Reduction of *E. coli* O157:H7 in Beef Feedlot Cattle using Varying Doses of a Direct-Fed Microbial

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Research Summary

This study was led by Texas Tech University to evaluate supplementing finishing beef cattle with *Lactobacillus*-based direct-fed microbials as a pre-harvest food safety intervention strategy. Our objective was to evaluate the effects of three different doses of *Lactobacillus acidophilus* strain NP 51 and a combination treatment of NP51 and NP45 on prevalence of *Escherichia coli* O157 in finishing beef cattle. Three hundred steers were used in the study which was conducted at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction. Cattle received one of five treatments throughout the feeding period: treatments were based on high, medium, and low dose levels of NP51, a combination of NP51 and NP45, or no DFM (control). All DFM treatments included *Propionibacterium freudenreichii* to enhance animal performance. Individual rectal fecal samples were collected on arrival and every 28 days throughout the feeding period. Hide samples were also collected at the end of the feeding period to determine the prevalence of *E. coli* O157 hide carriage immediately prior to harvest. Cattle receiving a high level dose of NP51 were, overall, 77% less likely to shed *E. coli* O157 in their feces relative to those receiving the control diet. While supplementing cattle with lower doses of NP51 resulted in decreased fecal prevalence of the organism, using the high-level dose of NP51 was most effective. A lower percentage of cattle were carrying detectable *E. coli* O157 on their hide among those receiving DFM compared to the control groups. Supplementing cattle with *Lactobacillus acidophilus* strain NP51 based DFM continues to show promise as a pre-harvest food safety intervention strategy to control *E. coli* O157 in finishing beef cattle. The effectiveness of using the high-level dose of NP51 may make it most advantageous in improving food safety pre-harvest.

Objective of Research Proposal

The overall objective of this study was to evaluate the effects of three different doses of NP51 and the combination of NP51 and NP45 fed to finishing beef cattle on the prevalence of *E. coli* in the feces and on the hides. Additionally, animal performance as affected by the addition of these treatments, was also evaluated.

Experimental Procedures

Cattle and Treatment Assignments. Three hundred steers were received at the Texas Tech University Burnett Center for Beef Cattle Research and Instruction and were subject to routine processing. Individual body weights were used to sort the steers into twelve blocks. Within each weight block, steers were assigned to one of five treatments and designated to pens (five animals/pen) accordingly in a randomized complete block design. There were a total of twelve pens assigned to each of the five treatments (five pens/block). All cattle received a standard steam-flaked corn-based finishing diet (92% concentrate) throughout the feeding period. The DFM treatments included: 1) control - no added DFM; 2) HNP51 – high dose of NP 51 at 1 x 10^9 CFU/steer daily; 3) MNP51 – NP51 at 1 x 10^8 CFU/steer daily; 4) LNP51 -- low dose of NP 51 at 1 x 10^7 CFU/steer daily; or 5) NP51+45 -- NP51 at 1 x 10^9 CFU/steer daily and NP45 at 1 x 10^9 CFU/steer daily. All four treatments containing NP51 also contained *Propionibacterium freudenreichii* at 1 x 10^9 CFU per steer daily. The DFM were provided by Nutrition Physiology Corp., Indianapolis, IN. The cultures were packaged in aluminum foil packets with each packet containing the desired daily amount of product for each treatment group. The cultures were mixed with 2.5 L of distilled water and the culture/water mixture was poured onto the cattle's diet.

Sample Collection. To determine the prevalence of *E. coli* O157, fecal and hide samples were collected from the cattle as they were restrained in a handling chute. Fecal samples were collected from each animal on the first day of the feeding period, and every subsequent 28 days until cattle were marketed for harvest. Cattle were marketed in blocks according to when the majority of cattle within a block(s) had reached a body weight and composition desired to achieve a USDA quality grade of Choice. On the day of harvest, both individual fecal and hide samples were collected from the live animal at the feedlot.

Fecal and hide samples were collected using techniques to avoid cross contamination. Rectal fecal samples were collected by persons wearing an arm-length plastic sleeve; a new sleeve was used for each sample. Individually labeled specimen containers were used to transport fecal samples. Hide swab samples were taken using a sponge which was swabbed over approximately a 600-cm^2 area of the perineum on the right side of each animal. The hide swab sponges were hydrated with Butterfield's Phosphate Diluent (BPD). Following swabbing, the sponges were placed in pre-labeled sample bags containing BPD. All samples were placed in a cooler with ice and transported to a laboratory at Texas Tech University for further processing.

Microbial Analysis. Methods adopted from Laegreid et. al., (*2*) were used to analyze the presence of *E. coli* O157 in fecal and hide samples. The methods were based on the immunomagnetic separation procedure followed by selective plating, biochemical testing, and latex agglutination tests for confirmation of isolates as *E. coli* O157.

Animal Performance. At each 28 d sampling period, steers were individually weighed and those weights were used to determine average daily gain for that 28 d period. Moreover, at this time, feedbunks were cleaned and a total dry matter intake was obtained for the 28-d period. Feed intake data were used in conjunction with gain data to calculate efficiency. At harvest, complete carcass data including, hot carcass weight, backfat thickness, longissimus muscle area, percentage of kidney pelvic and heart fat, and percentage of carcasses grading USDA Choice, were obtained.

Statistical Analysis. Descriptive statistics were generated for the prevalence of *E. coli* O157 in feces and on hides and presented in tabular or graphic formats. The data were formally analyzed using the GLIMMIX macro for SAS (SAS Inst., Inc., Cary, NC; available at http://ewe3.sas.com/techsup/download/stat/). A binomial response variable was constructed for each pen of cattle and was considered the dependent variable of interest. Single time-point analysis was performed on samples collected at arrival (feces) and at harvest (feces, hides, and presence either in feces or on hides). In these models, block was considered a random variable. Fecal samples collected over time were analyzed using repeated measures methodologies and first order auto-regressive

covariance matrices were used to model within pen dependency. Time was included in the model of longitudinal data in addition to treatment; block by treatment and block by time were included as random variables in addition to block. The longitudinal analysis only included post exposure samples (i.e., it did not include those samples collected at feedlot arrival). Where significant variation was detected ($P < 0.10$), contrasts were constructed to separate treatment means, compared the average of LNP51, MNP51, and HNP51 with the control, and evaluate a linear effect of dose on *E. coli* O157 prevalence.

Results

Fecal Prevalence. The overall prevalence of cattle shedding *E. coli* O157 at the initiation of the trial was 14.7% (44 of 300 fecal samples collected). There was not a difference in the prevalence of fecal shedding across treatment groups at this point ($P = 0.20$). Two animals were removed from the study before the collection of samples on day 28 for reasons unrelated to the treatments.

Overall, cattle receiving HNP51, MNP51, and LNP51 had a lower ($P < 0.01$) prevalence of *E. coli* O157 fecal shedding throughout the feeding period compared with cattle receiving the control diet (Figure 1). There was also a linear effect of NP51 dose level ($P < 0.01$), demonstrating a linear decrease in prevalence with increasing NP51 dose. The least squares mean estimates of average *E. coli* O157 fecal prevalence throughout the feeding period were 23.9, 10.5, 9.9, 6.8, and 17.3% for cattle receiving the control, LNP51, MNP51, HNP51, and NP51+45 diets, respectively (Figure 2). Averaged over time, cattle receiving HNP51 were 77% less likely to shed detectable *E. coli* O157 relative to those receiving the control diet ($P < 0.01$; Table 1). Cattle receiving LNP51 and MNP51 were 63 and 66% less likely to shed *E. coli* O157, respectively, relative to the controls ($P < 0.01$; Table 1). A decrease in prevalence for cattle receiving NP51+45 relative to the controls was not detected ($P = 0.15$; Table 1).

The proportion of cattle in each treatment group that shed a detectable level of *E. coli* O157 at any point in the feeding period differed significantly ($P < 0.02$; Figure 3). Sixty-nine percent of the cattle in the control group shed detectable *E. coli* O157 on at least one sampling date, whereas the proportion of cattle ever classified as *E. coli* O157 positive was 60, 55.9, 43.3, and 78.3% among those receiving LNP51, MNP51, HNP51, and NP51+45, respectively. Among those cattle classified as *E. coli* O157 positive, the average number of sample dates they were shedding detectable *E. coli* O157 was 2.4, 1.6, 1.5, 1.5,

and 1.8 among cattle receiving the control, LNP51, MNP51, HNP51, and NP51+45 diets, respectively.

At harvest, least squares mean estimates of *E. coli* O157 fecal prevalence were 31.7, 12.5, 17.4, 8.2, and 41.6% among cattle receiving the control, LNP51, MNP51, HNP51, and NP51+45, respectively (Figure 4). There was a linear effect (P = 0.03) across NP51 treatments at harvest, with the group receiving HNP51 having the lowest prevalence of fecal shedding of *E. coli* O157. Cattle receiving HNP51 were 80% less likely to be shedding detectable *E. coli* O157 at harvest than the controls (P < 0.01; Table 2). Those receiving LNP51 were 69% less likely to be shedding *E. coli* O157 than the controls (P = 0.03; Table 2), whereas prevalence among cattle receiving MNP51 was marginally different from the controls (P = 0.12; Table 2). *E. coli* O157 fecal prevalence at harvest among those receiving NP51+45 was not different from the controls (P = 0.37; Table 2).

Hide Prevalence. Numerical differences were observed in the percentage of cattle found to be carrying *E. coli* O157 on their hides between treatment groups. Least squares mean estimates of the percentage of positive hide samples at harvest were 8.7, 5.9, 4.8, 3.4, and 8.6% among cattle receiving the control, LNP51, MNP51, HNP51, and NP51+45 respectively. Cattle receiving HNP51 were 62% less likely to be carrying *E. coli* O157 on their hides than the controls; however this estimate was only marginally significant (P = 0.12; Table 2). Hide prevalence of *E. coli* O157 among those receiving LNP52, MNP51, and NP51+45 was not different (P = 0.46, 0.25, and 0.99, respectively) from those receiving the control diet (Table 2).

Overall Prevalence. Least square mean estimates of the proportion of cattle carrying *E. coli* O157 at harvest, based on either fecal or hide sample culture results, were 38.4, 14.8, 18.3, 10.3, and 42% among cattle receiving the control, LNP51, MNP51, HNP51, and NP51+45, respectively. Cattle receiving HNP51, MNP51, and LNP51 were 81, 65, and 72% less likely to be classified as "positive" at harvest than the controls (P = 0.01, 0.03, and 0.01, respectively; Table 2.) The proportion of cattle receiving NP51+45 classified as "positive" was not different than the controls (P = 0.73).

Performance/Carcass Data. No differences for period DMI ($P \geq 0.28$) or total DMI ($P \geq 0.52$) were detected among treatments. Period data and overall data

for ADG in this experiment revealed no differences ($P \geq 0.12$) among treatments. The F:G for the entire feeding period as well as carcass-adjusted F:G did not differ ($P \geq 0.12$) among treatments; however, F:G for d 0 to 56 tended to respond quadratically to NP51 dose level ($P = 0.09$), with the best F:G noted for the H treatment during this period. A linear effect of NP51 dose level also was detected for d 0 through 84 period ($P = 0.10$) for F:G, with superior F:G for the H treatment compared with the L and M treatments. The pre-planned orthogonal contrast for H vs. H+ approached significance ($P = 0.11$) for d 0 to 28.

Dressing percent was significantly greater ($P = 0.01$) for the control vs. the average of all other treatments, and dressing percent tended ($P = 0.15$) to be affected by NP51 dose, with a lower dress as dose level increased. Longissimus muscle (LM) measured at the split lean surface at the 12^{th} and 13^{th} ribs tended to differ ($P = 0.12$) for control vs. DFM treatments, and there was a trend ($P = 0.15$) for a quadratic effect of NP51 dose on LM. No other differences in other carcass components was detected.

Conclusions and Discussion

Previously, the findings of two past studies conducted at Texas Tech University indicated that cattle receiving a 1×10^9 CFU/steer daily dose of NP51 were approximately 50% less likely to be shedding *E. coli* O157 compared to animals receiving no DFM (*1, 3*). Likewise, in the current study, cattle receiving a diet supplemented with the high-level dose of NP51 had the lowest prevalence of shedding *E. coli* O157. However, the findings of this study indicate that feeding lower doses of NP51 may also be effective in decreasing the prevalence of fecal shedding of *E. coli* O157 during the feeding period.

The higher prevalence of *E. coli* O157 shedding observed among cattle receiving NP51 plus NP45 compared to those cattle receiving only NP51 may indicate that the two organisms are antagonistic. This finding was also observed in a previous study in which decreased efficiency was detected in cattle receiving both of these organisms (*3*). There were no detrimental effects of the DFM treatments used in these studies on animal performance

Supplementing cattle with *Lactobacillus acidophilus* strain NP51 based DFM continues to show effectiveness as a pre-harvest food safety intervention strategy to control *E. coli* O157 in finishing beef cattle. While supplementing cattle with lower doses of NP51 or NP51 combined with NP45 resulted in lower

prevalence of the organism, the effectiveness of using the high-level dose of NP51 may make it most advantageous in improving food safety pre-harvest.

Literature Cited

1. Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. Killinger-Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *Journal of Food Protection* 66:748-754.

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3. Younts-Dahl, S. M., M. L. Galyean, G. H. Loneragan, N. A. Elam, and M. M. Brashears. 2003. 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. *Journal of Food Protection* In Press.

TABLE 1. *Odds ratios (OR), 95% confidence intervals (CI), and probability (*P*) values for the likelihood of detectable fecal shedding E. coli O157 throughout the feeding period within treatment groups compared with the control group*.

Treatment	OR	95% CI	*P* values
LNP51[a]	0.37	0.21 to 0.68	0.002
MNP51[b]	0.34	0.19 to 0.62	<0.001
HNP51[c]	0.23	0.12 to 0.44	<0.001
NP51+45[d]	0.66	0.37 to 1.17	0.152

[a] Diet supplemented with a low-level dose (1 x 10^7 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*.

[b] Diet supplemented with a medium-level dose (1 x 10^8 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*.

[c] Diet supplemented with a high-level dose (1 x 10^9 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*

[d] Diet supplemented with a high-level dose (1 x 10^9 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^6 CFU/steer daily of strain NP45 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*

Table 2. *Odds ratios (OR), 95% confidence intervals (CI), and probability (*P*) values for the likelihood of detectable E. coli O157 carriage based on culture results for fecal, hide, or either fecal or hide samples at harvest within treatment groups compared with the control group*.

Sample Type	Treatment	OR	95% CI	*P* values
Fecal	LNP51[a]	0.31	0.10 to 0.90	0.033
	MNP51[b]	0.44	0.16 to 1.24	0.119
	HNP51[c]	0.20	0.06 to 0.64	0.008
	NP51+45[d]	1.53	0.59 to 3.98	0.371
Hide	LNP51[a]	0.67	0.22 to 1.98	0.455
	MNP51[b]	0.52	0.16 to 1.64	0.254
	HNP51[c]	0.38	0.11 to 1.32	0.124
	NP51+45[d]	0.99	0.36 to 2.74	0.991
Fecal or hide	LNP51[a]	0.28	0.10 to 0.75	0.013
	MNP51[b]	0.35	0.13 to 0.92	0.034
	HNP51[c]	0.19	0.06 to 0.54	0.003
	NP51+45[d]	1.16	0.48 to 2.81	0.729

[a] Diet supplemented with a low-level dose (1×10^7 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1×10^9 CFU/steer daily of *Propionibacterium freudenreichii*.

[b] Diet supplemented with a medium-level dose (1×10^8 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1×10^9 CFU/steer daily of *Propionibacterium freudenreichii*.

[c] Diet supplemented with a high-level dose (1 x 10^9 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*

[d] Diet supplemented with a high-level dose (1 x 10^9 CFU/steer daily) of *Lactobacillus acidophilus* strain NP51 and 1 x 10^6 CFU/steer daily of strain NP45 and 1 x 10^9 CFU/steer daily of *Propionibacterium freudenreichii*.

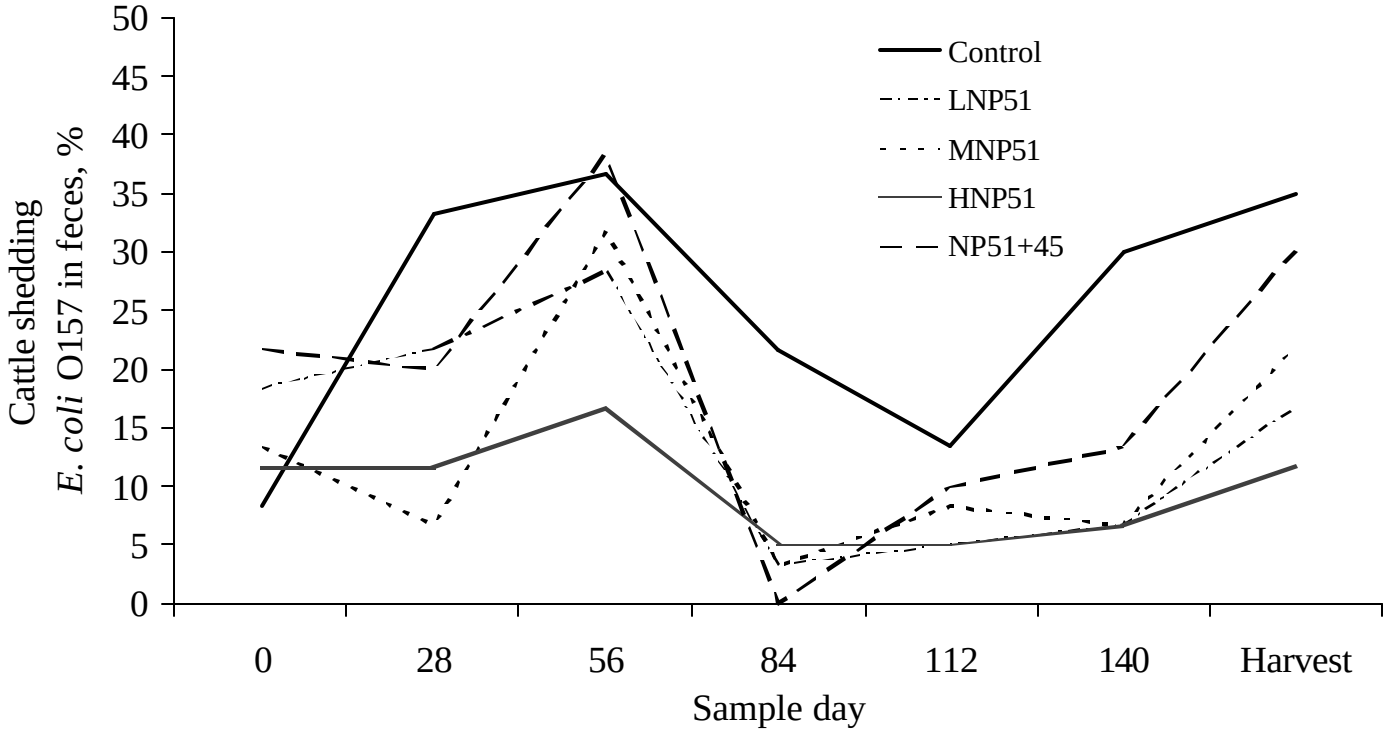

Figure 1. Percentage of cattle shedding a detectable level of *E. coli* O157 in their feces within each treatment group on sampling dates over the feeding period.

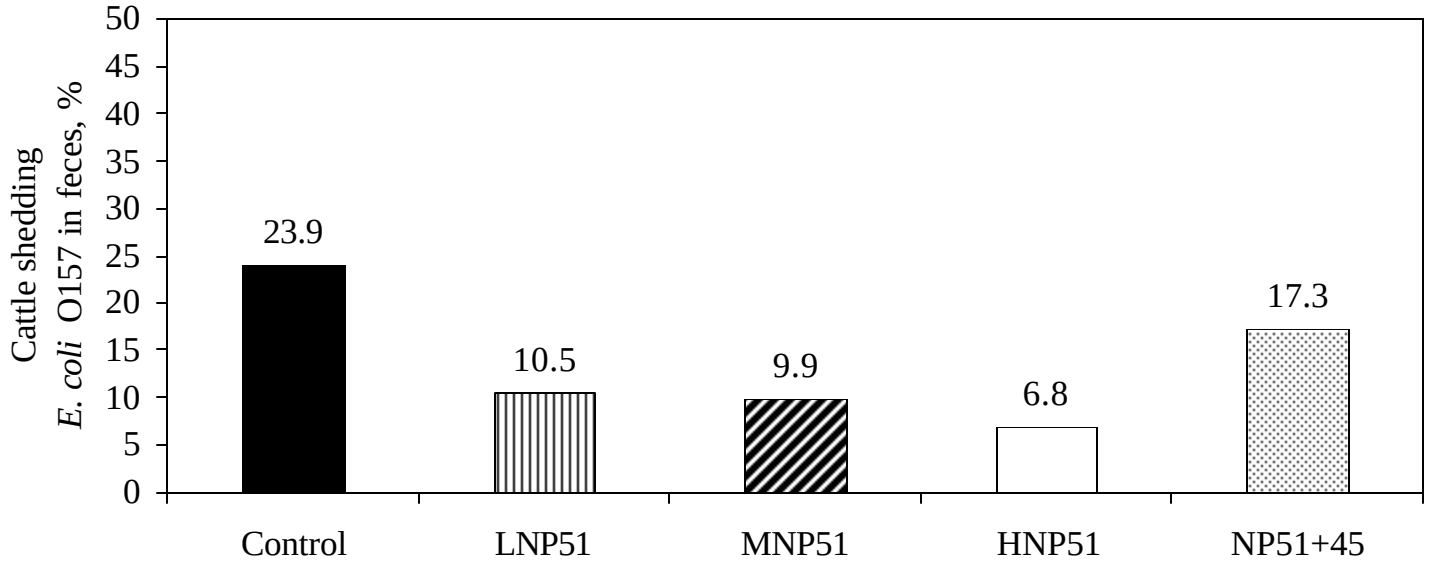

Figure 2. Least square mean estimates of the percentage of cattle shedding a detectable level of *E. coli* O157 in their feces by treatment group when prevalence was averaged over the feeding period.

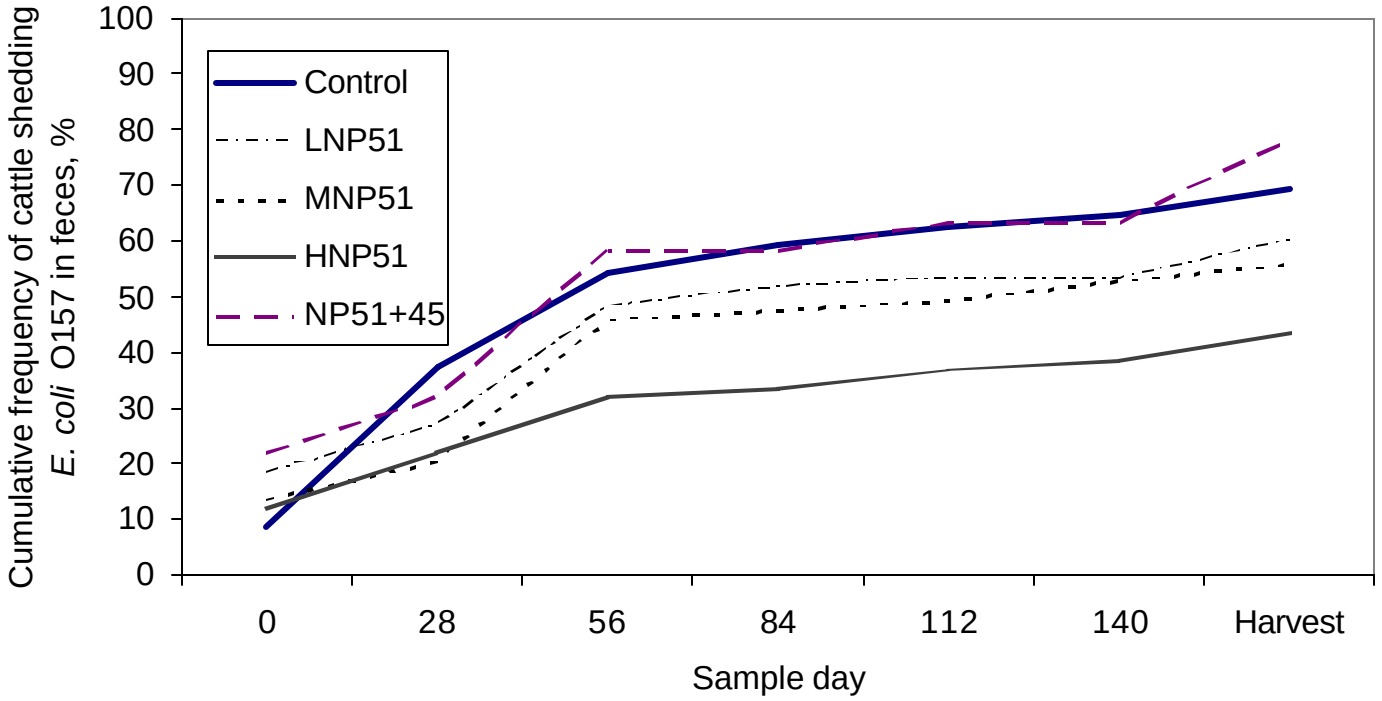

Figure 3. Cumulative proportion of cattle shedding a detectable level of *E. coli* O157 in their feces within each treatment group by sample date.

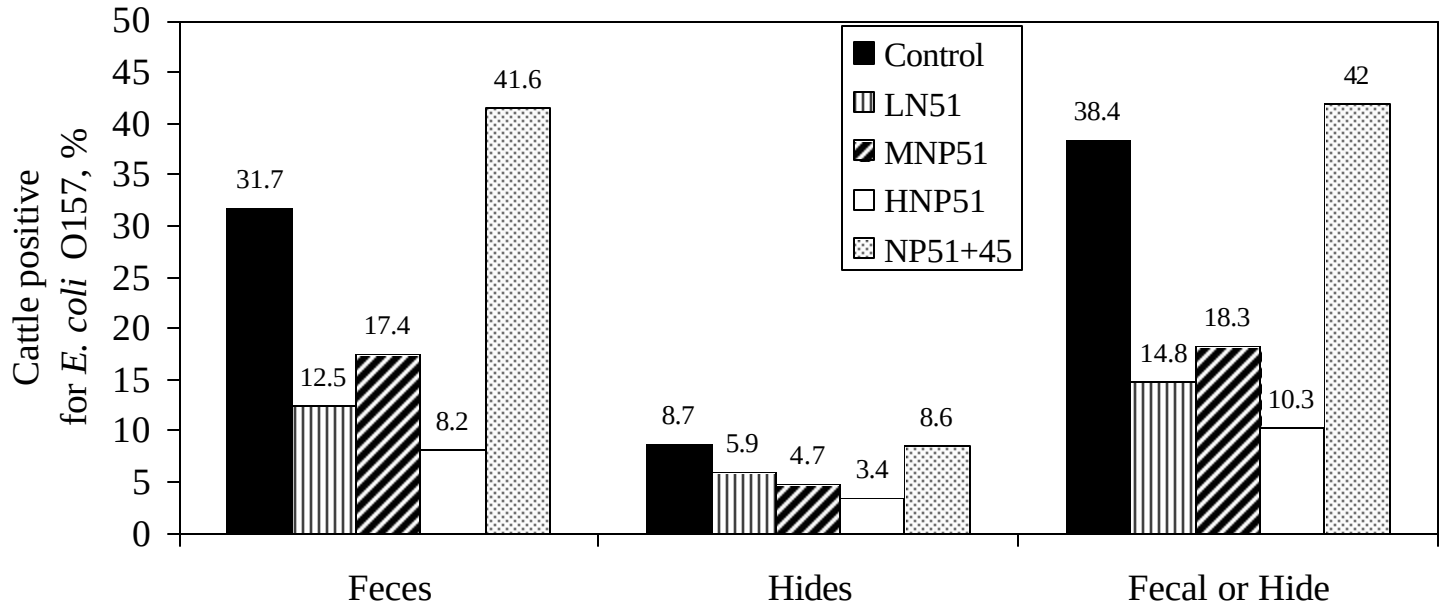

Figure 4. Least square mean estimates of the percentage of cattle with an *E. coli* O157 positive fecal, hide, and either fecal or hide sample by treatment group on the day of harvest.