Journal of Food Protection, Vol. 66, No. 5, 2003, Pages 748–754

Prevalence of *Escherichia coli* O157:H7 and Performance by Beef Feedlot Cattle Given *Lactobacillus* Direct-Fed Microbials†

M. M. BRASHEARS,[1]* M. L. GALYEAN,[1] G. H. LONERAGAN,[2] J. E. MANN,[1] AND K. KILLINGER-MANN[1]

[1]*Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas 79409; and* [2]*Division of Agriculture, West Texas A&M University, Canyon, Texas 79016, USA*

MS 02-312: Received 12 September 2002/Accepted 3 November 2002

ABSTRACT

Fecal shedding of *Escherichia coli* O157:H7, the prevalence of *Escherichia coli* O157:H7 in pens and on carcasses and hides, and cattle performance as a result of daily dietary supplementation with *Lactobacillus*-based direct-fed microbials (DFMs) were evaluated in a feeding trial involving 180 beef steers. Steers were evaluated for shedding of *E. coli* O157:H7 by an immunomagnetic separation technique on arrival at the feedlot, just before treatment with the DFMs, and every 14 days thereafter until slaughter. Composite pen fecal samples were collected every 14 days (alternating weeks with animal testing), and prevalence on hides and carcasses at slaughter was also evaluated. Feedlot performance (body weight gain and feed intake) was measured for the period during which the DFMs were fed. Gain efficiency was calculated as the ratio of weight gain to feed intake. *Lactobacillus acidophilus* NPC 747 decreased ($P < 0.01$) the shedding of *E. coli* O157:H7 in the feces of individual cattle during the feeding period. *E. coli* O157:H7 was approximately twice as likely to be detected in control animal samples as in samples from animals receiving *L. acidophilus* NPC 747. In addition, DFM supplementation decreased ($P < 0.05$) the number of *E. coli* O157:H7–positive hide samples at harvest and the number of pens testing positive for the pathogen. Body weight gains (on a live or carcass basis) and feed intakes during the DFM supplementation period did not differ among treatments. Gain efficiencies on a live-weight basis did not differ among treatments, but carcass-based gain/feed ratios tended ($P < 0.06$) to be better for animals receiving the two DFM treatments than for control animals. The results of this study suggest that the feeding of a *Lactobacillus*-based DFM to cattle will decrease, but not eliminate, fecal shedding of *E. coli* O157:H7, as well as contamination on hides, without detrimental effects on performance.

Escherichia coli O157:H7 was first recognized as a foodborne pathogen in 1982 following two outbreaks associated with undercooked meat products *(11, 15).* Since then, numerous outbreaks have been directly or indirectly associated with beef products. Beef products can become contaminated with pathogenic *E. coli* and other pathogens associated with the intestinal tract of the live animal in several ways. Direct contact with the fecal material, ingesta, or hide could result in a contaminated product. Additionally, dust created in the processing plant environment during the slaughter process can settle on carcasses and result in contamination. Recent studies have indicated that the hides of animals can be a source of contamination of the carcasses *(3, 9).* Although a number of interventions are effective in decreasing the pathogen load in the processing environment, reduction of the pathogen load at the preharvest stage is also important.

Early studies indicated that the prevalence of *E. coli* O157:H7 was relatively low (<5%) *(4).* With improved methods of detection, however, it has become evident that the prevalence of the pathogen in the feedlot environment is higher than previously reported. Recent estimates of the prevalence of *E. coli* O157:H7 in the feces of feedlot cattle include 15.7% *(4),* 28% *(5),* and 23% *(13).* Furthermore, it has been reported that 72% of pens and 100% of feedlots in one study *(13)* contained at least one *E. coli* O157:H7–positive animal. Because there is a direct correlation between positive animal samples and positive carcass samples *(5),* preharvest intervention strategies should be developed and implemented to decrease the pathogen load.

Several treatments, including dietary modifications (high-roughage versus high-energy diets), fasting, vaccinations, sodium chlorate treatment, bacteriophage therapy, and treatment with colicins, have been investigated as preharvest intervention methods for beef feedlot cattle. Zhao et al. *(16)* reported that the use of direct-fed microbials (DFMs) might be effective in decreasing *E. coli* O157:H7 in cattle. These investigators fed cattle a combination of nonpathogenic *E. coli* and *Proteus mirabilis* and reported that *E. coli* O157:H7 was detected in treated animals for only 9 to 17 days, whereas the control animals were *E. coli* O157:H7 positive for 32 days.

Lactic acid bacteria (LAB) inhibit pathogens in laboratory media and in foods; however, their ability to decrease pathogens in beef feedlot cattle has not previously been reported. Brashears et al. *(2)* reported that LAB isolates from cattle were effective in decreasing *E. coli* in laboratory media, ruminal fluid, and manure, indicating that they might be effective in decreasing the pathogen load in the live animal and, ultimately, in the feedlot environment.

* Author for correspondence. Tel: 806-742-2469; Fax: 806-742-2427; E-mail: mindy.brashears@ttu.edu.

† Manuscript number T-5-437, College of Agricultural Sciences and Natural Resources, Texas Tech University.

TABLE 1. *Composition of the 90% concentrate experimental diet*

Ingredient	% of DM
Alfalfa hay, ground	4.96
Cottonseed hulls	5.04
Steam-flaked corn	64.60
Dry-rolled corn	10.15
Cottonseed meal	4.82
Molasses	4.12
Fat (yellow grease)	2.93
Urea	0.90
Premix[a]	2.48

[a] The composition (DM) of the premix was as follows: cottonseed meal, 23.9733%; high-calcium limestone, 42.1053%; dicalcium phosphate, 1.0363%; potassium chloride, 8.0000%; magnesium oxide, 3.5587%; ammonium sulfate, 6.6667%; salt, 12.0000%; cobalt carbonate, 0.0017%; copper sulfate, 0.1572%; iron sulfate, 0.1333%; ethylenediamine dihydroiodide, 0.0025%; manganese oxide, 0.2667%; selenium premix (0.2% Se), 0.1000%; zinc sulfate, 0.8251%; vitamin A (650,000 IU/g), 0.0122%; vitamin E (275 IU/g), 0.1260%; Rumensin (176.4 g/kg), 0.6750%; Tylan (88.2 g/kg), 0.3600%. Concentrations given for sources of vitamins A and E, Rumensin (monensin; Elanco Animal Health), and Tylan (tylosin; Elanco Animal Health) are on a 90% DM basis.

The objectives of this study were to determine the efficacy of *Lactobacillus*-based DFMs fed to feedlot cattle as a daily feed supplement in reducing the shedding of *E. coli* O157:H7 in the feces of the live animal and the prevalence of the pathogen on hides and to determine the effect of DFMs on animal performance.

MATERIALS AND METHODS

Source of cattle. One hundred eighty-five steers of British breeding (primarily Angus, Hereford, and Angus × Hereford) were purchased at an auction in Pratt, Kans., and transported to the Texas Tech University Burnett Center. On arrival, each steer's body weight (BW) was measured and each steer received a uniquely numbered ear tag, a vaccination with a clostridial vaccine (UltraChoice 7, Pfizer Animal Health, Exton, Pa.), a vaccination with a modified-live IBR-BVD-PI3-BRSV vaccine (Bovishield 4 + Lepto; Pfizer Animal Health), and treatment down the back line with cydectin (Dectomax, Pfizer Animal Health). Steers were sorted into 37 concrete pens (2.9 by 5.6 m; five steers per pen) with partially slotted floors and offered a 65% concentrate starter diet for 5 days, a 70% concentrate diet for the following 5 days, and then an 80% concentrate diet.

All cattle were weighed 12 days after their arrival to initiate the preliminary phase of the experiment. At this time, a growth promoter (120 mg of trenbolone acetate and 24 mg of estradiol; Revalor S Intervet, Millsboro, Del.) was implanted in the right ear of each steer. Pen assignments did not change. On day 15, all cattle were switched to the final 90% concentrate diet (Table 1). Each steer was weighed after 49 days, and individual fecal samples were obtained for the isolation of *E. coli* O157:H7.

Treatment and pen assignments. The experimental design was a randomized complete block, with pen as the experimental unit (12 pens for each of three treatments, with five steers per pen). Cattle were blocked by BW without respect to previous shedding of *E. coli* O157. Five steers were designated extra cattle. The data for the 180 selected steers were then sorted by BW from lightest (block 1) to heaviest (block 12), with 15 steers per block. Within each block, steers were randomly assigned a control treatment (90% concentrate diet with carrier [lactose] dissolved in water and added to the diet before feed delivery), an NPC 747 treatment (90% concentrate diet with 1×10^9 CFU of *Lactobacillus acidophilus* NPC 747 per steer, mixed in water and added to the diet before feed delivery), or an NPC 750 treatment (90% concentrate diet with 1×10^9 CFU of *Lactobacillus cristatus* NPC 750 per steer, mixed in water and added to the diet before feed delivery). Blocks were assigned to three contiguous pens at the Burnett Center, and treatments were assigned randomly to pens within blocks.

Each DFM treatment (lactose for the control treatment) was prepared by a commercial culture company (Nutrition Physiology Corporation, Indianapolis, Ind.) under proprietary culture conditions. The treatments were prepackaged in aluminum foil packets. Each packet was color-coded to correspond to a treatment, with green indicating the control treatment, red indicating the NPC 747 treatment, and yellow indicating the NPC 750 treatment. One packet supplied the desired dose of microbial culture (or the equivalent quantity of carrier for the control treatment) for the 12 pens of cattle receiving each treatment. For each treatment, the contents of the packet were mixed with 2.5 liters of distilled water in a plastic sprinkler can, and then the contents of the sprinkler can were poured on the diet (1,500 lb; approximately 680 kg) as it was mixed in a feed mixer–delivery unit. Three sprinkler cans, each color-coded for one of the three treatments, were used. The culture company has performed proprietary studies indicating that this method results in uniform mixing of the culture as well as uniform delivery to the animals. Cultures were fed daily.

Experimental diets. The composition of the 90% concentrate diet is shown in Table 1. These data reflect adjustments for the average dry matter (DM) content of feed ingredients while DFMs were fed. Each diet contained the same intermediate premix (Table 1), which supplied protein, various minerals and vitamins, monensin (33 mg/kg, DM basis; Rumensin-80, Elanco Animal Health, Indianapolis, Ind.), and tylosin (8.8 mg/kg, DM basis; Tylan-40, Elanco Animal Health).

Management, feeding procedures, weighing procedures, and carcass data collection. Each feed bunk of the 36 pens was evaluated visually at approximately 0700 to 0730 h daily. The quantity of feed remaining in each bunk was estimated, and the suggested daily allotment of feed for each pen was recorded. The three treatment diets were mixed in a 1.27-m^3-capacity paddle mixer (Marion Mixer, Marion, Iowa). Once the total quantity of feed for a given treatment was mixed, the batch was released from the mixer and delivered by a drag chain conveyer to a self-propelled feed mixer–delivery system (Roto-Mix 84-8, Dodge City, Kans.). After feed had been delivered and the mixer unit was operating, the contents of the sprinkler can for a given treatment were poured onto the diet. After mixing for approximately 4 to 5 min, the quantity of feed allotted to each of the 12 pens receiving a given treatment was then weighed to the nearest 0.45 kg with the use of the load cells and indicator on the mixer-delivery unit. The feeding order of treatment diets throughout the experiment was control, NPC 747, and NPC 750. Clean-out of the mixer-delivery unit was monitored closely to avoid cross-contamination of diets, and at least one batch of a diet from another experiment (equivalent to the control diet) was mixed between the NPC 747 and NPC 750 treatment diets.

DM determinations for ingredients used in the experimental diets were carried out every 2 weeks throughout the experiment.

In addition, samples of mixed feed delivered to feed bunks were taken weekly throughout the experiment. Samples of feed taken from the bunks were composited for the entire preliminary period and for each interval for which cattle were weighed after the DFM treatments were initiated. These feed samples were ground to pass a 2-mm screen in a Wiley mill and analyzed for DM, ash, crude protein, acid detergent fiber, Ca, and P *(1)*.

Feed bunks were cleaned, and unconsumed feed was weighed (±0.045 kg) at intervals corresponding to intermediate weigh dates throughout the trial. The DM contents of these feed bunk weigh-back samples was determined in a forced-air oven by drying for approximately 20 h at 100°C. The DM intake (DMI) for each pen was calculated by multiplying the DM content of the delivered feed by the total feed delivery to each pen, with correction for the DM of any feed weighed back from each pen.

All BW measurements taken during the experiment were obtained with a single-animal scale set on four load cells. The scale was calibrated with 453.5 kg of certified weights (Texas Department of Agriculture, Austin, Tex.) before use. Intermediate BW measurements were taken every 28 days after the initiation of DFM feeding to assess the performance of the cattle and the shedding of *E. coli* O157:H7 on a regular basis. Fecal grab samples were collected during regularly scheduled BW measurements, at 14-day intervals between the regularly scheduled BW measurements, and when cattle were shipped to slaughter. Because the cattle were blocked by BW, blocks were deemed to have reached USDA (U.S. Department of Agriculture) Choice grade quality after different periods of treatment. All 180 cattle in the study were shipped for slaughter to the Excel Corp. facility in Plainview, Tex. Steers in blocks 11 and 12 were shipped after 45 days of treatment, those in blocks 9 and 10 were shipped after 55 days of treatment, those in blocks 5 through 8 were shipped after 64 days of treatment, those in blocks 3 and 4 were shipped after 84 days of treatment, and those in blocks 1 and 2 were shipped after 108 days of treatment. Routine carcass measurements included hot carcass weight (the weight of the carcass immediately after slaughter); longissimus muscle area; marbling score; percentage of kidney, pelvic, and heart fat; fat thickness between the 12th and 13th ribs; USDA yield grade; and USDA quality grade.

Microbiological measurements. Fecal samples were taken directly from the rectum of each animal on arrival at the feedlot (day 49) and every 14 days following the start of DFM supplementation. Approximately 50 to 100 g of sample was aseptically collected with a sterile glove. Pen-level composite fecal samples from the floor of the pen were also taken every 14 days, alternating weekly with individual animal samples. A composite sample consisted of five fresh fecal pats taken from the floor of each pen.

When cattle were harvested immediately after stunning, sterile gauze pads (5.1 by 5.1 cm) saturated with sterile distilled water were used to sample a 450-cm^2 area of the ventral brisket. A 10-g sample was placed in 20 ml of sterile 2% brilliant green bile and held for transport to the laboratory. Fecal grab samples were taken directly from the rectum of the animal after stunning. All animals in the study were sampled.

Carcasses were sampled before chilling and before any intervention treatments. A sterile Speci-Sponge (Nasco, Fort Atkinson, Wis.) was hydrated with sterile Butterfield's phosphate diluent. Sponges were used to sample the carcasses at three locations (brisket, flank, and round) as described by the USDA *(6)*. All samples were kept cool (with special care being taken to prevent freezing) in an ice chest during transport from the farm or the plant.

A sensitive assay involving immunomagnetic separation was used to isolate *E. coli* O157:H7 within 2 to 6 h after collection *(5)*. Ninety milliliters of GN-VCC broth (GN broth with 8 μg of vancomycin per ml, 50 ng of cefixime per ml, and 10 μg of cefsulodin per ml) was inoculated with 10 g of feces or with 10 ml of exudate from the sponge samples and incubated for 6 h at 37°C. *E. coli* cells were subjected to immunomagnetic separation by mixing 1 ml of the culture described above with 20 μl of anti-O157:H7 beads (Dynal, Lake Success, N.Y.) for 30 min at room temperature. Beads were washed three times in phosphate-buffered saline–Tween 20, and 50 μl of the bead-bacteria mixture was spread onto CT-SMAC plates (sorbitol MacConkey agar plates containing 50 ng of cefixime per ml and 2.5 μg of tellurite per ml) and streaked for isolation. Plates were incubated overnight at 37°C. To verify the purity of colony selection, 3 to 10 sorbitol-negative colonies (depending on the number of colonies on the plate) were picked and streaked for isolation on CT-SMAC. Plates were incubated overnight at 37°C. A single colony from the CT-SMAC plate described above was selected and inoculated on MacConkey agar, on Fluorocult agar, and in MacConkey broth. Broth was incubated overnight at 37°C. Methylumbelliferyl-β-glucuronide–negative, lactose-positive colonies were selected, and indole, triple sugar iron, and Voges-Proskauer tests were conducted on selected colonies. Colonies that were indole-positive, A/A (glucose and lactose and/or sucrose fermentation), or K/A (glucose fermentation only, peptone catabolized) plus gas and Voges-Proskauer–negative were boiled (by using cells from the MacConkey broth described above) and tested for the O157:H7 antigen with a latex agglutination kit (Remel, Lenexa, Kans.). Colonies were subcultured on blood agar. The H7 agglutination and API 20 tests were conducted for O157:H7-positive cells for confirmation. For final confirmation, polymerase chain reaction analysis for the O157:H7 antigen was carried out with a Dupont BAX system (Dupont Qualicon, Wilmington, Del.).

Statistical analyses. All analyses were performed with the use of computational routines of SAS (SAS Institute Inc., Cary, N.C.). All performance and carcass data were analyzed with pen as the experimental unit. A randomized complete block design was used, and computations were carried out with the GLM procedure of SAS. The effect of treatment and block were included in the model for pen-based data. Carcass data were entered on an individual-animal basis and analyzed with a model that included effects of treatment, block, and block × treatment. Block × treatment was specified as the error term for the testing of treatment effects with carcass data. Two orthogonal contrasts were used to test treatment effects: (i) the control treatment versus the average for the NPC 747 and NPC 750 treatments and (ii) the NPC 747 treatment versus the NPC 750 treatment. Carcass quality grade data were analyzed by chi-square techniques (FREQ procedure of SAS) with the use of individual-animal data.

Microbiological data were entered into an electronic spreadsheet, and descriptive statistics were generated. Analytical modeling was carried out with the use of a macro developed for the generalized linear modeling of mixed models with categorical response variables. A binomial error distribution was used in each of the models. Longitudinal data were modeled with the use of repeated-measures methods for mixed models *(8)*. First-order autoregressive matrices were used to model within-pen covariance. Because of a lack of model convergence, the samples collected from blocks 1 and 2 immediately before slaughter were excluded from the repeated-measures analyses. Time was treated as a continuous variable, and block was treated as a random variable.

Because cattle were harvested at four different times at in-

TABLE 2. *Effects of live cultures of* Lactobacillus acidophilus *NPC 747 and acidophilus NPC 750 on performance of finishing beef steers*

Item	Treatment[a]			SE[b]	Contrast[c]	
	Control	NPC 747	NPC 750		Control vs others	747 vs 750
Initial BW (kg)	470.4	466.7	466.9	1.08	0.013	0.895
Final BW (kg)	579.5	576.6	582.0	3.13	0.971	0.235
Adjusted final BW (kg)[d]	576.9	578.9	582.0	3.49	0.418	0.525
Daily gain (kg)						
Day 0 to day 28	1.85	1.94	1.96	0.068	0.207	0.782
Day 0 to end[e]	1.59	1.58	1.66	0.046	0.569	0.278
Adjusted day 0 to end[d]	1.56	1.62	1.67	0.048	0.144	0.462
Daily DMI (kg/steer)						
Day 0 to day 28	9.08	9.20	9.23	0.120	0.352	0.846
Day 0 to end[e]	9.32	9.22	9.46	0.113	0.875	0.146
Gain/feed ratio						
Day 0 to day 28	0.203	0.210	0.213	0.0063	0.297	0.734
Day 0 to end[e]	0.170	0.172	0.175	0.0033	0.443	0.439
Adjusted day 0 to end[d]	0.167	0.176	0.177	0.0038	0.060	0.822

[a] Control, standard TTU Burnett Center 90% concentrate diet with carrier (lactose) mixed in water and added to the diet at the time of feeding; NPOC 747, control plus 1×10^9 CFU of *L. acidophilus* NPC 747 per animal; NPC 750, control plus 1×10^9 CFU of *L. acidophilus* NPC 750 per animal. Average time on feed, 70 days.

[b] Pooled standard error of treatment means ($n = 12$ pens per treatment).

[c] Observed significance level for the orthogonal contrasts.

[d] Adjusted final BW was calculated as hot carcass weight/average dress of 62.41%. Adjusted daily gain was calculated as (adjusted final BW − initial BW)/days on feed. Adjusted feed/gain was the ratio of daily DMI to adjusted daily gain.

[e] Days from 0 to end varied among blocks: blocks 1 and 2 = 108 days; blocks 3 and 4 = 84 days; blocks 5 through 8 = 64 days; blocks 9 and 10 = 55 days; and blocks 11 and 12 = 45 days.

tervals of approximately 1 to 2 weeks, a dummy variable was created for each harvest. This variable was forced in the model as a random classification variable.

The number of positive samples per animal over time was modeled by logistic regression techniques. Block was treated as a random variable. Furthermore, a dummy variable was created for each steer and given a value of 1 when that animal had at least one positive fecal sample and a value of 0 otherwise. This dummy variable was then modeled with the use of logistic regression techniques.

Maximum-likelihood confidence limits were computed. The antilog of the parameter estimates was calculated to produce odds ratios and 95% confidence intervals. Treatment groups receiving DFMs were compared with those receiving the control treatment. If an overall type 3 test of treatment was associated with a P value of ≤ 0.10, a further analysis was performed to compare the NPC 747 and NPC 750 treatment groups.

RESULTS AND DISCUSSION

Performance data. The analyzed nutrient content of the 90% concentrate diet fed to cattle during the treatment period was generally in close agreement with formulated values. Averaged over the treatment feeding period, the diet contained (DM basis) 12.7% crude protein, 4.5% ash, 8.8% acid detergent fiber, 0.56% Ca, and 0.30% P.

By chance in the allotment process, initial BWs differed slightly among the three treatment groups (Table 2), with control cattle being approximately 3.6 kg heavier ($P \leq 0.013$) than those in the NPC 747 and NPC 750 treatment groups when the DFM treatments were initiated. However, final BWs (after an average of 70 days of treatment) and final BWs calculated on the basis of hot carcass weight divided by the average dressing percentage (adjusted final BW) did not differ among the three treatments. The calculation of final BW on the basis of hot carcass weight divided by a common dressing percentage was performed in an effort to decrease the effect that differences in gastrointestinal tract fill and/or weight of gastrointestinal tract tissues might have on the final BW data.

No differences were noted among treatments for average daily gain (ADG) based on either live weight ($P > 0.20$) or carcass adjusted weight ($P > 0.14$). Moreover, no differences in DMI were detected among treatments. Gain efficiencies based on live weight gain did not differ ($P > 0.43$) among treatments for the overall feeding period, but gain efficiencies based on carcass adjusted gain tended ($P < 0.06$) to be greater for the two DFM treatments than for the control treatment. Results of previous research conducted at the Burnett Center *(7)* indicated that the addition of live cultures of *L. acidophilus* 45 and/or *L. acidophilus* 51 plus *Propionibacterium freudenreichii* (PF-24) to feed increased ADG by 2.2 to 5.4% for growing and finishing steers compared with a control diet. On average, for the three DFM treatments used in that study, ADG was increased by 4.3% ($P < 0.06$) relative to that resulting from the control treatment. The DFM used in that study also increased daily DMI to a level slightly above that for the control treatment, but differences were not significant. Rust et al. *(12)* fed cattle microbial culture treatments similar to those used by Galyean et al. *(7)* and reported

TABLE 3. *Effects of live cultures of* Lactobacillus acidophilus *NPC 747 and* L. acidophilus *NPC 750 on carcass characteristics of finishing beef steers*

Item[a]	Treatment[b]			SE[c]	Contrast[d]	
	Control	NPC 747	NPC 750		Control vs others	747 vs 750
Hot carcass wt (kg)	360.2	361.4	363.4	2.18	0.418	0.525
Dressing %	62.14	62.65	62.43	0.227	0.164	0.486
LM area (cm^2)	83.68	86.64	85.42	1.032	0.072	0.426
Fat thickness (cm)	1.19	1.19	1.27	0.041	0.485	0.139
KPH (%)	1.98	1.94	2.01	0.028	0.810	0.106
Yield grade	3.17	3.03	3.20	0.072	0.560	0.107
Marbling score[e]	428.7	422.7	403.7	7.57	0.109	0.090
USDA grade						
Choice (%)	60.00	53.33	46.67	—	—	—
Select (%)	40.00	46.67	53.33	—	—	—

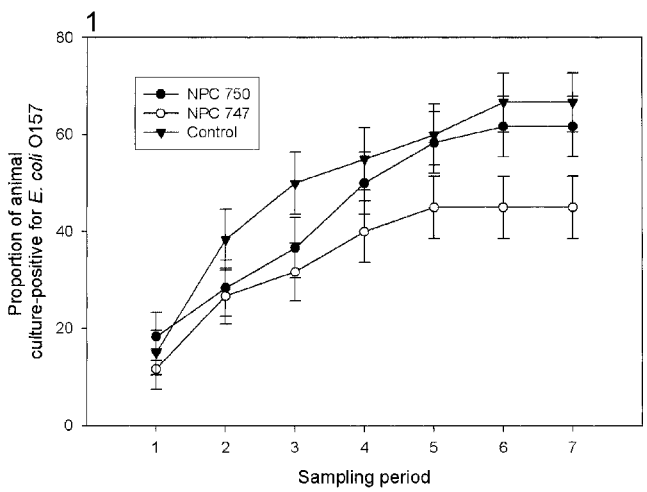
[a] LM, longissimus muscle; KPH, kidney, pelvic, and heart fat.
[b] Control, standard TTU Burnett Center 90% concentrate diet with carrier (lactose) mixed in water and added to the diet at the time of feeding; NPOC 747, control plus 1×10^9 CFU of *L. acidophilus* NPC 747 per animal; NPC 750, control plus 1×10^9 CFU of *L. acidophilus* NPC 750 per animal. Average time on feed, 70 days. Distributions of USDA Choice and USDA Select plus Standard carcasses did not differ among treatments ($P > 0.34$).
[c] Pooled standard error of treatment means ($n = 12$ pens per treatment).
[d] Observed significance level for orthogonal contrasts.
[e] 300 = slight; 400 = small; 500 = modest.

a 6.9% increase in ADG and a 7.3% improvement in feed/gain ratio with DFM treatments relative to those obtained with a control treatment.

Calculated net-energy-for-maintenance and net-energy-for-gain concentrations *(10)* for the treatment diets (data not shown) suggested that cattle on the two DFM diets converted DMI to gain at approximately the same efficiency as control cattle. Results obtained by Galyean et al. *(7)* and Rust et al. *(12)* are not directly comparable to the present results because of differences in strains of *Lactobacillus,* the feeding of *Propionibacterium,* and shorter treatment periods in the present study, but both studies suggest that the addition of DFMs to feed has no negative effects and potentially positive effects on performance.

Carcass data. Carcass data are shown in Table 3. No major differences in carcass data were noted among the three treatments groups. Control steers tended ($P < 0.072$) to have slightly smaller longissimus muscle areas than cattle in the NPC 747 and NPC 750 treatment groups, and steers in the NPC 750 treatment group tended ($P < 0.09$) to have lower marbling scores than those in the NPC 747 treatment group. Galyean et al. *(7)* reported that with the exception of hot carcass weight, which was greater for cattle fed DFM than for controls, carcass characteristics were not greatly affected by the feeding of various *Lactobacillus* cultures plus *P. freudenreichii* PF-24 to finishing steers.

***E. coli* O157:H7.** *E. coli* O157:H7 was isolated from 155 (12.0%) of the 1,290 fecal samples collected from an-



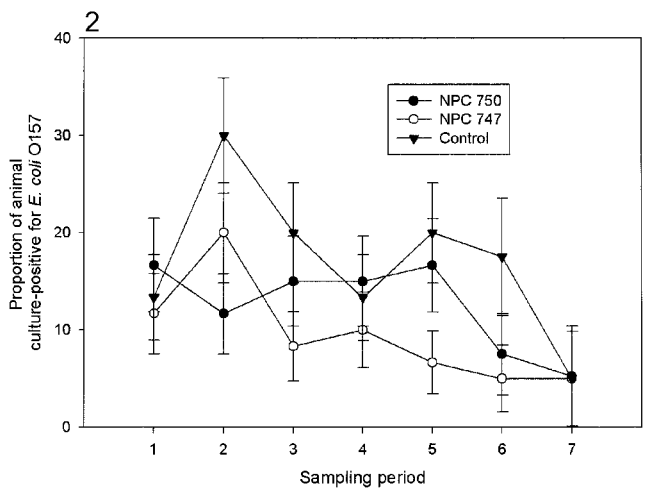


FIGURE 1. *Cumulative proportions of steers that were culture positive for* E. coli *O157:H7 by treatment group and by sampling period.*

FIGURE 2. *Proportions of steers that were culture positive for* E. coli *O157:H7 by treatment group and by sampling period.*

TABLE 4. *Overall type 3 tests, odds ratios, confidence intervals (CIs), and* P *values for time of sample collection and pairwise comparisons of treatment groups*

Test	Odds ratio	CI	*P*
Treatment	—	—	0.021
Time	0.89	0.79–1.00	0.056
NPC 750 vs control	0.70	0.45–1.10	0.122
NPC 747 vs control	0.51	0.32–0.82	0.006
NPC 750 vs NPC 747	1.38	0.85–2.26	0.195

imals. Averaged over time, the prevalence of *E. coli* O157:H7 varied with treatment group ($P = 0.02$); the bacterium was detected in 45.0, 60.0, and 65.0% of animals receiving NPC 747, NPC 750, and control diets, respectively (Fig. 1). More culture-positive animals were identified in the control group than in the NPC 747 group ($P = 0.03$). Averaged over time, *E. coli* O157:H7 was 49.0% less likely to be detected in animals receiving the NPC 747 treatment than in control animals ($P < 0.01$; Table 4). No differences between the prevalence of culture-positive animals in the NPC 750 group and that in the control group ($P = 0.122$) or between that in the NPC 747 group and that in the 750 group ($P = 0.195$) were observed when data were averaged over time.

The proportion of fecal samples from which *E. coli* O157:H7 was cultured tended to decrease over time ($P = 0.06$; Fig. 2). On average, each subsequent sampling period was associated with an 11.1% decrease in the odds of isolating *E. coli* O157:H7 (Table 4). During the third sampling period, *E. coli* O157:H7 was isolated from 20.6% of cattle (37 cattle), compared with 5.1% (3 cattle) for the eighth sampling period.

For culture-positive animals, the frequency of *E. coli* O157:H7 detection varied among treatments ($P = 0.01$). *E. coli* O157:H7 was detected less frequently in culture-positive animals receiving NPC 750 ($P = 0.02$) and NPC 747 ($P = 0.06$) than in control animals (Table 4). A difference between the frequency of the isolation of *E. coli* O157:H7 from culture-positive animals for the NPC 747 group and that for the NPC 750 group was not detected ($P = 0.77$). The estimated numbers of positive samples for typical culture-positive steers were 1.39, 1.41, and 1.73 for animals receiving NPC 750, NPC 747, and the control diet, respectively.

The NPC 747 treatment resulted in decreased ($P = 0.03$) detection of *E. coli* O157:H7 averaged over time. There was also evidence that the NPC 750 treatment was effective, but not as effective as the NPC 747 treatment. Even though the NPC 747 treatment decreased shedding, this effect was not as evident for the animal-level fecal samples when the animals went to slaughter, because the prevalence at slaughter had decreased to levels (<10%) that were too low for the detection of statistical levels of significance. However, the feeding of DFMs may be of benefit when carcass contamination results primarily from *E. coli* O157:H7 on hides and the pathogen load on hides is determined by prior exposure to *E. coli* O157:H7 in feces. In

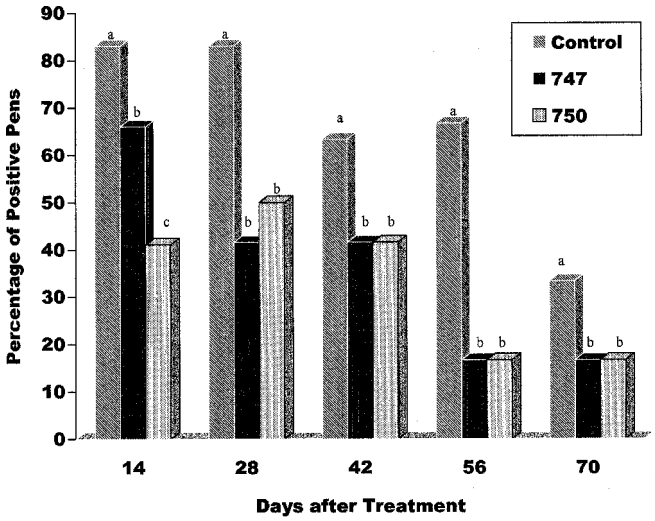


FIGURE 3. *Prevalence of* E. coli *O157:H7 in feedlot pens (12 per treatment group) by sampling period. Columns with different letters in a sampling period are significantly different* ($P < 0.05$).

the present study, the inclusion of NPC 747 in the diet was associated with a decrease in the number of animals testing positive for *E. coli* O157:H7 by culture at least once, and fewer positive samples were obtained from culture-positive animals in the NPC 747 treatment group than from culture-positive control animals.

On arrival at the feedlot, all animals were tested for *E. coli* O157:H7 in fecal grab samples; only three animals tested positive for the pathogen at this time. After the cattle had been on feed for the preliminary 49-day period, fecal grab samples for 12% of the animals contained the pathogen. The prevalence of the detection of the pathogen ranged from 7 to 12% during the middle of the DFM feeding period to <5% near harvest. These data are similar to prevalence estimates previously reported by the National Animal Health Monitoring System *(14).*

On the basis of fecal samples collected at the slaughter plant, very few animals tested positive for the pathogen just before slaughter. Only 1.67% of the animals receiving the NPC 747 treatment tested positive at slaughter, whereas 5% of those receiving the NPC 750 treatment tested positive and 6.67% of the control cattle tested positive. As noted above, these differences were not significant, most likely because of the low prevalence levels.

Although very few animals tested positive for the pathogen at slaughter, significant ($P < 0.05$) differences at each treatment interval were detected by pen testing, indicating that the treatments might affect the environmental contamination in the feedlot (Fig. 3). On the basis of composite fecal samples taken from the floors of the pens, 33.3 to 83% of the control pens were *E. coli* O157:H7 positive, depending on the sampling time. The NPC 747 treatment resulted in 16.6 to 66% of the pens testing positive, whereas the NPC 750 treatment resulted in 16.6 to 50% of the pens testing positive. At each sampling interval, fewer ($P < 0.05$) *E. coli* O157:H7–positive samples were obtained from the NPC 750 and NPC 747 treatment pens than from the control pens.

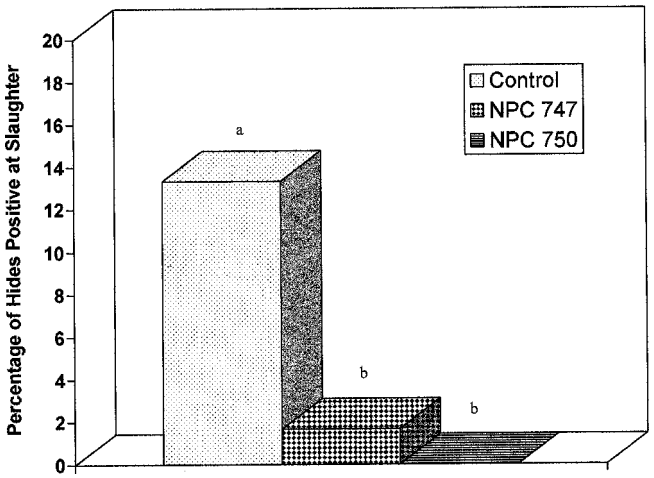


FIGURE 4. *Percentages of hides that were* E. coli *O157:H7–positive at slaughter by treatment group. Columns with different letters in a sampling period are significantly different (*$P < 0.05$*).*

Postharvest. The decreases in *E. coli* O157:H7 in the environment at the feedlot presumably translated into decreases in positive hide samples obtained from the processing plant. At harvest (Fig. 4), differences in the prevalences of positive hides were detected ($P < 0.05$); 13.33% of hide samples from control animals tested positive, whereas fewer ($P < 0.04$) hide samples from animals receiving the NPC 747 and 750 treatments tested positive (1.66 and 0%, respectively). None of the hot carcasses tested positive for the pathogen.

On the basis of observations from the present study, the supplementation of cattle feed with certain DFMs (NPC 747 and NPC 750) decreases the shedding of *E. coli* O157:H7 in the feces of finishing beef cattle. Overall, the NPC 747 treatment was the most effective in decreasing the shedding of *E. coli* O157:H7. At slaughter, significant differences were not detected for individual animals because of the low prevalence estimates for all three treatment groups. However, the prevalence levels for the samples collected from the feedlot pens differed ($P < 0.05$) throughout the feeding period, indicating that environmental contamination might be decreased by supplementation of the animals' feed with these two DFMs. Evidence of this environmental reduction is supported by the hide data, which indicate a significant decrease ($P < 0.04$) in the amount of *E. coli* O157:H7 on hide samples. Because recent studies have indicated that the hide can be a primary source of contamination of the final product *(3, 9),* a reduction in hide contamination could potentially decrease resultant contamination of the carcasses.

Under the conditions of this experiment, the feeding of the NPC 747 and NPC 750 cultures to cattle had few significant effects on feedlot performance and carcass characteristics. Long-term studies on the effects of the DFMs on feedlot performance are needed; however, the present results indicate that the feeding of these DFMs to cattle to decrease fecal shedding of *E. coli* O157:H7 would not negatively affect the performance of finishing beef steers.

ACKNOWLEDGMENT

The authors thank the American Meat Institute Foundation for financial support of this project.

REFERENCES

1. Association of Official Analytical Chemists. 1990. Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington, D.C.
2. 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.
3. Castillo, A., J. S. Dickson, R. P. Clayton, M. M. Lucia, and G. Acuff. 1998. Chemical dehairing of bovine skin to reduce pathogenic bacteria and bacteria of fecal origin. *J. Food Prot.* 61:623–625.
4. 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:H7 infection in man. *Epidemiol. Infect.* 111:439–447.
5. Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmarie, and W. W. Lagreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157:H7 prevalence in feces, hides, and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA* 97:2999–3003.
6. Federal Register. 1996. Pathogen reduction: hazard analysis and critical control point (HACCP) systems: final rule. USDA-FSIS. *Fed. Regist.* 61:38805–38989.
7. Galyean, M. L., G. A. Nunnery, P. J. Defoor, G. B. Salyer, and C. H. Parsons. 2000. Effects of live cultures of *Lactobacillus acidophilus* (strains 45 and 51) and *Propionibacterium freudenreichii* PF-24 on performance and carcass characteristics of finishing beef steers. Burnett Center Internet progress report no. 8. Texas Tech University. Available at: http://www.asft.ttu.edu/burnett_center/progress_reports/.
8. Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76:1216–1231.
9. Midgley, J., and P. Desmarchelier. 2001. Pre-slaughter handling of cattle and Shiga toxin-producing *Escherichia coli* (STEC). *Lett. Appl. Microbiol.* 32:307–311.
10. National Research Council. 1996. Nutrient requirements of beef cattle, 7th ed. National Academy Press, Washington, D.C.
11. Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, G. S. Olcott, L. M. Johnson, N. T. Hargett, P. A. Blake, and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype O157:H7. *N. Engl. J. Med.* 308:681–685.
12. Rust, S. R., K. Metz, and D. R. Ware. 2000. Effects of Bovamine™ rumen culture on the performance and carcass characteristics of feedlot steers. *J. Anim. Sci.* 78(Suppl. 2):82. (Abstract.)
13. Smith, D., M. Blackford, S. Younts, R. Moxley, J. Gray, L. Hungerford, T. Milton, and T. Klopfenstein. 2001. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and the characteristics of the cattle or conditions of the feedlot pen. *J. Food Prot.* 64:1899–1903.
14. U.S. Department of Agriculture. 2001. National Animal Health Monitoring System. *Salmonella* and *E. coli* O157:H7 in United States feedlots. Info sheet. U.S. Department of Agriculture, Washington, D.C.
15. Wells, J. G., D. R. Davis, L. K. Wachmuth, L. W. Riley, R. S. Remis, R. Sokolow, and G. D. Morris. 1983. Laboratory investigation of hemorrhagic colitis outbreaks associated with a rate *Escherichia coli* serotype. *J. Clin. Microbiol.* 18:512–520.
16. Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1996. Prevalence of enterohemorrhagic Escherichia coli O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.* 61:1290–1293.