SURVIVAL OF FECAL COLIFORMS IN FRESH AND STACKED BROILER LITTER

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Primary Audience: Researchers, Poultry Growers, Regulatory Personnel

SUMMARY

Fecal coliforms are indicator bacteria that are used for determining water quality. Poultry growers may be implicated when fecal coliforms are found in surface waters following runoff events from broiler litter-amended pastures and hayfields. This study determined the numbers of fecal coliforms in fresh and stacked broiler litter. In 1998, 10 of 20 fresh and all 19 stacked broiler litter samples from eight different Georgia counties contained less-than-detectable numbers of fecal coliforms (< 10 fecal coliforms per g of dry weight litter). In 1999, all 13 interior and 12 of 13 exterior samples of stacked litter from one South Carolina and two Georgia counties contained less-than-detectable numbers of fecal coliforms. When high numbers of fecal coliforms (> 10,000,000 fecal coliforms per g of dry weight litter) were added to five different broiler litter samples, numbers of fecal coliforms declined to below detectable levels within 8 days. When water was added to two of the five stacked litter samples, survival of fecal coliforms did not increase, but survival did increase when the temperature was lowered from 28 to 18 °C. The data suggest that poultry growers should consider stacking broiler litter for a reasonable period of time (> 8 days) to eliminate fecal coliforms in runoff from landspread broiler litter.

Key words: Bacteria, *Escherichia coli*, moisture, poultry litter, runoff, temperature, water content
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DESCRIPTION OF PROBLEM

In 1998, the United States produced 7.93 billion broilers [1]. Assuming that each broiler produces 1.46 kg of dry weight litter (a mixture of bird excreta, feathers, waste feed, and bedding materials) during a normal growing cycle [2], these broilers produced 11.58 billion kg of litter in the United States. Most of this litter is applied to land [3]. Broiler litter is a good source of plant nutrients, especially nitrogen and phosphorus, and therefore applying this litter to land is considered good agricultural practice. However,

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there is the potential for this litter to contribute fecal coliforms to streams and other water sources through surface runoff, particularly where land area is limited and poultry growers apply more than the recommended rate.

Fecal coliforms normally inhabit the intestinal tract of warm-blooded animals; their presence in soil and water indicates that the soil or water was contaminated by fecal material. Thus, these bacteria provide an accepted means of assessing soil and water quality [4]. Fecal coliforms consist of several genera of bacteria from the family Enterobacteriaceae that can grow in a selective medium at 44.5 °C for 24 hr. A good correlation exists between the presence of fecal coliforms in water and the potential presence of intestinal pathogens. In one of many studies of this correlation, when numbers of fecal coliforms exceed 2,000 per 100 mL of water, the likelihood of bacterial pathogens in the water is 98.1% [5]. Because of this correlation, each state in the United States decides on an acceptable level of fecal coliforms in its waters. For the State of Georgia, the water quality standard for recreational waters is 200 fecal coliforms per 100 mL based on a geometric mean of four samples collected over a 30-day period [6].

In poultry litter, numbers of fecal coliforms decrease with time. In a study of four different reused broiler litter samples, initial counts of fecal coliforms were 5.07, 5.21, 4.64, and 5.91 $\log_{10}$ colony-forming units (CFU) per g dry weight, and these numbers declined an average of over 96% after 2 to 16 wk to 3.65, 3.78, 3.20, and 4.47 $\log_{10}$ CFU per g dry weight, respectively [7]. Numbers of fecal coliforms decrease further if the litter or manure is composted. Of 64 samples of composted litter, Martin *et al.* [8] observed that only three contained quantifiable coliforms. In another study, poultry litter, combined with layer manure and then composted, contained *Escherichia coli*, a fecal coliform, on Day 1 but not on Days 22 and 57 [9]. These reports suggest that fresh poultry litter is a possible source of fecal coliforms, and that composting of litter can essentially eliminate these bacteria.

Studies of fresh broiler litter- and layer manure-amended field plots suggest that contamination of runoff water by fecal coliforms is a problem. Typically only runoff water is observed because poultry manures are often surface-applied with little or no incorporation into the soil. In a study of runoff water from soil amended with 25-wk-old broiler litter, counts of total coliforms ranged from near zero to 10^5 CFU per mL from plots amended with 5.6, 11.2, 22.4, 67.2, and 89.2 metric tons of fresh poultry litter per hectare [10]. Although the counts were not extremely high, they did exceed recommended water quality standards (*e.g.*, [6]). Edwards and Daniel [11] observed fecal coliforms in runoff from plots each amended with 5 metric tons of broiler litter or layer manure per hectare. Counts of fecal coliforms were 6.40 $\log_{10}$ CFU per 100 mL from the layer manure-amended plots, and 6.52 $\log_{10}$ CFU per 100 mL from the broiler litter-amended plots. These counts also exceeded water quality standards (*e.g.*, [6]). Similar results were observed in other studies of fecal coliforms in runoff from poultry litter-amended plots [12, 13, 14]. However, Hartel [15] was unable to detect fecal coliforms in runoff from Georgia hayfields amended with 6 metric tons of broiler litter per hectare.

The literature suggests that fresh poultry litter contributes fecal coliforms to runoff and that storage of poultry litter reduces fecal coliform numbers. However, there is little information on numbers of fecal coliforms in broiler litter under stackhouse conditions that poultry growers typically employ. Therefore, we conducted a study to determine fecal coliform numbers under these conditions. Because studies suggest that moisture and temperature are among the two most important factors affecting fecal coliform survival [16], we also conducted survival experiments to determine the effect of temperature and moisture on fecal coliform survival in stacked broiler litter samples. In this manner, we determined the potential for stacked broiler litter to contribute fecal coliforms to runoff if the litter was landspread.

Materials and Methods

SAMPLE COLLECTION AND PROCESSING

There were two samplings. In the first sampling, 20 fresh and 19 stacked litter samples were collected from eight different Georgia counties from March 30 to September 15, 1998. In all

cases, fresh litter samples were obtained within 2 wk of removal of the birds from the broiler house and in no case was the litter stacked. In the case of stacked litter, the age was estimated by the growers. In the second sampling, 13 stacked litter samples were collected from one South Carolina and two Georgia counties from August 31 to September 24, 1999. For each sampling, county extension agents selected farms that represented typical poultry operations. Because stackhouses are a relatively recent development in Georgia and South Carolina, stackhouse conditions varied widely, from litter that was well-protected from the weather to litter that was exposed to moisture from the sides or underneath from the soil. Litter samples were obtained from stackhouses that represented this range of conditions. In the first sampling, the fresh or stacked litter was sampled aseptically by placing a hand in an inverted Ziploc bag and obtaining a random grab sample from the top 5 cm of the stack exterior. Each extension county agent wore sterile disposable gloves. The Ziploc bag was turned rightside-out and sealed. This procedure was repeated 18 times. In the second sampling, the same method was used, except two samples were obtained, one from the top 5 cm of stack (exterior sample) and another 15 to 30 cm inside the stack (interior sample). In the case of the interior samples, 12 samples were obtained from each stack. All samples from both sampling periods were processed immediately or stored at room temperature (20 to 22 °C) until processed. All were processed within 72 hr. All 12 or 18 samples from each fresh or stacked litter were composited in a large Ziploc bag and mixed manually from the outside for 2 min to ensure a representative sample. The percentage moisture in each litter was determined gravimetrically by drying three replicate litter samples overnight at 105 °C. The pH of the litter was determined as described by Plank [17].

NUMBERS OF FECAL COLIFORMS IN SAMPLES OF FRESH AND STACKED LITTER

Numbers of fecal coliforms in samples of fresh and stacked litter were determined as described in the latest edition of *Standard Methods for the Examination of Water and Wastewater* [18]. To determine the ability of fecal coliforms to survive in different samples of stacked litter, two fecal coliform isolates were obtained from layer manure. Layer manure, collected on a sheet of sterile aluminum foil held under cages of defecating hens, was used instead of broiler litter to avoid any possibility of fecal coliform contamination from non-poultry sources (*e.g.*, rodent feces). The RiboPrinter System (Qualicon Inc., Wilmington, DE) identified these isolates as different ribotypes of *Escherichia coli* [19].

Survival of fecal coliforms was determined in broiler litter samples from Banks (23.0% water content), Barrow (38.4% water content), Clarke (23.0% water content), Hall (27.8% water content), and Morgan (46.1% water content) Counties, Georgia [20]. The incubation temperature was 28 °C. In addition, the effect of a 10 °C decrease in temperature (18 °C) and, separately, a 10% increase in water content, was conducted in the broiler litter samples from Barrow and Clarke Counties [20]. The lower temperature was selected because die-off rates of fecal coliforms, within a range of 5 to 30 °C, are halved for every 10 °C drop in temperature [16]. The increased water content was selected because it was consistent with the normal upper range for water content of most broiler litter [21]. Data were analyzed by SAS [22].

RESULTS AND DISCUSSION

NUMBERS OF FECAL COLIFORMS IN SAMPLES OF FRESH AND STACKED LITTER

In 1998, ten of the 20 fresh litter samples contained between 3.06 and 6.66 $\log_{10}$ CFU of fecal coliforms per g dry weight, while the remaining ten samples had numbers of fecal coliforms below the limit of detection (< 1.00 $\log_{10}$ CFU per g dry weight; Table 1). The data suggest that numbers of fecal coliforms in fresh broiler litter were highly variable. The reason for this variability is unclear. In contrast to the fresh litter samples, all 19 stacked litter samples had numbers of fecal coliforms below the limit of detection.

In 1999, 12 of 13 exterior and all 13 interior samples had numbers of fecal coliforms below the limit of detection (Table 2). Therefore, the 1999 results are almost identical to 1998 stacked litter results. One exterior sample did contain a

TABLE 1. Counts of fecal coliforms on the exterior (top 5 cm) of 20 fresh and 19 stacked broiler litter samples from nine different Georgia counties. All fresh litter samples were < 2 wk old and were not stacked. The samples were collected from March 30 to September 15, 1998. Low limit of detection, < 10 colony-forming units (CFU) per g dry weight. All numbers are the average of duplicate dilutions. Where a count is greater than the low limit of detection, the error equals ± 1 SEM

FRESH LITTER		STACKED LITTER		
County of origin	Fecal coliforms	County of origin	Age[A]	Fecal coliforms
	$\log_{10}$CFU per g dry wt		weeks	$\log_{10}$CFU per g dry wt
Banks #1	<1.00	Banks #1	ND	<1.00
Banks #2	6.66 ± 0.09	Banks #2	8	<1.00
Banks #3	5.44 ± 0.07	Banks #3	9	<1.00
Banks #4	4.91 ± 0.05	Banks #4	9	<1.00
Barrow #1	6.04 ± 0.08	Barrow #1	ND	<1.00
Dawson #1	4.20 ± 0.11	Barrow #2	ND	<1.00
Dawson #2	<1.00	Barrow #3	4	<1.00
Dawson #3	4.08 ± 0.11	Barrow #4	6	<1.00
Jackson #1	<1.00	Barrow #5	8	<1.00
Jackson #2	<1.00	Barrow #6	ND	<1.00
Jackson #3	<1.00	Habersham #1	8	<1.00
Jackson #4	<1.00	Habersham #2	12	<1.00
Jackson #5	<1.00	Habersham #3	20	<1.00
Madison #1	<1.00	Habersham #4	52+	<1.00
Madison #2	2.50 ± 0.11	Morgan	ND	<1.00
Madison #3	3.34 ± 0.20	Oconee #1	ND	<1.00
Madison #4	<1.00	Oconee #2	ND	<1.00
Madison #5	6.44 ± 0.20	Oconee #3	ND	<1.00
Madison #6	3.06 ± 0.11	Oconee #4	ND	<1.00
Morgan	<1.00			

[A]ND, grower could not estimate age.

low number of fecal coliforms. It is possible that wild animals (*e.g.*, rodents) contributed to this count, especially since detectable fecal coliforms were not observed in the interior of the stack. In summary, 44 of 45 total stacked litter samples from growers had below-detectable numbers of fecal coliforms.

SURVIVAL OF FECAL COLIFORMS IN STACKED LITTER

In stacked litter samples from Banks, Hall, and Morgan Counties, numbers of fecal coliforms decreased significantly from > 7.00 $\log_{10}$ CFU per g dry weight of litter to below the limit of detection within 8 days (Fig. 1). Similar results were observed for Clarke County (Fig. 2). When the water content of the Clarke County litter was increased from 23.0 to 33.0%, numbers of fecal coliforms decreased significantly to below the limit of detection within 4 days. In contrast, reducing the temperature in Clarke County litter from 28 to 18 °C significantly extended fecal coliform survival. Under these conditions, numbers of fecal coliforms decreased significantly from > 7.00 to 2.87 $\log_{10}$ CFU per g dry weight after 16 days. When the experiment was repeated in a Barrow County litter sample at 28 °C and water contents of 38.4 and 48.4%, numbers of fecal coliforms decreased significantly from > 7.00 $\log_{10}$ CFU per g dry weight of litter to below the limit of detection within 1 day (data not shown). At a water content of 38.4% and temperature of 18 °C, numbers of fecal coliforms decreased significantly from > 7.00 $\log_{10}$ CFU per g dry weight of litter to below the limit of detection within 2 days (data not shown). Therefore, the patterns of fecal coliform die-off were similar in broiler litter samples from both Clarke and Barrow Counties.

POSSIBLE FACTORS AFFECTING FECAL COLIFORM SURVIVAL IN STACKED LITTER SAMPLES

The most important factors affecting survival of fecal coliforms are temperature, moisture, pH, nutrient supply, and solar radiation

[23]. Because stackhouses are covered, solar radiation is an unlikely factor. Also, with the exception of the interior stacked litter samples from Oconee #7 (pH 4.78) and #8 (pH 4.88), the pH range of 1999 samples was between 6.47 and 7.91. Broiler litter is well-buffered [24] and survival of pathogens is adversely affected only outside the pH range of 5.8 to 8.4 [25]. For these reasons, pH is an unlikely factor. Litter pH would be a factor for the interior samples of Oconee #7 and #8. This low pH is likely the result of acid production from nitrogen mineralization [26], and because mineralization is a slow process, it is not surprising that these are the oldest two litter samples.

The factor most likely affecting fecal coliform survival in stacked litter samples was temperature. As a result of spontaneous heating, maximal temperatures in stacked litter are typically in the range of 43 to 60 °C [24], and die-off rates of fecal coliforms increase with increasing temperature [16]. Conversely, when the temperature is lowered, die-off rates of fecal coliforms decrease. This explains the higher numbers of fecal coliforms in the survival experiment when the temperature was lowered from 28 to 18 °C.

The effect of moisture was unclear. Although increased moisture is associated with greater fecal coliform survival in soil [16], wet broiler litter is almost free of fecal coliforms [27]. In our survival experiment, increasing the water content by 10% did not increase survival. More research is needed to understand this factor. In the case of the survival experiment where both temperature and moisture were kept constant, numbers of fecal coliforms declined below detectable levels within 8 days. In this case, nutrient supply was the likely dominant factor because of the inability of fecal coliforms to

TABLE 2. County origin, age, pH, water (moisture) content, and fecal coliform counts on the exterior (top 5 cm) and in the interior (depth of 15 to 30 cm) of 13 stacked broiler litter samples collected from August 31 to September 24, 1999. The litter samples were from Georgia (Gilmer and Pickens Counties) and South Carolina (Oconee County). Low limit of detection, < 10 colony-forming units (CFU) per g dry weight. All numbers are the average of duplicate dilutions. Where a count is greater than the low limit of detection, the error equals ± 1 SEM

COUNTY ORIGIN	EXTERIOR/INTERIOR	AGE	pH	WATER CONTENT	FECAL COLIFORMS
		weeks		%	$\log_{10}$ CFU per g dry wt
Gilmer #1	Exterior	6	7.72	16.5	2.89 ± 0.03
	Interior		7.65	28.5	<1.00
Gilmer #2	Exterior	8	7.66	12.0	<1.00
	Interior		7.91	36.8	<1.00
Gilmer #3	Exterior	9	7.80	20.8	<1.00
	Interior		7.59	39.4	<1.00
Oconee #1	Exterior	1	7.83	31.7	<1.00
	Interior		6.97	32.0	<1.00
Oconee #2	Exterior	2	7.81	18.3	<1.00
	Interior		6.67	46.6	<1.00
Oconee #3	Exterior	2	7.98	16.0	<1.00
	Interior		6.97	26.6	<1.00
Oconee #4	Exterior	2	7.69	21.5	<1.00
	Interior		6.47	26.3	<1.00
Oconee #5	Exterior	3	7.91	22.1	<1.00
	Interior		6.47	23.4	<1.00
Oconee #6	Exterior	3	7.82	23.7	<1.00
	Interior		6.55	27.0	<1.00
Oconee #7	Exterior	12	7.15	20.2	<1.00
	Interior		4.78	31.9	<1.00
Oconee #8	Exterior	104	6.03	13.7	<1.00
	Interior		4.88	18.3	<1.00
Pickens #1	Exterior	4	7.81	33.2	<1.00
	Interior		7.77	38.1	<1.00
Pickens #2	Exterior	4	7.70	23.1	<1.00
	Interior		7.70	29.4	<1.00

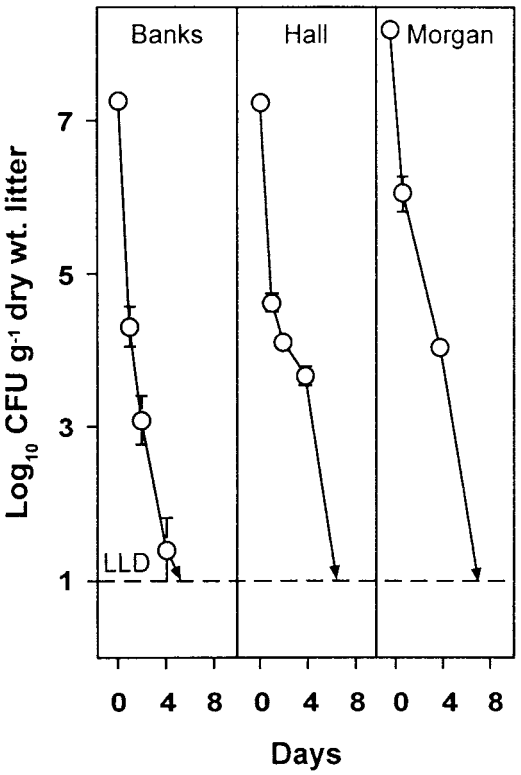

FIGURE 1. Survival of fecal coliforms in broiler litter from Banks (23.0% water [moisture] content), Hall (27.8% water content), and Morgan (46.1% water content) Counties, Georgia. All samples were kept at a constant incubation temperature of 28°. LLD, low limit of detection (< 10 fecal coliforms per g^{-1} dry weight of litter). Error bars, ± 1 SEM. Where error bars do not appear, the symbol was larger than the error.

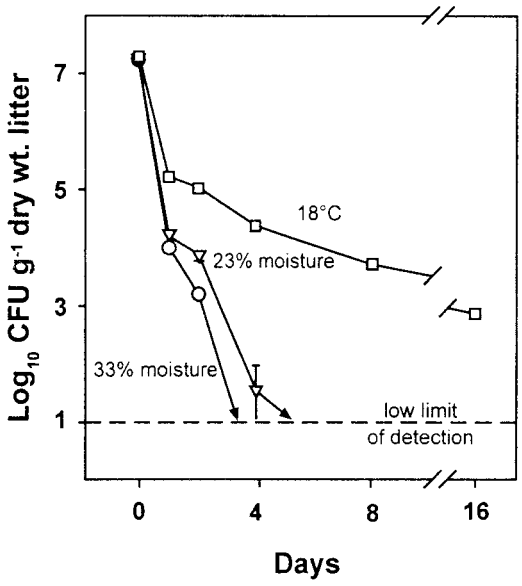

FIGURE 2. Survival of fecal coliforms in broiler litter from Clarke County (pH 8.0), Georgia, under conditions of 23.0% water (moisture) content and a constant incubation temperature of 28 °C (triangles). In the same litter, the water content was increased to 33.0% (circles), or the temperature was decreased to 18 °C (squares). Low limit of detection is < 10 fecal coliforms per g^{-1} dry weight of litter. Error bars, ± 1 SEM. Where error bars do not appear, the symbol was larger than the error.

reduce their metabolic rate to meet the low availability of usable organic carbon [28].

The reduction of fecal coliform numbers observed in our survival study was considerably faster than the reduction observed in reused litter by Kelley *et al.* [7]. This may be because their study was conducted in open-top, plywood bins in a broiler house and the litter may have become cross-contaminated with fecal coliforms from other sources (*e.g.*, feed, feathers, dust, and soil in the broiler house). In contrast, our experiment was conducted in presterilized, loosely capped dilution bottles.

Since most water testing in the State of Georgia uses fecal coliforms as an indicator of water contamination, it would be useful to determine the minimum time necessary to reduce fecal coliforms below detectable levels in stacked broiler litter throughout the year. Our data suggest that poultry growers should consider stacking broiler litter for a reasonable period of time (> 8 days) to eliminate the potential for litter to contribute fecal coliforms to runoff if the litter is land-spread. However, possible seasonal variation (*e.g.*, better fecal coliform survival with cool weather) still needs to be considered in this determination.

Conclusions and Applications

1. Ten of 20 fresh litter samples contained between 3.06 and 6.66 $\log_{10}$ CFU of fecal coliforms per g dry weight, while the remaining 10 samples had numbers of fecal coliforms below the limit of detection (< 1.00 $\log_{10}$ CFU per g dry weight).

2. Of 45 stacked broiler litter samples collected from poultry growers in eight counties from Georgia and South Carolina, 44 contained below-detectable numbers of fecal coliforms. The one exception contained low numbers of fecal coliforms (2.89 $\log_{10}$ CFU per g of dry weight litter) and may have been contaminated by wild animals.
3. When fecal coliforms were added to five stacked broiler litter samples from five different Georgia counties, numbers of fecal coliforms decreased from > 7.00 $\log_{10}$ CFU per g of dry weight litter to below detectable levels within 8 days.
4. Survival of fecal coliforms did not increase when the water content of two stacked litter samples was increased by 10%, but survival did increase when the temperature was lowered from 28 to 18 °C.
5. Poultry growers should consider stacking broiler litter for a reasonable period of time (> 8 days) to eliminate fecal coliforms in runoff from landspread broiler litter.

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18. See [4] for reference. Briefly, numbers of fecal coliforms were determined by placing a 1-g wet weight sample in 99 mL of sterile 0.1% Bacto-Peptone (Difco Laboratories, Detroit, MI) contained in a 160-mL dilution bottle. After 1 min of shaking, a 10-mL sample was transferred to another 160-mL bottle containing 90 mL of sterile 0.1% Bacto-Peptone. Samples of the 10^{-1} and 10^{-2} dilutions were filtered through sterile 0.45-μm filters (Nalge Nunc International, Rochester, NY), and the filters were placed on mFC agar plates (Difco). In addition, samples of the 10^{-2} dilution were spread directly onto mFC agar plates with a Spiral Plater (Spiral Systems, Cincinnati, OH). Plates were sealed inside quadrupled Ziploc freezer bags (to ensure dry conditions) and were incubated submerged in a water bath at 44.5 ± 0.2 °C. After 24 hr, blue colonies (fecal coliforms) were counted with a bacterial colony counter. All dilutions were done in duplicate.

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20. The isolates were grown overnight on duplicate plates of tryptic soy agar (Difco) at 37 °C. Lab acculturation of the isolates was avoided by freezing a loopful of each isolate at −70 °C in 700 μL of buffer (NaCl, 8.5 g L^{-1}; K_2HPO_4, 0.35 g L^{-1}; and KH_2PO_4, 0.65 g L^{-1}), 100 μL of dimethyl sulfoxide (Fisher Scientific, Fair Lawn, NJ), and 100 μL of glycerol (J.T. Baker, Phillipsburg, NJ). To inoculate a stacked litter, each isolate was grown on tryptic soy agar overnight at 37 °C and a small loopful of each was resuspended in phosphate buffer (K_2HPO_4, 0.35 g L^{-1}; and KH_2PO_4, 0.65 g L^{-1}). Dilution bottles, each containing 10 g wet weight of litter, were inoculated with sufficient diluent to give a final concentration of approximately 7.00 $\log_{10}$ CFU of fecal coliforms per g dry weight of litter. To keep the humidity high and avoid desiccation, bottles were placed in Ziploc bags with water added to surround the base of the bottles. The Ziploc bags were sealed and incubated at 28 °C. Bags were opened periodically to allow for air exchange. Three bottles were selected at 0, 1, 2, 4, 8, and 16 days, and dilutions

performed as previously described except the diluent was reduced to 90 mL for the initial dilution, and a 10^{-3} dilution was added for the spiral plating. To change the water content from 23.0 to 33.0% for the Clarke County stack litter, a 1-mL sample of sterile distilled water was added to each 10 g of litter contained in a 160-mL milk dilution bottle, and the litter was thoroughly mixed with an ethanol flame-sterilized spatula. The same procedure was used to increase the water content of the Barrow County litter from 38.4 to 48.4%. Counts of fecal coliforms were determined as previously described (see [18]).

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