Journal of Food Protection, Vol. 74, No. 6, 2011, Pages 912–918
doi:10.4315/0362-028X.JFP-10-484

Naturally Colonized Beef Cattle Populations Fed Combinations of Yeast Culture and an Ionophore in Finishing Diets Containing Dried Distiller's Grains with Solubles Had Similar Fecal Shedding of *Escherichia coli* O157:H7

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MS 10-484: Received 6 November 2010/Accepted 4 February 2011

ABSTRACT

Beef steers ($n = 252$) were used to evaluate the effects of dietary supplement on fecal shedding of *Escherichia coli* O157:H7. Seven pens of 9 steers (63 steers per treatment) were fed diets supplemented with or without yeast culture (YC) or monensin (MON) and their combination (YC × MON). YC and MON were offered at 2.8 g/kg and 33 mg/kg of dry matter intake, respectively. Environmental sponge samples (from each pen floor, feed bunk, and water trough) were collected on day 0. Rectal fecal grab samples were collected on days 0, 28, 56, 84, 110, and 125. Samples were collected and pooled by pen and analyzed for presumptive *E. coli* O157:H7 colonies, which were confirmed by a multiplex PCR assay and characterized by pulsed-field gel electrophoresis (PFGE) typing. On day 0, *E. coli* O157:H7 was detected in 7.0% of feed bunk samples and 14.3% of pen floor samples but in none of the water trough samples. The 71.4% prevalence of *E. coli* O157:H7 in fecal samples on day 0 decreased significantly ($P < 0.05$) over time. *E. coli* O157:H7 fecal shedding was not associated with dietary treatment ($P > 0.05$); however, in cattle fed YC and YC × MON fecal shedding was 0% by day 28. Eight *Xba*I PFGE subtypes were identified, and a predominant subtype and three closely related subtypes (differing by three or fewer bands) accounted for 78.7% of environmental and fecal isolates characterized. Results from this study indicate that feeding YC to cattle may numerically decrease but not eliminate fecal shedding of *E. coli* O157:H7 at the onset of treatment and that certain *E. coli* O157 subtypes found in the feedlot environment may persist in feedlot cattle.

The gastrointestinal tract of beef cattle is recognized as the major natural reservoir for Shiga toxin–producing *Escherichia coli* O157:H7, a human pathogen responsible for nearly 70,000 foodborne illnesses each year in the United States *(38)*. During hide removal at harvest, beef carcasses can become contaminated with *E. coli* O157:H7 from coming into contact with hides that are contaminated with fecal matter *(18, 19, 50)*. Elevated *E. coli* O157:H7 fecal shedding has been reported in cattle fed distiller's grains, an ethanol fermentation by-product *(15, 27–30, 52, 57)*. In a recent survey, approximately 83% of beef nutritionists reported formulating finishing diets to include ethanol by-products *(56)*. Therefore, strategies that effectively reduce *E. coli* O157:H7 fecal shedding in beef cattle at the preharvest stage are needed.

Monensin (MON), an antimicrobial classified as an ionophore, is routinely fed to growing and finishing beef cattle in rations used for growth promotion and control of coccidiosis *(48)*. However, growing public concern regarding antimicrobial resistance driven by the use of antimicrobials for growth promotion in agriculture *(44)* has prompted consumer demand for beef that has been raised without the use of ionophores *(22, 55)*. Ionophores have been shown to have no effect or to exacerbate *E. coli* O157:H7 fecal shedding in ruminants *(16, 24, 37)*.

Yeast culture (YC) is a potential candidate for a natural preharvest intervention to control *E. coli* O157:H7 fecal shedding in cattle. YC products, such as those made with *Saccharomyces cerevisiae,* often are thought to act as a prebiotic and thus are classified as nondigestible ingredients (such as a carbohydrate) that cannot be hydrolyzed or absorbed by the upper digestive tract and may stimulate growth or activity of the natural microflora in the gastrointestinal tract *(21)*. Callaway et al. *(10)* hypothesized that prebiotics may provide the native microflora with a competitive advantage, allowing them to displace and exclude pathogenic bacteria from the gastrointestinal tract. The objective of this study was to evaluate dietary effects of YC and MON and their combination on *E. coli* O157:H7 shedding in feedlot cattle fed finishing diets with dried distiller's grains with solubles (DDGS). The hypothesis was that if YC reduced *E. coli* O157:H7 fecal shedding, there would be contradictory results when both YC and MON were fed to cattle.

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TABLE 1. *Ingredients and nutrient composition of cattle diets throughout the study*

	% of total mixed ration on a dry matter basis[a]							
	Transition[b]				Finishing[c]			
Ingredients and nutrients	CON	YC	MON	YC × MON	CON	YC	MON	YC × MON
Ingredients								
Alfalfa hay	9.3	9.3	9.3	9.3	5.5	5.5	5.5	5.5
Corn silage	12.6	13.2	12.6	13.2	9.0	9.0	9.0	9.0
Flaked corn	57.8	56.9	57.7	56.9	63.5	63.5	63.5	63.5
DDGS[d]	18.1	18.3	18.1	18.3	19.7	19.7	19.7	19.7
Limestone	1.5	1.5	1.5	1.5	1.6	1.6	1.6	1.6
Cornmeal[e]	0.49	0.01	0.48		0.31	0.01	0.31	
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral oil	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Trace mineral premix[f]	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Vitamin E	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016
Vitamin A	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Yeast culture[g]		0.526		0.526		0.311		0.311
Monesin[h]			0.015	0.015			0.016	0.016
Nutrients[i]								
Dry matter	71.28	71.49	73.38	73.36	74.75	74.76	75.52	74.85
CP	12.75	13.18	13.28	12.78	14.09	13.90	13.96	13.77
NPN	0.40	0.31	0.26	0.29	0.61	0.40	0.33	0.35
NDF	17.98	18.08	17.50	18.16	16.78	16.55	17.15	17.37
Ether extract	5.28	5.35	5.16	5.05	5.70	5.41	5.66	5.71
Calcium	0.72	0.66	0.59	0.65	0.82	0.80	0.83	0.73
Phosphorus	0.39	0.40	0.40	0.38	0.39	0.39	0.39	0.38
Sulfur	0.26	0.30	0.27	0.28	0.24	0.26	0.26	0.27

[a] Treatments were a basal control (CON) diet, diet supplemented with yeast culture (YC; Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA), diet supplemented with monensin (MON; Rumensin, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and diet supplemented with YC and MON (YC × MON).

[b] Transition ration was fed on days 0 to 8 of the trial.

[c] Finishing ration was fed on days 9 through 125 of the trial.

[d] DDGS, dried distiller's grains with solubles.

[e] Both the YC and MON were offered in the total mixed ration in place of an equal amount of cornmeal on a dry matter basis.

[f] Formulated to provide the following on a dry matter basis: 0.70% Ca, 0.39% P, 0.78% K, 0.25% Mg, 0.25% S, 0.25% NaCl, 75 mg/kg Zn, 85 mg/kg Fe, 10 mg/kg Cu, 25 mg/kg Mn, 0.20 mg/kg Co, 0.25 mg/kg I, and 0.12 mg/kg Se.

[g] YC was offered at the manufacturer's recommended inclusion rate of 56 g·animal^{-1}·day^{-1} during the transition phase and decreased to 28 g·animal^{-1}·day^{-1} during the finishing phase (2.8 g/kg of dry matter intake).

[h] Final concentration of MON was 11 mg/kg of dry matter intake during the transition phase and increased to 33 mg/kg of dry matter intake during the finishing phase.

[i] Nutrient values were determined from ration samples taken every 7 days throughout the study.

MATERIALS AND METHODS

Study design. All sampling techniques and animal use and handling procedures were preapproved by the Colorado State University Animal Care and Use Committee (approval 08-092A-01). Cross-bred yearling beef steers ($n = 252$) consuming high-concentrate finishing rations at the Southeast Colorado Research Center (SECRC; Lamar, CO) were enrolled in a longitudinal study to probe the effect of YC, MON, and YC × MON on fecal shedding of *E. coli* O157:H7. Steers were sorted and housed in 28 dirt-surface pens (nine animals per pen), where every second pen shared a water trough along the fence line. Each pen was randomly assigned to one of four dietary treatments: (i) basal control (CON) diet, (ii) diet supplemented with YC (*Saccharomyces cerevisiae,* Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA), (iii) diet supplemented with MON (Rumensin, Elanco Animal Health, Greenfield, IN), and (iv) diet supplemented with both YC and MON. Finishing diets contained 19.7% DDGS on a dry matter basis (Table 1) and were formulated to meet or exceed nutrition requirements of finishing beef cattle *(42).* YC was offered at the manufacturer's recommended inclusion level of 56 g·animal^{-1}·day^{-1} (as fed) during the transition phase and then decreased to 28 g·animal^{-1}·day^{-1} (as fed) during the finishing phase (or 2.8 g/kg of dry matter intake). MON was offered at the inclusion level of 11 mg/kg of dry matter intake during the transition phase and then increased to 33 mg/kg of dry matter intake for the finishing phase *(6).* Both YC and MON were offered in the total mixed ration in place of an equal amount of cornmeal on a dry matter basis. Both water and diets were offered ad libitum throughout the study.

Environmental sponge samples were collected on day 0 from the floor, feed bunk, and water trough of each pen housing animals enrolled in the study. Pen floor and feed bunk samples were collected with sterile sponges hydrated with 10 ml buffered peptone water (BioPro Enviro-Sponge Bags, International BioProducts

Inc., Redmond, WA) by vigorously passing sponges over the sample site surface and then placing sponges into sterile bags. Pen floor samples were taken from the surface next to the water trough (approximately 60 by 60 cm). Approximately 300 ml of water was collected from each trough into a sterile Whirl-Pak bag (Nasco, Modesto, CA). Rectal fecal grab samples were collected from all animals on days 0, 28, 56, 84, 110, and 125 as described previously *(11)*. All samples were transported on ice to the Center for Meat Safety and Quality (Colorado State University, Fort Collins), stored at 4°C, and microbiologically analyzed within 24 h.

Microbiological analysis of environmental and fecal samples for *E. coli* O157:H7. Fecal samples (10 g) from each animal housed in the same pen were combined to create a 90-g composite sample representing each pen. Fecal samples were microbiologically analyzed to isolate and detect *E. coli* O157:H7 as detailed previously *(11)*. The entire (90-g) composite fecal sample for each pen was placed in a 1,627-ml Filter-Pak bag (Nasco) with 810 ml (10:1 dilution) of phosphate-buffered tryptic soy broth (TSB-PO_4; BD, Franklin Lakes, NJ). Fecal slurries were homogenized by hand massaging and incubated for 2 h at room temperature (25 ± 2°C)and then for 6 h at 42°C. After incubation, fecal slurries were stored up to 12 h at 4°C for analysis by immunomagnetic bead separation (IMS) using the Pathatrix protocol (Matrix MicroScience, Inc., Golden, CO). The fecal slurry bags were placed in the Pathatrix warming pots (preheated to 37°C), the Pathatrix apparatus was inserted into the filtered side of each fecal slurry bag, and 50 μl of anti-O157 immunomagnetic beads (Dynabeads, Invitrogen, Oslo, Norway) was added to the connector tubing. The samples were circulated for 60 min at 37°C, and the beads were then washed with phosphate-buffered saline (PBS). After IMS, 50 μl of the beads plus PBS was plated in duplicate onto modified sorbitol MacConkey agar (BD) supplemented with 20 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (mSMAC). The mSMAC plates were incubated for 24 ± 2 h at 37°C.

Environmental samples were prepared in a different manner. Water samples were passed over a 150-ml bottle-top filter unit (0.45-μm pore size; Corning Inc., Corning, NY) to concentrate *E. coli* O157:H7 cells. Environmental sponge samples from the pen floors and feed troughs and the filters from the bottle-top units were then transferred to sterile filter Whirl-Pak bags (Nasco) and combined with 90 ml of TSB-PO_4. After incubation, pen floor and feed trough samples were stored at 4°C until they were subjected to the IMS procedure as described by Barkocy-Gallagher et al. *(2, 3)* using anti-O157 immunomagnetic beads (Dynabeads). After IMS, 50 μl of each environmental sample was plated on mSMAC plates as described above.

Molecular confirmation and subtyping of *E. coli* O157:H7 isolates. After incubation, up to three colonies with morphology typical of *E. coli* O157:H7 were selected from each plate and confirmed as *E. coli* O157:H7 with a multiplex PCR assay. Presumptive *E. coli* O157:H7 colonies were screened for the presence of *eaeA*, *fliC*$_{h7}$, *rfbE*, *stx*$_1$, and *stx*$_2$ using primers and amplification conditions described previously *(25)*. A sample that contained an *E. coli* isolate carrying at least one Shiga toxin gene and *rfbE* was deemed positive for *E. coli* O157:H7. One isolate from each *E. coli* O157:H7–positive sample was characterized by pulsed-field gel electrophoresis (PFGE) typing using the standardized Centers for Disease Control and Prevention PulseNet protocol *(46)*. PFGE patterns obtained were analyzed and compared using the Bionumerics version 3.5 software package (Applied Maths, Saint-Matins-Latem, Belgium). Similarity clustering analyses were performed using the unweighted pair group matching algorithm and the Dice correlation coefficient *(26)*.

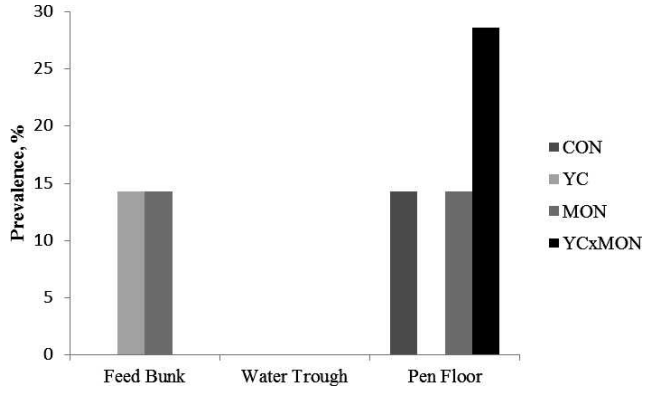

FIGURE 1. *Prevalence of* Escherichia coli *O157:H7 in environmental samples (feed bunk, water trough, and pen floor) collected on day 0 of the trial before cattle were introduced to pens. Pens of steers were fed diets supplemented with yeast culture (YC), monensin (MON), or both additives (YC × MON).* E. coli *O157:H7 was not detected in any samples taken from water troughs.*

Statistical analysis. Fecal shedding results were analyzed as a 2 × 2 factorial, completely randomized block design with repeated measures. Pens of cattle served as the experimental unit, and composite fecal samples from each pen served as the sample unit. Data were analyzed using the GLIMMIX procedure as implemented in Statistical Analysis Software (version 9.1.3; SAS Institute, Cary, NC). The final model for *E. coli* O157:H7 fecal shedding included the first order interactions of main effects of treatment (i.e., control, YC, MON, and YC × MON), sampling day, and treatment × sampling day interactions. The model also included a random statement to account for body weight block and a repeated measures statement because all pens were sampled on multiple days. The three-way interaction of YC × MON × sampling day was included in the final model because the data would not converge otherwise. Differences were computed by including the PDIFF statement in the model. A chi-square test as implemented in the frequency procedure in SAS was performed to evaluate the association between dietary treatment and the presence or absence of *E. coli* O157:H7 in feces. Individual comparisons for frequency differences within each day were analyzed using Fisher's exact test in SAS. The level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

There was no effect ($P > 0.05$) of treatment on daily feed intakes, which were 10.4, 10.2, 10.4, and 9.7 kg of dry matter per day for CON, YC, MON, and YC × MON treatments, respectively (data not shown). Before cattle were exposed to housing pens and treatment diets (day 0), *E. coli* O157:H7 was isolated from 7.0% of feed bunk samples and 14.3% of pen floor samples (Fig. 1). Before the initiation of dietary treatments on day 0, 71.4% of the pens had at least one animal that shed *E. coli* O157:H7 in their feces (Fig. 2). Overall, 19.6% (33 of 168) of composite fecal samples were positive for *E. coli* O157:H7. Cumulatively, 75.0% (21 of 28) of treatment pens produced at least one positive sample over the course of the study. Although no difference ($P > 0.05$) in fecal shedding of *E. coli* O157:H7 was associated with dietary treatment, the average pen prevalence of cattle shedding *E. coli* O157:H7 decreased significantly over time ($P < 0.01$). Additionally, YC-fed cattle (both YC and YC × MON treatment groups) numerically cleared *E. coli*

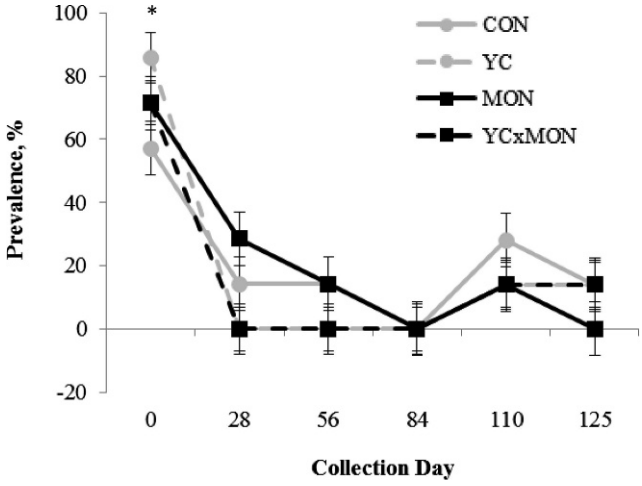

FIGURE 2. *Prevalence (mean and standard deviation) of* Escherichia coli *O157:H7 from day 0 (pretreatment) through day 125 in composite fecal samples of finishing beef steers (seven pens). Steers were fed diets supplemented with yeast culture (YC), monensin (MON), or both additives (YC × MON). No difference in fecal prevalence of* E. coli *O157:H7 due to treatment was found (*$P > 0.05$*); however, day of sample collection did have a significant effect (*$P < 0.01$*). Fecal shedding of* E. coli *O157:H7 was significantly reduced across all treatments on day 28 compared with day 0 (*). Error bars represent the standard error of the mean calculated for each dietary treatment for each sample collection day.*

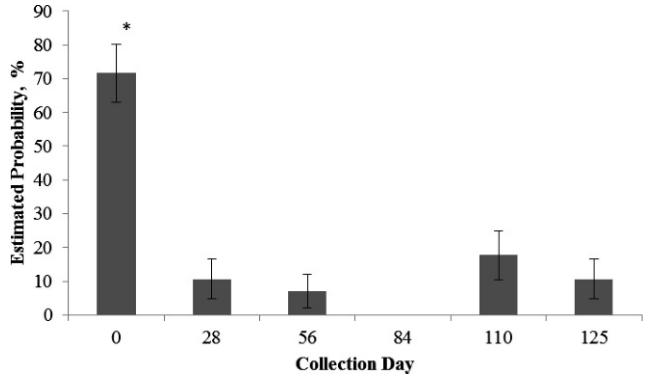

FIGURE 3. *Estimated probability of* E. coli *O157:H7 fecal shedding in cattle on each sample collection day across all treatments. Error bars represent the standard error of the mean calculated for each estimated probability. Significant differences (*) were detected for the probability of encountering* E. coli *O157:H7 in the feces on different collection days (*$P < 0.01$*).*

O157:H7 by day 28 and remained free of *E. coli* O157:H7 through day 84, at which time fecal shedding of this pathogen numerically increased for all treatment groups (Fig. 2). Estimated probability of encountering the pathogen decreased ($P < 0.01$; Fig. 3) over time in pooled pen samples.

Eight unique *Xba*I PFGE subtypes were identified from 39 *E. coli* O157:H7 isolates representing each positive environmental and fecal sample throughout this study (Table 2). One predominant PFGE subtype (subtype A) accounted for 22% of all isolates characterized (Table 2). PFGE subtypes B, C, and F differed from subtype A by three or fewer bands and thus were considered closely related to the predominant subtype *(54)*. Cumulatively, subtype A and the closely related subtypes B, C, and F accounted for 78.7% of *E. coli* O157:H7 isolates characterized by PFGE typing. These four closely related subtypes were detected in the pens and feces of cattle from all treatment groups, but subtype A was the only subtype that recurred throughout this longitudinal study (Table 3).

Based on previous studies and the high initial prevalence of *E. coli* O157:H7 colonization within the population of cattle enrolled in this study, we hypothesized that *E. coli* O157:H7 fecal shedding would increase over the course of the study in the basal control group. However, the data did not support this hypothesis; the prevalence of *E. coli* O157:H7 decreased over the course of the study across all treatment groups. We did not expect a synergistic effect on *E. coli* O157:H7 fecal shedding attributable to simultaneous ingestion of YC and MON *(49)*. We hypothesized that YC would reduced *E. coli* O157:H7 fecal shedding, and contradictory results would be obtained when YC was fed with MON. However, this hypothesis was not supported by the results, and further studies are needed to elucidate the relationship between YC and MON in finished cattle diets. The lack of an effect of the MON treatment was expected and is in agreement with previous reports *(12, 16, 20, 24)*.

DDGS in the feed did not stimulate proliferation and shedding of *E. coli* O157:H7 in the current study; shedding was low in the CON treatment group throughout the study. In other studies, feeding cattle fermented by-products (distiller's or brewer's grains) has been associated with increased fecal shedding of *E. coli* O157:H7 *(15, 27–30, 52, 57)*. We speculated that the low final prevalence of *E. coli* O157:H7 fecal shedding observed in the current study could be attributed to antimicrobial residues in the DDGS, which may have antagonized the growth of *E. coli* O157:H7. As part of the ethanol production process, some antimicrobials such as penicillin G, streptomycin, tetracycline, monensin, and virginiamycin have been used to suppress bacterial growth *(1, 14)*. Although there is no evidence to suggest that the DDGS used in the current study contained antimicrobials or antimicrobial residues at levels sufficient to destroy the pathogens in the gastrointestinal tract of these cattle, such residues may explain the lack of *E. coli* O157:H7 prevalence observed after day 0. Other properties of the DDGS, such as a dense population of *Lactobacillus* spp. *(43)*, also may have had a negative effect on *E. coli* O157:H7. In the fuel alcohol industry, lactobacilli are the most common and persistent bacterial contaminants *(4, 40)*. Because the study design lacked a treatment group that received no DDGS, comparisons with or without DDGS in the diet were not made, and no conclusions regarding DDGS can be clearly drawn. Studies evaluating the effects of lactobacilli in distiller's grains on the fecal prevalence of *E. coli* O157:H7 in feedlot cattle are needed.

Lactobacillus acidophilus, when offered in the diet as a direct-fed microbial, has been useful in some studies for reducing fecal shedding of *E. coli* O157:H7, thus having potential for preharvest reduction of this pathogen *(7, 8, 17, 35, 36, 53)*. Although both YC and *L. acidophilus* are considered direct-fed microbials, the direct mode of action

TABLE 2. *Pulsed-field gel electrophoresis (PFGE) characterization of* E. coli *O157:H7 isolates from environmental and fecal samples across all sample collection days*

PFGE subtype	No. of bands different from subtype A	No. of isolates[a]	No. of pens
A		22	8
B	2	1	1
C	3	12	9
D	>7	4	2
E	4	1	1
F	2	2	1
G	>7	1	1
H	>7	4	1
Total		47	

[a] Number of combined fecal and environmental samples throughout the study that were positive for *E. coli* O157:H7. Environmental samples were collected on day 0 (pretreatment) from each treatment pen floor, feed bunk, and automatic water trough. Fecal samples were collected from every animal by rectal palpation on days 0, 28, 56, 84, 110, and 125.

of YC versus *L. acidophilus* is likely different. Whereas *L. acidophilus* secretes a bacteriocin (colicin) that inhibits or directly interacts with the expression of virulence-related genes in *E. coli* O157:H7 *(39),* YC may provide the native microflora with nutritive constituents that inhibit colonization of cattle by *E. coli* O157:H7 *(10).* Other preslaughter interventions also have been proposed *(10, 35, 51).* Some potential strategies include (but are not limited to) competitive exclusion applications using native cultures from the gut of a healthy animal transferred to the naïve gut of young animals, antibiotics (e.g., neomycin sulfate), bacteriophages (bacterial viruses), treatment with sodium chlorate, vaccinations, diet shifts, and washing of animals before slaughter.

After day 0, the prevalence of *E. coli* O157:H7 fecal shedding was low throughout the study, with only a subtle spike in all treatment groups on day 110. Khaitsa et al. *(31)* described three distinct prevalence phases of fecal shedding of *E. coli* O157:H7 in finishing beef steers: preepidemic, epidemic, and postepidemic. These phases were characterized by a pattern of low initial prevalence, followed by dramatically high incidence, and then by a second low prevalence period, respectively. This pattern indicates the presence of time-dependent risk factors that could contribute to fecal shedding of *E. coli* O157:H7 *(31).* In the current study, this pattern could explain the initial peak in prevalence observed on day 0, the dramatic decrease on day 28, and then the subtle spike on day 110. The cattle probably entered the trial during an epidemic phase, which was followed by a postepidemic phase when treatments began. This theory coincides with the incidence of the predominant related subtypes A and C that were found in both the feces of cattle and environmental samples on day 0. These subtypes may have been unique to and persistent at the facilities at SECRC, and the cattle could have been naturally infected with these subtypes upon entering the feed yard (but not their treatment pens) the month before the trials began. This hypothesis is supported by previous findings indicating that the finishing unit rather than introduction of new cattle was the source of *E. coli* O157:H7, particularly when there was persistence of the pathogen on environmental surfaces *(32).* Another explanation for the decrease in *E. coli* O157:H7 prevalence over the course of the study may have been the small number of cattle in the pens *(5, 9).*

The current study was conducted from June through October, months when a seasonal increase in detection of *E. coli* O157:H7 would be expected *(23, 45).* However, *E. coli* O157:H7 fecal shedding patterns observed in this study were inversely related to changes in the weather. During the trial, the average temperature was 19.4, 23.9, 27.8, 22.2, and 15.6°C for June, July, August, September, and October, respectively *(41).* These changes in temperature may be relevant for explaining the subtle spike in *E. coli* O157:H7 prevalence on or before day 110.

The reason for the introduction of genetically diverse *E. coli* O157:H7 subtypes D and H on day 110 is not clear. The subtypes differed from the predominant subtype by more than seven bands and were distinctly different from each other. These two unique subtypes were present in CON, YC,

TABLE 3. *Distribution of pulse-field gel electrophoresis (PFGE)* E. coli *O157:H7 subtypes for each dietary treatment on each collection day*[a]

	PFGE subtypes isolated from:				
Collection day	Environment	CON	YC	MON	YC × MON
0	A, C	A, C, G	A, C, E	A, B, C, F	A
28		A	ND	A	ND
56		NA	ND	NA	ND
84		ND	ND	ND	ND
110		D	D	H	A
125		NA	NA	ND	NA

[a] Treatments were a basal control (CON) diet, diet supplemented with yeast culture (YC; Diamond V XP, Diamond V Mills, Inc., Cedar Rapids, IA), diet supplemented with monensin (MON; Rumensin, Elanco, Division of Eli Lilly and Company, Greenfield, IN), and diet supplemented with YC and MON (YC × MON). Environmental samples were collected only on day 0 (pretreatment) from each treatment pen floor, feed bunk, and automatic water trough. Fecal samples were collected from every animal in each treatment group by rectal palpation on days 0, 28, 56, 84, 110, and 125. ND, *E. coli* O157:H7 was not detected, therefore PFGE analysis was not conducted. NA, *E. coli* O157:H7–positive isolates were detected but not analyzed by PFGE.

and MON cattle, and the only similarity between the cattle shedding these strains, aside from weather, was the basal diet. Subtypes D and H may have been introduced from the diet or during handling or delivery of feed, which also could account for the sudden appearance of these subtypes on day 110. Colonization of cattle by *E. coli* O157:H7 from contaminated feed or water sources has been reported *(13, 33, 34)*.

The small number of sample collection days resulted in low statistical power to detect differences between dietary inclusions of YC and MON, and diluted levels of *E. coli* O157:H7 in pooled fecal samples may have affected our ability to detect differences due to dietary treatment. The sensitivity of the detection methods decreases when *E. coli* O157:H7–positive fecal samples are mixed with *E. coli* O157:H7–negative samples *(47)*.

Feeding YC and MON, either individually or in combination, did not have a significant effect on *E. coli* O157:H7 fecal shedding in feedlot cattle consuming DDGS. Fecal shedding of *E. coli* O157:H7 was low (<10%) across all treatment groups throughout the study, which may have affected our ability to identify significant differences. Although the results of our research agreed with earlier reports concerning MON, there was no evidence to support the hypothesis that DDGS stimulated shedding of *E. coli* O157:H7 in the control group of cattle. We did obtain some evidence that supplementing the diets of finishing cattle with YC could more rapidly decrease, but not eliminate, fecal shedding of *E. coli* O157:H7 after initial application of the feed additive. PFGE characterization of environmental and fecal isolates revealed the dominance of several closely related strains, suggesting that feedlots may act as reservoirs of *E. coli* O157:H7 strains and may contribute to the dissemination and maintenance of this pathogen on the farm. Additional research is needed to confirm an inhibiting effect of YC on *E. coli* O157:H7 in cattle. More research is needed to evaluate various dosage levels of YC formulated with different ration components (e.g., with and without DDGS) and the introduction of the feed additive into the diet at different times before slaughter.

ACKNOWLEDGMENTS

This project was funded by the Colorado State University (CSU) Center for Meat Safety and Quality. We are indebted to the staff and graduate research assistants in the Department of Animal Sciences at CSU who volunteered assistance for this project.

REFERENCES

1. Aquarone, E. 1960. Penicillin and tetracycline as contamination control agents in alcoholic fermentation of sugarcane molasses. *Appl. Microbiol.* 8:263–268.
2. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
3. Barkocy-Gallagher, G. A., K. K. Edwards, X. Nou, J. M. Bosilevac, T. M. Arthur, S. D. Shackelford, and M. Koohmaraie. 2005. Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. *J. Food Prot.* 68: 2264–2268.
4. Bayrock, D. P., K. C. Thomas, and W. M. Ingledew. 2003. Control of *Lactobacillus* contaminants in continuous fuel ethanol fermentations by constant or pulsed addition of penicillin G. *Appl. Microbiol. Biotechnol.* 62:498–502.
5. Berry, E. D., J. E. Wells, T. M. Arthur, B. L. Woodbury, J. A. Nienaber, T. M. Brown-Brandl, and R. A. Eigenberg. 2010. Soil versus pond ash surfacing of feedlot pens: occurrence of *Escherichia coli* O157:H7 in cattle and persistence in manure. *J. Food Prot.* 73:1269–1277.
6. Berthiaume, R., I. Mandell, L. Faucitano, and C. Lafreniere. 2006. Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants. 1. Feedlot performance, carcass quality, and production costs. *J. Anim. Sci.* 84:2168–2177.
7. Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. Killinger-Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *J. Food Prot.* 66:748–754.
8. Brashears, M. M., D. Jaroni, and J. Trimble. 2003. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. *J. Food Prot.* 66:355–363.
9. Brown-Brandl, T. M., E. D. Berry, J. E. Wells, T. M. Arthur, and J. A. Nienaber. 2009. Impacts of individual animal response to heat and handling stress on *Escherichia coli* and *E. coli* O157:H7 fecal shedding by feedlot cattle. *Foodborne Pathog. Dis.* 6:855–864.
10. Callaway, T. R., R. C. Anderson, T. S. Edrington, R. O. Elder, K. J. Genovese, K. M. Bischoff, T. L. Poole, Y. S. Jung, R. B. Harvey, and D. J. Nisbet. 2003. Preslaughter intervention strategies to reduce food-borne pathogens in food animals. *J. Anim. Sci.* 81(E, Suppl. 2):E17–E23.
11. Carlson, B. A., K. K. Nightingale, G. L. Mason, J. R. Ruby, W. T. Choat, G. H. Loneragan, G. C. Smith, J. N. Sofos, and K. E. Belk. 2009. *Escherichia coli* O157:H7 strains that persist in feedlot cattle are genetically related and demonstrate an enhanced ability to adhere to intestinal epithelial cells. *Appl. Environ. Mircobiol.* 75:5927–5937.
12. Dargatz, D. A., S. J. Wells, L. A. Thomas, D. D. Hancock, and L. P. Garber. 1997. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot.* 60:466–470.
13. Davis, M. A., D. D. Hancock, D. H. Rice, D. R. Call, R. DiGiacomo, M. Samadpour, and T. E. Besser. 2003. Feedstuffs as a vehicle of cattle exposure to *Escherichia coli* O157:H7 and *Salmonella enterica*. *Vet. Microbiol.* 95:199–210.
14. Day, W. H., W. C. Serjak, J. R. Stratton, and L. Stone. 1954. Antibiotics as contamination control agents in grain alcohol fermentations. *J. Agric. Food Chem.* 2:252–258.
15. Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith, and M. D. Salman. 2005. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. *Foodborne Pathog. Dis.* 2:70–76.
16. Edrington, T. S., T. R. Callaway, K. M. Bischoff, K. J. Genovese, R. O. Elder, R. C. Anderson, and D. J. Nisbet. 2003. Effect of feeding the ionophores monensin and laidlomycin propionate and the antimicrobial bambermycin to sheep experimentally infected with *E. coli* O157:H7 and *Salmonella typhimurium*. *J. Anim. Sci.* 81:553–560.
17. Elam, N. A., J. F. Gleghorn, J. D. Rivera, M. L. Galyean, P. J. Defoor, M. M. Brashears, and S. M. Younts-Dahl. 2003. Effects of live cultures of *Lactobacillus acidophilus* (strains NP 45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass and intestinal characteristics, and *Escherichia coli* O157 shedding of finishing beef steers. *J. Anim. Sci.* 81:2686–2698.
18. Elder, R. O., J. E. Kenn, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Lagried. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides, and carcass of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
19. Fox, J. T., D. G. Renter, M. W. Sanderson, A. L. Nutsch, X. Shi, and T. G. Nagaraja. 2008. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. *J. Food Prot.* 71: 1761–1767.

20. Garber, L. P., S. J. Wells, D. D. Hancock, M. P. Doyle, J. Tuttle, J. A. Shere, and T. Zhoa. 1995. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. *J. Am. Vet. Med. Assoc.* 207:46–49.
21. Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* 125:1401–1412.
22. Grannis, J., and D. Thilmany. 2000. Marketing opportunities for natural beef products in the intermountain west. Colorado State University Cooperative Extension, Agricultural Marketing Report 2000. Available at: http://dare.agsci.colostate.edu/extension/pubs.html. Accessed 2 November 2007.
23. Hancock, D. D., T. E. Besser, D. H. Rice, D. E. Herriott, and P. I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.* 118:193–195.
24. Herriott, D. E., D. D. Hancock, E. D. Ebel, L. V. Carpenter, D. H. Rice, and T. E. Besser. 1998. Association of herd management factors with colonization of dairy cattle by Shiga toxin–positive *Escherichia coli* O157. *J. Food Prot.* 61:802–807.
25. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
26. Hunter, S. B., P. Vauterin, M. A. Lambert-Fair, M. S. Van Duyne, K. Kubota, L. Graves, D. Wrigley, T. Barrett, and E. Ribot. 2005. Establishment of a universal size strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J. Clin. Microbiol.* 43:1045–1050.
27. Jacob, M. E., J. T. Fox, J. S. Drouillard, D. J. Renter, and T. G. Nagaraja. 2008. Effects of dried distiller's grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations of cattle. *Appl. Environ. Microbiol.* 74:38–43.
28. Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2008. Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. *J. Anim. Sci.* 86:1182–1190.
29. Jacob, M. E., Z. D. Paddock, D. G. Renter, K. F. Lechtenberg, and T. G. Nagaraja. 2010. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 76:7238–7242.
30. Jacob, M. E., G. L. Parsons, M. K. Shelor, J. T. Fox, J. S. Drouillard, D. U. Thomson, D. G. Renter, and T. G. Nagaraja. 2008. Feeding supplemental dried distiller's grains increases faecal shedding of *Escherichia coli* O157 in experimentally inoculated calves. *Zoonoses Public Health* 55:125–132.
31. Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley. 2003. Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.* 66:1972–1977.
32. Lahti, E., R. Olli, 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.
33. LeJeune, J. T., T. E. Besser, and D. D. Hancock. 2001. Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl. Environ. Microbiol.* 67:3053–3057.
34. LeJeune, J. T., T. E. Besser, N. L. Merrill, D. H. Rice, and D. D. Hancock. 2001. Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *J. Dairy Sci.* 84:1856–1862.
35. LeJeune, J. T., and A. N. Wetzel. 2007. Preharvest control of *Escherichia coli* O157:H7 in cattle. *J. Anim. Sci.* 85(E, Suppl.): E73–E80.
36. Lema, M., L. Williams, and D. R. Rao. 2001. Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157:H7 in lambs by feeding microbial feed supplement. *Small Ruminant Res.* 39:31–39.
37. McAllister, T. A., S. J. Bach, K. Stanford, and T. R. Callaway. 2006. Shedding of *Escherichia coli* O157:H7 by cattle fed diets containing monensin or tylosin. *J. Food Prot.* 69:2075–2083.
38. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCraig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illnesses and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
39. Medellin-Pena, M. J., J. H. Wang, R. Johnson, S. Anand, and M. W. Griffiths. 2007. Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 73:4259–4267.
40. Narendranath, N. V., S. H. Hynes, K. C. Thomas, and W. M. Ingledew. 1997. Effects of lactobacilli on yeast-catalyzed ethanol fermentations. *Appl. Environ. Microbiol.* 63:4158–4163.
41. National Oceanic and Atmospheric Administration. 2008. National Weather Service climate services. Available at: http://www.weather.gov/climate/index.php. Accessed 30 December 2008.
42. National Research Council. 2000. Nutrient requirements of beef cattle, 7th rev. ed. National Academies Press, Washington, DC.
43. Pederson, C., H. Jonson, J. E. Lindberg, and S. Roos. 2004. Microbiological characterization of wet wheat distiller's grain, with focus on isolation of lactobacilli with potential as probiotics. *Appl. Environ. Microbiol.* 70:1522–1527.
44. Pew Commission on Industrial Farm Animal Production. 2006. Putting meat on the table: industrial farm animal production in America. The Pew Charitable Trusts and John Hopkins Bloomberg School of Public Health. Available at: http://www.ncifap.org/reports.html. Accessed April 2009.
45. Rasmussen, M. A., and T. A. Casey. 2001. Environmental and food safety aspects of *Escherichia coli* O157:H7 infections in cattle. *Crit. Rev. Microbiol.* 27:57–73.
46. Ribot, E. M., M. A. Fair, R. Gautom, D. N. Cameron, S. B. Hunter, B. Swaminathan, and T. J. Barrett. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Food. Pathog. Dis.* 3:59–68.
47. Sanderson, M. J., J. M. Sargeant, and T. G. Nagaraja. 2005. Effect of pooling bovine fecal samples on the sensitivity of detection of *E. coli* O157:H7. *Vet. Microbiol.* 110:125–130.
48. Schelling, G. T. 1984. Monensin mode of action in the rumen. *J. Anim. Sci.* 58:1518–1527.
49. Steer, T., H. Carpenter, K. Tuohy, and G. R. Gibson. 2000. Perspectives on the role of the human gut microbiota and its modulation by pro and prebiotics. *Nutr. Res. Rev.* 13:229–254.
50. Stephens, T. P., T. A. McAllister, and K. Stanford. 2009. Perineal swabs reveal the effect of super shedders on the transmission of *Escherichia coli* O157:H7 in commercial feedlots. *J. Anim. Sci.* 87:4151–4160.
51. Stevens, M. P., P. M. van Diemen, F. Dziva, P. W. Jones, and T. S. Wallis. 2002. Options for the control of enterohaemorrhagic *Escherichia coli* in ruminants. *Microbiology* 148:3767–3778.
52. Synge, B. A., M. E. Chase-Topping, G. F. Hopkins, I. J. McKendrick, F. Thomson-Carter, D. Gray, S. M. Rusbridge, F. I. Munro, G. Foster, and G. J. Gunn. 2003. Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows. *Epidemiol. Infect.* 130:301–312.
53. Tabe, E. S., J. Oloya, D. K. Doetkott, M. L. Bauer, P. S. Gibbs, and M. L. Khaitsa. 2008. Comparative effect of direct-fed microbials on fecal shedding of *Escherichia coli* O157:H7 and *Salmonella* in naturally infected feedlot cattle. *J. Food Prot.* 71:539–544.
54. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelson, B. A. Murray, D. A. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacteria strain typing. *J. Clin. Microbiol.* 33:2233–2239.
55. Thompson, P., C. Harris, D. Holt, and E. A. Pajor. 2007. Livestock welfare product claims: the emerging social index. *J. Anim. Sci.* 85:2354–2360.
56. Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedlot consulting nutritionists: the 2007 Texas Tech University survey. *J. Anim. Sci.* 85:2772–2781.
57. Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, M. N. Guerini, V. H. Varel, T. M. Arthur, J. M. Bosilevac, H. C. Freetly, T. L. Wheeler, C. L. Ferrell, and M. Koohmaraie. 2009. Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. *J. Food Prot.* 72:1624–1633.