Salmonella Typhimurium in chicken manure reduced or eliminated by addition of LT1000

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Primary Audience: Food Safety Researchers and Regulators, The Poultry Industry (worldwide)

SUMMARY

Poultry are normally reared on bedding materials such as wood shavings or rice hulls. Poultry litter reuse for multiple flocks has become economically important in modern broiler production. However, this practice results in the litter serving as a reservoir of numerous microbial organisms, including, yeasts, molds, multiple types of viruses, and bacterial pathogens such as *Salmonella*, *Escherichia*, *Campylobacter*, *Clostridium*, *Staphylococcus*, and *Pseudomonas*. The foodborne pathogens are of particular importance for poultry producers. During the preharvest feed withdrawal period, consumption of contaminated litter and feces by the birds can lead to infection of the upper gastrointestinal tract with *Salmonella*, which presents substantial problems at processing. The current study was conducted to determine whether the use of a liquid bacterial product (LBP), such as LT1000, could reduce the load of *Salmonella* Typhimurium in poultry manure. The LBP was added to sterile poultry manure then challenged with 10^8 cfu/mL of *Salmonella* Typhimurium. The concentration of *Salmonella* Typhimurium was measured over 9 d or until the *Salmonella* Typhimurium was no longer detected. In 91% of the trials, *Salmonella* Typhimurium was completely eliminated within 9 d. This demonstrates that the LBP used in the current study has the potential to substantially improve the overall microbiological safety of used poultry litter.

Key words: *Salmonella* Typhimurium, poultry, manure, litter, effective microorganism

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DESCRIPTION OF PROBLEM

Salmonella is one of the most frequently isolated foodborne pathogens associated with human illness and has been estimated to cause over a million illnesses each year in the United States [1], costing over $14 billion [2]. Approximately 95% of human cases of salmonellosis are foodborne in origin [3] and frequently linked to the consumption of poultry products [4, 5]. The *Salmonella* bacterium is commonly found within the gastrointestinal tract of chickens and on finished retail poultry products [6–8].

Additionally, animal manure has been effectively used as fertilizer for centuries, and poultry waste is the most desirable of the organic fertilizers because of its high nitrogen content [9]. However, it is also source of some major human pathogens, such as *Salmonella, Staphylococcus*, and *Campylobacter*, all of which have the potential

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to cause food safety issues [10]. Chinivasagam et al. [11] detected *Salmonella* in 83% of farms that reuse litter and 68% of farms that dispose of litter after utilization by a single flock of broilers.

Due to rising costs and the difficulty of procuring bedding material, especially wood shavings, it has become a common practice for broiler producers to grow-out multiple flocks of broilers on the same litter. Using the same litter for multiple grow-outs can cause many problems for poultry producers, including disease outbreaks, higher litter moisture, and increased NH_3 production. One tactic for dealing with these issues is to leave the poultry house free of birds for 2 or more months, as this will allow for the reduction of bacteria due to desiccation within the litter [12]. However, leaving poultry houses empty for an extended time is not a realistic option due to the economic losses for the producer. Another tactic is for poultry producers is to use litter amendments, such as Poultry Litter Treatment [13], which has been shown to reduce pH, NH_3, and bacterial load [14].

Approximately 44 million tons of poultry manure was produced in the United States in 2008 [14]; in addition, the US poultry industry must meet stringent new performance standards proposed by the USDA-Food Safety Inspection Service aimed at reducing *Salmonella* in poultry [15]. Preharvest *Salmonella*-reduction strategies, such as prebiotics, probiotics, competitive exclusion, and bacteriophage treatment, have all been attempted with varying degrees of success [16, 17]. In-house windrowing and partial house cleanout are 2 approaches designed to aid in the reuse of litter for an extended period of time [18]. On-farm composting and in-house windrowing has been underutilized in the past; but, with the lack of viable alternatives to accommodate the increased practice of reutilization of litter for several flock rotations, in-house windrowing is becoming the method of choice for making organic wastes safe before application to land. In-house windrowing is a composting technique that uses grade blades on tractors, skid-steer loaders, or specially designed aeration equipment to pile litter into one or multiple conical piles (windrows) that extend the length of a poultry house and incubate for a period of 10 d or more. The technique requires the piles be turned during the incubation period to rotate cooler litter from the outside of the pile to the higher temperatures generated internally for effective composting. Zakia et al. [19] found that composting reduced the *Salmonella* spp. 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 d. Despite its increased utilization, the process of in-house windrowing is still far from an ideal alternative for poultry producers.

A liquid bacterial product (**LBP**; LT1000) composed of 3 groups of microbes—yeast (*Saccharomyces cerevisae*), photosynthetic bacteria (*Rhodopseudomonas palustris*), and lactic acid bacteria (*Lactobacillus casei*)—is purported to work synergistically to modify the surrounding microbial environment, encouraging the breakdown of ammonia and enhancing the efficacy of composting [20]. A slightly different formulation of this material, EM•1, has been shown to be safe for consumption and to enhance the immune response in chickens [21]. The object of the current study was to evaluate the efficacy of this LBP to reduce the concentration of *Salmonella* Typhimurium in poultry manure.

MATERIALS AND METHODS

All procedures in this study were approved by the USDA-Agricultural Research Service-Southern Plains Agricultural Research Center Institutional Animal Care and Use Committee (IACUC protocol # 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 NRC recommendations for nutrients [22]. The manure was collected and stored in sealed containers at 4°C. All manure collected over a period of 3 wk was combined, aliquoted into 500-mL polypropylene containers, autoclaved at 121°C for 20 min, and stored at 4°C until use. Manure was used in these tests to ensure a very consistent test matrix. The LBP (LT1000) material was provided by TeraGanix Inc. [23] and maintained at room temperature per the manufacturer's directions. The colony-forming units per milliliter

Table 1. Alteration in *Salmonella* Typhimurium concentration over time

Trial (cfu/mL)	Positive control	Days postinoculation		
		5	7	9
1	8.70[1]	5.18	0.00	0.00
2	8.70	8.16	6.64	5.00
3	8.60	7.10	6.50	0.00
4	8.62	7.43	6.50	0.00
Mean ± SD	8.66 ± 0.05[a]	6.97 ± 1.27[b]	4.91 ± 3.27[b]	1.25 ± 2.50[b]

[a,b]Values with different superscripts differ significantly as analyzed by ANOVA and Bonferroni's test ($P < 0.05$).

[1]Log_{10} transformed mean (cfu/mL) of 3 replicates, except trial 1, which had only 2 replicates.

of the indicator bacteria, *L. casei*, within the LBP was determined for each sample experiment by spread-plating a serial dilution of the stock material onto de Man, Rogosa, Sharpe agar [24] and maintained in a high-CO_2 environment at 37°C for 48 h before counting colonies. The *Salmonella* Typhimurium was obtained from the USDA-Agricultural Research Service-Southern Plains Agricultural Research Center microbial collection after confirmation by agglutination testing and 16s rRNA sequencing.

The *Salmonella* Typhimurium was cultured on tryptic soy agar at 37°C for 24 h; harvested and resuspended for use in experimentation in PBS to an optical density of ~0.7 at 620 nm. The final inoculum concentration was determined by serial dilution onto tryptic soy agar plates. An aliquot of 42 µL of LBP (1 gal/1,000 ft^2) mixed with 1 mL of tryptic soy broth (**TSB**) was added to 10 g of autoclaved poultry manure in each of 3 sterile 300-mL plastic tubs (treated). A tub of poultry manure without LBP served as a positive control for *Salmonella* Typhimurium growth (control). The 4 samples tubs were placed in an incubator at 37°C with normal atmospheric air. Every day for the duration of the experiment, 1 mL of TSB only was added to the manure and gently mixed to maintain moisture levels within the manure similar to those found in commercial poultry facilities (i.e., at or below 30%). On d 3, 100 µL of *Salmonella* Typhimurium inoculum with a mean concentration of 4.42×10^8 cfu/mL ($\pm 0.52 \times 10^8$) diluted in 1 mL of TSB was added to each of the 4 tubs. The manure was sampled for *Salmonella* Typhimurium and *L. casei* every other day for 9 d or until no *Salmonella* Typhimurium was detected, following the culture methods described above. The experiment was replicated 4 times.

Data were analyzed using commercially available statistical software [25]. Descriptive statistics were generated using the mean and standard deviation and presented in table formats. Comparison of treatment effect was analyzed by ANOVA followed by a Bonferroni's Multiple Comparison Test.

RESULTS AND DISCUSSION

We report here on the efficacy of a LBP (LT1000) to reduce *Salmonella* Typhimurium levels within poultry manure. *Salmonella* Typhimurium was eliminated from 91% of the manure samples within 9 d after the addition of LBP. In trial 1, the treatment marker bacteria (*L. casei*) could not be detected in 1 of the samples at d 9; therefore, this sample was not included in the final data analysis. Both of the remaining treated samples in trial 1 demonstrated elimination of *Salmonella* Typhimurium at d 5 and 7, respectively. The treated samples in trials 2 to 4 showed between 1- to 3-log reductions by d 5 and 7, respectively. By d 9, 10 of the 11 samples had eliminated *Salmonella* Typhimurium; the remaining sample did, however, show a 3-log reduction in *Salmonella* Typhimurium.

The concentration of *Salmonella* Typhimurium significantly ($P < 0.05$) decreased over time (Table 1) in the samples where LBP was present. In 3 of the 4 trials, no *Salmonella* Typhimurium could be detected in the manure by either d 7 or 9 in the study. In trial 2, however, the load of *Salmonella* Typhimurium in the tub was only reduced, not eliminated. The concentration level of the marker bacteria (*L. casei*) was also dynamic over the course of the trials. The *L. casei* levels consistently declined over 9 d in 3 of the 4 trials (Table 2). However, in trial 3, the *L. ca-*

Table 2. Alteration in *Lactobacillus casei* concentration over time

Trial (cfu/mL)	Positive control	Days postinoculation		
		5	7	9
1	8.00[1]	7.00	6.00	5.85
2	8.00	7.39	7.22	6.85
3	7.78	7.00	8.12	6.00
4	7.78	7.15	7.15	6.70
Mean ± SD	7.89 ± 0.13[a]	7.14 ± 0.18[a]	7.12 ± 0.87[a]	6.35 ± 0.50[b]

[a,b]Values with different superscripts differ significantly as analyzed by ANOVA and Bonferroni's test ($P < 0.05$).

[1]Log_{10} transformed mean (cfu/mL) of 3 replicates, except trial 1, which had only 2 replicates.

sei levels fluctuated over the course of the trial. The cause of the difference in growth patterns is unclear. Statistical analyses indicated that no significant differences ($P < 0.05$) could be found between the mean *L. casei* levels in the control and treated samples until d 9, where a reduction in *L. casei* concentration was detected in all 4 trials.

Other approaches to reducing *Salmonella* Typhimurium levels within poultry manure or litter have had mixed results. Williams et al. [26] reported that the addition of sodium bisulfate actually led to an increase in survivability of *Salmonella* Typhimurium. Larrison et al. [27] examined 2 litter treatments, one with an acidifier and one without, and reported that neither treatment was effective in reducing *Salmonella* colonization. Stringfellow et al. [28] found that quick lime and steam pasteurization were effective at controlling *Salmonella* Typhimurium in poultry litter; however, steam pasteurization is time consuming and requires specialized equipment. Furthermore, to enhance the performance of quick lime, water must be added to the litter. This increased moisture can lead to excess production of ammonia and other associated problems. Additionally, studies by Bennett et al. [29, 30] with day-of-hatch chicks showed that lime levels in excess of 5% (wt/vol) caused mild but obvious ocular and respiratory irritation. Vicente et al. [31] found that Poultry Guard litter amendment, a litter acidifier, significantly reduced *Salmonella enteritidis* levels in broiler chicks at 11 d post-treatment; however, this apparent reduction did not hold up over time, as no significant difference was noted between the treated and control chicks at 21 d post-treatment. Our study did not measure the persistence of the *L. casei* present in the manure past 9 d, but there was a decrease in its concentration over that time. Future studies should evaluate the longevity of LBP constituents and effectiveness in the litter. Based on a comparison of the efficacy to control of *Salmonella* Typhimurium in poultry manure or litter of LBP to the other approaches from the literature, LBP offers promise to provide an effective, easy, and safe means of controlling *Salmonella* Typhimurium in the boiler production arena.

CONCLUSIONS AND APPLICATIONS

1. An LBP administered at a level of 1 gal/1,000 ft^2 significantly ($P < 0.05$) reduced *Salmonella* Typhimurium in poultry manure over 9 d in a laboratory study. This material is easy to incorporate into litter, safe for poultry and humans, and requires no specialized equipment.
2. Further research should to be conducted on the usefulness, efficacy, and the persistence of this LBP under commercial broiler production conditions.

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