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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

A One Year Study of Newly Constructed Broiler Houses for the Prevalence of *Campylobacter*

K.N. Eberle¹, J.D. Davis², J.P. Purswell³, H.M. Parker¹, C.D. McDaniel¹ and A.S. Kiess¹

¹Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA

²Ag and Bio Engineering, Mississippi State University, Mississippi State, MS 39762, USA

³USDA-ARS, Poultry Research Unit, Mississippi State, MS 39762, USA

Abstract: In 2009, the USDA Food Safety and Inspection Service announced the development of new pathogen reduction performance standards for *Salmonella* and *Campylobacter* both on-farm and in the processing plant. The objective of this study was to evaluate the prevalence and distribution of *Campylobacter* in 3 newly constructed broiler houses for the first 4 flocks placed. Litter and fecal samples were collected from each house at 0, 28 and 48 d of production. Samples were serially diluted and spread onto Campy Cefex agar plates. Two 40 mL water samples were collected each production day and filtered through a 0.45 µm membrane before being placed onto a Campy Cefex agar plate. All plates were purged with a microaerophilic gas and incubated for 36 h at 42°C. Individual plates were screened for characteristic *Campylobacter* colonies and suspect colonies were confirmed using a latex agglutination kit. An additional 50 g of litter was collected from the evaporative cooling inlets, middle and tunnel ventilation fans to determine litter moisture and pH. Inside and outside temperatures were also collected. Out of 2300 litter, 900 fecal and 45 water samples, only 5, 6 and 1 of the collected samples, respectively, were confirmed *Campylobacter* positive. The middle of the house contained a higher litter moisture level (37%) than the evaporative cooling inlet end (33%) and tunnel ventilation fan end (34%) ($p < 0.05$). Litter pH was not different across days, locations or flocks. Temperature averaged 26.8°C inside and 27.6°C outside. In conclusion, the newly constructed houses did not show a high prevalence of *Campylobacter*. Litter moisture was at levels conducive for *Campylobacter* growth. The high litter pH and low temperatures, along with other on-farm management strategies and the fact the broiler houses were brand new, may have suppressed *Campylobacter*'s ability to colonize the litter.

Key words: *Campylobacter*, poultry, broiler house, food safety

INTRODUCTION

As of 2007, Americans on average consume 86.5 lb of chicken; a significant increase from the 1970s when chicken consumption was on average 40.2 lb (American Meat Institute, 2009). The major increase in poultry product consumption brings with it an increased awareness in food safety concerns for both consumers and poultry producers. *Campylobacter*, a bacterium known to be found in poultry, is the most frequent cause of bacterial gastroenteritis, also known as campylobacteriosis (Newell *et al.*, 2003). Campylobacteriosis is estimated to affect over 2.4 million people and cost around \$1.2 billion dollars annually. These costs are a result of physician visits, medical supplies, hospital services and medications as well as productivity loss from poorly performed or missed work (Economic Research Service, USDA, 2000; Center for Disease Control, 2008).

Only in the last 25 years has campylobacter been recognized as an important cause of human food-borne illness. Disease control studies have demonstrated that

50% to 70% of these illnesses are attributed to consuming contaminated poultry and poultry products (Keener *et al.*, 2004). The manner in which poultry is raised and slaughtered leaves little possibility for the complete elimination of *Campylobacter* on broiler carcasses (Oosterom *et al.*, 1983; Genigeorgis *et al.*, 1986). On-farm, broilers may be colonized by *Campylobacter* from contaminated feed, litter, or water and other vectors like rodents, wild birds, or humans (Shanker *et al.*, 1990; Stern *et al.*, 1997 and Quinones-Ramirez *et al.*, 2000). In the plant, raw carcasses may be contaminated by birds, equipment, workers or the air in the plant (Oosterom *et al.*, 1983). By reducing the number of bacteria on the farm, it may be possible to significantly lower the possibility for contamination in the plant.

In July of 1996, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) implemented the Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems final rule with the goal of improving food safety. In this

rule, FSIS established standards designed to reduce the occurrence and levels of pathogenic organisms on meat and poultry products in addition to reducing the incidences of food-borne illness associated with the consumption of those products (United States Department of Agriculture, Food Safety and Inspection Service, 1996). Also in this rule, FSIS discusses the farm-to-table strategy for the control of food safety hazards throughout the entire process of animal production. Establishing pathogen-specific standards for both on-farm and processing facilities have allowed for the direct measurement of pathogen management and reduction. While new standards for monitoring *Salmonella* and *Campylobacter* were implemented in July of 2011 for processed chicken and turkey (United States Department of Agriculture, Food Safety and Inspection Service, 2011), there is limited information available on the prevalence and distribution of *Campylobacter* on the farm. Therefore, the objective of this study was to investigate the prevalence of *C. jejuni* in 3 newly constructed broiler houses.

MATERIALS AND METHODS

House: A newly constructed broiler farm in Mississippi was used for sampling in this experiment. Sampling occurred in 3 of the 6 newly built houses (Fig. 1). The houses were solid side wall tunnel

ventilated houses, measuring 500 ft. in length and 50 ft. in width. Evaporative cool pads were located on the first 100 ft of the far west side of the houses. Each house contained 6 drinker lines and 3 feeder lines. The water was supplied by a well located on the west side, at the front of all the houses on the farm. Each house was surrounded by gravel and grass. Rice hulls were the chosen bedding material; all houses were filled with the first flock and top-dressed between every flock thereafter.

Samples and sampling scheme: All litter, fecal and water samples were collected on day 0, 28 and 48 of a typical grow out cycle over 4 flocks in each of the 3 houses. The pH and percent moisture samples were not collected until day 28 of the second flock in each house. The reason for the delay in pH and percent moisture samples was due to all microbial samples being negative up to that flock. Approximately 50 g of broiler litter was collected in a 2" x 2" x 2" area every 50 ft down the length of the house at 3 different locations and every 5 ft across the house at 100 ft, 250 ft and 400 ft down the house (Fig. 2), for a total of 51 litter samples per house per sampling time. These particular locations were chosen from preliminary data performed prior to this study (unpublished data). All litter samples were placed in sterile, labeled Whirl-pak® bags (Nasco, USA). Fecal samples were collected from random birds at

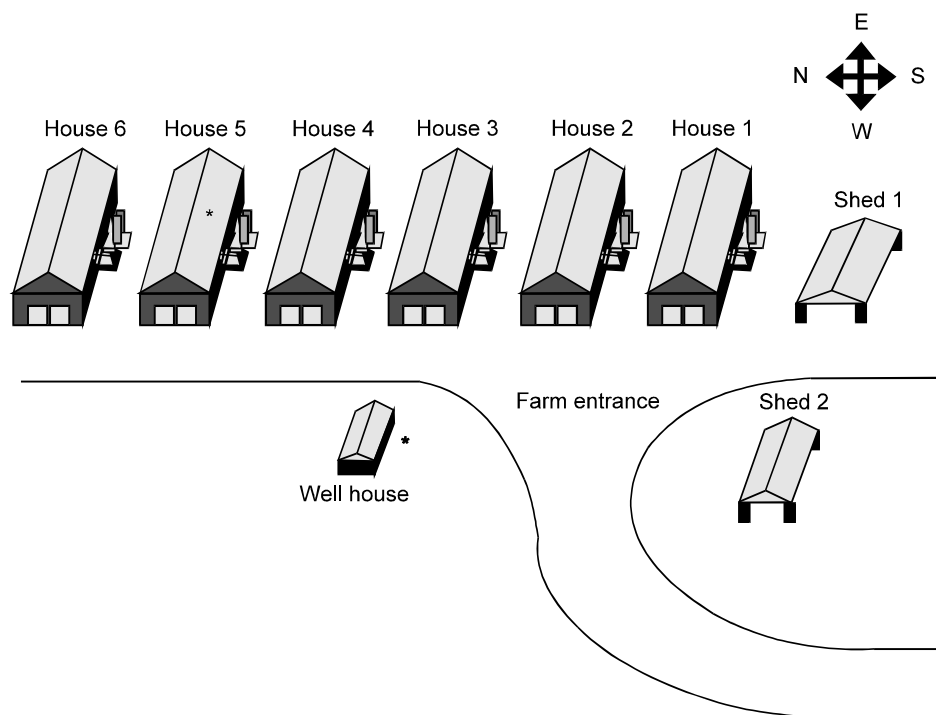


Fig. 1: An illustration of the poultry farm. The farm contained a total of 6 houses. Each house measured 500 ft in length and 50 ft in width. There was 30 ft of space between each house and 100 ft between house 4 and the well house. Samples were collected from houses 2, 4 and 6. *A weather station was placed outside beside the well house to obtain outside temperatures and inside house 4 to obtain inside temperatures

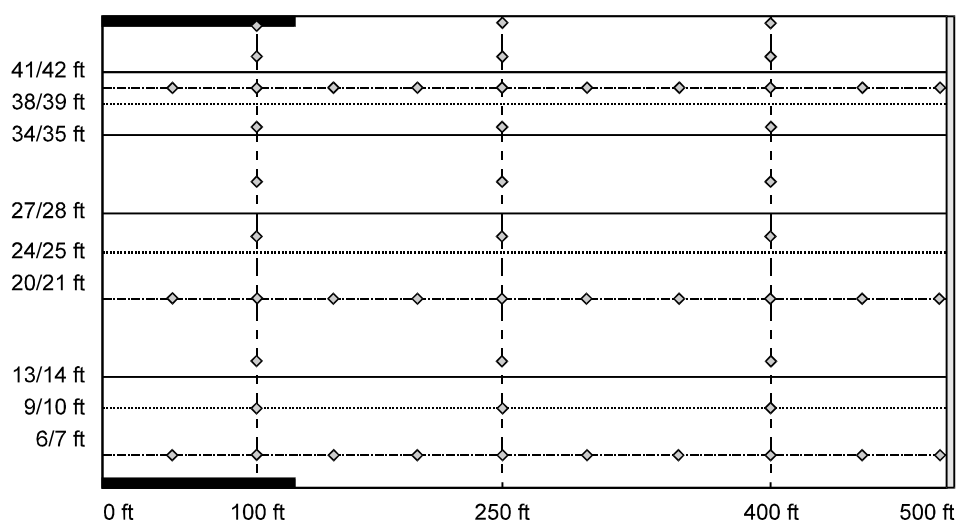


Fig. 2: Sampling scheme for litter collection. A total of 51 litter samples were collected across the width and down the length of each house. A total 10 litter samples were collected every 5 ft across the width of the house at 100 ft, 250 ft and 400 ft down the length of the house. An additional 7 litter samples were collected every 50 ft down the length of the house at 5 ft, 19 ft and 40 ft across the width of the house. ◆ represents litter samples, _ represent water lines, ... represents feed lines, - represents evaporative cool pads, - represents fans, an - • - represents sample lines

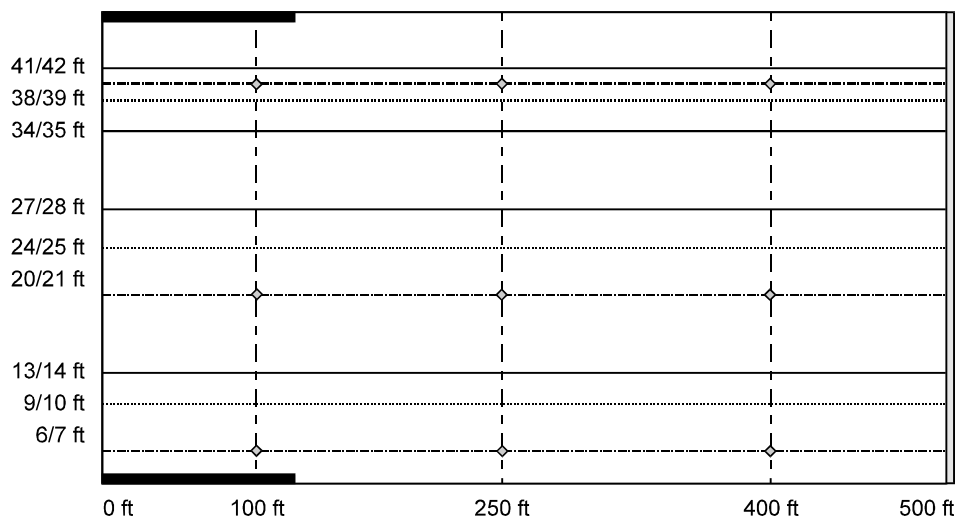


Fig. 3: Sampling scheme for fecal collection. A total of 20 fecal samples were collected across the width and down the length of each house. A total 2 birds were caught and feces expressed at 3 locations at each of 5 ft, 19 ft and 40 ft across the width of the house and at 100 ft, 250 ft and 400 ft down the length of the house. An additional 2 birds were randomly selected from the house for fecal samples. ◆ represents litter samples, _ represent water lines, ... represents feed lines, - represents evaporative cool pads, - represents fans, an - • - represents sample lines

corresponding cross-points of the litter sample pattern across and down the house (Fig. 3). Fecal material was expressed from the cloaca into sterile Whirl-pak® bags (Nasco, USA). Fecal collection was repeated at each cross-point (18 total) and twice more with birds chosen randomly throughout the house. Water samples from the

well house and the evaporative cooling pad tanks were collected in sterile 50 ml conical tubes (Becton Dickinson and Company, Franklin Lakes, NJ). The outside of the evaporative pads were dry swabbed and swabs were placed in 'Port-A-Cul' Collection and Transport Systems transport media (Becton Dickinson

and Company, Franklin Lakes, NJ). All samples were immediately placed on ice after collection for transport back to the laboratory. A total of 2, 300 litter, 900 fecal and 45 water samples were collected over the duration of this experiment.

Microbial analysis: For litter and fecal samples, approximately 10 g and 1 g, respectively, were weighed and diluted ten-fold into buffered peptone water. Samples were then stomached for 30 s at 135 rpm in a Brinkmann/Seward 400°C Stomacher® (Fisher Scientific, Pittsburgh, PA). After stomaching, samples were serially diluted and 100 µL of each dilution was spread, in duplicate, on Campy Cefex agar plates. For each water sample from both the well house and evaporative cooling pad tanks, 40 ml was vacuumed through 0.45 µm cellulose nitrate membranes which were positioned upside down on Campy Cefex agar plates. The evaporative cooling pad swab was removed from the transport media and directly streaked on a Campy Cefex agar plate. Using an Anoxomat Mart II system (Mart Microbiology B. V., Netherlands), all plates were flushed with a microaerophilic gas mixture (80% N₂, 10% CO₂, 5% H₂ and 5% O₂) and placed in a 20 cubic foot Precision Model 815 low temperature incubator (Thermo Scientific, Marietta, OH) at 42 C for 36 h. At 24 h, filters from water samples were removed and plates were flushed a second time with the microaerophilic gas mixture and incubated for an additional 12 h before confirmation. After the incubation period, plates with colonies suspected to be *C. jejuni* were confirmed using an Scimedx CAMPY (jcl)TM *C. jejuni*, *C. coli*, *C. laridis* Latex Agglutination Assay (Scimedx Corporation, Denville, NJ). Plates that were confirmed positive for *Campylobacter* were set aside and counted to determine the total number of colonies on the plate.

Percent moisture: Litter from each section of the house were separated into three separate samples and weighed out to approximately 15g to obtain an initial wet weight. Samples were placed in a 40GC series lab oven (Quincy Lab Inc, Chicago, IL) at 105°C for 24 h. Dried samples were removed from the oven and weighed again to determine a dry weight. The percent moisture was calculated using the following equation (Department of Sustainable Natural Resources, 1990):

$$MC (\%) = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \quad (1)$$

Where:

W₁ = Weight of tin (g)

W₂ = Weight of moist soil+tin (g)

W₃ = Weight of dried soil+tin (g)

pH: Approximately 10g of litter from each location within the house was weighed and placed into a 200 mL beaker. Distilled water (100 mL) was added to the beaker and mixed for approximately 5 min (AOAC, 1995). An Accumet excel XL60 pH probe (Fisher Scientific, Pittsburgh, PA) was then placed in the litter and water combination to obtain the pH reading.

Temperature: Outside temperatures were measured using a weather station (Onset Computer Corp., Pocasset, MA) located at the west end of the farm by the well house. A thermistor sensor attached to a data logger (ON-901-44008, Omega Engineering, Stamford, CT) was used to collect data in house 4 of the three houses sampled.

Statistical analysis: A randomized complete block design with a split-split plot over time was used to analyze litter moisture and pH data. The houses were assigned as blocks and the treatment was location within a house. Flock represented split plots and age of the flock represented split-split plots. The means were separated using Fishers Protected LSD. Means were considered significant at p<0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Three newly constructed broiler houses were investigated for levels of *C. jejuni* in litter, birds and surrounding environment. Litter, fecal and water samples were collected as well as percent moisture, pH and temperature both inside and outside the houses. Litter, fecal and water samples were collected over all four flocks, while pH and percent moisture samples were not collected until day 28 of grow-out for flock two. Out of 2, 300 litter, 900 fecal and 45 water samples, only 5, 6 and 1 of the collected samples respectively were confirmed *Campylobacter* positive. There were not enough positive *Campylobacter* samples to show any statistical significance of its growth in the water, birds or on the litter. The low number of positive *Campylobacter* samples may have been due to our sampling of new broiler houses which may have not had enough time to establish a strong litter microflora.

Litter moisture was determined to increase with flock age with Day 0 having much lower litter moisture (31%) than Day 28 (36%) and Day 48 (41%) (p<0.05, Fig. 4). There was a very small difference in average litter moisture across houses 4 (37%) and 6 (35%) (p<0.05, Fig. 5). Litter moisture was also different depending on location, with the tunnel ventilation end containing a lower level of moisture (34%) than the evaporative cooling inlet end (36%) and the middle (38%, p<0.05, Fig. 6). A national survey found the ideal average moisture content for litter to be around 25% (Terzich *et*

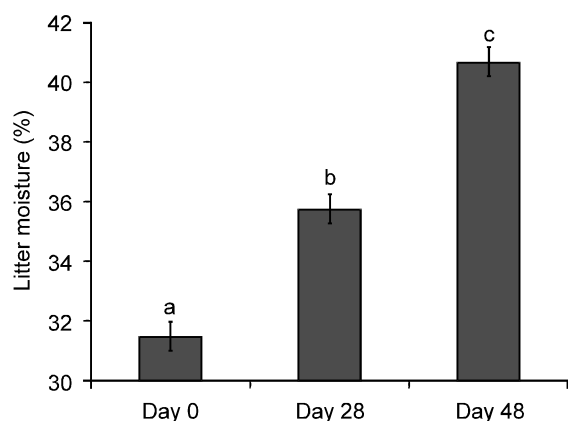


Fig. 4: Average litter moisture (% moisture) by flock age. For every flock age in each of 3 flocks, litter was sampled from 3 locations in each of 3 broiler houses (n = 27 samples per age). Columns with different superscripts represent differences in litter moisture ($P < 0.05$)

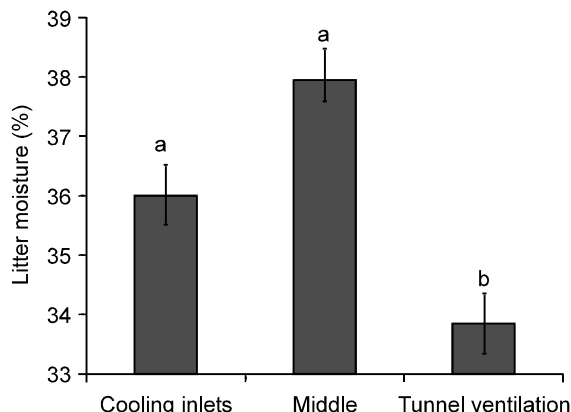


Fig. 6: Average litter moisture (% moisture) by location within broiler houses. For every location within 3 broiler houses, litter from 3 flocks was sampled at each of 3 broiler ages (n = 27 samples per location). Columns with different superscripts represent differences in litter moisture ($P < 0.05$)

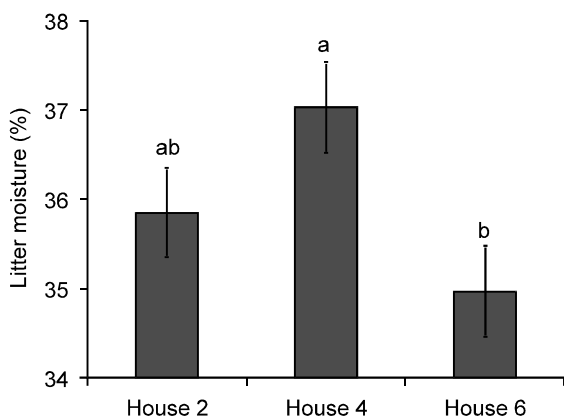


Fig. 5: Average litter moisture (% moisture) by broiler house. For every house, litter from 3 flocks was sampled from 3 locations at each of 3 broiler ages (n = 27 samples per house). Columns with different superscripts represent differences in litter moisture ($P < 0.05$)

et al., 2000). Before chick placement (Day 0), litter moisture will mostly likely be high due to the addition of fresh litter or top dressing by means of cut pine shavings or rice hulls which can hold gallons of excess water (Czarick and Fairchild, 2006). A decrease in litter moisture over flock age, as seen in Fig. 4, may be due to the temperature and ventilation changes that occur as a flock matures and body weight fluctuates. Initial heating of the house must occur in order to insure proper temperatures of the chicks during the first few days of placement (Bell and Weaver, 2001). Brooders heat the litter as well as the air, removing moisture from both as the temperature rises. As the birds get older and their

body weight and body heat rapidly increases, it is important to have proper ventilation to cool the birds to maintain feed consumption and reduce mortality (Bucklin *et al.*, 1998). Like heating, the movement of air can also remove moisture from the litter. As air is drawn down the length of the house the air temperature increases allowing moisture to be picked up and then removed from the house by the ventilation fans. Given that warmer air can hold more moisture than cool air, the litter near the fan end will be drier when compared to litter in other locations within the house (Czarick and Fairchild, 2003). Bird concentration can have an effect on how the air travels down a house. Higher concentrations of birds in a particular area may allow for the exposure of litter to the air, resulting in higher litter moisture percentages in the middle of the house, as demonstrated in Fig. 6 (Czarick and Fairchild, 2003).

An interaction was discovered for litter pH between flock, day and location. pH for Flock 2 was higher in the cooling inlet area on Day 48 than the tunnel ventilation area on Day 28 ($p < 0.05$, Fig. 7). The cooling inlet also was significantly different than the other two locations on Day 48 in Flock 2. Flock 3 results showed that on Day 0, the tunnel ventilation pH was significantly lower than the other two locations ($p < 0.05$, Fig. 8). Litter pH on Day 48 of Flock 3 leveled out, continuing into Flock 4 which showed no significant changes of pH due to location and day (Fig. 9).

Broiler litter ranges from pH 9.0 to 10.0 (Blake and Hess, 2001). The optimum pH for *Campylobacter* growth is 6.5 to 7.5 but the bacteria can grow from pH 4.9 to 9.0 (Doyle, 1989). In this study, the pH ranged from 8.09 to 9.30 which is right at the edge of the pH range for normal *Campylobacter* growth. While the pH in this study was

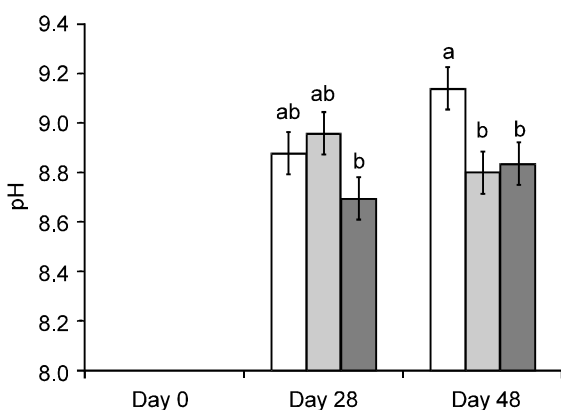


Fig. 7: Interaction of flock, day of age and location for litter pH. For Flock 2, samples were obtained from 3 houses (n = 3). Dark grey columns represent cooling inlet end, white columns represent the middle of the house and light grey columns represent tunnel ventilation exit end. Columns with different superscripts represent differences in litter pH ($P < 0.05$)

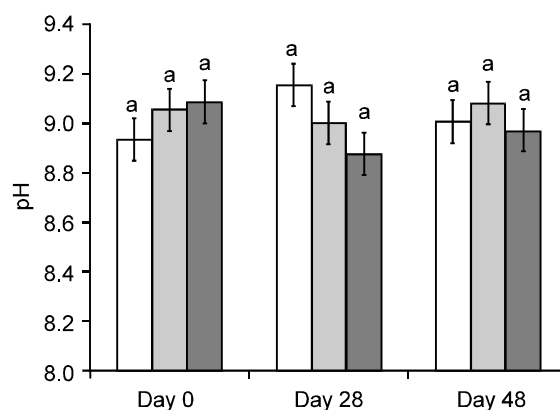


Fig. 9: Interaction of flock, day of age and location for litter pH. For flock 4, samples were obtained from 3 houses (n = 3). Dark grey columns represent cooling inlet end, white columns represent the middle of the house and light grey columns represent tunnel ventilation exit end. Columns with different superscripts represent differences in litter pH ($P < 0.05$)

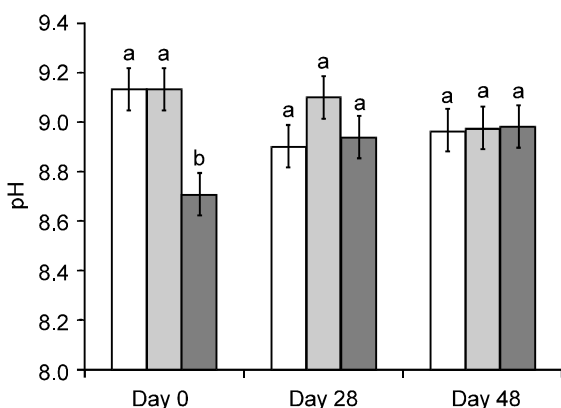


Fig. 8: Interaction of flock, day of age and location for litter pH. For Flock 3, samples were obtained from 3 houses (n = 3). Dark grey columns represent cooling inlet end, white columns represent the middle of the house and light grey columns represent tunnel ventilation exit end. Columns with different superscripts represent differences in litter pH ($P < 0.05$)

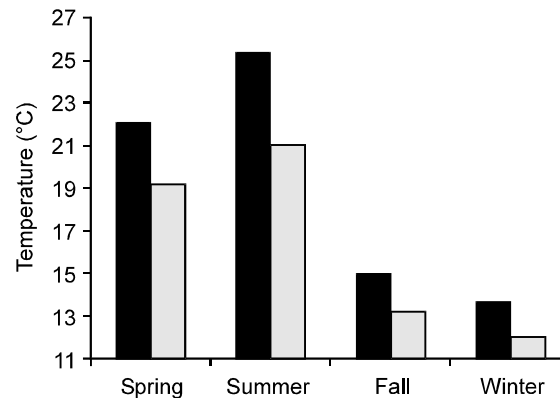


Fig. 10: Seasonal low temperature inside and outside broiler house 4. Black columns represent inside temperatures and light grey columns represent outside temperatures. Spring, summer, fall and winter were considered the months of March through May, June through August, September through November and December through February, respectively

found to be different over flocks, locations and days, physiologically the small changes in pH may not have been significant enough to allow for *Campylobacter* growth. Another explanation for the lack of *Campylobacter* presence may correspond with the amount of excreta being deposited on the litter because the birds eat and drink more as they grow. pH, as well as ammonia levels, increase as nitrogenous compounds, like urea and uric acid, are mineralized by microbes in the litter (Rothrock *et al.*, 2008). As litter pH

increases, ammonia levels increase (Blake and Hess, 2001). From Day 28 to Day 48 over multiple flocks, ammonia levels noticeably increased within the houses. Temperature was measured over the duration of the study and averaged into seasons: Spring (March, April, May), summer (June, July, August), fall (September, October, November) and winter (December, January, February). Figures 10 and 11 illustrate the changes in average high and low temperatures over the seasons. *Campylobacter* incidence, in both broilers and humans,

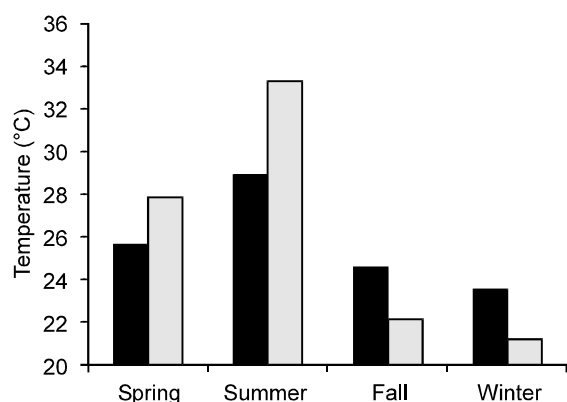


Fig. 11: Seasonal high temperature inside and outside broiler house 4. Black columns represent inside temperatures and light grey columns represent outside temperatures. Spring, summer, fall and winter were considered the months of March through May, June through August, September through November and December through February, respectively

has been found to follow a seasonal pattern, suggesting that climatic factors may have a role in colonization (Patrick *et al.*, 2004; Tam *et al.*, 2005). Patrick *et al.* (2004) observed a steep increase in *Campylobacter* prevalence in humans and broilers at a temperature range of 8°C to 20°C. Due to the lack of *Campylobacter* presence, the possibility of climate influence could not be observed in this study.

Insects and rodents were not huge factors on this new farm. Rodent feces, presumably mice, were found in the utility rooms attached to the houses but were never seen. Flies were found outside the houses during the summer and fall months but were never found in the houses themselves. Litter beetles and worms did not appear in the litter until Flock 4. The absence of these particular vectors in the houses may suggest that they did not play a role in initial *Campylobacter* transmission but may be carriers after a farm becomes established with this bacterium.

Conclusion: The newly constructed houses did not show a high prevalence of *Campylobacter*. Litter moisture was at levels conducive for *Campylobacter* growth. However, the high litter pH and low temperatures, along with other on-farm management strategies and the fact the houses were brand new, may have suppressed *Campylobacter*'s ability to colonize the litter.

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