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Abstract
This research evaluated what ammonia concentration causes pullets or young laying hens to show an aversion. Because previous studies had shown conflicting data for laying hen aversion at 25 ppm ammonia, this aversion was tested with pullets and young laying hens in the current study. The test birds used in this study had not previously experienced elevated ammonia concentrations and thus their natural aversion could be determined. The evaluation of aversion was done using a newly developed Environmental Preference Test Chamber (EPTC). The birds were monitored to determine the percentage of occupation time (POT) under a higher ammonia concentration (25 ppm) vs. a low ammonia concentration (<10 >ppm). The POT was measured over the total day (24 hr) as well as the lighted period only. Feed usage was also monitored as a possible indicator of aversion. Although none of the differences in POT or feed usage were significantly different, both lighted day POT and feed usage showed a trend of relationship (p= 0.13, p=0.16 respectively). This ongoing study aims to determine the birds' aversion as part of a larger study to determine if ammonia or thermal discomfort drives preference.

Keywords
Ammonia, laying hen, preference test, animal welfare

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Abstract. *This research evaluated what ammonia concentration causes pullets or young laying hens to show an aversion. Because previous studies had shown conflicting data for laying hen aversion at 25 ppm ammonia, this aversion was tested with pullets and young laying hens in the current study. The test birds used in this study had not previously experienced elevated ammonia concentrations and thus their natural aversion could be determined. The evaluation of aversion was done using a newly developed Environmental Preference Test Chamber (EPTC). The birds were monitored to determine the percentage of occupation time (POT) under a higher ammonia concentration (25 ppm) vs. a low ammonia concentration (<10 ppm). The POT was measured over the total day (24 hr) as well as the lighted period only. Feed usage was also monitored as a possible indicator of aversion. Although none of the differences in POT or feed usage were significantly different, both lighted day POT and feed usage showed a trend of relationship (p= 0.13, p=0.16 respectively). This ongoing study aims to determine the birds' aversion as part of a larger study to determine if ammonia or thermal discomfort drives preference.*

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Introduction

The health and welfare of laying hens is affected by the air quality at the bird level. Ammonia is a major pollutant that affects the air quality in laying hen houses (Carlile, 1984). Ammonia has the potential to have negative impacts on both health and production of laying hens (Anderson et al., 1964; Charles and Payne, 1966).

Research has shown that concentrations of 25 ppm or greater are aversive to hens (Kristensen et al., 2000; Jones et al., 2005). However in a preliminary test in the environmental test chamber (EPTC) when ammonia concentrations of 25 ppm and <10 ppm were applied the higher ammonia concentration did not cause the laying hens to spend significantly more time in chambers with lower ammonia concentration (Green and Xin, 2008).

The United Egg Producers (UEP) has set forth guidelines for laying hen welfare including atmospheric ammonia concentrations. Under these guidelines ammonia concentrations should "ideally be less than 10 ppm and should not exceed 25 ppm, but temporary excesses should not adversely affect birds health" (UEP, 2002). For humans ammonia exposure limits have been set at 25 ppm and 50 ppm by US National Institute of Occupational Safety and Health (NIOSH) and US Occupational Safety and Health Administration (OSHA), respectively, for 8-hr daily time weighted average (NIOSH, 2005; OSHA, 2006).

Although ammonia has the potential to be harmful to both humans and laying hens, the objective of this study is to determine if laying hens prefer an ammonia concentration of <10 ppm rather than 25 ppm. Preference chambers like the EPTC provide a means to ask an animal what conditions are aversive to the animal.

Method and Materials

EPTC and the Experimental Birds

The experiment was run with the EPTC originally developed by Green and Xin (2008) and refined by the authors. The EPTC had four compartments, where each compartment was accessible by the two compartments adjacent to it. Compartments had separate air supplies with airflow rates ranging from 9.2 to 11.1 m^3/hr. Ammonia could be injected into individual compartments to provide different ammonia levels. The EPTC had doorways in the passages between compartments to limit air exchange between compartments. Each compartment was divided to provide an area for stimulus birds, which remained in an individual compartment, as well as an area for a test bird with access to other compartments. Figure 1 provides an overview of the EPTC. Each test bird was recorded 24 hours per day using video recording software; however an infrared (IR) detection system was used to determine in which compartments the birds resided. The IR detection system was verified using the video recording over 24 hours, with the average difference by chamber equal to 0.93% of the time. Table 1 shows the percentage of time spent in each compartment, or percentage of occupation time (POT), calculated for both the IR detectors and video. The full details of the EPTC were described by Green and Xin (2008). A few modifications were made to the chamber; the weight of the swinging door was too heavy for the pullets to operate, hence a vinyl strip door was cut into 2 cm wide strips and doubled over to replace the heavier door. Also, the IR detectors were modified to take readings every 2 seconds instead of every 5 seconds in an effort to identify more short duration movements.

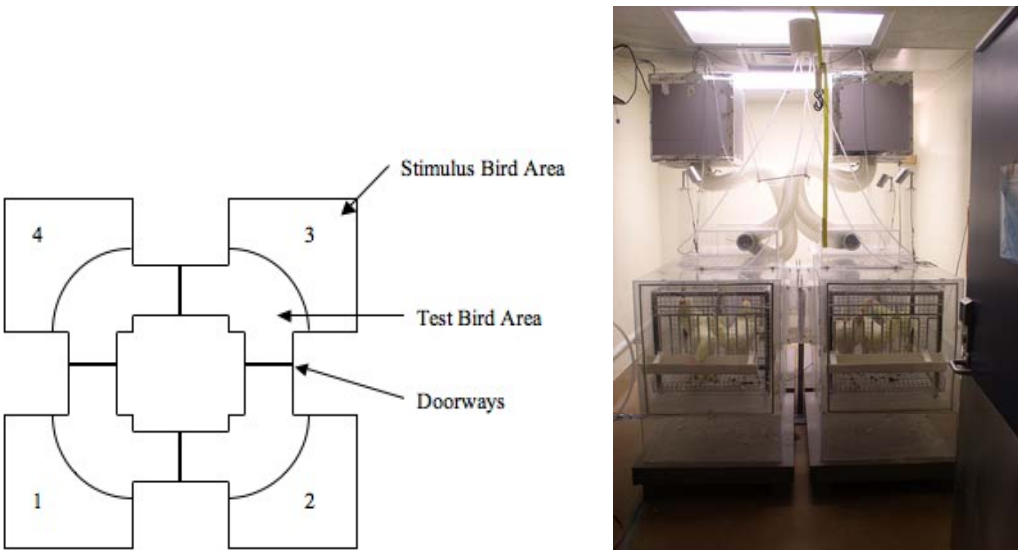

Figure 1. shows top view of the EPTC with the four compartments labeled and a picture with the side view of compartments 1 and 2.

Table 1. Percentage of occupation time (over 24 hours) in each compartment calculated using infrared (IR) detection system and by reviewing video images. The average difference was 0.93%.

	Compartment 1	Compartment 2	Compartment 3	Compartment 4
IR	14.60	13.08	66.49	5.83
Video	14.99	11.52	67.96	5.54

This experiment was run with eight (8) Hy-Line W-36 white leghorns at 10 to 24 weeks of age. The pullets for this study were provided by a commercial farm, from a pullet house with belt manure removal system. Because the pullets were from a belt house, it is assumed they had not been exposed to high ammonia levels (previous exposure <10 ppm) (Liang et al., 2005). The pullets were acclimated in 21°C and <5 ppm ammonia environmental conditions for a few weeks prior to testing. The pullets followed the lighting regime suggested by Hy-Line (2007). Table 2 shows the lighting program used for the study. The housing during acclimation consisted of two adjoining chambers with an identical door to the ones joining the EPTC compartments. To improve efficiency of the experiment while maintaining the quality, the compartments were modified to allow the test bird access to two cages (vs. four). By doing so, two test birds could be run at once. The last four test birds were run with this arrangement.

Individual feeders in each compartment were weighed at the beginning and end of the treatment periods to determine the feed use. Although feeders were modified with wire screens to prevent raking of feed, there were still losses visible on the manure pan. Feed use for stimulus birds was approximately half of that for the test bird. The stimulus birds' average feed usage followed the expected intake documented by Hy-Line (2007). Because the stimulus birds were the same age and from the same flock, it is assumed the difference in feed usage is due mainly to feed loss by the test bird.

Table 2. The lighting regime used for the pullets and young laying hens

Bird age (wk)	Light Level (Lux)	Light Duration (hr)
8-17	7	12
18	7	13
19	7	13.5
20	7	14
21	7	14.5
22	7	15
23	7	15.5
24	7	16

Experimental Design

The test birds were randomly selected for trials in the EPTC. The birds were first provided baseline condition of fresh air (<5 ppm ammonia). The birds were then provided different target ammonia conditions, which consisted of two compartments of 25 ppm ammonia and two compartments of fresh air (<10 ppm ammonia). The treatments were assigned in a randomized block. The birds were given at least 3 hours to acclimate to the EPTC and observed to confirm progressions through all of the compartments. Data were taken for 3 days with baseline conditions, 2 days with an ammonia treatment applied, and 2 days with the ammonia treatment switched to the opposite compartments. The decision to apply high ammonia to all four compartments in two treatment periods was to avoid any issues with baseline preferences toward specific compartments. When the EPTC was modified to provide access to only two compartments, the ammonia treatment was applied to one compartment and then the second. Again the order of treatments was assigned in a randomized block. When the treatments were switched, manure was removed, feed was weighed and replenished, and eggs were removed as needed.

Two situations of ammonia concentrations for the compartments are shown in Figures 2 and 3. Figure 2 shows a typical day's ammonia readings, where the concentrations were fairly stable, except around 11:30 AM where the compartments were opened to add feed for the stimulus birds. Figure 3 demonstrates an issue with the compartments. Due to the modified doors in the passageways, the test birds were able to remain in the doorway, which allows the air to move between compartments. This was more an issue in the dark periods as the test birds tended to remain in one place until the lights came back on. As was shown above, the first few test birds were on a lighting schedule of 12 hours light and 12 hours dark. If these early test birds were in the passageway for the entire 12 hour dark period it not only caused compartments on both sides of the passageway to have the similar ammonia concentrations, but the algorithm used to calculate location would assume the test bird was in the last compartment she was recorded in. This could provide up to 50% of the total time (24 hour) in chamber as a high ammonia choice, even if the bird had pushed her head into the low ammonia chamber.

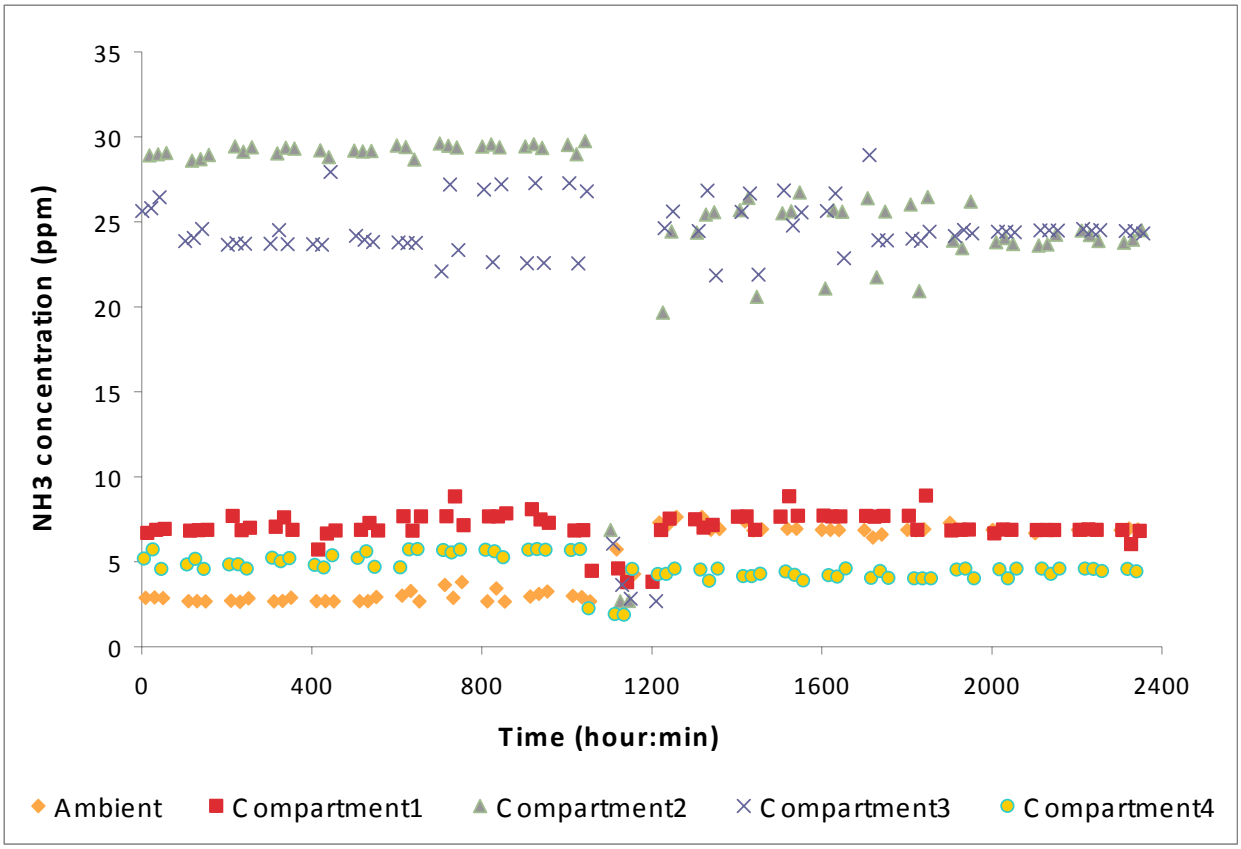

Figure 2. A typical ammonia concentration in each of the compartments and the ambient. The unusual, lower values around 11:30 were due to feed being added to the stimulus birds in all compartments.

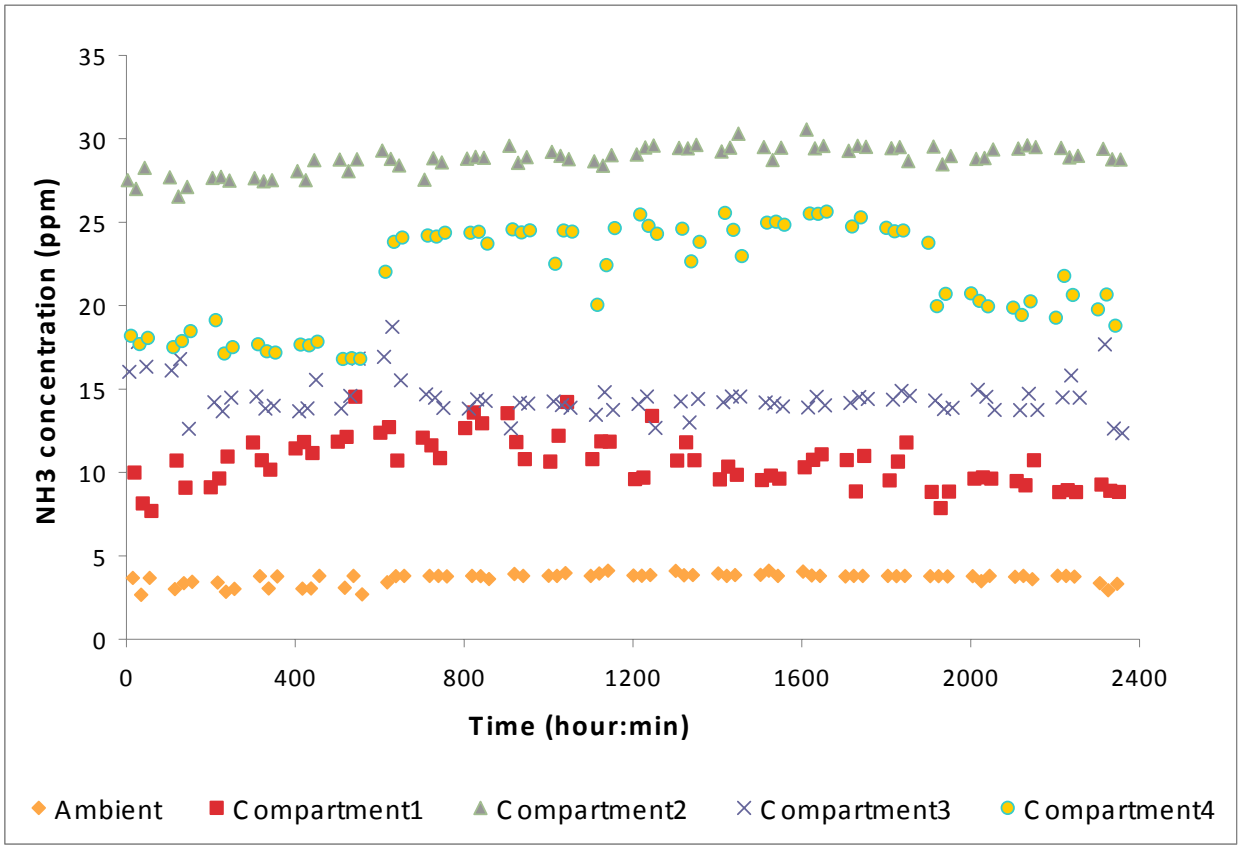

Figure 3. A problematic ammonia concentration in the compartments, where compartment 3 should be <10 pm ammonia, but during the night the bird chose to sleep in the passageway between compartments 3 and 4 (verified by video). This prolonged period of the test bird in the doorways caused significant cross-contamination between compartments.

The percentage of occupation time (POT) in each compartment was calculated using data from the IR sensors. The sensor output was processed to create a summary data set with total time and POT in each compartment and feed utilized by the test bird in each compartment. The summary data set was analyzed using SAS PROC T-TEST to determine if the high and low ammonia levels were significantly different. Effects were considered significant at α=0.05. As was mentioned above, there were some issues with the test birds spending significant periods in the passages between compartments. Because the birds tend to be inactive at night and much more active during lighted hours, data were analyzed on a whole day and lighted period basis. The rationale was that each test bird would more likely position themselves in the doorway for long periods when it was dark. Analyzing the lighted periods separately would take away the bias due to the birds not being willing to move at night as well as ambiguous data where the bird remained in the passageway not entirely in either compartment.

Results and Discussion

Because the test birds were run with two compartments at the higher ammonia level (nominally 25 ppm) and two compartments at the lower level (<10 ppm), the POT in the compartments at each level could be compared. If the birds do prefer the lower ammonia condition, POT in compartments should be greater when the compartments are at <10 ppm ammonia. As well, the corresponding feed usage should be higher in the low ammonia condition. Table 3 shows the difference in POT between the low ammonia and the high ammonia conditions. For both

POT and feed usage, it is expected that if the test bird is avoiding the higher ammonia conditions the difference will be positive. The POT data had 9 negative differences out of 24 readings. The average of the 24 total day differences was 1.1% of total time with a standard error (SE) of 3.2%. The POT data for the lighted period had 8 negative values. The average difference was 8.3% with a SE of 4.3%. For the feed usage data 8 of the 24 data points were negative. The difference in feed use was 0.5 ($\pm$0.35 SE) g day^{-1}compartment^{-1}.

The differences in the feed use data were inputted into SAS and a simple t-test ($p=0.16$) was run. In order to evaluate the POT data, the data had to be modified. For the analysis with four chambers, the two chambers with similar ammonia treatment were combined. The POT data from the combined chambers (half of the tests) could then be compared to the data from the birds with access to only two chambers (remaining half of the tests). The combined data provided two POT differences for each test bird. The POT between compartments summed to 100%, which caused the differences between the first ammonia treatment and the second to be equal and opposite for the compartment(s). When the difference was calculated as the low condition minus high condition, the differences were equal. Therefore, only one of the two differences was used in the t-test analysis. During lighted hours the POT was not significantly different ($p=0.13$). As well, the POT for total day was not significantly different ($p=0.75$). Although neither feed nor lighted period POT data were significantly different, they did indicate some trend of aversion.

There have been a few studies evaluating ammonia aversion. None of the studies has found significant differences in feed usage. Though foraging, a behavior indicator of feeding, was found to occur significantly more in low ammonia conditions (Kristensen et al., 2000). In terms of POT, studies by Kristensen et al. (2000) and Jones et al. (2005) showed significantly less time in higher ammonia conditions (25 and 45 ppm). A previous study performed in the EPTC did not find higher ammonia levels (25 ppm) leading to less POT (Green and Xin, 2008). None of the previous studies looked at lighted day length in addition to the 24-hour day period. However the preference chamber used by Kristensen et al. and Jones et al. was monitored by video, which meant even in dark periods some light was provided (<1 lux). The report by Jones et al. (2005) also noted that 25 percent of the hens showed no aversion to ammonia at 25 ppm. If the birds used in the current study responded with a similar number of non-aversion responses, more birds may be needed to strengthen statistical analysis.

Table 3. Difference in percentage of occupation time (POT) and feed usage as ammonia level was varied from low (<10 ppm) to high (25 ppm). If the test birds are avoiding high ammonia all values are expected to be positive. Bold vales are the mean and standard error (SE) for each bird as well as an overall mean and SE.

Test Bird	Compartment	Difference in POT for 24-hour day* (<10ppm-25ppm)	Difference in POT for lighted hours only (<10ppm-25ppm)	Difference in feed usage (<10ppm-25ppm)
1	1	-16.1	-9.1	-0.06
1	2	15.7	25.0	0.76
1	3	-27.9	-30.6	-1.53
1	4	3.8	3.5	0.33
1	**Mean (SE)**	**-6.1 (9.8)**	**-2.8 (11.6)**	**-0.13 (0.5)**
2	1	-12.1	36.3	2.48
2	2	2	54.3	2.5
2	3	4.6	15.2	-0.57
2	4	-9.9	-2.8	-0.03
2	**Mean (SE)**	**-3.9 (4.2)**	**25.8 (9.4)**	**1.10 (0.81)**
3	1	6.1	10.3	0.67
3	2	-7.6	-16.1	-0.14
3	3	22.4	27.7	2.23
3	4	-24	-33.4	-3.02
3	**Mean (SE)**	**-0.8 (9.9)**	**-2.9 (13.6)**	**-0.07 (1.10)**
4	1	-14	-7.3	0.02
4	2	0.5	1.4	-0.15
4	3	14.6	26.0	0.16
4	4	0.1	17.3	0.67
4	**Mean (SE)**	**0.3 (5.8)**	**9.4 (7.5)**	**0.18 (0.18)**
5	1	2.5	-1.0	0.52
5	2	2.4	-1.0	-3.69
5	**Mean (SE)**	**2.5 (N/A)**	**-1.0 (N/A)**	**-1.59 (2.1)**
6	3	-5.4	5.3	0.94
6	4	-5.4	5.3	0.45
6	**Mean (SE)**	**-5.4 (N/A)**	**5.3 (N/A)**	**0.70 (0.25)**
7	1	3	3.0	1.28
7	2	3	3.0	1.72
7	**Mean (SE)**	**3 (N/A)**	**3.0 (N/A)**	**1.50 (0.22)**
8	3	33.9	33.9	2.97
8	4	33.9	33.9	3.83
8	**Mean (SE)**	**33.9 (N/A)**	**33.9 (N/A)**	**3.40 (0.43)**
Overall	**Mean (SE)**	**1.1 (3.2)**	**8.3 (4.3)**	**0.51 (0.35)**

* The 24-hr day consisted of 12- to 16-hr light and 12- to 8-hr darkness, depending on bird age (see table 2)

Conclusion

The data acquired from the EPTC trials at 25 ppm and <10 ppm ammonia showed no significant difference for both feed usage and percentage of occupation time (POT) under the two ammonia conditions. However, the feed usage difference tends to show a possible correlation (p=0.16). The same is true with the difference in POT during the lighted period of the day (p=0.13). This study is ongoing; all the findings noted here are considered preliminary. More test birds will be used to strengthen the statistics of the lighted-period POT and feed usage differences.

Plan for Further Study

Future the experiment will continue to run in the EPTC with test birds. Additional replicates will be run at 25 ppm ammonia. The study will then be run with higher ammonia levels (50 ppm) to identify aversion at significant levels.

Acknowledgements

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References

Anderson, D.P., C.W. Beard, and R.P. Hanson. 1964. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Dis. 8: 369–379.

Carlile, F. S. 1984. Ammonia in Poultry Houses: A Literature Review. World's Poult. Sci. J. 40: 99-113.

Charles, D.R., and C.G. Payne. 1966. The influence of graded levels of atmospheric ammonia on chickens. II. Effects on the performance of laying hens. Br. Poult. Sci. 7: 189–198.

Green, A.R., and H. Xin. 2008. Development of a Novel Environmental Preference Test System for Laying Hens and Its Initial Application to Assess Hen Aversion to Atmospheric Ammonia. ASABE Paper No. 084451. St. Joseph, Mich.: ASABE.

Hy-Line. 2007. Hy-Line Commercial Management Guide. W-36. 2007-2008. West Des Moines, IA.

Jones, Emma K.M., Christopher M. Wathes, and A. John F. Webster. 2005. Avoidance of atmospheric ammonia by domestic fowl and the effect of early experience. Appl. Anim. Behav. Sci. 90: 293–308.

Kristensen, H.H., L.R. Burgess, T.G.M. Demmers, and C.M. Wathes. 2000. The preferences of laying hens for different concentrations of atmospheric ammonia. Appl. Anim. Behav. Sci. 68: 307–318.

Liang, Y., H. Xin, E. F. Wheeler, R. S. Gates, J. S. Zajaczkowski, P. Topper, H. Li and K. D. Casey. 2005. Ammonia emissions from U.S. laying hen houses in Iowa and Pennsylvania. *Transactions of the ASAE* 48(5): 1927-1941.

National Institute of Occupational Safety and Health (NIOSH). 2005. Available at: http://www.cdc.gov/niosh/npg/npgd0028.html. Accessed April 2009.

Occupational Safety and Health Administration (OSHA). 2006. Available at: http://www.osha.gov/dts/chemicalsampling/data/CH_218300.html. Accessed April 2009.

United Egg Producers (UEP). 2002. United Egg Producers' Animal Husbandry Guidelines. Available at: http://www.uepcertified.com/program/guidelines/1999. Accessed February 2009.