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Abstract

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Keywords

dairy manure, *Salmonella*, *E. coli* O157:H7, pathogen deactivation

Disciplines

Medicine and Health Sciences | Veterinary Medicine

Accelerating the Deactivation of *Salmonella enterica* Serovar Newport and *Escherichia coli* O157:H7 in Dairy Manure by Modifying pH or Temperature

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Abstract: To assess methods for control of disease-causing bacteria in animal manures prior to field application, we manipulated the temperature or adjusted pH of dairy manure to high (3.5 to 5) or low (10 to 12) values with aluminum sulfate or hydrated lime, and inoculated the manure with *Salmonella enterica* serovar Newport or *Escherichia coli* O157:H7, then incubated the manure at ambient temperature. At pH ≤ 4.2 , *S. Newport* was eliminated within 6 days; however at pH > 4.2 *S. Newport* was suppressed only temporarily and recovered to concentrations near the unamended controls. pH required to eliminate *E. coli* O157:H7 was ≤ 4.5 . Both pathogens were killed by pH ≥ 11.0 . The pathogens were eliminated within 2 weeks when inoculated manure was incubated at 37°C, whereas at 22°C and 4°C, the organisms persisted for much longer periods. *S. Newport* survived for over 300 days at 4°C, which has implications for manure spreading in colder seasons.

Keywords: Dairy manure, *Salmonella*, *E. coli* O157:H7, pathogen deactivation.

1. INTRODUCTION

Movement of zoonotic pathogenic microorganisms from animal production systems into the human food chain through land-spreading of animal manures can potentially lead to foodborne illness. Incidences of foodborne diseases have generated any number of media headlines. The Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, estimate that the total burden of foodborne illness is 48 million cases annually in the U.S., 128 thousand hospitalizations, and 3 000 deaths [1]. Shiga toxin-producing strains of *Escherichia coli*, including O157:H7 (*E. coli* O157:H7), are estimated to cause 265 thousand cases of foodborne illness per year in the US. *Salmonella enterica* serovar Newport (*S. Newport*) has in the past decade increased its presence in dairy cattle, and has been characterized as an emerging disease-causing agent [2]. Together, nontyphoidal strains of *Salmonella* and *E. coli* O157:H7 are among the top five pathogens leading to the greatest number of hospitalizations for foodborne illness. Recent high-profile cases have been reported as due to consuming pathogen-contaminated sprouts in Germany, and lettuce and spinach in the U.S.

Dairy cattle are important reservoirs for both pathogenic *E. coli* and *Salmonella* [3, 4]. Dairy manures when land-applied are a point of entry for the pathogenic organisms in

the agroecosystem, where they have been found to survive for months. Using environmental sentinel chambers [5], which allow equilibration of pathogens with ambient environmental moisture and gas exchange conditions while preventing their dissemination with semi-permeable membranes, Toth *et al.* [6] found that *S. Newport* survived for > 9 months in a grass field and > 6 months in a dairy effluent lagoon. The general pattern of *S. Newport* deactivation in the dairy farm environment was a log-linear decline with log reduction times (time for 10-fold deactivation) of 14 to 20 days in the soil and 7 days in the lagoon, followed by longer-term survival at low and variable concentrations. However, in a static dairy solids compost pile, the pathogens were killed by temperatures exceeding 60°C within one day [6].

Reducing pathogen loads on the farm prior to field spreading of manure can be a potential useful complement to adhering to proper hygiene in the human food chain, in addition to following prescribed food production practices such as a Hazard Analysis and Critical Control Points protocols [7]. Various manure treatments have been suggested as ways to decrease pathogens in manure, for example, through lowering manure pH [8] or increasing pH [9]. In the present study, manure pH adjustment was achieved using two chemicals commonly used in dairy production, aluminum sulfate (alum, $Al_2(SO_4)_3 \cdot 14 H_2O$) and hydrated lime ($Ca(OH)_2$). Alum is used in food animal production systems to bind soluble manure phosphorus [10, 11], and hydrated lime has been shown to reduce the population of mastitis-causing bacteria when applied on mattresses or to bedding [12]. Another treatment option

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effectively killing pathogenic bacteria is manure composting [6, 13], a process largely driven by thermal deactivation. This paper reports the survival and die-off characteristics of *S. Newport* and *E. coli* O157:H7 in dairy manure when treated with alum, hydrated lime, or with temperature adjustment in laboratory incubation studies. The results illustrated an accelerated deactivation of these pathogens at higher temperature or when the pH was adjusted to certain threshold levels.

2. MATERIALS AND METHODOLOGY

2.1. Manure and Manure Treatments

Manure from healthy, lactating Holstein cows was obtained from the floor of a free-stall dairy immediately following excretion. The cows were fed a total mixed ration including corn (maize) silage, grass hay, corn grain and protein mix. The bulk manure samples, consisting largely of feces plus possibly small amounts of urine and wood shavings bedding material, were stored frozen at -20°C until laboratory usage. Manure samples averaged 0.143 g g⁻¹ dry matter.

Alum or hydrated lime used to lower or increase pH of dairy manure were in their technical grade formulations. Bulk manure samples with pH values of 7.0 to 7.3 were amended with alum to achieve pH in the range 3.5 to 6, or with hydrated lime to reach the pH range 8 to 12. Following chemical amendment, initial pH was measured on 1:1 ratio manure:deionized water mixtures, then 400 g amended manure samples in sterile, 1 L Nalgene beakers were inoculated with the pathogens *S. Newport* or *E. coli* O157:H7 at a target rate of 7 log₁₀ colony-forming units (CFU) g⁻¹ manure, approximating a concentration shed by a heavily-infected animal [14]. Treatment beakers were covered with parafilm to retard moisture loss and incubated at ambient temperature with periodic sampling over a time course of 30 to 40 days. There were three replications of each pH or temperature treatment; an uninoculated beaker served for periodic monitoring of pH changes over the course of the incubation.

Preliminary trials were performed with pH adjusted to 4, 5 or 6 with alum and pH 8, 9 or 10 with hydrated lime, designed to estimate approximate pH values effective in pathogen suppression. To determine if addition of alum or hydrated lime altered the temperature of the inoculated manure, temperature was monitored over the first 120 min following chemical addition. The chemicals had no effect on manure temperature. The main experiments were designed to determine pH lethal to the pathogens (indicated by elimination of the pathogens within 1 week without regrowth thereafter), with pH adjusted to 3.5 to 5 and 10 to 12. On a per-kilogram manure basis, 31, 39, 45, 48, and 55 g alum were used to reach pH values of 4.9, 4.5, 4.2, 4.0, and 3.6, respectively; corresponding amounts of hydrated lime were 4.3, 4.7, 5.5, 5.7, and 7.2 g for pH of 10.0, 10.3, 10.8, 11.0, and 12.0.

For the temperature adjustment trials, 400 g inoculated manure samples in sterile Nalgene beakers in triplicate were incubated in the dark at 4°C in a constant-temperature chamber, 22°C at ambient room temperature, and 37°C in an incubator.

2.2. Bacteria Inoculation and Enumeration

E. coli O157:H7 (ATCC standard 43895) was obtained from Penn State University's *E. coli* Reference Center. The strain was positive for Shiga toxins 1 and 2, and labeled with green fluorescence protein (*gfp*; [15]). To propagate, a small amount of pure culture from a slant tube was transferred aseptically to 40 mL of Luria-Bertani broth (LB) in centrifuge tubes and incubated 24 hr at 37°C. Following incubation, the tubes were centrifuged 20 min at 2 700 × *g*. The supernatant was decanted, the pellet washed with dilute buffered peptone water (BPW, 0.1%), then the bacteria were resuspended with vortexing in 40 mL BPW.

S. Newport (SRC isolate 0007-33) of dairy cattle origin was obtained from the University of Pennsylvania's Salmonella Reference Center. Propagation was similar to that for *E. coli* O157:H7, except that the pure culture was incubated in 40 mL brain-heart infusion (BHI) broth for 24 hr, then 1 mL of the initial inoculum was added to 40 mL BHI, incubated a further 24 hr, centrifuged and resuspended in 40 mL physiological saline solution (PSS; 8.5 g NaCl L⁻¹). Pathogen inocula were stored at 4°C until use in the survival trials.

For each sampling during the pH or temperature adjustment trials, inoculated manure was homogenized thoroughly with a sterile wooden spatula, then a 2 g subsample was added to a 50 mL centrifuge tube with 18 mL sterile PSS, and the tube agitated vigorously by hand then shaken 6 min at 150 min⁻¹. For enumeration, aliquots were serially diluted in PSS at 1:10 ratios to bracket the expected countable concentration. *S. Newport* was plated on xylose-lysine-deoxycholate (XLD) agar and *E. coli* O157:H7 on MacConkey's agar with sorbitol. Fluorescing *E. coli* colonies were counted on a transilluminator under ultraviolet light at 350 nm. Thirty to 300 colony-forming units (CFU) per plate were considered optimal numbers for enumeration. After the pathogens were no longer detectable by direct plating (detection limit approximately 100 CFU g⁻¹), a modification of the most-probable number method (MPN) was used [16]. Plate counts were converted to log₁₀ CFU g⁻¹ manure.

2.3. Data Analysis

Pathogen die-off as a function of pH or temperature treatments was modeled as a linear first-order deactivation using PROC GLM in SAS [17]. Differences in deactivation rate parameters were tested by covariance analysis in PROC GLM, and mean separation at P=0.05 was determined by Fisher's protected lsd test where appropriate, following least-squares analysis of variance.

3. RESULTS

3.1. Chemical Amendment Effects on Manure pH

The initial pH in the unamended control was 7.0, which varied little over the 14 days of monitoring, ranging from 6.9 to 7.1. In the treatments receiving higher rates of alum (achieving initial pH of ≤4.2), the pH values increased by up to 1 unit but did not approach the circumneutral pH of the control (Fig. 1). Similarly, at the higher hydrated lime applications (pH ≥11), pH declined by approximately

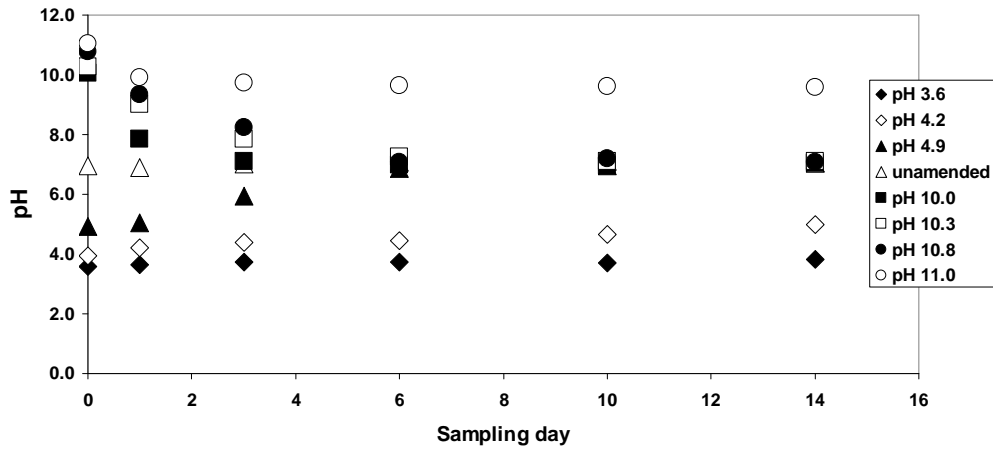


Fig. (1). Time trends in pH of dairy manure amended with alum or hydrated lime.

1.3 units through day 3, remaining steady thereafter. At these highest and lowest pH treatments, all enteric bacteria activity was strongly suppressed or halted. However, at intermediate rates, manure pH gradually normalized to the pH of the unamended control within six days; the trend and time course were similar in *S. Newport* and *E. coli* O157:H7. Biotic activity of the microbial communities in the manure and high organic matter content were likely the causes of the convergence of pH at the intermediate treatment rates.

3.2. pH Effects on Suppression of *S. Newport* and *E. coli* O157:H7

Preliminary trials established that at pH 4 there was a significant reduction in *S. Newport* in dairy manure, with

smaller and transient effects at pH 5 and 10 (data not presented). The main experiments were designed to identify suppressing and lethal pH values. In the pH 3.6 and 4.2 treatments, *S. Newport* concentrations showed some suppression within 2 hrs of the addition of alum; the pathogen died off completely by day 6 (Fig. 2A). At pH 4.5 and 5.0, *S. Newport* showed an initial reduction by up to 2.2 log units, but with a quick rebound to near the concentration in the unamended control by day 7 (Fig. 2A). After this rapid-change phase, *S. Newport* at pH 4.5 and 5.0 declined in a log-linear pattern at rates of 0.10 to 0.12 day⁻¹, not significantly different from the unamended control (Table 1). This decline continued over the period of observation to day 30.

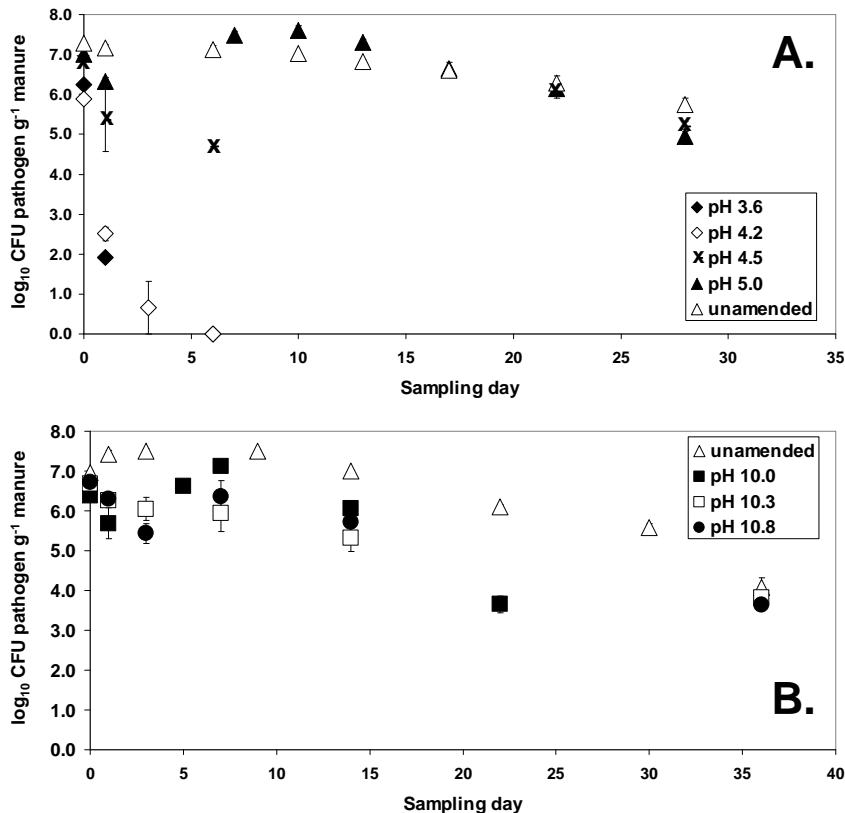


Fig. (2). *S. Newport* response to low pH (alum, A) and high pH (hydrated lime, B) amendment in dairy manure. Error bars represent ± 1 standard error of the mean. Inoculated *S. Newport* was immediately eliminated by the pH 11 and 12 treatments, not detected at the initial pathogen enumeration within 2 hours of hydrated lime application.

Table 1. Regression Equation Parameters for the Log-Linear Decline Phase of *E. coli* O157:H7 and *S. Newport* Response to Modification of Dairy Manure pH

pH Treatment	Initial pH	Slope	Y-Intercept	
<i>S. Newport</i>	3.6	– ^a	–	
Low pH	4.2	–	–	
	4.5	-0.098	8.05	
	5.0	-0.119	8.69	
	7.3 (unamended)	-0.117	8.56	
<i>S. Newport</i>	7.0 (unamended)	-0.112b	8.58b	
	High pH	10.0	-0.247c	9.07b
	10.3	-0.072ab	6.39a	
	10.8	-0.058a	7.02a	
	11.0	–	–	
	12.0	–	–	
<i>E. coli</i> O157:H7	4.0	–	–	
	Low pH	4.5	–	–
		4.9	-0.017a	4.76a
	7.0 (unamended)	-0.077b	7.96b	
<i>E. coli</i> O157:H7	7.0 (unamended)	-0.078	7.95b	
	High pH	10.0	-0.048	3.26a
		10.3	-0.073	3.47a
		10.8	-0.075	3.57a
		11.0	–	–
12.0	–	–		

^aDashes indicate pH adjustment treatments for which no linear regression equation could be generated.

Values for the same regression parameter, bacterial species and pH treatment followed by the same letter are not significantly different at the 5% probability level by Fisher's protected LSD test following least-squares analysis of variance.

With hydrated lime raising manure pH to 11 or 12, *S. Newport* was completely eliminated in the samples collected on the day of treatment application. At pH 10.0, 10.3 or 10.8, *S. Newport* concentration declined by about 2 log units in the first 3 days as compared to the unamended control (Fig. 2B). After the initial decline, *S. Newport* concentration returned to near the control concentration in the pH 10 treatment whereas in the pH 10.3 and 10.8 treatments the pathogen tended to be lower than in the control, and did not match those in the control until day 36.

For *E. coli* O157:H7, pH 4.0 and 4.5 treatments effectively deactivated the pathogen as its concentration fell under the detection limit one day after the alum addition. At pH 4.9, *E. coli* O157:H7 concentration was reduced by about 2.5 log₁₀ in the first day but it remained at that level for the rest of the experiment (Fig. 3A). As with the *S. Newport* experiments, the pH 11 and 12 treatments were immediately lethal to *E. coli* O157:H7 (Fig. 3B), with none detected at the initial sampling time within 2 hrs of hydrated lime addition. At pH 10.0, the initial measured concentration (6.5 log₁₀ CFU g⁻¹) was not different from the unamended control, whereas in the pH 10.3 and 10.8 treatments, initial

concentrations were 5.2 and 5.1 log₁₀ CFU g⁻¹, respectively. Between days 1 and 5, *E. coli* O157:H7 at pH 10.0 to 10.8 declined gradually to 3 log₁₀ CFU g⁻¹ and did not exhibit regrowth through day 14, after which they could not be detected against the strong background growth of native manure microorganisms.

In practical terms, amounts of aluminum sulfate required to eliminate pathogens (i.e., mass of chemical to achieve lethal pH adjustment) from our dairy manure samples was 45 g alum kg⁻¹ manure for *S. Newport* and 39 g kg⁻¹ for *E. coli* O157:H7; comparable amounts of hydrated lime were 5.7 g kg⁻¹ for both pathogens. Hydrated lime is clearly preferable on a mass-required basis, not considering possible increases in manure ammonia volatilization at high pH. If we use an economic analysis similar to that in Diez-Gonzalez *et al.* [18], assuming annual manure production per lactating cow to be 20 Mg [19] and current price per ton \$130 USD [20], 120 kg hydrated lime would be needed to eliminate *Salmonella* and *E. coli*. This corresponds to a cost of \$16.80 per cow per year, or <5 cents per day. If we calculate the amount of hydrated lime required to achieve pH 10, which reduced *E. coli* O157:H7 by >3 log units (1000-fold), cost would be 3.5 cents per cow per day. Amendment of dairy manures with hydrated lime may be a simple, low-cost means to lessen pathogen loads on-farm and before land-application of manures, and adds another technique to the toolkit needed to assure healthy animal and plant products in the human food sector.

At intermediate pH, the lack of complete elimination of pathogens with some regrowth after the initial suppression of *S. Newport* and *E. coli* O157:H7 is a matter for concern. Both pathogens can cause disease at very low dose rates. Humphrey [21] reported infective doses of *Salmonella* sp. in foodstuffs to be in the range of 1 to 100 CFU. While infective dose of *E. coli* O157:H7 is not definitely known, Paton and Paton's review [22] suggests a similar infective dose of 100 CFU or less. Particularly in the part of the population at higher risk of foodborne illness such as the very young, elderly, and those with compromised immune systems, temporary suppression of the pathogens by manure treatments without complete deactivation may not eliminate the possibility of contracting a foodborne disease if manures are allowed to contaminate the food production system.

3.3. Temperature and *S. Newport* and *E. coli* O157:H7 Deactivation

S. Newport and *E. coli* O157:H7 responded similarly to temperature treatments except for the first few days of the experiment, in which *E. coli* O157:H7 showed 0.3 to 1 log unit increase through day 7 at all three temperature treatments (Fig. 4A), while *S. Newport* either did not change or declined in concentration over the first 7 days of the incubation (Fig. 4B). At all three temperatures tested, both pathogens exhibited a log-linear decline phase, with deactivation rate constants between the two pathogens not significantly different at the 5% probability level (Table 2). This log-linear decline was observable in *E. coli* O157:H7 until day 62 at 22°C and day 91 at 4°C, after which strong background growth of native manure microorganisms prevented further detection of the inoculated *E. coli*. For *S. Newport*, the log-linear deactivation continued in the 22°C

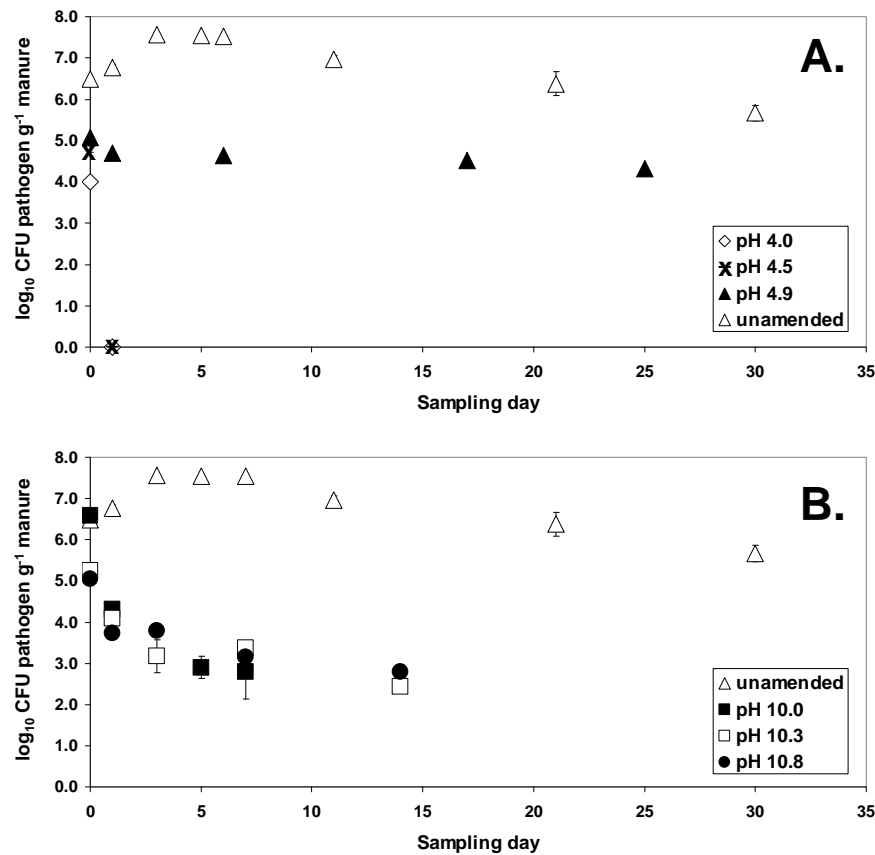


Fig. (3). *E. coli* O157:H7 response to low pH (alum, **A**) and high pH (hydrated lime, **B**) amendment in dairy manure. Error bars represent ± 1 standard error of the mean. Inoculated *E. coli* O157:H7 was immediately eliminated by the pH 11 and 12 treatments, not detected at the initial pathogen enumeration within 2 hours of hydrated lime application.

treatment until the organism could no longer be detected for two sampling times (last observation on day 63). At 4°C, *S. Newport* declined in a steady log-linear fashion to day 161, but persisted at low concentrations until day 309. At 37°C, both pathogens disappeared rapidly, and were not found past day 7 for *E. coli* O157:H7 and day 13 for *S. Newport*. Log reduction times (LRT, the inverse of the slope of the linear portion of the deactivation curve, and variously referred to as D-value or DRT in the literature) for the two pathogens, were 1.4 to 2.5 days at 37°C, 10.4 to 12.9 days at 22°C, and 33.0 to 34.5 days at 4°C.

4. DISCUSSION

A number of published reports have examined pH effects on suppression of pathogens in growth media. In a review paper on *Salmonella* response to environmental stress, Foster and Spector [23] suggested that minimum pH for growth in nutrient media was 4.3 for *Salmonella* Typhimurium and 4.6 for *E. coli*. This is comparable to our findings of lethal pH 4.2 for *S. Newport* and 4.5 for *E. coli* O157:H7 in inoculated dairy manure. In an early paper, Chung and Goepfert [24] noted that the minimum pH for growth of *Salmonella* serovar anatum in acidified nutrient broth ranged from 4.05 to 5.70. They found that at sub-lethal pH *Salmonella* was suppressed by 2 to 3 log units, followed by recovery and regrowth, which is similar to the observations in the present study. Studies on acid tolerance in *Salmonella* have shown that the pathogen can be induced to survive at pH as low as 3.3 if they are first exposed to intermediate acid conditions.

This tolerance is DNA-mediated involving several genes [25].

To render biosolids and sewage sludges safe for land application, the U.S. Environmental Protection Agency has mandated use of a variety of treatments to achieve adequate reduction in pathogens from the biosolids materials (EPA Part 503 Biosolids Rule; [26]). The recommended alkaline treatment (lime stabilization) is for increasing the pH to >12 for 72 hrs or longer. There have been studies of chemical amendments to sewage sludge for suppression of pathogen loads, Vinnerås *et al.* [27] found that urea effectively deactivated *E. coli* and *Salmonella*. Allievi *et al.* [9] noted that enteric bacteria in sewage sludges were eliminated within 2 weeks following treatment with NH₄OH or KOH, and resultant pH values of 10 to 10.5, whereas acidification of sludge to pH 4 had an equivalent, though slightly more rapid effect on pathogen elimination. Wong *et al.* [28] and Wong and Selvam [29] treated swine manure or biosolids with lime, calcium oxide or alkaline coal combustion byproducts. With an initial pH of 12, *Salmonella* and total coliforms were removed from the biosolids within 2 hrs, and *Salmonella* and *E. coli* were eliminated in swine manure within 8 days.

Several studies have reported the effects of various chemical treatments on suppressing pathogens in animal manure. Ottoson *et al.* [30] treated bovine manure with urea or aqueous ammonia and suggested effective treatment rates and exposure times for eliminating *Salmonella typhimurium*

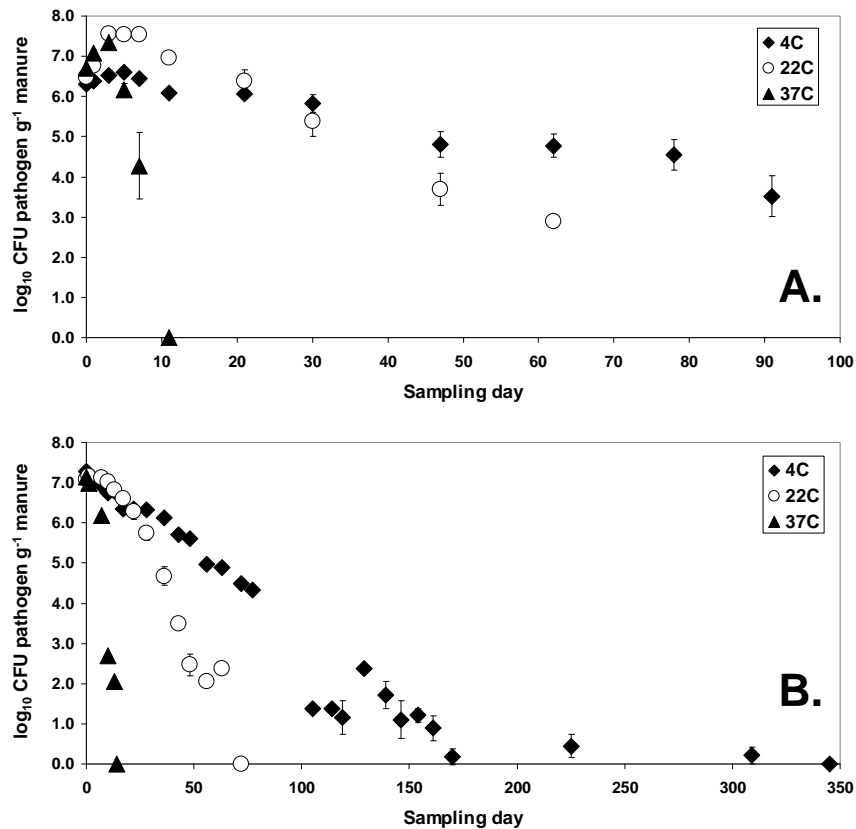


Fig. (4). *E. coli* O157:H7 (A) and *S. Newport* (B) survival response to incubation temperature in dairy manure. Error bars represent ± 1 standard error of the mean.

to be 1 week with 0.5% ammonia treatment (pH 9.7) and 2 weeks with 2% urea (pH 9.2) at 14°C. However, at 4°C the required exposure time was 1 month. Arthurs *et al.* [31] tested different carbonate-generating chemicals to determine their ability to reduce *E. coli* viability in dairy manure slurries. Sodium carbonate at an application rate of 16 $g\ kg^{-1}$ reduced *E. coli* from 6 $\log_{10}\ g^{-1}$ to the detection limit of 1 $\log_{10}\ g^{-1}$ within 5 days. Although the threshold pH for pathogen deactivation was lower in their study (≈ 10.0) than that in the present study (>10.8), Arthurs *et al.* also noted a similar effect on *E. coli* at intermediate pH, between 9 and 10, which suppressed *E. coli* by up to 3 log units without complete elimination. Park and Diez-Gonzalez [32]

inoculated dairy manure adjusted to pH 9.5 with NaOH, then supplemented the pH-adjusted manure with ammonium chloride, urea or sodium carbonate. *E. coli* O157:H7 and *Salmonella* Typhimurium concentrations were reduced by up to 6 $\log_{10}\ g^{-1}$. In general, modifying manure or biosolids materials to pH in the range 10 to 12 is shown in published studies as well as the present research to be effective in rapid elimination of several pathogen species, although the lethal pH and rate of pathogen destruction may vary.

Reports in the literature agree that the duration of pathogen survival in animal manures and biowastes is generally greater at low temperatures than at high ones, although deactivation rates vary by species and experimental

Table 2. Regression Equation Parameters for the Log-Linear Decline Phase, Comparison Between *E. coli* O157:H7 and *S. Newport* Responses to Incubation of Dairy Manure at Three Temperatures

Temperature	Pathogen Species	Slope	Y-Intercept	R ²	Log Reduction Time (Days) ^a
4°C	<i>E. coli</i> O157:H7	-0.0290	6.64a	0.97	34.5
	<i>S. Newport</i>	-0.0303	6.47a	0.89	33.0
22°C	<i>E. coli</i> O157:H7	-0.0773	7.92a	0.98	12.9
	<i>S. Newport</i>	-0.0963	7.89a	0.94	10.4
37°C	<i>E. coli</i> O157:H7	-0.7377	9.66b	0.82	1.4
	<i>S. Newport</i>	-0.3957	7.80a	0.89	2.5

^aLog reduction time is the number of days over which pathogen concentration declines by 10-fold. It is calculated as the inverse of the deactivation rate constant, the slope of the linear regression equation.

Values for the same regression parameter and temperature followed by the same letter are not significantly different at the 5% probability level by Fisher's protected lsd test following least-squares analysis of variance.

conditions. Himathongkham *et al.* [33] incubated cow manure inoculated with *Salmonella* serovar typhimurium and *E. coli* O157:H7 at 6 to 8 log₁₀ CFU g⁻¹ and found decimal reduction times of 1.7 to 3.8 days, 9.4 to 13.5 days, and 18.6 to 20.3 days at 37, 22 and 4°C, respectively. Hutchison *et al.* [34] studied the survival of four pathogen species in large tanks outdoors under summer and winter temperatures, and determined the seasonal LRT values. Log reduction times for *Salmonella* in stored dairy manure over winter (average temperature 4.3°C) were 7.3 to 11.8 days, and 6.8 to 8.9 days in summer (average temperature 12.4°C).

Raising manure temperature may be an effective way to reduce potential pathogen loadings to the environment. For example, manure composting is a frequently-employed means to reduce pathogen concentrations before land application. Temperatures of 55°C maintained for 2 to 3 days is generally considered to be sufficient to suppress or eliminate most pathogens [35]. At sub-lethal temperatures, however, evidence for effective deactivation of pathogens is equivocal. Fremaux *et al.* [36] and Wang *et al.* [37] noted survival of *E. coli* O157:H7 for several months at 35 to 37°C, whereas in the present study *S. Newport* and *E. coli* O157:H7 were not found after 14 days incubation at 37°C.

5. CONCLUSION

Using hydrated lime or aluminum sulfate, the foodborne pathogens *Salmonella* serovar Newport and *E. coli* O157:H7 could be effectively eliminated from dairy manure by altering the manure's pH regime to ≤4.2 to 4.5, or ≥11. Amendment with hydrated lime may be a better practical option since the amount needed to achieve a given pH is less than with alum. Similarly, the pathogens were deactivated within 2 weeks by increasing the temperature of inoculated manure to 37°C. Meanwhile, spreading manure in cold seasons in temperate climate conditions can have an elevated risk of pathogen dissemination.

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CONFLICT OF INTEREST

Declared none.

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