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Inclusion of Dried or Wet Distillers' Grains at Different Levels in Diets of Feedlot Cattle Affects Fecal Shedding of *Escherichia coli* O157:H7[▽]

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Our objectives were to evaluate the prevalence of *Escherichia coli* O157:H7 in cattle fed diets supplemented with 20 or 40% dried distillers' grains (DG) (DDG) or wet DG (WDG) and assess whether removing DG from diets before slaughter affected fecal shedding of *E. coli* O157:H7. Eight hundred forty steers were allocated to 70 pens (12 steers/pen). Treatments were no DG (control), 20% DDG or WDG, and 40% DDG or WDG, and each was replicated in 14 pens. In phase 1, eight floor fecal samples were collected from each pen every 2 weeks for 12 weeks for isolation of *E. coli* O157:H7 and detection of high shedders. In phase 2, half of the pens with DG were transitioned to the no-DG control diet, and pen floor fecal samples were collected weekly from all pens for 4 weeks. During phase 1, prevalence of *E. coli* O157:H7 was 20.8% and 3.2% for high shedders. The form of DG had no significant effect on fecal *E. coli* O157:H7 shedding. The prevalence levels of *E. coli* O157:H7 and the numbers of high shedders were not different between diets with 0 or 20% DG; however, cattle fed 40% DG had a higher prevalence and more high shedders than cattle fed 0 or 20% DG ($P \leq 0.05$). During phase 2, overall and high-shedder prevalence estimates were 3.3% and <0.1%, respectively, and there were no differences between those for different DG forms and inclusion levels or when DG was removed from diets. The form of DG had no impact on *E. coli* O157:H7; however, fecal shedding was associated with the DG inclusion level.

Cattle are asymptomatic reservoirs for *Escherichia coli* O157:H7, a food-borne pathogen associated with gastrointestinal disease in thousands of Americans each year. The organism colonizes the hindgut of cattle (18, 27) and is shed in cattle feces. Once shed, *E. coli* O157:H7 can contaminate food and water, creating a food safety risk (20). Contamination of beef products occurs during slaughter and is associated with the prevalence of *E. coli* O157:H7 in feces and on the hides of cattle at harvest (5, 8, 12).

The prevalence of *E. coli* O157:H7 in cattle is associated with many factors, including season, geographic location, and diet. Previous work has shown that cattle fed diets containing distillers' grains (DG), an ethanol fermentation coproduct, have a higher prevalence of *E. coli* O157:H7 than cattle fed diets without DG (10, 28). Distillers' grains are a valuable feed commodity for cattle producers, and use of these coproducts has increased with the expansion of the ethanol industry (14, 17). Distillers' grains for use in cattle diets are available in wet (WDG) or dry (DDG) form. The association between feeding DG and *E. coli* O157:H7 prevalence has been shown with both forms (10, 28), but no study has directly compared the two forms. The levels of DG supplementation in cattle diets generally range from 10 to 50% (dry matter basis) depending on whether the coproduct is used as a protein or energy source. As a protein supplement, DG is included at 10 to 15%; as an energy source, the DG level is generally dictated by coproduct availability and grain price (14). There is some indication that *E. coli* O157:H7 prevalence is different for cattle fed different levels of DG (19). However, no study has specifically evaluated the relationship between *E. coli* O157:H7 prevalence and DG inclusion level. Evaluation of these two factors (form and inclusion level) is important for furthering our understanding of the association between DG and *E. coli* O157:H7 in cattle.

We also were interested in determining whether removing the DG component of the diet would lower fecal prevalence of *E. coli* O157:H7. Such a strategy may lead to potential mitigation options and would provide further evidence of a positive association between feeding DG and *E. coli* O157:H7 prevalence in cattle. In this two-phase study, our objectives were to (i) concurrently evaluate the effect of DG inclusion level and form on *E. coli* O157:H7 prevalence in feedlot cattle and (ii) determine if removing DG from cattle diets subsequently reduces the fecal prevalence of *E. coli* O157:H7.

MATERIALS AND METHODS

Phase 1: animals, treatments, and sampling. The study was conducted during summer and fall (May through October) 2009. Eight hundred forty steers (body weight [BW] = 414 ± 131 kg) were randomly allocated to 1 of 70 pens (12 steers/pen) in a commercial research feedlot in Nebraska. Cattle were fed high-grain, corn-based finishing diets (Table 1), and one of five treatments was randomly assigned once within a block of five pens. Treatments were 0% DG (control), 20% DDG, 20% WDG, 40% DDG, and 40% WDG. Each treatment was replicated in 14 pens, and adjacent pens shared common fence lines. Diets were formulated to meet nutritional requirements and were designed to be as similar as possible, differing only in level and form of DG inclusion. Cattle began final finishing diets after a 1-month transition and were fed once per day with continual access to water. After cattle began final finishing diets, eight fresh pen floor fecal samples were collected from each pen once every 2 weeks for 12 weeks. Care was taken to avoid contamination of samples from other feces or from pen floors, and efforts were made to collect each sample from a different

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TABLE 1. Composition of treatment diets

Item	Control	% of composition in:			
		DDG		WDG	
		20%	40%	20%	40%
Ingredients (as-fed basis)					
Cracked corn	82.9	68.2	48.9	51.8	29.7
Soybean meal	2.03	0.0	0.0	0.0	0.0
Ground alfalfa	7.63	7.79	7.93	5.91	4.82
Dried distillers' grain	0.0	18.4	37.5	0.0	0.0
Wet distillers' grain	0.0	0.0	0.0	38.1	62.0
Low-protein liquid supplement	0.0	5.60	5.70	4.25	3.46
High-protein liquid supplement	7.49	0.0	0.0	0.0	0.0
Chemical components (dry-matter basis)					
Dry matter	80.7	82.1	83.2	65.2	55.3
Crude protein	10.1	15.9	20.9	19.8	25.3
Soluble protein	5.87	4.38	5.72	4.91	6.06
Acid detergent fiber	5.08	7.63	10.5	10.6	14.2
Neutral detergent fiber	10.4	15.0	20.0	18.4	23.5
Crude fat	4.69	6.17	7.57	8.21	10.4
Sulfur	0.16	0.27	0.42	0.34	0.47
Metabolic energy, Mcal/kg of feed	3.16	3.22	3.21	3.35	3.43

animal within that pen by observing individual animals defecating. Samples were collected with plastic spoons, placed in Whirl Pack bags (Nasco, Fort Atkinson, WI), and transported on ice to the laboratory.

Phase 2: animals, treatments, and sampling. After cattle were fed phase 1 finishing diets for 12 weeks, DG was removed from half (7 of 14 pens) of each of the DG-fed treatments to initiate phase 2 of the study. Two consecutive blocks of five pens (see description for phase 1 above) were used to create one block for phase 2. Within the new block of 10 pens, one pen receiving 20% DDG, 20% WDG, 40% DDG, or 40% WDG was randomly selected to transition to the control (no-DG) diet. First, half of the pens in the 40% DDG or WDG group were changed to 20% DDG or WDG, with a concurrent increase in the cracked corn grain. One week later, the 20% DDG or WDG was replaced with corn grain; at the same time, half of the pens in the 20% DDG or WDG group were changed to 0% DG. Therefore, phase 2 had nine treatments: 0% DG (control), 20% DDG, 20% WDG, 40% DDG, 40% WDG, 20% DDG-removed (REM), 20% WDG-REM, 40% DDG-REM, and 40% WDG-REM. After cattle had been acclimated to new diets (2 weeks), eight pen floor fecal grab samples were collected from each pen once weekly for 4 weeks.

Detection of *E. coli* O157:H7. Approximately 1 g of fecal sample was placed into 9 ml of Gram-negative (GN) broth (Difco; BD, Sparks, MD) supplemented with cefixime (0.5 mg/liter), cefsulodin (10 mg/liter), and vancomycin (GN_{ccv}; 8 mg/liter). Samples were vortexed and incubated for 5.5 h at 37°C, after which manual immunomagnetic bead separation (IMS) (Dynabeads; Invitrogen Corp., Carlsbad, CA) was performed on 1 ml of enrichment. After IMS, samples were plated onto sorbitol-MacConkey agar supplemented with cefixime (0.5 mg/liter) and potassium tellurite (CT-SMAC; 2.5 mg/liter). Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies were streaked onto blood agar plates (Remel, Lenexa, KS). After an overnight incubation at 37°C, colonies were tested for indole production and latex agglutination for the O157 antigen (Oxoid, Remel), and positive isolates were further characterized by multiplex PCR identifying the *rfbE* (O157), *eae* (intimin), stx_1 (Shiga toxin 1), stx_2 (Shiga toxin 2), *hlyA* (hemolysin), and *fliC* (flagella) genes (2).

In addition to detection by enrichment and selective plating, fecal samples were categorized into low and high shedders of *E. coli* O157:H7 by a semiquantitative direct plating method (8, 22). Briefly, a swab of fecal suspension in GN_{ccv} broth (prior to enrichment) was spread onto a top quadrant of a CT-SMAC plate. A loop was used to streak from the quadrant for isolation, and plates were incubated for approximately 20 h at 37°C. Up to six sorbitol-negative colonies were picked from CT-SMAC plates, transferred to blood agar plates, incubated at 37°C overnight, evaluated for indole production and latex agglutination for the O157 antigen, and confirmed by multiplex PCR as described above. Following the semiquantitative and IMS methods, samples were categorized as high-shedder positive (positive isolate obtained from a direct-streak plate) and enrichment positive (positive isolate obtained only with the IMS method).

Statistical analysis. Pen-level fecal prevalence and prevalence of cattle shedding *E. coli* O157:H7 at high concentrations (high shedders) were the outcomes of interest in this study. Descriptive statistics on prevalence outcomes were assessed prior to multivariable analysis. Data were then analyzed with generalized linear models for count data using Poisson and negative binomial distributions with log links in PROC GENMOD of SAS (version 9.1; Cary, NC). A negative binomial model was used only when there was evidence of overdispersion in the Poisson model. A pen was the experimental unit, and the count of positive samples (or count of high shedders) was the outcome, which was offset by the natural log of the number of samples processed for each pen. A repeated (random) effect was included in all models to account for the lack of independence among samples within pens over time. Score statistics for generalized estimating equations were used to assess the statistical significance of effects for the DG form, inclusion level, collection day, and first-order interactions. Data from phase 2 were analyzed in different, but similarly specified, models. In addition to variables evaluated in phase 1 models, phase 2 models included a dichotomous variable indicating whether DG was removed from diets. When phase 2 data were analyzed, *E. coli* O157:H7 outcomes from cattle fed only the control diet were not included because the objective was to evaluate the effect of removing DG on *E. coli* O157:H7 prevalence estimates. Results were considered statistically significant at a P value of <0.05.

RESULTS

Phase 1. A total of 3,350 fecal samples (six collections over 12 weeks) that included 670 from control cattle, 671 from the 20% DDG group, 666 from the 20% WDG group (669 for determining high-shedder prevalence), 672 from the 40% DDG group, and 671 from the 40% WDG group were obtained. The overall fecal prevalence of *E. coli* O157:H7 during phase 1 was 20.8% (695 of 3,350). Model-adjusted estimates of cumulative prevalence were 15.5, 19.2, 18.6, 25.6, and 24.7% for pens of cattle fed control, 20% DDG, 20% WDG, 40% DDG, and 40% WDG diets, respectively (Fig. 1). Collection day was associated with *E. coli* O157:H7 fecal prevalence ($P < 0.01$). The level at which DG was included in cattle diets was associated with the fecal prevalence of *E. coli* O157:H7 ($P = 0.05$), but DG form was not associated with prevalence ($P = 0.8$). Pens of cattle fed 20% DG (wet or dried) did not have an *E. coli* O157:H7 prevalence statistically different than that of

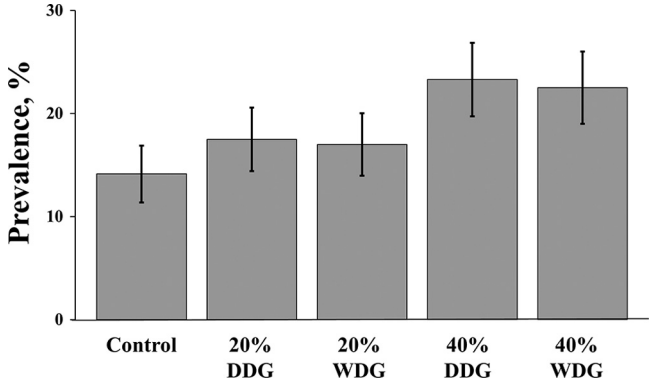

FIG. 1. Model-adjusted cumulative prevalence estimates (and 95% confidence intervals) of fecal *Escherichia coli* O157:H7 shedding in pens of cattle fed diets with 0, 20, or 40% WDG or DDG for 12 weeks. Prevalence estimates for cattle fed WDG and DDG were not statistically different; however, cattle fed 40% WDG or DDG had a higher fecal prevalence of *E. coli* O157:H7 than cattle fed 0 or 20% ($P \leq 0.05$).

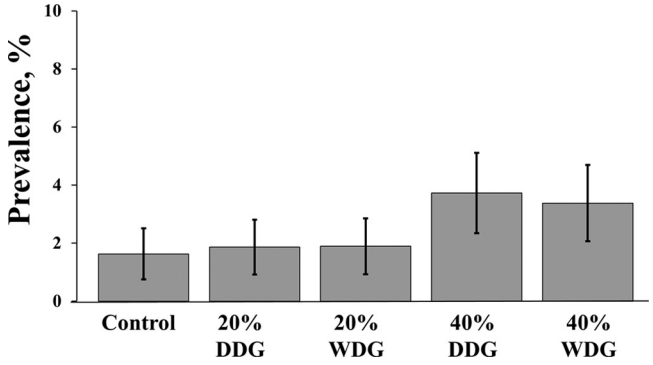

FIG. 2. Model-adjusted cumulative prevalence estimates (and 95% confidence intervals) of *Escherichia coli* O157:H7 high shedders in pens of cattle fed diets with 0, 20, or 40% WDG or DDG for 12 weeks. After analysis, WDG and DDG types were not statistically different; however, cattle fed 40% WDG or DDG had a higher high-shedder prevalence than cattle fed 0 or 20% ($P < 0.01$).

cattle fed the control diet ($P = 0.4$); however, the prevalence in cattle fed 40% DG (wet or dried) differed from the prevalence for cattle fed control ($P = 0.02$) or 20% DG ($P = 0.05$) diets. The interaction of DG form and inclusion level was not statistically significant ($P = 0.9$).

The overall prevalence of *E. coli* O157:H7 high shedders during phase 1 was 3.2% (107 of 3,353), and model-adjusted prevalence estimates were 2.1, 2.4, 2.4, 4.8, and 4.3% for cattle fed control, 20% DDG, 20% WDG, 40% DDG, and 40% WDG diets, respectively (Fig. 2). Similar to overall fecal shedding results, collection day was associated with high-shedder prevalence ($P < 0.01$). The level of DG inclusion was associated with *E. coli* O157:H7 high-shedder prevalence ($P < 0.01$), but the DG form ($P = 0.7$) and the interaction ($P = 0.7$) were not significant. The prevalence of high shedders was higher among cattle fed diets with 40% DG than among those fed no DG ($P < 0.01$) or 20% DG ($P < 0.01$); the prevalence of high shedders was not different between cattle fed control and 20% DG diets ($P = 0.6$).

Phase 2. The overall fecal prevalence of *E. coli* O157:H7 during phase 2 was 3.3% (60 of 1,792), and <0.1% (1 of 1,792) of pen floor samples were considered high shedders. The prevalence of *E. coli* O157:H7 varied by collection day during phase 2 ($P < 0.01$). The model-adjusted estimates of cumulative prevalence were 0.7, 2.6, 3.7, and 3.7 for cattle fed 20% DDG, 20% WDG, 40% DDG, and 40% WDG diets, respectively, and 4.8, 1.9, 1.9, and 3.0% for cattle fed 20% DDG-REM, 20% WDG-REM, 40% DDG-REM, and 40% WDG-REM diets, respectively. The inclusion level and form of DG were not associated with *E. coli* O157:H7 prevalence during this phase. In addition, DG removal from treatments was not associated with prevalence ($P = 0.7$).

DISCUSSION

These results confirm the previous observation that feeding cattle wet or dried DG can be associated with increased *E. coli* O157:H7 prevalence. This study also showed that not only overall prevalence but also high-shedder prevalence was dependent on DG inclusion level. Previous studies documenting the relationship between *E. coli* O157:H7 prevalence and feeding DG differed in the inclusion level (10 to 50%) and form of DG (wet or dried) fed to cattle; further, the magnitude of the reported association has not been consistent (10, 11, 28). Although both dried and wet DG have been studied, the two have not been directly compared in terms of their impact on *E. coli* O157:H7 prevalence. The current study was designed to concurrently evaluate DG inclusion level and form (dried or wet) to understand how previous inconsistencies in prevalence may be related to diet composition. In this study, there was no difference between the two forms of DG and no interaction between inclusion level and DG form; thus, we conclude that DG form (wet or dry) does not affect *E. coli* O157:H7 prevalence.

The amount of DG included in cattle diets varies; most studies use 40 to 50% DG on a dry-matter basis as the upper limit (6, 9, 14). The optimum inclusion level for animal performance ranges between 15 and 30% and is related to additional factors, including primary grain type, source, and grain processing (3, 14). Grain price and availability as well as location of DG plants also influence the level of DG fed to cattle (26). A previous study evaluating the efficacy of an *E. coli* O157:H7 vaccine included DG in cattle diets at different levels (0, 10, 20, 30, 40, and 50%) and indicated that the inclusion level may be associated with *E. coli* O157:H7 prevalence (19). We also observed an association between the DG inclusion level and *E. coli* O157:H7 prevalence; at 20% DG inclusion, there was no significant increase in *E. coli* O157:H7 prevalence compared with that in cattle not fed DG; however, the fecal prevalence of cattle fed 40% DG was significantly higher than that of cattle fed 0 or 20% DG.

Different DG inclusion levels as well as variability in nutrient components between DG sources (13, 24) may explain the inconsistencies among previous studies regarding the magnitude of association between *E. coli* O157:H7 and DG in the diet. Because *E. coli* O157:H7 is found primarily in the hindgut of cattle (18, 27), it is likely affected by changes in hindgut ecology, such as pH, volatile fatty acid (VFA) concentrations, and nutrient availability. Distillers' grains are low in starch

content, so the amount of starch reaching the hindgut is less than that in a grain-fed animal. On the other hand, more protein is likely to reach the hindgut, because DG protein is less fermentable in the rumen (14). Fecal pH and VFA concentrations have been used as indicators of hindgut fermentation (16). Previous work has shown that total VFA concentration in feces decreases linearly with increasing levels of DG fed to cattle (25). Wells et al. (28) evaluated *E. coli* O157:H7 shedding in cattle fed 40% DG diets and observed no relationship with fecal pH; however, concentrations of L-lactate and total VFA were higher in *E. coli* O157:H7-positive fecal samples than in *E. coli* O157:H7-negative fecal samples. In that study, differences in fecal pH and VFA concentrations between cattle fed 0 and 40% WDG were not examined but may have provided additional information on the differences between diets. We did not measure fecal pH, L-lactate, or VFA concentrations in the present study; however, it is likely that cattle fed increasing amounts of DG have different physiological conditions and microbial populations in the hindgut.

In addition to the fecal prevalence of *E. coli* O157:H7 being positively associated with feeding 40% DG to cattle, we also observed that a greater number of cattle fed these diets shed the organism at high concentrations. This observation is in agreement with the study reported by Wells et al. (28), who showed that the number of samples that were quantifiable (shedding higher concentrations) for *E. coli* O157:H7 was higher for cattle fed 40% WDG than for cattle fed control diets. Although the significance of cattle shedding high concentrations of *E. coli* O157:H7 is not fully understood, there is evidence that, at harvest, the prevalence of high shedders is positively associated with hide and carcass contamination (8, 12). It also has been proposed that high shedders are responsible for increased transmission of *E. coli* O157:H7 within a cohort (1, 15), possibly by increasing the concentration of the organism in the environment (25). Although the reason cattle shed high concentrations of *E. coli* O157:H7 is not known, we surmise that more cattle shedding higher concentrations of the organisms increase the food safety risk. It is not known why DG feeding increases high shedders, but it has been suggested that DG may directly stimulate growth of *E. coli* O157 (10). Our results, however, seem to suggest that the mechanism may be similar to that promoting *E. coli* O157:H7 at normal concentrations because the trends between inclusion level and both fecal prevalence and high-shedder prevalence were similar.

Dried DG differs from wet DG in that it is dehydrated, which enables easier transportation of the product to cattle producers (14). Despite potential differences in nutrient content between WDG and DDG, the prevalence and concentration of *E. coli* O157:H7 in cattle fed similar amounts of either form appeared unaffected. This might indicate that the observed association with DG may be more a function of starch displacement than of added nutrients. This hypothesis has been proposed by others evaluating the effect on *E. coli* O157:H7 prevalence due to diet regimens that alter the availability of starch in the hindgut (11). Fox et al. (7) reported a higher prevalence of *E. coli* O157:H7 in cattle fed steam-flaked corn than in cattle fed dry-rolled corn and hypothesized that the difference may be related to the amount of starch reaching the hindgut, which would be higher in dry-rolled than in steam-flaked corn diets. The effect of starch in the hindgut of cattle on *E. coli* O157:H7 remains unknown, yet differences in starch content seem common in diets that alter *E. coli* O157:H7 shedding.

The prevalence of *E. coli* O157:H7 in cattle during phase 2 of this study was quite low (3.3%), which limited our ability to effectively evaluate whether removal of DG had any effect on fecal prevalence of *E. coli* O157:H7. We anticipated that the prevalence and concentration of *E. coli* O157:H7 in cattle fed diets from which the DG was removed would decrease to control values seen in phase 1, providing further evidence for the positive association between DG and *E. coli* O157 prevalence. Instead, the prevalence in all treatments dropped significantly, as did the number of high shedders. Although there was no statistical difference in the prevalence levels between cattle fed DG diets and those fed diets from which the DG was removed, these results should be interpreted with caution. Samples for phase 2 were collected in late fall; the fact that we observed low prevalence during this time may not be surprising considering the seasonal prevalence of *E. coli* O157:H7, which typically peaks during summer months (4, 21, 23). We transitioned cattle from diets containing DG to control finishing diets over 2 weeks to prevent disruption in intake and maintain rumen health. Even if we had observed a reduction of *E. coli* O157:H7 shedding, it would be challenging to develop widespread adoption of such an intervention strategy, particularly because of the risk of reduced animal performance.

This study confirmed that feeding DG to cattle is associated with increased fecal shedding of *E. coli* O157:H7 and an increased number of cattle shedding the organism at high concentrations. The form of DG (wet or dry) had no impact on *E. coli* O157:H7 prevalence; however, fecal shedding of *E. coli* O157:H7 was dependent on the DG inclusion level.

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