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

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Distribution of Bacteria at Different Poultry Litter Depths[1]

K.J. Barker[1], J.L. Purswell[2], J.D. Davis[3], H.M. Parker[1], M.T. Kidd[1], C.D. McDaniel[1] and A.S. Kiess[1]
[1]Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA
[2]United States Department of Agriculture, Agriculture Research Service (USDA-ARS)
Poultry Research Unit, Mississippi State, MS 39762, USA
[3]Department of Agricultural and Biological Engineering, Mississippi State University,
Mississippi State, MS 39762, USA

Abstract: A common practice in the commercial broiler industry is to reuse litter over multiple broiler flocks. Over time the bacterial populations in the reused litter increases but how those organisms are spatially distributed throughout the litter bed is unclear. Therefore, the goal of this project was to investigate the distribution of bacteria at three different depths of litter. Litter samples were collected from three commercial broiler houses on three different farms. Four samples from each house were collected using clear PVC pipes which were driven through the litter bed to the clay floor. Each pipe was transported up-right to the lab, where they were cut into three sections (top, middle and bottom) exposing the litter for processing. Litter from each section was serially diluted in peptone and streaked onto either tryptic soy agar or Levin eosin methylene blue agar plates. Plates were incubated under the appropriate atmospheric condition for 24 h at 37°C. After 24 h, plates were counted for total aerobes, anaerobes and coliforms. Results of this study indicate a significant difference ($p<0.05$) in bacterial counts between the different sections of the litter. The middle and bottom sections had significantly lower anaerobe and coliform counts compared to the bacterial counts in the top sections. In conclusion, the results suggest that the middle and bottom section of litter provide a less favorable environment for bacterial growth than the top section.

Key words: Poultry, bacteria, litter depth, disease

INTRODUCTION

In the commercial broiler industry, it is common practice to grow multiple flocks of broilers on the same litter, also known as built-up litter (Vizzier-Thaxton *et al*., 2003). By using the same litter for multiple flock grow-outs, the level of bacteria in the litter increases, which in return increases the risk of disease outbreaks (Macklin *et al*., 2008). In-house composting is a management practice being used to help reduce the level of bacteria in broiler litter. Research has proven in-house composting to be an effective method of killing the infectious laryngotracheitis virus (Giambrone *et al*., 2008) and reducing aerobic and anaerobic bacteria (Macklin *et al*., 2006). In-house composting works by allowing the established litter microflora to degrade the organic material which in turn produces heat as a by-product. By trapping this heat in a windrow, temperatures are reached and maintained that are detrimental to the survival of the bacteria, including pathogens (Lavergne *et al*., 2006).

During the process of piling litter into windrows, litter is redistributed allowing portions of the bottom layer of the litter bed to end up on the surface of the windrow and eventually becoming a part of the top layer of the litter bed. At the surface of a windrow, the temperature required to eliminate or reduce bacterial loads are not achieved like they are at the interior core of the windrow (Jeffrey *et al*., 1998). This can become a major issue for poultry producers who are using built-up litter and experiencing disease outbreaks. For example if clostridium, an anaerobic pathogen, responsible for causing necrotic enteritis, were to reside deep within the litter bed. This pathogen could be re-introduced to the surface during the piling process and eventually come into contact with day old chicks causing a disease outbreak. Therefore, the objective of this study was to determine the distribution of aerobic, anaerobic and coliform bacteria in commercial poultry litter at three different depths.

MATERIALS AND METHODS

Houses: Three commercial broiler farms (farm 1, farm 2, and farm 3) containing birds that were greater than 5 wks of age were selected for sampling at the following times: 58d, 39d and 47d of age, respectively. All houses contained litter that had been used for multiple grow-outs and had a least one flock grown out since its last composting.

Sample collection: Litter samples were collected from one randomly selected broiler house on each of the three farms. Within each house, four random litter

Corresponding Authro: A.S. Kiess, Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA

samples were collected. Samples were collected using clear PVC pipe (ALSCO Industrial Products, Inc, Lithia Springs, GA), measuring 2 inches in diameter and 24 inches in length. The pipes were driven into the clay floor of the litter bed, so that each pipe contained all layers of the litter bed. Once the samples were collected, the bottom of each pipe was sealed using pieces of duct tape and then transported up-right to the lab for processing. At the lab, each pipe was divided into three equal sections: Top, middle and bottom. To determine each section, the depth of the litter bed was measured for each individual pipe. The litter bed depth was considered to be from the top of the litter down to the clay interfaced which was approximately 7-9 in for the three farms sampled. The three sections (top, middle and bottom) were determined as 33% of the total depth or approximately 3 in per section. The PVC pipe was cut two times using a tubing cutter. The first cut exposed the top section of the litter, the second cut exposed the middle section and the remainder was considered the bottom section of the litter bed. Litter from each section was emptied into sterile Whirl-pak® bags (Nasco, Fort Atkinson, WI).

Microbiology: Aerobic, anaerobic, and coliform bacteria populations were evaluated from each sample. In total, four samples from each broiler house were evaluated for microbial population. To process the samples, 10 g of litter was removed from each Whirl-pak® bag after the bag had been hand massaged to thoroughly mix the contents. The 10 g sample was then diluted 10 fold using sterile buffered peptone water. The diluted samples were placed in a Brinkmann/Seward 440C Stomacher® (Fisher Scientific, Marietta, GA) for 30 s at 130 rpm. After stomaching, the diluted sample was serially diluted using sterile buffered peptone water until a final dilution of $1:10^{12}$ was obtained. From each serial dilution, 0.1 mL was then spread plated onto two different media: tryptic soy agar and Levine eosin methylene blue agar. The dilutions were plated in quadruplicate on the tryptic soy agar and in duplicate on the Levin eosin methylene blue agar. Half of the tryptic soy agar plates and all of the Levin eosin methylene blue plates were incubated in a Precision Thelco 6DM incubator (Thermo Fisher Scientific, Marietta, GA) under aerobic conditions at 37°C for 24 h. To obtain anaerobic bacteria counts, the other tryptic soy plates were placed into Mart anaerobic chambers (Mart® Microbiology B.V. The Netherlands) and flushed with a microaerophilic gas mixture (80%N, 10% CO_2 and 10% H_2) using the Mart Anoxomat AN2CTS Mark II System (Mart® Microbiology B.V. The Netherlands). Chambers were then placed into a 20 ft^3 Precision Model 815 low temperature incubator (Thermo Fisher Scientific, Marietta, GA) for 24 h, at 37°C. After 24 h plates were counted, recorded and the average bacteria counts for each media was determined.

Statistics: Data were analyzed with a generalized randomized complete block design, in which each farm was a block, with each treatment being replicated three times. Each replication consisted of four litter samples. The GLM procedure of SAS was used and means were separated with Fisher's protected LSD at the .05 level (Steel and Torrie, 1980).

RESULTS

The results of this study indicate that, although the three farms contained birds of varying ages, there were no significant differences among farms in respect to the level of aerobic and coliform bacteria in their litter (data not shown). However, the level of anaerobic bacteria was different between farms ($p<0.0001$, Fig. 1). Farm 1 had fewer anaerobic bacteria in the litter (6.71±0.083 log CFU/g litter) than farms 2 and 3 (7.0±0.083 log CFU/g and 7.02±0.083 log CFU/g litter, respectively). The level of anaerobic bacteria in litter between farms 2 and 3 was not different from one another ($p>0.05$).

When evaluating the concentration of aerobic bacteria throughout the different depths of the litter bed, differences were apparent ($p<0.027$, Fig. 2). The results indicated that the top layer of the litter bed harbored more aerobic bacteria (7.59±0.097 log CFU/g litter) than the bottom layer of the litter bed (7.18±0.097 log CFU/g litter). There were no differences between the top and middle layer of the litter bed or between the middle and bottom layers of the litter bed.

There were differences in the concentration of anaerobic bacteria throughout the different depths of the litter bed ($p<0.0001$, Fig. 3). The top layer of the litter bed had a higher concentration of anaerobes (7.57±0.093 log CFU/g litter) when compared to the middle and bottom layers of the litter bed (6.42±0.093 log CFU/g and 6.38±0.093 log CFU/g litter, respectively). However, there was no difference in the concentration of anaerobes between the middle and bottom sections of the litter.

The concentration of coliforms in the different depths of the litter bed followed the same profile as the anaerobic bacteria (Fig. 4). The middle and bottom layers of the litter bed had significantly less ($p<0.044$) coliforms (6.59±0.13 log CFU/g and 6.37±0.13 log CFU/g litter, respectively) than the top layer of the litter bed (7.17±0.13 log CFU/g litter). On the other hand, there were no differences between the middle or bottom sections of the litter.

DISCUSSION

To properly manage built-up litter and prevent the outbreak of disease, it is necessary to understand how bacteria interact with the litter environment. The average concentration of bacteria in broiler litter has been established. Halbrook *et al.* (1951) determined that built-up litter was populated with enterococci, lactobacilli and coliforms. The level of bacteria located in the built-up

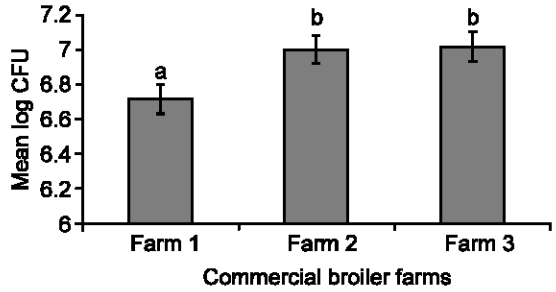

Fig. 1: Mean Log Colony Forming Units (CFU) for anaerobic bacteria at 3 commercial broiler farms. Each farm housed birds at least 5 weeks old; Farm 1: 58 days of age, Farm 2: 39 days of age, Farm 3: 47 days of age. At each farm 4 litter samples were collected for processing. [a-b]Means with different letters are significantly different at p<0.0001

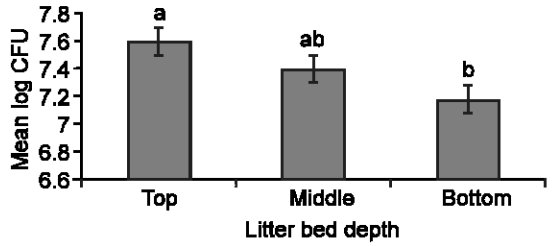

Fig. 2: Mean Log Colony Forming Units (CFU) for aerobic bacteria at each litter bed depth. Four samples were collected from 3 commercial broiler houses and divided into either a top, middle or bottom section. [a-b]Means with different letters are significantly different at p<0.027

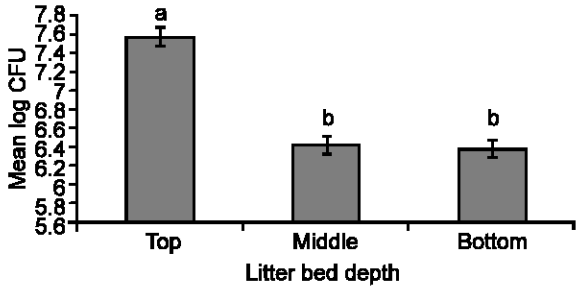

Fig. 3: Mean Log Colony Forming Units (CFU) for anaerobic bacteria at each litter bed depth. Four samples were collected from 3 commercial broiler houses and divided into either a top, middle or bottom section. [a-b]Means with different letters are significantly different at p<0.0001

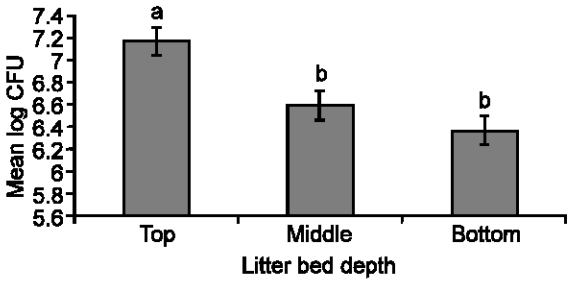

Fig. 4: Mean Log Colony Forming Units (CFU) for coliform bacteria at each litter bed depth. Four samples were collected from 3 commercial broiler houses and divided into either a top, middle or bottom section. [a-b]Means with different letters are significantly different at p<0.044

litter was determined to be less than the concentration of bacteria isolated in litter that had been changed weekly or after 1-8 wk of use (Halbrook *et al*., 1951). Another investigation by Lovett *et al*. (1971) evaluated the bacterial populations in poultry litter. Results indicated that *Escherichia coli* and coliforms were a constant inhabitant of the litter. Although, Salmonella was never isolated, the investigators did find that *Arizona* and *Bethesda-Ballerup* could be frequently isolated (Lovett *et al*., 1971).

Others have evaluated the microbial population of litter through molecular techniques. Several bacterial species have been identified in broiler litter by Lu *et al*. (2003) using 16s rRNA and functional gene markers. Some of the identified bacteria include: *Clostridium*, *Corynebacterium*, *Denitrobacter*, *Globicatella*, *Staphylococci* and *Bordetalla* (Lu *et al*., 2003). Other investigators have evaluated the bacteria composition of different bedding materials. Fries and colleagues (2005) determined that the dynamics of populations were different between the bedding material used before chicks were placed in the house. After chicks were placed on the bedding material there was very little difference between bacterial populations (Fries *et al*., 2005). Gram-positive bacteria were determined to increase after birds were placed and they remained high after the birds were removed. On the other hand concentrations of gram-negative bacteria were low while the birds were in the house and remained low after they were removed (Fries *et al*., 2005). All of the previously referenced studies provide information to producers that allow them to adopt the necessary management strategies to reduce the bacterial populations in their litter, but how those bacteria are distributed throughout the litter bed is still uncertain.

In the current study the concentration of aerobes, anaerobes and coliforms were highest in the top layer of litter and decreased with increasing litter depth. This outcome is consistent with increased deposition of fecal droppings containing high levels of intestinal bacteria on the litter surface. The organisms have an abundant source of energy through spilled feed and organic bedding material. It was expected that the environment

becomes more anaerobic at greater litter depths and that the population of anaerobic bacteria would increase with litter depth. However, our results demonstrate that the lower litter layers contained fewer bacteria regardless of type. Therefore, the mixing, turning and moving of litter between flocks through windrowing and/or decaking should not increase the surface concentration of bacteria or increase disease outbreaks by pathogenic bacteria or viruses due to concentrations in the lower litter.

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