Dietary Supplementation with *Lactobacillus*- and *Propionibacterium*-Based Direct-Fed Microbials and Prevalence of *Escherichia coli* O157 in Beef Feedlot Cattle and on Hides at Harvest

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ABSTRACT

The objective of this study was to describe the prevalence of *Escherichia coli* O157 in the feces and on the hides of finishing beef cattle fed a standard diet and those fed diets supplemented with direct-fed microbials. Two hundred forty steers received one of four treatments throughout the feeding period: (i) control: no added microbials; (ii) HNP51: high dose of *Lactobacillus acidophilus* strain NP 51 (10^9 CFU per steer daily) and *Propionibacterium freudenreichii* (10^6 CFU per steer daily); (iii) HNP51 +45: high dose of NP 51 (10^8 CFU per steer daily), *P. freudenreichii* (10^6 CFU per steer daily), and *L. acidophilus* NP 45 (10^6 CFU per steer daily); or (iv) LNP51 +45: low dose of NP 51 (10^6 CFU per steer daily), *P. freudenreichii* (10^6 CFU per steer daily), and NP 45 (10^6 CFU per steer daily). Samples were collected from each animal and analyzed for the presence of *E. coli* O157 using immunomagnetic separation methods on day 0 (feces), 7 days before harvest (feces), and at harvest (feces and hide). At the end of the feeding period, cattle receiving HNP51 were 57% less likely to shed detectable *E. coli* O157 in their feces than were the controls (P < 0.01). For animals receiving HNP51 +45 and LNP51 +45, fecal prevalence did not differ from that of the controls. The prevalence of positive hide samples was least among cattle receiving HNP51 +45 (3.3%); these animals were 79% less likely (P < 0.06) to have a positive hide sample than were the controls (prevalence = 13.8%). There was poor agreement of the culture results between fecal and hide samples collected from the same animal (κ = 0.08; confidence interval = −0.05 to 0.2). Cattle supplemented with a high dose of NP 51 had reduced *E. coli* O157 prevalence in both fecal and hide samples, indicating that this treatment may be an efficacious preharvest intervention strategy.

*Escherichia coli* O157 has become a public health concern because this organism can be a foodborne pathogen, creating possibly life-threatening complications (4, 20). Cattle have been identified as a reservoir for *E. coli* O157 (11, 23). Epidemiological studies have focused on gaining an understanding of the distribution, prevalence, and potential risk factors associated with *E. coli* O157 among feedlot cattle and their environments. Recent reports have indicated a higher prevalence of *E. coli* O157 among finishing beef cattle than described in earlier studies, in which the prevalence was estimated to be relatively low at 2.3 to 6.1% (12). Elder et al. (9) reported a prevalence of *E. coli* O157 of 28% among fecal samples collected from feedlot cattle during summer months. In other epidemiological studies, 15.7 and 23% of feedlot cattle were shedding *E. coli* O157 in their feces when the prevalence was averaged over time (6, 21). In multiple studies, researchers have concluded that *E. coli* O157 is widely distributed among feedlot cattle and in their environment (9, 10, 13, 21); in one study the organism was present in 100% of 73 cattle feedlots (22). The increase in reported prevalence may be a result of improvements in methodologies used to isolate the pathogen from feces and the environment.

Because of the high prevalence, decreasing *E. coli* O157 in feedlot cattle is an important part of food safety efforts throughout the beef supply chain. The development of economically feasible intervention strategies that are effective against foodborne pathogens is a priority for the beef industry. Proposed intervention strategies have included varying the grain concentrate or roughage composition of the diet (7, 14–16, 18), including specific dietary ingredients or supplements such as cottonseed (10) or competitive exclusion products (3, 24), using animal drinking water treatments such as sodium chlorate (1, 5), and vaccination (19). In addition to determining the efficacy of each proposed intervention on the prevalence of *E. coli* O157, it is also important to consider the economic feasibility for the producer, including effects of the treatment on animal health and performance.

Our previous data (2) indicated that supplementation with direct-fed microbials (DFM), specifically two different strains of *Lactobacillus acidophilus*, substantially decreased the presence of *E. coli* O157 in the feces and on the hides of finishing beef cattle. One particular strain (NP 51, formally known as NPC 747) decreased the likelihood of fecal...
sheding of *E. coli* O157 by approximately 50% during the course of the feeding period. Those cattle receiving the DFM-supplemented diets also had improved feed efficiency when final body weight was calculated from hot carcass weight and the overall average dressing percentage. The objective of the present study was to describe the prevalence of *E. coli* O157 among groups of finishing beef cattle fed a standard diet and those supplemented with DFM: *L. acidophilus* and *Propionibacterium freudenreichii*. Combination treatments were used to determine whether additional inhibition of *E. coli* O157 or improved performance were obtained. Fecal and hide samples from individual animals were analyzed for the presence of *E. coli* O157 at three times during the feeding period. The effect of the DFM on animal performance also was evaluated and has been reported elsewhere (8).

**MATERIALS AND METHODS**

**Cattle, treatments, and pen assignments.** Two hundred sixty steers of primarily British breeding were received at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction. The steers had an arrival body weight of 286.7 kg and were obtained from a single source. Following arrival, the steers were subjected to routine processing (8). Before the initiation of the study, 240 of the 260 steers were selected based on uniformity and body weight and were sorted into 12 blocks based on weight. Within each weight block, steers were assigned randomly to one of four dietary treatments and designated to pens accordingly (four pens per block, five animals per pen). A total of 12 pens were assigned to each of the four treatments. All cattle received a standard steam-flaked corn-based finishing diet (92% concentrate) (7) with or without supplemental DFM throughout the feeding period. The treatments were based on different combinations of two strains of *E. coli* (NP 51 and NP 45, formally known as NPC 750) and *P. freudenreichii* (Nutrition Physiology Corporation, Indianapolis, Ind.). The control diet (CON) had no added DFM; only a carrier (lactose) mixed with water was added to the diet. One treatment group received a diet (HNPS1) supplemented with a high-level (concentration) dose of NP 51 (10⁸ CFU per steer daily) and *P. freudenreichii* (10⁹ CFU per steer daily). Another treatment group received a diet (HNPS1 + 45) containing the high-dose level of NP 51 (10⁹ CFU per steer daily) plus NP 45 (10⁸ CFU per steer daily) and *P. freudenreichii* (10⁹ CFU per steer daily). The fourth group of cattle received a diet (LNP51 + 45) supplemented with a lower dose of NP 51 (10⁸ CFU per steer daily), NP 45 (10⁷ CFU per steer daily), and *P. freudenreichii* (10⁹ CFU per steer daily). The treatment cultures were prepackaged in aluminum foil packets and provided by Nutrition Physiology Corporation. Each packet contained enough culture to supply the desired daily dose of the DFM to 12 pens of cattle. The contents of the packet for each treatment were mixed with 2.5 liters of distilled water in a plastic sprinker can, after which the contents of the sprinker can were poured onto the diet as it was mixed in a self-propelled feed mixer and delivery unit (Rotomix 84-8, Rotomix, Dodge City, Kans.; approximate capacity of 700 kg). Separate sprinker cans were designated for each treatment to avoid cross-contamination. Detailed descriptions of the diets used and feeding management have been provided elsewhere (8).

**Sampling plan.** Fecal samples were collected from each animal at three times during the feeding period. Samples were collected on the first day of the feeding period to determine the overall prevalence of cattle shedding *E. coli* O157 in their feces before application of treatments. Fecal samples also were collected 7 days before the cattle were shipped for harvest and again on the day of shipment. Hide swab samples were collected from each animal on the day of harvest. The cattle were marketed according to when they were projected to reach a body weight and composition needed to achieve a U.S. Department of Agriculture quality grade of Choice, which resulted in three different harvest days.

**Sample collection.** Fecal samples were collected directly from the rectum of each animal as they were restrained in a handling chute. The personnel collecting samples wore latex gloves and arm-length plastic sleeves to obtain the samples. A new sleeve was used to collect each sample. Each fecal sample was placed in an individually labeled specimen cup. Hide swab samples were obtained using a sterile sponge hydrated with Butterfield’s phosphate diluent. The sponge was swabbed over a 600-cm² area of the perineum area on the right side of each steer and placed in a prefilled sample bag containing fresh diluent. All samples were placed in a cooler with ice and transported approximately 20 km to the laboratory for further processing.

**Microbial analysis.** The presence of *E. coli* O157 in fecal samples was determined using methods adapted from those of Faerch et al. (17). Ten grams of feces from each fecal sample was placed in 90 ml of GN broth (BD Diagnostics, Franklin Lakes, N.J.) containing 8 µg/ml vancomycin, 59 µg/ml ceftizoxime, and 10 µg/ml cefsulodin and then incubated for 6 h at 37°C. One milliliter of this enriched culture was mixed with 20 µg of Dynal O157 beads (Dynal, Lake Success, N.Y.) for 30 min at room temperature for the immunomagnetic separation of *E. coli* O157 cells. The beads were subsequently washed three times in phosphate-buffered saline containing Tween 20. The bead-bacteria mixture (50 µl) was spread onto sorbitol MacConkey plates (BD Diagnostics) containing 50 ng/ml vancomycin and 2.5 µg/ml tellurite. Plates were incubated at 37°C overnight. Individual sorbitol-nondegrading colonies were inoculated into MacConkey agar, Fluorocult agar, and MacConkey broth. The broth was incubated overnight at 37°C. Methylumbelliferyl-β-D-glucuronidase-negative, lactose-positive colonies were selected and subjected to indole, triple sugar iron agar, and Voges-Proskauer tests. Colonies that were positive for indole and negative for A/A (glucose and lactose and/or sucrose fermentation) or K/A (glucose fermentation only, peptone catalyzed) plus gas and the Voges-Proskauer tests were boiled (using cells from the MacConkey broth above) and tested for the O157 antigen using a latex agglutination kit (Remel, Lenexa, Kans.) for confirmation.

**Statistical analyses.** The prevalence of animals shedding *E. coli* O157 in their feces was determined at each time point that samples were collected. Prevalence of hide carriage of *E. coli* O157 was determined for samples collected at harvest. The number of pens in each treatment group that had at least one steer positive for fecal shedding or hide carriage of *E. coli* O157 also was determined. The kappa statistic was calculated to determine the percentage of agreement beyond chance between fecal and hide sample culture results for the same animal on the day of harvest.

To evaluate differences in *E. coli* O157 pen-level prevalence among the treatment groups, the data were analyzed as a binomial distribution using the GENMOD procedure of SAS (SAS Institute, Cary, N.C.). A repeated-measures statement was included in the analysis for the fecal samples collected over time. Weight block and marketing date also were evaluated as sources of variation in the model.
Diets were supplemented as follows: HNP51 = Lactobacillus acidophilus strain NP 51 (10^6 CFU per steer daily) plus Propionibacterium freudenreichii (10^6 CFU per steer daily); HNP51 + 45 = NP 51 (10^6 CFU per steer daily) plus NP 45 (10^6 CFU per steer daily) plus P. freudenreichii (10^6 CFU per steer daily); LNP51 + 45 = NP 51 (10^6 CFU per steer daily) plus NP 45 (10^6 CFU per steer daily) plus P. freudenreichii (10^6 CFU per steer daily).

**RESULTS AND DISCUSSION**

E. coli O157 was recovered from the feces of 5 of the 240 steers (2.0%) at the beginning of the feeding period in April. During the course of the study, four animals were removed from the trial for causes unrelated to treatments (8). At the end of the feeding period, the prevalences of steers shedding detectable E. coli O157 within treatment groups compared with the control group were 23.3 and 18.3% at 7 days before harvest and on the day of harvest, respectively, whereas those cattle receiving LNP51 were 79% less likely to carry E. coli O157 in their feces (Table 1). The prevalence of fecal shedding of E. coli O157 among those steers fed HNP51 at 7 days before harvest and on the day of harvest were 13.6 and 13.3%, respectively...
E. coli O157 based on the culture results for either hide or fecal samples, the results would agree in only 8% of the classifications. Positive hide samples and fecal samples were in agreement only 8% of the time. However, this result was not unexpected. Hides are contaminated from many sources, including the pen floor, feces from other animals, dust, and various other environmental sources, whereas fecal grab samples reveal the E. coli O157 status of individual animals. Shedding of the pathogen in the feces also varies over time. The hides may be more representative of cumulative contamination and may represent animals that were previously shedding the pathogen. These results indicate that fecal status of an animal is not a good predictor of hide status and vice versa.

Based on culture results from fecal samples collected 7 days before harvest, 32 of the total 48 pens (66.7%) had at least one animal shedding a detectable level of E. coli O157 in their feces. Nine (75%) of the positive pens were in the control group. Eight pens (66.7%) each were positive for the HNP51 and LNP51+45 groups. Among pens in the HNP51+45 group, seven (58.3%) were positive for at least one animal shedding E. coli O157 at 7 days before harvest (Fig. 3).

The overall prevalence of pens positive for E. coli O157 based on individual fecal samples collected on the day of harvest was 64.6% (31 of 48 pens). Six HNP51 pens (50%) were positive, whereas nine (75%) of the pens of animals on the control diet were positive (Fig. 3). Eight pens (66.7%) among those holding animals in the HNP51+45 and LNP51+45 groups had at least one animal shedding the organism on the day of harvest.

Thirteen of the 48 pens (27.1%) had at least one animal with an E. coli O157–positive hide swab sample on the day of harvest. Only one of the HNP51+45 group of pens (8.3%) had an animal with a positive hide sample on the day of harvest (Fig. 3). Half the pens (50%) in the control group had at least one animal with a positive hide sample, whereas there were three positive pens (25%) in the HNP51 and LNP51+45 groups.

The prevalence of fecal shedding of a detectable level of E. coli O157 among the cattle in this study was consistent with recent prevalence estimates reported by others (8, 20). In the present study, cattle receiving diets supplemented with NP 51 at a dose of $10^9$ CFU per steer daily had a decreased prevalence of E. coli O157 compared with that of steers receiving no DFM. More specifically, cattle receiving HNP51 were 57% less likely to be shedding E. coli O157 in their feces than were the control cattle. This finding is comparable to the findings of our previous study (2) in which cattle supplemented with NP 51 at a dose of $10^9$ CFU per steer daily were 50% less likely to shed the organism. However, in the current study, diets supplement-
ed with the high dose of NP 51 in combination with NP 45 were less effective in decreasing the fecal prevalence of \(E.\ coli\) O157, perhaps indicating that the two organisms might be antagonistic. Those animals receiving the high-dose supplements of NP 51 also had the lowest prevalence levels of \(E.\ coli\) O157–positive hide samples, although there was poor agreement between fecal and hide sample culture results for the same animal. Hide contamination may result from direct contact with other animals in the pen or from environmental exposure and may not be an accurate indicator of fecal shedding.

Given the results of the present study and of our previous research, supplementing cattle diets with NP 51 at \(10^8\) CFU per animal daily as a preharvest intervention strategy could effectively decrease \(E.\ coli\) O157 prevalence among groups of finishing beef cattle. Decreasing \(E.\ coli\) O157 both in the feces and on the hides of finishing cattle could decrease the potential for carcass contamination and improve food safety.

REFERENCES


