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Effect of *Lactobacillus acidophilus* Strain NP51 on *Escherichia coli* O157:H7 Fecal Shedding and Finishing Performance in Beef Feedlot Cattle†

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ABSTRACT

A 2-year study was conducted during the summer months (May to September) to test the effectiveness of feeding *Lactobacillus acidophilus* strain NP51 on the proportion of cattle shedding *Escherichia coli* O157:H7 in the feces and evaluate the effect of the treatment on finishing performance. Steers (n = 448) were assigned randomly to pens, and pens of cattle were assigned randomly to NP51 supplementation or no supplementation (control). NP51 products were mixed with water and applied as the feed was mixed daily in treatment-designated trucks at the rate of 10^9 CFU per steer. Fecal samples were collected (n = 3,360) from the rectum from each animal every 3 weeks, and *E. coli* O157:H7 was isolated by standard procedures, using selective enrichment, immunomagnetic separation, and PCR confirmation. The outcome variable was the recovery of *E. coli* O157:H7 from feces, and was modeled using logistic regression accounting for year, repeated measures of pens of cattle, and block. No significant differences were detected for gain, intakes, or feed efficiency of control or NP51-fed steers. The probability for cattle to shed *E. coli* O157:H7 varied significantly between 2002 and 2003 (P = 0.004). In 2002 and 2003, the probability for NP51-treated steers to shed *E. coli* O157:H7 over the test periods was 13 and 21%, respectively, compared with 21 and 28% among controls. Over the 2 years, NP51-treated steers were 35% less likely to shed *E. coli* O157:H7 than were steers in untreated pens (odds ratio = 0.58, P = 0.008). This study is consistent with previous reports that feeding NP51 is effective in reducing *E. coli* O157:H7 fecal shedding in feedlot cattle.

Escherichia coli O157:H7 is an important cause of foodborne illness and death in humans *(1)*. The disease is quite often severe, characterized by hemorrhagic colitis, and in a small percentage of cases, hemolytic uremic syndrome *(1)*. Beef cattle are important reservoirs of *E. coli* O157:H7 *(8, 18)* and, consequently, this organism is responsible for important economic losses associated with beef production *(12)*. Contamination of beef carcasses with *E. coli* O157:H7 has been linked to the presence of this organism on hides and in feces of cattle at the time of harvest *(2, 8, 9)*. Preharvest intervention strategies aimed at reducing carriage and shedding of *E. coli* O157:H7 in feces of beef feedlot cattle during the finishing phase of beef production may prove beneficial in reducing carcass contamination at harvest and further controlling the incidence of foodborne disease outbreaks *(8)*.

Increased emphasis in the area of preharvest food safety has led to increased interest in identifying preharvest management strategies to reduce the prevalence of *E. coli* O157:H7 associated with cattle at harvest. Included in these are direct-fed microbials (DFMs), antibiotics, bacteriophages, vaccination, diet change, and good management practices in regards to pen, feed bunk, and water trough maintenance *(6)*. Unfortunately, only a few of these strategies have been shown to reduce carriage and fecal shedding of *E. coli* O157:H7 in feedlot cattle.

Supplementing cattle with DFM products has shown promise as a preharvest intervention *(3, 20, 21)*. Further, there is an increased interest in the effect of DFMs on finishing performance due to concern regarding the use of antibiotics and other growth stimulants in the animal feed industry *(6, 14)*. *Lactobacillus acidophilus* strain NP51 (also known as NPC747), when used as a DFM, has significantly decreased *E. coli* O157:H7 fecal shedding and hide contamination, without reduction in performance in feedlot cattle *(3, 20)*. The objective of this clinical trial was to test the effectiveness of feeding *L. acidophilus* strain NP51 on the proportion of cattle shedding *E. coli* O157:H7 in feces and finishing performance in a larger scale study that extended over 2 years.

MATERIALS AND METHODS

Study design. A feedlot finishing experiment was conducted in two phases during the summers (May to September) of 2002 and 2003. The experimental design was a randomized complete block design with sampling group and initial body weight (BW) as the blocking factor. Treatments were assigned randomly to pens, consisted of a DFM product (*L. acidophilus* strain NP51) or no supplementation (control), and were given every day of the

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experiment. Fecal samples were collected from the rectum of each animal every 21 days starting on day 0 and extending through the duration of the finishing period.

In 2002, steers were sampled one block per week in 3-week experimental periods, resulting in one pretreatment sampling and five separate test period samplings. The pretreatment sample was collected on day 0, whereas the five test-period samplings were collected on days 21, 42, 63, 84, and 105. Cattle within each block were started on trial on the same day; however, a block of cattle was started on trial every week for three consecutive weeks. All blocks of cattle were harvested on the same day which resulted in 116, 122, and 129 days on feed, respectively.

In 2003, steers were sampled in one block per day on two consecutive days every 21 days, resulting in one pretreatment sampling and six separate test period samplings. The pretreatment sample was collected on day 0, whereas the six test-period samplings were collected on days 21, 42, 63, 84, 105, and 126. Cattle within each block were started on trial on the same day and harvested 127 days later.

In 2002, 192 steer calves had an average initial BW of 349 kg. Initial BW was the average of BW taken for two consecutive days following a 5-day limit-fed period. The limit-fed diet in 2002 consisted of 50% alfalfa hay:50% wet-corn gluten feed (dry matter) and fed at 2% BW for five consecutive days to reduce variation in initial weight due to gut fill. Following the initial weigh day, steers were stratified by BW, blocked by weight and sampling day into three blocks, and assigned randomly within block using a random number generator to 24 pens (eight head per pen). Pens within each block were assigned randomly to either control or NP51. On the second day of the initial weigh period (day 0), cattle were sorted into their respective pens and fed the finishing diet with NP51 or without a DFM product (control). The finishing diet dry matter composition was 55% high-moisture corn, 35% wet-corn gluten feed, 5% corn silage, 2% alfalfa hay, 2% supplement, and 1% water (water used to mix NP51).

In 2003, 256 steer calves had an average initial BW of 367 kg. Initial BW was obtained following the same 5-day limit-fed protocol described above. Steers were stratified by BW, assigned randomly to two blocks, and assigned randomly within block using a random number generator to 24 pens (8 steers per pen in 16 pens within one block and 16 steers per pen in 8 pens in the second block). Within each block, pens were assigned to one of four dietary treatments consisting of different proportions of dry-rolled corn, corn silage, corn bran, steep, molasses, and a dry supplement as the basal feed ingredients. DFM product treatment was balanced across block and dietary treatment and assigned randomly. On the second day of the 2-day weigh period (day 0), cattle were sorted into their respective pens. DFM product treatments included NP51 supplementation and control supplementation.

In each year of the study, the NP51 product was mixed with water and applied to the feed truck mixing box and fed at a rate of 10^9 CFU per steer daily. In both years, steers were fed once daily. Separate feed trucks were used to feed the control and NP51 diets to eliminate the chance of cross contamination.

Performance measures collected included average daily gain, dry matter intake measured on a daily basis, and feed efficiency. Gain is based on final BW minus initial BW divided by days fed. Final BW was based on hot carcass weight collected at a commercial abbatoir the day of slaughter divided by a dressing percentage of 63.5%. Feed efficiency is a common measure calculated as a ratio of average daily gain/dry matter intake with units of kg/kg. Slaughter time was based on visual appraisal when cattle contained an average of 1.2 cm of back fat. On the day of slaughter, hot carcass weight was recorded. Following a 24-h chill, 12th rib fat was measured and U.S. Department of Agriculture (USDA) marbling scores and USDA yield grade data recorded from a trained USDA grader. USDA marbling scores are indicative of intramuscular fat at the 12th rib and recorded on a scale of 300 = slight marbling (USDA Select quality grade), 400 = small marbling (USDA low Choice quality grade), and 500 = modest marbling. USDA yield grade measures are based on a scale of 1 to 5. USDA yield grade is an estimate of boneless, closely trimmed retail cuts, with a yield grade 1 having more retail yield than yield grade 5, which is much less lean and more fat.

Sample collection. In both years, fecal samples were collected directly from the rectum of each animal as they were restrained in a cattle processing chute. The personnel collecting samples wore latex gloves and an arm length plastic sleeve, and a new sleeve was used to collect each sample. Each fecal sample was placed in an individually labeled specimen cup. All samples were placed in a cooler with ice packs and immediately transported to the laboratory. Laboratory personnel were blinded to treatments and field investigators were blinded to test results until after the study was completed.

Microbial analysis. Fecal samples were analyzed for presence of *E. coli* O157:H7, using procedures previously described *(19)*. Isolates that collectively were sorbitol nonfermenting, lactose-fermenting, negative for β-glucuronidase activity, and positive for the O157 antigen were tested in a five–primer pair multiplex PCR assay *(16)*. The multiplex PCR detected genes for *E. coli* O157 ($rfbE_{O157:H7}$), H7 ($fliC_{h7}$), Shiga toxins 1 (stx_1) and 2 (stx_2), and intimin (eae_{O157}) *(16)*. Detection of genes for O157, H7, and at least one other target in the assay were considered to be confirmation of an isolate as *E. coli* O157:H7.

Statistical analysis. The effect of NP51 was tested by modeling the probability of detecting *E. coli* O157:H7 from feces using the logit link function in a binomial distribution multivariable generalized estimation equation model (Proc GENMOD, SAS Institute, Inc., Cary, N.C.). A first-order autoregressive correlation structure was defined to account for repeated measures of pens over time, sampling blocks, and year. The models were fashioned with a manual forward selection process with subsequent backwards elimination so that variables in the model were significant at $\alpha \leq 0.05$, using the Score statistic for Type 3 generalized estimation equation analysis. Fixed effects tested in the model were NP51, test period, block, and year. Two-way interactions between these variables were also tested.

Least-square means of the parameter estimates from the multivariable logistic models were used to estimate adjusted probabilities for fixed effects of NP51, test period, block, and year. The relative risk for NP51 supplementation was calculated from the adjusted probabilities. Treatment efficacy of NP51 was calculated as (1 − relative risk).

The effect of NP51 on feedlot performance and carcass characteristics were evaluated statistically as a randomized complete block design with the MIXED procedure of SAS. Pen was the experimental unit, NP51 treatment was the fixed effect, and block within year was treated as random effects. Performance data from both years were pooled, and year was included in the final analysis as a random effect. Average daily gain, dry matter intake, feed efficiency, hot carcass weight, 12th rib fat thickness, USDA marbling score, and USDA yield grade were reported by NP51 treatment.

TABLE 1. *Multivariable logistic regression model of the probability to detect* E. coli *O157:H7 from feces*

Variable	Unit	Parameter	Odds ratio	95% confidence interval		*P* value
Intercept		−2.829				
NP51	Yes	−0.545	0.58	0.41	0.82	0.008
	No	Ref.[a]	1.00	Ref.		
Year	2002	−0.537	0.58	0.44	0.78	0.004
	2003	Ref.	1.00	Ref.		
Block	1	−0.509	0.60	0.37	0.99	0.02
	2	−0.818	0.44	0.27	0.71	
	3	Ref.	1.00	Ref.		
Test period	1	−0.207	0.81	0.48	1.38	0.01
	2	−1.256	0.28	0.16	0.52	
	3	−0.635	0.53	0.33	0.84	
	4	−1.309	0.27	0.16	0.45	
	5	−0.157	0.85	0.52	1.39	
	6	Ref.	1.00	Ref.		

[a] Ref., reference group.

RESULTS

No steers were removed from the study in either year of the trial. In 2002, steers in NP51 and control pens were fed for an average of 122 days. In 2003, steers in NP51 and control pens were fed for an average of 127 days.

***E. coli* O157:H7.** In total, *E. coli* O157:H7 was isolated from 672 of the 3,360 fecal samples collected from steers in both years. In 2002, the pretreatment probability of detecting *E. coli* O157:H7 in the feces of steers in control and NP51 pens was 32.3 and 22.9%, respectively. In 2003, the pretreatment probability of detecting *E. coli* O157:H7 in the feces of steers in control and NP51 pens was 30.5 and 37.5%, respectively. Pretreatment probability was not different ($P = 0.89$) among DFM product treatments for either year. In 2002 and 2003, average pretreatment probability was 31 and 34%, respectively.

In 2002, *E. coli* O157:H7 was isolated from 62 (12.9%) of 480 fecal samples from steers supplemented with NP51, and from 102 (21.3%) of 480 fecal samples from control steers. In 2003, *E. coli* O157:H7 was isolated from 146 (19.0%) of 768 fecal samples from steers supplemented with NP51, and from 218 (28.4%) of 768 fecal samples from control steers.

The factors explaining the probability for steers to test positive during the study period for *E. coli* O157:H7 in the multivariable logistic regression model were year, block, test period, and DFM product treatment (Table 1). The probability of recovering *E. coli* O157:H7 from the feces during the study period differed ($P = 0.004$) between 2002 and 2003. Accounting for other variables in the model, the odds of detecting *E. coli* O157:H7 in feces of steers fed in 2002 was 0.58 times the odds of detecting *E. coli* O157:H7 in feces of steers fed in 2003. The model-adjusted probability to detect *E. coli* O157:H7 during the study period was 0.17 (standard error [SE] = 0.017) and 0.26 (SE = 0.020) for 2002 and 2003, respectively. There was no interaction ($P = 0.86$) between DFM product treatment and year. Block ($P = 0.02$) and test period ($P = 0.01$) were also associated with the probability to detect *E. coli* O157:H7 from feces. There was no interaction between test period and DFM product treatment ($P = 0.77$).

Supplementing cattle with NP51 reduced the probability of recovering *E. coli* O157:H7 from the feces during the study period (adjusted odds ratio = 0.58, $P = 0.008$). The model-adjusted probability to detect *E. coli* O157:H7 from feces during the two study periods was 0.17 (SE = 0.022) for cattle fed NP51 and 0.26 (SE = 0.017) for cattle fed the control diet. Therefore, over the 2 years, cattle within NP51-treated pens of steers were 35% less likely to shed *E. coli* O157:H7 in feces compared with control pens of steers.

TABLE 2. *Effects of* Lactobacillus acidophilus *NP51 on performance and carcass characteristics of finishing beef steers; year was included in the final analysis as a random effect*

Item	Treatment[a]: Control	NP51	SEM[b]	*P* value[c]
Steers	224	224	—	—
Pens	24	24	—	—
Initial body weight (kg)	358	358	8.8	0.12
Final body weight (kg)[d]	566	568	3.7	0.54
ADG (kg)[e]	1.67	1.69	0.05	0.45
DMI (kg/day)[f]	11.42	11.31	0.12	0.29
Feed efficiency[g]	0.147	0.150	0.006	0.11
Hot carcass weight (kg)	360	361	2.4	0.54
Fat thickness (cm)	1.24	1.24	0.01	0.81
Yield grade[h]	2.66	2.55	0.06	0.06
Marbling score[i]	490	487	8.0	0.63

[a] Control, carrier (lactose) mixed in water and added to the diet at the time of feeding; NP51, control plus 10^9 CFU of *L. acidophilus* NP51 per animal daily. Average time on feed, 122 days for 2002 and 127 days for 2003. Number of steers, 192 for 2002 and 256 for 2003.

[b] Pooled SE of treatment means.

[c] *P* value for overall *F*-test statistic for treatment.

[d] Final body weight (BW) was calculated as hot carcass weight/average dressing of 63.5%.

[e] ADG, daily gain calcuated as (final BW − initial BW)/days on feed.

[f] DMI, daily dry matter intake, in kilograms per day.

[g] Feed efficiency is the ratio of ADG to DMI, as kg/kg.

[h] USDA yield grade on a scale of 1 to 5, with leaner carcasses with greater retail of yield of meat scoring lower.

[i] USDA marbling score with 300 = slight or USDA Select, 400 = small or USDA low Choice, and 500 = modest or USDA average Choice.

Feedlot performance. The effects of the four diets fed in 2003 were tested. Diet was not associated ($P = 0.24$) with the probability of recovering *E. coli* O157:H7 in the feces in this study.

Feedlot performance and carcass characteristics outcomes are presented in Table 2. Average initial BW was not different ($P = 0.12$) among NP51 and control steers, and averaged 358 kg. Carcass-adjusted final live BW was not different ($P = 0.54$) among NP51 and control steers.

Supplementation of NP51 had no effect on average daily gain ($P = 0.45$) or daily dry matter intake ($P = 0.29$). Feed efficiency (gain/intake) for steers supplemented with NP51 and controls were 0.150 and 0.147, respectively ($P = 0.11$). Hot carcass weight, fat thickness, and USDA marbling score were not different ($P > 0.54$) among NP51 and control steers. Yield grade for NP51 and control steers averaged 2.55 and 2.66, respectively ($P = 0.06$).

DISCUSSION

Differences in the probability to detect *E. coli* O157:H7 by test period and year were observed. This is consistent with previous longitudinal studies that show the proportion of cattle shedding *E. coli* O157:H7 can vary greatly over time *(13, 16)*. In a study designed to describe and explain the ecology of *E. coli* O157:H7 by time and place in commercial beef feedlots, large differences in the proportion of pens of cattle classified as positive for *E. coli* O157:H7 by feeding season, weeks within season, and feed yard were observed *(19)*. Probability to detect *E. coli* O157:H7 varies by time and place and the results of this study were consistent with that finding, and the reason for collecting samples for five and six periods.

The probability for cattle to shed *E. coli* O157:H7 among control pens was higher than those of the NP51-treated pens. There was no interaction between DFM product treatment and either study period or year of study, indicating the effect of NP51 was consistent over the feeding period and the 2 years of study.

Our study indicates that NP51-treated pens of steers were 35% less likely to shed *E. coli* O157:H7 in feces than were untreated pens of steers. Other researchers *(3, 20)* have found greater reductions in detectable levels of *E. coli* O157:H7 isolated from feces in response to NP51 supplementation. In one study, *E. coli* O157:H7 was 49% less likely to be detected in the feces of animals receiving NP51, compared with control animals *(3)*. In another, cattle supplemented with NP51 were 57.5% less likely to shed detectable levels of *E. coli* O157:H7 in the feces, when compared with control animals *(20)*. In both studies, NP51 was fed at the same level (10^9 CFU per steer per day) as in our study. Although we observed less benefit of NP51 supplementation than previously reported, this study is consistent in suggesting NP51 is effective at reducing the probability of detecting *E. coli* O157:H7 in feces of commercially fed cattle. Several terms have been used to describe microorganisms used for health and performance benefits. Probiotics are defined as live microorganisms, which, when administered in an adequate amount, confer a health benefit on the host *(11)*. DFMs have been defined as feed products that contain a source of live, naturally occurring microorganisms and, when used to compete with a particular organism, may be referred to as competitive exclusion products *(3, 4, 7)*. Competitive exclusion involves the use of live microbial cultures that exhibit antagonistic effects against specific groups of organisms, resulting in a decrease in their numbers in the intestinal tract *(3, 4)*.

The mechanisms by which DFMs, such as *Lactobacillus* spp., could potentially compete with *E. coli* O157:H7 in the intestine are numerous. Ogawa et al. *(15)*, who conducted studies in infant rabbits experimentally infected with *E. coli* O157:H7, noted several mechanisms by which lactobacilli could exert competitive exclusion effects against this organism; they produce volatile fatty acids; can enhance specific and total IgA secretion when used as an oral adjuvant; enhance the secretion of specific antibodies against Stx1, Stx2, and Stx-producing *E. coli* cells; and have other effects. Sherman et al. *(17)*, using an intestinal epithelial cell line (T84 cells) found that certain strains of *Lactobacillus* spp., including those of *L. acidophilus* and *L. rhamnosus*, reduce intestinal epithelial cell injury after exposure to enterohemorrhagic *E. coli* O157:H7 and enteropathogenic *E. coli* O127:H6. In that study, the probiotic *Lactobacillus* strains reduced the binding of the *E. coli* O157:H7 and *E. coli* O127:H6 to the epithelial cells, and reduced the number of foci of rearrangements of alpha-actin, which was indicative of a reduction in the number of attaching-effacing lesions.

We observed little advantage to feeding NP51 with regard to important feedlot performance outcomes, and this finding is consistent with that of other studies *(3, 7)*. One study reported no differences in daily gain or dry matter intake among treatments *(3)*. However, in that study, carcass-adjusted feed efficiencies were 0.176 for NP51 treated cattle and 0.167 for control cattle ($P = 0.06$) *(3)*. In a second study evaluating NP51, authors concluded that there were no differences for final BW, carcass-adjusted final BW, dry matter intake, or daily gain between cattle fed NP51 or no DFM *(7)*. Additionally, feed efficiency for the entire feeding period, as well as carcass adjusted feed efficiency, did not differ ($P > 0.18$) among treatments *(7)*. We observed a 2% difference in feed efficiency ($P = 0.11$) when cattle were supplemented with the DFM product. Although not significant in this study, a 2% difference in feed efficiency would be meaningful when supplementing a DFM product in the ration. In a review of six research trials ($n = 1{,}249$ cattle), Krehbiel et al. *(14)* reported that feeding combinations of lactic acid- and propionic acid-producing bacteria to feedlot cattle might improve growth rate by 2.6% ($P = 0.12$). However, in a large-scale commercial finishing study ($n = 3{,}539$ steers and heifers), Greenquist et al. *(10)* reported supplementation of *L. acidophilus* (NP51) and *Propionibacterium freudenreichii* had no measurable affect on growth performance ($P = 0.27$). In the current study, only the *L. acidophilus* strain (NP51) was fed, which may influence performance response.

The results of the present study demonstrate supplementing feedlot cattle with 10^9 CFU per steer daily with *L. acidophilus* (NP51) significantly reduced the probability of fecal shedding of *E. coli* O157:H7. Given these results and those of previous studies, we conclude that NP51 effectively reduces the shedding of *E. coli* O157:H7 in beef feedlot cattle and may be a useful preharvest intervention strategy.

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