December 2005

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APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2005, p. 7974–7979 Vol. 71, No. 12
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.12.7974–7979.2005

Effects of Common Forage Phenolic Acids on *Escherichia coli* O157:H7 Viability in Bovine Feces

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Received 5 May 2005/Accepted 27 August 2005

Ruminant animals are carriers of *Escherichia coli* O157:H7, and the transmission of *E. coli* O157:H7 from cattle to the environment and to humans is a concern. It is unclear if diet can influence the survivability of *E. coli* O157:H7 in the gastrointestinal system or in feces in the environment. Feces from cattle fed bromegrass hay or corn silage diets were inoculated with *E. coli* O157:H7, and the survival of this pathogen was analyzed. When animals consumed bromegrass hay for <1 month, viable *E. coli* O157:H7 was not recovered after 28 days postinoculation, but when animals consumed the diet for >1 month, *E. coli* O157:H7 cells were recovered for >120 days. Viable *E. coli* O157:H7 cells in feces from animals fed corn silage were detected until day 45 and differed little with the time on the diet. To determine if forage phenolic acids affected the viability of *E. coli* O157:H7, feces from animals fed corn silage or cracked corn were amended with common forage phenolic acids. When 0.5% *trans*-cinnamic acid or 0.5% *para*-coumaric acid was added to feces from silage-fed animals, the *E. coli* O157:H7 death rate was increased significantly (17-fold and 23-fold, respectively) compared to that with no addition. In feces from animals fed cracked corn, *E. coli* O157:H7 death rates were increased significantly with the addition of 0.1% and 0.5% *trans*-cinnamic acid (7- and 13-fold), 0.1% and 0.5% *p*-coumaric acid (3- and 8-fold), and 0.5% ferulic acid (3-fold). These data suggest that phenolic acids common to forage plants can decrease viable counts of *E. coli* O157:H7 shed in feces.

Escherichia coli O157:H7 is a food-borne pathogen associated with hemorrhagic colitis and hemolytic-uremic syndrome in humans, and outbreaks are commonly associated with contaminated meat products from cattle (35). Numerous studies have demonstrated the shedding of *E. coli* O157:H7 from cattle in feces (5, 11, 23, 29, 38) and have reported the bovine gastrointestinal system to be a reservoir for the pathogen (24, 32). Improved meat-processing techniques and testing procedures appear to have decreased recent incidents of contaminated beef products reaching the public (4), but outbreaks associated with fecal contamination of water and produce have been documented (31).

Beef animal production typically involves the concentration of animals in feedlot pens and the provision of energy-rich diets (grain and/or grain silage) to maximize animal performance. Manure collected from cattle operations is often stored and later applied to the land as a means of disposal as well as a source of nutrients for agricultural crops. However, studies have shown that *E. coli* O157:H7 can survive in manure for >3 months after defecation (1, 2, 21, 26). The ability of this bacterium to survive in animal manure is both an environmental and a food safety concern.

Efforts to decrease the shedding and persistence of *E. coli* O157:H7 in cattle manure are ongoing, but few viable interventions have been identified. The feeding of hay to feedlot cattle may decrease bacteria such as *E. coli* O157:H7 from being shed (3, 7), but a specific mechanism to explain a decrease has not been documented. Treatment of manure or animal waste during storage or prior to land application has the potential for decreasing manure-borne pathogens, thereby reducing the risk of water and crop contamination (18, 31, 37). However, concerns pertaining to the cost and environmental impact of treatments are important considerations prior to their implementation by producers. Therefore, a variety of both economical and effective manure treatments need to be developed and tested.

Plant carboxylic phenols have been shown to inhibit certain pathogens (9, 28, 33, 39), and in grasses used as cattle forages, their concentrations range from 2 to 10 g/kg (6, 19). These compounds are synthesized from phenylalanine via a cinnamic acid intermediate to phenylpropanoid compounds (19), and the predominant phenylpropanoid compounds in many forages are *para*-coumaric acid and ferulic acid (12, 25, 30). The phenolic acids are often conjugated to sugars in plants and may play a role in protecting plants from pathogens (12, 30). However, the antimicrobial effects of these plant compounds against pathogens in the complex fecal or manure environment are unknown. The objectives of this study were to evaluate the survival of *E. coli* O157:H7 in feces from cattle fed different types of diets and to determine if amendments with *trans*-cinnamic, coumaric, or ferulic acid affected the survival of this pathogen in cattle feces. Information garnered from this study will identify candidate plant compounds with antimicrobial activity in the fecal environment that may be useful as dietary additives or manure treatments.

MATERIALS AND METHODS

Fecal sample collection. Fecal samples were collected at 4-week intervals from beef steers fed growing diets as part of a separate feed efficiency study. The growing diets were either bromegrass hay (100% ground bromegrass hay [as fed]) or corn silage (88% corn silage, 9% cracked corn, 3% soybean meal [as fed]) fed ad libitum. Mineral blocks and water were accessible at all times. The

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animals were composite animals from Angus, Hereford, Pinzgauer, and Red Poll cattle bred from populations at the U.S. Meat Animal Research Center, USDA-ARS (MARC; Clay Center, NE). Prior to the provision of experimental diets, animals were adapted to outdoor concrete-surfaced pens and fed corn silage for 4 weeks. Following the adaptation period, the animals were blocked by weight (range, 150 to 250 kg) into dietary treatment groups. Rectal fecal samples were collected from steers on both diets at 4, 8, 12, and 16 weeks, using sterile gauntlet gloves. After collection, the fresh fecal samples were transported to the lab, and feces from six animals (~200 g/animal) were compiled for each diet and mixed well for laboratory study.

In subsequent studies to determine the potential for forage phenolic acids to decrease the viability of *E. coli* O157:H7 in bovine feces, fresh feces were collected from cattle feedlot pens located at MARC. The pens have packed dirt floors with concrete aprons extending from the feed trough, where most fecal samples were collected. The beef steers (approximately 250 to 450 kg, or 9 to 10 months of age) were being fed either corn silage (MARC feedlot growing diet; 87.5% corn silage and 12.5% cracked corn [base diet]) or cracked corn (MARC feedlot finishing diet; 69% cracked corn and 31% corn silage [base diet]). The corn silage and cracked-corn base diets were supplemented, as fed, with a 2.5% or 4.5% complete liquid supplement to provide additional dietary nitrogen (as urea), vitamin A, vitamin E, calcium, phosphorus, and salt. Feces were collected on four occasions over an 8-week period (June to August) from representative pens. A minimum of eight undisturbed fecal samples (~2 kg of feces total) were collected, with little soil contamination, and were compiled for each experiment.

Bacterial cultures. *Escherichia coli* O157:H7 Str^r strains 43985 and MARC S-1 were maintained throughout the experimental periods on tryptic soy agar plates supplemented with 250 μg/ml streptomycin. Prior to inoculation, colonies were picked and grown overnight at 37°C in tryptic soy broth supplemented with 250 μg/ml streptomycin. The cultures were then removed from the incubator and allowed to sit at room temperature for at least 24 h to acclimate to laboratory temperatures.

Fecal inoculations and incubations. The fecal samples were analyzed prior to inoculation with the laboratory strains and did not contain indigenous Str^r bacteria at the lowest dilution. To determine the survival of *E. coli* O157:H7 in feces from cattle fed the growing diets, laboratory-acclimated cultures of *E. coli* O157:H7 Str^r strains 43985 and MARC S-1 were diluted in buffered peptone water to yield approximately 10^8 cells for each culture in a 2-ml cocktail, as determined by plate counts. In preliminary trials with cattle feces, a 2-ml inoculation cocktail volume per 100 g of feces consistently yielded 5.9 ± 0.1 $\log_{10}$ streptomycin-resistant cells per g feces after being mixed in sterile Whirl-Pak bags (NASCO, Fort Atkinson, WI) by hand massage for at least 5 minutes, and this protocol was followed in all subsequent experiments. The inoculated feces were placed into deep cell culture dishes (100 × 25 mm) and maintained upright and loosely covered in the laboratory at room temperature throughout the experiment. Incubations for each diet treatment were performed in duplicate.

To determine if phenolic acid compounds common to forage plants have an antimicrobial capacity, fecal composites were collected from pens housing animals consuming either a corn silage or cracked-corn diet, inoculated (as described above) with *E. coli* O157:H7 strains, and treated with phenolic acid compounds common to forages. *Trans*-cinnamic, *para*-coumaric, and ferulic acids were added individually as dry compounds to 200 g of inoculated feces at 0.1 or 0.5% of the wet fecal weight in separate plastic bags. Each bag with amended inoculated feces was mixed well by hand massage for 10 min. The fecal samples were then placed into deep culture dishes and maintained loosely covered in the laboratory at room temperature throughout the experiment. Fecal samples were collected on four occasions for either the corn silage or corn diets, and incubations for each treatment were performed in duplicate.

Viable cell counts. Viable counts were determined by counting the CFU of streptomycin-resistant bacterial cells plated onto MacConkey sorbitol agar containing 250 μg/ml streptomycin. The CFU were determined at time zero with freshly inoculated feces and at the same relative time each assay day thereafter by serial dilution of a 1-g fecal sample. Aliquots (100 μl) of the appropriate dilutions were spread onto individual plates. Colonies were counted after the plates were incubated overnight at 37°C. Colonies were visually typical of *E. coli* O157:H7 and were confirmed to be *E. coli* O157 by agglutination using anti-O157 antibodies (*Escherichia coli* O157 test kit; Oxoid Ltd., Hampshire, England) if questionable. The inoculated feces were assayed until no cells were recovered from the lowest dilution (10^{-1}) for at least two consecutive assays. In cases where rapid death was observed, no viable cells were found for up to 14 days after death. The viable counts were transformed to $\log_{10}$ equivalents for analysis. The detection limit was 1.5 $\log_{10}$ CFU per g feces.

Chemicals and analyses. Chemicals and antimicrobials were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). Bacterial growth media and buffers were Difco brand (Becton-Dickinson Company, Sparks, MD). The fecal pH was determined with 0.5 g of freshly composited feces suspended in 2.5 ml of distilled water.

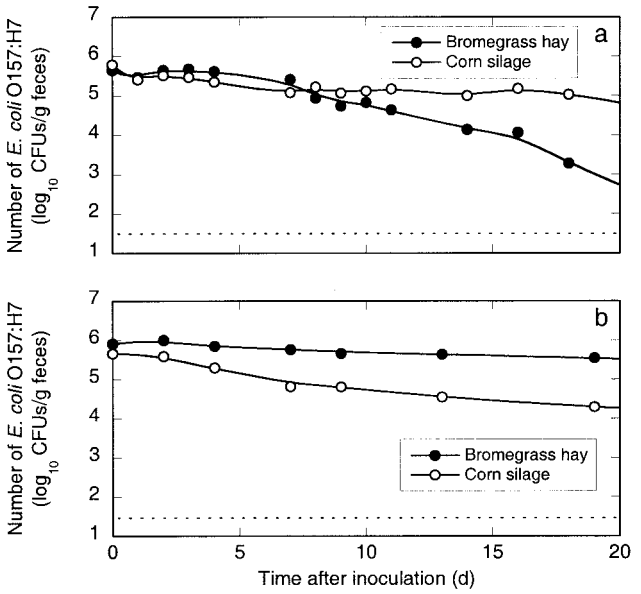

FIG. 1. Effect of diet and time on diet on the viability of *E. coli* O157:H7 in inoculated feces. Fecal samples were collected from animals consuming either bromegrass hay (●) or corn silage (○) for 4 weeks (a) or 8 weeks (b). The dotted line denotes the limit of detection for viable counts. Slopes (not shown in figure) were fit to the decreases in viable counts and represent the death rates ($\log_{10}$ CFU/g feces/day) of the inoculated strains.

Statistics. All incubations were performed in duplicate for each fecal collection or treatment, and the coefficients of variation for duplicate pairs were <10%. Means and standard errors of the means are reported for all treatment effects. The statistical significance of treatment means were determined using Student's *t* test, and linear regressions were fit using least-square means by KaleidaGraph for the Macintosh, version 3.5x (Synergy Software, Reading, PA).

RESULTS

Dietary effects. Viable *E. coli* O157:H7 Str^r cells were recovered at $>10^5$ CFU per g feces directly following inoculation into feces from cattle fed either bromegrass hay or corn silage for 4 weeks. These viable counts declined with time in the feces but decreased faster in feces from animals fed bromegrass hay (bromegrass hay feces) (Fig. 1a). Viable *E. coli* O157:H7 cells were no longer detected after 28 days in the bromegrass hay feces, whereas the corn silage feces sustained viable counts for 42 days (data not shown).

Feces from animals on each diet for 8, 12, and 16 weeks were also collected and inoculated with *E. coli* O157:H7 Str^r cells. When animals were fed the same diets for 8 weeks (Fig. 1b), the viable counts in bromegrass hay feces decreased slowly (Fig. 1b) and were detected longer (over 100 days) than those in the corn silage feces (45 days). Similar results were observed in feces from animals consuming the diets for 12 and 16 weeks (data not shown). The fecal pH was always lower in feces from animals fed corn silage than in those from animals fed bromegrass hay (6.36 versus 7.31, respectively) and was not significantly affected by the time on the diet (data not shown).

The time-dependent decreases in viable cells were nearly

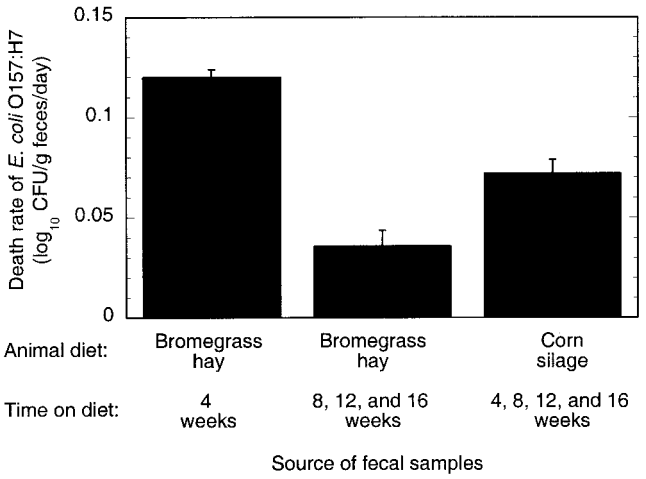

FIG. 2. Death rates ($\log_{10}$ CFU/g feces/day) of *E. coli* O157:H7 cells inoculated into feces from animals consuming bromegrass hay for 4 weeks (first column), bromegrass hay for 8, 12, and 16 weeks (second column), or corn silage for 4, 8, 12, and 16 weeks (third column). All data presented are averages with respective standard errors, and the averages are significantly different from each other ($P < 0.05$).

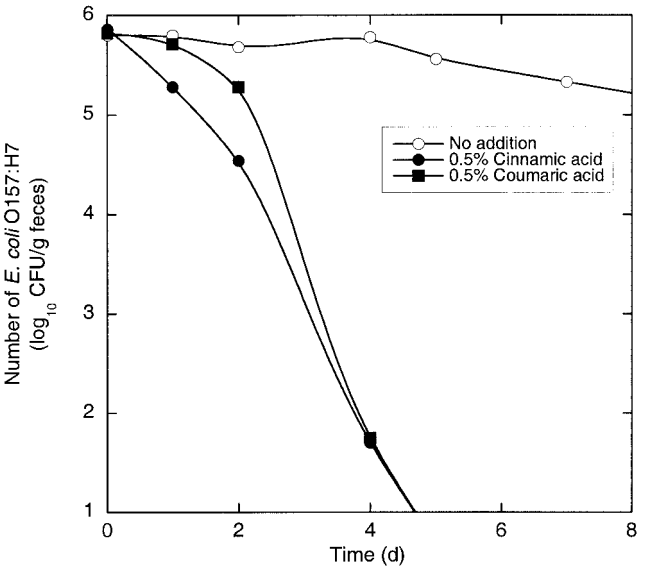

FIG. 3. Recovery of *E. coli* O157:H7 over time from inoculated feces following no treatment or treatment with 0.5% *trans*-cinnamic acid or 0.5% *para*-coumaric acid. Fecal samples were collected from animals consuming a corn silage diet. Slopes (not shown in figure) were fit to the decreases in viable counts and represent the death rates ($\log_{10}$ CFU/g feces/day) of the inoculated strains.

linear when expressed in $\log_{10}$ form ($R^2 > 0.7$). The slopes of the lines represent death rates for *E. coli* O157:H7 ($\log_{10}$ CFU/g feces/day) and are shown in Fig. 2. The death rate was 0.12 $\log_{10}$ CFU/g feces/day in feces from animals consuming bromegrass hay for only 4 weeks. In fecal samples from animals consuming bromegrass hay for 8, 12, and 16 weeks, the *E. coli* O157:H7 death rates were not significantly different from each other (average rate, 0.036 $\log_{10}$ CFU/g feces/day; $P > 0.5$) but were significantly lower ($P < 0.01$) than the *E. coli* O157:H7 death rate in feces collected on week 4. The *E. coli* O157:H7 death rates in feces from animals consuming corn silage were not significantly affected by the time on the diet (4, 8, 12, or 16 weeks), and the average death rate was 0.074 $\log_{10}$ CFU/g feces/day, a value significantly different from the observed rates for bromegrass hay feces ($P < 0.05$).

Forage phenolic acid treatments. In experiments with inoculated feces from animals fed corn silage, the loss in viable *E. coli* O157:H7 Str^r cells in untreated feces (no phenolic acid addition) was similar to the previous results (Fig. 3 versus Fig. 1), and viable cells were recovered for at least 40 days. The loss of viable *E. coli* O157:H7 cells was greater in the presence of 0.5% *trans*-cinnamic acid or 0.5% *para*-coumaric acid, and no viable cells were detected after approximately 4 days (Fig. 3). The *E. coli* O157:H7 death rates were determined from the linear decreases in viable counts over time, and 0.5% *trans*-cinnamic and *para*-coumaric acids increased the *E. coli* O157:H7 death rate 17-fold ($P < 0.05$) and 23-fold ($P < 0.01$), respectively (Fig. 4). A lower level (0.1%) of *trans*-cinnamic or *para*-coumaric acid or ferulic acid at 0.5% was not as effective, and these amendments did not significantly increase ($P > 0.1$) the *E. coli* O157:H7 death rate (fourfold, less than twofold, and less than twofold, respectively) in experiments with corn silage feces. The addition of *trans*-cinnamic, *para*-coumaric, or ferulic acid at 0.1 or 0.5% to bovine feces did not affect the fecal pH in any of the treatments (data not shown).

Viable *E. coli* O157:H7 cells in inoculated, untreated feces from animals fed cracked corn were not recovered after 20 days and decreased in number faster than cells in corn silage feces (Fig. 5 versus Fig. 3). In the presence of 0.5% *trans*-cinnamic or 0.5% *para*-coumaric acid, the decrease in viable *E. coli* O157:H7 cells in feces from animals fed cracked corn was rapid, and in the presence of 0.5% *trans*-cinnamic acid, no viable cells were observed after 2 days (Fig. 5). The *E. coli* O157:H7 death rates were determined from the decreases in linear viable counts over time, and the addition of 0.5% *trans*-cinnamic acid and 0.5% *para*-coumaric acid increased the death rate 13-fold ($P < 0.01$) and 8-fold ($P < 0.01$), respectively, compared to that in inoculated, untreated feces (Fig. 6).

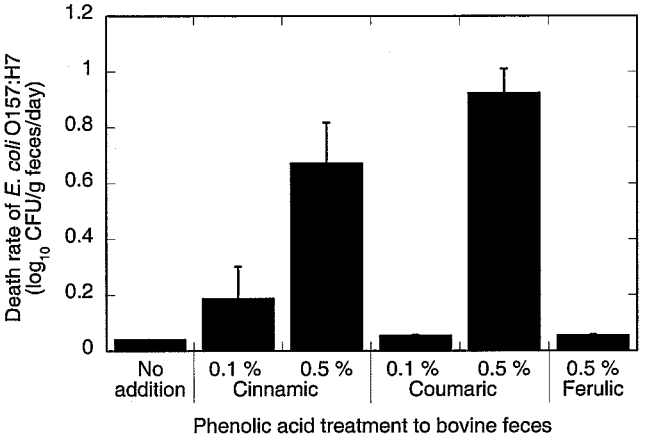

FIG. 4. Effects of added *trans*-cinnamic, *para*-coumaric, and ferulic acids on the death rate of *E. coli* O157:H7 in feces from animals fed corn silage. All data presented are averages with respective standard errors from four experiments.

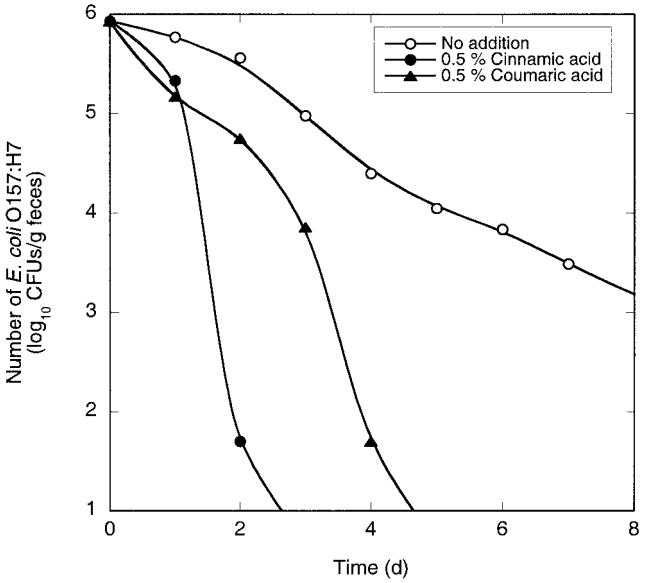

FIG. 5. Recovery of *E. coli* O157:H7 over time from inoculated feces following no treatment or treatment with 0.5% *trans*-cinnamic acid or 0.5% *para*-coumaric acid. Fecal samples were collected from animals consuming a cracked-corn diet. Slopes (not shown in figure) were fit to the decreases in viable counts and represent the death rates ($\log_{10}$ CFU/g feces/day) of the inoculated strains.

The *E. coli* O157:H7 viable counts decreased with a lower level (0.1%) of *trans*-cinnamic or *para*-coumaric acid, and the apparent death rates were significantly higher (threefold [$P < 0.01$] and sevenfold [$P < 0.01$], respectively) than that in inoculated, untreated feces. The addition of 0.5% ferulic acid also significantly increased the *E. coli* O157:H7 death rate nearly threefold ($P < 0.01$) compared to that in inoculated, untreated feces. The addition of *trans*-cinnamic, *para*-coumaric, or ferulic acid at 0.1 or 0.5% to bovine feces did not affect the fecal pH in any of the treatments (data not shown).

Fecal pH and *E. coli* O157:H7 viability. The fecal pH was lowest with the cracked-corn diet and highest with the bromegrass hay diet (data not shown). Lower fecal pHs were associated with higher death rates, but overall the fecal pH had a minimal effect on the death of *E. coli* O157:H7 cells in inoculated, untreated feces (Fig. 7). In treatments with phenolic acids, the initial fecal pH affected the antimicrobial activities of *trans*-cinnamic and ferulic acids. The effect of fecal pH was greatest with 0.5% *trans*-cinnamic acid (Fig. 7), suggesting that the bactericidal effect of *trans*-cinnamic acid on *E. coli* O157:H7 is influenced by the fecal pH. Similar, albeit weaker, death rate/pH relationships were observed with 0.1% *trans*-cinnamic acid and 0.5% ferulic acid additions, whereas no apparent death rate/pH relationship was observed with 0.1 or 0.5% *para*-coumaric acid (data not shown).

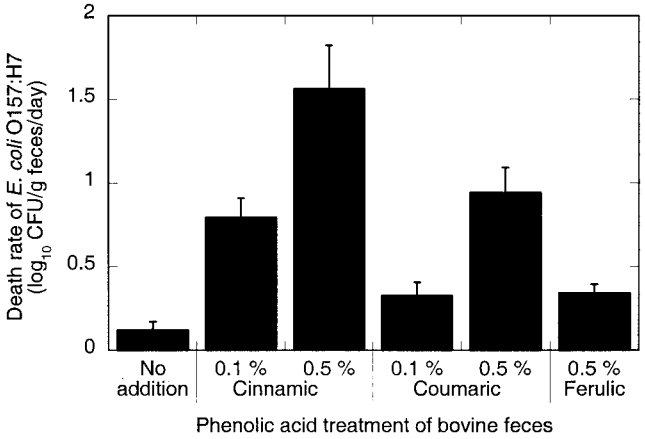

FIG. 6. Effects of added *trans*-cinnamic, *para*-coumaric, and ferulic acids on the death rate of *E. coli* O157:H7 in feces from animals fed cracked corn. All data presented are averages with respective standard errors from four experiments.

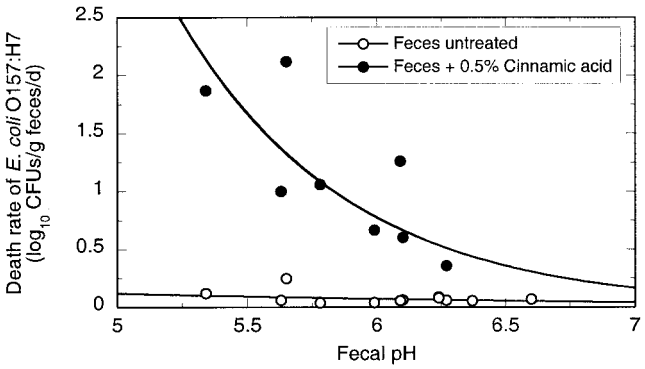

FIG. 7. Effect of initial fecal pH on viability of *E. coli* O157:H7 in untreated bovine feces (○) and in bovine feces treated with 0.5% *trans*-cinnamic acid (●). Feces were collected from animals consuming either corn silage (pH 5.9 to 6.3) or cracked corn (pH 5.3 to 5.8).

DISCUSSION

Escherichia coli O157:H7 is a zoonotic pathogen commonly found in the feces of farm animals, in particular ruminant animals (14). Ruminants are grazing animals, but in confinement they are fed a variety of diets, such as hays (dried grass or legumes), corn silage (chopped and ensiled corn plants), and/or grain (corn or barley). In experiments with confined cattle fed bromegrass hay or corn silage, we not only observed significant differences among diets with regard to the death rates of *E. coli* O157:H7 in the feces from these animals, but we also saw differences among times on the diets with feces from cattle fed hay. When animals were fed corn silage, the death rates of *E. coli* O157:H7 in feces were similar regardless of the time on the diet (up to 16 weeks) (Fig. 2). However, in feces from cattle fed bromegrass hay, *E. coli* O157:H7 death rates were associated with the time on the diet. In feces from animals consuming bromegrass hay for <1 month, the death rate was nearly fourfold higher than the observed death rate in feces when animals were on the bromegrass hay diet for >1 month.

The feeding of hay (timothy-grass hay) was proposed early as a dietary intervention to possibly control *E. coli* O157:H7 shedding (7). Reductions in acid-resistant *E. coli* levels in feces were noted when animals were fed hay for <2 weeks, but no specific mechanism to explain these effects has been reported (3). Other reports did not observe a benefit in feeding hays

(timothy-grass or alfalfa-legume) over longer periods to sheep (21, 22) or cattle (15, 36), and in these experiments, *E. coli* O157:H7 was persistently shed in the feces of pathogen-dosed animals fed hay. Based on our results with bromegrass hay in relation to published observations, any observed anti-*E. coli* O157:H7 benefits of hay feeding may disappear as the ruminant animal fully adapts to the hay diet, which may explain these discrepancies.

Plants have bound phenolic acids that are released to help control bacterial pathogen invasion (8, 12, 30), and in vitro studies have shown that plant phenolic acids can rapidly kill many human-pathogenic bacteria (13, 20, 28, 39). In forages, phenolic acids are derived from cinnamic acid and are often conjugated to carbohydrates or carbohydrate structures (12, 25, 30). These compounds, commonly known as hydroxycinnamic acids, include *para*-coumaric, ferulic, synapic, and caffeic acids. The phenolic acid content can vary in individual amounts among plant species, but the total extractable phenolics may be >1% of the plant dry matter (6, 19). The effect of these compounds in the gastrointestinal system may not be fully appreciated.

In ruminants that are fully adapted to forage diets, plant phenolic acids are metabolized in the rumen (6, 25). However, information about phenolic acid metabolism in the rumens of animals fed grain is lacking. A previous report (22) suggested that *E. coli* O157:H7 was shed less in feces from animals with corn diets than in those from animals with hay diets, but the animals fed corn were also fed alfalfa, and it should be noted that the fiber content of the feces of these animals was actually higher than that for animals fed hay. Animals on grain diets have little capacity to digest fiber in the rumen (34), and as a consequence, when they are fed hay, more fiber with phenolic acids passes into the lower gastrointestinal system, where liberated phenolic acids would have the greatest effect on *E. coli* O157:H7 populations.

Typically in feedlots in the United States, beef cattle are fed high-energy diets based on corn, and *E. coli* O157:H7 can persist in the manures. To determine if forage phenolic acids could affect the viability of *E. coli* O157:H7 in feces from animals fed corn silage or cracked corn, we added *trans*-cinnamic, *para*-coumaric, or ferulic acid to cattle feces inoculated with *E. coli* O157:H7. The phenolic acid treatments decreased the viable cell counts of *E. coli* O157:H7 in feces from corn silage or cracked-corn diets in a dose-dependent response. However, the most prominent effect was in the feces from animals fed cracked corn, and the effectiveness differed between the phenolic acids.

Cinnamic acid was the most effective compound tested in feces, and its effectiveness was related to the fecal pH (Fig. 7); a similar response of *trans*-cinnamic acid to pH was observed previously against *Listeria monocytogenes* in pure culture (39). A higher dose (0.5%) of *para*-coumaric acid reduced the number of *E. coli* O157:H7 cells in feces from the corn silage and cracked-corn diets similarly, but the lower dose (0.1%) was only effective in feces from animals fed cracked corn. Ferulic acid (0.5%) was more effective in feces from animals fed cracked corn, which is similar to the response obtained with 0.1% *para*-coumaric acid. These dietary differences may be related to higher ferulic and coumaric acid contents in corn silage than in corn grain (12) and to selection for gastrointestinal microorganisms capable of partially degrading these phenolic acids in the feces. Nevertheless, these compounds still had significant antimicrobial capabilities in the feces.

Escherichia coli O157:H7 is generally recognized as a problem in feces from concentrated animal operations where animals are fed low-forage/high-energy diets. In our work, the death of *E. coli* O157:H7 was fastest in the feces of animals fed a high-corn diet (Fig. 4 and Fig. 6), and this effect seemed to be associated with a lower fecal pH (Fig. 7). Nonetheless, viable counts were recovered from the feces of animals on the diets for appreciably long periods of time (>20 days), and this would be a problem for manure management. Considering that *E. coli* O157:H7 can persist as a contaminant in soil and on crops for months after manure application or irrigation (16, 17, 27), the reduction or elimination of *E. coli* O157:H7 in production animal systems would be ideal.

To our knowledge, the plant phenolic acids have not been associated with decreased pathogen survival in feces. In our initial study with bovine diets and the viability of *E. coli* O157:H7 in the feces, we did not anticipate that the viability would be affected by the time on the diet, and as a consequence, we did not sample these feces for phenolic acids. Recognizing the differences with the time on the diet, the adaptation of ruminants to forages, and the ability of plant phenolic acids to affect pathogens in in vitro studies (13, 20, 28, 39) led us to analyze the effects of phenolic acids in bovine feces. The fact that we observed significant effects with concentrations as low as 0.1% of the phenolic acids, an amount less than that found in many forages, suggests a potential role for controlling pathogens in the gastrointestinal system.

The treatment of stored animal manures with plant essential oils has decreased the amounts of pathogens (37), and our work suggests that phenolic acids may also be useful for similar treatments. In addition, extrapolation of our work with bovine feces to the gastrointestinal system suggests that supplementation of plant phenolic acids to the diets of feedlot animals may be an exploitable intervention. However, more research needs to be done to determine the feasibility of this approach. Recent works by Duncan et al. (9, 10) have demonstrated the potential of dietary esculin and esculitin, which are plant coumarin compounds, to decrease viable *E. coli* O157:H7 in rumen fluid and colonic contents. The study of plant phenolic acid metabolism in ruminants has been limited to forage-adapted animals (6, 25), and limited information is available about their metabolism in animals fed feedlot diets. Future studies will need to determine the stability of phenolic acids in animals fed grain and then determine their dietary potential to reduce pathogen shedding from feedlot cattle.

ACKNOWLEDGMENTS

We acknowledge the secretarial assistance of Jackie Byrkit and the technical assistance of Dee Kucera, Cindy Felber, and Jane Long. We also acknowledge Calvin Ferrell for allowing access to animals from the feed efficiency study.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

1. **Avery, S. M., A. Moore, and M. L. Hutchison.** 2004. Fate of *Escherichia coli* originating from livestock faeces deposited directly onto pasture. Lett. Appl. Microbiol. **38:**355–359.
2. **Bolton, D. J., C. M. Byrne, J. J. Sheridan, D. A. McDowell, and I. S. Blair.** 1999. The survival characteristics of a non-toxigenic strain of *Escherichia coli* O157:H7. J. Appl. Microbiol. **86:**407–411.
3. **Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet.** 2003. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. J. Dairy Sci. **86:**852–860.
4. **Centers for Disease Control and Prevention.** 2004. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food-selected sites, United States, 2003. Morb. Mortal. Wkly. Rep. **53:**338–343.
5. **Chapman, P. A., C. A. Siddons, D. J. Wright, P. Norman, J. Fox, and E. Crick.** 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* O157 infections in man. Epidemiol. Infect. **111:**439–447.
6. **Cremin, J. D., Jr., K. R. McLeod, D. L. Harmon, A. L. Goetsch, L. D. Bourquin, and G. C. Fahey, Jr.** 1995. Portal and hepatic fluxes in sheep and concentrations in cattle ruminal fluid of 3-(4-hydroxyphenyl)propionic, benzoic, 3-phenylpropionic, and trans-cinnamic acids. J. Anim. Sci. **73:**1766–1775.
7. **Diez-Gonzalez, F., T. R. Callaway, M. G. Kizoulis, and J. B. Russell.** 1998. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science **281:**1666–1668.
8. **Draughon, F. A.** 2004. Use of botanicals as biopreservatives in foods. Food Technol. **58:**20–28.
9. **Duncan, S. H., H. J. Flint, and C. S. Stewart.** 1998. Inhibitory activity of gut bacteria against *Escherichia coli* O157 mediated by dietary plant metabolites. FEMS Microbiol. Lett. **164:**283–288.
10. **Duncan, S. H., E. C. Leitch, K. N. Stanley, A. J. Richardson, R. A. Laven, H. J. Flint, and C. S. Stewart.** 2004. Effects of esculin and esculetin on the survival of *Escherichia coli* O157 in human faecal slurries, continuous-flow simulations of the rumen and colon and in calves. Br. J. Nutr. **91:**749–755.
11. **Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid.** 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA **97:**2999–3003.
12. **Faulds, C. B., and G. Williamson.** 1999. The role of hydroxycinnamates in the plant cell wall. J. Sci. Food Agric. **79:**393–395.
13. **Friedman, M., P. R. Henika, and R. E. Mandrell.** 2003. Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. J. Food Prot. **66:**1811–1821.
14. **Gansheroff, L. J., and A. D. O'Brien.** 2000. *Escherichia coli* O157:H7 in beef cattle presented for slaughter in the U.S.: higher prevalence rates than previously estimated. Proc. Natl. Acad. Sci. USA **97:**2959–2961.
15. **Hovde, C. J., P. R. Austin, K. A. Cloud, C. J. Williams, and C. W. Hunt.** 1999. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl. Environ. Microbiol. **65:**3233–3235.
16. **Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang.** 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J. Food Prot. **67:**1365–1370.
17. **Islam, M., J. Morgan, M. P. Doyle, and X. Jiang.** 2004. Fate of *Escherichia coli* O157:H7 in manure compost-amended soil and on carrots and onions grown in an environmentally controlled growth chamber. J. Food Prot. **67:**574–578.
18. **Jiang, X., J. Morgan, and M. P. Doyle.** 2003. Thermal inactivation of *Escherichia coli* O157:H7 in cow manure compost. J. Food Prot. **66:**1771–1777.
19. **Jung, H., and G. C. Fahey, Jr.** 1983. Nutritional implications of phenolic monomers and lignin: a review. J. Anim. Sci. **57:**206–225.
20. **Kim, H.-O., S.-W. Park, and H.-D. Park.** 2004. Inactivation of *Escherichia coli* O157:H7 by cinnamic aldehyde purified from *Cinnamomum cassia* shoot. Food Microbiol. **21:**105–110.
21. **Kudva, I. T., K. Blanch, and C. J. Hovde.** 1998. Analysis of *Escherichia coli* O157:H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol. **64:**3166–3174.
22. **Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and C. J. Hovde.** 1997. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ. Microbiol. **63:**3878–3886.
23. **Lahti, E., O. Ruoho, L. Rantala, M. L. Hanninen, and T. Honkanen-Buzalski.** 2003. Longitudinal study of *Escherichia coli* O157 in a cattle finishing unit. Appl. Environ. Microbiol. **69:**554–561.
24. **Laven, R. A., A. Ashmore, and C. S. Stewart.** 2003. *Escherichia coli* in the rumen and colon of slaughter cattle, with particular reference to *E. coli* O157. Vet. J. **165:**78–83.
25. **Lowry, J. B.** 1990. Metabolic and nutritional significance of the cell-wall phenolic acid fraction, p. 119–126. *In* P. J. Harris (ed.), Microbial and plant opportunities to improve lignocellulose utilization by ruminants. Elsevier, New York, N.Y.
26. **McGee, P., D. J. Bolton, J. J. Sheridan, B. Earley, and N. Leonard.** 2001. The survival of *Escherichia coli* O157:H7 in slurry from cattle fed different diets. Lett. Appl. Microbiol. **32:**152–155.
27. **Natvig, E. E., S. C. Ingham, B. H. Ingham, L. R. Cooperband, and T. R. Roper.** 2002. *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. Appl. Environ. Microbiol. **68:**2737–2744.
28. **Olasupo, N. A., D. J. Fitzgerald, M. J. Gasson, and A. Narbad.** 2003. Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Lett. Appl. Microbiol. **37:**448–451.
29. **Omisakin, F., M. MacRae, I. D. Ogden, and N. J. Strachan.** 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. Appl. Environ. Microbiol. **69:**2444–2447.
30. **Ou, S., and K.-C. Kwok.** 2004. Ferulic acid: pharmaceutical functions, preparation and application in foods. J. Sci. Food Agric. **84:**1261–1269.
31. **Pell, A. N.** 1997. Manure and microbes: public and animal health problem? J. Dairy Sci. **80:**2673–2681.
32. **Rasmussen, M. A., W. C. Cray, Jr., T. A. Casey, and S. C. Whipp.** 1993. Rumen contents as a reservoir of enterohemorrhagic *Escherichia coli*. FEMS Microbiol. Lett. **114:**79–84.
33. **Reinders, R. D., S. Biesterveld, and P. G. Bijker.** 2001. Survival of *Escherichia coli* O157:H7 ATCC 43895 in a model apple juice medium with different concentrations of proline and caffeic acid. Appl. Environ. Microbiol. **67:**2863–2866.
34. **Russell, J. B., and D. B. Wilson.** 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. **79:**1503–1509.
35. **USDA-APHIS.** 1997. Presented at CEAH, Fort Collins, Colo.
36. **Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja.** 2004. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. Appl. Environ. Microbiol. **70:**5336–5342.
37. **Varel, V. H., and D. N. Miller.** 2001. Plant-derived oils reduce pathogens and gaseous emissions from stored cattle waste. Appl. Environ. Microbiol. **67:**1366–1370.
38. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
39. **Wen, A., P. Delaquis, K. Stanich, and P. Toivonen.** 2003. Antilisterial activity of selected phenolic acids. Food Microbiol. **20:**305–311.