Effects of Lime Amendment on Antibiotic Resistance in Beef Cattle Manure of Open Feedlots

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Summary with Implications

The objective of this study was to evaluate the effectiveness of lime amendment on the reduction of antimicrobial resistant bacteria and antimicrobial resistance genes in beef cattle manure in open feedlots. Hydrated lime was uniformly applied to the surface of feedlot pen floor 1 day prior to cattle harvest at a rate of 0.36 lb/ft^2 and samples were collected over time. Collected samples were analyzed for change in pH and levels of antimicrobial resistant bacteria and antimicrobial resistance genes. Lime amendment elevated the pH of pen floor surface materials to pH > 12 for 4 hours and then pH > 11 for another 24 hours. Lime amendment reduced the concentration of generic and resistant Escherichia coli by 1–2 log for up to 4 hours. The abundance of antimicrobial resistance genes, such as tet(X) and tet(O), decreased by 1–2 order of magnitude with lime amendment. Results indicate that lime addition reduced the concentrations of antimicrobial resistant bacteria and antimicrobial resistance genes in pen surface materials from open beef feedlot pens.

Introduction

The proliferation of antimicrobial resistance is an emerging global health concern. In livestock agriculture, antimicrobials are used to control and treat infections in animals. Livestock manure application has been identified as a potential pathway for environmental exposure of antimicrobial resistance, as manure contains antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs). ARGs are the genetic determinants that make bacteria resistant to antimicrobials. Hence, mitigating antimicrobial resistance in manure at feedlots is important in preventing the potential spread of antimicrobial resistance into the environment and into meat packing plants.

Alkaline stabilization is a process recommended by the US Environmental Protection Agency to treat biosolids from municipal wastewater treatment plants. During alkaline stabilization, pathogens in biosolids are significantly reduced due to elevated pH and treated biosolids can then be safely land applied. To meet the requirements of Class A biosolids, a pH of 12 or above needs to be maintained for 72 hours and a temperature of 125°F or above be maintained for at least 12 hours. To meet the requirements for Class B biosolids, a pH of 12 or above needs to be maintained for 2 hours. Alkaline stabilization has not been previously used to mitigate antimicrobial resistance in manure from feedlot pen surfaces. Hence, the objective of this study was to evaluate the effectiveness of alkaline stabilization on the reduction of ARB and ARGs in beef feedlot pen surface manure.

Procedure

The study was conducted at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE in June 2020. Ten pens, each containing ten finishing beef cattle, were included in this study. Five pens were designated randomly for lime amendment and five pens as control which did not receive lime application. In the lime amendment pens, lime was applied uniformly using a skid loader at a rate of 0.36 lb/ft^2 on a 30 ft × 63 ft pen surface area the day before cattle were shipped for harvest.

Pen floor material samples were collected from the amendment and control pens. Within each pen, samples were collected from 3 different sections of the pen and three locations within each section using sterile spoons. On the first sampling day, samples were collected immediately after lime application (0.5 hours), and then at 2 hours, 4 hours, and 6 hours. Additional samples were collected on subsequent days at 24 hours, 48 hours, and 72 hours. Collected samples were stored on ice in coolers, transported back to the laboratory, and processed within a day.

Manure pH was determined by adding appropriate weight of manure sample to water in a 1:2 w:v ratio [manure sample (w): deionized water (v)] and measured using the Orion 3-star pH meter (Thermo Scientific, Waltham, MA, USA). Moisture content of manure samples was determined gravimetrically by oven-drying at 105°C for 24 h.

For ARB analysis, samples were enumerated to determine the abundance of total culturable *Escherichia coli* and *Enterococci*, as well as their resistant subpopulations. Manure samples were diluted 1:10 (*w*/*v*) in phosphate buffered tryptic soy broth (Becton Dickson, Sparks, MD; TSB-PO_4). The suspension was plated for bacterial enumeration using an Eddy Jet 2 spiral plater with spiral counting grids (IUL, S.A., Barcelona, Spain). *E. coli* was enumerated on CHROMagar plates amended with no antibiotic, 20 mg L^{-1} azithromycin or 32 mg L^{-1} tetracycline. Similarly, *Enterococci* was enumerated on Slanetz-Bartley agar containing no antibiotic, 32 mg L^{-1} tylosin, or with 32 mg L^{-1} tetracycline. The CHROMagar plates were incubated at 37°C for 24 hours while the Slanetz-Bartley plates were incubated at 37°C for 4 hours followed by 48 h at 44°C. Blue colonies on CHROMagar plates were enumerated as *E. coli* colonies, while brown and maroon colonies on Slanetz-Bartley plates as *Enterococci* colonies. The colonies counted were converted to colony forming units (CFU) per g sample on a dry weight basis.

DNA was extracted from the manure samples for ARG measurement. To avoid the negative impacts of high pH on the DNA extraction efficiency, the pH of samples collected from lime amended pens

Table 1. Effects of lime amendment and sampling time on bacteria concentration in beef cattle feedlot pen floor surface materials

		[a]Time (hour)							*P*-value		
		0.5	2	4	6	24	48	72	Lime × time	Lime	Time
Tetracycline[R] *Enterococci*	Lime	1.53	2.18	2.03	2.09	1.79	1.67	1.71	0.35	0.10	<0.01
	Control	1.47	2.69	2.47	2.54	2.01	1.50	1.81			
Tylosin[R] *Enterococci*	Lime	1.27	2.11	2.27	2.67	2.06	1.54	1.88	0.05	<0.01	<0.01
	Control	2.29	2.80	2.73	2.92	2.17	1.67	2.16			
Total *Enterococci*	Lime	1.57	2.46	2.46	2.83	2.46	1.90	2.39	<0.01	<0.01	<0.01
	Control	2.91	3.40	3.23	3.41	2.50	2.10	2.91			
Tetracycline[R] *E. coli*	Lime	1.87	1.70	1.97	2.04	1.51	2.06	1.74	<0.01	<0.01	<0.01
	Control	2.68	3.00	2.71	3.12	2.44	2.40	2.07			
Azithromycin[R] *E. coli*	Lime	2.03	2.08	2.33	3.05	2.82	3.16	3.08	<0.01	0.01	<0.01
	Control	3.76	3.59	3.64	3.73	3.42	3.47	3.39			
Total *E. coli*	Lime	2.17	2.14	2.22	2.99	3.01	3.19	2.19	<0.01	0.01	<0.01
	Control	3.85	3.71	3.82	4.01	3.41	3.44	2.14			

[a] Mean ARB concentration (Log CFU g^{-1} manure dry weight)

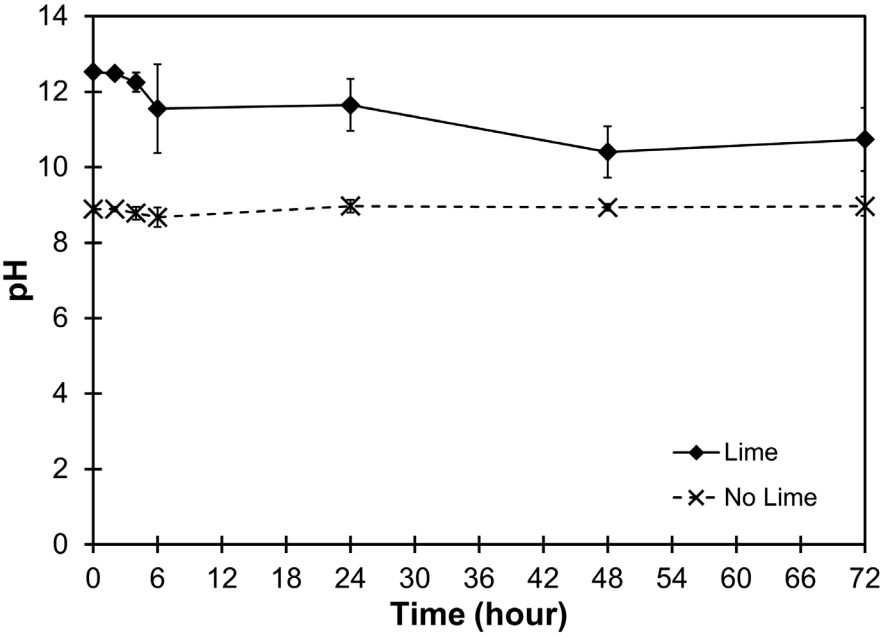

Figure 1. Effect of lime amendment on the pH of feedlot pen floor surface materials.

was adjusted to neutral pH prior to DNA extraction using 5× concentrated phosphate buffer saline. DNA was extracted using the Powersoil Powerlyzer DNA extraction kit (Qiagen, Germantown, MD). Extracted DNA was used for ARG analysis of macrolide resistance genes [*erm*(B), *erm*(C), and *erm*(F)], tetracycline resistance genes [*tet*(D), *tet*(O), and *tet*(X)], beta-lactam resistance gene [*bla*TEM], as well as the 16S rRNA gene and the Class 1 integron integrase gene *intI1*. Results from ARG concentrations was quantified in duplicate, the average concentration was used for statistical analysis and is reported as log copy number (CN) per gram of manure.

Data were analyzed using the GLIMMIX procedure SAS (SAS Institute, Cary NC). Repeated measures analysis of variance (rANOVA) was conducted to evaluate the impacts of lime amendment on the concentration of ARB and ARGs in beef cattle manure across time. Least significant difference (LSD) tests were used to determine significance of the differences among treatment conditions if treatment method was found to be significant according to rANOVA. Cattle performance and mass balance data for these pens are reported in the *2022 Nebraska Beef Cattle Report*, pp. 86–90.

Results

The pH of the pen floor surface materials reached 12.5 after lime application and pH was maintained at 12 and higher for 4 hours. After that, the pH decreased slightly and was maintained at 11.5 until 24 hours after lime application. Thereafter, pH was maintained above 10.0 until 72 hours after lime amendment (Figure 1). The pH from the control pens averaged at 8.9 throughout the sampling period.

Significantly lower concentrations of bacteria were recovered from the lime amended plots than from the control plots ($P < 0.05$, Table 1), with the exception of tetracycline resistant *Enterococci*. For both total and resistant bacteria, the log concentration was lower in manure from pens that were amended with lime (Figure 2). Lime amendment reduced ARB especially when the pH was above 11. Lime amendment reduced the concentration of total and tylosin resistant *Enterococci* by 1–2 orders of magnitude for up to 6 hours. After 6 hours, the distinction became smaller. The abundance of resistant and total *E. coli* was significantly reduced by lime amendment

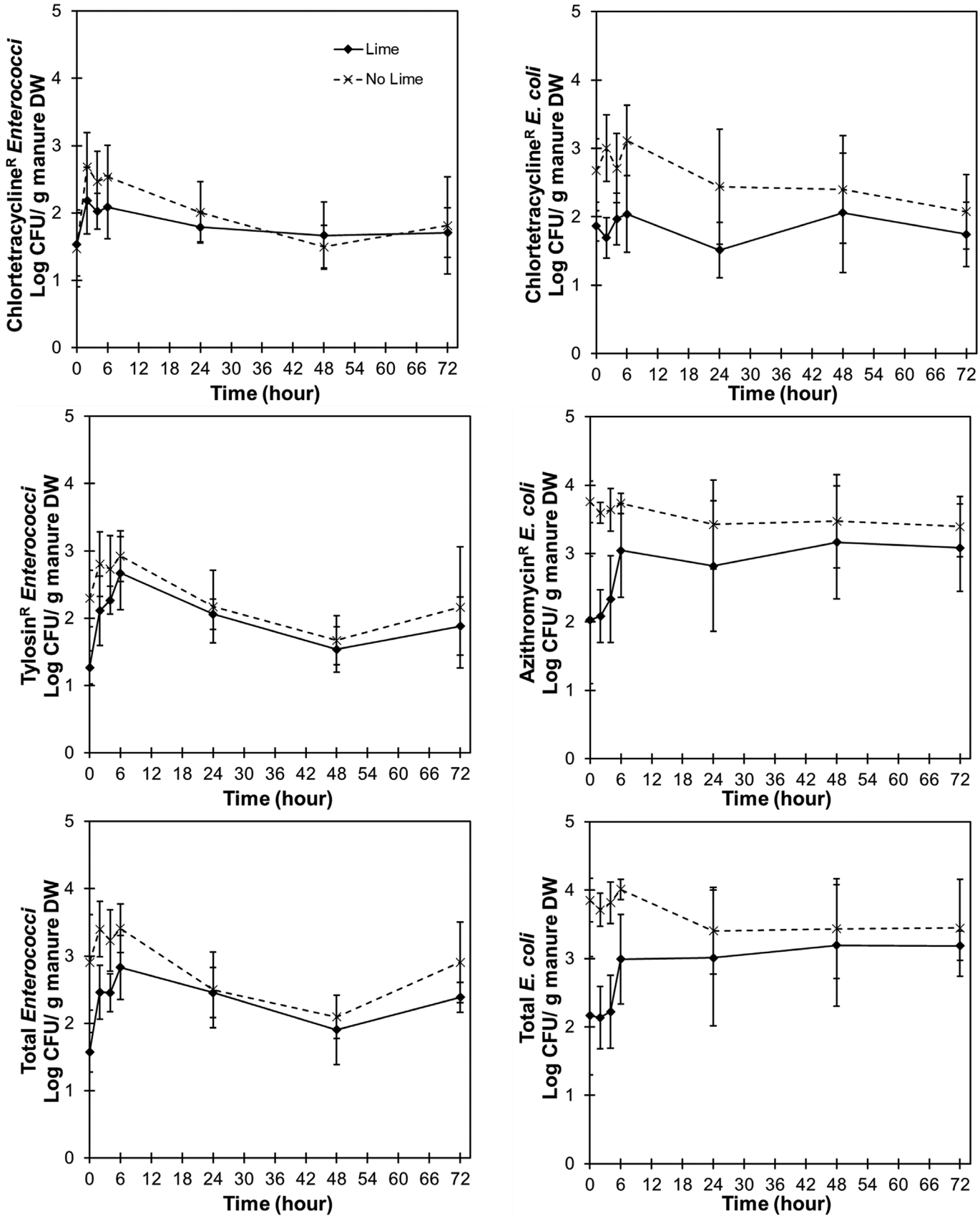

Figure 2. Effects of lime amendment on A) Tetracycline^R *Enterococci*, (B) Macrolide^R *Enterococci*, (C) Total *Enterococci*, (D) Tetracycline^R *E. coli*, (E) Macrolide^R *E. coli* and (F) Total *E. coli* as a function of time.

Table 2. rANOVA and LSD tests on the effects of lime amendment and sampling time on ARG concentrations in beef cattle feedlot pen floor surface materials

		[a]Time (hour)							*P*-value		
		0.5	2	4	6	24	48	72	Lime × time	Lime	Time
16S rRNA	Lime	11.15	10.69	10.89	11.34	11.16	11.32	11.15	0.29	0.06	0.38
	Control	11.60	11.59	11.63	11.59	11.64	11.55	11.58			
erm(B)	Lime	5.91	5.90	5.78	4.94	5.90	5.74	5.92	0.02	0.05	0.01
	Control	4.59	4.89	4.99	4.99	4.69	5.95	5.88			
erm(C)	Lime	6.41	6.29	6.75	6.73	6.64	7.00	6.73	0.30	0.07	0.02
	Control	7.08	7.11	7.21	7.00	7.35	7.33	7.33			
erm(F)	Lime	7.80	7.13	7.31	7.23	6.87	7.23	7.39	0.01	0.90	0.03
	Control	7.03	7.18	7.52	6.71	7.27	7.79	7.55			
tet(D)	Lime	3.76	3.58	3.41	3.05	3.22	3.15	3.64	0.09	0.25	0.40
	Control	2.61	3.73	3.11	2.96	3.46	3.01	3.71			
tet(O)	Lime	6.49	6.66	7.15	7.74	7.14	7.76	6.95	0.23	0.06	0.16
	Control	7.53	7.32	7.66	7.35	7.42	7.74	7.76			
tet(X)	Lime	6.63	6.35	6.57	6.86	6.49	6.71	6.68	0.76	0.06	0.64
	Control	6.76	6.83	6.93	6.93	7.00	7.22	6.94			
blaTEM	Lime	5.16	5.09	5.61	5.66	5.49	5.62	5.44	0.86	0.30	0.68
	Control	5.98	5.63	6.23	5.71	5.92	5.78	5.73			
*IntI*1	Lime	8.23	8.13	8.52	8.73	8.55	8.74	8.31	0.05	0.03	0.03
	Control	8.65	8.70	8.73	8.63	8.86	8.88	8.93			

[a] Mean absolute gene abundance (Log copies g^{-1} manure dry weight)

by about 2 orders of magnitude for up to 6 hours. Similar to *Enterococci*, the distinction between treatment and control for *E. coli* also decreased after the initial hours.

The rANOVA results revealed significant effects of lime amendment for the *intI1* gene (Table 2, $P = 0.03$). At the $P < 0.10$ level, the concentrations of 16S rRNA, *erm*(B), *erm*(C), *tet*(O) and *tet*(X) were significantly impacted by lime amendment compared to control. All genes had significantly lower concentrations in the lime amendment pens compared to the control pens, except for the *erm*(B) gene.

Conclusion

Land application of animal manure can potentially introduce antimicrobial resistance to the environment. Alkaline stabilization through addition of hydrated lime was tested for its effectiveness on the reduction of ARB and ARGs in pen floor surface materials. The pH ≥ 12 was attained as required by the US Environmental Protection Agency for pathogen reduction. Lime amendment was able to significantly reduce the levels of total and resistant *E. coli* and *Enterococci* in pen floor surface materials. The effects of lime amendment on ARGs were less pronounced, although the ARG concentrations in lime treated pens were generally lower than those in control pens. Although the lime amendment on pen floor surface resulted in pH elevation that meets the alkaline stabilization specification for class B biosolids, fecal indicator bacteria were still present in beef cattle manure at substantial levels. Further research is needed to determine how lime amendment may affect other properties of manure, such as nutrient levels.

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