Feeding Crude Glycerin Decreases Fecal Shedding of *E. coli* O157:H7 in Growing Cattle

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Introduction

Crude glycerin is a byproduct of ethanol production and is used as a carbohydrate source for cattle feed. Glycerin levels in previous studies have ranged from 0 to 20% of diet dry matter, and concentrations of 8% or less generally improve feedlot performance. At even low levels of glycerin, however, the activity of cellulolytic bacteria is depressed, ultimately leading to poorer fiber digestion. This observation suggests that glycerin may affect a specific population of bacteria in the gut. Crude glycerin can account for 8 to 10% of the weight of dried distillers grains with solubles, because it is one of the primary end-products when yeast ferments sugars to produce ethanol. Addition of 25% dried distillers grains with solubles to a feedlot diet increased the prevalence of *Escherichia coli* O157:H7 in feces of cattle. These observations led us to question whether glycerin might be the component of distillers grains responsible for the increases in prevalence of *E. coli* O157:H7 that often are observed in cattle fed distillers grains. To address this question, we added glycerin to diets of growing cattle and subsequently evaluated fecal shedding of *E. coli* O157:H7.

Experimental Procedures

We added three levels of crude glycerin, 0, 4, or 8%, to growing diets containing dry-rolled corn, corn silage, alfalfa hay, and corn steep liquor (Table 1). We formulated all diets so they would be isonitrogenous.

Each treatment was represented by 16 pens, each containing 7 to 8 heifers. We obtained fecal samples by fecal grab at the chute once a week for 6 weeks. Fecal samples were kept on ice until analysis. We weighted approximately 1 gram of feces and placed it in 9 mL Gram Negative broth (Difco, Inc., Corpus Christi, TX) with cefixime (0.05 mg/L), cefsulodin (10 mg/L), and vancomycin (8mg/L; GNccv) for a 6-hour incubation at 104°F. We added 1 mL of GNccv to a sterile tube containing 20 µL of *E. coli* O157-specific beads and subjected it to immunomagnetic separation. We then resuspended the resulting *E. coli* O157 beads in 100 µL of phosphate buffer and plated them onto a selective agar for *E. coli* O157:H7 for an overnight incubation at 98°F. After incubation, we picked up to 6 non-sorbitol fermenting colonies and tested them for indole production. We further analyzed indole-positive colonies using an O157 antigen agglutination kit. We considered colonies positive for agglutination and indole production as *E. coli* O157:H7.

Results and Discussion

The crude glycerin used in this experiment contained 81.5% glycerol. After statistical analysis, we concluded that no interaction occurred between sampling date and the levels of crude glycerin ($P > 0.2$). Sampling date, however, did have an effect ($P < 0.01$), as shown in Figure 1. For the first 2 weeks, percentages of samples that tested positive for *E. coli* O157:H7 were 1.3 and 0.8%, respectively. The prevalence then increased to

a peak of 8.8% during the fourth week, then stabilized around 4.3 to 5.8% during the final 2 weeks of the experiment. We also observed an effect of glycerin inclusion levels ($P < 0.01$; Figure 2). Fecal incidence rates of *E. coli* O157:H7 were 5.8, 4.3, and 2.4% for heifers fed 0, 4, and 8% glycerin, respectively. The prevalence we observed in heifers fed 4% glycerin tended to differ from that of cattle fed 8% glycerin ($P = 0.06$), but was not different from that of cattle fed the diet with 0% glycerin. Glycerin previously has been shown to inhibit the activity of cellulolytic bacteria in the rumen. Consequently, changes in fecal prevalence of *E. coli* O157:H7 observed in this study might be explained by alterations in gastrointestinal flora, with higher levels of glycerin producing a less favorable environment for the proliferation of pathogenic *E. coli* O157:H7.

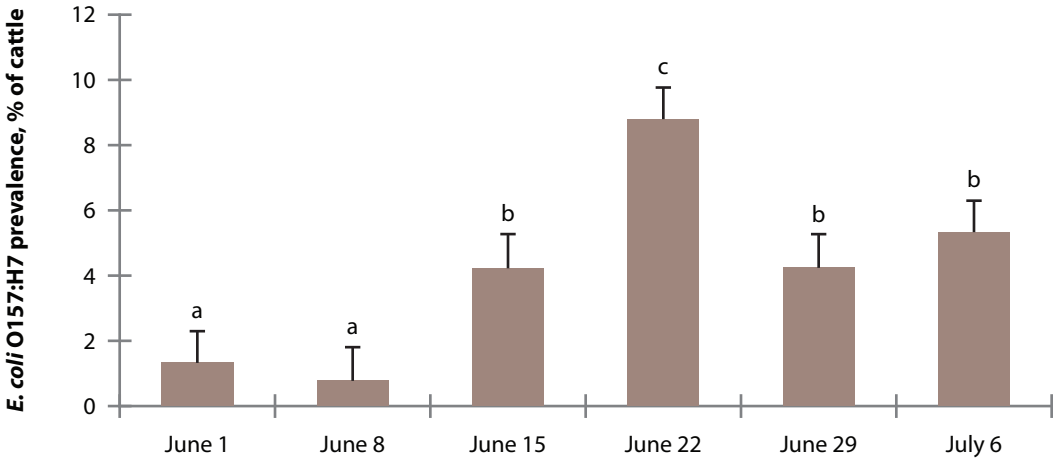
Implications

Our goal in this study was to determine if feeding glycerin would affect shedding of *E. coli* O157:H7 in cattle feces. Our results demonstrated that increasing levels of crude glycerin decreased the prevalence of *E. coli* O157:H7, and that this could be a useful pre-harvest strategy for controlling the shedding of pathogenic *E. coli* in cattle.

Table 1. Composition of experimental diets (dry basis)

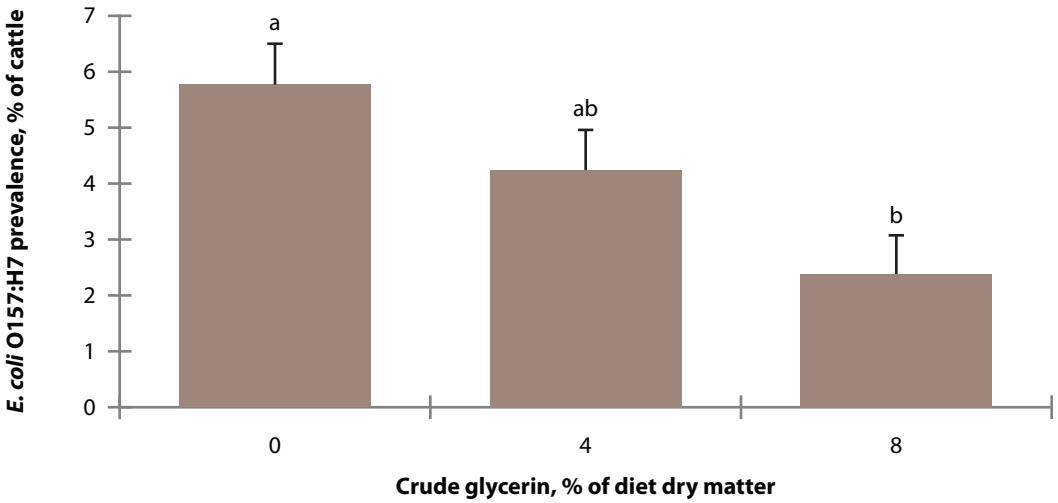
Ingredients, %	0% glycerin	4% glycerin	8% glycerin
Corn silage	60	60	60
Wet corn gluten feed	35	30.2	25.4
Crude glycerin	-	4	8
Soybean meal	-	0.8	1.6
Limestone	1.6	1.6	1.6
Urea	0.4	0.4	0.4
Vitamin/mineral premix[1]	0.3	0.3	0.3
Feed additive premix[2]	2.7	2.7	2.7
Nutrient composition			
Dry matter, %	43.3	43.7	44.2
Crude protein, %	13.0	12.5	12.1
Neutral detergent fiber, %	36.2	34.7	33.2
Calcium, %	0.75	0.75	0.75
Phosphorus, %	0.51	0.47	0.42

[1] Formulated to provide (dry basis) 0.1 ppm cobalt, 10 ppm copper, 0.6 ppm iodine, 60 ppm manganese, 0.25 ppm selenium, 60 ppm zinc, 0.3% salt, 1,000 IU/lb vitamin A in the total diet.
[2] Provided 300 mg of Rumensin (Elanco Animal Health, Greenfield, IN) per heifer daily in a ground corn carrier.



[abc] Means without a common superscript differ ($P < 0.05$).

Figure 1. *E. coli* O157:H7 prevalence in growing cattle fed crude glycerin.



[ab] Means without a common superscript differ ($P < 0.05$).

Figure 2. Effect of crude glycerin levels fed to growing cattle on *E. coli* O157:H7 prevalence.