In-House Windrow Composting and Its Effects on Foodborne Pathogens

K. S. Macklin,[1] J. B. Hess, and S. F. Bilgili

Department of Poultry Science, Auburn University, Auburn, AL 36849-5416

Primary Audience: Broiler Managers, Researchers

SUMMARY

Control of foodborne pathogens at the farm is a growing concern that is being addressed in the industry. Several methods have shown varying effectiveness in reducing pathogens on the farm, one of which is in-house windrow composting. In this experiment, used litter was obtained and samples were taken to determine the baseline levels of *Campylobacter*, *Clostridium perfringens*, and *Salmonella*. From these samples, no *Salmonella* or *Campylobacter* was detected and baseline counts for *C. perfringens* were determined. This litter was then formed into 3 windrow compost piles. Temperature probes were placed so as to measure the internal and external temperatures, with the data being recorded hourly. From each compost pile, 3 samples were removed and inoculated with *Campylobacter*, *C. perfringens*, and *Salmonella*. These 9 inoculated samples were then wrapped in cheesecloth, and 2 were placed in the interior and the other 1 was placed in the exterior of each compost pile. After 7 d, the inoculated samples were collected and tested to determine the number of inoculated bacteria that had survived. In all the samples (composted and uncomposted), there was a significant reduction in all of the bacteria measured. *Salmonella* was completely eliminated from the samples that were composted, whereas it was still recoverable from the uncomposted samples. The results show that in-house composting of litter is an effective way of reducing, and in some cases eliminating, foodborne pathogens in a poultry house.

Key words: *Salmonella*, *Clostridium perfringens*, *Campylobacter*, in-house compost

2008 J. Appl. Poult. Res. 17:121–127

doi:10.3382/japr.2007-00051

DESCRIPTION OF PROBLEM

There is growing concern among consumers about the safety of their food. Within the poultry industry, there are currently several methods for reducing foodborne pathogens, but these are primarily in place at the processing plant. Thus, broiler farms will require greater scrutiny if foodborne pathogens are to be reduced. Several methods for reducing bacteria at the poultry farm have been reported [1, 2]. In-house composting may reduce bacterial numbers, contamination in the processing plant, and eventually pathogens on the final product.

Clostridium perfringens, *Salmonella* spp., and *Campylobacter* spp. are commonly associated with the intestinal microflora of the chicken. These bacteria can produce foodborne illness if they are present in the final processed product. Under appropriate circumstances, *C. perfringens*

[1]Corresponding author: mackls@auburn.edu

can produce necrotic enteritis or gangrenous dermatitis in the bird as well. Annually, it is projected that approximately 4 million people in the United States develop foodborne illness from 1 of these 3 bacteria [3].

In-house composting of litter between flocks, to reduce microbial load, is currently being performed by some broiler growers. The current report focuses on the use of in-house composting for reducing foodborne pathogens on the poultry farm.

MATERIALS AND METHODS

In-House Compost

Pine shavings bedding that had been used by 3 previous flocks at a density of 1.16 ft^2/bird was used. After flock removal, the litter was tested to determine the amounts of *Salmonella*, *Campylobacter*, and *C. perfringens*. After testing this litter for the presence of these 3 foodborne pathogens, the litter was transported to a pen-style house at the Auburn University Research Farm. This particular house had cement floors and a dropped ceiling. At this house, 3 piles were created. The piles were approximately $1 \times 1 \times 1$ m in size. After creation, each pile was inoculated with the bacteria described below and allowed to compost for 7 d. During that time, each compost pile had ambient, surface, and internal temperatures monitored hourly by using a data logger [4]. Ambient temperature was taken by placing a probe at a distance of 1 m from each litter pile. Surface temperature was measured by placing a probe on the surface of the litter. Internal temperatures were taken at 2 points in each pile, 1 being at a depth of approximately 25 cm and the other at a depth of at least 50 cm. Uncomposted samples were collected from three 3×3 m pens that had a litter depth of 8 cm and were located in the same pen house as the compost piles. Moisture content of the litter was also determined by mixing 5 g of internal sample and 5 g of exterior sample from each pile.

Determination of Initial Campylobacter spp., C. perfringens, and Salmonella spp. Levels

Litter was collected from each pen that was to be used in this experiment. *Campylobacter* and *Salmonella* detection was performed by using blood-free Bolton's broth (**BB**) [5] and tetrathionate TT Hajna broth [5] for *Campylobacter* and *Salmonella*, respectively. The enriched samples were streaked in triplicate onto the following media. Modified charcoal cefoperazone deoxycholate agar [5] and modified campy cefex agar [5, 6] were used for *Campylobacter*; these media were then incubated under microaerobic [7] conditions at 37°C for 24 to 48 h. *Salmonella* recovery was performed by using xylose Lys tergitol 4 agar [5] that was incubated under aerobic conditions at 37°C for 24 h. Suspect colonies of *Campylobacter* or *Salmonella* were confirmed by the following methods. *Clostridium perfringens* isolation was performed by serially diluting the litter in sterile PBS [8], then plating it onto egg yolk-free tryptose-sulfite-cycloserine [5] agar and oleandomycin polymyxin sulfadiazine agar [5]. These plates were incubated anaerobically for 24 h at 37°C. Suspect colonies were counted manually by using a Quebec colony counter [9] and were confirmed by the method described below.

Litter Microbiology

From the collected litter populations aerobic, anaerobic, and enteric bacteria were enumerated. This was performed by diluting the samples 1:10 in sterile filter bags with sterile PBS. These bags were then placed in a stomacher [10] for 1 min. After being stomached, this 1:10 dilution was serially diluted in sterile PBS, then spiral plated in triplicate onto 3 different media types by using a DW Scientific spiral plater [11]. The media used were plate count agar (**PCA**) [5], reduced trypticase soy agar containing 5% sheep red blood cells (**RBA**) [12], and MacConkey agar (**MA**) [5] for aerobic, anaerobic, and enteric bacteria, respectively. The plates were than incubated either aerobically (PCA and MA) or anaerobically (RBA) [13] at 37°C for 24 h. Colonies were quantified on a digital plate reader [14] and the average bacterial count for each plate was obtained by using the standard software associated with this plate reader.

Moisture Content

Determination of moisture content was performed by weighing 1 g of litter and placing this into a drying oven overnight at 150°C. The

following day, the dried samples were allowed to cool in a desiccator and then weighed. For each sample, this was performed in triplicate. From the difference between the original weight and the new dried weight, the percentage of moisture was determined.

Origin of Tested Bacteria

The bacteria used were all originally isolated from chickens. The 3 *Campylobacter* spp. consisted of 2 *Campylobacter jejuni* and 1 *Campylobacter coli*, all of which were recovered from a processing plant. The 3 *C. perfringens* isolates were recovered from birds that had necrotic enteritis. The 5 *Salmonella* spp. isolates consisted of 3 processing plant isolates (2 of which were *S.* Enteritidis and 1 of which was *S.* Typhimurium) and 2 that were isolated from a poultry house (*S.* Kentucky and *S.* Heidelberg).

Inoculation of Campylobacter spp., C. perfringens, and Salmonella spp.

The 11 bacteria used in this experiment were grown in tryptic soy broth [5] at 37°C under the appropriate conditions. From each tryptic soy broth tube, approximately 10^8 cfu were collected, combined, and brought up to a final volume of 5 mL. This number was confirmed by diluting and plating the contents of each tube on RBA. This bacterial solution was dripped onto 20 g of poultry litter, then wrapped in cheesecloth and tied with string. A total of 9 inoculated poultry litter samples were created this way. Each of the 3 piles received 3 inoculated litter samples. One was placed on the surface of the litter; this was the uncomposted sample. The other 2 samples were placed at a depth of approximately 25 cm and at least 50 cm into the piled litter; these were the composted samples.

Recovery of Bacteria

After 7 d, the inoculated litter samples were recovered. Serial dilutions were performed by using PBS and, from these dilutions, selective media were plated in triplicate. The following media were used to isolate the inoculated bacteria. *Campylobacter* spp. was isolated by using modified charcoal cefoperazone deoxycholate agar and modified campy cefex agar, *C. perfringens* was recovered by using tryptose-sulfite-cycloserine agar and oleandomycin polymyxin sulfadiazine agar, and *Salmonella* spp. recovery was done with xylose Lys tergitol 4 agar. Recovery of noninoculated bacteria was performed by using PCA, RBA, and MA for the recovery of aerobic, anaerobic, and enteric bacteria, respectively. These media were then incubated at 37°C in their respective environments for 24 h, after which time counts were performed. The media that were used to recover characteristic colonies of the inoculated bacteria were counted manually by using a Quebec colony counter. The 3 media used to recover the noninoculated bacteria were counted by using a digital plate reader. At the same time as direct plating was being performed, all of the samples were enriched in case direct plating did not yield any countable plates. *Campylobacter* was enriched with BB, *C. perfringens* with chopped meat media, and *Salmonella* with tetrathionate TT Hajna broth. All of the enrichments were incubated at 37°C in their respective environments. Suspect colonies were tested to verify that they were the target bacteria.

Identification of Campylobacter spp.

For each suspect colony, a wet mount was performed and observed under a phase contrast microscope [15] for typical helical bacteria. If positive, an additional test for catalase and oxidase activity [16] was performed to confirm presumptive isolates. These presumptive *Campylobacter* spp. samples were then subjected to automated ribotyping for identification.

Identification of C. perfringens

Suspect colonies were streaked onto RBA and incubated for 24 h at 37°C under anaerobic conditions. If after this time double-zone hemolysis had developed, a presumptive identification of *C. perfringens* was made.

Identification of Salmonella spp.

Suspect colonies were streaked onto MA and allowed to incubate overnight. If characteristic colonies grew, a presumptive identification of *Salmonella* was made. These presumptive *Salmonella* samples were confirmed by automated ribotyping.

Automated Ribotyping

Ribotyping was performed according to the manufacturer's instructions [17]. Briefly, all the tested bacteria were grown overnight on brain heart infusion agar [5]. Cells were harvested and transferred to a microcentrifuge tube containing buffer. An aliquot was transferred to the sample carrier and then heat treated. After heat treatment, lysozyme, restriction enzyme, buffers, gel, and transfer membrane were loaded into the ribotyping machine. The sample carrier, buffer, restriction enzymes (*Pvu*II and *Pst*I for suspect *Salmonella* and *Campylobacter*, respectively), and all reagents were components of a kit designed for the RiboPrinter Microbial Characterization System [17]. The RiboPrinter System identifies the bacterial genus and species level through the analysis of genomic fragments containing the rRNA generated by restriction digestion of ribosomal RNA operons [18, 19].

Statistical Analysis

Because of the similarity in counts between the 2 depths of composted litter, the data were pooled and simply called compost. The resulting colony-forming units per gram counts for the different media types were pooled and analyzed by using GLM. If significant differences were detected at $P < 0.05$, then the means were separated by using Tukey's multiple comparison test [20].

RESULTS AND DISCUSSION

Composting of litter after it has been removed from a broiler house has been performed for several years. The resulting product is known to contain significantly less bacteria than uncomposted litter and is safe to use as a soil amendment [21–23]. Other studies [24, 25] have shown that in-house composting is an effective method of reducing overall bacterial numbers. Given the length of time that in-house composting is performed (typically 5 to 7 d), it is not as thorough at destroying bacteria as traditional composting. However, it is still an effective method of reducing bacterial numbers in a broiler house between flocks [1]. The reduction of foodborne pathogens such as *Salmonella*, *Campylobacter*, and *C. perfringens* from a poultry farm would reduce the burden of removing these pathogens at the processing plant. Of these 3, the removal of *Salmonella* is paramount, because federal guidelines regulating this pathogen have recently been tightened [26].

For composting to be effective in eliminating microorganisms such as bacteria, the temperature must be at least 50°C and this temperature must be maintained for at least 24 h [27, 28]. A traditional compost pile is turned every few days; this reintroduces oxygen into the compost pile. The presence of oxygen allows aerobic bacteria to further break down the organic material in the pile and to generate heat. In the current study, turning was not performed. If it had been, there might have been a greater reduction in overall bacterial numbers because of the internal temperature being maintained for a longer period of time. Turning was not performed because in-house composting must be practical and beneficial for the typical grower.

The temperature data in Figure 1 show that an average internal temperature of 50°C+ began 18 h after initiation and was maintained for approximately 32 h. The significance of attaining this temperature is important not only for killing bacteria, but also because at this temperature most viruses, fungi, and parasite eggs are killed [27, 28]. It is interesting to note that the average temperature of the exterior of the pile was 5 to 10°C higher than the ambient temperature. These external temperatures are not high enough to kill any microorganisms; however, this warming does increase the amount of ammonia generated. This increase in ammonia may have been responsible for reducing the bacterial levels from the surface of the compost pile, as noted in Tables 1 and 2. The average moisture content among the 3 compost piles was 25%. This percentage of moisture is typical of what the authors have observed in a commercial broiler operation in Alabama.

Initial, 7-d composted, and 7-d uncomposted total aerobic, anaerobic, and enteric bacterial counts are presented in Table 1. There was a significant decrease in the amounts of aerobic and anaerobic bacteria present in composted samples compared with the initial samples. Additionally, composted litter had significantly lower counts of anaerobic and enteric bacteria when compared with uncomposted litter. In the composted pile, there was slightly less aerobic

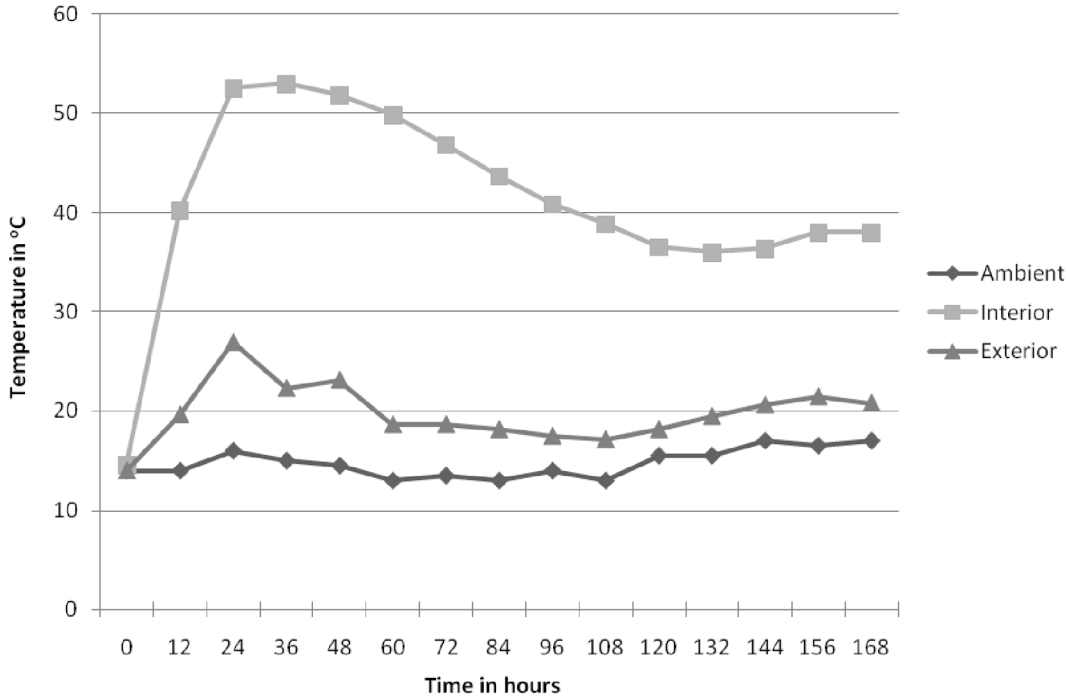

Figure 1. Internal, external, and ambient temperatures associated with in-house composting. Temperature readings were taken every hour, and the averages of the 6 internal probes and 3 exterior probes used in this trial are shown. Only 1 probe was used to determine the ambient temperature.

bacteria than in the uncomposted litter; however, this difference was not determined to be statistically different. The reductions noted here are similar to what has been observed previously [1].

Table 2 shows the total amounts of *Salmonella*, *Campylobacter*, and *C. perfringens* present in the inoculated samples. The 7-d uncomposted samples had detectable levels of *Salmonella* and *C. perfringens* when direct plating was used; however, *Campylobacter* was not detected in any sample even after enrichment in BB. Inoculated samples recovered from the inside of the compost pile had no detectable *Salmonella* or *Campylobacter*, because even after enrichment they were not recovered. *Clostridium perfringens* was recovered from 5 of the 6 interior samples and was recoverable only after the samples were enriched. The slight reduction in *C. perfringens* between the composted and uncomposted samples was not statistically significant. Although in this study the number of viable *C. perfringens* seemed small, when considered in

Table 1. Bacterial levels recovered from litter before composting and after 7 d of being either composted or not composted

Treatment	Aerobic (log_{10} cfu/g)	Anaerobic (log_{10} cfu/g)	Enteric (log_{10} cfu/g)
By treatment			
Initial levels	11.497[a]	9.411[a]	4.192[ab]
Uncomposted	10.116[b]	8.726[a]	5.912[a]
Compost	9.503[b]	3.058[b]	1.918[b]
Probability			
Treatment	<0.001	<0.001	0.004

[a,b]Letter differences signify that there was a difference in that column after a GLM was performed, followed by Tukey's multiple comparison test, at the *P*-value shown.

Table 2. Bacterial levels that were inoculated into the respective treatments and the counts at 7 d postchallenge

Treatment	*Salmonella* (log_{10} cfu/g)	*Campylobacter* (log_{10} cfu/g)	*Clostridium perfringens*[1] (log_{10} cfu/g)
By treatment			
Initial levels	10.186[a]	11.575[a]	9.753[a]
Uncomposted	1.897[b]	0[b]	1.441[b]
Compost	0[c]	0[b]	0.833[b]
Probability			
Treatment	<0.001	<0.001	<0.001

[a–c]Letter differences signify that there was a difference in that column after a GLM was performed, followed by Tukey's multiple comparison test, at the *P*-value shown.
[1]Final *C. perfringens* is the total number of bacteria that were found in the sample. This number includes the *C. perfringens* that were initially found within the samples.

the context of a broiler house, the total decrease in *C. perfringens* could be economically important.

Automated ribotyping was performed on 8 randomly selected suspect *Salmonella* isolates. All 8 isolates were confirmed as the *S.* Kentucky that was used in the inoculum. Given that colony morphology was the same among the suspect *Salmonellae*, it was assumed that all of the recovered *Salmonellae* were *S.* Kentucky. This observation is not completely surprising, because this isolate was originally an environmental isolate collected from a poultry house. Because of this, it is probable that it had adapted to become more resistant to environmental factors than the other *Salmonella* used in this study.

The results of this study show that in-house composting is an effective method for reducing bacterial numbers in litter. In addition, the common foodborne pathogen *Salmonella* was highly likely to be eliminated during the process. *Clostridium perfringens* is a spore former, and this ability to form a spore may have made this bacterium more resistant to composting than the other 2 bacteria that were studied.

CONCLUSIONS AND APPLICATIONS

1. We demonstrated that significant reductions in *Salmonella* spp. can be achieved by performing in-house composting.
2. The reduction of *Salmonella* at the farm may reduce their presence at the processing plant by preventing the colonization of these bacteria in newly placed chicks.
3. The reduction of anaerobic and aerobic bacteria in the in-house composted samples implies that these organisms are more susceptible to this procedure. This also implies that the procedure could decrease the numbers and likelihood of foodborne pathogens in a flock and positively influence bird health and performance.

REFERENCES AND NOTES

1. Macklin, K. S., J. B. Hess, S. F. Bilgili, and R. A. Norton. 2006. Effects of in-house composting of litter on bacterial levels. J. Appl. Poult. Res. 15:531–537.

2. Pope, M. J., and T. E. Cherry. 2000. An evaluation of the presence of pathogens on broilers raised on poultry litter treatment-treated litter. Poult. Sci. 79:1351–1355.

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

4. Squirrel SQ800 data logger, Grant Instruments (Cambridge) Ltd., Cambridgeshire, UK.

5. Difco Laboratories, Detroit, MI.

6. Oyarzabal, O. A., K. S. Macklin, R. S. Miller, and J. M. Barbaree. 2005. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. Appl. Environ. Microbiol. 71:3351–3354.

7. The gas mixture was composed of 10% CO_2, 5% O_2, and 85% N_2.

8. Final concentrations 137 m*M* NaCl, 10 m*M* phosphate, 2.7 m*M* KCl, pH 7.4.

9. Darkfield Quebec colony counter, Reichert Analytical Instruments, Depew, NY.

10. Microbiology International, Fredrick, MD.

11. Don Whitley Scientific Limited, West Yorkshire, UK.

12. Becton Dickinson, Sparks, MD.

13. The gas mixture was composed of 5% CO_2, 5% H_2, and 90% N_2.

14. Microbiology International, Fredrick, MD.

15. Optiphot-2, Nikon, Melville, NY.

16. The catalase and oxidase test was performed following the manufacturer's instructions; Becton Dickinson, Sparks, MD.

17. RiboPrinter Microbial Characterization System, Qualicon, Wilmington, DE.

18. Bruce, J. 1996. Automated system rapidly identifies and characterizes microorganisms in food. Food Technol. 50:77–81.

19. Oscar, T. P. 1998. Identification and characterization of *Salmonella* isolates by automated ribotyping. J. Food Prot. 61:519–524.

20. SPSS Inc. 2003. SPSS for Windows, Release 12.0. SPSS Inc., Chicago, IL.

21. Hartel, P. G., W. I. Segars, J. D. Summer, J. V. Collins, A. T. Phillips, and E. Whittle. 2000. Survival of fecal coliforms in fresh and stacked broiler litter. J. Appl. Poult. Res. 9:505–512.

22. Tiquia, S. M., J. H. C. Wan, and N. F. Y. Tam. 2002. Microbial population dynamics and enzyme activity during composting. Comp. Sci. Util. 10:150–161.

23. Brodie, H. L., L. E. Carr, and P. Condon. 2000. Poultry litter composting comparisons. Biocycle 41:36–40.

24. Macklin, K. S., J. B. Hess, S. F. Bilgili, J. P. Blake, and R. A. Norton. 2004. Bacterial counts associated with composting litter between flocks. Pages 177–180 in Proc. Natl. Poult. Waste Manage. Symp., Memphis, TN. Fed. Anim. Sci. Soc., Savoy, IL.

25. Lavergne, T. K., M. F. Stephens, D. Schellinger, and W. A. Carney Jr. 2004. Making poultry litter safe for reuse: in-house pasteurization can save money for producers. La. Agric. 47:4.

26. Bennett, P. 2006. Compliance Guideline for Controlling Salmonella in Poultry. First Edition, August 2006. http://www.fsis.usda.gov/PDF/Compliance_Guideline_Controlling_Salmonella_Poultry.pdf Accessed Jan. 2007.

27. Dumontet, S., H. Dinel, and S. B. Baloda. 1999. Pathogen reduction in sewage sludge by composting and other biological treatments: A review. Biol. Agric. Hort. 16:409–430.

28. Jones, P., and M. Martin. 2003. A review of the literature on the occurrence and survival of pathogens of animals and humans in green compost. The Waste and Resource Action Programme. WebMD http://www.wrap.org.uk/downloads/LitReviewPathogensAnimalHumanCompost.b1895775.pdf Accessed Jan. 2008.

Acknowledgments

Funded by the US Poultry and Egg Association (Tucker, GA).