

# **Feeding Supplemental Dried Distiller's Grains Increases Fecal Shedding of *Escherichia coli* O157 in Experimentally Inoculated Calves**

**M. E. Jacob<sup>1</sup>, G. L. Parsons<sup>2</sup>, M. K. Shelor<sup>2</sup>, J. T. Fox<sup>1</sup>, J. S. Drouillard<sup>2</sup>, D. U. Thomson<sup>3</sup>,  
D. G. Renter<sup>1</sup>, and T. G. Nagaraja<sup>1</sup>**

<sup>1</sup>Department of Diagnostic Medicine and Pathobiology

<sup>2</sup>Department of Animal Sciences and Industry

<sup>3</sup>Department of Clinical Sciences

Kansas State University, Manhattan, Kansas 66506

## **Keywords:**

Distiller's grains, *E. coli* O157, cattle, fecal shedding

## **Correspondence:**

T. G. Nagaraja  
Department of Diagnostic Medicine/Pathobiology  
College of Veterinary Medicine  
Kansas State University  
Tel: 785-532-1214  
Fax: 785-532-4851  
e-mail: [tnagaraj@vet.k-state.edu](mailto:tnagaraj@vet.k-state.edu)

One Table

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## 1 **Summary**

2 *Escherichia coli* O157 is an important foodborne pathogen and asymptomatic cattle serve as  
3 major reservoirs for human infection. We have shown a positive association between feeding  
4 distiller's grains and *E. coli* O157 prevalence in feedlot cattle. The objective of this study was to  
5 determine the effect of feeding dried distiller's grain (DDG) on fecal shedding of *E. coli* O157 in  
6 calves experimentally inoculated with *E. coli* O157. Holstein calves (5 per treatment group), fed  
7 steam-flaked corn-based high-grain diets supplemented with 0% (control) or 25% DDG, were  
8 orally inoculated with a five-strain mixture ( $6 \times 10^9$  CFU/calf) of nalidixic acid-resistant (*Nal<sup>R</sup>*)  
9 *E. coli* O157. Fecal samples were taken three times per week for six weeks to determine the  
10 prevalence and concentration of *Nal<sup>R</sup> E. coli* O157. At the end of the study (day 43), calves were  
11 euthanized and necropsied. Ruminal, cecum, colon, and rectal contents, and rectoanal mucosal  
12 swab (RAMS) samples were collected at necropsy to determine *Nal<sup>R</sup> E. coli* O157 concentration.  
13 There was a trend for an interaction between treatment and fecal sampling day. The  
14 concentration of *Nal<sup>R</sup> E. coli* O157 in the feces was significantly higher in fecal samples from  
15 calves fed DDG compared to control calves on days 35, 37, 39, and 42. At necropsy, the  
16 concentration of *Nal<sup>R</sup> E. coli* O157 was higher in the cecum ( $P = 0.01$ ), colon ( $P = 0.03$ ), and  
17 rectum ( $P = 0.01$ ) from calves fed DDG compared to control animals. The number of sites at  
18 necropsy positive for *Nal<sup>R</sup> E. coli* O157 was higher in calves fed DDG compared to calves in the  
19 control treatment ( $P < 0.001$ ). Our results indicate that *E. coli* O157 gut persistence and fecal  
20 prevalence increased in calves fed DDG, which potentially have important implications for food  
21 safety.

22

## 23 **Introduction**

24 Shiga-toxin producing *Escherichia coli* O157 is a major foodborne pathogen, and ruminants,  
25 particularly cattle, are primary reservoirs (Bach et al., 2002; Gyles, 2007; Hussein, 2007). The  
26 organism colonizes the hindgut and is shed in the feces, which serves as a major source of  
27 contamination of food products (Callaway et al., 2003). In a review of *E. coli* O157 outbreaks  
28 between 1982 and 2002, Rangel et al. (2005) reported that the pathogen caused 73,000 illnesses  
29 in the US annually. Transmission route for the outbreaks included foodborne (52%), person to  
30 person (14%), waterborne (9%), animal contact (3%), laboratory-related (0.3%), and unknown  
31 (21%). Among the foodborne outbreaks 38% was attributed to ground beef and 21% to produce  
32 (Rangel et al., 2005). Diet has been implicated as a factor in the prevalence of *E. coli* O157 in  
33 cattle (Van Baale et al., 2004; Dewell et al., 2005; Fox et al., 2007a). Cattle orally inoculated  
34 with *E. coli* O157 have been used to assess dietary impacts on fecal shedding. Van Baale et al.  
35 (2004) reported that *E. coli* O157 was shed for a longer duration in orally challenged calves fed a  
36 forage-based diet, compared to a grain-based diet. Another challenge study reported that a  
37 higher number of steers were *E. coli* O157 positive when fed barley grain compared to those fed  
38 corn grain (Buchko et al., 2000).

39 Distiller's grains (DG), a co-product of ethanol production by yeast fermentation of  
40 enzymatically hydrolyzed grain starch, is an effective feed supplement in finishing cattle diets  
41 (Firkins et al., 1985). The co-product can be fed as dry (DDG) or wet (WDG), with or without  
42 dehydration to remove the moisture, respectively. Distiller's grains constitute the components of  
43 cereal grains that remain after starch fermentation, effectively concentrating the non-starch  
44 nutrients (fiber, protein and lipid) approximately three-fold (Spiehs et al., 2002). The use of DG  
45 has been shown to improve daily gain in beef cattle (Ham et al., 1994; Al-Suwaiegh et al., 2002)

46 and increase milk yield and feed efficiency in dairy cattle (Kleinschmit et al., 2006). Distiller's  
47 grain usage in cattle is likely to increase with further expansion of the ethanol industry.

48 Previously, natural prevalence studies have indicated a positive association between *E. coli*  
49 O157 prevalence and feeding brewers grain or distiller's grains (both WDG and DDG) in feedlot  
50 cattle (Synge et al., 2003; Dewell et al., 2005; Jacob et al., 2006, 2008). Therefore, an  
51 experimental inoculation study was deemed necessary to confirm the positive association  
52 between *E. coli* O157 prevalence and feeding supplemental DG. The objective of this study was  
53 to compare the fecal shedding patterns and concentration of *E. coli* O157 in orally inoculated  
54 calves fed diets with or without DDG from corn grain. We hypothesized that feeding DDG  
55 would increase fecal prevalence and concentration of *E. coli* O157.

56

## 57 **Materials and Methods**

### 58 **Animals and treatment groups**

59 Ten Holstein bull calves (BW = 90.7 ± 22.7 kg) were randomly assigned to one of two dietary  
60 treatments (five calves per treatment group): steam-flaked corn (SFC) based high grain diets with  
61 0% (control) or 25% DDG. Calves were fed a series of four step-up diets (stepped-up  
62 approximately every 5 days) with their respective diets for approximately three weeks, during  
63 which time they were housed in two outdoor pens with a common fence line. Once calves were  
64 adjusted to the final diet {0 or 25% DDG, steam-flaked corn (84 or 61%), alfalfa hay (6%), corn  
65 steep liquor (6%), and a vitamin and mineral supplement (4 or 2%)} they were moved to  
66 individual pens (5' × 10') within a biosafety level-2 facility and allowed to acclimate for one  
67 week prior to oral inoculation with *E. coli* O157. Calves in each group were housed in five  
68 consecutive pens per isle (one treatment per isle) and each pen contained walls tall enough to

69 prevent physical contact between adjacent animals. Diets were fed once daily and all calves had  
70 access to fresh water. Pens were scraped and cleaned once daily.

71

### 72 ***Escherichia coli* O157 inoculation**

73 Calves were orally dosed with a five-strain mixture of Shiga-toxin producing *E. coli* O157  
74 isolated from beef feedlots (Sargeant et al., 2003) and made resistant to 50 µg/ml nalidixic acid  
75 (*Nal<sup>R</sup> E. coli* O157). Isolates were plated on blood agar (BAP; Remel, Lenexa, KS) and grown  
76 overnight at 37°C. A single colony was selected, inoculated into 10 ml of tryptic soy broth  
77 (TSB; Difco; BD, Sparks, MD) and incubated overnight at 37°C. One ml of TSB was inoculated  
78 into 100 ml of TSB and incubated for 7 h at 37°C. All strains were pooled together after  
79 incubation and 1 ml of pooled culture was serially diluted ten-fold in peptone water (Remel) and  
80 spread plated (4 plates per dilution) onto sorbitol-MacConkey agar (Difco) plates with cefixime  
81 (0.5 mg/L), potassium tellurite (2.5 mg/L), and nalidixic acid (50 µg/ml; ctn-SMAC) to  
82 determine the concentration of *Nal<sup>R</sup> E. coli* O157. Each calf was dosed orally, via gastric tube,  
83 with 5 ml of pooled culture mixed with 200 ml of 1% sterile skim milk (Oxoid, Basingstoke  
84 Hampshire, UK). The final inoculum for each calf contained  $6.0 \times 10^9$  CFU of *Nal<sup>R</sup> E. coli*  
85 O157.

86

### 87 **Fecal sampling and detection or enumeration of *Nal<sup>R</sup> E. coli* O157**

88 Fecal samples were collected from each animal before inoculation (day 0) to determine the  
89 prevalence of wild-type *E. coli* O157, as described previously (Greenquist et al, 2005). Briefly,  
90 one gram of fecal sample was enriched in 9 ml of Gram Negative broth (Difco) with cefixime  
91 (0.5 mg/L), cefsoludin (10 mg/L), and vancomycin (8 mg/L; GNccv). Immunomagnetic bead

92 separation (Dynal, Inc., New Hyde Park, NY) was performed and samples were plated onto  
93 sorbitol-MacConkey agar (Difco) plates with cefixime (0.5 mg/L), potassium tellurite (2.5  
94 mg/L). Six sorbitol-negative colonies were picked and colonies were tested for indole  
95 production and O157 antigen latex agglutination (Oxoid).

96 After oral challenge with *Nal<sup>R</sup> E. coli* O157 (day 0), fecal samples were collected from each  
97 calf three times a week (Monday, Wednesday, and Friday). At each sampling, control calves  
98 were sampled before DDG-supplemented calves and personnel changed suits (Tyvek, DuPont;  
99 Wilmington, DE) between treatment groups. Foot baths with bleach were used and gloves were  
100 changed between each pen and isle. Fecal samples were collected by rectal palpation, placed in  
101 sterile bags, and transported to the laboratory immediately. A tube containing 9 ml GNccv was  
102 weighed, approximately 1 g of fecal sample was added, and the tube was re-weighed to  
103 determine the weight of the sample. One ml of fecal suspension was serially diluted ten-fold in  
104 buffered peptone water. One hundred microliters of appropriate dilutions were plated, in  
105 triplicate, on ctn-SMAC to determine the concentration of *Nal<sup>R</sup> E. coli* O157. Plates were  
106 incubated overnight at 37°C and the sorbitol-negative colonies were counted. The remaining  
107 GNccv broth was enriched for 6 h at 37 °C, after which, 1 ml was removed and inoculated into  
108 another 9 ml tube of GNccv broth (secondary enrichment) and incubated for 16 to 18 h at 37°C.  
109 If colonies were not recovered by direct plating, 100 µl of secondary enrichment was plated onto  
110 ctn-SMAC. A colony from each direct or enrichment plate was picked, plated on BAP and  
111 tested for indole production and O157 antigen latex agglutination.

112 At the termination of the study (day 43), all calves were euthanized and necropsied.  
113 Contents from the rumen, cecum, colon, and rectum were collected and the concentration of *Nal<sup>R</sup>*  
114 *E. coli* O157 was determined as above. Also, the rectum was cut open, the mucosa was rinsed

115 with water to remove visible fecal material and the area, 3 to 5 cm proximal to the rectoanal  
116 junction, was swabbed with a foam-tipped applicator (RAMS; VWR International, Buffalo  
117 Grove, IL, catalog #10812-022; Fox et al., 2007b). Swabs were immediately placed in 3 ml of  
118 GNccv broth, transported to the laboratory, vortexed and processed for detection of *Nal<sup>R</sup> E. coli*  
119 O157 by direct plating or enrichment as above.

120

### 121 **Statistical analysis**

122 Fecal concentrations of *Nal<sup>R</sup> E. coli* O157 were log<sub>10</sub> transformed before data analyses. If *Nal<sup>R</sup>*  
123 *E. coli* O157 was not detected by direct plating, but enrichment was positive, the lowest  
124 detectable concentration was assigned, accounting for sample weight (10<sup>1</sup> CFU/g). The  
125 concentration in RAMS samples was not determined because the size of the inoculum was  
126 unknown; therefore, swabs were considered positive (direct plating or enrichment) or negative.  
127 For live animal fecal collections (day 2 through 42), differences in *Nal<sup>R</sup> E. coli* O157  
128 concentrations between treatment groups (control or DDG), sampling day and treatment ×  
129 sampling day effects were analyzed using the MIXED procedure in SAS (Version 9.1, SAS  
130 Institute, Cary, NC). Sampling day and the treatment × sampling day effects were included  
131 anticipating *a priori* that fecal concentrations would be high the first few days after inoculation,  
132 and then gradually decreasing with any treatment effects occurring only after 7-10 days post-  
133 inoculation. The MIXED procedure also was used to detect differences in *Nal<sup>R</sup> E. coli* O157  
134 concentration in gut contents collected at necropsy with treatment, site (rumen, cecum, colon,  
135 and rectum), and treatment × site effects included in the final model. For both live animal and  
136 necropsy sample analyses, animal was included as a repeated effect. Two way comparisons were  
137 conducted using least squares means estimates.

138 The prevalence (positive by direct plating or by enrichment) of *Nal<sup>R</sup> E. coli* O157 in live  
139 animals was analyzed using logistic regression in GENMOD of SAS. This analysis compared  
140 the cumulative prevalence for each animal between day 21 (the first study day in which at least  
141 one animal was negative for *Nal<sup>R</sup> E. coli* O157) and the termination of the study (day 42).  
142 Treatment was included as the explanatory variable. Initially, a repeated measures analysis was  
143 used to assess the probability of an animal being fecal-positive on each sample day from days 21  
144 to 42, but this model did not converge. Prevalence of *Nal<sup>R</sup> E. coli* O157 in necropsy samples  
145 also was analyzed using logistic regression in GENMOD of SAS. The cumulative prevalence of  
146 *Nal<sup>R</sup> E. coli* O157 at the five necropsy sites for each calf was the evaluated response and  
147 treatment was included as an explanatory variable.

148

## 149 **Results**

### 150 **Fecal shedding of *E. coli* O157**

151 Fecal samples from all calves were negative for *E. coli* O157 prior to oral inoculation. One calf  
152 in the control diet was treated with Banamine<sup>®</sup> (Schering Plough Animal Health Corp., Summit,  
153 NJ) over a one-week period (on days 5 through 8 and day 11 after inoculation) because of high  
154 body temperature and loss of appetite. Normal body temperature and appetite were restored after  
155 treatment and the calf was kept in the study.

156 Two days after oral inoculation with *Nal<sup>R</sup> E. coli* O157, all calves were positive by direct  
157 plating. All calves in both treatments were positive for *Nal<sup>R</sup> E. coli* O157 through sampling day  
158 18, after which the cumulative prevalence of *Nal<sup>R</sup> E. coli* O157 (between days 21 and 42) was  
159 higher in DDG-fed calves compared to calves in the control group ( $P < 0.0001$ ). Sampling day  
160 had a significant effect on the concentration of *Nal<sup>R</sup> E. coli* O157 in fecal samples ( $P < 0.001$ ).

161 The treatment  $\times$  day interaction ( $P = 0.07$ ) was sufficient to warrant further investigation,  
162 therefore, the differences between treatments were examined on each collection day (Fig. 2). On  
163 day two, the *Nal<sup>R</sup> E. coli* O157 concentration was higher in fecal samples from calves fed no  
164 DDG than from calves fed DDG ( $P < 0.01$ ). However, there were no differences in the  
165 concentrations of *Nal<sup>R</sup> E. coli* O157 between treatment groups after day two until day 35 of the  
166 study. The *Nal<sup>R</sup> E. coli* O157 concentrations in fecal samples were higher in calves fed DDG  
167 compared to control calves (Fig 1) on days 35 ( $P = 0.02$ ), 37 ( $P = 0.03$ ), 39 ( $P = 0.02$ ), and 42 ( $P$   
168  $< 0.01$ ).

169

#### 170 ***E. coli* O157 in gut contents and on rectoanal mucosa**

171 All calves in the DDG group were positive for *Nal<sup>R</sup> E. coli* O157 in at least one site in the gut  
172 (rumen, cecum, colon, rectum, or RAMS) at necropsy compared to only three of five control  
173 calves (Table 1). The number of sites at necropsy positive for *Nal<sup>R</sup> E. coli* O157 was higher in  
174 calves fed DDG compared to calves in the control treatment ( $P < 0.001$ ). Of the five calves in  
175 the DDG group, four were positive in all samples collected at necropsy and the fifth calf was  
176 positive only in cecal contents. In two of three control calves, *Nal<sup>R</sup> E. coli* O157 was only  
177 isolated from the rectal contents or on the rectoanal mucosa (Table 1). For ruminal contents,  
178 four of five calves fed DDG were positive for *Nal<sup>R</sup> E. coli* O157, whereas none of the control  
179 calves were positive. In addition, four of five DDG calves were culture positive for *Nal<sup>R</sup> E. coli*  
180 O157 in RAMS compared to only one calf in the control group (Table 1).

181 Feeding DDG increased ( $P < 0.01$ ) *Nal<sup>R</sup> E. coli* O157 concentrations in gut contents  
182 collected at necropsy (Fig. 3). The *Nal<sup>R</sup> E. coli* O157 concentrations in DDG-fed calves were  
183 higher than control calves in cecal ( $P = 0.01$ ), colonic ( $P = 0.03$ ), and rectal ( $P = 0.01$ ) contents.

184 Because all ruminal content samples were positive only after enrichment (approx.  $10^1$  CFU/g),  
185 low concentrations were calculated and no statistical differences were evident between treatment  
186 groups.

187

## 188 **Discussion**

189 Supplementation of calves fed high-grain diets with 25% DDG significantly increased the  
190 cumulative prevalence and concentration of *E. coli* O157 in feces following oral inoculation with  
191 *Nal<sup>R</sup> E. coli* O157. This may imply that the organism can persist and colonize the gut more  
192 readily in calves fed DDG compared to no DDG control calves. Necropsy data further supports  
193 *E. coli* O157 persistency in the gut of DDG-supplemented calves. Because of the preferential  
194 colonization of *E. coli* O157 on the recto-anal mucosa (Naylor et al., 2003), the RAMS technique  
195 was developed to swab the rectoanal junction and was shown to be more sensitive than fecal  
196 samples from cattle in detecting *E. coli* O157 (Rice et al., 2003; Greenquist et al., 2005). The  
197 RAMS technique was used in our study only at necropsy and four of five calves supplemented  
198 with DDG were positive, while only one control calf was RAMS positive. There is evidence to  
199 suggest that mucosal carriage of *E. coli* O157 at the rectoanal junction is associated with long-  
200 duration fecal shedding and high concentration of fecal excretion (Cobbold et al., 2007; Davis et  
201 al., 2006; Lim et al., 2007; Low et al., 2005).

202 This study confirms our previous observations from two natural prevalence studies that have  
203 shown a positive association between DG supplementation and *E. coli* O157 prevalence (Jacob et  
204 al., 2006, 2008). In the first study, we observed that the prevalence of *E. coli* O157 in fecal  
205 samples was higher from cattle fed 25% WDG compared to cattle fed diets without WDG on one  
206 of two collection days during the 150-day feeding period (Jacob et al., 2006). In the second

207 feedlot study, cattle ( $n = 379$ ) were fed SFC-based diets supplemented with 0 or 25% DDG  
208 (similar to the present study), and the cumulative prevalence of *E. coli* O157 from pen floor fecal  
209 samples was higher ( $P = 0.01$ ) in DDG-fed cattle compared to control cattle throughout the 12-  
210 week finishing period (Jacob et al., 2008). These two studies suggested that positive associations  
211 between DG supplementation and prevalence of *E. coli* O157 occurred with dry or wet DG.  
212 Synge et al. (2003) in their investigation of management factors influencing the shedding of *E.*  
213 *coli* O157 by beef cows in Scotland observed that the housed cows supplemented with DG  
214 exhibited a higher shedding rate than cows not given DG. Additionally, Dewell et al. (2005)  
215 reported, in a multi-state epidemiological feedlot study, that the odds of an *E. coli* O157 positive  
216 fecal sample, were six times higher in cattle fed brewer's grains, a fermentative co-product  
217 similar to DG, than in cattle without brewer's grains.

218 The mechanism to explain the relationship between *E. coli* O157 and supplementation of  
219 distiller's grains is not known. It appears that DG may contain components that directly or  
220 indirectly stimulate growth of *E. coli* O157. Indirect stimulation is probably mediated via the  
221 impact of DG on ruminal or hindgut microbial populations. We have shown that addition of  
222 DDG to *in vitro* fermentations with ruminal or fecal (representing hindgut microbial  
223 fermentation) microbial inoculum resulted in a higher concentration of inoculated *Nal<sup>R</sup> E. coli*  
224 O157 after 24 h incubation compared to fermentations without added DDG (Jacob et al., 2008).  
225 Growth stimulation observed within a short *in vitro* incubation (24 h) suggests that the more  
226 likely reason for an association is the direct effect of DG on *E. coli* O157. The DG supplement  
227 includes the non-starch fractions of the corn grain, as well as yeast cells and their metabolic  
228 intermediates and end products from ethanol production. Therefore, the factor(s) in DG  
229 responsible for stimulation of *E. coli* O157 may be associated with the yeast (cells or products)

230 or unfermented fractions of the grain. The soluble fraction obtained after separation of solids,  
231 generally by centrifugation, is often condensed by heating and added to the DG as condensed  
232 distiller's solubles (Rausch and Belyea, 2005). The soluble fraction is more likely to contain  
233 yeast fermentation intermediates and products, other than volatile compounds, such as ethanol,  
234 which are removed during distillation or dehydration (Fron et al., 1996). Analyses of distiller's  
235 soluble fractions from several sources have identified lactate, fumarate, malate, and succinate as  
236 components (Fron et al., 1996). Although the effect of these organic acids on *E. coli* O157 is not  
237 known, it is well known that they stimulate the growth of certain ruminal bacteria, particularly  
238 the lactate fermenting bacteria, such as *Selenomonas ruminantium* and *Megasphaera elsdenii*  
239 (Martin, 1998). Bach et al. (2003) have previously reported that the yeast *Saccharomyces*  
240 *cerevisiae* subsp. *boulardii* inhibited *E. coli* O157 strains in batch culture fermentations;  
241 however, inhibition did not occur in continuous culture.

242 Much of the attention DG has received is focused on three major macronutrients, protein,  
243 fiber, and lipids, because of their usefulness as protein or energy sources for livestock. We  
244 cannot rule out the possibility of unfermented macro or micronutrients of DG providing direct  
245 stimulation of *E. coli* O157. More likely, the effect of macronutrients is mediated via alteration  
246 of the ruminal or hindgut microbial ecosystem. Because DG replaces a portion of cereal grain of  
247 the diet, the reduction in dietary starch content also contributes to changes in ruminal or hindgut  
248 fermentation. Fox et al. (2007a) showed that grain processing (dry-rolling verse steam-flaking)  
249 impacted the prevalence of *E. coli* O157, possibly because of differences in the ability of starch  
250 to reach the hindgut. Grain processing methods, such as steam-flaking, increase ruminal  
251 digestibility of the grain and reduce the amount of starch reaching the hindgut (Huntington,  
252 1997). An altered hindgut ecology impacting the growth of competing microorganisms, an

253 increased hindgut pH, or other mechanisms associated with decreased starch flow to the hindgut  
254 may be a means for the increased *E. coli* O157 concentration or prevalence (Fox et al., 2007a).  
255 Also, the impact of DG or condensed distiller's products on ruminal microbial population and  
256 their fermentation products could influence *E. coli* O157. The altered ruminal microbial  
257 fermentation is evidenced by increased molar proportion of propionic acid in DG supplemented  
258 cattle (Ham et al., 1994) or increased cultural counts of lactate-utilizing bacteria with a two-fold  
259 increase in the *in vitro* lactic acid fermentation in steers supplemented with condensed distiller's  
260 solubles (Fron et al., 1996). An altered rumen ecosystem promoting growth and persistence of  
261 *E. coli* O157 is supported by our observation of the presence of *Nal<sup>R</sup>* *E. coli* O157 in ruminal  
262 contents from four out of five calves fed DDG in this study.

263 The primary colonization of *E. coli* O157 in ruminants is the hindgut (Grauke et al., 2002;  
264 Van Baale et al., 2004), specifically the rectoanal mucosal junction (Naylor et al., 2003).  
265 Although reasons for persistence or colonization in the hindgut are undetermined, suggested  
266 rationale includes a more hospitable environment for *E. coli* colonization and proliferation  
267 compared to the rumen (Van Baale et al., 2004; Fox et al., 2007a). In our experiment, all DDG  
268 calves were culture positive in one or more regions of the hindgut (cecum, colon, rectum or  
269 rectoanal mucosa) and the three calves in the control group that were positive for *E. coli* O157  
270 were negative in the rumen but positive in the hindgut. In DG-supplemented cattle, more  
271 nutrients, particularly bran and protein, could possibly flow into the hindgut and alter the  
272 microbial ecosystem in favor of growth and persistence of *E. coli* O157. Previous studies have  
273 shown that forage feeding is positively associated with *E. coli* O157 in ruminants (Kudva et al.,  
274 1997; Van Baale et al., 2004), possibly because of altered hindgut ecology. Van Baale et al.  
275 (2004) reported that forage-fed cattle inoculated with *E. coli* O157:H7 shed the organism in the

276 feces for a longer duration than grain-fed calves. Increased protein degradation in the hindgut  
277 because of increased flow of protein could possibly elevate pH (increased ammonia production)  
278 thus favoring growth and persistence of *E. coli* O157.

279 In conclusion, this study confirmed previous observations that feeding cattle DG was  
280 positively associated with *E. coli* O157 shedding. The mechanism by which this occurs is  
281 unknown; however, we hypothesize that altered hindgut ecology or a component in DG that  
282 stimulates *E. coli* O157 proliferation and colonization may be responsible for this positive  
283 association. Further research is needed to delineate the mechanism responsible for this  
284 association. Given the growing trend for use of DG in cattle finishing diets, our finding of a  
285 positive association with prevalence of *E. coli* O157 is significant and has potentially important  
286 ramifications for food safety.

287

## 288 **Acknowledgements**

289 Published as contribution no. 08-68-J Kansas Agric. Exp. Station. This work was supported in  
290 part by the College of Veterinary Medicine intramural funds under Sustained Momentum for  
291 Investigators with Laboratories Established (SMILE). Authors extend thanks to Elita Balridge,  
292 Miguel Barrios, Dr. Bart Carter, Nathan Hoffman, Mark Minihan, Dr. Sally Olson, Xiaorong  
293 Shi, Ashley Thornton, Neil Wallace, and other personnel from the KSU Beef Cattle Research  
294 Center, the KSU College of Veterinary Medicine Animal Resource Facility and the KSU College  
295 of Veterinary Medicine Necropsy Laboratory for their help with this project.

296

297 **References**

- 298 Al-Suwaiegh, S., K. C. Fanning, R. J. Grant, C. T. Milton, and T. J. Klopfenstein. 2002.  
299 Utilization of distillers grains from the fermentation of sorghum or corn in diets for finishing  
300 beef and lactating dairy cattle. *J. Anim. Sci.* 80, 1105-1111.  
301
- 302 Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2002.  
303 Transmission and control of *Escherichia coli* O157:H7: A Review. *Can. J. Anim. Sci.* 82,  
304 475-490.  
305
- 306 Bach, S. J., T. A. McAllister, D. M. Veira, V. P. J. Gannon, and R. A. Holley. 2003. Effects of a  
307 *Saccharomyces cerevisiae* feed supplement on *Escherichia coli* O157:H7 in ruminal fluid *in*  
308 *vitro*. *Anim. Feed Sci. Technol.* 104, 179-189.  
309
- 310 Buchko, S. J., R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira. 2000. The effect of  
311 different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. *J. Food Prot.*  
312 63, 1467-1474.  
313
- 314 Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet. 2003. Forage  
315 feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. *J. Dairy Sci.*  
316 86, 852-860.  
317
- 318 Cobbold, R. N., D. D. Hancock, D. H. Rice, J. Berg, R. Stilborn, C. J. Hovde, and T. E. Besser.  
319 2007. Rectoanal junction colonization of feedlot cattle by *Escherichia coli* O157:H7 and its

320 association with supershedders and excretion dynamics. *Appl. Environ. Microbiol.* 73, 1563-  
321 1568.

322

323 Davis, M. A., D. H. Rice, H. Sheng, D. D. Hancock, T. E. Besser, R. Cobbold, and C. J. Hovde.  
324 2006. Comparison of cultures from rectoanal-junction mucosal swabs and feces for detection  
325 of *Escherichia coli* O157 in dairy heifers. *Appl. Environ. Microbiol.* 72, 3766-3770.

326

327 Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos,  
328 K. E. Belk, G. C. Smith, and M. D. Salman. 2005. Prevalence of and risk factors for  
329 *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. *Foodborne Pathog.*  
330 *Dis.* 2, 70-76.

331

332 Firkins, J. L., L. L. Berger, and G. C. Fahey. 1985. Evaluation of wet and dry distiller's grains  
333 and wet and dry gluten feeds for ruminants. *J. Anim. Sci.* 60, 847-860.

334

335 Fox, J. T., B. E. Depenbusch, J. S. Drouillard, and T. G. Nagaraja. 2007a. Dry-rolled or steam-  
336 flaked grain-based diets and fecal shedding of *Escherichia coli* O157 in feedlot cattle. *J.*  
337 *Anim. Sci.* 85, 1207-1212.

338

339 Fox, J. T., X. Shi, and T. G. Nagaraja. 2007b. *Escherichia coli* O157 in the rectoanal mucosal  
340 region of cattle. *Foodborne Path. Dis.* In Press

341

342 Fron, M., H. Madeira, C. Richards, and M. Morrison. 1996. The impact of feeding condensed  
343 distillers byproducts on rumen microbiology and metabolism. *Anim. Feed Sci. Technol.* 61,  
344 235-245.

345

346 Grauke, L. J., I. T. Kudva, J. W. Yoon, C. W. Hunt, C. J. Williams, and C. J. Hovde. 2002.  
347 Gastrointestinal tract location of *Escherichia coli* O157:H7 in ruminants. *Appl. Environ.*  
348 *Microbiol.* 68, 2269-2277.

349

350 Greenquist, M. A., J. S. Drouillard, J. M. Sargeant, B. E. Depenbusch, X. Shi, K. F. Lechtenberg,  
351 and T. G. Nagaraja. 2005. Comparison of rectoanal mucosal swab cultures and fecal  
352 cultures for determining prevalence of *Escherichia coli* O157:H7 in feedlot cattle. *Appl.*  
353 *Environ. Microbiol.* 71, 6431-6433.

354

355 Gyles, C. L. 2007. Shiga toxin-producing *Escherichia coli*: An overview. *J. Anim. Sci.* 85(E.  
356 suppl.), E45-E62.

357

358 Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain, and R. P. Huffman.  
359 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles  
360 as a source of protein and energy for ruminants. *J. Anim. Sci.* 72, 3246-3257.

361

362 Huntington, G. B. 1997. Starch utilization by ruminants: from basics to the bunk. *J. Anim. Sci.*  
363 75, 852-867.

364

365 Hussein, H. S. 2007. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in  
366 beef cattle and their products. J. Anim. Sci. 85(E. Suppl.), E63-E72.  
367

368 Jacob, M. E., J. T. Fox, J. S. Drouillard, and T. G. Nagaraja. 2008. Effects of dried distiller's  
369 grain on cattle fecal prevalence and growth of *Escherichia coli* O157 in batch culture  
370 fermentations. Appl. Environ. Microbiol. 74 (*in press*).  
371

372 Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, and T. G. Nagaraja. 2006. Effects of  
373 feeding wet corn distiller's grains with solubles and monensin and tylosin on the prevalence  
374 and antimicrobial susceptibility of *E. coli* O157 in feedlot cattle. J. Anim. Sci. 84(2), 81.  
375

376 Kleinschmit, D. H., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of  
377 various sources of corn dried distillers grains plus solubles for lactating dairy cattle. J. Dairy  
378 Sci. 89, 4784-4794.  
379

380 Kudva, I. T., C. W. Hunt, C. J. Williams, U. M. Nance, and C. J. Hovde. 1997. Evaluation of  
381 dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl. Environ.  
382 Microbiol. 63, 3878-3886.  
383

384 Lim, J. Y., J. Li, H. Sheng, T. E. Besser, K. Potter, and C. J. Hovde. 2007. *Escherichia coli*  
385 O157:H7 colonization at the rectoanal junction of long-duration culture-positive cattle. Appl.  
386 Environ. Microbiol. 73, 1380-1382.  
387

388 Low, J. C., I. J. McKendrick, C. McKenchnie, D. Fenlon, S. W. Naylor, C. Currie, D. G. E.  
389 Smith, L. Allison, and D. L. Gally. 2005. Rectal carriage of enterohemorrhagic  
390 *Escherichia coli* O157 in slaughtered cattle. *Appl. Environ. Microbiol.* 71, 93-97.  
391

392 Martin, S. A. 1998. Manipulation of ruminal fermentation with organic acids: A review. *J.*  
393 *Anim. Sci.* 76, 3123-3132.  
394

395 Naylor, S. W., J. C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick,  
396 D. G. E. Smith, and D. L. Gally. 2003. Lymphoid follicle-dense mucosa at the terminal  
397 rectum in the principle site of colonization of enterohemorrhagic *Escherichia coli*  
398 O157:H7 in the bovine host. *Infect. Immun.* 71, 1505-1512.  
399

400 Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology  
401 of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.*  
402 11:603-609.  
403

404 Rausch, K. D. and R. L. Belyea. 2005. Coproducts from bioprocessing of corn. ASAE Paper  
405 No. 057041. St. Joseph, Mich.:ASAE. [http://www.ddgs.umn.edu/articles-proc-storage-](http://www.ddgs.umn.edu/articles-proc-storage-quality/2005-Rausch-%20Coproducts%20from%20bioprocessing--.pdf)  
406 [quality/2005-Rausch-%20Coproducts%20from%20bioprocessing--.pdf](http://www.ddgs.umn.edu/articles-proc-storage-quality/2005-Rausch-%20Coproducts%20from%20bioprocessing--.pdf).  
407

408 Rice, D. H., H. Q. Sheng, S. A. Wynia, and C. J. Hovde. 2003. Rectoanal mucosal swab culture  
409 is more sensitive than fecal culture and distinguishes *Escherichia coli* O157:H7-colonized  
410 cattle and those transiently shedding the same organism. *J. Clin. Microbiol.* 41, 4924-4929.

411

412 Sargeant, J. M., M. W. Sanderson, R. A. Smith, D. D. Griffin. 2003. *Escherichia coli* O157 in  
413 feedlot cattle feces and water in four major feeder-cattle states in the USA. *Prev. Vet Med.*  
414 61, 127-135.

415

416 Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database from distiller's dried  
417 grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J.*  
418 *Anim. Sci.* 80, 2639-2645.

419

420 Syngé, B.A., M. E. Chase-Topping, G. F. Hopkins, I. J. McKendrick, F. Thomson-Carter, D.  
421 Gray, S. M. Rusbridge, F. I. Munro, G. Foster, G. J. Gunn. 2003. Factors influencing the  
422 shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows.  
423 *Epidemiol. Infect.* 130:301–312.

424

425 Van Baale, M. J., J. M. Sargeant, D. P. Gnad, D. M. DeBey, K. F. Lechtenberg, and T. G.  
426 Nagaraja. 2004. Effect of forage or grain diets with or without monensin on ruminal  
427 persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl. Environ. Microbiol.* 70,  
428 5336-5342.

429 **Table 1.** Prevalence of nalidixic acid-resistant *Escherichia coli* O157 in gut contents and on the  
 430 rectoanal mucosa at necropsy in calves fed high-grain diets with 0 or 25% dried distiller's grains  
 431 (DDG).

Calf #	DDG, % of diet	Gut contents				Rectoanal mucosa	Total number of gut locations positive (%)
		Rumen	Cecum	Colon	Rectum		
1	0	-	+	+	+		
2	0	-	-	-	-	+	
3	0	-	-	-	-	-	5 of 25 (20)
4	0	-	-	-	-	-	
5	0	-	-	-	+	-	
6	25	+	+	+	+	+	
7	25	+	+	+	+	+	
8	25	+	+	+	+	+	21 of 25 (84)
9	25	+	+	+	+	+	
10	25	-	+	-	-	-	

432 + = Positive for nalidixic acid-resistant *E. coli* O157 by direct plating or by enrichment.

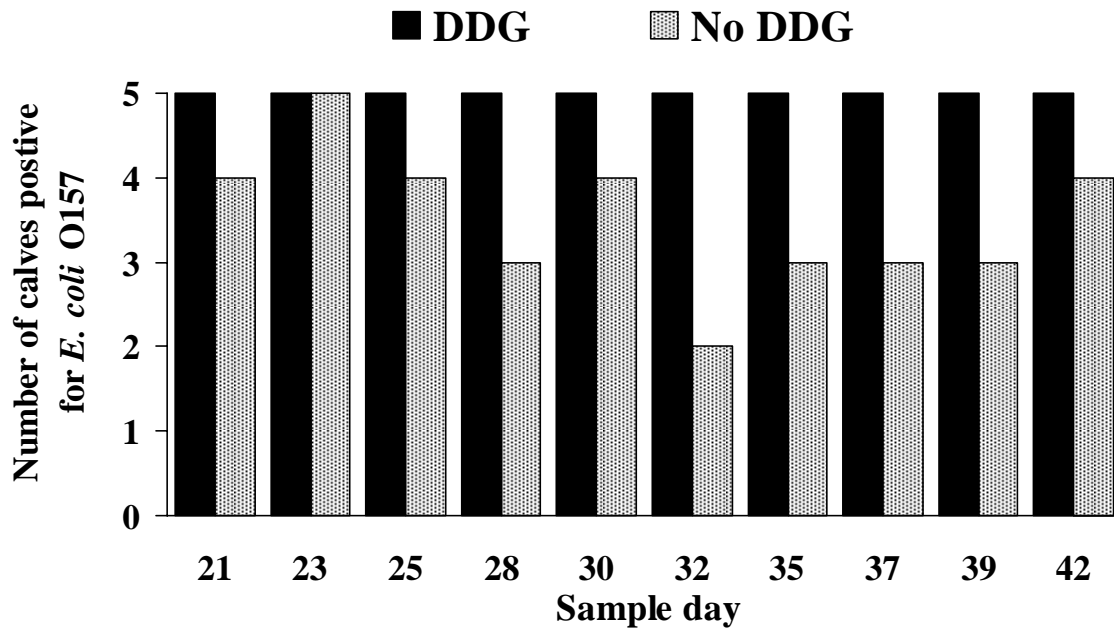
433 - = Negative for nalidixic acid-resistant *E. coli* O157 by enrichment.

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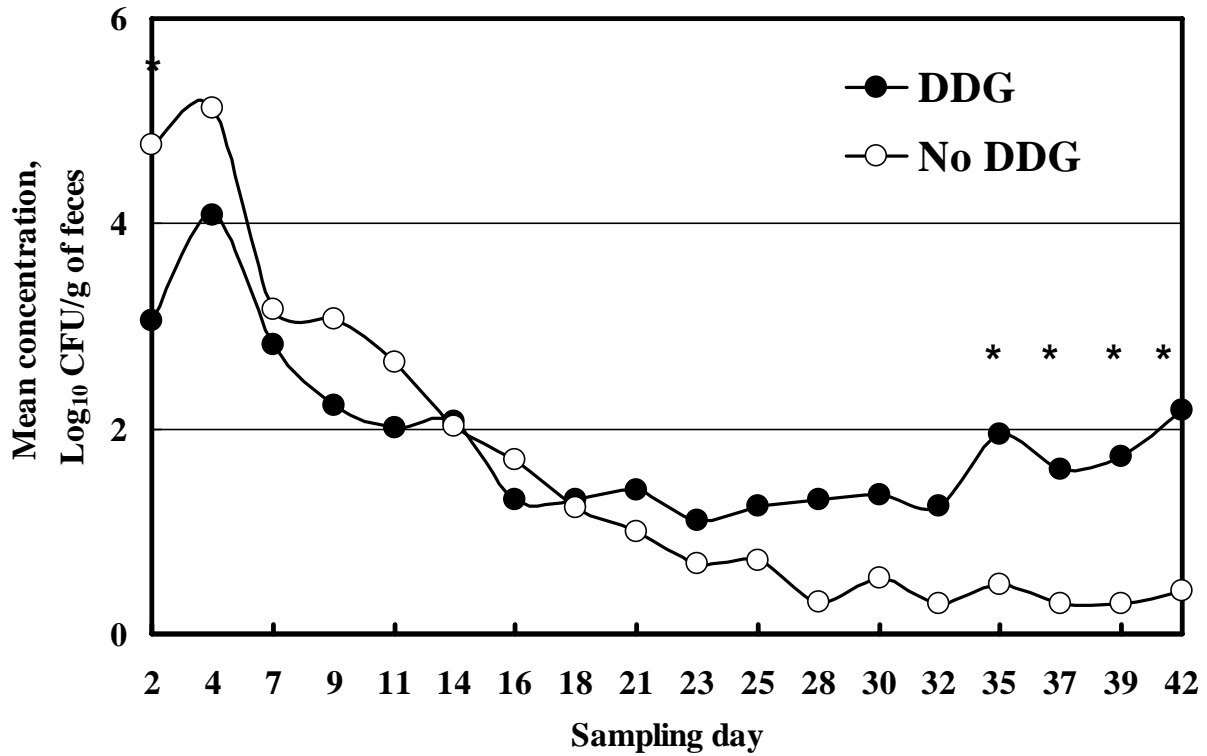
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436

437 **Fig 1.** The number of fecal samples positive for nalidixic acid-resistant *Escherichia coli* O157  
438 from calves fed high-grain diets with (n=5) or without (n = 5) 25% supplemental dried distiller's  
439 grains (DDG) between study days 21 and 42. The cumulative prevalence between days 21 and  
440 42 was higher ( $P < 0.001$ ) in calves fed DDG compared to control calves.

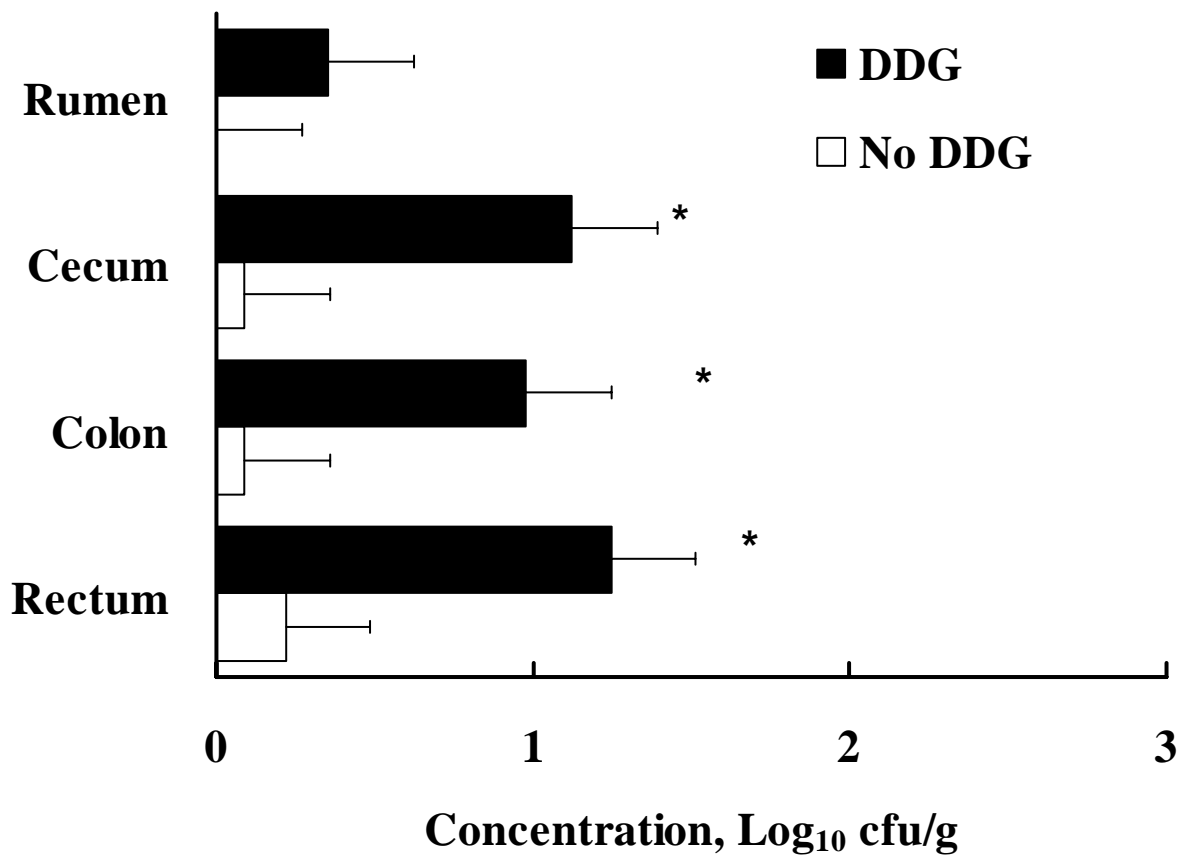


441 **Fig. 2.** The mean log<sub>10</sub> concentration of nalidixic acid-resistant *Escherichia coli* O157 in fecal  
442 samples of calves (n = 5 per treatment) fed high-grain diets with or without 25% supplemental  
443 dried distiller's grains (DDG) across sample days. The concentration was significantly different  
444 between treatments on days 2, 35, 37, 39, and 42 ( $P < 0.05$ ).



445

446 **Fig. 3.** The mean  $\log_{10}$  concentration and SE of nalidixic acid-resistant *Escherichia coli* O157 in  
447 gut contents at necropsy from calves fed high-grain diets with or without 25% supplemental  
448 dried distiller's grains (DDG; five calves per treatment). \* Indicates statistical difference  
449 between calves fed DDG and those fed the control diet ( $P < 0.05$ ).



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