (l) – estimated volume of oxygen consumed by ethanol lamp, assumes behaves as an ideal gas;

$PVCO_2$ (l) – estimated volume of carbon dioxide produced by ethanol lamp; assumes carbon dioxide behaves as an ideal gas;

RCO_2 (%) – recovery of carbon dioxide.

APPENDIX 5.

SAS PROGRAMS DEVELOPED TO ANALYZE THE DATA SETS IN TWO LEVELS: THE 'FULL MODEL' THAT ACCOUNTED FOR EFFECTS OF AGE, MANURE ACCUMULATION TIME AND STOCKING DENSITY AND INTERACTIONS VS. THE 'REDUCED' MODEL THAT ONLY ACCOUNTED FOR STOCKING DENSITY EFFECTS

SAS Program for the Full Model Analysis

```
/* read in data */
data amonia;
input age batch day density cage y;
cards;
1      1      1      1      1      0.257
1      1      1      1      2      0.031
1      1      1      2      1      0.035
...
5      2      6      2      2      123.011
run;

/* log transform the response */
data amonia;
      set amonia;
      ly = log(y);
run;
proc sort data=amonia;
by density batch day age cage ly;
run;
proc univariate data=amonia plot;
var ly;
by density;
run;

/* fit the model */
proc mixed data=amonia method=reml nobound;
      class age batch day density cage;
      model ly = batch age|day|density / ddfm=satterthwaite residual
outp=amonia_out;
      random cage cage*age;
      lsmeans age*day*density / adjust=tukey;
run;
proc glimmix data=amonia;
      class age batch day density cage;
      model ly = batch age|day|density / ddfm=satterthwaite;
      random cage cage*age;
```

```
      lsmeans age*day*density / lines adjust=tukey;
run;

/* Assess the model fit using the residuals. */
proc gplot data=amonia_out;
      plot studentresid*pred;
run;
proc gplot data=amonia_out;
plot predictedly*day;
run;
proc univariate data=amonia_out;
      var studentresid;
      qqplot;
run;
proc print data=amonia_out;
run;
```

SAS Program for the Reduced Model

```
/* read in data */
data amonia;
input day age batch density cage y;
cards;
1     1     1     1     1     0.313
1     1     1     1     2     0.037
1     1     1     2     1     0.037
...
6     5     2     1     2     118.655
6     5     2     2     1     127.483
6     5     2     2     2     149.466
run;
data amonia1;
set amonia;
      if day=1 and age=3;
run;

/* fit the model */
proc glm data=amonia1;
      class batch density cage;
      model y=batch density batch*density;
      random cage*density*batch;
      means batch density / tukey lines cldiff alpha=0.05;
      LSMeans batch*density / adjust=tukey-krammer pdiff cl alpha=0.05;
run;
proc mixed data=amonia1 method=reml nobound;
      class batch density cage;
      model y = batch density batch*density / ddfm=satterthwaite;
      random cage;
      lsmeans density batch / pdiff adjust=Tukey;
run;

proc print data=amonia1;
run;
```

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