Research Note

Reduction of *Escherichia coli* O157 and *Salmonella* in Feces and on Hides of Feedlot Cattle Using Various Doses of a Direct-Fed Microbial

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ABSTRACT

In this study, the effectiveness of direct-fed microbials at reducing *Escherichia coli* O157 and *Salmonella* in beef cattle was evaluated. Steers ($n = 240$) received one of the following four treatment concentrations: control = lactose carrier only; low = 1×10^7 CFU per steer daily *Lactobacillus acidophilus* NP51; medium = 5×10^8 CFU per steer daily *L. acidophilus* NP51; and high = 1×10^9 CFU per steer daily *L. acidophilus* NP51. Low, medium, and high diets also included 1×10^9 CFU per steer *Propionibacterium freudenreichii* NP24. Feces were collected from each animal at allocation of treatment and found to have no variation ($P = 0.54$) between cohorts concerning *E. coli* O157 recovery. Feces and hide swabs were collected at harvest and analyzed for the presence of *E. coli* O157 by immunomagnetic separation and *Salmonella* by PCR. No significant dosing effects were detected for *E. coli* O157 recovery from feces at the medium dose or from hides at the medium and high doses. *E. coli* O157 was 74% ($P < 0.01$) and 69% ($P < 0.01$) less likely to be recovered in feces from animals receiving the high and low diets, respectively, compared with controls. Compared with controls, *E. coli* O157 was 74% ($P = 0.05$) less likely to be isolated on hides of cattle receiving the low dose. No significant dosing effects were detected for *Salmonella* recovery from feces at the medium and low doses or from hides at any doses. Compared with controls, *Salmonella* was 48% ($P = 0.09$) less likely to be shed in feces of cattle receiving the high dose. No obvious dose-response of *L. acidophilus* NP51 on recovery of *E. coli* O157 or *Salmonella* was detected in our study.

Escherichia coli O157 and *Salmonella* account for approximately 1.5 million cases of human disease, 19,000 hospitalizations, and 650 deaths annually in the United States *(21)*. *E. coli* O157 was first associated with undercooked ground beef as a foodborne pathogen in two 1982 outbreaks *(27)*, and two human diseases associated with this pathogen are hemorrhagic colitis and hemolytic-uremic syndrome *(15, 27)*. When humans are exposed to Shiga-like toxin-producing *E. coli* (stx *E. coli*), 38 to 61% develop hemorrhagic colitis *(23)*, and 3 to 7% (sporadic cases) or 20% (epidemic forms) develop into hemolytic-uremic syndrome *(20)*. *Salmonella* (Typhimurium, Enteritidis, Newport, Heidelberg, and Javiana made up a majority of the serotypes) is the leading bacterial cause of foodborne infection, when compared with *Campylobacter, E. coli* O157, *Listeria, Shigella, Vibrio,* and *Yersinia,* among humans in the United States *(33)*. The number of cases of foodborne illnesses in humans per year associated with *E. coli* O157 has decreased, but the number of cases in humans per year of *Salmonella* cases has increased in the United States from 1998 to 2002 *(8)*.

Cattle are a major reservoir for *E. coli* O157 *(4, 34–36)*, and a direct correlation between pathogen-positive an-

imal samples (fecal and hide) with carcass contamination has been observed at the lot level *(14)*. The National Animal Health Monitoring System *(22)* division of the U.S. Department of Agriculture reported that feedlot cattle fed for a longer time appear to have a higher tendency to be positive for *Salmonella* than animals fed for a shorter time period. *Salmonella* (most commonly serotype Typhimurium) has been reported to be carried by healthy animals at slaughter *(17, 18, 28)* and has been associated with outbreaks in beef products (most commonly serotypes Newport, Typhimurium, and Thompson) *(7, 9, 19, 30, 37)*. This information warrants preharvest intervention strategies that will effectively reduce both *E. coli* O157 and *Salmonella* (most commonly serotypes Newport, Typhimurium, and Thompson) due to the connection between positive animals and contaminated beef, to provide consumers with safer beef products.

Some preharvest intervention strategies that have been investigated for the reduction of *E. coli* O157 in beef cattle are supplementation with sodium chlorate *(1, 6)*, vaccination *(25, 26)*, and direct-fed microbials (DFMs *(4, 29, 35, 36, 38)*). Studies in our laboratory demonstrate that DFM supplementation does not affect cattle performance *(4, 13)* during reducing *E. coli* O157 load. DFMs (approximately $0.015 to $0.02 per head per day of feeding) as an inter-

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vention of *E. coli* O157 can be beneficial as a cost-effective treatment comparable to Tasco (estimated at $3.75 to $4.25 per head for 14 days prior to slaughter), bacteriophage (<$1.00 per head as a one-time treatment), and sodium chlorate (estimated at $0.30 per head for the feeding period *(11)*).

The novelty of the current research is that we observed the efficacy of a DFM on the reduction of *Salmonella*. Therefore, the objective of this study was to evaluate the effects of three different doses of DFMs on *E. coli* O157 and *Salmonella* prevalence in beef cattle feces and hides at slaughter.

MATERIALS AND METHODS

Cattle, pen assignments, and treatments. Two hundred sixty-nine steers of British × Continental breeding were received at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction (Lubbock). The steers were obtained from a single source with an average arrival body weight of 350.6 kg. All cattle were taken through the Burnett Center working facilities for initial processing, which included (i) the placement of a uniquely numbered ear tag in the left ear; (ii) an individual body weight measurement; (iii) a vaccination with a modified live virus containing types 1 and 2 bovine viral diarrhea viruses, bovine herpes virus type 1, bovine respiratory syncytial virus, and parainfluenza 3 virus (Titanium 5, Agri-Labs, Des Moines, Iowa) and a multivalent clostridial toxoid containing Vision 7 with Spur (Intervet, Millsboro, Del.); and (iv) a deworming with moxidectin (Cydectin Pour-on, Ft. Dodge Animal Health, Overland Park, Kans.).

Prior to the initiation of the study, 240 of the 269 steers were chosen on the basis of body weight and uniformity and sorted into 12 blocks on the basis of weight. Pen assignments were designated by randomly assigning one of four dietary treatments to 20 steers within each weight block (four pens per block, five animals per pen). There were a total of 12 pens assigned to each of the four treatments (48 total pens). Cattle within each block were housed in four contiguous partially slotted-floor concrete pens (2.9 by 5.6 m).

All animals received a standard steam-flaked corn-based finishing diet (92% concentrate in their final ration) typically fed on the High Plains with or without supplemental DFMs throughout the feeding period beginning 17 May 2005. The four treatments were based on different concentrations (CFU per steer daily) of *Lactobacillus acidophilus* strain NP 51. Treatments included (i) control: lactose carrier only (C); (ii) low: 1×10^9 CFU of *Propionibacterium freudenreichii* (NP 24) plus 1×10^7 CFU of *L. acidophilus* (NP 51) per steer daily (L); (iii) medium: 5×10^9 CFU of *P. freudenreichii* (NP 24) plus 5×10^8 CFU of *L. acidophilus* (NP 51) per steer daily (M); and (iv) high: 1×10^9 CFU of *P. freudenreichii* (NP 21) plus 1×10^9 CFU of *L. acidophilus* (NP 51) per steer daily (H). The treatment cultures were prepackaged in aluminum foil packets provided by Nutrition Physiology Corp. (Indianapolis, Ind.). Each packet contained enough culture to supply the desired daily dose of the DFMs to 12 pens of cattle (60 steers). The content of one packet was added to 1 kg of dried, ground corn and thoroughly mixed with air-dried, ground corn (as-fed basis) in a plastic bucket with a screw-on lid. The corn-culture mixture was tumbled approximately 25 times and then added to the feed that had been batched for a given treatment in a self-propelled feed mixer and delivery unit (Rotomix 84-8, approximate capacity = 700 kg; Rotomix, Dodge City, Kans.). Blocks 10 to 12, 5 to 9, and 1 to 4 were on DFM supplementation for 123, 137, and 151 days, respectively (an average of 137 days),

and were sent to the abattoir on 20 September 2005, 4 October 2005, and 18 October 2005, respectively.

Sampling scheme and collection. Fecal grab samples were collected from the rectum of each animal upon allocation to each treatment group to evaluate the overall fecal prevalence of cattle shedding *E. coli* O157 before treatments were given to ensure that there were no differences in prevalence across treatments. Fecal and hide swab samples were collected from each steer prior to shipment to the abattoir to provide a snapshot at harvest time for the evaluation of the efficacy of the DFM treatments compared with the control cohort. Cattle were shipped to the abattoir when the group was expected to have sufficient finish to grade U.S. Department of Agriculture Choice.

Fecal samples were collected directly from the rectum of each animal while they were restrained in a handling chute. Latex gloves and arm-length plastic palpation sleeves were worn by collection personnel to obtain these fecal samples. A new sleeve was used to collect each sample, and the feces for each steer were placed in an individually labeled specimen cup unique to that animal. Hide swab samples were taken from the right side of the perineum area of each animal by swabbing a 100-cm² area with a sterile sponge prehydrated with 25 ml of Butterfield's phosphate diluent. The sponges were placed in a prelabeled sample bag unique to each steer. All samples were placed in a cooler with ice and transported to the food microbiology laboratory at Texas Tech University (approximately 20 km) for microbial analysis where laboratory personnel were blinded to the treatment allocated.

***E. coli* O157 detection.** Ten grams of feces from each of the samples was placed in 90 ml of gram-negative broth containing vancomycin (8 mg/liter), cefixime (0.05 mg/liter), and cefsulodin (10 mg/liter) and incubated for 6 h at 37°C *(16)*. Ten milliliters of diluent from each of the hide samples was placed in 90 ml of tryptic soy broth (TSB) and incubated for 2 h at 25°C and then for 6 h at 42°C *(2)*. One milliliter of each of the above preenrichments was subjected to immunomagnetic separation by mixing with 1 ml of phosphate-buffered saline Tween 20, pH 7.4 (Sigma-Aldrich, St. Louis, Mo.), and 20 µl of *E. coli* O157 Dynabeads (Dynal, Lake Success, N.Y.). An automatic immunomagnetic separation machine was used according to manufacturer's instructions to wash the beads (Dynal). After bead washing, 50 µl of the bead-bacteria mixture was spread plated onto CHROMagar O157 (CHROMagar Microbiology, Paris, France). Suspect colonies (mauve) were subjected to latex agglutination containing anti–*E. coli* O157 antibodies (Remel, Lenexa, Kans.).

***Salmonella* detection.** One milliliter or gram of diluent or feces from each sample (hide or feces) was placed in 9 ml of TSB and incubated at 37°C overnight. Preenrichment cultures were then subjected to PCR detection of *Salmonella* with the BAX system as recommended by the manufacturer (DuPont Qualicon, Wilmington, Del.).

***E. coli* O157 enumeration.** Enumeration of fecal-positive harvest samples that had enough feces to enumerate (59 samples were enumerated of the 82 *E. coli* O157–positive samples) was performed by a most-probable-number (MPN)–immunomagnetic separation technique *(31)*. Serial dilutions of the samples were made in triplicate by a three-tube MPN dilution scheme in gram-negative broth. Tubes were subjected to 6 h of incubation at 37°C, and the presence of *E. coli* O157 was determined in each tube by immunomagnetic separation detection and confirmation as described above. The upper limits of the MPN were decreased in this study compared with Stephens et al. *(32)* because a 3 × 3 MPN dilution scheme was used rather than a 3 × 5 MPN. All

TABLE 1. *Odds ratios (OR), relative risk (RR), 95% confidence intervals (CI) for odds ratios, and* P *values for the likelihood of detectable fecal shedding, hide carriage, and either fecal shedding or hide carriage of* E. coli *O157 within treatment groups compared with the control (C) group at harvest*

Treatment	OR	RR	95% CI	*P* values
	Fecal shedding of *E. coli* O157			
High[a]	0.26	0.50	0.11–0.59	<0.01
Medium[b]	0.65	0.84	0.30–1.39	0.26
Low[c]	0.31	0.57	0.14–0.70	<0.01
	Hide carriage of *E. coli* O157			
High	0.45	0.49	0.13–1.44	0.17
Medium	0.36	0.40	0.10–1.25	0.11
Low	0.26	0.30	0.07–1.03	0.05
	Fecal shedding or hide carriage of *E. coli* O157			
High	0.30	0.56	0.14–0.66	<0.01
Medium	0.57	0.80	0.27–1.21	0.14
Low	0.30	0.56	0.14–0.66	<0.01

[a] 10^9 P. freudenreichii NP24 and 1×10^9 L. acidophilus NP51 CFU per steer daily.

[b] 10^9 P. freudenreichii NP24 and 5×10^8 L. acidophilus NP51 CFU per steer daily.

[c] 10^9 P. freudenreichii NP24 and 1×10^7 L. acidophilus NP51 CFU per steer daily.

MPNs were calculated by a freely available MPN calculator *(10)*. The sensitivity of the MPN method was assumed to be >0.3 MPN/g of feces.

Statistical analysis. Data were imported into a commercially available software package for analysis (SAS, System for Windows Release 9.1.3, SAS Institute, Cary, N.C.). Descriptive statistics were generated to determine the proportion of animals in which *E. coli* O157 or *Salmonella* was detected in feces, on hides, and either in feces or on hides (any). The standard errors of the proportions mentioned above were hand calculated by the formula described by Brayer *(5).*

Categorical data were analyzed by logistic regression techniques on a pen-level basis, and pen prevalence was averaged. Odds ratios (ORs) and confidence intervals were calculated to compare the exposed cohorts with the unexposed cohort (referent). Multiple comparisons of least-squares means were used to evaluate dose efficacy. Contrasts were constructed to evaluate the response variable across DFM doses compared with controls.

Enumeration data were log transformed to control statistical variance. Where the estimate of concentration was below the detection limit, it was arbitrarily set at 3 MPN/g prior to log transformation. Data were analyzed by linear regression techniques and least-squares means, and the 95% confidence interval was generated. Multiple comparisons of least-squares means were performed.

RESULTS AND DISCUSSION

There was no statistical variation among cohorts ($P = 0.54$) in the likelihood of *E. coli* O157 receovery from fecal samples collected at allocation to treatment. Compared with controls, *E. coli* O157 was 74 (high; $P < 0.01$), 35 (medium; $P = 0.26$), and 69% (low; $P < 0.01$) less likely to be shed in feces (Table 1 and Fig. 1). The low treatment may have been more effective in reducing the prevalence
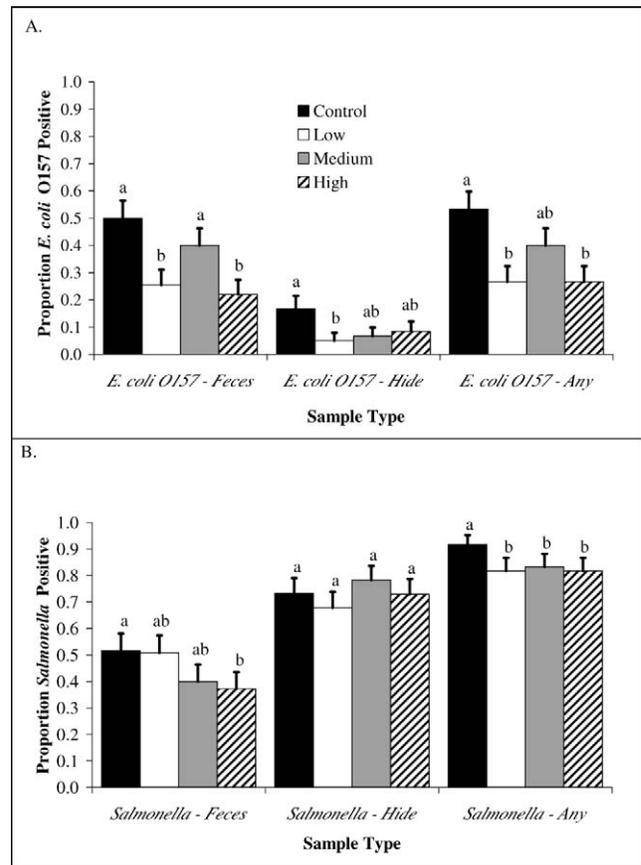

FIGURE 1. *Proportion of market-ready feedlot cattle from which* E. coli *O157 (A) or* Salmonella *(B) was detected in feces, on hides, and either in feces or on hides (any). Treatments: control = lactose carrier only; low = 10^9* P. freudenreichii *NP24 and 1×10^7* L. acidophilus *NP51 CFU per steer daily; medium = 10^9* P. freudenreichii *NP24 and 5×10^8* L. acidophilus *NP51 CFU per steer daily; and high = 10^9* P. freudenreichii *NP24 and 1×10^9* L. acidophilus *NP51 CFU per steer daily. Error bars represent standard error of the proportion. Columns with differing letters within each sample type vary significantly ($P < 0.10$).*

of *E. coli* O157 in feces at the pen level when compared with the high and medium concentrations of DFMs, because one steer in each cohort shed *E. coli* O157 at a concentration >110 MPN/g of feces (with the control cohort as a referent). The animal in the high concentration treatment may not have been shedding *E. coli* O157 in feces at the magnitude of the steer in the medium cohort. Compared with controls, *E. coli* O157 was 55 (high; $P = 0.17$), 64 (medium; $P = 0.11$), and 74% (low; $P = 0.05$) less likely to be isolated on hides. Compared with controls, *E. coli* O157 was 70 (high; $P < 0.01$), 43 (medium; $P = 0.14$), and 70% (low; $P < 0.01$) less likely to be shed in feces or isolated on hides. ORs were a good estimation for relative risk (RR) concerning *E. coli* O157 shedding in feces, isolated on hides, and shedding in feces or isolated on hides. When the odds of a particular event occurring is rare, ORs are generally a good estimator of RR. ORs have been documented to be a safe estimator of RR when used in logistic regression *(12)* (Table 1).

Compared with controls, *Salmonella* was 48 (high; *P*

TABLE 2. *Odds ratios (OR), relative risk (RR), 95% confidence intervals (CI) for odds ratios, and* P *values for the likelihood of detectable fecal shedding, hide carriage, and either fecal shedding or hide carriage of* Salmonella O157 *within treatment groups compared with the control (C) group at harvest*

Treatment	OR	RR	95% CI	*P* values
Fecal shedding of *Salmonella*				
High[a]	0.52	0.71	0.25–1.11	0.09
Medium[b]	0.62	0.79	0.29–1.31	0.20
Low[c]	0.90	0.95	0.43–1.89	0.78
Hide carriage of *Salmonella*				
High	0.86	0.96	0.37–1.99	0.72
Medium	1.10	1.02	0.46–2.66	0.83
Low	0.68	0.89	0.30–1.55	0.35
Fecal shedding or hide carriage of *Salmonella*				
High	0.32	0.88	0.09–1.12	0.07
Medium	0.33	0.88	0.09–1.16	0.08
Low	0.32	0.88	0.09–1.12	0.07

[a] 10^9 *P. freudenreichii* NP24 and 1×10^9 *L. acidophilus* NP51 CFU per steer daily.

[b] 10^9 *P. freudenreichii* NP24 and 5×10^8 *L. acidophilus* NP51 CFU per steer daily.

[c] 10^9 *P. freudenreichii* NP24 and 1×10^7 *L. acidophilus* NP51 CFU per steer daily.

$= 0.09$), 38 (medium; $P = 0.20$), and 10% (low; $P = 0.78$) less likely to be shed in feces. Compared with controls, *Salmonella* was 14 (high; $P = 0.72$) and 32% (low; $P = 0.35$) less likely to be isolated on hides. Compared with controls, *Salmonella* was 110% (medium; $P = 0.83$) more likely to be isolated on hides. Compared with controls, *Salmonella* was 68 (high; $P = 0.07$), 67 (medium; $P = 0.08$), and 68% (low; $P = 0.07$) less likely to be shed in feces or isolated on hides. ORs were a good estimation for RR concerning *Salmonella* shedding in feces and isolated on hides but were not a good estimation for RR for *Salmonella* shedding in feces or isolated on hides (Table 2).

The only significant ($P = 0.04$) linear relationship in dose of *L. acidophilus* strain NP51 detected was fecal detection of *E. coli* O157 decreased from high to low dose, but the high and low cohorts were not statistically different ($P = 0.69$) for fecal shedding of *E. coli* O157. The medium cohort had one animal that was shedding *E. coli* O157 at a concentration >110 MPN/g of feces; thus, it is possible that this animal was a high shedder (>10^4 CFU/g of feces (24)), which may have contributed to the medium cohort not being as effective in reducing the prevalence of *E. coli* O157 in feces at the pen level, when compared with the high and low cohorts (with the control cohort as a referent). The high and control cohorts also had an animal that shed *E. coli* O157 >110 MPN/g of feces, but these animals may not have been shedding this pathogen at the magnitude of the high shedder in the medium cohort. On average, cattle exposed to *L. acidophilus* strain NP51 were less likely ($P < 0.05$) to have *E. coli* O157 recovered from feces, hides, or either from feces or hides. *Salmonella* was also less like-

ly ($P = 0.05$) on average to be recovered either from feces or hides.

Enumeration of *E. coli* O157–positive fecal samples from the exposed cohorts (high, medium, and low cohorts had 12, 19, and 11 samples that had enough feces to enumerate, respectively; 92.31, 79.17, and 73.33% of the total positive samples in each cohort, respectively) did not vary statistically ($P = 0.40$) from the unexposed cohort (17 samples had enough feces to enumerate; 56.67% of the total positive samples in the control cohort). The least-squares means for the high, medium, low, and control cohorts were 0.23, 0.24, -0.10, and -0.16 log MPN/g of feces, respectively. These data are not consistent with data we previously collected that demonstrated cattle supplemented with a DFM concentration containing 1×10^9 CFU of *L. acidophilus* strain NP51 and 1×10^9 CFU of *P. freudenreichii* (NP24) per steer daily (0.9 log MPN/g) was statistically different ($P < 0.05$) from the unexposed cohort (3.2 log MPN/g (32)). This inconsistency in data could be attributed to the use of a 3×3 MPN in the current study, which reduces the upper limits of the MPN when compared with the 3×5 MPN that was used in Stephens et al. (32) findings. The high, medium, and control cohorts all had one sample that was >110 MPN/g of feces of *E. coli* O157, resulting in 8.33, 5.26, and 5.88% of the total samples enumerated in each cohort, respectively. These samples slightly increased the least-squares means for the enumeration of *E. coli* O157 in the control and medium cohorts. Another reason for the contradiction in results between these studies could have been caused by the sensitivity of the MPN method used being >0.3 MPN/g of feces. There were four, four, one, and six enumerated samples that were <0.3 MPN/g of feces of *E. coli* O157 in the high, medium, low, and control cohorts, resulting in 33.33, 21.05, 9.09, and 35.26% of the total samples enumerated, respectively. These results would have greatly decreased the least-squares means in the high, medium, and control cohorts and would have slightly decreased the least-squares means in the low cohort. Another issue is that a small percentage (56.67%) of the control cohort samples had enough feces to enumerate. Had we been able to enumerate more of the *E. coli* O157–positive fecal samples from the control cohort, we might have found animals shedding high amounts of this pathogen in their feces.

We confirmed the findings of other studies conducted in our laboratory, that *L. acidophilus* NP51 (also known as NPC747) at 1×10^9 cells per animal per day effectively reduces the prevalence of *E. coli* O157 in harvest-ready cattle feces and hides. Our laboratory has reported a 49 (3), 58 (35), 80 (36), and 51% (32) reduction of *E. coli* O157 shedding in feces of cattle receiving *L. acidophilus* NP51 at this concentration compared with unexposed cohorts. In this study, we detected a 74% reduction in pathogen recovery from feces. Our laboratory has reported an 88 (3), 67 (35), and 62% (36) reduction of *E. coli* O157 prevalence on hides of cattle receiving *L. acidophilus* NP51 at this concentration compared with the unexposed cohort. In this study, we detected a 55% reduction in pathogen recovery on hides. Although not identical to previously reported

data, our current results support the role of *L. acidophilus* NP51 as an effective preharvest intervention. Younts-Dahl et al. *(36)* reported a 69 and 72% reduction of *E. coli* O157 prevalence in feces and in feces or on hides, respectively, when compared with controls. In this study, we detected a 69 and 70% reduction in pathogen recovery in feces and in feces or on hides, respectively, when compared with an unexposed cohort.

In this study, we demonstrated that doses of *L. acidophilus* NP 51 supplementation effect reduction of *E. coli* O157 and *Salmonella* in feces, on hides, and in feces or on hides of feedlot cattle. It appears from this research that the low (1×10^7 CFU per animal per day) and high (1×10^9 CFU per animal per day) concentrations of *L. acidophilus* NP51 supplementation are effective for *E. coli* O157 reduction in feces, on hides, and in feces or on hides of feedlot cattle. For reductions in *Salmonella,* the high concentration (1×10^9 CFU per animal per day) of *L. acidophilus* NP51 supplementation appears to be the most effective of the three doses in feces and in feces or on hides of feedlot cattle.

To our knowledge, this is the first demonstration of the effect of this DFM on *Salmonella* concentrations in feces, on hides, and in feces or on hides. *Salmonella* has become important to the cattle industry because of the association of this pathogen with outbreaks (most commonly serotypes Newport, Typhimurium, and Thompson) in beef products *(7, 9, 19, 30, 37).* Therefore, it is advantageous to determine the efficacy of an intervention strategy commonly used for *E. coli* O157 on the reduction of *Salmonella* in feces and on hides of feedlot cattle. Further research is warranted to observe the efficacy of DFMs on the reduction of pathogenic *Salmonella* strains rather than observing generic *Salmonella.*

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