

Impact of Reducing the Level of Wet Distillers Grains Fed to Cattle Prior to Harvest on Prevalence and Levels of *Escherichia coli* O157:H7 in Feces and on Hides[†]

J. E. WELLS,* S. D. SHACKELFORD, E. D. BERRY, N. KALCHAYANAND, J. M. BOSILEVAC, AND T. L. WHEELER

U.S. Department of Agriculture, Agricultural Research Service, U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

MS 11-160: Received 29 March 2011/Accepted 27 May 2011

ABSTRACT

Cattle fed finishing diets with wet distillers grains with solubles (WDGS) have been shown to harbor increased *Escherichia coli* O157:H7 populations in the feces and on the hides. To determine if feeding a lower level of WDGS at the end of the feeding period reduces *E. coli* O157:H7 load at harvest, 608 heifers were sorted into one of five treatments and fed 0, 40, or 70% WDGS (dry matter basis). For three of the treatments, WDGS was reduced midway through the study. Treatment 0W0W heifers (positive control) were fed a corn grain-based diet continuously, and 40W40W heifers (negative control) were fed 40% WDGS continuously. Heifers subjected to treatments 40W0W, 40W15W, and 70W15W were fed either 40 or 70% WDGS for the first 56 days and switched to 0 or 15% WDGS, respectively, for the last 56 days. Prior to the switch in diets, animals fed diets with 40 or 70% had higher prevalence and percent enumerable fecal samples for *E. coli* O157:H7. After the dietary switch, animals fed 40W0W, 40W15W, and 70W15W diets had fecal prevalence and percent enumerable samples (33.4 and 6.3%, 31.0 and 9.7%, and 34.9 and 8.4%, respectively) similar to those of animals fed 0W0W diets (10.2 and 3.2%, respectively; $P > 0.05$), whereas animals fed 40W40W had the highest fecal prevalence and percent enumerable samples (70.1 and 29.2%, respectively; $P < 0.05$). Similar relationships between the treatments were observed for hide samples. Time after dietary switch was important, as animals fed lower levels had significantly lower fecal prevalence and percent enumerable samples after 56 days, but not after 28 days. The study indicates that cattle can be switched to lower levels of dietary WDGS (15% or less) 56 days prior to harvest to significantly reduce *E. coli* O157:H7 in feces and on hides.

The potential role for diet to affect *Escherichia coli* O157:H7 in cattle has been debated (6, 10). In recent years, the inclusion of distillers grains in cattle diets has been associated with higher prevalence for *E. coli* O157:H7 in feces (11, 12), and in the study reported by Wells et al. (20), feeding finishing cattle diets with 40% wet distillers grains with solubles (WDGS) was associated not only with higher prevalence but also with a greater number of animals with high levels in feces and higher prevalence on the hides for *E. coli* O157:H7. The mechanism for dietary distillers grains in altering fecal *E. coli* O157:H7 is not known, but feeding high levels of WDGS resulted in higher levels for total *E. coli* in feces of finishing cattle (20).

Interventions to reduce *E. coli* O157:H7 during beef harvest have been very effective to reduce pathogen load on carcasses, but in the feedlot, interventions have largely been ineffective to date (6). Because of the potential for higher

levels of *E. coli* O157:H7 with cattle fed distillers grains, identifying an effective intervention prior to harvest is a priority for cattle fed these diets in particular. Considering that animals fed diets with dry-rolled corn had lower prevalence than cattle fed diets with WDGS (20), it appeared that a simple intervention for cattle fed WDGS would be to switch diets to predominantly corn prior to harvest, reducing the level of WDGS. A recent report by Jacob et al. (13) tested a similar approach in cattle fed distillers grains, but in that study fecal prevalence for *E. coli* O157:H7 was low and no significant effect of a dietary switch was observed. The goal of this research was to determine if switching animals from diets with high levels (40 or 70%) of WDGS to diets with lower levels (0 or 15%) of WDGS and more corn would reduce the *E. coli* O157:H7 load. A secondary goal was to determine if the dietary shift resulted in immediate reductions for *E. coli* O157:H7, or if longer feeding times would be required before significant reductions in fecal *E. coli* O157:H7 were observed. Feeding WDGS and other distillers grains products are economically important factors affecting cattle operations, and better feed management practices that sustain lower operating costs and minimize the presence of *E. coli* O157:H7 at harvest will provide a safer, more affordable food supply.

* Author for correspondence. Tel: 402-762-4174; Fax: 402-762-4209; E-mail: jim.wells@ars.usda.gov.

[†] Product names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. USDA is an equal opportunity provider and employer.

MATERIALS AND METHODS

Animals and diets. All animal procedures were reviewed and approved by the U.S. Meat Animal Research Center (USMARC) Animal Care and Use Committee.

The study consisted of two separate experiments with identical treatments conducted in Spring–Summer 2009 (experiment 1) and repeated along a similar timeline in Spring–Summer 2010 (experiment 2). A total of 608 spring-born heifers (337 for year 1 and 271 for year 2) were identified after weaning for this project. For each year, the heifers were from USMARC cattle populations; they were blocked by dam line, sire line, sire (if known), and weaning weight and assigned to one of five dietary treatment groups. The treatment groups consisted of the following: (i) 0W0W, pens provided diets without WDGS for 224 days; (ii) 40W40W, pens provided diets with 40% WDGS (dry matter [DM] basis) for 224 days; (iii) 40W0W, pens provided diet with 40% WDGS (DM basis) for 168 days followed by diet with 0% WDGS for 56 days; (iv) 40W15W, pens provided diet with 40% WDGS (DM basis) for 168 days followed by diet with 15% WDGS for 56 days; and (v) 70W15W, pens provided diet with 70% WDGS (DM basis) for 168 days followed by diet with 15% WDGS for 56 days. For comparative purposes, with regard to *E. coli* O157:H7 population measurements and experimental design, the 0W0W treatment served as a positive control and the 40W40W treatment served as a negative control.

Throughout the study, animals were provided ad libitum access to the respective diets. The study was initiated in November of each year, with 337 heifers in year 1 and 271 heifers in year 2 assigned each year to 1 of 10 pens with 27 to 33 heifers per pen (two pens per dietary treatment each year). Heifers were sorted to pens and treatments according to the blocking scheme described above. Prior to the study, all animals were acclimated to the feedlot and fed an alfalfa hay and corn silage diet for 28 days. Animals were sorted into treatment pens, where animals were fed a corn silage-based growing ration (20) for 112 days with 0, 40, or 70% WDGS (DM basis). Animals were adapted to finishing diets (dry-rolled corn based diet as described previously by Wells et al. (20)) that were formulated with 0, 40, or 70% WDGS, where WDGS replaced corn in the diet on an equal DM basis. Finishing rations with 0, 40, or 70% WDGS were fed for 56 days before selected pens were switched to lower levels of WDGS, 0, 15, or 40% WDGS, with the balance consisting of dry-rolled corn. Adaptation to lower levels of WDGS in the diet was accomplished over a 10-day period with diets formulated with intermediate levels of WDGS fed for 3 to 4 days. All diets were formulated to meet or exceed the National Research Council recommendations for growing and finishing beef steers (14). For reporting purposes, the day of the dietary switch is reported as day 0. In both experiments, fecal and hide samples were collected from all animals on day -56 (start of the finishing phase) and on days 0, 28, and 56 after the dietary switch, but in experiment 2, additional samples were collected from all animals on day 42.

Sample collection and bacterial analyses. Samples of feces and hides were collected as described previously (20). Each animal was restrained in a squeeze chute to collect the samples. The fecal sample (at least 10 g) was collected as a rectal grab sample with a clean gauntlet glove (NASCO, Ft. Atkinson, WI) for each animal. Feces were immediately transferred to a clean closable plastic bag (Envision, Wichita, KS) for transport to the laboratory. The hide sample was collected with a sterile sponge (NASCO) premoistened with 20 ml of sterile buffered peptone water (Difco, BD, Sparks, MD) through an open-access panel in the squeeze chute. An area of approximately 1,000 cm² behind the left shoulder was wetted with

distilled water and then sampled by hand with a clean latex glove each time with 10 up-down strokes of the sponge (5 strokes per each side of sponge), and the sample sponge was returned to the sterile sponge bag. Samples were continuously transported to the laboratory in batches within 20 min after sampling for immediate processing.

A 10-g (± 0.1 g) amount of each fecal sample was placed into a sterile filter bag (NASCO) with 90 ml of sterile tryptic soy broth (TSB; Difco, BD) containing 100 mM potassium phosphate buffer (5) (18 mM KH₂PO₄ and 82 mM K₂HPO₄, pH 7.2; Sigma Chemicals, St. Louis, MO) and mixed well by hand massage. A 500- μ l aliquot was removed to a sterile 2-ml cluster biotube (Simport Corp., Beloell, QC, Canada) for enumeration (detection limit for *E. coli* O157:H7 is 200 CFU/g of feces). For the hide sponge samples, samples were mixed in the bag by hand squeezing, a 250- μ l aliquot was removed to a sterile 2-ml cluster biotube (Simport Corp.) for enumeration (detection limit for *E. coli* O157:H7 is 40 CFU/100 cm²), and then 80 ml of TSB was added and mixed again by hand. All samples with added media were enriched by incubation for 2 h at 25°C followed by 6 h at 42°C. Samples were then held at 4°C overnight for immunomagnetic separation.

To enumerate *E. coli* O157 organisms in each sample (described previously by Brichta-Harhay et al. (8)), 50 μ l of each fecal and hide aliquot was plated onto ntCHROMO157 agar (CHROMO157 agar, DRG International, Mountainside, NJ) supplemented with 5 mg of novobiocin per liter (Sigma Chemicals) and 2.5 mg of potassium tellurite per liter (Sigma Chemicals) by use of an Autoplate 4000 spiral-plater (Spiral Biotech Inc., Norwood, MA). Plates were air dried and incubated overnight at 42°C. Presumptive colonies (up to five per sample) were tested by latex agglutination with DrySpot *E. coli* O157 (Oxoid Ltd., Basingstoke, UK). Agglutination-positive colonies were counted and confirmed by PCR assay for combination of O157, H7 flagella, intimin, and Shiga toxin 1 and 2 genes (9).

To determine *E. coli* O157 prevalence, filter bags with enriched fecal samples and sponge bags with enriched hide samples were mixed by hand massage, and 0.5 ml was removed to an individual well in deep-well 96-well blocks. Each well was preloaded with 0.5 ml of phosphate-buffered saline with Tween (PBS-Tween; Sigma Chemicals) and 20 μ l (for fecal samples) or 5 μ l (for hide samples) of anti-O157 immunomagnetic beads (Invitrogen Corp., Carlsbad, CA). Magnetic beads were mixed with enriched sample for 15 min at room temperature on a vibrating MicroMixer Mx4 (FinePCR, Seoul, Korea). The beads were removed from the sample, washed twice in PBS-Tween, and eluted into 100 μ l of PBS-Tween in a Kingfisher 96 robotic processor (ThermoLifeSciences/Fisher Scientific, St. Louis, MO). A 50- μ l aliquot of the bead-bacteria complex was plated onto ntCHROMO157 agar. Plates were incubated overnight at 37°C. Presumptive colonies were tested by latex agglutination with DrySpot *E. coli* O157 (Oxoid). At least two positive colonies were picked per plate and confirmed by PCR assay as described above.

Statistical analyses. Bacterial counts in feces were transformed based on the count, the plated volume, and the dilution factor of sample and reported in CFU per gram of feces. Bacterial counts on hides were transformed based on the count and the plated volume and are reported in CFU per 100 cm². *E. coli* O157:H7 prevalence in each pen was determined as a proportion of positives to total samples for each sample day and reported as a percentage. The prevalence data were analyzed as a split-plot in time with pen as the experimental unit. An initial model was used with all interactions, and after step-down analyses, the final model

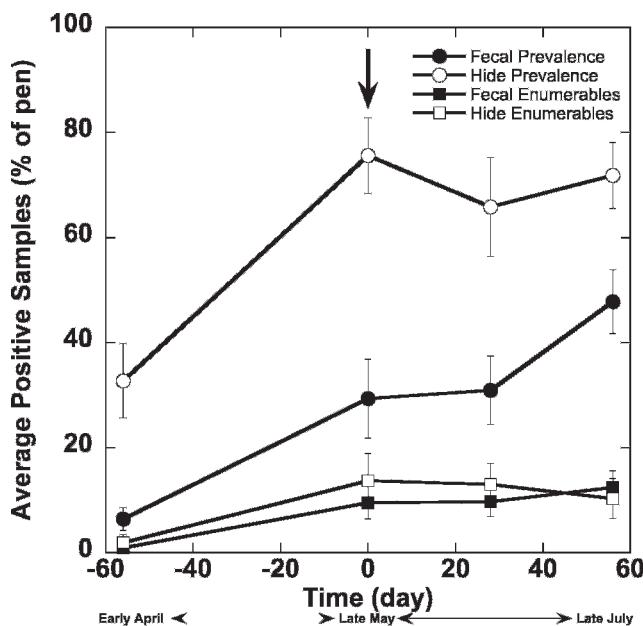


FIGURE 1. Levels and prevalence for *E. coli* O157:H7 in feces and on hides for all animals across all treatments over the course of the 2-year study for finishing cattle from day -56 in April to day 56 in July. All feedlot heifers were converted from growing rations to their respective finishing diets on day -56, and the arrow denotes the time in the study (designated day 0) at which the selected treatment groups fed high levels of WDGS in the diet were switched to lower levels of WDGS in the diets. Bars on each sample point denote standard errors of the means for each sample day averaged for both years.

included the effects of dietary treatment, year of study, sample day, and pen nested within dietary treatment. Dietary treatment was tested with pen nested within dietary treatment as the error term. Least-squares means and pooled standard errors of the means are presented in the text, tables, or figures where appropriate. Where noted, pooled prevalence data at specific times for both years were analyzed by the chi-square test. For all statistical analyses, differences were considered significant when the type I error probabilities were 0.05 or less and were considered tendencies when the probabilities were between 0.05 and 0.10. Statistical analyses were conducted using the GLM procedure in SAS (SAS Institute Inc., Cary, NC) except for the chi-square analyses (16).

RESULTS AND DISCUSSION

In each year of this study to determine if dietary manipulation would affect *E. coli* O157:H7, animals were started on finishing rations with and without WDGS on similar schedules, and samples were collected from all animals in May to determine if diet affected the *E. coli* O157:H7 load. Six pens of animals on high levels of WDGS were then switched to diets with 0 or 15% WDGS to determine if a reduction in WDGS would reduce the *E. coli* O157:H7 load by day 28 or 56 after the dietary switch. Overall for the entire study period, fecal and hide prevalence averaged 36.0 and 71.1% positive, respectively, and percent fecal and hide enumerable samples averaged 10.5 and 12.3%, respectively. The effect of time on the *E. coli* O157:H7 parameters for animals in this study is shown in Figure 1, indicating that the average *E. coli* O157:H7 load

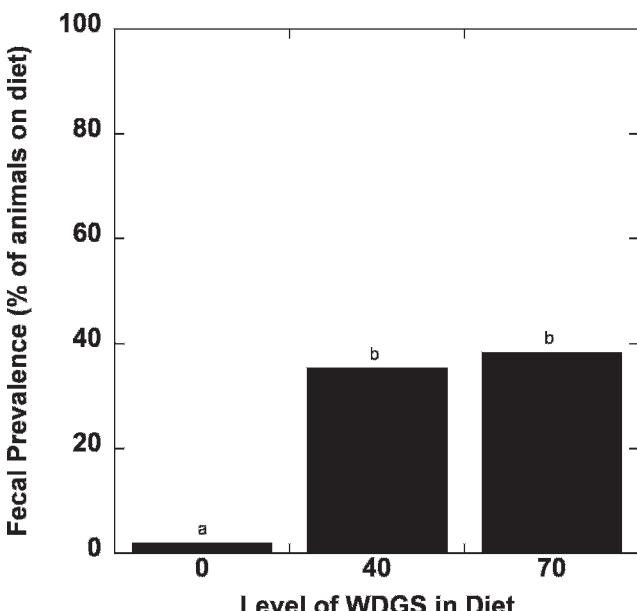


FIGURE 2. Average fecal prevalence of *E. coli* O157:H7 for animals on day 0 being fed finishing diets with 0, 40, or 70% WDGS in the diet. Means that are significantly different ($P < 0.05$) are denoted by different letters above the bars, as determined by chi-square analysis.

was high throughout the course of the study and the study should have potential to observe dietary effects. As expected, fecal prevalence was lowest in April (day -56, before the dietary switch) and increased with time for samples collected in May (day 0, day of the switch) to July (day 56, after the dietary switch), demonstrating the typical seasonality for *E. coli* O157:H7. Similar results for fecal prevalence were observed previously over similar points in time (20).

To determine if diets with high levels of dietary WDGS had an effect on the *E. coli* O157:H7 load before the dietary switch, data were analyzed by chi-square analyses for the samples collected on day 0. Animals were fed 0, 40, or 70% WDGS for 56 days of the finishing phase and sampled on day 0 prior to any dietary switch in the finishing phase to determine if diets containing high levels of WDGS affected the *E. coli* O157:H7 load. The animals being fed 0% WDGS finishing diet had lower fecal prevalence for *E. coli* O157:H7 than did the animals fed 40 and 70% WDGS finishing diets (1.9% versus 35.4 and 38.4%, respectively; $P < 0.05$; Fig. 2), and similar differences were measured with percent fecal enumerable levels (≥ 200 CFU/g of feces) of *E. coli* O157:H7 (0.0% versus 12.8 and 9.1%, respectively; $P < 0.05$; data not shown). Previous research has indicated that distillers grains fed at 20 to 40% of the diet resulted in higher fecal prevalence for *E. coli* O157:H7 (11–13, 20), and the differences between 0 and 40% WDGS dietary treatments observed in this study are comparable to those reported previously (20). In this study, no significant difference was observed between animals fed the 40 or 70% WDGS finishing diets on day 0 of this study ($P > 0.1$), indicating that dietary WDGS levels greater than 40% may

not result in further increases for the shedding of *E. coli* O157:H7.

After day 0 sampling, some pens of animals that had been fed 40 or 70% WDGS were switched to diets with 0% (40W0W) or 15% (40W15W and 70W15W) WDGS to determine if shedding subsequently would be reduced by lower levels of WDGS in the diet, compared with animals maintained on a 40% (40W40W) WDGS diet. Following the dietary switch, samples were collected on days 28 and 56 for year 1 and days 28, 42, and 56 for year 2 of the study. When the data for both years were pooled across sampling times and analyzed, animals fed 40% WDGS continuously (40W40W) had the highest overall fecal prevalence, whereas the animals fed 0% WDGS before or after the switch (0W0W) had the lowest overall fecal prevalence (70.1 versus 10.2%, $P < 0.05$; Fig. 3A). The pens of animals switched from high levels of WDGS to lower levels of WDGS in the diet (40W15W, 40W0W, and 70W15W diets) had lower fecal prevalence than did the pens maintained on 40% WDGS diet ($P < 0.05$). Compared with the pens fed corn continuously with the 0W0W diet, the pens switched to the lower levels of dietary WDGS numerically had higher fecal prevalence for *E. coli* O157:H7, but these differences were not statistically significant ($P > 0.05$). It should be noted that the fecal prevalence tended to be higher for 40W0W and 70W15W than for the 0W0W diet ($P < 0.1$).

Fecal samples were enumerated by direct plating immediately after sampling, and the percentage of animals with enumerable levels in the feces was determined for each pen. Similar to the fecal prevalence, the percentage of animals with enumerable levels in the feces was analyzed for each diet pooled across sampling times after the dietary switch (Fig. 3B). Animals fed the 0W0W diet had an average of 3.2% of enumerable fecal samples per pen, whereas the animals fed the 40W40W diet had an average of 29.9% enumerable fecal samples per pen ($P < 0.05$). The relative difference between the percentages of *E. coli* O157:H7 enumerable samples for the corn grain diet and for the 40% WDGS diet was similar to that reported previously (20). As shown in Figure 3B, the average pen fecal enumerable samples were lower in the pens of animals switched to lower levels of WDGS in the diet (40W15W, 40W0W, and 70W15W) than in the pens maintained on 40% WDGS with the 40W40W diet ($P < 0.05$). The pens of animals switched to lower levels of WDGS had average enumerable levels between 6.3 and 9.7%, and these values were not significantly different ($P > 0.05$) from those obtained from the animals fed corn continuously on the 0W0W diet.

Overall hide prevalence for *E. coli* O157:H7 was high (Fig. 1), as would be expected for the typical summer seasonal increase in prevalence (4, 20). Overall hide prevalence for *E. coli* O157:H7 after the dietary switch for samples pooled across all samplings ranged from 45.1 to 96.0% for the five dietary treatments (Fig. 4A). Animals fed the corn diet continuously with the 0W0W diet had a lower hide prevalence (45.1%) than that of the animals fed 40% WDGS continuously with the 40W40W diet (96.0%; $P <$

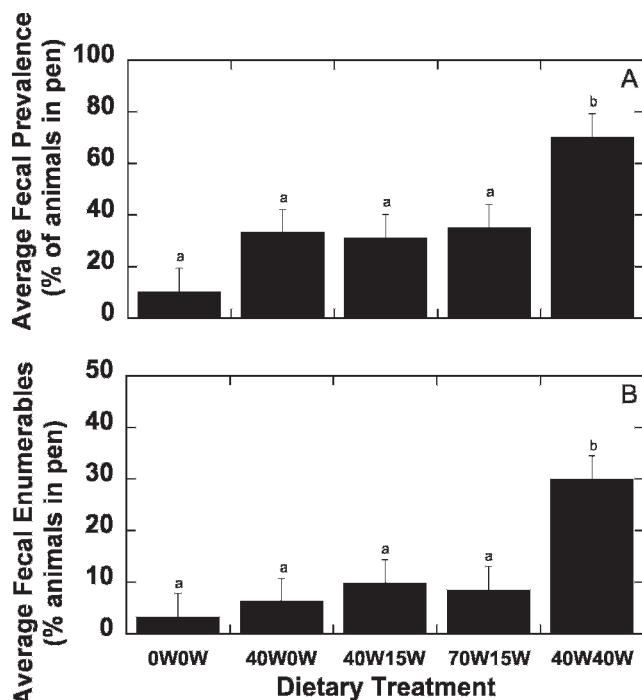


FIGURE 3. *E. coli* O157:H7 prevalence (A) and percent enumerable (B) in samples of feces for pens of finishing cattle fed treatment rations after dietary switch on day 0. Treatment diet 0W0W was a corn grain diet fed continuously during the finishing phase and serves as a negative control. Treatment diet 40W40W was a 40% WDGS diet fed continuously and serves as a positive control. Treatments 40W0W, 40W15W, and 70W15W were fed 40% (40W) or 70% (70W) WDGS before day 0 and 0% (0W) or 15% (15W) after day 0. Means are calculated from pooled samples across days 28 to 56 for each treatment, and bars on each sample point denote standard errors of the means for each treatment.

0.05). The animals switched to the lower levels of WDGS (40W15W, 40W0W, and 70W15W diets) had hide prevalence ranging from 68.0 to 74.7%, but these values were not significantly different ($P > 0.05$) from those seen in animals fed the corn (0W0W) or 40% WDGS (40W40W) diets. The percentage of hide samples with enumerable levels of *E. coli* O157:H7 was 3.7% for the pens of animals fed the 0W0W diet (Fig. 4B) and 34.6% for the pens of animals fed the 40W40W diet ($P < 0.05$). In contrast to the hide prevalence data, pens of animals switched to lower levels of WDGS (40W15W, 40W0W, and 70W15W diets) had significantly lower percentages for *E. coli* O157:H7 enumerable hide samples than did animals continuously fed 40% WDGS with the 40W40W diet ($P < 0.05$). These pens of animals switched to the lower amounts of WDGS had percent hide enumerable samples ranging from 4.3 to 9.0%, and these values, while numerically higher than those for the pens fed corn continuously with the 0W0W diet, were not significantly different, nor were tendencies observed ($P > 0.1$). Hides are an important source of *E. coli* O157:H7 transmission to the carcass at harvest (1, 2, 15), and significant reductions in hide enumerable counts at the feedlot may reduce the *E. coli* O157:H7 load at harvest (2, 3). Reducing WDGS levels to 15% or lower before slaughter may be beneficial in this respect.

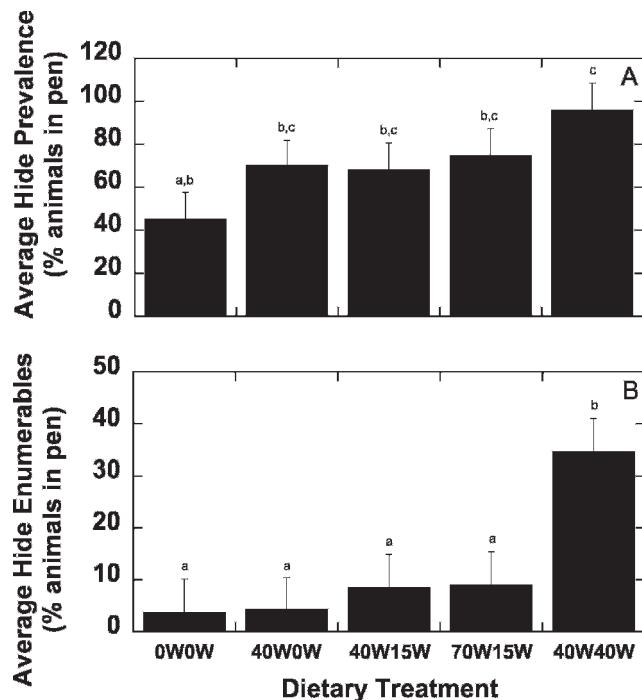


FIGURE 4. *E. coli* O157:H7 prevalence (A) and percent enumerable (B) in samples from hides for pens of finishing cattle fed treatment rations after dietary switch on day 0. Treatment diet 0W0W was a corn grain diet fed continuously during the finishing phase and serves as a negative control. Treatment diet 40W40W was a 40% WDGS diet fed continuously and serves as a positive control. Treatments 40W0W, 40W15W, and 70W15W were fed 40% (40W) or 70% (70W) WDGS before day 0 and 0% (0W) or 15% (15W) after day 0. Means are calculated from pooled samples across days 28 to 56 for each treatment, and bars on each sample point denote standard errors of the means for each treatment.

To determine the length of time necessary after the dietary switch from high WDGS to mostly corn grain before there is an effect on the *E. coli* O157:H7 load, data were analyzed individually by chi-square analyses for the samples collected on day 28 or 56. The effect of time after dietary switch can be seen in Figure 5, and it appears that the dietary switch to less WDGS in the diet had little effect on fecal *E. coli* O157:H7 prevalence or enumerables by day 28 compared with the 40W40W diet with 40% WDGS (Fig. 5A), and the fecal prevalences for animals on the 40W0W, 40W15W, and 70W15W diets were still higher than for the 0W0W diet ($P < 0.05$). However, by day 56, the pens switched to lower levels of dietary WDGS (40W0W, 40W15W, and 70W15W) had fecal prevalence similar to those of the pens fed the 0W0W diet, and animals on all of these diets showed significantly less prevalence than the animals fed the 40W40W diet. For samples collected on day 56, the percentages of enumerable samples for the animals switched to lower levels of WDGS were significantly lower than for the animals continuously fed 40% WDGS with the 40W40W, and all of these treatments showed equal to or less than the percentages of enumerable samples for the animals fed corn continuously with the 0W0W diet. In year 2 of the study, we collected data on day 42, and fecal *E. coli* O157:H7 prevalence and enumerables

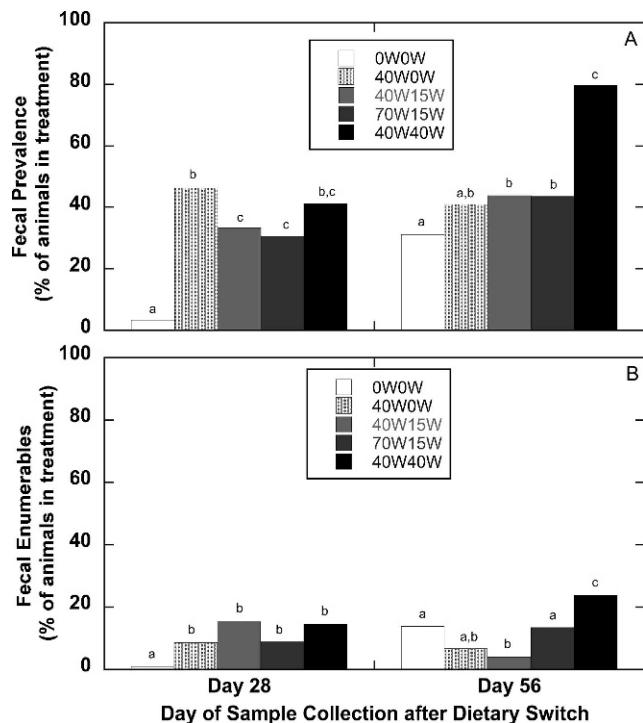


FIGURE 5. Average fecal prevalence of *E. coli* O157:H7 (A) and percentages of samples with enumerable levels for *E. coli* O157:H7 (B) on days 28 and 56 after the dietary switch. Treatment diet 0W0W was a corn grain diet fed continuously during the finishing phase and serves as a negative control. Treatment diet 40W40W was a 40% WDGS diet fed continuously and serves as a positive control. Treatments 40W0W, 40W15W, and 70W15W were fed 40% (40W) or 70% (70W) WDGS before day 0 and 0% (0W) or 15% (15W) after day 0. Means that are significantly different ($P < 0.05$) are denoted by different letters above the bars, as determined by chi-square analysis.

averaged 86.9 and 52.9%, respectively, for the 40W40W diet (data not shown); the animals switched to lower levels of WDGS with the 40W0W, 40W15W, and 70W15W diets had 80% or better reductions in prevalence and enumerables compared with the animals fed 40W40W ($P < 0.05$), indicating that possible benefits could be observed prior to 56 days after the dietary switch. Because we only have the day 42 data for year 2, we have reservations about making recommendations on a dietary switch without additional studies to validate a feeding period shorter than 56 days.

Hide prevalence and enumerable levels for *E. coli* O157 for days 28 and 56 are shown in Figure 6, and for each sample collection date, the hide prevalence for animals fed corn continuously (0W0W diet) was nearly 50% less than for animals fed 40% WDGS (40W40W) continuously ($P < 0.05$). Hide enumerables were also lower for samples from animals fed the 0W0W diet compared with the 40W40W diet on both day 28 and day 56 ($P < 0.05$). Compared with the animals fed the 40% WDGS diet continuously, by day 28 the dietary switch to lower levels of WDGS resulted in lower hide prevalence values for 40W0W, 40W15W, and 70W15W treatments ($P < 0.05$). On day 28, the percentages of hides with enumerable levels for 40W0W and 70W15W was significantly lower, and the percentage for the

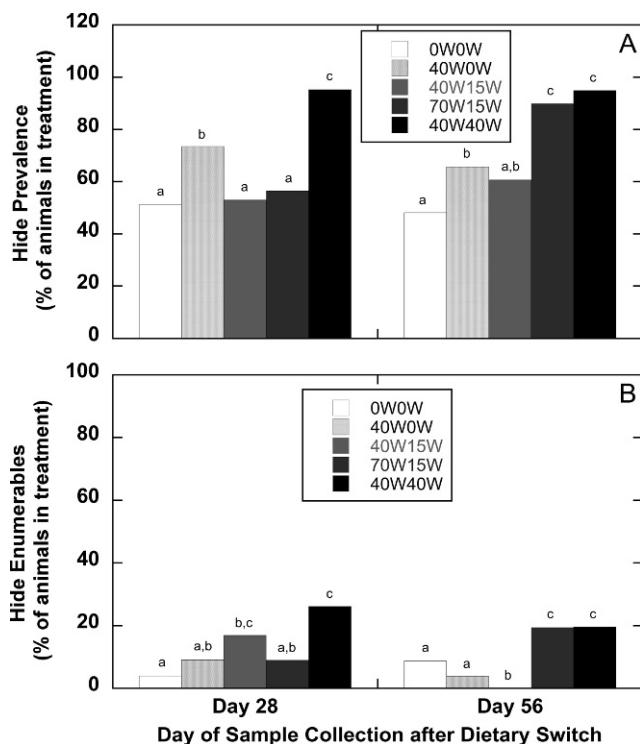


FIGURE 6. Average hide prevalence of *E. coli* O157:H7 (A) and percentages of samples with enumerable levels for *E. coli* O157:H7 (B) on days 28 and 56 after the dietary switch. Treatment diet 0WOW was a corn grain diet fed continuously during the finishing phase and serves as a negative control. Treatment diet 40W40W was a 40% WDGS diet fed continuously and serves as a positive control. Treatments 40WOW, 40W15W, and 70W15W were fed 40% (40W) or 70% (70W) WDGS before day 0 and 0% (0W) or 15% (15W) after day 0. Means that are significantly different ($P < 0.05$) are denoted by different letters above the bars, as determined by chi-square analysis.

40W15W treatment tended ($P < 0.1$) to be lower than that of 40W40W. By day 56, the dietary switch treatments 40WOW and 40W15W maintained lower hide prevalence and enumerable values than those observed with the 40W40W diet, but prevalence and enumerable levels for the 70W15W diet had increased. These results were similar to those of the 40W40W diet, even though the animals on 70W15W exhibited lower fecal prevalence and enumerable levels (Fig. 5). In year 2, we collected data for hide *E. coli* O157:H7 prevalence and enumerables on day 42, and in these samples both hide prevalence and enumerables were lower for all treatments groups of animals switched to lower levels of distillers grains compared with the animals fed 40% WDGS continuously ($P < 0.05$). Overall, switching to lower levels of WDGS in the diet appears to reduce the *E. coli* O157:H7 load on the hide before similar reductions on feces are observed in the feedlot. Although the hide is a significant source of transmission at harvest (1–3, 15), recontamination of the hide can occur during transport and lairage from the feces of shedding cattle.

Based on this and prior reports (13, 20), feeding high levels of WDGS (40 to 70%) to cattle significantly increases the fecal *E. coli* O157:H7 prevalence and percentage of animals shedding *E. coli* O157:H7 at enumerable levels

compared with feeding a corn grain diet without WDGS. Reducing WDGS to 0 or 15% for 56 days, but not 28 days, reduced fecal prevalence and enumeration, and with the exception of one treatment, hide prevalence and enumeration. However, there is little evidence to indicate a reduction in WDGS would rapidly reduce *E. coli* O157:H7 shedding. In support, Jacob et al. (13) removed distillers grains from cattle diets (40% reduced to 0%) and no differences in *E. coli* O157:H7 prevalence for fecal pats were observed in the 4-week study. Likewise, we did not observe a significant difference after WDGS was reduced or removed from the diet for 28 days, but we did observe a significant difference by 56 days. Collectively, the lack of a significant effect as a result of the dietary switch by day 28 suggests that the high *E. coli* O157:H7 load associated with feeding high levels of WDGS may be extrinsic to the gastrointestinal system of the animal. In a recent report by Berry et al. (7), when animals were moved to clean pens and in smaller groups, the fecal prevalence for *E. coli* O157:H7 was reduced nearly 50% by day 28 and 90% by day 56. The pen environment may play an important role in sustaining the prevalence, considering that previous publications have shown how *E. coli* O157:H7 can survive better in manures and feces from cattle fed less corn and more WDGS (17–20).

Based on the data presented here, feeding WDGS at levels of 40% and higher can increase the prevalence and levels of *E. coli* O157:H7 in feces and on hides. Switching animals to diets with 0 or 15% WDGS resulted in lower prevalence and levels of *E. coli* O157:H7 in the feces and on the hides after 56 days. Considering that WDGS is often a less expensive alternative to corn, switching animals fed high levels of WDGS to finishing diets with 15% WDGS prior to harvest may be an economically viable and effective food safety solution compared with eliminating WDGS from the diet altogether. From the results of this study, a dietary period of 56 days for feeding the lower levels of WDGS may be an acceptable strategy to reduce the *E. coli* O157:H7 load prior to shipping for harvest.

ACKNOWLEDGMENTS

We thank Dee Kucera, Shannon Ostdiek, Bruce Jasch, Sidney Bidleman, Frank Reno, Greg Smith, and Lisa Baker for their technical assistance, and Donna Griess for her secretarial assistance. We also thank Patty Beska, Kathy Mihm, and Pat Tammen for assistance in sample collection and the USMARC feedlot crew, in particular, Chad Engle, B. J. Johnson, Randy Scott, and Fred Ehrman, for their dedicated assistance in care, handling, and management of the animals over the 2 years of this study.

REFERENCES

- Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, D. A. King, S. D. Shackelford, T. L. Wheeler, and M. Koohmariae. 2008. Source tracking of *Escherichia coli* O157:H7 and *Salmonella* contamination in the lairage environment at commercial U.S. beef processing plants and identification of an effective intervention. *J. Food Prot.* 71:1752–1760.
- Arthur, T. M., D. M. Harhay, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmariae. 2010. Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. *Meat Sci.* 86:32–37.
- Arthur, T. M., J. E. Keen, J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, X. Nou, and M.

- Koohmaraie. 2009. Longitudinal study of *Escherichia coli* O157:H7 in a beef cattle feedlot and the role of high shedders in hide contamination. *Appl. Environ. Microbiol.* 75:6515–6523.
4. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
5. Barkocy-Gallagher, G. A., K. K. Edwards, X. Nou, J. M. Bosilevac, T. M. Arthur, S. D. Shackelford, and M. Koohmaraie. 2005. Methods for recovering *Escherichia coli* O157:H7 from cattle fecal, hide, and carcass samples: sensitivity and improvements. *J. Food Prot.* 68: 2264–2268.
6. Berry, E. D., and J. E. Wells. 2010. *Escherichia coli* O157:H7 recent advances in research on occurrence, transmission, and control in cattle and the production environment. *Adv. Food Nutr. Res.* 60:67–117.
7. Berry, E. D., J. E. Wells, T. M. Arthur, B. L. Woodbury, J. A. Nienaber, T. M. Brown-Brandl, and R. A. Eigenberg. 2010. Soil versus pond ash surfacing of feedlot pens: occurrence of *Escherichia coli* O157:H7 in cattle and persistence in manure. *J. Food Prot.* 73: 1269–1277.
8. Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and faecal samples using direct plating methods. *J. Appl. Microbiol.* 103:1657–1668.
9. Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. *J. Appl. Microbiol.* 87:867–876.
10. Jacob, M. E., T. R. Callaway, and T. G. Nagaraja. 2009. Dietary interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. *Foodborne Pathog. Dis.* 6:785–792.
11. Jacob, M. E., J. T. Fox, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2008. Effects of dried distillers' grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. *Appl. Environ. Microbiol.* 74:38–43.
12. Jacob, M. E., J. T. Fox, S. K. Narayanan, J. S. Drouillard, D. G. Renter, and T. G. Nagaraja. 2008. Effects of feeding wet corn distillers grains with solubles with or without monensin and tylosin on the prevalence and antimicrobial susceptibilities of fecal foodborne pathogenic and commensal bacteria in feedlot cattle. *J. Anim. Sci.* 86:1182–1190.
13. Jacob, M. E., Z. D. Paddock, D. G. Renter, K. F. Lechtenberg, and T. G. Nagaraja. 2010. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 76:7238–7242.
14. National Research Council. 1996. Nutrient requirements of beef cattle. National Academy Press, Washington, DC.
15. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
16. Uitenbroek, D. G. 1997. SISA-two by two table. Available at: <http://www.quantitativeskills.com/sisa/statistics/twoby2.htm>. Accessed 8 February 2011.
17. Varel, V. H., J. E. Wells, E. D. Berry, and D. N. Miller. 2010. Manure odor potential and *Escherichia coli* concentrations in manure slurries of feedlot steers fed 40% corn wet distillers grains. *J. Environ. Qual.* 39:1498–1506.
18. Varel, V. H., J. E. Wells, E. D. Berry, M. J. Spiehs, D. N. Miller, C. L. Ferrell, S. D. Shackelford, and M. Koohmaraie. 2008. Odorant production and persistence of *Escherichia coli* in manure slurries from cattle fed 0, 20, 40, or 60% wet distillers grains with solubles. *J. Anim. Sci.* 86:3617–3627.
19. Wells, J. E., E. D. Berry, and V. H. Varel. 2005. Effects of common forage phenolic acids on *Escherichia coli* O157:H7 viability in bovine feces. *Appl. Environ. Microbiol.* 71:7974–7979.
20. Wells, J. E., S. D. Shackelford, E. D. Berry, N. Kalchayanand, M. N. Guerini, V. H. Varel, T. M. Arthur, J. M. Bosilevac, H. C. Freely, T. L. Wheeler, C. L. Ferrell, and M. Koohmaraie. 2009. Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with solubles. *J. Food Prot.* 72:1624–1633.