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Soil versus Pond Ash Surfacing of Feedlot Pens: Occurrence of *Escherichia coli* O157:H7 in Cattle and Persistence in Manure†

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ABSTRACT

Reducing *Escherichia coli* O157:H7 in cattle and their manure is critical for reducing the risk for human foodborne and waterborne illness. The objective of this study was to evaluate the effects of soil and pond ash surfaces for feedlot pens on the prevalence, levels, and/or persistence of naturally occurring *E. coli* O157:H7 and total *E. coli* in cattle (feces and hides) and manure. Cattle (128 beef heifers) were sorted among 16 pens: 8 surfaced with soil and 8 surfaced with pond ash. The prevalence of *E. coli* O157:H7 in feces decreased ($P < 0.0001$) during the study from 57.0% on day 0 to 3.9% on day 84 but did not differ ($P \geq 0.05$) between cattle on soil and on pond ash pens at any sampling period. The prevalence of the pathogen on hides and in feedlot surface material (FSM) also decreased ($P < 0.0001$), with no effect of soil or pond ash surface ($P \geq 0.05$). Similarly, levels of *E. coli* in FSM did not differ ($P \geq 0.05$) at any sampling period, and there were no clear trends for survival differences of *E. coli* O157:H7 or *E. coli* in FSM between pond ash and soil surfaces, although *E. coli* populations survived at 5.0 log CFU/g of FSM on the pen surfaces 6 weeks after the cattle were removed. These results indicate that housing cattle on pens surfaced with pond ash versus pens surfaced with soil does not affect *E. coli* O157:H7 in cattle or their manure.

Cattle are an important reservoir of *Escherichia coli* O157:H7, a pathogen that causes an estimated 73,000 cases of human illness annually *(34)*. In infected persons, *E. coli* O157:H7 commonly causes bloody diarrhea, and in susceptible populations this pathogen can cause hemolytic uremic syndrome and death *(41)*. Although human disease caused by this organism is commonly linked to consumption of undercooked ground beef and raw milk *(13, 16, 38)*, numerous recent *E. coli* O157:H7 infection outbreaks have been associated with produce and water *(12, 27, 35, 38)*. Feces and manure are the primary vehicles of food and water contamination with this pathogen. Feces in cattle production or lairage environments can contaminate cattle hides, which in turn can contaminate beef carcasses with *E. coli* O157:H7 at harvest *(3, 33)*. Manure containing this pathogen may contaminate food crops when applied as a soil amendment. Runoff from livestock production or manure-amended soils can contaminate water supplies used for drinking, recreation, or irrigation of food crops *(12, 27, 28, 35)*. Although the mammalian gastrointestinal tract is the primary habitat of *E. coli*, this bacterium can survive for long periods in manure, feedlot surface material (FSM), and soils *(5, 9, 11, 50)*. For example, *E. coli* O157:H7 survived in bovine feces for 56 days at 22°C and 49 days at 37°C despite the rapid reduction in moisture content and water activity of the feces *(50)*. *E. coli* was recoverable from pasture soils up to 162 days after natural deposition by cattle *(5)*. In previous work, we found that *E. coli* can persist at levels as high as 10^3 CFU/g of soil for 171 days after feedlot runoff discharge onto a bromegrass vegetative treatment area *(11)*. In this same study, 30% of soil samples were positive for *E. coli* O157:H7 at 171 days.

Manure content influences the effect of water on *E. coli* O157:H7 in feedlot soils, and with appropriate moisture and manure content the pathogen can both survive and multiply in FSM *(9)*. Dietary components of cattle feed can affect both the levels of *E. coli* O157:H7 shed in feces and the ability of *E. coli* O157:H7 to survive in the resulting manure, and longer survival in manure may be a contributing factor to the increased prevalence of this pathogen in cattle given certain feedstuffs *(47, 52)*. Thus, reducing the levels and survivability of *E. coli* O157:H7 in manure could both reduce contamination of food and water and limit additional contamination of animals in the production environment, thereby reducing the risk of human illness caused by this pathogen.

Effective control of this organism will require an understanding of factors that impact levels and persistence of *E. coli* O157:H7 in manure and the production environment. Pond ash is a low-cost by-product of coal combustion for electricity generation *(1)*. This material provides a hard, stable surface when packed into layers and

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has been examined as an alternative feedlot pen floor surface *(24, 53)*. One advantage of this stable pen surface is that accumulated manure is more easily removed from it than it is from soil-surfaced pens *(37, 53)*. In soil-surfaced pens, accumulated manure is mixed with soil by the action of the animals' hooves, especially when conditions are muddy, and further soil may be removed along with the manure when the pens are scraped and cleaned. Because there is less mixing with soil, manure from hard-surfaced pens has a lower ash content and therefore greater value for land application and energy production *(44, 53)*. Woodbury et al. *(53)* found that FSM from soil-surfaced pens contained approximately four times more ash than did FSM from pond ash–surfaced pens. FSM from the pond ash–surfaced pens had a higher percentage of total solids and volatile solids and a lower moisture content than that from soil-surfaced pens *(53)*. The pH was higher, and calcium and magnesium concentrations were greater in FSM from pond ash–surfaced than from soil-surfaced pens *(24)*. Differences in the concentrations of numerous trace metals in FSM and in runoff from pond ash and soil pen surfaces also have been noted *(48)*. These compositional differences in FSM between the two types of pen surfaces may impact the bacteria originating from bovine feces that are deposited and accumulated on the pen surface during the finishing period and in turn may affect hide contamination and pathogen carriage in cattle. The specific objectives of this study were to determine whether (i) populations and persistence of naturally occurring *E. coli*, including *E. coli* O157:H7, differ in bovine manure from feedlot pens with soil and pond ash surfaces and (ii) the prevalence of naturally occurring *E. coli* O157:H7 in feces and on hides differs for cattle housed in soil-surfaced or pond ash–surfaced feedlot pens.

MATERIALS AND METHODS

Feedlot pens and cattle. All animal procedures were reviewed and approved by the U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee. Details of the feedlot pens were reported by Brown-Brandl et al. *(15)*. The study was conducted at the 6,000-head capacity USMARC feedlot near Clay Center, NE from May to September 2006 in 16 pens (7.3 m wide by 20.7 m long), of which 8 were surfaced with soil and 8 were surfaced with pond ash as described previously *(24, 53)*. Pairs of pens sharing a water trough were surfaced with the same material, and these pen pairs were arranged such that alternating pairs in a row had soil or pond ash surfaces. Wooden barriers were installed along the bottoms of shared fences to prevent movement of FSM between the pens.

The study was part of a larger work that examined the effects of heat and handling stresses on fecal shedding of *E. coli* O157:H7 by fed beef cattle *(15)* and included 128 finishing heifers of four beef breeds: Angus, Charolais, MARC I (a five-breed composite of Charolais, Braunvieh, Limousin, Angus, and Hereford), and MARC III (a four-breed composite of Pinzgauer, Red Poll, Hereford, and Angus). The heifers were born in spring 2005 and weaned in fall 2005, when they were brought to the feedlot and housed in pens that were separate from the experimental pens. One week before the experiment, natural *E. coli* O157:H7 fecal shedding status (both presence and levels) and weight was determined for each animal (mean weight, 401.7 $\pm$ 35.4 kg) using the procedures described below. At this time, 54% of the animals were shedding *E. coli* O157:H7, and 24 of the 128 animals were shedding the pathogen at enumerable levels ($\geq$200 CFU/g of feces). Heifers were blocked by breed, weight, and preexperiment *E. coli* O157:H7 shedding status and assigned to one of the two pen surface treatment groups, soil or pond ash. Each pen had eight heifers (two of each breed), one or two animals that were shedding enumerable levels of *E. coli* O157:H7, and similar numbers of animals that were positive and negative for the pathogen as determined after fecal sample enrichment. Each heifer had 19.0 m^2 of pen space. The cattle were provided ad libitum access to a standard feedlot diet of corn and corn silage that was fed twice daily throughout the 84-day finishing period. Animals were weighed on a 28-day schedule.

***E. coli* O157:H7 in bovine feces and on hides.** Hide and rectal fecal samples were collected from each animal upon placement in the experimental pens on 15 May 2006 (day 0), on 12 June and 10 July 2006 (days 28 and 56) when animals were removed for weighing, and on 7 August 2006 (day 84) when the animals were harvested. Rectal fecal samples were obtained directly from each animal with a clean shoulder-length glove that was changed for each sample. Sterile sponges (Nasco, Ft. Atkinson, WI) premoistened with 20 ml of buffered peptone water (BPW; Difco, Becton Dickinson, Sparks, MD) were used to collect hide samples by swabbing an approximately 1,000-cm^2 area behind the left shoulder *(52)*. Samples were transported to the laboratory within 1 h for processing. Both the presence and levels of *E. coli* O157:H7 were determined in each hide and fecal sample.

For fecal samples, 10 g of feces was placed into a sterile filtered sample bag (Nasco) with 90 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) containing 100 mM potassium phosphate buffer *(8)*. The bag contents were mixed by hand massage, and a 1-ml aliquot was removed for *E. coli* O157:H7 enumeration by spiral plating. For hide samples, the sponge in the sample bag was hand massaged, a 250-μl aliquot was removed for *E. coli* O157:H7 enumeration, 80 ml of TSB was added, and the bag contents were mixed again by squeezing. Both fecal and hide-sponge samples were enriched by incubation for 2 h at 25°C and then 6 h at 42°C *(7)* and held overnight at 4°C.

The sample aliquots removed for *E. coli* O157:H7 enumeration were plated onto CHROMagar O157 (DRG International, Mountainside, NJ) containing 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (ntCHROM) with an Autoplate 4000 spiral plater (Spiral Biotech, Inc., Norwood, MA) *(10, 14)*. The ntCHROM plates were incubated for 22 to 24 h at 42°C *(14)* and examined for suspect colonies, which were tested with *E. coli* O157 latex agglutination reagents (Oxoid Limited, Basingstoke, UK). Presumptive *E. coli* O157:H7 colonies were counted, isolated, and confirmed by multiplex PCR assay for the *E. coli* O157:H7 genes *eaeA, slt-I, slt-II, fliC,* and *rfbE (26)*. The lower limits of detection were 200 CFU/g for fecal samples and 40 CFU/100 cm^2 for hide samples.

To determine the presence of *E. coli* O157:H7, 1-ml aliquots of the enriched fecal and hide samples were subjected to immunomagnetic separation using 20 μl of anti-*E. coli* O157 Dynabeads (Invitrogen Corp., Carlsbad, CA), and 50 μl of the concentrated bead suspension was plated onto plates of ntCHROM and sorbitol MacConkey agar (Difco, Becton Dickinson) containing 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (ctSMAC). Both ntCHROM and ctSMAC plates were incubated at 37°C for 22 to 24 h before examination. Presumptive *E. coli* O157:H7 colonies were tested for agglutination and confirmed by PCR assay as described above.

***E. coli* O157:H7 and *E. coli* in FSM.** Four FSM samples were collected from each pen before placing the animals in the pens on day 0, again on days 28 and 56 when animals were removed for weighing, and on day 84 when the animals were harvested. For collection of the four samples, the pens were divided into four regions of approximately equal size along the length of the pen. Using a new clean glove for each region, FSM (ca. 500 g) was collected by compositing multiple grab samples taken across the width of the pens (i) just off the feedbunk apron, (ii) in the region that included the water trough, (iii) near the midline of the pen, and (iv) at the foot of the pen. Samples were placed in separate sealable plastic bags for transport to the laboratory and immediate processing.

The collected FSM samples were mixed well before further subsampling. Ten grams of each sample was measured into separate sterile filtered sample bags (Nasco), 90 ml of TSB was added to each bag, and the bag contents were mixed well by hand. A 1-ml aliquot was removed from each bag and placed in a sterile tube for enumeration of *E. coli,* and the remaining sample mixtures were enriched by incubation at 37°C for 7 h and then held at 4°C overnight. The retained volumes of the initial 10^{-1} sample dilutions in TSB were diluted further as necessary in 2% BPW and spiral plated onto CHROMagar ECC (DRG International) for enumeration of *E. coli*. The CHROMagar ECC plates were incubated for 22 to 24 h at 37°C, and blue *E. coli* colonies were counted.

To determine the presence of *E. coli* O157:H7, 500 μl of the enriched FSM sample was added to 500 μl of phosphate-buffered saline with Tween (Sigma, St. Louis, MO) and 20 μl of anti-*E. coli* O157 Dynabeads. After 30 min of shaking incubation and concentration into a 100-μl volume, 50 μl of the eluted beads was plated onto ntCHROM and ctSMAC plates, incubated at 37°C for 22 to 24 h, and examined for suspect colonies. Presumptive *E. coli* O157:H7 colonies were tested for agglutination, isolated, and confirmed by PCR assay as described above.

Persistence of *E. coli* O157:H7 and *E. coli* in FSM. The survival of *E. coli* O157:H7 and *E. coli* in FSM from the soil and pond ash pen surfaces was examined in FSM collected on days 28 and 56. On each of these days, after the removal of the 10-g FSM samples for the initial analyses, 250-g subsamples of each of the four FSM samples from each pen were pooled into a clean sealable plastic bag and mixed well, and 800 g of these pooled subsamples was weighed into separate clean plastic pans (one pan per pen). Pans were loosely covered with lids to slow moisture loss and incubated at room temperature (18 to 22°C) for 4 weeks. The FSM pans were sampled weekly by first mixing the FSM with a new clean utensil and then measuring out 10 g for *E. coli* O157:H7 and *E. coli* analyses as described above for FSM. At the same times, additional samples were processed for determination of pH, moisture content, organic matter content, and ash content. Water content of FSM was determined by mass loss after drying overnight at 105°C. Organic matter and pH determinations were done as described by Berry and Miller *(9).*

Persistence of *E. coli* O157:H7 and *E. coli* in FSM was further examined by determining survival on the surfaces of the soil and pond ash pens after removal of the cattle on 7 August 2006. The pens were sampled weekly for 6 weeks by collection of four FSM samples per pen as described above. The presence and levels of *E. coli* O157:H7 and *E. coli* and the moisture content, organic matter content, ash content, and pH of the FSM were determined as described above.

Statistical analyses. The unit of observation was the individual animal. *E. coli* populations were converted to log CFU per gram of feces or FSM for statistical analyses. *E. coli* O157:H7 prevalence was determined as a proportion of samples positive for *E. coli* O157:H7 to the total samples for a given sample type (feces, hides, or FSM) for each sampling day and reported as a percentage. Analysis of variance, with surface type and time (week) as the main effects, and the Tukey-Kramer multiple comparisons test were performed on the converted bacterial population data and the FSM composition data using InStat (version 3.00, GraphPad Software, San Diego, CA). Differences in the prevalence of *E. coli* O157:H7 in FSM, in feces, and on hides and the numbers of fecal samples containing enumerable *E. coli* O157:H7 (samples with ≥200 CFU/g of feces or ≥40 CFU/100 cm^2 of hide) were assessed by using the two-tailed Fisher exact test *(45).* For all analyses, differences were considered significant when P values were less than 0.05.

RESULTS AND DISCUSSION

Pond ash is coal fly ash that has been flushed to evaporative storage ponds after combustion of the coal and then removed and dewatered for disposal *(1, 53).* Pond ash and other coal combustion products have been used to surface pens in cattle feedlots and have allowed better pen management and concentration of pen manure *(44, 53).* In addition to manure management benefits, the use of pond ash as a surface for cattle feedlot pens may offer other advantages when compared with soil surfaces. By providing a more solid base during times of high precipitation, pond ash may alleviate some of the problems associated with muddy cattle pens, including loss of traction, energy expended by wading through mud, and stress leading to immune system suppression, thereby improving animal performance and health *(30, 37, 39).* Furthermore, in feedlot pens that are less muddy, cattle may be cleaner and have lower prevalence of *E. coli* O157:H7. In a large study that included more than 3,000 cattle in five midwestern U.S. feed yards, Smith et al. *(42)* found that the pens with the highest percentages of animals shedding *E. coli* O157:H7 were muddy at the time of sample collection. These authors reasoned that muddy conditions, in comparison to normal pen conditions, may facilitate fecal-oral transmission of the pathogen. However, hard surfacing materials such as concrete can have adverse affects on cattle hooves and legs, and additional research regarding these potential disadvantages of pond ash are needed *(20, 49).* In the present study, animal health was evaluated daily, and no differences were noted in the health of cattle in pens with pond ash surfaces compared with those in pens with soil surfaces. No cattle were removed during the study.

On day 0, all 16 pens contained cattle that were positive for *E. coli* O157:H7 based on fecal and hide samples. Pen-level fecal prevalence was 37.5 to 87.5% (mean, 55.6%) for soil pens and 37.5 to 87.5% (mean, 59.4%) for pond ash pens. Pen-level hide prevalence was 75 to 100% (mean, 87.5%) for soil pens and 87.5 to 100% (mean, 95.2%) for pond ash pens.

The prevalence of *E. coli* O157:H7 in feces and on hides of cattle in the soil and pond ash pens is shown in Table 1. *E. coli* O157:H7 prevalence in feces was not different (P = 0.72) when animals were moved into the pens on day 0, with 55.6% (35 of 64) of the heifers on the soil pens and 59.4% (38 of 64) of the heifers on the pond ash pens shedding the pathogen in their feces. In addition, on day 0, similar numbers of animals on both types of pen surface were shedding *E. coli*

TABLE 1. E. coli *O157:H7 (prevalence and percentage of enumerable samples) in feces and on hides of cattle from feedlot pens surfaced with soil or pond ash*[a]

Measurement	Sampling day	Feces		Hide	
		Soil pens	Pond ash pens	Soil pens	Pond ash pens
E. coli O157:H7 prevalence (% positive)	0	55.6 AX	59.4 AX	87.5 AW	95.2 AX
	28	26.6 AY	39.1 AX	68.8 AX	82.8 AX
	56	9.4 AZ	6.3 AY	50.0 AY	39.1 AY
	84	1.6 AZ	6.3 AY	6.3 AZ	17.2 AZ
Enumerable *E. coli* O157:H7 (% of total samples)[b]	0	26.6 AX	18.8 AX	45.3 AX	54.7 AX
	28	6.3 AY	7.8 AXY	9.4 AY	4.7 AY
	56	4.7 AY	1.6 AY	3.1 AYZ	0 AY
	84	0 AY	3.1 AY	0 AZ	0 AY

[a] $n = 64$. Within sample type (feces or hide) and row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within measure (*E. coli* O157:H7 prevalence or enumerable *E. coli* O157:H7) and column, values followed by different letters (W, X, Y, and Z) are significantly different ($P \leq 0.05$).

[b] Percentage of samples with ≥200 CFU/g of feces or ≥40 CFU/100 cm^2 of hide.

O157:H7 at levels of 200 CFU/g of feces or greater (17 of 64 animals on soil pens and 12 of 64 animals on pond ash pens; $P = 0.40$; Tables 1 and 2). The overall percentage of *E. coli* O157:H7–positive fecal samples decreased ($P < 0.0001$) during the study from 57.0% on day 0 (15 May 2006) to 3.9% on day 84 (7 August 2006). During this same time period, the number of animals shedding enumerable levels of *E. coli* O157:H7 also decreased ($P < 0.0001$) and did not differ ($P \geq 0.05$) between pen surface type at any sampling period (Table 1). From a high of 17 of 64 heifers on soil pens shedding enumerable *E. coli* O157:H7 on day 0, 4 were doing so on day 28, 3 on day 56, and 0 on day 84. The number of heifers on pond ash pens shedding enumerable *E. coli* O157:H7 decreased from 12 on day 0 to 5 on day 28, 1 on day 56, and 2 on day 84. Additionally, for both pen surface types, the number of animals shedding higher *E. coli* O157:H7 levels decreased during the study period (Table 2). On day 0, of the 29 animals shedding enumerable levels of *E. coli* O157:H7 in their feces, 2 were shedding 10^6 CFU/g; on day 84, only 2 animals were shedding enumerable levels of approximately 200 CFU/g of feces. This decrease in *E. coli* O157:H7 fecal shedding was reflected by the concomitant decrease in hide contamination (Table 1). The overall prevalence of *E. coli* O157:H7 on hides was 90.6% on day 0 and decreased ($P < 0.0001$) during the study to 11.7% on day 84. However, *E. coli* O157:H7 prevalence in feces or on hides did not differ ($P \geq 0.05$) between cattle on soil and cattle on pond ash at any sampling period during the study.

The *E. coli* O157:H7 fecal prevalence on day 0 was high and likely a reflection of the typical seasonality of prevalence of this pathogen in cattle; prevalence predictably increases during the warmer months and is often highest in the late summer and early fall *(2, 4, 6, 46)*. Barkocy-Gallagher et al. *(6)* found that fecal prevalence of *E. coli* O157:H7 in beef cattle at harvest was 12.9% during the summer, 6.8% in the fall, 0.3% in the winter, and 3.9% in the spring. Similarly, fecal prevalence of *E. coli* O157:H7 in yearling beef cattle and cull cows in Alberta, Canada was highest in the summer at 19.7%, 4.7% in the fall, 0.7% in the winter, and 4.9% in the spring *(46)*. Our initial overall prevalence of 55.6% is in agreement with *E. coli* O157:H7 prevalence in cattle in this feedlot that has been reported previously *(4, 52)*. In a study conducted from September 2004 to May 2005, Arthur et al. *(4)* found *E. coli* O157:H7 fecal prevalence of greater than 60% in 5 of 10 feedlot pens for cattle sampled during the fall and ca. 90% and greater prevalence in 1 of these same pens for cattle sampled in April and May. Average pen prevalence of *E. coli* O157:H7

TABLE 2. *Distribution of* E. coli *O157:H7 enumerable counts in feces of cattle from feedlot pens surfaced with soil or pond ash*

Pen surface	*E. coli* O157:H7 level (CFU/g)	No. of animals shedding *E. coli* O157:H7 at the indicated concn			
		Day 0	Day 28	Day 56	Day 84
Soil	<200	47	60	61	64
	200–999	2	1	1	
	1,000–9,999	8	2	2	
	10,000–99,999	5	1		
	100,000–999,999	2			
Pond ash	<200	52	59	63	62
	200–999	2	3	1	2
	1,000–9,999	5	2		
	10,000–99,999	3			
	100,000–999,999	0			
	1,000,000–9,999,999	2			

TABLE 3. E. coli *O157:H7 prevalence and levels of* E. coli *in feedlot surface material (FSM) from feedlot pens surfaced with soil or pond ash*[a]

Sampling day	*E. coli* O157:H7 (% positive samples)		*E. coli* (log CFU/g of FSM)	
	Soil pens	Pond ash pens	Soil pens	Pond ash pens
0[b]	3.1 AX	0 AX	1.43 AX	1.83 AX
28	78.1 AY	90.6 AY	5.76 AY	6.06 AY
56	28.1 AZ	9.4 AX	6.59 AZ	6.44 AY
84	0 AX	6.3 AX	6.40 AYZ	6.76 AY

[a] $n = 32$. Within bacterial category (*E. coli* O157:H7 or *E. coli*) and row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within each column, values followed by different letters (X, Y, and Z) are significantly different ($P \leq 0.05$).

[b] FSM samples collected on day 0 were taken before the cattle were moved into the pens.

in feces was highest (>40%) in late June for a study conducted from October 2007 to June 2008 *(52)*.

Thus, the decrease in prevalence of *E. coli* O157:H7 during the summer months that we observed in the current study is atypical. We previously hypothesized that this decrease in prevalence is due to the small number of cattle in the pens *(15)*. Several studies have revealed an association between the presence of animals shedding high levels of *E. coli* O157:H7 (10^3 to 10^4 CFU/g of feces) and increased transmission of the pathogen, resulting in higher prevalence of fecal shedding and/or hide contamination in the pen or herd *(4, 17–19, 32, 43)*. Results of these studies suggest that even very few cattle shedding high levels of the pathogen can be responsible for a large proportion of the total *E. coli* O157:H7 contamination of the pen and other cattle and that targeting preharvest interventions to reduce the levels of shedding by these animals may be an effective approach for reducing this pathogen in cattle and beef. The levels of *E. coli* O157:H7 shed by cattle in feces typically are below the detection level for most enumeration procedures *(14, 36, 43)*. High-level shedders (super shedders) of *E. coli* O157:H7 have been defined as those animals that excrete $>10^4$ CFU/g of feces, but host and other environmental factors that result in the generation of a super shedder are not well understood *(17)*. With only eight heifers in each of the pens, the probability of having and/or sustaining adequate numbers of animals shedding *E. coli* O157:H7 at a high level to maintain the carriage or contamination rate among the animals in the pen may have been low. In this regard, the results of our current work provide evidence of the important role of super shedders in the preharvest contamination of cattle, by demonstrating the reduction of *E. coli* O157:H7 prevalence in and on cattle and in their environment when high-level shedders are not present in the pen (Table 2).

Alternatively, *E. coli* O157:H7 prevalence in cattle has been reported to decrease with increasing animal age and/or the longer that the cattle are on feed. Some researchers have reported a higher prevalence of *E. coli* O157:H7 early in the feeding period for feedlot cattle, possibly as a response to the stresses of a new environment, dietary changes, commingling, or weaning, with subsequent decreases in prevalence over time *(21, 23, 25, 31)*. Fecal shedding of this pathogen by beef calves can increase after weaning *(23)*. However, other researchers have not found associations between *E. coli* O157:H7 fecal prevalence and days on feed *(22, 40, 42)* or have observed increases in prevalence during the feeding period or with increasing age *(29)*. Dewell et al. *(22)* examined feces of beef cattle in 15 pens at 12 feedlots at the end of the finishing period and within 36 h of shipping to slaughter and found *E. coli* O157:H7 pen-level prevalence ranging from 0.0 to 76.7%, indicating that factors other than days on feed can influence the occurrence of this pathogen in cattle. The heifers used in the present study were 12 to 14 months old and had been weaned and adapted to the feedlot and diet for several months before the beginning of the study. Thus, neither animal age nor days on feed are likely reasons for either the initially high *E. coli* O157:H7 prevalence or the subsequent reduction in prevalence that occurred during the study. In addition, the pens in this study were centrally located in a large 6,000-head feedlot. Thus, the observed decrease in *E. coli* O157:H7 prevalence suggests that vectors such as flies, vermin, and dust may be of minor consequence in sustaining long-term *E. coli* O157:H7 infections in cattle. Further study is needed to confirm the mechanism(s) of the decrease in *E. coli* O157:H7 prevalence in our study and to provide additional information that can be used to reduce this pathogen in cattle.

Because manure is an important source of the *E. coli* O157:H7 that can contaminate food, water, and food animals in the production environment, one goal of our work was to determine whether the levels or the overall survival of this pathogen differed in the FSM accumulated on the two different pen surface types during the cattle production cycle. FSM properties such as manure content, water content, pH, and water activity may combine to either reduce or promote survival and growth of *E. coli* O157:H7, thereby affecting the risk of pathogen contamination associated with this material *(9, 47, 50)*. Combinations of soil, manure, and water that resulted in aerobic conditions either allowed the growth or improved the survival of *E. coli* O157:H7 in FSM *(9)*. Levels of both indigenous *E. coli* and inoculated *E. coli* O157:H7 were reduced more rapidly in feces and manures from cattle fed diets higher in starch, where fermentation resulted in the accumulation of high concentrations of L-lactate and low pH *(47, 51)*. Ammonia concentrations also may impact the survival of *E. coli* O157:H7 in manures *(47)*. Reported differences in FSM from soil and pond ash feedlot pens include differences in moisture content, mineral content, and pH *(24, 44, 53)*. To determine whether *E. coli,* including *E. coli* O157:H7, prevalence and persistence differed in FSM from soil and pond ash feedlot pens, we examined (i) the prevalence of *E. coli* O157:H7 and populations of *E. coli* in FSM during the 84-day finishing period and (ii) the persistence of both *E. coli* O157:H7 and *E. coli* in FSM collected from the pen surfaces both during the finishing period and after the cattle were removed from the pens.

Samples from the pen surfaces were collected to determine the presence of *E. coli* O157:H7 on day 0 before cattle were placed in the pens. Of 32 samples collected from

TABLE 4. *Persistence of* E. coli *O157:H7 and* E. coli *in feedlot surface material (FSM) collected from soil or pond ash feedlot pens and stored for 4 weeks*[a]

Sampling day	Sample storage time (wk)	*E. coli* O157:H7 (% positive samples)		*E. coli* (log CFU/g of FSM)	
		Soil pens	Pond ash pens	Soil pens	Pond ash pens
28	0	87.5 AX	100 AX	5.98 AX	6.47 AX
	1	50.0 AX	87.5 AX	6.19 AX	5.74 AX
	2	37.5 AX	62.5 AX	5.89 AX	6.08 AX
	3	37.5 AX	87.5 AX	6.07 AX	6.48 AX
	4	25.0 AY	62.5 AX	3.96 AY	5.38 BX
56	0	25.0 AX	0 AX	6.40 AXYZ	6.62 AX
	1	25.0 AX	12.5 AX	7.25 AX	6.90 AX
	2	25.0 AX	0 AX	6.82 AXY	5.89 AXY
	3	25.0 AX	0 AX	5.79 AYZ	4.92 AYZ
	4	12.5 AX	0 AX	5.38 AZ	4.19 BZ

[a] $n = 8$. Within sampling day (day 28 or day 56), bacterial category (*E. coli* O157:H7 or *E. coli*), and row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within sampling day and column, values followed by different letters (X, Y, and Z) are significantly different ($P \leq 0.05$).

each pen type, only one sample from a soil-surfaced pen was positive for the pathogen (Table 3). After the cattle were on the pens for 28 days, prevalence of *E. coli* O157:H7 in FSM was 78.1% on soil pens and 90.6% on pond ash pens, but this difference was not significant ($P = 0.302$). The prevalence of the pathogen in FSM decreased during the finishing period, reflective of the decrease in *E. coli* O157:H7 fecal shedding by the cattle (Table 3). Within sampling periods, there was no effect of treatment (soil versus pond ash; $P \geq 0.05$) on *E. coli* O157:H7 prevalence in the FSM, and there was no effect of treatment ($P \geq 0.05$) on *E. coli* levels in the FSM. This result is consistent with previous observations that *E. coli* levels did not differ in feedlot soils or in runoff from pond ash and soil pen surfaces *(24)*.

Two studies were conducted to determine whether there were differences in the survival of *E. coli* O157:H7 and *E. coli* in FSM from pond ash and soil feedlot pens. For the in vitro study, on days 28 and 56 FSM samples were collected and pooled by pen, and prevalence of the pathogen and levels of *E. coli* were determined at weekly intervals for 4 weeks (Table 4). Within sampling period and week, there was no difference ($P \geq 0.05$) in *E. coli* O157:H7 prevalence in soil and pond ash FSM. For *E. coli* levels, treatment differences were seen after 4 weeks of incubation for day 28 and day 56 FSM. For day 28 FSM, *E. coli* levels were higher ($P < 0.05$) in FSM from pond ash pens than in FSM from soil pens. However, for day 56 FSM, *E. coli* levels were higher ($P < 0.05$) in FSM from soil pens than in FSM from pond ash pens, indicating that these differences were likely arbitrary. Although general trends indicated a reduction in *E. coli* O157:H7 prevalence and *E. coli* levels over time, the reductions seen after 4 weeks were not always significant, and there were no clear trends for increased or reduced survival in FSM from either pond ash or soil pens (Table 4). There was no effect of pen surface type (soil versus pond ash; $P \geq 0.05$) on pH, water content, organic matter content, or ash content of FSM (Table 5).

TABLE 5. *pH, water content, organic matter content (OM), and ash content of feedlot surface material (FSM) collected from soil or pond ash feedlot pens and stored for 4 weeks*[a]

Sampling day	Sample storage time (wk)	pH		Water (% wet wt)		OM (% dry matter wt)		Ash (% dry matter wt)	
		Soil pens	Pond ash pens	Soil pens	Pond ash pens	Soil pens	Pond ash pens	Soil pens	Pond ash pens
28	0	7.81 AX	7.96 AX	20.05 AX	23.48 AX	50.90 AX	47.63 AX	49.10 AX	52.37 AX
	1	8.15 AY	7.88 AX	20.34 AX	22.54 AX	49.57 AX	44.41 AX	50.43 AX	55.59 AX
	2	8.10 AXY	8.00 AX	19.68 AX	22.57 AX	49.46 AX	43.10 AX	50.54 AX	56.9 AX
	3	8.23 AY	8.19 AX	20.23 AX	23.96 AX	45.93 AX	40.27 AX	54.07 AX	59.73 AX
	4	8.16 AY	8.08 AX	21.74 AX	25.67 AX	45.44 AX	37.33 AX	54.56 AX	62.68 AX
56	0	7.53 AX	7.61 AX	38.79 AX	33.53 AXY	51.70 AX	49.50 AX	48.30 AX	50.50 AX
	1	7.66 AX	7.64 AX	41.79 AX	37.92 AX	49.92 AX	43.99 AXY	50.07 AX	56.01 AXY
	2	8.30 AY	8.34 AY	40.08 AX	32.66 AXY	44.31 AX	42.82 AXY	55.69 AX	57.18 AXY
	3	8.24 AY	8.32 AY	29.97 AXY	26.89 AXY	43.97 AX	41.09 AXY	56.03 AX	58.91 AXY
	4	8.15 AY	8.29 AY	23.78 AY	22.50 AY	43.46 AX	39.17 AY	56.54 AX	60.83 AY

[a] $n = 8$. Within sampling day (day 28 or day 56) and row, pH, water content, OM content, or ash content values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within sampling day and column, values followed by different letters (X, Y, and Z) are significantly different ($P \leq 0.05$).

TABLE 6. *Persistence of* E. coli *O157:H7 and* E. coli *in feedlot surface material (FSM) on soil or pond ash feedlot pens after removal of cattle from the pens*[a]

Time following removal of cattle (wk)	*E. coli* O157:H7 (% positive samples)		*E. coli* (log CFU/g of FSM)	
	Soil pens	Pond ash pens	Soil pens	Pond ash pens
0	0 AX	6.3 AX	6.40 AXY	6.70 AX
1	3.1 AX	0 AX	6.25 AXY	6.44 AXY
2	3.1 AX	3.1 AX	6.70 AX	6.68 AX
3	3.1 AX	0 AX	6.39 AXY	5.99 AY
4	0 AX	3.1 AX	6.04 AY	5.88 AY
6	0 AX	0 AX	4.98 AZ	4.98 AZ

[a] $n = 32$. Within bacterial category (*E. coli* O157:H7 or *E. coli*) and row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within each column, values followed by different letters (X, Y, and Z) are significantly different ($P \leq 0.05$).

For determination of *E. coli* persistence on pen surfaces, FSM samples were collected from the pens weekly for 6 weeks after the cattle were harvested on day 84. The prevalence of *E. coli* O157:H7 fecal shedding by the cattle by day 84 was low, and the pathogen was isolated from the FSM only sporadically (Table 6), with no effect of pen surface type ($P \geq 0.05$). There was no effect of pen surface type ($P \geq 0.05$) on *E. coli* levels on any week. Initial mean levels of *E. coli* in the FSM were 6.55 log CFU/g, and after 6 weeks levels had dropped by only ca. 1.5 log units to a mean of 4.98 log CFU/g of FSM. Similar to the results of the in vitro FSM study, *E. coli* persisted at these high levels in FSM in spite of exposure to sun, rain, and drying conditions on the feedlot pen surface (Table 7). Also as for the *in vitro* study, there were no effects of pen surface type (soil versus pond ash; $P \geq 0.05$) on pH, water content, organic matter content, or ash content of FSM.

Previous work revealed differences in properties such as pH, water content, or ash content in FSM from feedlot pens surfaced with soil or pond ash; however, such differences were not found in the present study (Tables 4 and 6). The discrepancies between the results from these studies may be attributed to differences in FSM sampling procedures. Gilley et al. *(24)* collected both unconsolidated and consolidated FSM from soil and pond ash feedlot pens, although the surface condition (unconsolidated or consolidated) did not affect the FSM properties measured. Woodbury et al. *(53)* scraped and piled the FSM from each pen before sampling. In contrast, we sampled FSM from the immediate surface of the feedlot pen, collecting primarily unconsolidated material. FSM samples collected at a greater depth and including material from the soil-manure or pond ash–manure interface may have revealed differences in FSM properties between the two pen surface types. In particular, sampling at the soil-manure interface might affect ash content of FSM *(53)*. We sampled FSM from the near surface because this material was in immediate contact with the cattle and because our primary interest was determining the persistence of *E. coli* O157:H7 in the FSM and the potential impact of this persistence on the prevalence of this pathogen in cattle.

In conclusion, further work is needed to determine the mechanism for the reduction of *E. coli* O157:H7 prevalence that we observed, which is in contrast to the increase in prevalence that is typically found in cattle during the warmer summer months. Such information may be useful for development of strategies to reduce this pathogen. We found no differences in either the prevalence or levels of *E. coli* O157:H7 in feces, on hides, or in FSM in the pens of cattle housed on soil or pond ash feedlot pens. As previously reported, both *E. coli* O157:H7 and *E. coli* can persist for several weeks in FSM; however, there were no differences in survival in FSM from the two types of pen surface. Results indicate that the use of pond ash as an alternative to soil for a feedlot pen surfacing material does not affect the prevalence of *E. coli* O157:H7 in cattle and the production environment.

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TABLE 7. *pH, water content, organic matter content (OM), and ash content of feedlot surface material (FSM) on soil or pond ash feedlot pens after removal of cattle from the pens*[a]

Time following removal of cattle (wk)	pH		H_2O (% of wet wt)		OM (% of dry matter wt)		Ash (% of dry matter wt)	
	Soil pens	Pond ash pens	Soil pens	Pond ash pens	Soil pens	Pond ash pens	Soil pens	Pond ash pens
0	7.88 AX	7.96 AX	27.75 AX	24.64 AX	46.39 AX	49.20 AX	53.61 AX	50.80 AX
1	7.69 AY	7.76 AY	38.54 AY	39.72 AY	43.46 AXY	44.27 AXY	56.54 AXY	55.73 AXY
2	8.11 AZ	8.11 AX	27.77 AX	27.25 AX	42.29 AXY	42.72 AXY	57.71 AXY	57.28 AXY
3	7.91 AX	7.96 AX	12.96 AYZ	13.27 AZ	42.76 AXY	40.32 AY	57.24 AXY	59.68 AY
4	7.87 AX	7.92 AXY	11.46 AZ	10.97 AZ	40.35 AXY	39.76 AY	59.65 AXY	60.24 AY
6	ND[b]	ND	18.49 AY	13.00 AZ	37.61 AY	39.07 AY	62.39 AY	60.93 AY

[a] $n = 32$. Within each row, pH, water content, OM content, or ash content values followed by different letters (A and B) are significantly different ($P \leq 0.05$). Within each column, values followed by different letters (X, Y, and Z) are significantly different ($P \leq 0.05$).
[b] ND, not determined.

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