

Multi-Microbial Compounds Eliminate or Reduce *Salmonella typhimurium* from One-third of Poultry Litter Samples Within 8 days

Cynthia L. Sheffield, Tawni L. Crippen and Ross C. Beier
US Department of Agriculture, Agricultural Research Service,
Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center,
College Station, TX 77845 Texas, USA

Abstract: Poultry are generally reared on bedding such as wood shavings, peanut or rice hulls. It has become economically important to reuse poultry litter for multiple flocks often resulting in litter serving as a reservoir of microbial such as *Salmonella*, *Escherichia* and *Campylobacter*. Previous research demonstrated that during the pre-harvest feed withdrawal period, bird consumption of contaminated litter can lead to infection of the upper gastrointestinal tract with *Salmonella* which presents significant problems during processing. This study examined efficacy of two commercially available compost enhancers CE1 and CE2) in reducing *Salmonella typhimurium* (ST) in poultry litter. After 8 days, CE1 had an average 6-log decrease in ST concentration and elimination of ST in one third of the samples. CE2 had an average 4-log decrease in ST concentration but did not eliminate ST from any of the samples. This suggests that both materials could potentially decrease the down-time required to substantially reduce the ST concentration in reused litter. Further, these materials are easily incorporated and safe for poultry and humans. This could provide both economic and food safety advantages to the broiler producers and enhance the overall food safety of poultry products.

Key words: *Salmonella typhimurium*, poultry, litter, microorganism, composting, safety

INTRODUCTION

One of the most frequently isolated food borne pathogens associated with human illness is *Salmonella* sp. Salmonellosis has been estimated to cause over a million illnesses each year in the United States (Scallan *et al.*, 2011) costing over \$14 billion USD (Scharff *et al.*, 2009). Approximately 95% of human cases of salmonellosis are food borne in origin (Mead *et al.*, 1999) and repeatedly linked to eating poultry products (Kimura *et al.*, 2004; Guo *et al.*, 2011). The *Salmonella* bacterium is routinely found within the gastrointestinal tract of chickens and on finished retail poultry products (Zhao *et al.*, 2001; Bailey and Cosby, 2005; Braden, 2006).

Furthermore, poultry litter/waste is the most desirable of the organic fertilizers because of its high nitrogen content (Moore *et al.*, 1995). Poultry litter/waste is also a potential source of human pathogens that can result in food safety issues, for example, *Salmonella*, *Staphylococcus* and *Campylobacter* (Terzich *et al.*, 2000). Research conducted by Chinivasagam *et al.* (2012) detected *Salmonella* on 83% of farms that reuse litter and only 68% of farms that dispose of litter after utilization by a single flock of broilers. In the united states in 2008 roughly 44 million tons of poultry

manure was produced (Bolan *et al.*, 2010). Currently the US poultry industry must meet stringent new performance standards put forth by the US Department of Agriculture Food Safety Inspection Service (USDA/FSIS). These standards are meant to aid in the reduction of *Salmonella* in poultry (Anonymous, 2011). Pre-harvest *Salmonella*-reduction strategies (i.e., prebiotics, probiotics, competitive exclusion and bacteriophage treatment) have all been utilized with variable degrees of success (Vandeplas *et al.*, 2010; Bucher *et al.*, 2012).

Because of rising costs and the limited supplies of bedding material, especially, high quality wood shavings it has become customary practice for broiler producers to grow-out multiple flocks using the same bedding material/litter. This practice may result in many complications for poultry producers including disease outbreaks, higher litter moisture and increased NH₃ production. One approach to dealing with some of these issues is to leave the poultry house free of birds for 2 or more months this will result in the reduction of bacteria due to desiccation within the litter (Lovanh *et al.*, 2007). Due to economic losses incurred by the producer the practice of leaving poultry houses empty for an extended time is not a realistic option. Another approach is to add

litter amendments such as poultry litter treatment (Jones-Hamilton Co. Walbridge OH) the efficacy of this approach is still up in the air. Shah *et al.* (2006) suggested that the application of litter amendments is only a short term solution, however, Bolan *et al.* (2010) demonstrated that these materials successfully reduce pH, NH₃ and bacterial load.

Two approaches designed to support the re-use of litter for an extended period of time are 1 in-house windrowing 2 partial house clean outs (Tabler and Wells, 2012; Williams *et al.*, 2012). In the past on-farm composting and in house windrowing has been under-utilized. But in the absence of other viable options in-house windrowing is becoming the method of choice to accommodate the increased practice of reutilization of litter for several flock rotations. In-house windrowing is a composting technique that employs specifically designed equipment or grade blades on tractors, skid-steer loaders to mound litter into several conical piles (windrows) that extend length wise down both sides of a poultry house. These mounds then age until the internal temperature reaches at least 135°F once this temperature is reached the mound is turned and this process is repeated over a period of 10 days or more. Turning the mound is critical to the success of the method as this allows for the rotation of the cooler litter from the outside of the pile to be internalized, so that, it can reach the higher temperatures generated internally for effective composting. Ahmed *et al.* (2012) demonstrated that composting reduced the Salmonella species count in poultry litter by 70.59%, however, to achieve this result, the compost required daily turning and was composted for a total of 35 days. The major problem with this approach is the cost resulting from down-time added labor and equipment there. Foreign-house windrowing is still far from an ideal alternative for poultry producers.

Two liquid compost enhancers (CE1 and CE2) were compared to determine their relative effectiveness in reducing the concentration of ST in poultry litter over an 8 days post-treatment period. The CE1 is composed of three groups of microbes yeast (*Saccharomyces cerevisiae*) photosynthetic bacteria (*Rhodospseudomonas palustris*) and lactic acid bacteria (*Lactobacillus casei*). The three microbes are purported to work synergistically to modify the surrounding microbial environment, encouraging the breakdown of ammonia and enhancing the efficacy of composting (Higa, 2013). Sheffield *et al.* (2014) demonstrated that CE1 is capable of eliminating ST in poultry feces within 8 days. A similar formulation of this material, EM•1® has been shown to be safe for consumption by chickens (Esatu *et al.*, 2012). The CE2 is composed of a highly concentrated liquid spore-based

Bacillus culture consortium consisting of six select strains (proprietary information) designed to accelerate the microbial process of composting.

MATERIALS AND METHODS

The experimental procedure used here was described by Sheffield *et al.* (2014). All procedures in this study were approved by the USDA-ARS-SPARC, Institutional Animal Care and Use Committee (IACUC protocol No. 09-12). The poultry manure used in this study was collected from mature single-comb white Leghorn hens obtained from the Texas A&M Poultry Research facility. They were housed individually in commercial layer cages and provided free access to water and balanced unmedicated corn-soybean based mash layer diet that met or exceeded the National Research Council recommendations for nutrients (National Research Council, 1994). The LT1000™ material was generously donated by TeroGanix, Inc. (Alto, Texas) and maintained at room temperature as per manufacturer's directions. The Microbe-Lift®/55X-SNF material was generously donated by Ecological Laboratories Inc. (Cape Coral, FL) and maintained at room temperature as per manufacturer's directions. The poultry derived *Salmonella typhimurium* (ST) was obtained from the USDA-ARS-SPARC microbial collection confirmed by agglutination testing and 16s rRNA sequencing.

Briefly, ST was cultured on Tryptic Soy Agar (TSA) at 37°C for 24 h. Bacteria were harvested and suspended in Phosphate Buffered Saline (PBS) to an Optical Density (OD) of 0.700±10% at 620 nm this yielded a suspension at approximately 10⁸ CFU/mL. The final inoculum concentration was determined by serial dilution on to TSA plates. Litter material was prepared by combining 10 g each of autoclaved poultry manure and autoclaved commercial poultry bedding material. This mixture was moistened with 5 mL of Tryptic Soy Broth (TSB) and placed in sterile 300 mL plastic tubs. One aliquot per tub of either (CE1-treated-120 µL) or (CE2-treated-840 µL) was diluted with 10 mL of TSB then added to the moistened litter mixture. The litter and treatment material were mixed thoroughly and then placed in an incubator at 37°C with normal atmospheric air for 48 h prior to the introduction of ST. This incubation was done to approximate the field practice of treating the litter 48 h prior to the introduction of birds. Every other day for the duration of the experiment TSB was added to the litter and gently mixed to maintain moisture levels similar to those found in commercial poultry facilities (i.e., at or below 30%). On 3 days, ST inoculum with a mean concentration of

$5.03 \times 10^8 \pm 2.93 \times 10^8$ diluted in 10 mL of TSB was added to each of the control and treatment tubs. The litter was sampled for ST every other day for 8 days following the culture methods described before. There were 3 replicate tubs per treatment for each experiment and the entire experiment was replicated three times. Data were analyzed using commercially available statistical software (JMP, SAS Institute, Cary, NC). Descriptive statistics were generated using the LS means differences Tukey HSD analysis.

RESULTS AND DISCUSSION

We report here on the efficacy of LT 1000™ (CE1) and microbe-Lift®/55X-S NF (CE2) to reduce ST levels within poultry litter. In 33% of the samples CE1 demonstrated elimination of ST at day 8. In the remaining samples CE1 exhibited an average 6-log decrease in ST concentration over 8 days (Table 1). The CE2 did not eliminate ST in any samples over the 8 days trial and had an average 4-log decrease in ST concentration (Table 2). Further, the average reduction within the initial 2 days post treatment period for CE1 and CE2 was 1.9 and 0.6-log, respectively. Over the next 6 days the reduction within CE1 was (1.11, 1.11 and 1.88-log, respectively) and within CE2 it was (1.28, 1.54 and 0.81-log, respectively) (Table 1 and 2).

Several other tactics to reduce ST levels within poultry litter have had varied results, and one without and reported that neither treatment was effective in reducing *Salmonella* colonization (Stringfellow *et al.*, 2010) determined that quick lime and steam pasteurization were effective at controlling ST in poultry (Williams *et al.*, 2012) reported that the addition of sodium bisulfate led to an increase in survivability of Larrison *et al.* (2010) examined two litter treatments one with an acidifier y litter, however, steam pasteurization is time consuming and requires specialized equipment. Additionally, to enhance the performance of quick lime water must be added to the litter which can lead to excess production of ammonia and associated problems. Studies by Bennett *et al.* (2003-2005), with day-of-hatch chicks showed that lime levels in excess of 5% (wt./vol.) caused obvious ocular and respiratory irritation. Vicente *et al.* (2007) found that a litter acidifier (poultry guard) significantly reduced *Salmonella enteritidis* levels in broiler chicks at 11 days post-treatment however this specious reduction did not hold up over time and at 21 days post-treatment there was no significant difference between the treated and control chicks.

Table 1: Concentration of salmonella typhimurium in litter treated with CE1

Trial	Positive control	Days post-treatment			
		2	4	6	8
1	7.94	5.83	4.55	4.16	3.24
2	7.79	5.74	3.97	3.07	1.13
3	8.18	6.62	6.32	4.27	1.52
Mean±SD	7.97*±0.20*	6.06*±0.49*	4.95*±1.23*	3.84*±0.66*	1.96*±1.12*

*b Values with different superscripts differ significantly, analyzed by least significant means different Tukey HSD ($p < 0.0185$). *Log₁₀ transformed mean (CFU/mL) of 3 replicates

Table 2: Concentration of salmonella typhimurium in litter treated with CE2

Trial	Positive control	Days post-treatment			
		2	4	6	8
1	7.94	7.27	6.05	4.18	4.11
2	7.79	7.67	5.35	4.98	3.43
3	8.18	7.16	6.86	4.48	3.69
Mean±SD	7.97*±0.20	7.37*±0.27	6.09*±0.76	4.55*±0.40	3.74*±0.35

Values not significantly different, analyzed by least significant means different Tukey HSD; *Log₁₀ transformed means (CFU/mL) of 3 replicates

CONCLUSION

Comparing the efficacy to control ST in poultry litter by either CE1 or CE2 to the former approaches from the literature, both materials provided substantial reductions in ST, however, CE1 was able to eliminate ST within 8 days post-treatment. CE1 appears to be a promising commercial material that is easily incorporated into litter, safe for poultry and humans and required no specialized equipment. Thus, CE1 may provide an easy, effective and safe means of controlling ST in the boiler production arena.

RECOMMENDATION

However, further research needs to be conducted on the usefulness, efficacy and the persistence of these materials under commercial broiler production conditions.

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