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Effects of Dried Distillers' Grain on Fecal Prevalence and Growth of *Escherichia coli* O157 in Batch Culture Fermentations from Cattle[▽][†]

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Distillers' grains (DG), a by-product of ethanol production, are fed to cattle. Associations between *Escherichia coli* O157 prevalence and feeding of DG were investigated in feedlot cattle (*n* = 379) given one of three diets: steam-flaked corn (SFC) and 15% corn silage with 0 or 25% dried distillers' grains (DDG) or SFC with 5% corn silage and 25% DDG. Ten fecal samples were collected from each pen weekly for 12 weeks to isolate *E. coli* O157. Cattle fed 25% DDG with 5 or 15% silage had a higher (*P* = 0.01) prevalence of *E. coli* O157 than cattle fed a diet without DDG. Batch culture ruminal or fecal microbial fermentations were conducted to evaluate the effect of DDG on *E. coli* O157 growth. The first study utilized microbial inocula from steers fed SFC or dry-rolled corn with 0 or 25% DDG and included their diet as the substrate. Ruminal microbial fermentations from steers fed DDG had higher *E. coli* O157 contents than ruminal microbial fermentations from steers fed no DDG (*P* < 0.05) when no substrate was included. Fecal fermentations showed no DDG effect on *E. coli* O157 growth. In the second study with DDG as a substrate, ruminal fermentations with 0.5 g DDG had higher (*P* < 0.01) *E. coli* O157 concentrations at 24 h than ruminal fermentations with 0, 1, or 2 g DDG. In fecal fermentations, 2 g DDG resulted in a higher concentration (*P* < 0.05) at 24 h than 0, 0.5, or 1 g DDG. The results indicate that there is a positive association between DDG and *E. coli* O157 in cattle, and the findings should have important ramifications for food safety.

Cattle are major reservoirs for *Escherichia coli* O157, a significant food-borne pathogen (19, 24, 32). Asymptomatic colonization by *E. coli* O157 in cattle occurs in the lower gastrointestinal tract, specifically the mucosal surface of the rectum (31), and the organism is shed in feces (2, 6). There are multiple variables that influence the prevalence and shedding of *E. coli* O157 in ruminants (2, 34, 37). One of these factors is diet (3, 5, 6, 10, 14, 40), which may suggest that diet influences the physiological environment of the gut and affects the survival and establishment of *E. coli* O157.

Different diet components have been evaluated to identify associations with *E. coli* O157 shedding. An epidemiologic study revealed a positive association between cattle receiving barley grain in their diet and *E. coli* O157 prevalence in feedlot cattle (8), which was confirmed by natural prevalence and challenge model studies (3, 5). Several studies have indicated that forage-fed cattle shed *E. coli* O157 in their feces for a longer duration than grain-fed cattle (21, 40). Increased amounts of starch reaching the hindgut can increase volatile fatty acid production and reduce the pH in the hindgut, altering the environment for growth and survival of the organism (14, 35).

The fermentation of cereal grains for ethanol production results in a coproduct called distillers' grains (DG), which can be used as a livestock feed. This coproduct is the "spent" fraction that remains after distillation of ethanol. The solid fraction, called wet distillers' grain (WDG) (approximately 30% dry matter [DM]), may be used as a feed or may be dehydrated to produce dried distillers' grains (DDG) (approximately 90% DM). After enzymatic fermentation of the starch portion of grain, the remaining fraction is concentrated in other nutrient components, including protein, fiber, and lipid (38). Because of the properties of the remaining nutrients (energy or protein), DG is well suited for ruminant diets and has been shown to increase daily weight gain in finishing cattle (20). The absence of starch and the presence of relatively high concentrations of ruminal escape protein and bran (fiber) components of DG are likely to impact the ecology of the hindgut. Dewell et al. (9) found that fecal samples from cattle fed brewers' grains, a fermentative product similar to DG, were more likely to be positive for *E. coli* O157 than fecal samples from cattle not fed brewers' grains. In a previous study (25), we observed that feedlot cattle fed WDG had a higher prevalence of *E. coli* O157 on one of two collection days; however, the positive association was apparent on both days. The objectives of this study were to determine the prevalence of *E. coli* O157 in feces of feedlot cattle fed grain diets supplemented with DG and to determine if DG stimulated growth of *E. coli* O157 using in vitro fermentations with a ruminal or fecal microbial inoculum.

MATERIALS AND METHODS

Study 1. (i) Animals, treatments, and sampling. Yearling heifers (*n* = 379) were allocated randomly to three treatment groups. The diets consisted of a combination of steam-flaked corn (SFC), corn DDG, and corn silage and were formulated to meet National Research Council requirements (30). Cattle were fed in feed bunks once daily so that only a trace amount of feed remained the following day. The treatments included SFC with 15% corn silage (DM basis),
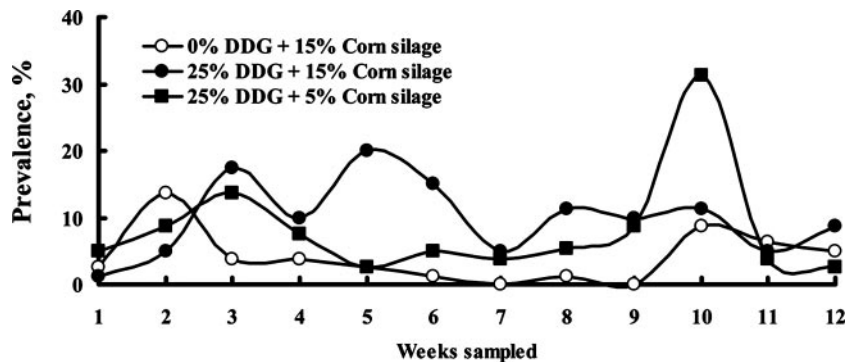
FIG. 1. Prevalence of *E. coli* O157 in pen floor fecal samples from cattle fed SFC-based high-grain diets with 5 or 15% corn silage and supplemented or not supplemented with 25% DDG.

SFC with 25% DDG and 15% corn silage, and SFC with 25% DDG and 5% corn silage. Each treatment was replicated in eight pens with 15 or 16 heifers per pen. Cattle were housed in dirt floor feedlot pens, and fence line water units were shared by adjacent pens. Ten freshly voided pen floor fecal samples were collected from each pen once each week for 12 weeks (August through October; $n =$ 2,877), placed in sterile bags, and immediately transported to the laboratory.

(ii) Isolation of *E. coli* O157. Fecal samples were cultured for *E. coli* O157 as described by Greenquist et al. (18). Briefly, samples were kneaded for 30 s, and approximately 1 g of feces was placed in 9 ml of gram-negative (GN) broth with cefixime (0.5 mg/liter), cefsoludin (10 mg/liter), and vancomycin (8 mg/liter). The GN broth tubes were enriched for 6 h at 37°C. After enrichment, immunomagnetic bead separation was performed with 1 ml of GN broth, and after this 50 μl was plated onto sorbitol-MacConkey agar with cefixime (0.5 mg/liter) and potassium tellurite (2.5 mg/liter) (ct-SMAC). The plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies were picked and transferred to blood agar plates (Remel, Lenexa, KS). Following incubation at 37°C for 16 to 18 h, colonies were tested for indole production and latex agglutination with the O157 antigen. *E. coli* O157 isolates were further characterized by multiplex PCR identifying the stx_1, stx_2, and *eae* genes (12). The following multiplex PCR program was used to amplify targets: initial denaturation at 95°C for 3 min, 30 cycles of 95°C for 20 s, 58°C for 40 s, and 72°C for 90 s and then a final elongation at 72°C for 5 min.

Study 2. (i) Animals and sample collection. Twelve ruminally cannulated Holstein steers (body weight, 359 ± 54 kg), allotted randomly in a 2 × 2 factorial arrangement of dietary treatments, were used as sources of ruminal fluid and fecal inocula for this study. The dietary factors were grain type (SFC or dry-rolled corn [DRC]) and level of DDG (0 or 25%). Steers were housed in individual pens with concrete floors and fed approximately 3% of their body weight once each day. The steers were adapted to their treatment diets for at least 3 weeks prior to sample collection. Ruminal fluid was collected from each steer, immediately strained through four layers of cheesecloth, and transported to the laboratory in a flask sealed with a butyl rubber stopper. Fecal samples were collected at the same time from each steer via rectal grab, placed in sterile bags, and transported to the laboratory. An equal volume of Ringer's solution was added to each fecal sample so that the DM content was approximately the same as that of ruminal fluid, and the sample was stomached for 30 s, strained through four layers of cheesecloth (29), and placed in a flask with a butyl rubber stopper.

(ii) Fermentation treatment and bacteriological procedures. Batch culture fermentations were set up with or without substrate in 70-ml serum bottles sealed with butyl rubber stoppers fitted with Bunsen valves. For each steer the substrate was 0.5 g of its whole diet, which was ground. Each bottle contained 50 ml of a fermentation mixture composed of 33 ml of McDougall's buffer (28) and 17 ml of the ruminal fluid or fecal microbial inoculum (ratio of buffer to inoculum, 2:1). The buffer and the inoculum were added under flowing oxygen-free CO_2 gas (22) to create and maintain an anaerobic environment within the bottle. The fermentations were then inoculated anaerobically (under flowing O_2-free CO_2) with 100 μl (approximately 10^3 CFU/ml of fermentation) of a five-strain mixture of *E. coli* O157 (strains 01-2-1863, 01-2-7443, 01-2-10004, 1-2-10530, and 01-2-12329 [36]) resistant to 50 μg/ml nalidixic acid (Nal^r). The fermentations were kept at 37°C in an orbital incubator (80 rpm) and sampled anaerobically (under flowing O_2-free CO_2) at 0, 6, 12, and 24 h to determine the concentrations of Nal^r *E. coli* O157. Samples were serially diluted in buffered peptone water (Sigma-Aldrich, St. Louis, MO), and 0.1 ml of an appropriate dilution was spread, in triplicate,

onto ct-SMAC plates with nalidixic acid (50 μg/ml) (15). Ruminal fluid and fecal microbial fermentations were set up in duplicate and repeated on a different day using new samples collected from the same animals.

Study 3. Two ruminally cannulated Holstein steers adapted to high-grain diets served as donors for collection of ruminal fluid and fecal samples. The steers, fed SFC with 6% alfalfa hay and supplemented with either 0 or 25% DDG, were adapted to their diets for a minimum of 2 weeks prior to sample collection. They were housed individually in dirt floor pens and were bunk fed ad libitum once daily. Fermentations with a ruminal fluid or fecal microbial inoculum were set up as described above, except that DDG was added as a substrate at levels of 0, 0.5, 1, and 2 g per fermentation. Samples were removed at 0, 6, 12, and 24 h to determine the concentrations of Nal^r *E. coli* O157 as described above. Fermentations were repeated on a different day using new samples collected from the same steers.

Statistical analysis. In study 1, the prevalence of *E. coli* O157 was analyzed using logit models in PROC GENMOD of SAS (v. 9.1; SAS, Cary, NC); the numerator was the number of positive samples per pen, and the denominator was the total number of samples per pen (1). Diet and week were investigated as explanatory effects, and pen was included as a repeated effect. In studies 2 and 3, analyses were conducted using the MIXED procedure of SAS to determine differences in the log_{10} concentration of Nal^r *E. coli* O157. In study 2, the fixed effects included diet, substrate (ground feed included or not included in the fermentation), DDG (present or not present in the feed), and hour. The effects evaluated in study 3 included DDG substrate concentration (0, 0.5, 1, or 2 g), hour, and presence of DDG in the animal diet. When appropriate, linear contrast statements were included to account for the linear increase in the amount of substrate. Experiment repetition was included as a random effect for both studies 2 and 3.

RESULTS

Prevalence of *E. coli* O157 in feedlot cattle (study 1). The mean prevalence of *E. coli* O157 in pen floor samples throughout the 12-week study was 7.4% (213 of 2,877 samples). The mean weekly *E. coli* O157 prevalence for cattle fed SFC-based high-grain diets with 5 or 15% corn silage and supplemented or not supplemented with 25% DDG are shown in Fig. 1. The prevalence ranged from 2.9% (weeks 1 and 7) to 17.1% (week 10), but week was not significantly associated with *E. coli* O157 prevalence ($P > 0.05$). Analysis of the data showed that diet tended to be associated with *E. coli* O157 prevalence in cattle ($P = 0.06$); therefore, dietary effects were examined further. The prevalence of *E. coli* O157 was higher ($P = 0.01$) in cattle fed SFC with 25% DDG and 15% corn silage than in cattle fed SFC with no DDG and 15% corn silage (Fig. 2). Likewise, cattle fed SFC with 25% DDG and 5% corn silage had a higher ($P = 0.01$) prevalence of *E. coli* O157 than cattle fed SFC with no DDG and 15% corn silage. However, no difference ($P >$
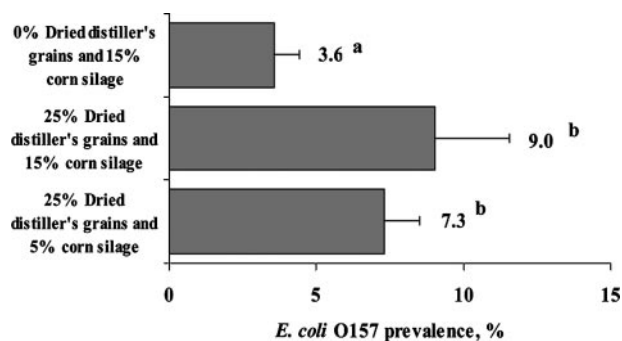
FIG. 2. Cumulative prevalence of *E. coli* O157 in pen floor fecal samples from cattle fed SFC-based high-grain diets with 5 or 15% corn silage and supplemented or not supplemented with 25% DG. The error bars indicate standard errors of the means. Bars with different superscripts are significantly different (*P* < 0.05).

FIG. 3. Concentrations of Nal^r *E. coli* O157 cultured from in vitro ruminal microbial fermentations (study 2). Ruminal fluid inocula were obtained from donor steers fed diets containing either DRC (○) or SFC (●). The difference in the Nal^r *E. coli* O157 concentration between the DRC and SFC diets was significant at 24 h (*P* < 0.01).

0.5) in prevalence was observed between cattle fed DDG with 5% corn silage and cattle fed DDG with 15% corn silage (Fig. 2). The presence of virulence genes in *E. coli* O157 isolates is shown in Table 1. As expected, all the isolates were positive for the *eae* gene and contained either the *stx*$_1$ gene or the *stx*$_2$ gene. Diet had no influence on the prevalence of the *eae*, *stx*$_1$, and *stx*$_2$ genes in *E. coli* O157 isolates.

Growth of *E. coli* O157 during in vitro fermentations with ground complete diets with or without DG as the substrate (study 2). Ruminal fluid fermentations from cattle fed SFC had a higher concentration of Nal^r *E. coli* O157 than fermentations from cattle fed DRC, but the difference was significant only at 24 h (*P* < 0.01) (Fig. 3). The ruminal microbial fermentations from cattle fed DDG had higher Nal^r *E. coli* O157 concentrations at 24 h than the ruminal microbial fermentations from cattle fed no DDG (*P* < 0.05) when substrate was not included in the fermentation (Table 2). However, if substrate was included in ruminal fluid microbial fermentations, there were no differences in Nal^r *E. coli* O157 concentrations between fermentations containing DDG and fermentations not containing DDG (*P* > 0.4). In fecal microbial fermentations, DDG had no effect on the concentration of Nal^r *E. coli* O157, regardless of substrate inclusion (Table 2). There were, however, differences in Nal^r *E. coli* O157 concentrations between cattle fed DRC and cattle fed SFC. This effect was statistically significant only when substrate was excluded from
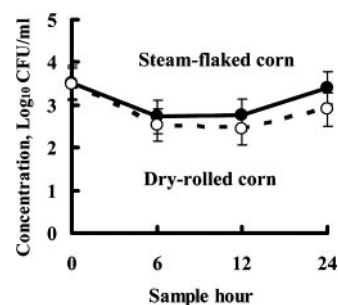
the fermentation. The concentrations of Nal^r *E. coli* O157 in fecal fermentations were higher for cattle fed SFC than for cattle fed DRC (*P* < 0.01) at the 12- and 24-h sampling times.

Growth of *E. coli* O157 during in vitro fermentation with DDG substrate (study 3). In vitro fermentations with DDG as a substrate revealed several statistical differences in the concentration of Nal^r *E. coli* O157 in both ruminal fluid and fecal microbial fermentations. In the 24-h samples from ruminal fluid fermentations, the concentration of Nal^r *E. coli* O157 was significantly higher when 0.5 g of DDG substrate was included than when no DDG substrate (*P* < 0.01), 1 g of DDG substrate (*P* < 0.001), or 2 g of DDG substrate (*P* < 0.001) was included (Table 3). The concentration of Nal^r *E. coli* O157 at 24 h was higher when no DDG substrate was included in the ruminal fluid fermentations than when 1 of DDG substrate (*P* < 0.01) or 2 of DDG substrate (*P* < 0.001) was included. The fecal microbial fermentation results revealed a different response for the Nal^r *E. coli* O157 concentration when different concentrations of DDG substrate were included (Table 3). Inclusion of 2 g of DDG substrate in fecal fermentations resulted in an Nal^r *E. coli* O157 concentration at 24 h that was higher than the concentration when no DDG substrate (*P* < 0.05), 0.5 g of DDG substrate (*P* < 0.05), or 1 g of DDG substrate (*P* < 0.01) was included. No other differences in the Nal^r *E. coli* O157 concentration were significant in 24-h fecal microbial fermentations (Table 3). No difference in the Nal^r *E. coli* O157 concentration was found between the donor steers whose diet was not supplemented with DDG and the donor steers whose diet was supplemented with 25% DDG using either a ruminal fluid or fecal microbial inoculum (*P* > 0.4).

DISCUSSION

It has been suggested that the potential association between feeding cattle DG or other fermentation by-products and *E. coli* O157 prevalence should be examined (32). Recently, a multistate epidemiologic study conducted in feedlots showed that the odds of obtaining an *E. coli* O157-positive fecal sample were six times higher if cattle were fed brewers' grains, a fermentative product similar to DG (9). The authors suggested that the increased odds of obtaining an *E. coli* O157-positive sample may have been due to components of the brewers' grain or management factors associated with brewers' grain

TABLE 1. Numbers and percentages of *E. coli* O157 isolates positive for the *eae* gene and Shiga toxin-producing genes (*stx*$_1$ and *stx*$_2$)

Virulence gene(s)	No. (%) of *E. coli* O157 isolates positive with the following diet[a]:		
	No DDG + 15% corn silage (*n* = 39)	25% DDG + 15% corn silage (*n* = 96)	25% DDG + 5% corn silage (*n* = 78)
eae	39 (100.0)	96 (100.0)	78 (100.0)
stx$_1$ only		3 (3.1)	2 (2.6)
stx$_2$ only	27 (69.2)	71 (74.0)	58 (74.4)
stx$_1$ and *stx*$_2$	12 (30.8)	22 (22.9)	18 (23.1)

[a] Isolates were obtained from a prevalence study which allocated feedlot cattle to treatments with or without 25% DDG and 5 or 15% corn silage.

TABLE 2. Mean concentrations of Nal[r] *E. coli* O157 and standard errors of the means in ruminal fluid or fecal microbial fermentations at 24 h (study 2)

Microbial inoculum	Substrate included in fermentations[a]	DDG in diet[b]	Concn of Nal[r] *E. coli* O157 (log$_{10}$ CFU/ml)	SEM (log$_{10}$ CFU/ml)	*P* value
Ruminal fluid	Yes	Yes	2.57	0.24	0.43
		No	2.79		
	No	Yes	3.91	0.24	0.04
		No	3.33		
Feces	Yes	Yes	2.83	0.13	0.99
		No	2.83		
	No	Yes	2.76	0.13	0.78
		No	2.72		

[a] The substrate included in fermentations was the ground whole diet of steers that served as donors of ruminal fluid or feces for in vitro fermentations.
[b] DDG was included in the diet of steers at a concentration of 25%.

feeding. The results of the present study are in agreement with a previous study that showed that there was a higher prevalence of *E. coli* O157 in cattle fed DG than in cattle fed diets lacking DG (25). In that previous study, we sampled approximately 370 yearling heifers and concluded that the prevalence of *E. coli* O157 in fecal samples collected twice during a 150-day feeding period (days 122 and 136) from cattle fed WDG was higher than the prevalence in fecal samples collected from cattle not fed WDG. In contrast to the previous study, the present study allowed us to follow the prevalence of *E. coli* O157 in cattle throughout the 12-week finishing period. Interestingly, in the present study, the prevalence of *E. coli* O157 in cattle fed DDG with 5% corn silage was between the values obtained with two other diets (both containing 15% corn silage; one with DDG and one without DDG) at most sampling times. DG replaced a portion of the corn in these diets. Because the starch in DG has been utilized during fermentation, addition of the by-product to corn-based diets decreases the total starch content and increases the fiber (bran) content of the feed (38). Previous work has shown that feeding forage-based diets increases the duration of shedding and/or concentration of *E. coli* O157 in the feces compared to the values obtained for animals fed a grain-based diet (21, 26, 40). Possibly, feeding DG altered the hindgut environment favorably, either by decreasing the starch content (14) or by increasing the fiber content, causing a higher prevalence of *E. coli* O157. There was considerable week-to-week variation in the preva-

lence of *E. coli* O157 in this study. Such variation is not surprising because a number of factors influence prevalence (37). Also, there are a number of non-O157 serotypes that are becoming increasingly important as food-borne pathogens, and cattle have been shown to harbor many of these serotypes (4). It is important to determine whether DG has an influence on non-O157 serotypes; however, such analyses were not included in the current study.

Another explanation for the DG association with prevalence seen in this study is that DG contains some component(s) that stimulates *E. coli* O157 growth. To determine if DG can stimulate the growth of *E. coli* O157, in vitro fermentations were conducted. Both ruminal fluid and fecal microbial fermentations were used to examine this relationship. In vitro fermentations with a ruminal fluid or fecal microbial inoculum have been used to assess gut microbial activity and interactions and the digestibility of substrates (11, 29, 41). Chaucheyras-Durand et al. (7) used in vitro fermentations with ruminal fluid or fecal microbial suspensions to evaluate the effects of gut biotic and abiotic factors on the growth and survival of *E. coli* O157. They observed that growth of *E. coli* O157 in a ruminal fluid fermentation was inhibited by the resident microbial flora. The site of *E. coli* O157 colonization in cattle is the hindgut and not the rumen (17, 27, 33, 40). Although the reasons for the preference for the hindgut are not known, it is logical to surmise that the ecosystem of the hindgut is more hospitable to *E. coli* O157 than the rumen. In the intestine of ruminants, the microbial counts in the colon and rectum were higher than the counts at any other location (39). Fecal microbial suspensions used during in in vitro fermentation studies of sheep were shown to have microbial counts representative of the counts in the colon and rectum (7). In addition, the fermentation processes and microbial populations in the rumen differ from those in the hindgut (29), so fecal microbial fermentations may represent hindgut fermentation better than ruminal fermentations.

Feeding DG may alter the microbial populations in the rumen (16) and/or prevent depression of the ruminal pH that could occur normally with corn diets without DG, possibly because of the decreased starch content (13), both of which may enhance the viability of *E. coli* O157 in the rumen. In

TABLE 3. Mean concentrations of Nal[r] *E. coli* O157 and standard errors of the means in ruminal fluid or fecal microbial fermentations at 12 and 24 h (study 3)

DDG concn in fermentation (g)	Concn (log$_{10}$ CFU/ml) of Nal[r] *E. coli* O157[a]					
	Ruminal fluid fermentation			Fecal microbial fermentation		
	12 h	24 h	SEM	12 h	24 h	SEM
0	2.37*	3.45*	0.25	2.39*	2.24*	0.25
0.5	2.56*	4.07†	0.26	2.59*†	2.27*	0.25
1.0	2.14*	2.66#	0.25	2.67*†	2.17*	0.25
2.0	2.15*	2.38#	0.25	2.90†	2.76†	0.25

[a] Different symbols within a column indicate statistical significance ($P < 0.05$).

ruminal fluid microbial fermentations, when substrate was not included, the concentration of Nal^r *E. coli* O157 at 24 h was higher for cattle fed DDG than for cattle fed no DDG. The increase may have been due to a higher pH in fermentations containing DG than in fermentations without DG. Alternatively, there may have been a stimulatory component(s) present in the DG diet that allowed *E. coli* O157 to grow. Possibly, a masking effect occurred when substrate was included in the fermentations (increased amount of fermentation products and a consequent reduction in the pH), resulting in no difference between the two diets for these fermentations. Surprisingly, there was no DDG effect in the fecal microbial fermentations, whether substrate was included or not. This may have been because the stimulatory component(s) of the DG did not reach the hindgut in cattle fed DDG and thus was not present in the fecal microbial inoculum.

In addition to a DDG effect on ruminal fluid fermentations in study 2, the type of grain processing for the animal diet (steam flaked or dry rolled) was associated with the concentration of Nal^r *E. coli* O157 in both ruminal fluid and fecal microbial fermentations. Fermentations with inocula from cattle fed SFC had higher Nal^r *E. coli* O157 concentrations than fermentations with inocula from cattle fed DRC. Previous work showed that grain processing (specifically, steam-flaked versus dry-rolled grains fed to cattle) was related to the fecal shedding of *E. coli* O157 (14). Two grain types, wheat and sorghum, were used in that study, and steam flaking of both grains resulted in a higher prevalence of *E. coli* O157 than dry rolling. The in vitro fermentation results of our study are in agreement with this observation. Grain processing (heat, moisture, etc.) increases the digestibility of the grain within the rumen (23), thereby reducing the amount of starch reaching the hindgut and not allowing a increased volatile fatty acid content and a decreased pH at that location (35).

Study 3 evaluated addition of different amounts of DDG to ruminal fluid or fecal microbial fermentations. In both instances, addition of DDG was associated with higher concentrations of Nal^r *E. coli* O157. In ruminal fluid fermentations, the concentration of Nal^r *E. coli* O157 was greater at 24 h than at zero time, suggesting that DG stimulated growth of the organism. In the ruminal fluid fermentations, adding 0.5 g of DDG substrate stimulated the growth of Nal^r *E. coli* O157; however, when DDG was included at concentrations greater than 0.5 g, there was no apparent change. This may have been a result of a decreased pH associated with fermentations of DG, inhibiting *E. coli* O157. Unfortunately, the pH values were not determined in this study. Perhaps not surprisingly, the results for fecal microbial fermentations were quite different. In these fermentations there appeared to be a more "dose-dependent" response of Nal^r *E. coli* O157 concentrations to increases in the amount of DDG substrate. There was no stimulation of growth of *E. coli* O157 like that observed in the ruminal fluid fermentations; however, addition of 2 g of DDG did result in higher concentrations at 24 h than addition of other DDG doses, likely because of some protective or stimulatory component in DG.

In conclusion, our study revealed a positive association between the presence of DG in the feed and the fecal prevalence of *E. coli* O157 in cattle, which confirmed our preliminary observations. Our observations have potentially serious rami-

fications. DG are likely to become more available and a popular feed supplement because of the projected growth of ethanol as a biofuel. Feeding programs that result in increases in the prevalence of *E. coli* O157 are likely to be met with strong opposition by beef producers and especially by beef processors. The utilization of distillers' by-products as components of feedlot diets may depend in part on our ability to devise feeding strategies that do not compromise the perceived safety of beef products. Therefore, it is important to confirm the positive association and determine the mechanism of the association because such knowledge could lead to potential intervention strategies. In vitro fermentation studies provided some evidence that DG may actually stimulate the growth of *E. coli* O157. We hypothesize that there are two possible mechanisms for the *E. coli* O157 association with DG: (i) a decreased starch concentration (DG replaced 25% of the grain in this study) reaches the hindgut, affects the ecology, and favors the growth of *E. coli* O157 or (ii) DG has a stimulatory component(s) that enhances the growth of this organism. Further research is needed to test our hypotheses.

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