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Airflow Pattern in Broiler Houses as a Risk Factor for Growth of Enteric Pathogens

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Abstract: Increased moisture in litter may create favorable conditions for multiplication of enteric pathogens. Areas of reduced air-flow within a broiler house are at increased risk of having excess litter moisture. A cross-sectional study was conducted to assess the association between airflow patterns within a poultry house and litter *Salmonella* and fecal coliform distribution. Five commercial broiler houses in two geographical regions of the USA were sampled. Both conventional and tunnel ventilated houses were represented in the sample. Airflow was not uniform throughout the houses sampled. Airflow at three feet (91.2cm) above the litter surface was greater than the airflow at three inches (7.6cm) above the litter surface. Across a 30 site sampling grid each house had at least 1 region where the airflow velocity was significantly reduced when compared to other regions within the house at the same height. The Friedman two-way analysis of variance found an association between regions of reduced airflow within a poultry house and regions of increased coliform and *Salmonella* contamination. Across all houses , there was a significant association between low air flow regions within the poultry house and fecal coliform counts in excess of the sample median (3.635 cfu /10 g of litter) (p=0.0073). Those litter samples with median fecal coliform counts in excess of 3.635 cfu/ 10 g of litter were 16 times more likely to come from a low air-flow region within the poultry house (95% CI 8.001, 31.994).

Key words: Airflow, broiler house, risk factor, enteric pathogens

Introduction

Salmonellosis is the most frequently reported foodborne illness in the United States, and is the second most common foodborne illness worldwide (Aabo *et al.*, 1995). Controlling *Salmonella* and other enteric pathogens has thus become an important objective for the poultry industry from both public health and economic perspectives. Previous *Salmonella* research has indicated that control at the farm in broiler litter and layer manure could lower contamination in poultry products (Campbell *et al.*, 1982; Henzler *et al.*, 1998; Mallinson *et al.*, 1995). There are several indicator variables for environmental moisture. Studies on the association between environmental moisture and litter bacterial load have defined moisture as: diffuse wetness that can be felt as vapor in the atmosphere; condensed liquid on the surfaces of objects; and water activity (Aw) or equilibrium relative humidity (%ERH) which is the vapor pressure generated by the moisture present in a hygroscopic product and reflects the active part of moisture content or the part which, under normal circumstances, can be exchanged between the product and its environment (Campbell *et al.*, 1982; Henzler *et al.*, 1998; Mallinson *et al.*, 1995). High water parameters

such as these in litter facilitate the multiplication of enteric pathogens such as *Salmonella* and *E. coli*. Studies have shown that Aw greater than 0.90 at the broiler litter surface were associated with increased *Salmonella* prevalence in poultry houses and on carcasses of birds processed from these houses (Edel 1994; Mallinson *et al.*, 1995). These studies conclude that lower Aw levels at the litter surface are associated with lower *Salmonella* loads on carcasses. Therefore, the transmission of *Salmonella* from the farm to the processing plant and potentially to marketed carcasses may be diminished and controlled by implementing management strategies that reduce bacterial loads in the production environment.

Reduction of moisture within a poultry house can be achieved through adequate ventilation. One of the goals of tunnel ventilation systems is to reduce moisture in the poultry house environment (Choiniere *et al.*, 1988; Wierup, 1997). However, an association between rate and pattern of airflow within the poultry house and distribution of litter bacterial load has not been established. Increased airflow, especially in those regions of the house where the airflow may be relatively stagnant even with tunnel ventilation, can lower Aw

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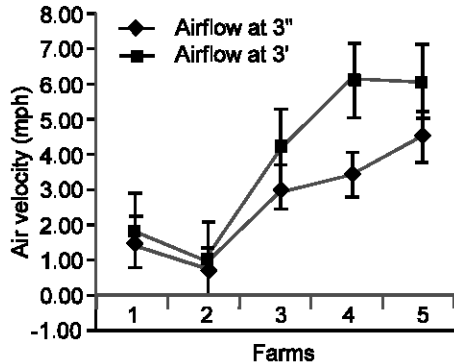
Fig. 1: Average airflow at 3 inches and 3 feet above litter surface for 5 commercial broiler houses in the Mid-Atlantic and Southern region of the United States.
Houses 1, 2 and 3 are from the Mid-Atlantic US
Houses 4 and 5 are from the Southern US
Houses 1, 4 and 5 had tunnel ventilation
Houses 2 and 3 had conventional ventilation

levels at the litter surface throughout the house. This may also indirectly reduce bacterial loads in litter and consequently reduce bacterial loads in the birds at processing. Identification of regions within the poultry house with reduced air-flow and increased *Salmonella* and fecal coliform (FC)multiplication can lead to the development of interventions, such as supplemental ventilation, to control bacterial growth in those "hot spots". Many studies have been shown that environments with low levels of bacterial contamination are associated both with a reduction in prime broiler production costs and *Salmonella*-negative carcasses at processing (Campbell *et al.*, 1982; Kingston, 1981; Mallinson *et al.*, 1995, Pomeroy *et al.*, 1989). This will enhance poultry health, productivity and product food safety.
The objectives of this study are:
1. To study airflow patterns inside broiler houses with different ventilation systems
2. To evaluate the relationship between airflow and the distribution of *Salmonella* and fecal coliforms in broiler house litter.

Materials and Methods
Study design: A cross-sectional study approach was used to assess the association between airflow within a poultry house and litter *Salmonella* and FC loads. Five commercial broiler houses at three different commercial poultry operations located on university farms in the Mid-Atlantic and Southern regions of the United States were visited once during the final week of a six-week grow-out period between April and June, 2004. Each house was sampled by six-drag swabs (DS) collected from the left, center and right sections of the poultry house. After

dragging, the swabs were returned to the transport containers, labeled and placed in an insulated foam box. Airflow within the poultry house was monitored using a sampling grid technique in which 30 evenly spaced intervals of the house were marked. Airflow was then measured at a height of 3 inches (7.6cm) above the litter surface and 3 feet (91.4cm) above the litter surface. Airflow patterns over each sampling site were measured using a digital Hygro-thermometer, anemometer, data-logging instrument (Pacer Industries, Inc., Chippewa Falls, WI) and digital thermo wind meter (Spectrum technologies, Inc., Plainfield, IL) according to the manufacturer's instructions. Each air velocity reading represented the maximum airflow at a particular location during a 30-s interval. From the collected airflow data, 2 maximum, 2 median, and 2 minimum airflow locations were marked for litter sample collection.
A 25-gram litter sample was collected at each of the 6 designated locations. Litter samples were transferred onsite into 50 ml centrifuge tubes containing 25 ml of 2% buffered peptone water (BPW). Tubes were labeled and sealed. Samples were placed in a styrofoam shipping box containing dry ice and sent to the laboratory via overnight courier. Drag swab and litter samples were processed within 24 hrs of collection. All litter samples were processed according to Mallinson *et al.* (1989). The frozen litter samples were thawed quickly in a hot water bath (41°C). The litter was weighed and transferred to 225 ml BPW, thoroughly shaken for 10 minutes, then filtered using a stomacher bag (Fisher brand filtra bag). A 45 ml aliquot of the filtrate was placed into a 50ml plastic tube and frozen at -70°C. The remaining filtrate was placed in a 400ml plastic bottle and placed in a 37°C incubator overnight.

***Salmonella* quantification:** Litter samples were pre-screened for *Salmonella* quantification as previously described. *Salmonella* screening was performed by qualitatively testing for this organism using filtrate. After primary and selective enrichment in BPW and Rappaport Vassiliatis (RV) broth, respectively, all samples were placed on Miller-Mallinson (MM) agar and incubated at 37°C. Plates were read after 24 and 48 hrs of incubation. Suspect *Salmonella* colonies were confirmed by biochemical tests with Triple Sugar Iron (TSI) and Lysine Iron (LIA) agar. Positive litter samples were quantified with a three tube serial dilution technique using the most probable number (MPN) calculation by the FDA-Bacterial Analytical Manual (BAM) method (Andrews and Hammack, 2001).

Fecal coliform quantification: FC quantification was performed by thawing 45-ml aliquot suspension of litter, which was serially diluted to 200μl, 20μl, 2 μl and 0.2 μl and filtered through a microbial monitor (Schleicher and Schuell MicroScience, Inc. USA Riviera Beach, FL) for
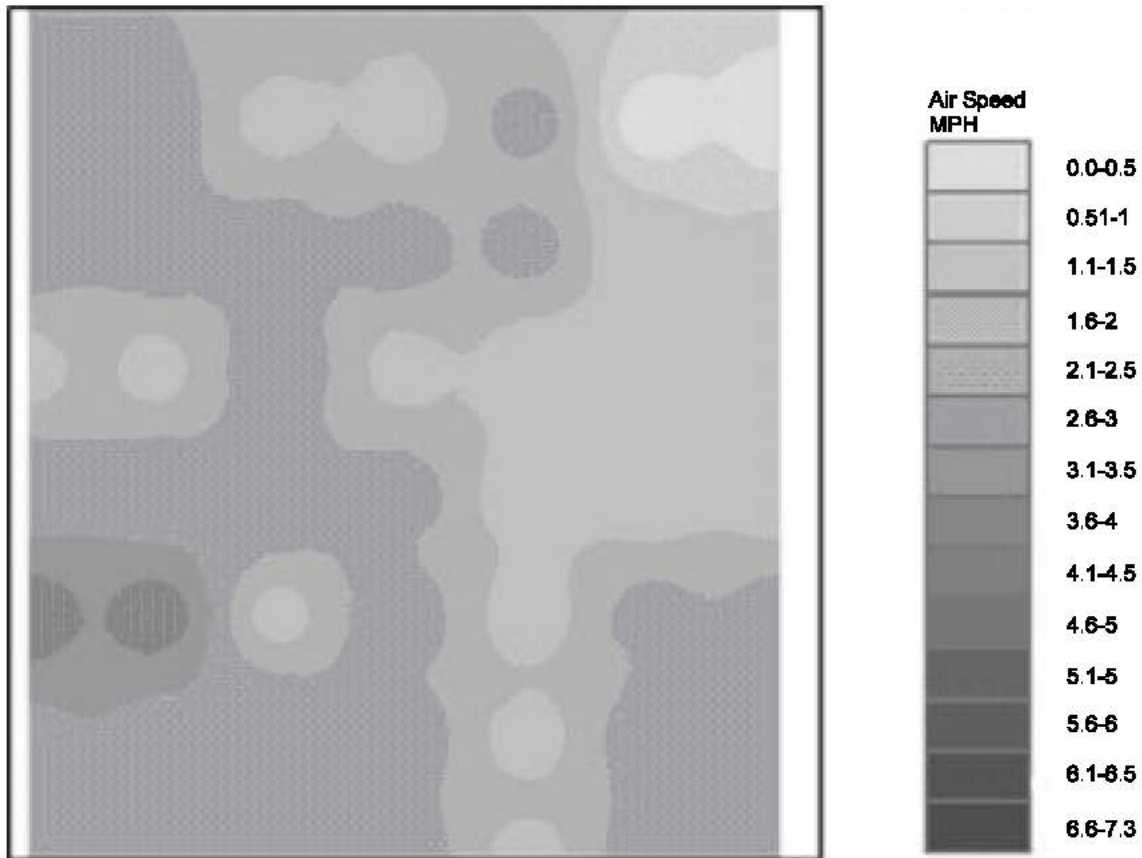
Fig. 2: Airflow pattern in commercial broiler House 1 with tunnel ventilation with fans running, located in the Mid-Atlantic region of the USA.
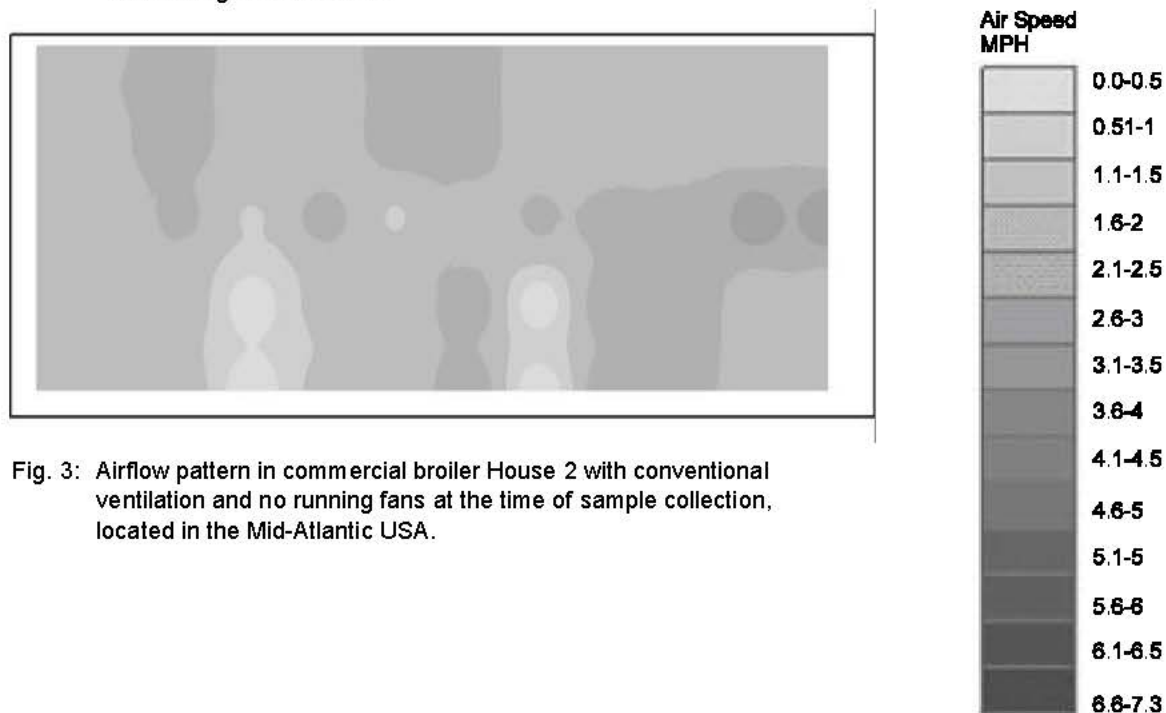

Fig. 3: Airflow pattern in commercial broiler House 2 with conventional ventilation and no running fans at the time of sample collection, located in the Mid-Atlantic USA.
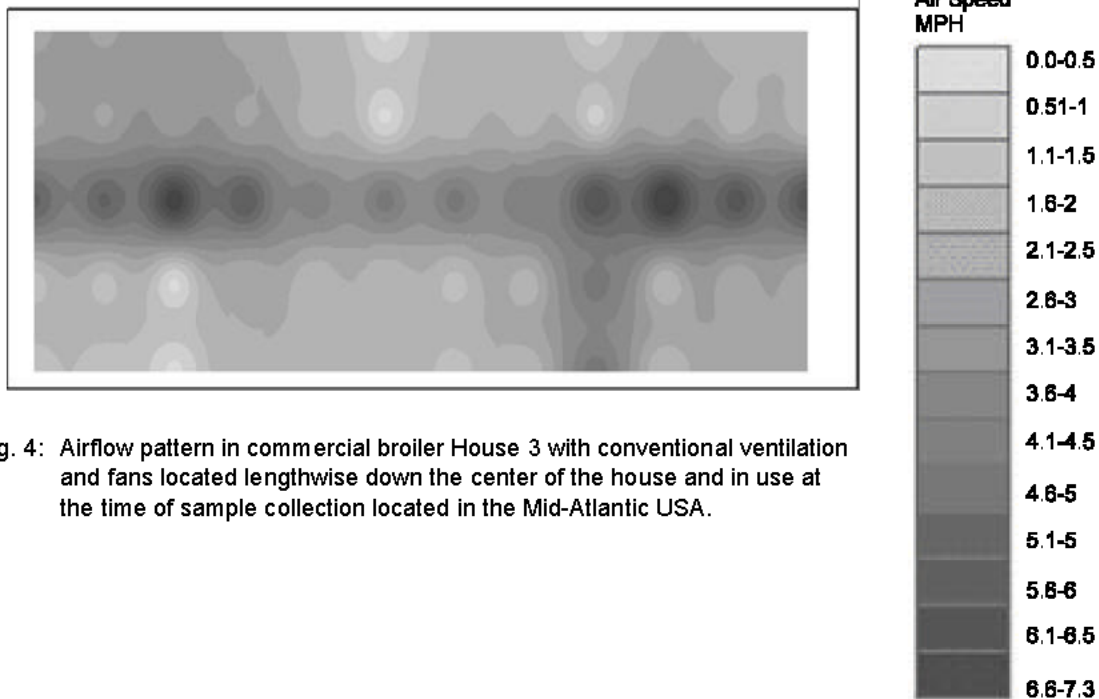
Fig. 4: Airflow pattern in commercial broiler House 3 with conventional ventilation and fans located lengthwise down the center of the house and in use at the time of sample collection located in the Mid-Atlantic USA.

each dilution. 2ml m-FC medium with rosolic acid, (Millipore Corp., Bedford, MA) was added to each monitor and incubated at 41°C for 24 hrs. Typical blue FC colonies were counted for quantification from each dilution and colony forming units (cfu) per 10 grams of litter sample was calculated based on number of colonies, dilution ratio, and litter sample weight.

Statistical analysis: Statistix-8 statistical software (Tallahassee, FL) was used to analyze the data. Raw and transformed data were tested for normality using the Shapiro-Wilkes test (Daniel, 1995). The Spearman Rank Correlation coefficient was used to compare the correlation of airflow at 3 inches above litter and FC counts with airflow at 3 feet above litter and FC counts (Daniel, 1995). The Friedman two-way analysis of variance by rank and simple regression analysis were used to model the relationship between airflow levels and the FC count in the broiler litter samples (Daniel, 1995). Simple regression analysis was also used to model the relationship between FC count and *Salmonella* MPN. Fisher's exact test was used to compare binary outcomes and to estimate odds ratios (Daniel, 1995).

Results
The results of descriptive analysis of airflow patterns in each house are detailed in Fig. 1. Briefly, airflow was not uniform throughout the houses sampled irrespective of ventilation system. Airflow at three feet (91.2cm) above the litter surface was greater than the airflow at three inches (7.6cm) above the litter surface in each house

and also statistically significant (p-value <0.0001) (Fig. 1). Across a 30 site sampling grid each house had at least 1 region where the airflow velocity was significantly reduced when compared to other regions within the house at the same height. See figures 2-6 for the airflow patterns in each house sampled.

Table 1 details the descriptive analysis of *Salmonella* MPN, and fecal coliform counts for litter samples from each house. *Salmonella* and FC counts were not normally distributed. These data were transformed using the natural log transformation. The natural log of the bacterial counts was used in the analysis. Overall the prevalence of *Salmonella* positive houses as determined by the DS method was 80% (4 of the 5 houses sampled) with 50% (15/30) swabs testing positive. By individual litter sample, the prevalence of *Salmonella* positive houses was 60% (3 of the 5 houses sampled) with 33.3% (10/30) litter samples testing positive.

The results of the Spearman Rank Correlation coefficient analysis of airflow velocity and FC counts were inconsistent across the houses. For houses 1 and 5 airflow velocity at a height of 3 feet (-0.8 and -0.6 respectively) was better correlated with FC count than velocity at 3 inches (-0.4 and -0.3) above the litter surface. For house 2 the correlation was greater at 3 inches (-0.8) than at 3 feet (-0.6) and in houses 3 and 4 there was no difference in the magnitude of the correlation coefficients at the 2 heights.

Across all 5 houses, the Friedman's two-way analysis of variance by rank found an association between low airflow and elevated FC rank when compared to
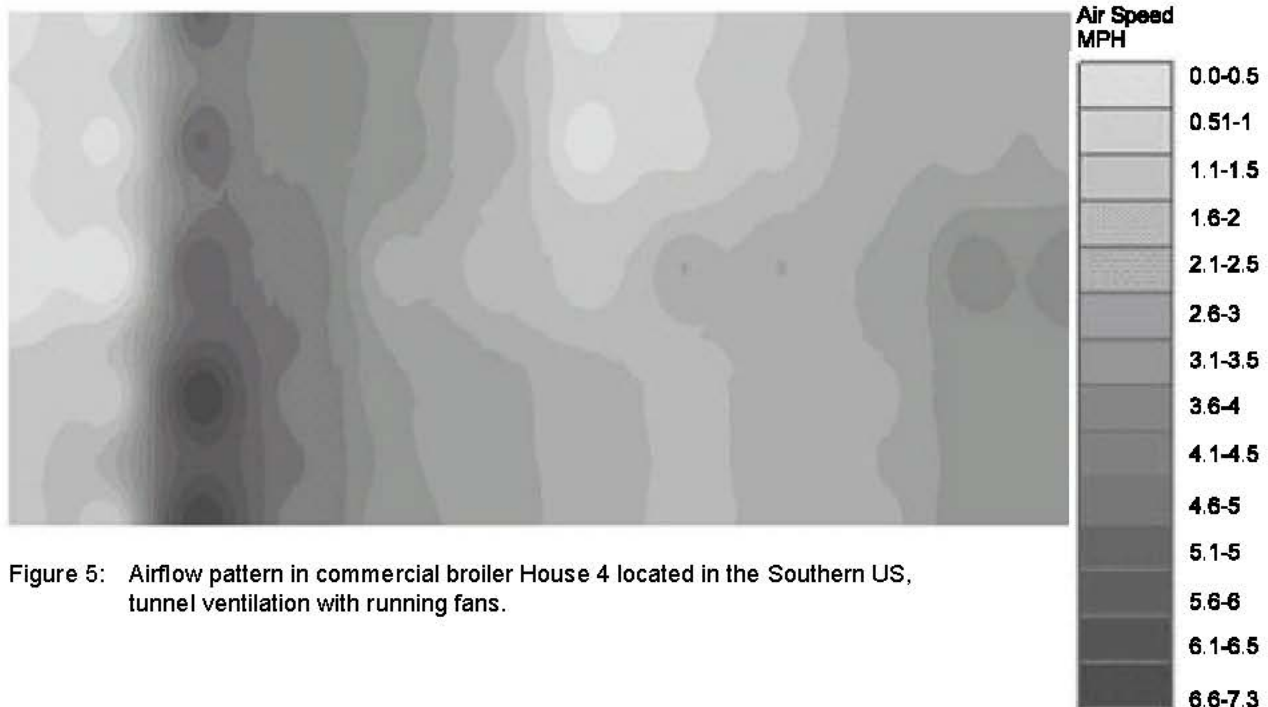
Figure 5: Airflow pattern in commercial broiler House 4 located in the Southern US, tunnel ventilation with running fans.

maximum airflow and FC rank. However, this difference was not statistically significant at α level 0.05 (p-value 0.0578). The median FC cfu for the entire sample was 3.635 cfu/10 g of litter. Overall, there was a significant association between low airflow regions within the poultry house and FC counts in excess of 3.635 cfu /10 g of litter (p=0.0073). Those litter samples with median FC counts in excess of 3.635 cfu / 10 g of litter are 16 times more likely to come from a low air-flow region within the poultry house (95% CI 8.001, 31.994).

At the level of the individual house there was an inverse association between airflow and FC counts in Farms 1, 2 and 3. The resulting relationship was a 3-4-fold reduction in FC count at those sites in which the airflow exceeded the median for the house when compared to those sites where the airflow was below the house median velocity (Table 2). Houses 2 and 3 had a statistically significant difference between FC counts from low airflow litter sampling sites and FC counts from high airflow litter sampling sites (p = 0.0276 and 0.0111 respectively). Fig. 7 illustrates the association between airflow velocity and FC count distribution for House 1.

Regression analysis of airflow rates directly over broiler litter surfaces, in House 1 where litter scattering had been controlled by use of pens, were observed to be significantly associated with FC counts of these surfaces (P = 0.0005, adjusted R^2=0.9120). The regression coefficient of -0.6892 indicates that for every 1mph increase in airflow there was a corresponding 0.6892 cfu decrease in the log FC count (Fig. 8). Regression analysis of the relationship between fecal coliform counts and *Salmonella* MPN using data from

Houses 1-3 indicates that there was a significant association between litter FC count and *Salmonella* MPN (p= 0.0001, adjusted R^2= 0.9108). The regression coefficient of 436.87 indicates that for every 1 cfu increase in fecal coliform count there was a 437 fold increase in *Salmonella* MPN (Fig. 9).

Discussion

As reported by Eriksson-De Rezende *et al.* (2001), the DS method was more sensitive for qualitatively determining the *Salmonella* status of a house than litter samples. The high percentage of negative litter culture results compared to the DS results suggests that *Salmonella* are not uniformly distributed throughout the litter surface. Airflow results indicated that areas exposed to higher ventilation rates were associated with lower FC levels and that this is perhaps due to lower litter moisture levels. The low prevalence of *Salmonella*-positive litter samples precluded analysis of an association between airflow and *Salmonella* load. However, the strong association between FC count and *Salmonella* MPN demonstrate that fecal coliforms can serve as reliable indicator organisms for houses at increased risk of Salmonella contamination.

Although ventilation practices varied widely between farms (number, placement, static pressure, running time of fans, and curtain setting), there was an association between the rate of airflow over a specific litter location and its bacterial load. The strength of the association varied across houses, the closest association between bacterial load and airflow was in Houses 1-3 which were located in the Mid-Atlantic region of the US. Houses 4
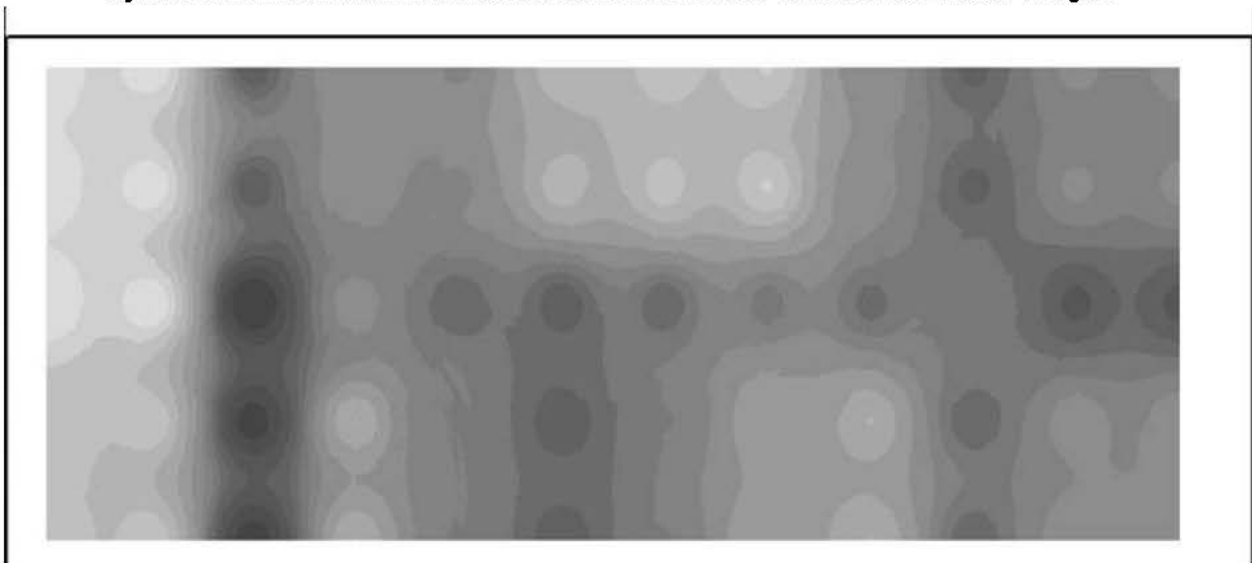
Fig. 6: Airflow pattern in commercial broiler House 5 located in the Southern US, tunnel ventilation with running fans
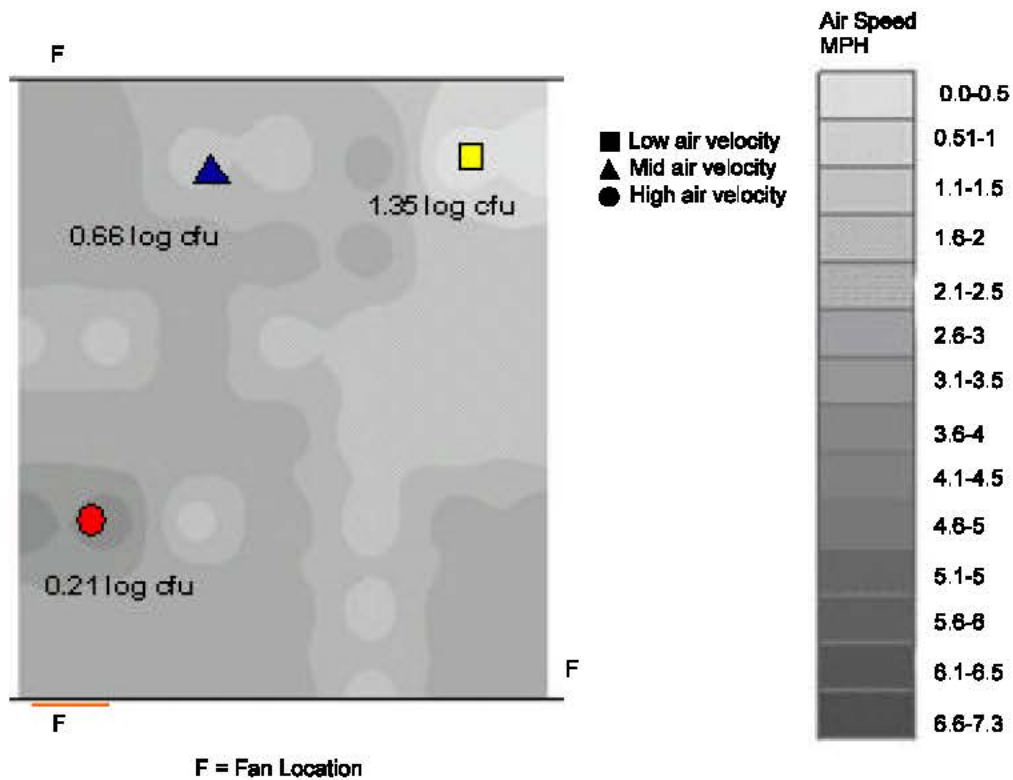

F = Fan Location

Fig. 7: Fecal Coliform counts and airflow pattern in House 1, a tunnel ventilated house.

and 5 were located in the Southern US where ambient temperature and relative humidity were much higher at the time the samples were collected compared to those in the Mid-Atlantic. To further analyze the impact of geographic location, weather conditions, and seasonal patterns, prospective studies need to be conducted with a larger sample size.

In contrast to houses that were ventilated by wind and propeller fans, tunnel ventilated houses had a greater and unvarying flow of air (3.3 to 4.8 mph) over the entire litter surface. It was also noted that, velocity at 3 inches above litter surface was lower than at 3 feet above the surface. Lacy *et al.* (1992) reported that tunnel ventilation had advantages of reduction of heat stress related mortality and improved feed conversion and these advantage could also be serve as an effective means of

Table 1: Mean *Salmonella* MPN and fecal coliform counts for 5 commercial broiler houses in the Mid-Atlantic and Southern USA

Farm#	Mean *Sal.* MPN	Range	Mean F C counts	Range
1	8.0	(1.61 – 22.48)	0.0	(0 – 0)
2	1.86	(0.32 – 4.58)	0.0	(0 – 0)
3	1.67	(0.43 – 4.0)	1.0	(0 – 5)
4	6.76	(0.7 – 15.2)	229.3	(0 – 889)
5	63.73	(3.46 – 285)	27×10^3	$(0 - 12.5 \times 10^3)$

Table 2: Association between airflow and fecal coliform counts in commercial broiler houses 1-3 in the Mid-Atlantic region of the United States

Farm #	Median Airflow (mph)	Median FC counts (millions)/10g	OR	95% C.I
1	2.5	2.5	3	0.8-100.9
2	0.52	0.7	4	2.6 -111.4
3	2.5	0.8	4	2.6-111.4

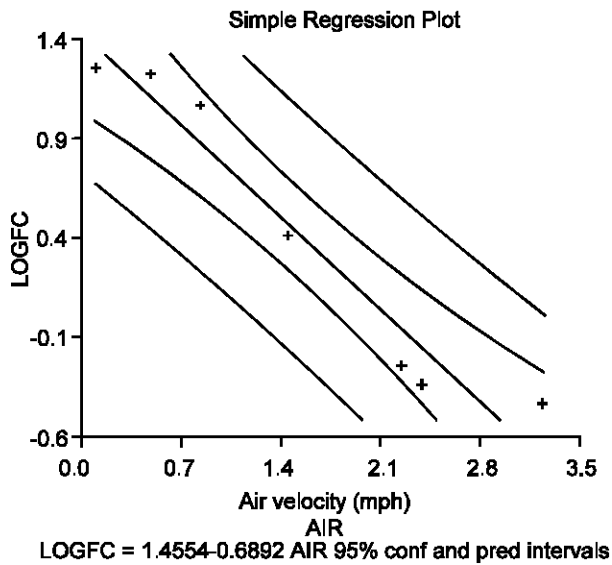LOGFC = 1.4554-0.6892 AIR 95% conf and pred intervals

Fig. 8: Simple regression plot to estimate fecal coliform counts by average airflow at 3 inches level in a controlled airflow house (p-value = 0.0005, adjusted R^2= 0.9120).

SALM = -1445.9 + 436.88 E 95% conf and pred intervals

Fig. 9: Simple regression plot to estimate Salmonella MPN by fecal coliform count (p-value<0.001, adjusted R^2= 0.9108).

removing excessive moisture from litter, thereby helping to suppress *Salmonella* and *E.coli* levels and risks. In naturally ventilated houses sidewall propeller fans, ceiling fans or perhaps even baffles may provide more homogenous airflow coverage over the litter bed (Bottcher *et al.*, 1995). This study provided more information that airflow over the litter surface and moisture levels within the litter represent potentially important poultry house parameters that need to be monitored to ensure a safe and healthy environment for broiler performance. While avoiding establishment of undesirable dry dusty conditions, the corrective management practices of identifying regions of relatively stagnant air flow and adding supplemental ventilation in those areas may prove to be a cost effective means of reducing contamination by enteric pathogens.
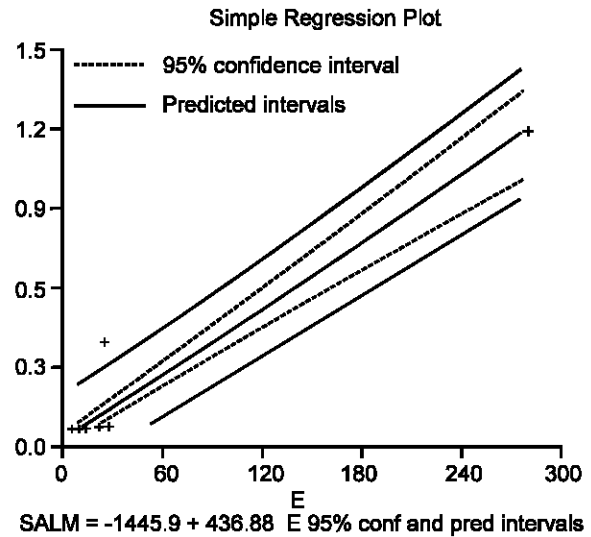
In short, the identification of ventilation designs and/or devices that ensure proper rates of minimal airflow is encouraged. Proper airflow, well known to reduce litter moisture reduction and heat stress, appears to have an additional role as the suppression of enteric pathogens.

Acknowledgement

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