Research Note

Prevalence and Enumeration of *Escherichia coli* O157 in Steers Receiving Various Strains of *Lactobacillus*-Based Direct-Fed Microbials

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ABSTRACT

The objective of this research was to evaluate the effect of daily dietary inclusion of specific strains of *Lactobacillus acidophilus* on prevalence and concentration of *Escherichia coli* O157 in harvest-ready feedlot cattle. Five hundred yearling steers were housed in pens of 10 animals each. At arrival, steers were randomly allocated to one of five cohorts. Four of the cohorts were fed various strains and dosages of *Lactobacillus*-based direct-fed microbials throughout the feeding period. Fecal samples were collected from the rectum of each animal immediately prior to shipment to the abattoir. *E. coli* O157 was detected using selective enrichment and immunomagnetic separation techniques. For positive samples, *E. coli* O157 concentration was estimated using a most-probable-number (MPN) technique that included immunomagnetic separation. Prevalence varied among the cohorts (*P* < 0.01). The prevalence in the controls (26.3%) was greater (*P* < 0.05) than that in cattle supplemented with *L. acidophilus* strains NP51, NP28, or NP51-NP35 (13.0, 11.0, and 11.0%, respectively). The greatest *E. coli* O157 concentration was also observed in the controls (3.2 log MPN/g of feces); this concentration was greater (*P* < 0.05) than that observed in positive animals receiving NP51, NP28, or NP51-NP35 (0.9, 1.1, 1.7 log MPN/g of feces, respectively). Specific strains of *Lactobacillus*-based direct-fed microbials effectively reduced the prevalence and concentration of *E. coli* O157 in harvest-ready cattle, whereas others did not. When using direct-fed microbials to reduce carriage of *E. coli* O157 in cattle, it is important to select appropriately validated products.

*Escherichia coli* O157:H7 reportedly causes an estimated 73,480 illnesses, 2,168 hospitalizations, and 61 deaths each year (7), which is of great concern in the United States. It is generally accepted that cattle are a major reservoir of this organism, and humans may be exposed through consumption of contaminated beef products, particularly undercooked ground beef. As a consequence, several interventions have been developed and implemented in abattoirs to prevent or remove bacterial contamination associated with beef carcasses. Despite the effectiveness of these interventions, it is becoming increasingly apparent that preharvest group-level prevalence of *E. coli* O157 is associated with the likelihood of carcass contamination within abattoirs. It is postulated, therefore, that reducing pathogen carriage in the preharvest environment (and in cattle presented for harvest) will reduce contamination of the final product.

A variety of preharvest interventions have been evaluated for their efficacy in reducing *E. coli* O157 prevalence in feedlot cattle. Direct-fed microbials, such as *E. coli*, *Proteus mirabilis*, and *Lactobacillus acidophilus*, have demonstrated efficacy both in vitro and in vivo. Zhao et al. (12) used various strains of nonpathogenic *E. coli* (17 strains) and *P. mirabilis* (1 strain) that had been tested for efficacy in vitro. The authors performed an in vivo challenge study by inoculating 15 calves with a five-strain mixture of *E. coli* O157:H7 at 10⁸ CFU/ml with (6 animals) or without (9 animals) probiotic at 10¹⁰ CFU/g of feed (12). The addition of these probiotic bacteria significantly reduced the concentration of *E. coli* O157 shed in the feces of cattle by days 15 and 18 posttreatment when compared with controls (12). Schamberger et al. (8) artificially inoculated calves with eight strains of colicinogenic *E. coli* (10⁷ and 10⁸ CFU/g of feed) and six strains of *E. coli* O157:H7 (10¹¹ CFU per calf). These results imply that daily administration of colicinogenic *E. coli* at 10⁸ CFU/g of feed can reduce fecal *E. coli* O157:H7 shedding (8).

In one study, *E. coli* O157 was 49% less likely to be recovered from steers receiving 10⁹ cells of *L. acidophilus* strain NP51 (also known as NPC747) daily compared with steers receiving the carrier only (2). In a subsequent study, the same daily dose of *L. acidophilus* NP51 resulted in an 80% reduction in *E. coli* O157 prevalence (11). However, not all strains or combinations of strains are effective. *L. acidophilus* NP35 (also known as NPC750) did not reduce *E. coli* O157 prevalence to the same extent as did strain NP51. Moreover, when the dose of *L. acidophilus* NP51 was reduced or when this strain was combined with *L. aci-
dophilus NP45, its effectiveness was not the same as it was for NP51 alone at $10^9$ cells per animal per day (10). Therefore, strain and dose are important considerations when selecting a potential preharvest intervention.

Although specific strains of *L. acidophilus* appear efficacious at reducing *E. coli* O157 prevalence, it is unclear whether these direct-fed microbials exert a substantive effect on the concentration of *E. coli* O157 carried by positive animals; i.e., more data are needed to determine whether the number of cells carried by cattle is reduced. When the number of *E. coli* O157 cells is reduced, fewer cells are available to contaminate carcasses. The objective of this study was to evaluate the effect of daily dietary inclusion of specific strains of *L. acidophilus* on prevalence and concentration of *E. coli* O157 in harvest-ready feedlot cattle.

**MATERIALS AND METHODS**

**Animals.** Five hundred sixty steers were eligible for inclusion in this study. Upon arrival, all steers were given a vaccine (Bovi-Shield 4, Pfizer Animal Health, New York, N.Y.) containing modified-live variants of bovine viral diarrhea virus, bovine herpesvirus 1, parainfluenza virus type 3, and bovine respiratory syncytial virus. Steers also were given a broad-spectrum anthelmintic (Dectomax, Pfizer) and a growth promotant (Synovex Choice, Fort Dodge Animal Health, Kansas City, Mo.). Of the 560 steers, 500 were randomly selected and divided by weight into 10 replicate groups. Within each group, steers were randomly allocated to one of five cohorts. The steers were housed in 50 outdoor dirt-floor pens of 10 animals each according to cohort and replicate allocation.

**Exposure.** The exposures of interest were various strains or combinations of strains of *L. acidophilus*. A lactose carrier was used in all *L. acidophilus*-based direct-fed microbial preparations. The daily doses of *L. acidophilus* strains were $10^9$ CFU strain NP28, $10^9$ CFU strain NP51, $10^6$ CFU strain NP35, and $10^6$ CFU each of strains NP51 and NP35 (NP51-NP35). Each exposure included $10^6$ CFU *Propionibacterium freudenreichii* NP24 to enhance animal performance (5). The unexposed cohort received only the lactose carrier. Personnel administering the products and those responsible for microbial culture and isolation were unaware of the exposure status of the cattle.

Treatments (including the lactose carrier for the unexposed cohort) were incorporated into the ration of the steers. Test articles were stored in frozen color-coded aluminum pouches. Each pouch contained sufficient test article for 100 animals, i.e., a daily dose for an entire cohort. The contents of each pouch were incorporated into 2.3 kg of ground corn using bench-top mixers, which were color coded to match the pouches. Ten percent aliquots of the mixture were then incorporated into 2.3-kg batches of ground corn so that 50 2.53-kg batches of test article were made each day. These batches were incorporated into the ration of each pen according to exposure classification. Steers were fed a high-concentration ration typical of those used in feedlots, except that the primary concentrate source was wheat instead of corn. The rations were delivered once a day to the steers.

**Sample collection and analyses.** Immediately before shipment to the abattoir, steers were moved through an animal handling facility and restrained. Fecal grab samples were aseptically collected from the rectum of each animal and placed in individually labeled specimen cups. Samples were shipped overnight in coolers containing wet ice to the food microbiology laboratory at Texas Tech University.

Upon arrival, 10 g of feces was inoculated into 90 ml of gram-negative broth containing 8 μg/ml vancomycin, 50 ng/ml cefixime, and 10 μg/ml cefsulodin and incubated for 6 h at 37°C. One milliliter of the broth culture was mixed with 20 μl of anti-O157 beads (Dynal, Lake Success, N.Y.) for 30 min at room temperature (25°C). Beads were washed three times, and 50 μl of the bead-bacteria mixture was streaked onto sorbitol MacConkey agar plates (CT-SMAC) supplemented with cefixime (50 ng/ml) and potassium tellurite (2.5 μg/ml) and incubated at 37°C for 18 to 24 h. Up to three typical non-sorbitol-fermenting colonies per plate were chosen and restreaked for isolation on CT-SMAC and incubated overnight at 37°C. Non-sorbitol-fermenting colonies were selected and inoculated onto MacConkey agar and Fluorocult *E. coli* O157:H7 agar (MUG). After incubation, MUG-negative colonies were transferred from the plates into tryptic soy broth, triple sugar iron agar, and MacConkey broth and incubated overnight under aerobic conditions at 37°C. Presumptive *E. coli* O157 colonies were confirmed with a commercial latex agglutination test (Remel, Lenexa, Kans.). Further confirmatory steps included gene detection (including genes encoding Shiga toxin production) with a commercially available PCR system (DuPont, Wilmington, Del.).

*E. coli* O157–positive samples for which there was a sufficient quantity of sample available were used with a most-probable-number (MPN) technique to determine *E. coli* O157 concentration (4, 9). Samples were serially diluted in GN broth in triplicate using a three-tube MPN dilution scheme. Tubes were incubated for 6 h at 37°C, and the presence of *E. coli* O157 was determined in each tube using immunomagnetic separation and confirmation as described above. Standard MPN statistical tables were used to determine the MPN per gram. The sensitivity of this method was 3 cells per gram of feces.

**Statistical analyses.** Data were imported into a commercially available software package (System for Windows Release 9.1.3, SAS Institute, Cary, N.C.). Descriptive statistics were generated, and categorical data, i.e., culture positive or negative, were analyzed using logistic regression techniques. The unexposed cohorts were set as the referent, and ratios of the odds of shedding in each exposed cohort relative to the unexposed cohort were calculated using logistic regression techniques. Maximum likelihood confidence intervals (CIs) were generated. Multiple comparison of means was performed to evaluate exposures. Model-predicted means and CIs were back-transformed to the normal scale, and relative risks were computed and compared with the relevant odds ratios.

Enumeration data were log transformed. Where the estimate of concentration was below the detection limit, it was arbitrarily recorded at 3 MPN/g before log transformation. Data were analyzed using linear regression techniques and least square means, and the 95% CIs were generated. Multiple comparison of least square means was performed.

**RESULTS AND DISCUSSION**

Overall prevalence of *E. coli* O157 was 16.6%. Prevalence in animals of the control cohort and in animals receiving *L. acidophilus* NP51, NP28, NP35, and NP51-NP35 was 26.3, 13.0, 11.0, 22.0, and 11.0%, respectively. Likelihood of pathogen recovery varied among the cohorts ($P < 0.02$; Fig. 1). *E. coli* O157 was 58.3% ($P = 0.02$), 64.0% ($P < 0.01$), and 64.0% ($P < 0.01$) less likely to be
recovered from those animals receiving NP51, NP28, and NP51-NP35, respectively, compared with controls (Table 1). The likelihood of *E. coli* O157 recovery from animals receiving NP35 (without NP51) did not differ significantly from that of the unexposed cohort (*P* = 0.51). Fewer animals among those receiving NP51 (*P* = 0.10), NP28 (*P* = 0.04), and NP51-NP35 (*P* = 0.04) were shedding *E. coli* O157 than were steers receiving NP35. The prevalence of *E. coli* O157 among animals receiving NP51, NP28, and NP51-NP35 did not vary significantly (*P* > 0.65 for all pairwise comparisons).

Of the 83 samples from which *E. coli* O157 was recovered, there was enough remaining for enumeration in 38 samples: 7, 8, 6, 9, and 8 samples from the control cohort and those receiving NP51, NP28, NP35, and NP51-NP35, respectively. Mean *E. coli* O157 concentration among positive animals was 1.86 log MPN/g of feces (range, 0.48 to 5.04 log MPN/g).

Concentration varied among treatment groups (*P* = 0.03; Fig. 1). Model-predicted means were 0.90, 1.14, 1.73, 2.30, and 3.17 log MPN/g for animals receiving NP51, NP28, NP51-NP35, and NP35 and the controls, respectively. Compared with controls, animals consuming NP51 (*P* < 0.01), NP28 (*P* = 0.01), and NP51-NP35 (*P* = 0.05) had lower concentrations of *E. coli* O157 among culture-positive animals. Concentration among positive animals receiving NP35 and controls did not differ significantly (*P* = 0.22).

We confirmed the findings of others that NP51 at 10⁹ cells per animal per day effectively reduces prevalence of *E. coli* O157 in harvest-ready cattle. Others have reported a 49% (2), 58% (10), and 77% (11) reduction in prevalence in cattle supplemented with this quantity of NP51 compared with controls fed typical feedlot diets. In this study, we detected a 51% reduction in pathogen prevalence.

Other *L. acidophilus* strains and combinations of strains also were effective at reducing pathogen prevalence. Strain NP28 (10⁹ cells per day) and a combination of strains NP35 and NP51 (both at 10⁸ cells per day) were effective and were associated with a 58% reduction in prevalence. However, strain NP35 alone had limited efficacy for *E. coli* O157 reduction. In a previous study, Brashears and co-workers (2) also found that NP35 was not as effective as NP51 at reducing *E. coli* O157 prevalence.

In this study, we utilized a novel enumeration method to estimate *E. coli* O157 concentration. To our knowledge, quantitative evaluation of direct-fed microbial efficacy in field conditions has not been reported previously. In addition to reducing the number of cattle from which *E. coli* O157 was recovered, specific strains (NP51 and NP28) or a combination of strains (NP51 with NP35) were associated with reduced *E. coli* O157 concentration in those animals that remained positive compared with controls. We observed a 1- to 2-log reduction in concentration. Among positive animals, *E. coli* O157 concentration was typically 90 to 99% lower in animals supplemented with certain direct-fed microbials than in controls. These estimates are based on relatively few samples (38), and further data are needed to validate the efficacy of *L. acidophilus* strains for reduction of *E. coli* O157 concentration.

We did not measure hide carriage of *E. coli* O157. When this has been done, similar efficacy has been observed (2, 11). Moreover, Loneragan and Brashears (6) reported that within-pen fecal prevalence is directly associated with hide carriage. Therefore, we anticipate that the efficacious strains evaluated in the present study would be associated with reduced carriage on hides. More data are needed to determine whether these *L. acidophilus* strains are associated with reduced concentration of *E. coli* O157 on hides.

The data reported here demonstrate that substantive preharvest control of *E. coli* O157 is possible, although no control will be 100% effective. Fewer feedlot cattle supplemented with specific strains of *L. acidophilus* will likely carry *E. coli* O157, and those that do will carry fewer cells. This reduced infection should make it easier for in-plant interventions to prevent and remove carcasses contamination during processing (1, 3). Our data also highlight the need to validate candidate direct-fed microbial strains because the efficacy associated with one does not necessarily predict the efficacy of another.

**ACKNOWLEDGMENT**

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