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Air Quality and Hen Health Status in Three Types of Commercial Laying Hen Houses

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Abstract. *Environmental conditions and bird health are important elements in assessment of animal welfare for laying hen housing systems, but limited information is available comparing different types of systems. Three types of laying hen houses – caged high-rise, caged manure-belt, and cage-free floor-raised – were monitored for temperature, relative humidity, carbon dioxide, and atmospheric ammonia during winter and summer conditions in Iowa. During winter conditions, temperature and ammonia concentrations were maintained at a more comfortable level for the caged facilities. During summer conditions, temperature showed the least rise above ambient for the cage-free facilities, and ammonia was maintained at similar levels for all housing types. Assessment of hen health status revealed differences in pathogen frequency between housing systems for winter and summer, but not conclusively in favor of one system over another. The results of this observational study indicate that each system may offer benefits during specific weather conditions. Further monitoring to quantify the benefits of each system should be completed.*

Keywords. Air quality, ammonia, temperature, *Campylobacter*, *Salmonella*, high rise, manure belt, floor raised, deep litter.

Introduction

Animal welfare issues are an increasing concern for the egg production industry in the USA and abroad. A segment of the US egg industry has begun modifying housing systems from conventional cages to alternative (non-caged) systems. This trend is more prevalent in Europe.

The housing system plays a critical role in welfare of laying hens, and various systems have been implemented throughout the world. Benefits vary for different housing schemes. Behavioral benefits of cage-free systems are well documented, as are disadvantages (van Emous et al., 2004). Caged systems offer opportunities for better management and reduce production costs. Important considerations for welfare also include environmental conditions (including air quality) and hen health, but these parameters are not well documented for different laying hen housing systems.

Different housing systems create unique management scenarios, and may result in different microclimates for the same weather. For example, to maintain a higher in-house temperature in winter, the ventilation rate must be decreased, which can result in increased atmospheric ammonia concentrations. To ensure good bird health and performance, it is recommended that atmospheric ammonia in poultry houses, including laying hen houses, should not exceed 25 ppm (UEP, 2006). Therefore, thermal comfort may be compromised to attain the recommended air quality. On the other hand, during summer conditions, it may be problematic for houses with high numbers of birds to provide sufficient ventilation to maintain comfortable temperatures, even at maximum ventilation rates.

Ammonia emissions from layer houses are different in high-rise housing, belt-system housing, and cage-free systems (Koerkamp, 1998). Ammonia emission from laying hen battery cage systems with manure storage beneath the cages (high-rise housing) is roughly 10-times higher than that from systems with belt drying and frequent removal of the manure (Liang et al., 2005).

Consequences of poor air quality include diminished production performance and poor bird health. Elevated concentrations of atmospheric ammonia in the poultry house will reduce feed intake and impede bird growth rate (Deaton et al., 1984, Charles and Payne, 1966a), decrease egg production (Charles and Payne, 1966b), damage the respiratory tract (Nagaraja et al., 1983), increase susceptibility to Newcastle Disease Virus (Anderson et al., 1964), increase the incidence of air sacculitis (Oyetunde et al., 1978) and keratoconjunctivitis (blind eye) (Faddoul et al., 1950) and prevalence of *Mycoplasma gallisepticum* (Sato et al., 1973). Egg quality may also be adversely affected by high levels of atmospheric ammonia as measured by reduced albumen height, elevated albumen pH, and contributing albumen liquefaction (Cotterill and Nordsog, 1954).

Environmental temperatures not only influence hen comfort and performance, but also affect other environmental conditions, such as ammonia and dust levels in poultry houses (Carlile, 1984). Air temperature and concentration of dust within a building vary with the size and activity of the birds, air space, humidity, and ventilation rate. Higher temperatures not only stimulate bacterial activity and ammonia production, but also facilitate the mass transfer of ammonia from the litter or manure to the air. Accordingly, a small increase in air temperature can significantly influence ammonia levels in intensive housing.

Health concerns impact not only the welfare of the birds, but also the microbial food safety of the consumer. *Campylobacter jejuni* and *C. coli* are frequently reported in clinically healthy live birds and poultry meat but infrequently in egg products (Altekruse *et al.* 2003, Kapperud *et al.*. 2003, Neal *et al.* 1995, Stern *et al.* 2003). However, antimicrobial resistance and the ability of *Campylobacter* to laterally transfer genes encoding antimicrobial resistance to other bacteria in the avian intestine are of emerging concern. *Campylobacte*r spp. causes nearly 2 million cases

of foodborne illness resulting in approximately 10,000 hospitalizations and approximately 100 deaths annually (Mead *et al.* 1998). *Salmonella* causes nearly 1,343,000 cases of foodborne illness resulting in approximately 15,000 hospitalizations and approximately 500 deaths annually (Mead *et al.,* 1998). To reduce human foodborne illness, on-farm pathogen reduction strategies strive to deliver poultry, meat, and eggs to the American consumer with low levels of *Campylobacter* and *Salmonella*.

Despite the emergence of alternative management systems, correlations of the prevalence of human foodborne pathogens with type of poultry housing yield conflicting results. On one hand, cages restrict bird movements (Vits *et al.,* 2005), which should reduce transmission of *Salmonella* and *Campylobacter* in the flock. Indeed, the reported relative risk of *Campylobacter* in French free-range broiler flocks exceeded that of birds with a limited fenced run (Huneau-Salaun *et al*., 2007). A survey of Danish flocks documented a higher *Campylobacter* prevalence in free-range chickens (100%) when compared to confined birds (36.7%, Heuer *et al.,* 2001). Similar observations have been made for Peruvian birds (Tresierra-Ayala *et al.,* 1995). On the other hand, studies have not always supported this theory. A survey of Swiss poultry indicated that the *Campylobacter* prevalence in feces of free-range birds (69%) was not significantly different from that of conventionally reared broilers (50%, Wittwer *et al.* 2005). Avrain *et al.* (2003) determined that *C. jejuni* prevalence was lower in free-range (31%) versus standard production (81.4%) broilers. Health parameters (white blood cell counts, etc) were also scored higher in birds housed in pens with access to green areas when compared with hens housed in conventional cages (Posadas-Hernandez *et al*. 2005). Egg risk factors were also negatively impacted by caging birds. The quality (eggshell thickness, egg weight, egg yolk color) and *Salmonella* contamination of eggs laid by caged hens was negatively impacted when compared to free-range birds especially under heat stress (Barbosa-Filho *et al.* 2006). Further, while eggs obtained from free-range hens exhibited a lower *Salmonella* penetration rate (6%) than eggs from hens in conventional battery cages (16%), a number of factors, including the strain of layer hens and diet were critical (Messens *et al.* 2007).

Epidemiological studies have found the prevalence of either *Salmonella* or *Campylobacter* varies with housing system, diet, season and age of birds. Methner *et al.* (2006) concluded that *Salmonella* prevalence was highest in layer hens in conventional cage systems (46.3%) and lowest in birds in free-range flocks (21.9%). In contrast, a California study reported fewer *Salmonella enteritidis* in caged birds (1.7%) than in free-range birds (50%) with a similar pattern overall for group D *Salmonella* prevalence in caged (1.5 per 10,000) and free-range (14.9 per 10,000) hens (Kinde *et al.* 1996). Likewise, significantly more *Salmonella* were isolated from floor pens than from batteries of caged layer hens (Geue and Schluter, 1998). *Salmonella* prevalence in non-caged barn layers (61.5%) and free range (54%) layers exceeded estimates for caged birds (34%) in the United Kingdom (Davies *et al.* 2001). Similarly, among the multiple risk factors for *Salmonella* infection in laying hens of the same age, confining birds to a cage lowered the risk of *Salmonella* when compared to free-ranging hens (Mollenhorst *et al.* 2005).

To fully assess the welfare of birds in a specific system, it is important to consider the system as a whole, including aspects of health, environment, behavior, biological functioning, handling and management practices, worker education and training, and economics. Few studies compare air quality at bird level in high-rise caged, manure-belt caged, and cage-free littered floor laying hen facilities. Information regarding hen health status and prevalence of foodborne pathogens in these housing systems yields conflicting reports. Therefore, the objective of this field observation research was to monitor the air quality and hen health status in these three types of housing systems, for both warm and cold climatic conditions. This paper summarizes the results of this monitoring, which may be used by decision makers to improve laying hen husbandry.

Materials and Methods

Four houses from each of three housing systems (caged high-rise, caged manure-belt, and cage-free littered floor) were selected based on farm access and availability. Environmental variables measured near bird level included: ammonia (NH_3) concentration, carbon dioxide (CO_2) concentration, air temperature, and relative humidity (RH). Each house was monitored continuously over a 24-hour period in winter and summer. All 12 houses in the study contained adult laying hens of various ages, but all chickens within a house were the same age (Table 1). Birds for health assessment were randomly selected from each house on the day of monitoring.

Data Collection

Environmental Conditions

Ammonia and CO_2 concentrations inside the barns were measured using portable monitoring units (PMUs) previously developed for monitoring layer building ammonia emissions (Xin et al, 2003). Each PMU contained two electro-chemical NH_3 monitors (PACIII, 0-200 ppm range NH_3, Drager, www.draeger.com) with data storage capability and one infrared CO_2 monitor (GMT220, 0-7000 ppm range, Vaisala, www.vaisala.com). Before placement in the monitored layer barns, the PMUs were calibrated with zero (N_2) and the proper span (NH_3+N_2 balance or CO_2+N_2 balance) calibration gases. During data collection, ammonia and CO_2 concentrations were measured at 30 min intervals over the 24 h data collection period. The 30 min interval consisted of 5 min air sampling within the house and 25 min fresh air purging. Air temperature and RH both inside and outside the barns were recorded at 5 min intervals during the 24 h data collection using programmable, portable temperature and RH loggers (H08-032-08, Hobo Pro, Onset Computer Company, www.onsetcomp.com).

Hen Health Status

Ten birds were randomly selected from each house for assessment of health status, tracheal condition and prevalence of *Campylobacter* and *Salmonella*. Blood samples were taken from each hen and sera from these samples were subsequently tested for the presence of antibodies against *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by the serum plate agglutination test. Birds were euthanized via injection of sodium pentobarbital, and a trachea sample was collected. Ceca and small intestine were collected, refrigerated (4^{o}C) and processed the next day for *Campylobacter* and *Salmonella*. Cecal and small intestine contents were squeezed into a sterile sampling bag (Whirl-Pak, Nasco, Ft. Atkinson, WI), weighed and buffered peptone water (Oxoid, Hampshire England) added to achieve a 10% w/v suspension. The suspension was homogenized (30 s) in a stomacher (Seward, Norfolk UK) on medium speed.

Tracheal analysis. Tracheas were fixed in 10% neutral buffered formalin, dehydrated in a graded series of ethanol, and embedded in paraffin. Sections were cut (4 u) and stained with hematoxylin and eosin and examined by light microscopy.

Detection and identification of *Campylobacter* spp. One milliliter of the homogenate was transferred to a 16 x 125 mm polystyrene round bottom tube containing 12.5 ml blood-free enrichment broth (Tran, 1998) and incubated aerobically (24 h, 42^{o}C). After incubation, 50 μl of the enrichment was streaked onto Campy-Cefex agar with Amphotericin B (2 mg/L) replacing sodium cycloheximide (Stern *et al,.* 1992). Campy-Cefex plates were incubated (48 h, 42^{o}C) microaerobically (5% O_2, 10% CO_2, 85% N_2) in a CO_2 water jacketed incubator (Forma Scientific, Waltham MA). Three presumptive *Campylobacter* colonies were streaked onto brain heart infusion agar supplemented with 0.6% yeast extract and 10% defibrinated sheep blood and incubated microaerobically (24 h, 42^{o}C). Presumptive *Campylobacter* isolates were

confirmed and speciated as *C. coli* or *C. jejuni* by PCR as previously described (Wesley *et al.* 2005). Data were also recorded as the number of birds dually infected with *C. jejuni*/*C. coli*.

For the summer samples, the BAX® real-time instrument was used for direct detection of *Campylobacter* (Q7RT *Campylobacter* kit, Dupont Qualicon, Wilmington, Delaware). This allowed for its comparison with conventional culture (direct and indirect plating) methods of *Campylobacter.* For real-time PCR analysis, protease (150 ul) was added to the lysis buffer (12 ml) and aliquoted (200 ul) to lysis tubes supplied by the manufacturer. An aliquot of the 10% cecal suspension (5 ul) was added to each tube containing lysis reagent and heated at 37°C (20 min) followed by 95°C for 10 minutes to inactivate the protease, as recommended by the manufacturer. Data generated by the BAX instrument identify only the genus *Campylobacter* without further speciation as either *C. jejuni* or *C. coli*. Conventional enrichment followed by plating to Campy-Cefex agar continued as described in winter. In addition, the cecal suspensions (10%) were directly plated (100 ul) to Campy- Cefex agar, incubated microaerobically and species determined by the multiplex PCR as described above.

Detection and identification of Salmonella. The buffered peptone water homogenate from the sample processing was incubated (24 h, 37°C) aerobically. Following incubation, 1 ml of the enrichment was transferred to 10 ml Tetrathionate Hajna broth (Becton Dickinson, Sparks MD) and incubated (24 h, 42°C) aerobically. Enrichments were screened for *Salmonella* using the BAX® system according to the manufacturer's recommendations with the addition of 50 μl BAX® system sample supplement prior to transferring the lysate to the PCR tubes.

For the summer, in addition to the real-time PCR system used in the winter sampling, the FSIS protocols for *Salmonella* isolation were incorporated into the study.

Statistical analysis. Two-factor repeated measures analyses were used in two different comparisons between Table 1 and Table 2 prevalence of *Campylobacter* data. The first comparison examined differences among birds under three manure management practices (cage-free floor litter, caged manure-belt, and caged high-rise) over two trials (winter and summer) using 4 replicates. The second comparison examined differences between caged and non-caged birds over winter and summer with an unequal number of replicates.

Description of Housing Types

Floor-Raised (FR) Houses

Four houses characterized by floors that were partially or fully available to the hens and covered with litter were studied at three separate sites (Site 1, Site 2, Site 3) within 16 km (10 mile) of one another (Table 1). The same houses were monitored for both winter and summer conditions and all houses incorporated cage-free production methods. Birds were allowed to move freely within the houses, or at least within a section of the house, and had access to a litter-covered floor. Hens in one house produced organic eggs and were allowed daily access to pasture under suitable weather conditions. All houses had automated feeding, water and egg collection and provided nest boxes for the hens to use during oviposition. Two houses had a partially slatted floor located along the center of the house and manure accumulated beneath the slatted floor was periodically removed. Three houses were naturally ventilated, and one was mechanically ventilated. Three houses had an east-west orientation, and one had a north-south orientation (Table 1).

Manure-Belt (MB) Houses

Five houses with manure belts were selected at one egg-production site (Site 4, Table 1). Each of these houses had one floor level with hens in cages and a manure belt beneath each row of

cages which extended the length of the house. All MB houses had similar floor plans, cage arrangements, and management. Four houses were monitored in winter, but two of those houses were unavailable during the following summer. Hence, summer monitoring included two houses monitored during the preceding winter and one house not included in the winter study.

High-Rise (HR) Houses

Four HR houses were selected at one egg-production site (Site 5, Table 1). Each house had two floor levels with hens in cages on the upper floor and manure falling through spaces between cage wires and accumulating beneath the cages on the lower floor. All HR houses had similar floor plans, cage arrangements, and management. Four HR houses were monitored in winter and four in summer, three of which were the same for both monitoring periods.

Monitoring Equipment Setup

For all housing types, a 3-location composite air sample was taken at one cross-section of each house for atmospheric monitoring of the bird microenvironment. Temperature and RH were monitored both inside and outside at each egg-production site.

For the FR system, three air sampling ports were placed near the birds' level across the house: one at the middle and two at one-fourth of the building width from each sidewall (fig.1). The cross-section was approximately 30 m (100 ft) from the one end of the house. Temperature/RH loggers were placed inside the building at each air sampling port and outside the building near the eave of each house.

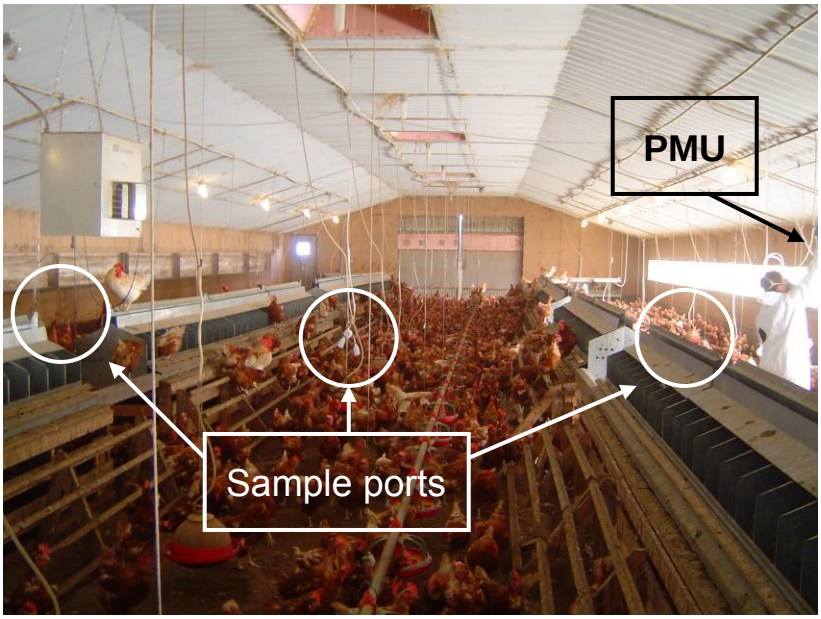


Figure 1: Monitoring configuration of floor-raised (FR) house.

For the HR and MB cage systems, sampling ports were placed inside three different cages across the house (fig. 2 and 3). Birds were removed from each cage prior to placement of the air sampling port and temperature/RH logger. All three sampling ports were centrally located in the cage tiers (fig. 4), with one near the middle row and two near one-fourth of the building width from each sidewall. The cross-section was taken approximately 30 m (100 ft) from the one end wall. Temperature and RH were monitored at the same locations within the cages, in the aisle near each cage location, and at one outdoor location on the facility site.

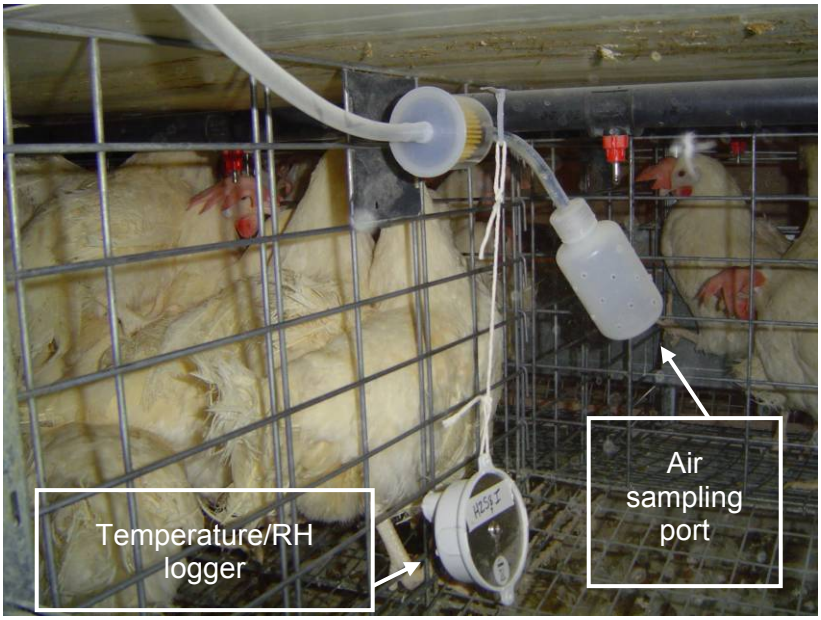


Figure 2: Sampling port at bird level inside cage.

Figure 3: Sampling port for high-rise (HR) house.

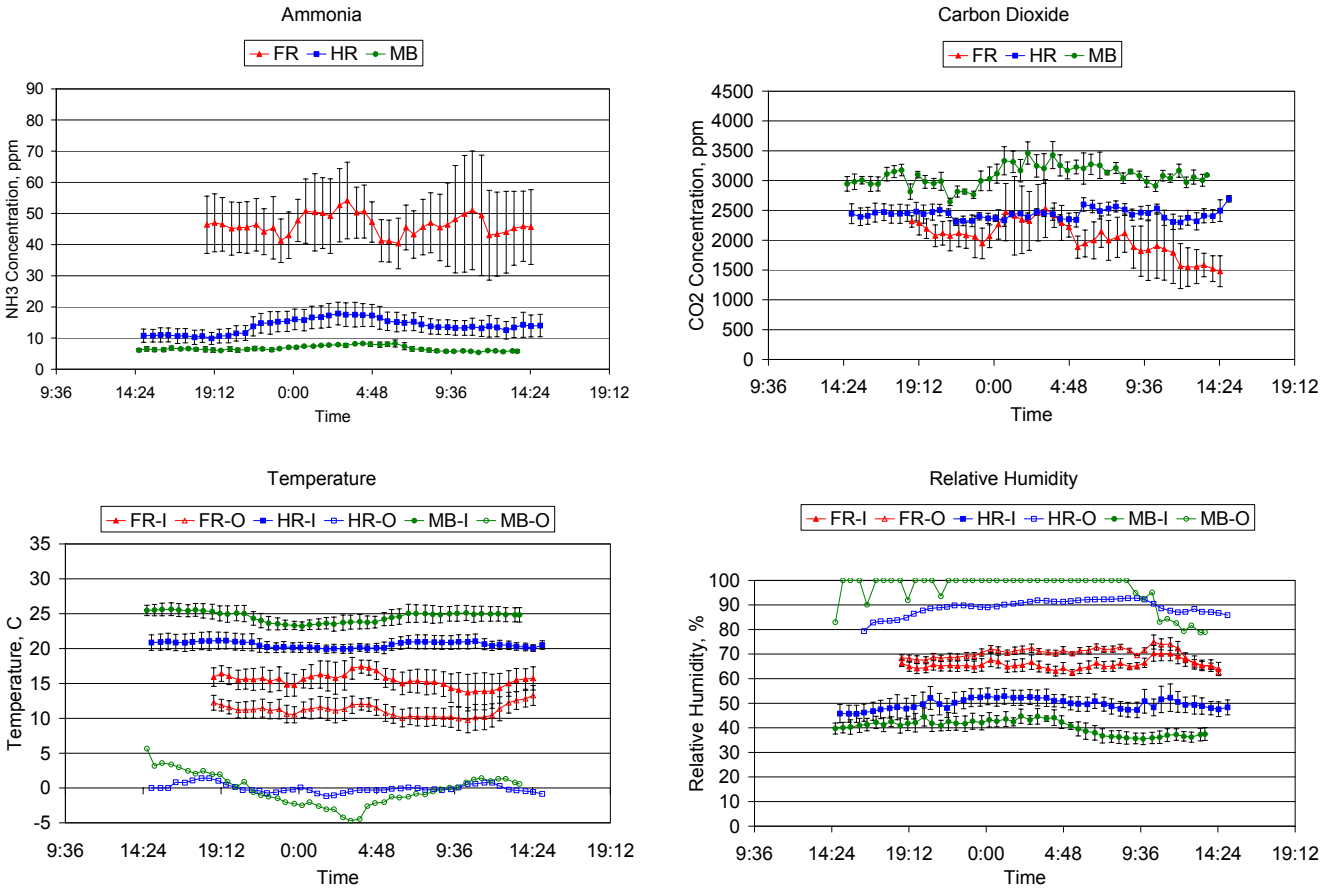
Figure 4: Sampling lines and aisle sensor for manure-belt (MB) house.

Results

Data were summarized for each house and combined into mean plots for each variable during each monitoring period.

Bird-Level Environment in Winter

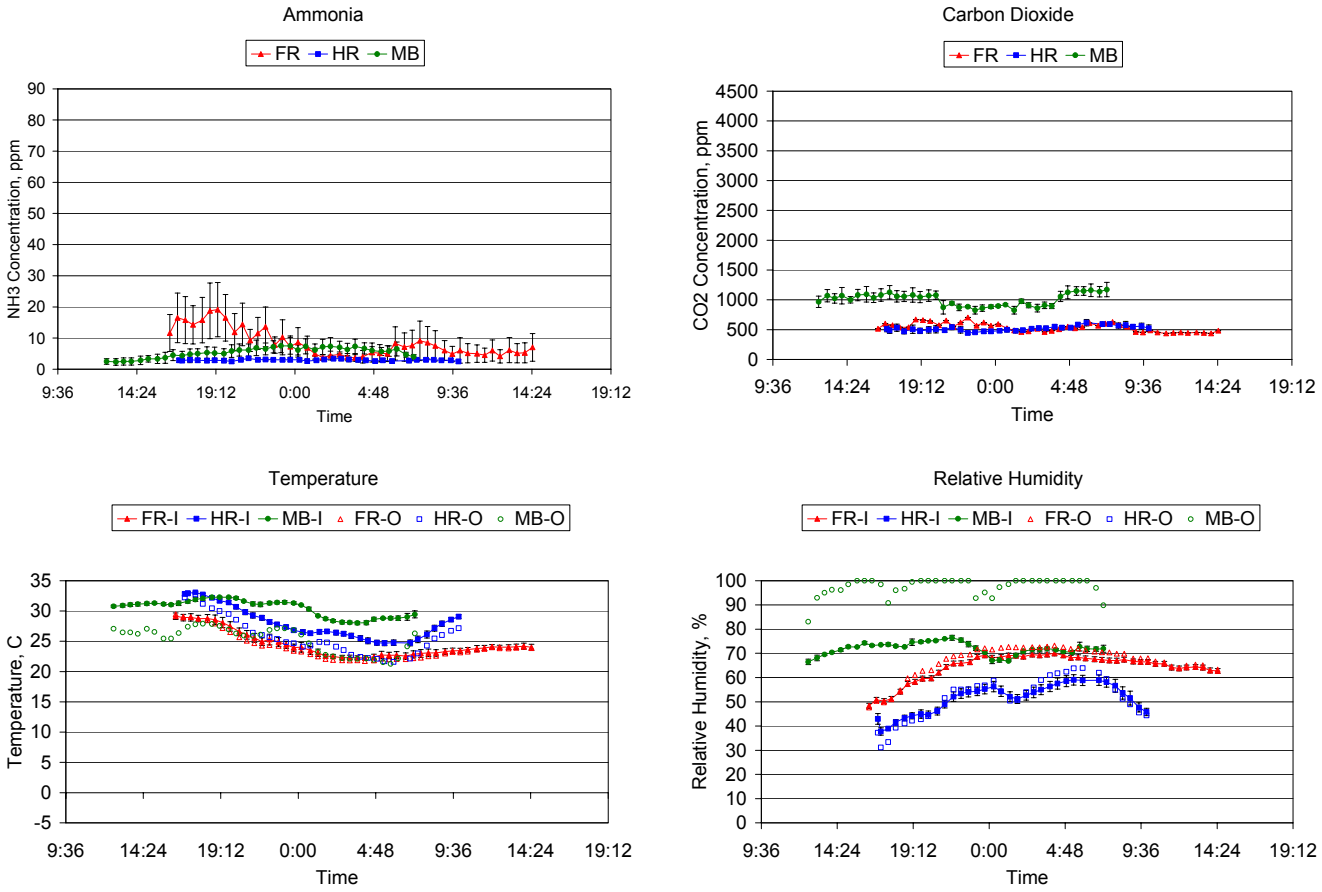
Table 2 summarizes mean, maximum and minimum values for each variable measured within the houses during a 24 h winter monitoring period. Figures 5-8 display means over 24 h for each variable for each house. Temperatures and ammonia concentrations remained within comfortable ranges during the entire monitoring period for the cage systems, but ammonia concentrations, on average, exceeded recommended levels for laying hens in the FR system. Ammonia concentrations were considerably higher in the FR houses than in the cage houses (mean of 46 ppm for FR vs. 14 ppm for HR and 7 ppm for MB). Maximum concentration in the FR houses reached or exceeded 85 ppm. Temperatures in the FR houses tended to fluctuate more, following the outside conditions, and the temperature at bird level was less than optimal, but still comfortable (mean ± SE of 15.5 ± 1.5 ^{o}C for FR vs. 20.6 ± 0.8 ^{o}C for HR and 24.6 ± 1.0 ^{o}C for MB, which were controlled at different setpoints). Interestingly, CO_2 concentrations tended to be lower in FR houses (mean ± SE, 2021 ± 199 ppm) than in the HR (2433 ± 95 ppm) or MB (3072 ± 36 ppm) cage systems, presumably a result of lower bird density and thus less CO_2 generation from bird respiration in the FR system.



Figures 5-8: Winter conditions: Mean (±SE) plot for NH_3, CO_2, temperature, and RH for floor-raised (FR), high-rise (HR), and manure-belt (MB) laying hen houses. For temperature and RH plots, I = inside and O = outside.

Bird-Level Environment in Summer

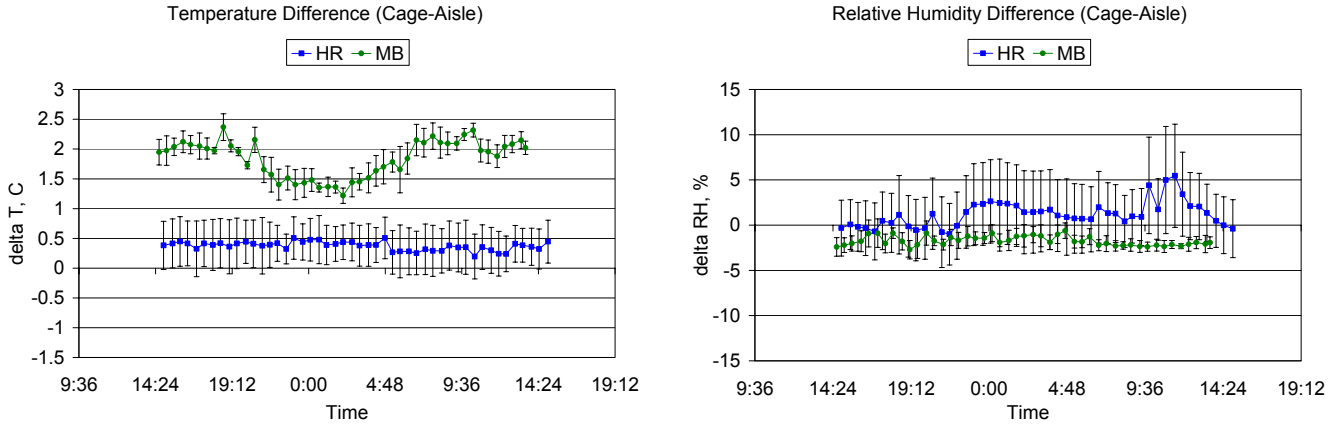
Table 3 summarizes mean, maximum and minimum values for each variable measured within the houses during a 24 h summer monitoring period. Figures 9-12 display means over 24 h for each parameter for each house. Ammonia concentrations were within the recommended level (daily maximum less than 25 ppm) for all houses, with the exception of FR3 and FR4, where maximum ammonia concentrations reached 42 and 29 ppm, respectively. All daily mean ammonia concentrations were below 25 ppm. Temperatures for the FR houses showed less rise over ambient than the cage houses (average rise or percent rise with respect to ambient: 0.3°C or 1% for FR, 1.2°C or 4% for HR, and 4.7°C or 18% for MB).



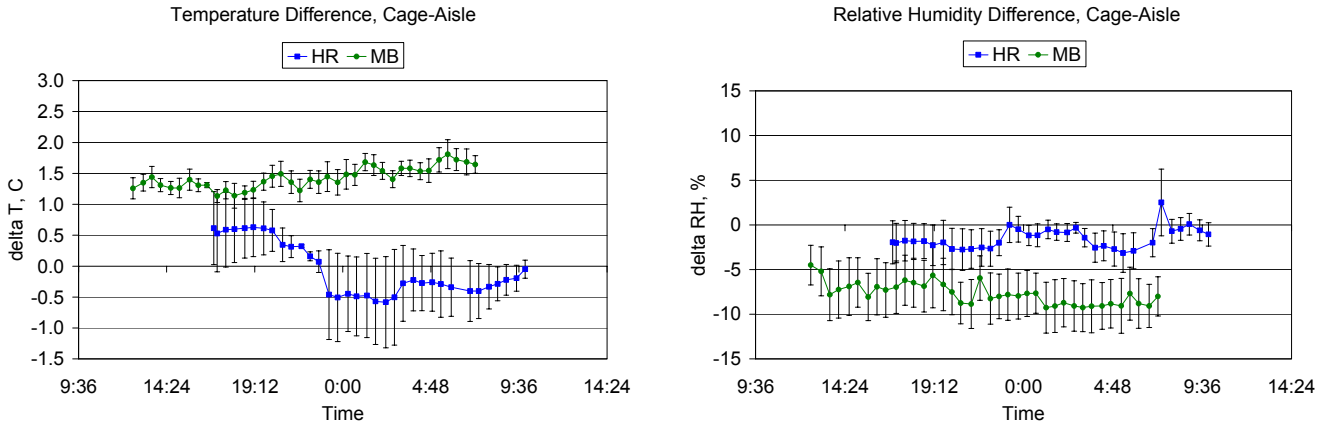
Figures 9-12: Summer conditions: Mean (±SE) plot for NH_3, CO_2, temperature, and RH for floor-raised (FR), high-rise (HR), and manure-belt (MB) laying hen houses. For temperature and RH plots, I = inside and O = outside.

Temperature Stratification between Cages and Aisles

Figures 13-16 display the difference in temperature between the aisle and inside cages. Air temperature tended to be higher inside cages than in the aisle during both winter and summer, especially for the MB houses. As expected, the differences were more apparent in winter than in summer due to lower ventilation rate in winter. The magnitude of the differences tended to be smaller in the HR houses than in the MB houses, even though the differences fluctuated more in the HR houses.



Figures 13-14: Winter conditions: Mean temperature (L) and relative humidity (R) difference between cage and aisle for high-rise (HR), and manure-belt (MB) laying hen houses.



Figures 15-16: Summer conditions: Mean temperature (L) and relative humidity (R) difference between cage and aisle for high-rise (HR), and manure-belt (MB) laying hen houses.

Hen Health Status

Serological tests detected antibodies against *Mycoplasma synoviae* and/or *Mycoplasma gallisepticum* in sera from all hens except in samples from hens in House FR2 (Table 4). Microscopic examination of hen tracheas revealed abnormally high numbers of lymphocytes within the lamina propria layer of the tracheal wall in birds from all houses except from hens in House FR2. Intact cilia were present on the respiratory surface of all birds from all houses.

Bacteriology results obtained for *Campylobacter* and *Salmonella* are presented in Table 5 for winter and Table 6 for summer. For winter conditions, *Campylobacter* spp. prevalence was higher in FR when compared with birds in HR houses (80.0% vs 37.5%, $P<0.05$), but there was no difference in *Campylobacter* prevalence between FR hens (37.5%) and hens from MB houses (62.0%). The prevalence of *C. coli* was higher in FR hens compared to hens from both HR and MB houses (55.0% vs. 25.0% or 25.6%, respectively, $P<0.05$). No such differences were seen when *Salmonella* prevalence was correlated with housing systems. Prevalence numbers were too low to perform χ^2 tests for birds dually infected with *C. jejuni/C.coli*. For summer conditions, results from conventional isolation of *Campylobacter*, showed lower prevalence of *Campylobacter* and *C. jejuni* for FR hens and MB hens than HR hens (27.5% and

20.0% vs. 65.0%; and 7.5% and 20.0% vs. 52.5%, respectively, P<0.01). This result may reflect the ability of some of the FR to move freely in the house and come into contact with infected birds. When winter and summer are compared, the prevalence of *Campylobacter* spp. in the FR birds was higher in winter than in summer (80.0% vs. 27.5%, P<0.05). Again, this may reflect the contact potential of the birds, thus facilitating transmission.

When real-time PCR was used to detect *Campylobacter* spp., the prevalence of *C.jejuni*/*C coli* (dual infection) was higher in FR birds when compared to MB birds (27.5% vs. 3.3%, P<0.01). The real-time format was only used in the summer trial thus precluding comparison of data with winter sampling. This system was in beta-testing in our (USDA-NADC) laboratory during this analysis, and these PCR data should be interpreted with caution.

Discussion

In general, environmental conditions recorded differed for all three housing types. There was much greater variability from house to house for the FR system flocks that were independently operated. This variability could have resulted from the multiple monitoring sites with different house configurations and different management styles. Variability would be expected to be less for houses located on the same site and operated under the same management, as was the case for the MB and HR houses.

Many differences were noted in ventilation of the houses. Simple operating adjustments could have been made that might have improved the conditions during the monitoring periods. For the naturally ventilated FR houses, addition and operation of minimum ventilation fans could have significantly reduced ammonia concentration during the night when side curtains were closed. Litter management likely had a significant impact on ammonia emissions, with drier litter lessening NH_3 volatilization. A thin layer of wood shavings was periodically spread over the litter in FR3, and this house showed much lower levels of ammonia in winter, even during the night when the curtains were closed. Orientation of the houses for natural ventilation (E-W) is critical in summer months when wind drives the air exchange. House FR3 was oriented N-S and had the poorest air quality during the summer study period. During winter, ventilation of the same house was likely enhanced by the chimneys located longitudinally along the center of the house.

For the HR houses, the curtain backed cages may block airflow, which could explain the temperature variation between cages and aisles. For the MB houses, some ventilation dead spots were noted during the summer, leading to poor air quality at these locations. We are uncertain if these stagnant areas were reflected in the measurements. The tunnel ventilation configuration used in the MB houses in this case could be difficult to control: the eave inlet dampers must be properly adjusted to achieve the relatively uniform air distribution along the length of the building. Otherwise airflow is higher closer to the fans located in the end walls. Even so, the temperature distribution was more uniform in the MB houses than in the HR houses, particularly during summer. Frequent (daily in this case) removal of manure in the MB houses greatly reduced ammonia concentrations.

Results of this monitoring study support the concept that the ventilation driving force varies with weather for all systems monitored. In winter months, ventilation to control ammonia levels is more critical for the health of birds and workers. In summer months, control of temperature rise is more critical for prevention of heat stress.

Temperature differences between cages and aisles likely resulted from several factors. The movement of air was likely impeded by cage fixtures and the presence of birds, and the birds contribute heat to their microenvironment that would not be detected by a thermostat located in the aisle. Additionally, because the cage temperature was monitored inside an empty cage

(recall, birds were removed prior to sensor placement), the microenvironment of individual birds may have experienced even greater differences. The observation that temperatures in cages were generally warmer than temperatures in the aisles suggests that it may be advisable to periodically monitor that difference, as its magnitude can be affected by the season and thus ventilation rate of the house. When appreciable differences exist, it may be prudent to adjust the temperature setpoint to truly reflect the microenvironment that the birds are experiencing. Alternatively, one may consider locating the temperature sensors of the controllers near the bird microenvironment, such as in empty cages adjacent to the birds.

The presence of antibodies against *Mycoplasma synoviae* (MS) or *Mycoplasma gallisepticum* (MG) in the sera of chickens indicates that flocks were positively infected by these pathogens. The immune response of hens to the presence of avian mycoplasmas colonizing respiratory epithelium of the trachea is manifested by the accumulation of lymphocytes within the underlying lamina propria. Hens in FR2 were not infected by MG or MS, did not mount an immune response, and consequently did not have significant numbers of lymphocytes in the tracheal wall during winter or summer. Because most hens in this study were infected with *Mycoplasma*, microscopic changes observed in the tracheas could not be distinguished from changes that might have resulted from exposure to ammonia or particulate matter in the air. High levels of ammonia have been associated with loss of cilia (Anderson, 1966; Nagaraja, 1983), but deciliation was not observed in the trachea of any chickens in this study.

Monitoring for bacterial foodborne pathogens showed seasonal differences between the housing systems. For winter, *Campylobacter* spp., specifically *C. coli,* prevalence was higher in the FR hens than in caged birds. Factors contributing to the higher prevalence in the FR birds may include: different breed of layer hen used in the FR houses, bird housing densities, and maintenance of the FR birds on organic (antibiotic-free) diets. Most significantly, during periods of inclement weather in the winter months, the FR birds were confined indoors which facilitates fecal-oral transmission of *Campylobacter* within the flock house.

In contrast, for the summer monitoring, the prevalence of *Campylobacter* spp. and *C. jejuni* was significantly lower in the FR birds when compared to the HR hens ($p<0.01$). The prevalence of *Campylobacter* spp. and *C. jejuni* was higher in HR birds than in MB hens ($p<0.01$). No such differences between the housing types were seen in the prevalence of *Salmonella*. When seasonality is evaluated, the prevalence of *Campylobacter* spp. was lower in the FR hens during the summer.

In the summer months, a real-time PCR assay for detection of *Campylobacter* was used to facilitate the screening of the large number of samples evaluated (Table 6). When real-time PCR was used, the prevalence of *C.jejuni*/*C coli* (dually infected birds) was higher in FR birds than in MB hens ($P<0.01$). No other significant differences are evident with results from PCR analysis. The real-time format was only used in the summer trial thus precluding comparison of data with winter sampling.

Results from this study should be regarded as observational only. Because monitoring was conducted at a system level, results could not be interpreted to specifically identify the source of differences. It also should be acknowledged that data from 24 h environmental monitoring would not be sufficient to yield incontrovertible conclusions regarding different housing types. Nevertheless, the data confirmed the initial hypotheses that under similar weather patterns, different environmental conditions may be observed in from different housing systems and different management schemes. Also, results show differences among housing systems for pathogen frequency, but results do not conclusively show that one system yields lower pathogen frequencies than another, as reported in the Netherlands (van Emous, 2004). Further studies should include multiple representations of each house type, encompassing different

management and varieties of housing configurations to better identify seasonal sources of variation.

Conclusions

Environmental conditions and bird health are important aspects to consider in assessment of animal welfare for housing systems. Limited information has been published regarding air quality at bird level and the health status of laying hens within different housing systems. For this study, we collected observational data to assess the bird-level air temperature, RH, carbon dioxide, and atmospheric ammonia for three housing scenarios: cage-free floor-raised, caged high-rise, and caged manure-belt. Health status was also compared for birds within each system. Environmental monitoring occurred over a period of 24 h during winter and summer in Iowa, and bird health was assessed for both periods. Differences in environmental conditions and/or pathogen frequency were observed among all three housing types during summer and winter conditions. Results of this observational study could not be used to identify the specific sources of benefits associated with each system. All houses were different in some aspect, and were operated under different management practices. Differences observed in air quality and pathogen frequency merit further research to quantify and identify sources of these differences and attempts should be made to incorporate additional measures of health and production.

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References

Anderson, D. P., C. W. Beard, and R. P. Hanson. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Dis. 8:3469-379. 1964.

Altekruse, S. F., and L. K. Tollefson. 2003. Human campylobacteriosis: a challenge for the veterinary profession. J. Am. Vet. Med. Assoc. 223:445-452.

Avrain L, Humbert F, L'Hospitalier R, Sanders P, Vernozy –Rozand C, and Kempf I. 2003. Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. Vet Microbiol 96:267-276.

Bailey JS, Cosby DE. 2005. *Salmonella* prevalence in free-range and certified organic chickens. J Food Prot. 68: 2451-2453.

Barbosa-Filho JAD, Silva MAN, Silva IJO, Coelho AAD. 2006. Egg quality of layers housed in different production systems and submitted to two environmental conditions. Revista Brasileira de Ciencia Avicola. 8(1):23-28.

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

Charles, D. R. and C. G. Payne. The influence of graded levels of atmospheric ammonia on chickens. I. Effects on respiration and on the performance of broilers and replacement growing stock. British Poultry Science 7:189-198. 1966a

Charles, D. R. and C. G. Payne. The influence of graded levels of atmospheric ammonia on chickens. II. Effects on the performance of laying hens. British Poultry Science 7:189-198. 1966b

Cotterill, O. and A. W. Nordsog. Influence of ammonia on egg white quality. Poultry Sci. 33:432. 1950.

Davies R and Breslin M. 2001. Environmental contamination and detection of *Salmonella enterica* serovar enteritidis in laying flocks. Vet Rec 149: 699-704.

Deaton, J. W., F. N. Reece, and B. D. Lott. Effect of atmospheric ammonia on pullets at point of lay. Poultry Science 63:384-385. 1984.

Faddoul, G. P. and R. C. Ringrose. Avian keratoconjunctivitis. Vet. Med. 45:492-493. 1950.

Geue L and Schluter H. 1998. A *Salmonella* monitoring programme in egg production farms in Germany. J Veterinary Medicine Series B. 45 (12): 95-103.

Heuer OE, Pedersen K, Andersen JS, and Madsen M. 2001. Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. Lett in Appl Micobiol 33: 269-274.

Huneau-Salaun A, Denis M, Balaine L, Salvat G. 2007. Risk factors for *Campylobacter* spp. colonization in French free-range broiler chicken flocks at the end of the indoor rearing period. Prev Vet Med 80 (1): 34-48.

Kapperud, G., G. Espeland, E. Wahl, A. Walde, H. Herikstad, S. Gustavsen, I. Tveit, O. Natas, L. Bevanger, and A. Digranes. 2003. Factors associated with increased and decreased risk of *Campylobacter* infection: a prospective case-control study in Norway. Am. J. Epidemiol. 158:234-242.

Kinde H, Read DH, Chin RP, Bivkford AA, Walker RL, Ardans A, Breitmeyer RE, Wiloughby D, Little HE. 1996. *Salmonella enteritidis*, phage type 4 infection in a commercial layer flock in southern California: bacteriologic and epidemiologic findings. Avian Diseases 40(3): 665-671.

Koerkamp, P. W. G. and R. Bleijenberg. Effect of type of aviary, manure and litter handling on the emission kinetics of ammonia from layer houses. British Poultry Sci. 39:379-392. 1998

Liang, Y., H. Xin, E. F. Wheeler, R. S. Gates, H. Li, J. S. Zajaczkowski, P. A. Topper, K. D. Casey, B. R. Behrends, D. J. Burnham, and Zajaczkowski. 2005. Ammonia Emissions from US Laying Hen Houses in Iowa and Pennsylvania. Transactions of the Asae 48:1927-1941.

Mead, P.S., L.Slutsker, V.Dietz, L.F. McCaig, J.S. Breesee, C.Shapiro, M.Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-625.

Messens W., Grijspeerdt K, DeReu K, DeKetelaere B, Mertens K, Bamelis F, Kemps B, DeBaerdemaeker J. 2007. Eggshell penetraton of various types of hens' eggs by Samonella enterica serovar Enteritidis. J Food Prot 70 (3): 623-728.

Methner, U, Diller R., Reiche R, and Bohland K. 2006. Occurrence of Salmonellae in laying hens in different housing systems and conclusion for the control. Berliner und Munchener Tierarztliche Wochenschfirt 119: (11/12_) 467-473.

Mollenhorst H, van Woudenbergh CJ, Bokkers EGM,de Boer IJM. 2005. Risk factors for *Salmonella enteritidis* infections in laying hens. Poultry Science 84(8):1308-1313.

Nagaraja, K. V., D. A. Emery, K. A. Jordan, J. A. Newman, and B. S. Pomeroy. Scanning electron microscopic studies of adverse effects of ammonia on tracheal tissues of turkeys. Am. J. Vet. Res. 44:1530-1536. 1983.

Neal, K. R., and R. C. Slack. 1995. The autumn peak in campylobacter gastro-enteritis. Are the risk factors the same for travel- and UK-acquired campylobacter infections? J. Public Health Med. 17:98-102.

Oyetunde, O. O., R. G. Thomson, and H. C. Carlson. Aerosol exposure of ammonia, dust, and *Escherichia coli* in broiler chickens. Can. Vet. J. 19:187-193. 1978.

Posadas-Hernandez E, Sanchez-Ramirez E, Avila-Gonzales E, Tellez-Isais G, Salmeron-Sosa DF. Behaviour of certain productive characteristics, stress and resistance to Salmonella enteritidis in semi-heavy poultry under two production systems. Veterinaria Mexicvo 36(2):205-215.

Sato, S., S. Shaya, and H. Kobayashi. Effect of ammonia on *Mycoplasma gallisepticum* infection in chickens. National Inst. Anim. Hlth Qt., Tokyo 13:45-53. 1973.

Stern, N. J., K. L. Hiett, G. A. Alfredsson, K. G. Kristinsson, J. Reiersen, H. Hardardottir, H. Briem, E. Gunnarsson, F. Georgsson, R. Lowman, E. Berndtson, A. M. Lammerding, G. M. Paoli, and M. T. Musgrove. 2003. *Campylobacter* spp. in Icelandic poultry operations and human disease. Epidemiol. Infect. 130:23-32.

Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55:514-517.

Tran, T. T. 1998. A blood-free enrichment medium for growing *Campylobacter* spp. under aerobic conditions. Lett. Appl. Microbiol. 26:145-148.

Tresierra-Ayala, Fernandez H, Bendayan, M. Pereya, G, Bernuy A. 1995. Isolation of thermotolerant *Campylobacter* species from two populations of chickens bred in confinement and at liberty. Revista de Saude Publica 29 (5): 389-392.

UEP. 2006. United Egg Producers Animal Husbandry Guidelines for US Egg Laying Flocks. 3rd Ed. www.uepcertified.com accessed June 2006.

Van Emous RA and Fikls-van Niekerk TGCM. 2004. Higher mortality in free-range aviary houses . World Poultry 20 (6):26-27.

Vits A, Weirtzenburger D, Distl O. 2005. Comparison of different housing systems for laying hens in respect to economic, health and welfare parameters with special regard to organized cages. Dtsch Tierarztl Wochenschrl 112:332-342.

Wesley IV, Muraoka WT, Trampel D and Hurd HS. 2005. The effect of perimarketing events on the prevalence of *Campylobacter jejuni* and *Campylobacter coli* in market-weight turkeys. Appl. Environ. Microbiol. 71: 2824-2831.

Wittwer M, Keller J, Wassenar TM, Stephan R, Howald D, Regula G, Bissig-Choisat B. 2005. Genetic diversity and antibiotic resistance patterns in a *Campylobacter* population isolated from poultry farms in Switzerland. Appl Environ Microbiol 71(6): 2840-2847.

Xin, H., A. Tanaka, T. Wang, R.S. Gates, E.F. Wheeler, K.D. Casey, A.J. Heber, J. Ni, and T. Lem. 2002. A Portable System for Continuous Ammonia Measurement in the Field. ASAE Paper no. 02-4168. Chicago, IL: ASAE.

Table 1. Description and characterization of the laying hen houses monitored in this field observational study.

		Floor-Raised				Manure belt					High-rise				
	House	FR1	FR2	FR3	FR4	MB1	MB2	MB3	MB4	MB5	HR1	HR2	HR3	HR4	HR5
	Site	1	1	2	3	4	4	4	4	4	5	5	5	5	5
	Ventilation	Natural	Mechanical, side inlets and fans	Natural, with chimneys	Natural	Mechanical, tunnel with lengthwise inlet					Mechanical, ceiling inlet with side fans in manure storage area				
	Orientation	E-W	E-W	N-S	E-W	E-W	E-W	E-W	E-W	E-W	E-W	E-W	E-W	E-W	E-W
	Manure management	Litter, wood shavings added once at start of flock	Litter, wood shavings added once at start of flock, partial slatted floor with auger removal	Litter, sawdust added every 2 weeks, partial slatted floor with auger removal	Litter, wood shavings added once at start of flock	Removed twice weekly					Removed between flocks				
WINTER 2006	Date monitored	Jan 10-11	Jan 10-11	Jan 10-11	Jan 10-11	Jan 16-17	Jan 16-17	Jan 16-17	Jan 16-17	DNM	Jan 21-22	Jan 21-22	Jan 21-22	Jan 21-22	DNM
	Bird age	76 weeks	36 weeks	32 weeks	53 weeks	39 weeks	98 weeks	45 weeks	109 weeks	DNM	42 weeks	46 weeks	93 weeks	142 weeks	DNM
	Flock size (initial)	3500	6000	8700	10,000	104,500	106,400	106,400	93,200	DNM	66,061	65,141	64,727	80,174	DNM
SUMMER 2006	Date monitored	Aug 7-8	Aug 7-8	Aug 7-8	Aug 7-8	DNM	DNM	Aug 1-2	Aug 1-2	Aug 1-2	DNM	Jul 24-25	Jul 24-25	Jul 24-25	Jul 24-25
	Bird age	43 weeks	67 weeks	63 weeks	36 weeks	DNM	DNM	76 weeks	32 weeks	50 weeks	DNM	99 weeks	22 weeks	72 weeks	39 weeks
	Flock size (initial)	3500	6000	8700	10,000	DNM	DNM	106,400	106,400	106,400	DNM	65,141	63,006	73,600	66,061
	House dimensions	40 x 160 ft	50 x 210 ft	40 x 300 ft	66 x 180 ft	60 x 520 ft	60 x 520 ft	60 x 520 ft	60 x 520 ft	60 x 520 ft	48 x 430 ft	48 x 430 ft	48 x 430 ft	48 x 430 ft	48 x 430 ft
	Bird housing	Cage-free, free range organic	Cage-free	Cage-free	Cage-free, Omega-3 diet	Caged	Caged	Caged	Caged	Caged	Caged,	Caged	Caged	Caged	Caged
	Birds per group (W/S)	3500	6000	8700	10,000	10	6	6	6	6	8	8	9/8	6/5	8
	Breed	?	?	?	?	W-36	W-36	W-36	W-36	W-36	W-36	W-36	W-36	W-36	W-36
	Molt history (W/S)	None	None	None	None	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?	?/?
	Space allowance, sq in per bird (W/S)	263 + daily pasture access	252	199	171	54	54	54	54	54	59	59	56/61	56/61	59
	Water treatment	peroxide	peroxide	peroxide	none, well-water	ozonated	ozonated	ozonated	ozonated	ozonated	unknown	unknown	unknown	unknown	unknown
	Feed anti-microbials	probiotic	probiotic	FastTrack	Oregano blend	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown	unknown

*DNM = did not monitor

W/S = Winter/Summer

Table 2. Winter conditions: 24-hour mean, maximum and minimum values for each laying hen house and resulting overall mean and standard error for each type of housing system.

24-h Means	**Floor-Raised**						**Manure Belt**						**High-Rise**					
	FR1	FR2	FR3	FR4	**Mean**	SE	MB1	MB2	MB3	MB4	**Mean**	SE	HR1	HR2	HR3	HR4	**Mean**	SE
NH3 Mean	59	57	20	50	**46**	9	6	8	7	6	**7**	0	8	10	20	17	**14**	3
Max	85	86	30	89	**72**	14	9	10	10	9	**9**	0	11	14	26	24	**18**	4
Min	45	46	3	20	**28**	10	4	6	6	5	**5**	0	7	9	16	8	**10**	2
CO2 Mean	2150	2376	1451	2108	**2021**	199	3122	2987	3037	3142	**3072**	36	2455	2260	2691	2326	**2433**	95
Max	2713	3159	2161	4261	**3073**	445	3986	3434	3469	3885	**3694**	141	2643	2678	2953	2643	**2729**	75
Min	1369	2091	884	919	**1316**	281	2507	2472	2713	2643	**2583**	57	2091	2056	2437	2056	**2160**	93
Temperature Mean	16.8	18.6	11.4	15.3	**15.5**	1.5	27.1	25.1	23.8	22.6	**24.6**	1.0	22.8	18.8	20.2	20.6	**20.6**	0.8
Max	17.8	19.5	14.9	20.7	**18.2**	1.3	28.3	26.5	25.3	23.8	**26.0**	1.0	24.7	19.3	21.1	21.3	**21.6**	1.1
Min	14.8	17.5	8.2	9.4	**12.5**	2.2	24.9	23.1	22.3	21.7	**23.0**	0.7	20.4	18.3	18.8	19.8	**19.3**	0.5
Rel. Humidity Mean	69	64	66	62	**65**	1	36	37	47	41	**40**	2	41	51	56	50	**50**	3
Max	72	79	72	69	**73**	2	44	41	54	46	**46**	3	45	56	62	64	**57**	4
Min	63	59	59	55	**59**	2	29	33	40	37	**34**	2	37	49	52	42	**45**	3
Ambient Temp Mean	11.9	13.4	8.0	11.6	**11.2**	1.1					**2.2**						**-0.1**	
Max	14.6	16.5	10.7	14.8	**14.1**	1.2					**21.3**						**1.4**	
Min	10.2	12.3	6.1	7.1	**8.9**	1.4					**-4.7**						**-1.2**	
Ambient rH Mean	71	69	70	67	**69**	1					**92**						**89**	
Max	76	83	76	71	**77**	2					**100**						**93**	
Min	60	64	60	59	**61**	1					**51**						**82**	
Temp. Rise Above Ambient	4.8	5.2	3.4	3.7	**4.3**	0.4	24.9	22.9	21.6	20.4	**22.5**	1.0	22.9	18.8	20.2	20.6	**20.6**	0.8

Table 3. Summer conditions: 24-hour mean, maximum and minimum values for each house and resulting overall mean and standard error for each type of housing system.

24-h Means	**Floor-Raised**						**Manure Belt**					**High-Rise**					
	FR1	FR2	FR3	FR4	**Mean**	SE	MB3	MB4	MB5	**Mean**	SE	HR2	HR3	HR4	HR5	**Mean**	SE
NH3 Mean	3	3	14	15	**9**	3	2	8	5	**5**	2	3	2	3	4	**3**	1
Max	6	6	42	29	**21**	9	4	14	7	**8**	3	5	3	7	8	**6**	1
Min	0	1	3	5	**2**	1	0	3	3	**2**	1	3	0	2	3	**2**	1
CO2 Mean	451	406	631	641	**532**	61	853	1043	1140	**1012**	73	541	442	475	621	**520**	40
Max	643	578	1059	1059	**835**	130	1059	1264	1435	**1253**	94	678	608	643	884	**703**	62
Min	368	333	368	438	**376**	22	643	884	884	**804**	70	473	368	403	508	**438**	32
Temperature Mean	24.0	25.1	25.2	25.5	**25.0**	0.3	30.0	31.0	30.3	**30.4**	0.3	30.1	28.8	28.3	28.7	**28.9**	0.4
Max	28.5	30.3	30.1	30.0	**29.7**	0.4	32.1	32.8	32.1	**32.3**	0.2	33.8	33.3	34.9	33.4	**33.9**	0.4
Min	21.3	22.4	21.9	22.8	**22.1**	0.3	27.4	28.4	28.0	**27.9**	0.3	25.9	24.1	24.3	23.9	**24.6**	0.5
Rel. Humidity Mean	66	61	62	62	**63**	1	73	71	71	**72**	1	46	47	53	52	**50**	2
Max	76	70	70	70	**71**	2	78	78	77	**78**	0	54	57	63	63	**59**	2
Min	50	42	46	46	**46**	2	66	64	65	**65**	1	35	37	37	39	**37**	1
Ambient Temp Mean	24.0	25.0	24.8	24.7	**24.6**	0.2				25.7						27.7	
Max	30.0	32.3	29.8	30.2	**30.6**	0.6				32.3						33.4	
Min	20.8	21.8	21.9	22.0	**21.6**	0.3				21.3						21.5	
Ambient rH Mean	66	60	67	65	**64**	2				94						48	
Max	77	71	76	73	**74**	1				100						64	
Min	46	37	48	45	**44**	2				62						31	
Temp. Rise Above Ambient	0.0	0.1	0.4	0.8	**0.3**	0.2	4.3	5.3	4.6	**4.7**	0.3	2.4	1.1	0.6	1.0	**1.2**	0.4

Table 4. *Mycoplasma synoviae* (MS) or *Mycoplasma gallisepticum* (MG) serology results from chickens in three different housing systems

		Floor-Raised				Manure Belt					High-Rise				
	(% positive)	FR1	FR2	FR3	FR4	MB1	MB2	MB3	MB4	MB 5	HR1	HR2	HR3	HR4	HR 5
Winter	**MS**	0	0	100	100	100	100	100	100	DNM	100	100	100	100	DNM
	MG	100	0	0	0	0	100	0	0	DNM	100	100	100	100	DNM
Summer	**MS**	0	0	100	0	DNM	DNM	100	100	100	DNM	20	100	100	80
	MG	0	0	0	0	DNM	DNM	0	0	0	DNM	100	100	100	100

*DNM = did not monitor

Table 5. Prevalence of *Campylobacter, C. coli*, *C. jejuni* and *Salmonella* in winter.

	Non-caged Floor-Raised (n=40)	Caged (n=79)		
		Manure Belt (n=40)	High-Rise (n=39)	Total (n=79)
Campylobacter	32 (80.0%)[a]	24 (62.0%)[a]	15 (37.5%)[b]	39 (49.4%)[b]
C. jejuni	7 (17.5%)	9 (23.0%)	4 (10.0%)	13 (16.5%)
C. coli	22 (55.0%)[a]	10 (25.6%)[b]	10 (25.0%)[b]	20 (25.3%)[b]
C. jejuni/C.coli	3 (7.5%)	5 (12.8%)	1 (2.5%)	6 (7.6%)
Salmonella	0 (0.0%)	2 (5.1%)	2 (5.0%)	4 (5.1%)

[a,b] Indicates a statistically significant difference ($P<0.05$)

Table 6. Prevalence of *Campylobacter, C. coli*, *C. jejuni* and *Salmonella* in summer based on conventional isolation techniques and real-time PCR detection.

	Conventional Isolation				Real-time PCR			
		Caged (n=70)				Caged (n=70)		
	Non-caged Floor-Raised (n=40)	Manure Belt (n=40)	High-Rise (n=30)	Total (n=70)	Non-caged Floor-Raised (n=40)	Manure Belt (n=40)	High-Rise (n=30)	Total (n=70)
Campylobacter	11 (27.5%)[a]	6 (20.0%)[a]	26 (65.0%)[b]	32 (45.7%)	26 (65.0%)	16 (53.3%)	29 (72.5%)	45 (64.3%)
C. jejuni	3 (7.5%)[a]	6 (20.0%)[a]	21 (52.5%)[b]	27 (38.6%)	12 (30.0%)	9 (30.0%)	15 (37.5%)	24 (34.3%)
C. coli	7 (17.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (7.5%)	7 (23.3%)	9 (22.5%)	16 (22.9%)
C. jejuni/C.coli	1 (2.5%)	0 (0.0%)	5 (12.5%)	6 (8.6%)	11 (27.5%)[a]	1 (3.3%)[b]	5 (12.5%)[a]	6 (8.6%)
Salmonella	3 (7.5%)	2 (6.7%)	1 (2.5%)	3 (14.3%)	3 (7.5%)	2 (6.7%)	1 (2.5%)	3 (4.3%)

[a,b] Indicates a statistically significant difference ($P<0.01$)