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Inactivation of Enterohemorrhagic *Escherichia coli* in Rumen Content- or Feces-Contaminated Drinking Water for Cattle

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Cattle drinking water is a source of on-farm *Escherichia coli* O157:H7 transmission. The antimicrobial activities of disinfectants to control *E. coli* O157:H7 in on-farm drinking water are frequently neutralized by the presence of rumen content and manure that generally contaminate the drinking water. Different chemical treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, butyric acid, sodium benzoate, and competing *E. coli*, were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm), ozone (22 to 24 ppm at 5°C), and competing *E. coli* treatment of water had minimal effects (<1 log CFU/ml reduction) on killing *E. coli* O157:H7 in the presence of rumen content at water-to-rumen content ratios of 50:1 (vol/wt) and lower. Four chemical-treatment combinations, including (i) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (treatment A); (ii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (treatment B); (iii) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (treatment C); and (iv) 0.1% lactic acid, 0.9% acidic calcium sulfate, and 100 ppm chlorine dioxide (treatment D); were highly effective (>3 log CFU/ml reduction) at 21°C in killing *E. coli* O157:H7, O26:H11, and O111:NM in water heavily contaminated with rumen content (10:1 water/rumen content ratio [vol/wt]) or feces (20:1 water/feces ratio [vol/wt]). Among them, treatments A, B, and C killed >5 log CFU *E. coli* O157:H7, O26:H11, and O111:NM/ml within 30 min in water containing rumen content or feces, whereas treatment D inactivated approximately 3 to 4 log CFU/ml under the same conditions. Cattle given water containing treatment A or C or untreated water (control) ad libitum for two 7-day periods drank 15.2, 13.8, and 30.3 liters/day, respectively, and cattle given water containing 0.1% lactic acid plus 0.9% acidic calcium sulfate (pH 2.1) drank 18.6 liters/day. The amounts of water consumed for all water treatments were significantly different from that for the control, but there were no significant differences among the water treatments. Such treatments may best be applied periodically to drinking water troughs and then flushed, rather than being added continuously, to avoid reduced water consumption by cattle.

Escherichia coli O157:H7 has emerged in the last 10 years as an important food-borne pathogen (12, 15, 22, 30, 32, 36), with an estimated 73,000 cases of *E. coli* O157 infection annually in the United States (23). Cattle are a major reservoir of *E. coli* O157:H7 (2, 4, 5, 7), and cattle water troughs are important sources of the pathogen on farms (2, 4, 5, 7, 17, 20, 21, 27, 28, 37). Studies have further revealed that when present in cattle drinking water, the pathogen was disseminated to other cattle consuming the contaminated water (19, 29).

Genomic subtyping by pulsed-field gel electrophoresis of *E. coli* O157:H7 isolates from farms revealed that a single O157:H7 strain was dominant among isolates from cohort and noncohort cattle, water, and other positive samples (i.e., from feed, flies, a pigeon, etc.) on a farm (29). This information demonstrates that drinking water is an important vehicle for disseminating *E. coli* O157:H7 on the farm and that methods for treatment of drinking water on farms are needed for reduction of the pathogen.

Studies indicate that *E. coli* O157:H7 can survive in cattle drinking water for a long time (up to 12 months) (13, 19, 29). A variety of treatments have been evaluated for efficacy in killing *E. coli* O157:H7 in drinking water contaminated with cattle feces (9, 10, 18, 24). The results revealed that most had minimal effects on killing the pathogen, in part because these treatments were neutralized by organic materials present in feces. The objective of this study was to identify practical treatments to eliminate or control *E. coli* O157:H7 in drinking water by simulating on-farm conditions.

MATERIALS AND METHODS

Bacterial strains. Five isolates of *E. coli* O157:H7, i.e., 932 (a human isolate), E009 (a beef isolate), E0018 (a cattle isolate), E0122 (a cattle isolate), and E0139 (a deer jerky isolate); five isolates of *E. coli* O26:H11, i.e., strains DEC10E (a cattle isolate), DEC9E (a cattle isolate), DEC10B (a cattle isolate), 3079-97 (a human isolate), and 3183-96 (a human isolate); and five strains of *E. coli* O111:NM, i.e., strains 3208-95 (a human isolate), 0944-95 (a cattle isolate), 3287-97 (a human isolate), 4543-95 (a cattle isolate), and 0073-92 (a cattle isolate), were used as five-strain mixtures specific to each serotype. To facilitate the enumeration of these bacterial isolates, all strains were selected for resistance to nalidixic acid (50 μg/ml) according to procedures described previously (37). Each strain was grown individually in 10 ml of tryptic soy broth (TSB) (Becton Dickinson Microbiology Systems, Sparks, MD) containing 50 μg of nalidixic acid (NA) (Sigma Chemical Co., St. Louis, MO) per ml (TSB-NA) for 16 to 18 h at 37°C with agitation (150 rpm). The bacterial cells were sedimented three times by centrifugation (4,000 × *g*; 20 min); washed in 0.1 M phosphate-buffered saline (PBS), pH 7.2; and resuspended in PBS. The cell suspensions were adjusted with PBS to an optical density at 630 nm of 0.5 (approximately 10^8 CFU/ml). Five strains of the same serotype were combined at approximately equal cell numbers, which, for each individual strain and the five-strain mixture, were enumerated on

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tryptic soy agar (TSA) and sorbitol MacConkey agar (SMA) or MacConkey agar plates (all obtained from Becton Dickinson Microbiology Systems).

Rumen contents and feces. Rumen contents or feces from three different cattle were combined and used as a mixture. Rumen contents were collected from beef cattle at slaughter, and feces were collected from cattle on a beef farm, held at 4°C, and used within 7 days. Different samples obtained from the same slaughterhouse or farm were used for different trials.

Treatment with competing bacteria. A five-strain mixture of *E. coli* O157:H7 at 10^5 CFU/ml and a mixture of three strains of competing bacteria (*E. coli* 271, 786, and 797) (37) antagonistic to *E. coli* O157:H7 at 10^7 CFU/ml were added to different flasks containing a mixture of water and rumen content at ratios of 100:1, 50:1, 25:1, 10:1, and 5:1 and held at 21°C.

Chlorine and chlorine dioxide treatments. Standard chlorine solutions obtained from HACH Company (Loveland, CO) were freshly diluted for each experiment in deionized water to the required concentration according to a method described previously (39). The free-chlorine concentrations in the diluted chlorine solutions were determined with a Digital Titrator (HACH Co.). The *E. coli* O157:H7 suspension (1 ml) at 10^8 to 10^9 CFU/ml was added to 199 ml of water containing rumen content at ratios of 100:1, 50:1, 25:1, and 10:1 (vol/wt) and 5 ppm chlorine solution (4 ppm chlorine is the maximum residual disinfectant level allowed in drinking water by the Environmental Protection Agency) at 21°C and stirred with a magnetic stir bar in a 500-ml Erlenmeyer flask. Studies with chlorine dioxide were conducted using similar procedures.

Ozone treatments. Ozone was produced by a laboratory scale ozone generator (model H-50; Hess Machine International, Ephrata, PA) equipped with an oxygen concentrator (model AS-12; AirSep, Buffalo, NY), and ozone concentrations (ppm) were measured by the indigo colorimeter method. Ozonated (22 to 24 ppm at 5°C) water was mixed within 5 min with rumen content at ratios of 100:1, 50:1, 25:1, 10:1, and 5:1. Milli-Q water (Milli-Q Synthesis A10; Millipore Corp., Billerica, MA) was used as the control. One milliliter of a mixture of five strains of *E. coli* O157:H7 (10^8 CFU/ml) was mixed with 199 ml of the ozonated water with rumen content at 5°C and sampled at 0 to 20 min.

Chemical treatments. Chemicals, including lactic acid (0.05 to 0.5%; Fisher Scientific, Fair Lawn, NJ), hydrogen peroxide (0.5%; Sigma Chemicals Inc., St. Louis, MO), sodium benzoate (0.1%; Fisher Scientific), acidic calcium sulfate (0.9 to 4.5%; Mionix Inc., Naperville, IL), caprylic acid (0.05 to 1.5%; Aldrich Chemicals Inc., Milwaukee, WI), butyric acid (0.5 to 4%; Aldrich Chemicals Inc.), propionic acid (0.5 to 4%; Sigma Chemicals Inc.), and chlorine dioxide (10 to 1,000 ppm; Aldrich Chemicals Inc.), were evaluated separately or as a combination. The concentrations used for each chemical evaluated were based on the results of previous studies conducted with the chemicals and *E. coli* O157:H7 in deionized water. The chemicals were diluted to appropriate concentrations in Milli-Q water (Milli-Q Synthesis A10; Millipore Corp.) initially tested with the pure cultures of *E. coli* O157:H7. The effective chemical or combination of different chemicals was further tested for killing effects at 21°C on *E. coli* O157:H7 in tap water containing rumen content at the different ratios described above.

Enumeration of nalidixic acid-resistant *E. coli*. At predetermined sampling times, 1.0 ml of the treated bacterial suspension was removed and mixed with 9.0 ml of neutralizing buffer (Becton Dickinson Microbiology Systems). Bacteria were serially (1:10) diluted in 0.1% peptone water, and 0.1 ml of each dilution was surface plated onto TSA containing 50 μg NA/ml (TSA-NA) and SMA containing 50 μg NA/ml (SMA-NA) in duplicate and incubated at 37°C for 24 h. Colonies typical of *E. coli* O157:H7 (sorbitol negative) were randomly picked from plates with the highest dilutions for confirmation of *E. coli* by biochemical tests (API 20E miniaturized diagnostic test; bioMérieux Vitek, Hazelwood, MO) and for confirmation of serogroup O157 by latex agglutination assay (Oxoid, Ogdensburg, N.Y.). Colonies of *E. coli* O26:H11 and O111:NM were confirmed as *E. coli* only by biochemical tests. For studies with butyric acid, caprylic acid, sodium benzoate, and acidic calcium sulfate, when *E. coli* O157:H7, O26:H11, and O111:NM were not detected by direct plating, a selective enrichment in TSB-NA was performed by incubating 25 ml of treatment suspension in 225 ml of TSB-NA for 24 h at 37°C and then plating 0.1 ml of enrichment culture in duplicate on TSA-NA and SMA-NA plates. Combinations of chemicals effective in killing *E. coli* O157:H7 were further evaluated in water containing a mixture of feces collected from three beef cattle at a ratio of 20:1 (vol/wt) according to the methods described above for treatment in water containing rumen content. All effective chemical combinations were further evaluated for their killing effects on *E. coli* O26:H11 and O111:NM using the same protocol described for studies of *E. coli* O157:H7, except for serological confirmation. Studies with all chemical and competing *E. coli* treatments were done in duplicate or triplicate; two replicates were plated per sample, and the results are reported as mean plus standard deviation.

Cattle selection and training for palatability evaluations. Twenty-one pregnant dairy heifers were selected to determine the palatability of drinking water treated with different chemical combinations. The heifers generally exceeded 454 kg body weight (BW). Prior to the study, the heifers were trained to use electronic Calan doors, which allowed each animal access to a specific water treatment in an individual water trough. Access to all other water sources was restricted, and all water for the heifers was provided through the Calan doors. The heifers were group fed in an area adjacent to the Calan doors. The heifers had access to individual free stalls and to an outside exercise paddock. Following adaptation to water consumption through the Calan doors, the cows entered a 3-week experimental period.

Palatability assay of different chemical combinations. The cows were assigned to one of four groups. Three of the groups contained five cows each; the fourth group contained six cows. The groups were randomly assigned to one of the four experimental treatments, to which they were exposed for 1 week. Following the first experimental week, all of the cows were given fresh water for a week to ensure that they were fully hydrated at the beginning of the next experimental week. This was necessary, because water intake was negatively affected during the first experimental week. For the start of the second experimental week, the groups were reassigned to treatments different from those during the first experimental week. The cattle were offered water ad libitum, and daily consumption was determined for each heifer. The amount of unconsumed water was determined each morning, the troughs were emptied, and fresh water and treatments were added. The heifers were weighed at the end of the trial.

Data analysis. The least-squares method of enterohemorrhagic *E. coli* counts (log unit CFU/ml) in samples of phosphate buffer-treated and chemical-treated solution was analyzed using the general linear model of the Statistical Analysis System (SAS Institute, Cary, NC). The value used for statistical analysis when treatments with chemicals yielded undetectable enterohemorrhagic *E. coli* by the direct-plating method was 1.6 log CFU/ml. Data from cattle studies were analyzed using the General Linear Models procedure of SAS. Included in the statistical model were cow group, treatment, and period. In addition, data collected during the control week between experimental weeks were used as a covariant to adjust for individual differences in intake while the cows were receiving the control treatment. Only data collected after the cows had stabilized their intake following the first experimental week were used for covariant analysis. Analyses of total water intake and water intake per metabolic body weight (MBW) were conducted. Paired *t* tests were used for mean separation, and comparisons of means were only within significant F tests.

RESULTS

Treatment of water containing rumen content with competing *E. coli* decreased *E. coli* O157:H7 cell numbers by 0.2 to 0.7 log CFU/ml by day 16 at 21°C (data not shown), whereas *E. coli* O157:H7 counts increased by 0.6 to 1.0 log CFU/ml in the control (no competing *E. coli*).

Chlorine at 5 ppm in water with no rumen content killed 10^6 to 10^7 CFU/ml of *E. coli* O157:H7 to undetectable levels (<1.7 log CFU/ml) in less than 1 min. However, the addition of rumen content to water at 100 parts water to 1 part rumen content (vol/wt) or more reduced the killing effect of added chlorine to ca. 1.5 log CFU/ml within 20 min. Little to no *E. coli* O157:H7 inactivation occurred in 50 parts water to 1 part rumen content (data not shown). Similar results were obtained with 22 to 24 ppm ozone at 5°C within 20 min (data not shown). Studies with ozone were conducted at 5°C instead of 21°C, because ozone rapidly dissipated from water when held at 21°C compared to 5°C. Ozonated water alone killed 10^6 CFU of *E. coli* O157:H7/ml to undetectable levels (<1.7 log CFU/ml) within 1 min. However, the addition of 100 parts ozonated water to 1 part rumen content (vol/wt) provided only a 0.5-log CFU/ml reduction, and at 50 parts ozonated water or less to 1 part rumen content (vol/wt), it was ineffective in killing the pathogen.

Lactic acid (0.05 to 0.5%), hydrogen peroxide (0.5%), sodium benzoate (0.1%), acidic calcium sulfate (0.9%), butyric

TABLE 1. *E. coli* O157:H7 counts in water containing rumen content (10:1 [vol/wt]) or feces (20:1 [vol/wt]) and treated with different chemical combinations at 21°C

Treatment	*E. coli* O157:H7 count (log CFU/ml) at min[a]:					
	0[b]	2	5	10	20	30
Rumen content contamination						
E. coli O157:H7 only (pH 8.2)	6.2 ± 0.7	6.1 ± 0.2	6.1 ± 0.1	6.0 ± 0.1	5.9 ± 0.3	5.9 ± 0.1
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 1.9)	5.7 ± 0.4	5.3 ± 0.1	4.4 ± 0.1	3.9 ± 0.2	2.8 ± 0.2	2.5 ± 0.1*
0.5% butyric acid (pH 4.0)	5.9 ± 0.1	5.9 ± 0.3	5.9 ± 0.2	5.8 ± 0.4	5.7 ± 0.4	5.8 ± 0.6**
1.5% butyric acid (pH 3.9)	6.0 ± 0.3	6.1 ± 0.2	6.0 ± 0.2	5.9 ± 0.1	5.8 ± 0.4	5.3 ± 0.3**
2% butyric acid (pH 3.8)	5.9 ± 0.5	4.6 ± 0.6	2.7 ± 0.3	2.5 ± 0.2	+	+*
4% butyric acid (pH 3.5)	3.3 ± 0.6	+	+	−	−	−*
0.05% caprylic acid (pH 7.8)	6.0 ± 0.3	6.0 ± 0	6.0 ± 0	5.9 ± 0.5	6.0 ± 0.1	5.9 ± 0.3**
0.1% caprylic acid (pH 5.1)	5.8 ± 0.3	5.4 ± 0.6	4.3 ± 0.2	+	+	+*
0.5% caprylic acid (pH 4.6)	2.2 ± 0.1	+	+	−	−	−*
0.1% sodium benzoate (pH 8.2)	5.9 ± 0.5	6.0 ± 0.1	6.0 ± 0.1	5.9 ± 0.4	6.0 ± 0.3	5.9 ± 0.1**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (pH 2.1)	5.8 ± 0.4	4.2 ± 0.1	+	+	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.1% sodium benzoate (pH 2.1)	6.7 ± 0.4	4.9 ± 0.2	2.8 ± 0.1	1.7 ± 0	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.0)	5.2 ± 0.1	−	−	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.1)	5.7 ± 0.3	4.3 ± 0.1	3.7 ± 0.2	3.4 ± 0.1	3.1 ± 0.3	2.9 ± 0.1*
Fecal contamination						
E. coli O157:H7 only (pH 8.5)	6.1 ± 0.4	6.1 ± 0.1	6.1 ± 0.3	6.0 ± 0.2	6.1 ± 0.1	6.1 ± 0.2
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 2.2)	5.5 ± 0.3	5.1 ± 0.1	4.6 ± 0.2	3.9 ± 0.3	2.1 ± 0.1	2.0 ± 0.1*
0.5% butyric acid (pH 4.5)	6.0 ± 0.4	6.0 ± 0.3	6.0 ± 0.1	6.1 ± 0.1	6.1 ± 0.2	6.1 ± 0.2**
0.05% caprylic acid (pH 7.1)	6.0 ± 0.5	5.6 ± 0.1	4.3 ± 0.3	2.3 ± 0.1	2.0 ± 0.1	2.0 ± 0.1*
0.1% sodium benzoate (pH 8.8)	5.7 ± 0.6	5.6 ± 0.2	5.4 ± 0.3	5.6 ± 0.3	5.5 ± 0.1	5.4 ± 0.2**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (pH 2.3)	5.8 ± 0.4	5.2 ± 0.5	3.6 ± 0.1	3.1 ± 0.2	2.6 ± 0.1	+*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.1% sodium benzoate (pH 2.2)	5.7 ± 0.6	4.0 ± 0.2	2.0 ± 0.1	1.7 ± 0	+	+*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.2)	4.9 ± 0.1	2.0 ± 0.1	+	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.3)	5.5 ± 0.4	3.0 ± 0.2	2.7 ± 0.1	2.5 ± 0.2	2.5 ± 0.1	2.0 ± 0.1*

[a] Results are means ± standard deviations of duplicate trials. An asterisk indicates a significant difference ($P < 0.05$), whereas two asterisks indicate no significant difference ($P > 0.05$). +, positive by enrichment culture (<1.7 log CFU/ml); −, negative by enrichment culture.

[b] The actual time zero may have been 3 to 7 seconds after treatment, because of the time required to remove an aliquot and suspend it in neutralizing buffer.

acid (0.5 to 1.5%), propionic acid (0.5 to 4%), chlorine dioxide (10 to 100 ppm), and 0.05% caprylic acid did not substantially reduce (<1.0 log CFU/ml) *E. coli* O157:H7 within 20 min when tested individually in water containing rumen content (100:1) at 21°C. However, increasing the concentration of butyric acid to ≥2% and that of caprylic acid to ≥0.1% resulted in substantial inactivation of *E. coli* O157:H7 (>5 log CFU/ml) within 20 min (Table 1). Unfortunately, these higher concentrations of butyric acid and caprylic acid were offensively odoriferous.

Several combinations of chemicals at different concentrations were evaluated subsequently for inactivation of more than 5 log CFU of *E. coli* O157:H7/ml within 20 min at 21°C in water containing large amounts of rumen content (10 parts water-1 part rumen content [vol/wt]). Three combinations, i.e., 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (A); 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (B); and 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (C), met these criteria (Table 1). A fourth chemical combination (D) comprised of 0.1% lactic acid, 0.9% acidic calcium sulfate, and 100 ppm chlorine dioxide reduced *E. coli* O157:H7 populations by 2.6 log CFU/ml within 20 min and by 5.0 log CFU/ml within 120 min (Table 1).

These four chemical combinations were tested for their antimicrobial effects on *E. coli* O26:H11 and *E. coli* O111:NM in water containing large amounts of rumen content (10:1 [vol/wt]). The results revealed that the same three combinations, A, B, and C, had similar antimicrobial activities (ca. 5-log CFU/ml reduction within 20 min at 21°C) against *E. coli* O26:H11 (Table 2) and *E. coli* O111:NM, except for treatment B, which required 30 min for a 5-log CFU/ml reduction (Table 3). Combination D reduced *E. coli* O26:H11 and *E. coli* O111:NM populations within 20 min by 3.3 and 3.0 log CFU/ml, respectively (Tables 2 and 3).

The antimicrobial activities of these four chemical combinations were further determined in water containing cattle feces at a ratio of 20:1 (vol/wt). The results revealed that combinations A, B, and C inactivated all three pathogens by more than 5 log CFU/ml within 30 min (Tables 1 to 3). Combination D

TABLE 2. *E. coli* O26:H11 counts in water containing rumen content (10:1 [vol/wt]) or feces (20:1 [vol/wt]) and treated with different chemical combinations at 21°C

Treatment	*E. coli* O26:H11 counts (log CFU/ml) at min[a]:					
	0[b]	2	5	10	20	30
Rumen content contamination						
E. coli O26:H11 only (pH 8.8)	5.5 ± 0.2	5.6 ± 0.3	5.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.2	5.5 ± 0.5
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 2.2)	5.0 ± 0.1	4.4 ± 0.2	3.7 ± 0.2	3.2 ± 0.4	2.4 ± 0.1	2.3 ± 0.1*
0.5% butyric acid (pH 4.4)	5.4 ± 0.1	5.4 ± 0.2	5.3 ± 0.2	5.4 ± 0.2	5.4 ± 0.1	5.2 ± 0.2**
0.05% caprylic acid (pH 7.0)	5.3 ± 0.2	5.4 ± 0.3	5.2 ± 0.1	5.4 ± 0.4	5.3 ± 0.2	5.4 ± 0.5**
0.1% sodium benzoate (pH 8.6)	5.6 ± 0.3	5.5 ± 0.1	5.4 ± 0.4	5.5 ± 0.2	5.5 ± 0.4	5.5 ± 0.3**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (pH 2.3)	5.3 ± 0.1	4.1 ± 0.2	+	+	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.1% sodium benzoate (pH 2.3)	5.2 ± 0.2	5.1 ± 0.1	3.6 ± 0.2	1.7 ± 0	+	+*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.3)	5.7 ± 0.4	−	−	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.2)	5.5 ± 0.1	5.1 ± 0.2	2.6 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1*
Fecal contamination						
E. coli O26:H11 only (pH 7.4)	5.4 ± 0.1	5.5 ± 0.1	5.4 ± 0.3	5.6 ± 0.2	5.5 ± 0.4	5.4 ± 0.2
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 2.1)	5.4 ± 0.2	4.7 ± 0.4	4.7 ± 0.1	4.5 ± 0.2	4.0 ± 0.1	2.0 ± 0.1*
0.5% butyric acid (pH 4.1)	5.5 ± 0.1	5.4 ± 0.2	5.4 ± 0.4	5.4 ± 0.3	5.5 ± 0.3	5.2 ± 0.4**
0.05% caprylic acid (pH 5.6)	5.5 ± 0.2	5.4 ± 0.1	5.5 ± 0.2	5.5 ± 0.3	5.3 ± 0.2	5.2 ± 0.4**
0.1% sodium benzoate (pH 7.8)	5.5 ± 0.1	5.4 ± 0.2	5.5 ± 0.2	5.4 ± 0.2	5.5 ± 0.1	5.4 ± 0.3**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (pH 2.1)	5.4 ± 0.3	3.3 ± 0.5	−	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.1% sodium benzoate (pH 2.1)	5.3 ± 0.4	+	+	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.0)	3.9 ± 0.5	1.7 ± 0	−	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.1)	4.9 ± 0.4	1.7 ± 0	+	+	+	−*

[a] Results are the means ± standard deviations of duplicate trials. An asterisk indicates a significant difference ($P < 0.05$), whereas two asterisks indicate no significant difference ($P > 0.05$). +, positive by enrichment culture (<1.7 log CFU/ml); −, negative by enrichment culture.

[b] The actual time zero may have been 3 to 7 seconds after treatment, because of the time required to remove an aliquot and suspend it in neutralizing buffer.

reduced *E. coli* O157:H7, O26:H11, and O111:NM cell numbers within 30 min by 3.5, 4.9, and 4.6 log CFU/ml, respectively (Tables 1 to 3).

The dairy heifers' intake of drinking water containing chemical combination A or C was significantly less than that of control water with no added chemicals (data not shown). The average amount of water (pH 6.7) consumed by the control group was 30.3 liters/day, whereas consumption rates of the treated waters ranged from 13.8 to 18.6 liters/day, which were significantly ($P < 0.01$) less than that of the control (data not shown).

DISCUSSION

Although studies have revealed that contaminated cattle drinking water is an important vehicle in the persistence and dissemination of *E. coli* O157:H7 on cattle farms, highly effective, practical methods to control the pathogen in drinking water have not been not available (19, 25). Estimates indicate that *E. coli* O157:H7 contaminates as many as 10% of drinking water troughs for cattle (13, 19). We evaluated several practical treatments for efficacy in killing *E. coli* O157:H7 when drinking water was heavily contaminated with rumen content or feces. These treatments included microbiological and chemical approaches.

Previous reports indicated that some competing bacterial strains produce antimicrobials that can reduce *E. coli* O157:H7 cell numbers in cattle (17, 26, 37). Our results from treating cattle drinking water with competing *E. coli* revealed that growth of *E. coli* O157:H7 was controlled, but the treatment had minimal effect on reducing *E. coli* O157:H7 populations. Hence, it is not a practical approach for treating drinking water to control the pathogen.

Chlorine is a highly effective treatment to kill pathogens such as *E. coli* O157:H7 in pure water (12, 22, 25). However, the efficacy of chlorine in killing *E. coli* O157:H7 is dependent on the purity of the water, and its bactericidal activity is reduced or eliminated in the presence of high levels of organic material, such as soil and feces (15, 39). Our studies determined that the addition of rumen content to water with 5 ppm chlorine reduced or eliminated the bactericidal effect of chlorine. Hence, chlorination at 5 ppm does not appear to be an effective treatment to control *E. coli* O157:H7 in drinking water for cattle. Similarly, 22 to 24 ppm ozone, which was the highest concentration that could be generated by the available equipment, was not practically effective in reducing *E. coli* O157:H7 cell numbers in water containing relatively low levels (100 parts water-1 part rumen content) of rumen content (11, 14, 35).

TABLE 3. *E. coli* O111:NM counts in water containing rumen content (10:1 [vol/wt]) or feces (20:1 [vol/wt]) and treated with different chemical combinations at 21°C

Treatment	*E. coli* O111:NM counts (log CFU/ml) at min[a]:					
	0[b]	2	5	10	20	30
Rumen content contamination						
E. coli O111:NM only (pH 8.7)	5.8 ± 0.3	5.9 ± 0.5	5.7 ± 0.2	5.7 ± 0.4	5.8 ± 0.3	5.8 ± 0.3
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 2.2)	5.5 ± 0.5	5.4 ± 0.3	4.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1*
0.5% butyric acid (pH 3.8)	5.5 ± 0.2	5.5 ± 0.2	5.5 ± 0.2	5.6 ± 0.3	5.6 ± 0.3	5.4 ± 0.2**
0.05% caprylic acid (pH 5.7)	5.6 ± 0.1	5.5 ± 0.2	5.7 ± 0.4	5.5 ± 0.3	5.8 ± 0.5	5.6 ± 0.2**
0.1% sodium benzoate (pH 8.6)	5.6 ± 0.3	5.6 ± 0.2	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.5	5.5 ± 0.2**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.5% butyric acid (pH 2.3)	5.7 ± 0.2	5.0 ± 0.1	2.8 ± 0.1	+	+	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.1% sodium benzoate (pH 2.3)	5.7 ± 0.5	5.3 ± 0.2	4.4 ± 0.2	3.6 ± 0.3	2.1 ± 0.1	+*
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.2)	4.4 ± 0.6	+	+	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.3)	5.8 ± 0.2	5.1 ± 0.3	3.3 ± 0.2	3.0 ± 0.1	2.8 ± 0.1	2.6 ± 0.2*
Fecal contamination						
E. coli O111:NM only (pH 7.7)	5.6 ± 0.1	5.7 ± 0.4	5.7 ± 0.3	5.6 ± 0.1	5.6 ± 0.2	5.6 ± 0.1
0.1% lactic acid + 0.9% acidic calcium sulfate (pH 2.2)	4.5 ± 0.5	3.1 ± 0.3	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0*
0.5% butyric acid (pH 4.1)	5.6 ± 0.2	5.6 ± 0.1	5.5 ± 0.4	5.6 ± 0.1	5.6 ± 0.3	5.6 ± 0.2**
0.05% caprylic acid (pH 5.5)	5.7 ± 0.3	5.5 ± 0.1	5.7 ± 0.4	5.5 ± 0.2	5.6 ± 0.3	5.7 ± 0.3**
0.1% sodium benzoate (pH 7.6)	5.6 ± 0.2	5.6 ± 0.1	5.6 ± 0.5	5.7 ± 0.4	5.6 ± 0.3	5.5 ± 0.1**
0.1% lactic acid + 0.9% acidic calcium sulfate + 0.05% caprylic acid (pH 2.0)	5.5 ± 0.1	2.3 ± 0.5	−	−	−	−*
0.1% lactic acid + 0.9% acidic calcium sulfate + 100 ppm chlorine dioxide (pH 2.0)	5.3 ± 0	2.7 ± 0.5	2.6 ± 0.1	1.9 ± 0.1	+	+*

[a] Results are the means ± standard deviations of duplicate trials. An asterisk indicates a significant difference ($P < 0.05$), whereas two asterisks indicate no significant difference ($P > 0.05$). +, positive by enrichment culture (<1.7 log CFU/ml); −, negative by enrichment culture.

[b] The actual time zero may have been 3 to 7 seconds after treatment, because of the time required to removean aliquot and suspend it in neutralizing buffer.

Some chemicals, such as 2% butyric acid and 0.1% caprylic acid, were effective in killing 5 log CFU *E. coli* O157:H7 in rumen content-contaminated water; however, they are not practical treatments because of cost constraints and objectionable odors. We evaluated a variety of chemicals in combination at different concentrations for their effects on inactivation of *E. coli* O157:H7 within 20 min in water heavily contaminated with rumen content. Four different combinations were highly effective, and all contained acidic calcium sulfate, which is a highly acidic methylated calcium sulfate, and lactic acid (38).

Several studies have revealed that application of organic acids, such as acetic, citric, and lactic acids, does not substantially reduce *E. coli* O157:H7 cell numbers in food, which may be explained by the exceptional acid tolerance of many strains of *E. coli* O157:H7 (6, 8, 16, 31, 33, 34). For example, Brackett et al. (8) evaluated the efficacies of warm (20°C) and hot (55°C) acetic, citric, and lactic acid sprays on the survival of *E. coli* O157:H7 on raw beef and determined that none of the acid treatments appreciably reduced *E. coli* O157:H7 on beef samples, nor were any of the acid treatments judged effective for practical uses. Glass et al. (16) studied the influence of pH adjusted with lactic acid or HCl on survival or growth of *E. coli* O157:H7 in tryptic soy broth and determined that the organism could grow in TSB at pH 4.5 to 9.0, adjusted with HCl. When TSB was acidified with lactic acid, the organism grew at pH 4.6 but not at pH 4.5 (16). *E. coli* O157:H7 can tolerate acidic conditions in a variety of fermented and acidified meats, such as during the processing of dry fermented sausage, in processed salami, and in acidified ground roast beef (1, 16).

Annamalai et al. (3) studied the antimicrobial effects of 35 and 50 mM caprylic acid on *Escherichia coli* O157:H7 at 39°C in rumen fluid (pH 5.6 and 6.8) from 12 beef cattle. The results revealed that treatments with caprylic acid at both pH values significantly reduced *E. coli* O157:H7 cell numbers. At pH 5.6, both concentrations of caprylic acid killed *E. coli* O157:H7 rapidly, reducing the pathogen count to undetectable levels at 1 min of incubation (>6.0 log CFU/ml). However, the killing effect was reduced in buffered rumen fluid. At pH 6.8, 50 mM caprylic acid reduced the *E. coli* O157:H7 population to undetectable levels at 1 min of incubation, whereas 35 mM caprylic acid reduced the pathogen by approximately 3.0 and 5.0 log CFU/ml at 8 and 24 h of incubation, respectively.

Our results revealed that three chemical combinations, i.e., 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.05% caprylic acid (A); 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.1% sodium benzoate (B); and 0.1% lactic acid, 0.9% acidic calcium sulfate, and 0.5% butyric acid (C), were highly effective at 21°C in reducing large cell numbers (10^5 CFU/ml) of *E. coli* O157:H7, O26:H11, and O111:NM to undetectable levels (by enrichment) within 30 min in water heavily contaminated with rumen content at a ratio of 10:1 (water/rumen content) or feces at a ratio of 20:1 (water/feces). Though the exact mechanism of these chemical combinations is not clear, we believe that multiple functions may be involved and that the killing effect may be significantly increased through different functions. However, drinking water treatments with acidic calcium sulfate as the base ingredient significantly depressed wa-

ter intake by cattle, and there were no significant differences in depressed water intake among the acidic calcium sulfate-based water treatments. The covariant was significant, but there were no differences among cow groups or between the two treatment periods. This implies that the covariant effectively removed variation among animals from the statistical analysis, that the randomly assigned groups were similar, and that the treatment effect was consistent between the two experimental periods. To ensure that treatment effects on water intake were not due to differences in cow body size, cow BW was converted to MBW ($BW^{0.75}$), and intake of water per MBW was calculated. The results revealed that treatment effects for water intake per MBW were similar to those for total water intake.

Practical application of such treatments on the farm would likely best be accomplished by periodic, such as daily, addition of the treatment, holding for 20 to 30 min, and then flushing to remove the disinfectant and residue and replacing the treated water with fresh water. On-farm studies are needed to validate and optimize this practice.

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