Effect of Crude Glycerin on Fecal Shedding of *Escherichia coli* O157 in Growing and Finishing Cattle

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ABSTRACT

Two experiments were conducted to evaluate the effects of crude glycerin feeding on *E. coli* O157 prevalence in feces of growing and finishing cattle. In study 1, crude glycerin was included at 0, 4 or 8% of dry matter in growing cattle diets comprised of 62.5% corn silage and 33, 28, or 24% wet corn gluten feed. Heifers (n = 368; initial body weight = 234 ± 3.2 kg) were housed in partially covered, concrete-surfaced pens with seven to eight animals per pen and 16 pens per treatment for the 90-day experiment. Study 2 was a finishing experiment with a 2 x 2 factorial treatment arrangement. Factor 1 consisted of the level of crude glycerin (0 or 2% of dry matter) and factor 2 was the presence or absence of a combination of 25% soybean hulls and 15% wet distiller's grains (WDG) as grain substitutes. Heifers (n = 232; initial body weight = 427 ± 8.8 kg) were housed in concrete-surfaced pens with seven to eight animals per pen and eight pens per treatment. Fresh fecal samples were taken from growing and finishing animals via rectal palpation. One gram of feces was incubated for 6 hr at 40ºC in Gram negative broth with cefixime (0.05 mg/L), cefsulodin (10 mg/L), and vancomycin (8 mg/L), and then subjected to immunomagnetic separation with *E. coli* O157 beads. Recovered beads were plated onto MacConkey agar with sorbitol, cefixime, and tellurite (CT-SMAC), and non-sorbitol fermenting colonies were tested for indole production and O157 antigen agglutination. Positive colonies for both tests were confirmed as *E. coli* using the API 20E kit. Fecal prevalence rates of *E. coli* O157 were affected by sampling day in the growing study ($P < 0.01$) but not in the finishing study ($P > 0.1$). Increasing levels of crude glycerin decreased incidence of *E. coli* O157 in growing cattle (linear effect, $P < 0.01$; 4.4, 3.2, and 1.8% for heifers fed 0, 4, and 8% glycerin, respectively), but had no significant effect in finishing cattle ($P > 0.05$) despite a strong tendency for a decrease in finishing cattle receiving corn-based diets ($P = 0.059$). There was no interaction between WDG inclusion and

glycerin level (P > 0.1) and no WDG effect on prevalence of *E. coli* O157 in finishing cattle (P > 0.1). Glycerin may be useful as a means for decreasing fecal prevalence of *E. coli* O157 in cattle, but effects may depend on the type of diet that is fed.

INTRODUCTION

Expansion of biofuels production has diverted cereal grains from livestock feed to energy feedstock, but, on the other hand, has yielded large quantities of co-products that can be used as animal feeds. Ethanol production generates wet or dried distiller's grains, as byproducts, which commonly constitute 10 to 50% of cattle diets in the United States, whilst maintaining acceptable animal performance and meat quality (NASS, 2007). Feeding distiller's grains has, however, been shown to increase prevalence of *E. coli* O157:H7 in cattle feces (Wells et al., 2009, Jacob et al., 2010, Wells et al., 2011), and its persistence in manure (Varel et al., 2008, Varel et al., 2010).

It has been suggested that changes in the hindgut environment create a more favorable environment for *E. coli* O157 (Berg et al., 2004, Fox et al., 2007, Klopfenstein et al., 2008), or that a compound in distiller's grain creates a selective niche for the foodborne pathogen (Dewell et al., 2005, Jacob et al., 2008, Viazis and Diez-Gonzalez, 2011). Analysis of distiller's grains has revealed the presence of 3.5 to 10% glycerin, which is formed by yeast during fermentation of carbohydrates (Wu, 1994; Kingsly et al., 2010). Crude glycerin is, also, a byproduct of the biodiesel industry, and is frequently fed to animals as an alternative source of energy (Hampy et al., 2008, Parsons et al., 2009, Gunn et al., 2010).

Glycerin has been shown to influence microbial populations in ruminants (Roger et al., 1992, Paggi et al., 2004, AbuGhazaleh et al., 2011), which is why we chose to focus on the effects of glycerin on *E. coli* O157 prevalence in cattle feces, and to determine if the impact of glycerin feeding would be influenced by feeding distiller's grains. For this purpose, we conducted two experiments: one in growing cattle fed diets containing 0, 4, or, 8 % of glycerin, and another in finishing cattle fed diets with and without added distiller's grains and with 0 or 2% crude glycerin.

MATERIALS AND METHODS

Animals and Experimental Designs

Procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

Study

Crossbred heifers (n = 368; initial body weight 234 ± 3.2 kg) were randomly assigned, at receiving, to growing diets containing corn silage, wet gluten feed, and one of three levels of crude glycerin: 0, 4 or 8% of diet dry matter (Table 1). Crude glycerin was added to the diet at the expense of wet gluten feed and in conjunction with soybean meal to keep diets isonitrogenous. Diets were fed once daily ad libitum. Each treatment was represented by 16 pens (36.5 m2) of cattle, each containing seven to eight animals. Approximately half of each pen, including the feed bunk, was covered by a corrugated steel roof.

Pens were equipped with fenceline feed bunks (3.65 linear m), and a fenceline water fountain was shared between two adjacent pens. Fecal samples were obtained from each animal in each pen by rectal palpation once each week for 6 weeks, over the months of June and July 2010, on days 55, 62, 69, 76, 83, and 90 after beginning of the treatment.

Study 2

Crossbred heifers (n = 232; initial body weight 427 ± 8.8 kg) were stratified by weight and randomly assigned (within strata) to the previously described pens, using a total of 32 pens containing seven to eight animals each. The study utilized a 2 x 2 factorial treatment arrangement, with factor 1 consisting of the level of crude glycerin added to the diet (0 or 2%, dry matter basis), and factor 2 consisting of type of basal diet (corn or corn plus soybean hulls and wet distiller's grains; Table 2). Pens within block

Table 1: *Composition of growing experimental diets (dry basis)*

	0% Glycerin	4% Glycerin	8% Glycerin
Ingredients, %			
Corn silage	62.50	62.50	62.50
Wet corn gluten feed	32.90	28.30	23.90
Crude glycerin[1]	-	3.70	7.50
Soybean meal	-	1.60	3.10
Limestone	0.50	0.50	0.50
Urea	0.70	0.90	0.90
Vitamin/mineral premix[2]	1.60	1.40	-
Feed additive premix[3]	2.50	2.50	2.50
Nutrient Composition, %			
Dry matter	43.30	43.70	44.20
Crude protein	12.80	12.50	11.90
Neutral detergent fiber	36.20	34.60	33.00
Crude fat	2.57	2.45	2.34
Calcium	1.00	1.10	1.10
Phosphorus	0.66	0.61	0.56
Glycerol	0.06	3.41	6.86

[1]Contained 14.3% moisture, 6.68% ash, 2.58% Na, 0.04% N, and less than 0.01% methanol.
[2]Formulated to provide 0.1 mg Co, 10 mg Cu, 0.6 mg I, 60 mg Mn, 0.25 mg Se, 60 mg Zn, and 2640 IU vitamin A per kg diet DM.
[3]Provided 300 mg of Rumensin (Elanco Animal Health, Greenfield, In) per animal daily in a ground corn carrier.

were randomly assigned to treatment, thus providing eight replicates per treatment. Cattle were fed once daily ad libitum. Heifers were given 8.33 mg Zilpaterol HCl (Intervet Inc., Millsboro, DE)/kg of diet for 21 d before harvest followed by a 3-d withdrawal period. Fecal samples were collected, during the month of August 2009, on days 160, 164, 168, 172, 176, 180 and 184 after beginning of the treatment. On each sampling day, fresh fecal pats were obtained from 5 randomly selected heifers in each pen.

Crude Glycerin Analysis

Crude glycerin used in the diets was analyzed by a commercial laboratory (SDK laboratories, Hutchinson, KS) for moisture using a Karl Fischer titration (method 966.2; AOAC, 1995), ash (method 942.05; AOAC, 1995), Na (method 956.01; AOAC, 1995), N (method 920.176; AOAC, 1995), and methanol (method 973.23; AOAC, 1995). Crude glycerin contained 14.3% moisture, 6.68% ash, 2.58% Na, 0.04% N, and less than 0.01% methanol. Composite samples of wet distiller's grains and corn silage also were analyzed by the commercial laboratory by HPLC for glycerol content (method 982.22; AOAC, 1995) and contained 7.2% and less than 0.1% of glycerol on a DM basis, respectively.

***Escherichia coli* O157 Isolation**

Samples were placed into plastic bags and stored on ice immediately after sampling, and rapidly transported 3 km to the Preharvest Food Safety Laboratory for analysis. Feces (1 g) were weighed and transferred to 9-mL of Gram negative broth (Difco, Franklin Lakes, NJ) containing 0.05 mg/L cefixime, 10 mg/L cefsulodin, and 8 mg/L vancomycin (GNccv) for a 6-hour incubation at 40°C. After incubation, samples from study 1 were thoroughly vortexed, and

Table 2: *Composition of finishing grain-based diets and byproduct-based diets with or without 2% crude glycerine (dry basis)*

	Grain-based Diets		Byproduct-based Diets	
	0% Glycerin	**2% Glycerin**	**0% Glycerin**	**2% Glycerin**
Ingredients, %				
Corn	80.60	78.20	46.60	44.20
Soybean hulls	-	-	25.00	25.00
Wet distiller's grains	-	-	15.00	15.00
Corn silage	6.00	6.00	6.00	6.00
Soybean meal	4.40	4.80	-	0.40
Alfalfa hay	3.00	3.00	3.00	3.00
Crude glycerin[1]	-	2.00	-	2.00
Limestone	1.70	1.70	1.40	1.40
Urea	1.20	1.20	0.40	0.40
Vitamin/mineral premix[2]	0.90	0.90	0.40	0.40
Feed additive premix[3]	2.20	2.20	2.20	2.20
Nutrient Composition, %				
Dry matter	76.10	76.30	64.60	64.70
Crude protein	14.80	14.80	14.70	14.70
Neutral detergent fiber[4]	11.90	11.80	28.90	28.80
Crude fat[5]	2.50	2.50	6.60	6.60
Calcium	0.70	0.70	0.90	0.90
Phosphorus	0.30	0.30	0.30	0.30
Glycerol	0.01	1.82	1.09	2.90

[1]Contained 14.3% moisture, 6.68% ash, 2.58% Na, 0.04% N, and less than 0.01% methanol
[2]Formulated to provide 0.1 mg Co, 10 mg Cu, 0.6 mg I, 60 mg Mn, 0.25 mg Se, 60 mg Zn, 2640 IU vitamin A, and 11 IU vitamin E per kg diet DM.
[3]Feed additive premix provided 300 mg of monensin (Elanco Animal Health, Greenfield, IN), 90 mg tylosin (Elanco), and 0.5 mg of melengestrol acetate (Pfizer Animal Health, Exton, PA) per animal daily in a ground corn carrier. Zilpaterol HCl (Intervet Inc., Millsboro, DE) was fed for 21 d before harvest at the rate of 8.33 mg/kg of diet DM, followed by a 3-d withdrawal period.
[4]NRC (2000) feed library NDF values for soybean meal were used in calculation of NDF content.
[5]NRC (2000) feed library fat values for soybean meal and soybean hulls were used in calculation of crude fat content.

1 mL of the inoculated GNccv broth was added to a sterile tube containing *E. coli* O157 specific Dynabeads (Invitrogen Dynal AS, Oslo, Norway), and subjected to immunomagnetic separation according to manufacturer's instructions. GNccv tubes from study 2 were pooled by pen after incubation (5 tubes/pen) and subjected to immunomagnetic separation using a Pathatrix device (Matrix Microscience, Life Technologies, Grand Island, NY). *Escherichia coli* O157 beads resulting from both immunomagnetic separations were resuspended in 100 µL of phosphate buffer and plated onto two MacConkey sorbitol plates containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L; CT-SMAC). Up to six non-sorbitol fermenting colonies were selected from the CT-SMAC plate and inoculated into 5 mL Tryptic soy broth (TSB). Colonies were grown overnight at 37°C and tested for indole production. Indole-positive colonies

were plated onto SMAC and further tested for O157 antigen agglutination (Oxoid, Hampshire, United Kingdom). Colonies positive for indole production and antigen agglutination were confirmed as *E. coli* by Gram staining and API 20E (Biomerieux, Durham, NC).

Statistical Analysis

Effect of glycerin inclusion on prevalence of *E. coli* O157 in study 1 was analyzed as binary data using Proc Glimmix of SAS (SAS Inst. Inc., Cary, NC). Levels of glycerin and sampling day were included in the model as fixed effects, while animal and pen were random effects. Linear and quadratic contrasts were analyzed for the different levels of glycerin. In study 2, effect of sampling day, glycerin, WDG and the combination of glycerin and WDG inclusion on prevalence of *E. coli* O157 were analyzed using Proc Glimmix. Glycerin levels, byproduct inclusion and sampling day were the fixed effects. A weight stratum was the blocking factor and pen the experimental unit. Statistical significance was considered for P-value of less than 0.05 and tendencies for P-value of less than 0.1.

RESULTS

Study 1

Animal performance data, average daily gain (ADG; $P = 0.767$), and dry matter intake (DMI; $P = 0.166$) were not affected by the inclusion of glycerin in the diets, although heifers were more efficient ($P < 0.011$) when fed glycerin (data not shown). There were no interactions between sampling day and glycerin levels ($P > 0.1$) on *E. coli* O157 prevalence. Therefore, only main effects are presented. Prevalence of *E. coli* O157 in fecal samples was influenced by sampling day ($P < 0.01$). Figure 1 represents shedding of the foodborne pathogen over the experimental period. Foodborne pathogen prevalence was low for the first 2 weeks, 1.3 and 0.8%, respectively. Prevalence then increased to 4.0% in the third week ($P < 0.021$), peaking at 8.4% during the fourth week ($P < 0.014$), and decreasing to 4.0% on the fifth week ($P < 0.014$), to stabilize at 5.0% during the final week of sampling ($P = 0.07$). Figure 2 illustrates the effect of glycerin on *E. coli* O157 prevalence. Fecal prevalence rates of *E. coli* O157 were 4.4, 3.2, and 1.8% for heifers fed 0, 4, and 8% glycerin, respectively (linear effect of glycerin, $P < 0.01$). The prevalence observed in heifers fed 8% glycerin was less than that of cattle fed 0% glycerin ($P < 0.05$), while prevalence in cattle fed 4% glycerin was not different from other treatments ($P > 0.18$).

Study 2

Animal performance data showed no interaction between glycerin levels and WDG inclusion (data not shown). There was no difference in DMI with inclusion of glycerin in diets ($P > 0.05$), but an increase in DMI in animals receiving diets containing WDG when compared to the one receiving diets containing no WDG ($P < 0.01$). ADG and G:F were not influenced by the inclusion of glycerin and/or WDG. E coli O157 prevalence in study 2 showed no overall sampling day effect ($P > 0.1$). *E. coli* O157 fecal shedding, illustrated in Figure 3, was highest at the first sampling (23%), d 160, but rapidly decreased to 8.2% on day 164 and 168 ($P > 0.1$). Prevalence reached its lowest point on day 172 (2.7%), which was significantly different from day 160 ($P < 0.04$). On day 176, *E. coli* O157 shedding again increased to 16.9%, which tended to be different from the previous sampling date ($P < 0.08$). For the last two sampling days, foodborne pathogen prevalence again declined to reach 2.7 and 8.2%, respectively.

Presence of a high number of *E. coli* O157 negative samples in this experiment did not allow us to statistically characterize the sampling day by glycerin level by WDG inclusion interactions. Analysis of the combination of glycerin inclusion and presence of WDG in the diet was, however, possible, and revealed the absence of an interaction ($P > 0.1$). WDG inclusion alone (Figure 4) had no effect on prevalence of *E. coli* O157 ($P > 0.1$).

Heifers fed diets without glycerin either based on corn or corn with soybean hulls

Figure 1: *E. coli O157 prevalence in feces of growing cattle at each sampling days. Letters above the bars represent the comparison among sampling days. Bars with different superscript are significantly different (P < 0.05). Sampling day effect, P < 0.001. SEM = 0.01.*

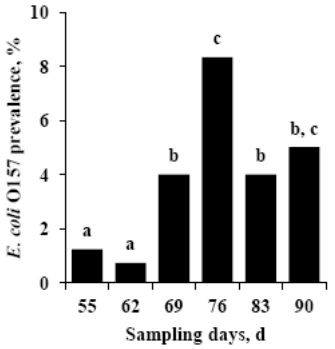


Figure 2: *E. coli O157 prevalence in feces of cattle fed growing diets containing 0, 4, and 8% crude glycerin. Letters above bars represent the comparison among glycerin levels. Bars with different superscripts are significantly different (P < 0.01). Glycerin level effect, P = 0.0052. Linear effect of glycerin level, P = 0.0012. SEM = 0.008.*

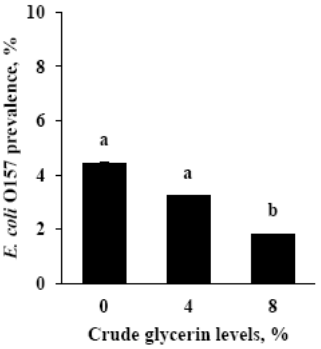


Figure 3: *E. coli O157 prevalence in feces of finishing cattle at each sampling day. Letters above bars represent comparisons among means between sampling days. Bars with different superscript are significantly different (P < 0.05). Sampling day effect, P = 0.1070. SEM = 0.08.*

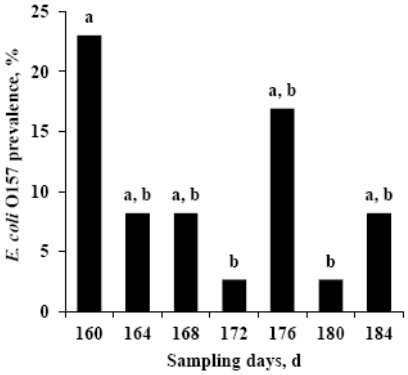
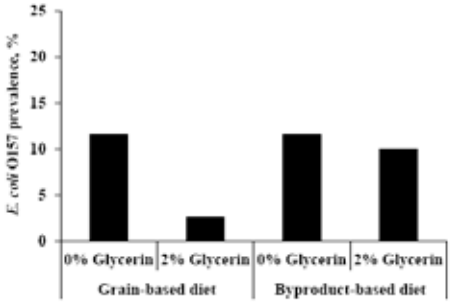


Figure 4: *E. coli O157 prevalence in feces of cattle fed a finishing diets based on grain or byproduct with or without 2% glycerin over the 24-day period. Byproduct inclusion effect, P > 0.1; Glycerin inclusion effect, P > 0.08; Glycerin inclusion*Byproduct inclusion interaction, P > 0.1. SEM = 0.041.*



and WDG presented 11.64% *E. coli* O157 positive isolates over the course of the experiment. Heifers fed corn diets with 2% glycerin had a 2.7% foodborne pathogen prevalence and animals fed corn with soybean hulls and WDG diets with 2% glycerin had a 10% prevalence; but the difference between these two treatments only tended to be significant (P = 0.096). There was a tendency for glycerin inclusion in the diet to decrease foodborne pathogen prevalence (P = 0.087), and foodborne pathogen prevalence, also, tended to differ between animals fed corn diets with and without glycerin (P = 0.059), with 2.7% and 11.6% prevalence, respectively. In diets containing soybean hulls and WDGS the difference in prevalence with the addition of 2% glycerin was not significant (10 vs. 11.6%, respectively; P > 0.10).

DISCUSSION

Increases in prevalence of *E. coli* O157 have frequently been associated with addition of distiller's grains, wet or dried, in feedlot diets (Dewell et al., 2005, Jacob et al., 2010). Nevertheless, explanations for this interaction are still unclear. It was first thought that replacement of part of the corn in the diet by dried distiller's grains with soluble (DDGS) or wet distiller's grains with soluble (WDGS) modified the amount of starch reaching the hindgut (Jacob et al., 2010), and in turn, increased pH and created a more hospitable environment for foodborne pathogens (Berg et al., 2004, Fox et al., 2007). In vitro and in vivo studies have failed to establish a clear relationship among pH, diets, and *E. coli* O157 prevalence (Depenbusch et al., 2009, Wells et al., 2009, Yang et al., 2010).

Similarly, it has been hypothesized that differences in concentrations of VFA and branched amino acids might explain changes in foodborne pathogen prevalence. Distiller's byproducts contain much higher levels of protein compared to corn (Klopfenstein et al., 2008), and only a portion of this protein is degraded in the rumen. Consequently, greater supplies of protein reach the hindgut, potentially yielding increased levels of VFA and branched amino acids that could influence foodborne pathogen survival. Definitive evidence supporting this hypothesis is, however, lacking (Huntington et al., 2006).

Concentration of L-lactate has also been proposed as a contributing factor, recognizing that L-lactate has antimicrobial properties (McWilliam Leitch and Stewart, 2002) and its rarefaction could benefit foodborne pathogen persistence in manure. Inclusion of WDGS in cattle diets has been shown to decrease significantly L-lactate concentration in feces compared to cattle fed corn (Varel et al., 2008, Wells et al., 2009). However, these concentrations, 3.72 mM on average, are far below the 50 to 200 mM concentrations that have been identified as having inhibitory effects, and cannot solely explain increased prevalence of *E. coli* O157 in feces. Other authors (Dewell et al., 2005, Jacob et al., 2008) suggested the presence of a compound in distiller's grains that would benefit, directly or indirectly, the foodborne pathogen. Previous publications have reported distiller's grains to contain between 3.5 and 10% glycerin on DM basis (Wu, 1994, Kingsly et al., 2010).

Glycerin is formed during yeast fermentation in the early stage of ethanol production. Its concentration in the final byproduct depends on ethanol process, grain type, yeast type, and treatment of distiller's grain used. In our experiment WDG consumed by finishing heifers contained 7.2% glycerol, thus contributing 1% glycerol to the total diet. Feedlot diets with higher inclusion levels of byproducts could contain significant amount of glycerin.

We hypothesized that glycerin would increase shedding of *E. coli* O157 in feces of cattle fed distiller's grains. Based on present results, inclusion of glycerin, in presence or absence of byproduct in the diet, did not increase foodborne pathogen prevalence. Addition of glycerin to growing diets significantly decreased *E. coli* O157 shedding, and numerically decreased it in grain-based finishing diets without altering animal performances. It is important to

acknowledge that in the growing study, we cannot dissociate effect of glycerin inclusion and removal of wet corn gluten feed on prevalence of *E. coli* O157. Based on these findings, glycerin could be a potential way to mitigate foodborne pathogen prevalence. Explanation of the mechanism behind *E. coli* O157 prevalence reduction is, however, not yet understood.

Glycerin is known to reduce cellulolytic activity in the rumen (Roger et al., 1992, Paggi et al., 2004). One percent excess glycerol inhibited growth and activity of Ruminococcus flavefaciens and Fibrobacter succinogens in an in vitro assay (Roger et al., 1992). Likewise, replacing 30% of the corn by glycerin, in a continuous fermenter, reduced DNA concentrations of Butyrivibrio fibrisolvens by preventing its attachment to fiber (AbuGhazaleh et al., 2011). Feeding studies corroborate with these observations, as apparent digestibility of NDF (Donkin et al., 2009, Parsons and Drouillard, 2010) was decreased by increasing amount of glycerin incorporated into cattle diets. In addition, glycerol was found to decrease proteolytic activity by 20% when added to culture media at concentration of 50 mM or more (Paggi et al., 1999). Bacterial protein synthesis was decreased by infusing glycerol into the rumen of bulls (Kijora et al., 1998). Overall, effects of glycerin on fiber and protein digestion within the rumen could be responsible for eliciting changes in nutrients flowing to the hindgut of animals, thus creating conditions that are less favorable for the foodborne pathogen. It could, as well, alter conditions within the gut to increase populations that effectively compete with *E. coli* O157.

Many microorganisms are able to use glycerol as a substrate, but only few of them can do so under anaerobic conditions. In the rumen, Selenomonas is one of the key glycerol fermenters, producing propionate, lactate, succinate and acetate (Krehbiel, 2008). Increase of glycerol supply to the rumen could potentially benefit Selenomonas and have a negative effect on *Escherichia coli*. Additionally, decline in rumen pH observed in vivo and in vitro with increasing levels of glycerin (Kijora et al., 1998, Lee et al., 2011) could be another factor explaining the reduction in *E. coli* O157 shedding. Effects of glycerin on pH have not, however, been consistent, and some experiments have failed to illustrate an effect of glycerol on pH (Rico et al., 2012) or, in contrary, observed an increase in pH (Parsons and Drouillard, 2010). Discrepancies in results may be explained in part by differences in basal diets or glycerol levels used among studies. Therefore, the relationship between *E. coli* O157 prevalence reduction by glycerin and pH is not definite and would need more attention.

Despite the mechanisms involved in reduction of foodborne pathogen shedding, addition of glycerin efficiently reduced percentage of feces positive for *E. coli* O157 in finishing animals fed the grain-based diet. We speculate that numerical differences observed in *E. coli* O157 prevalence in grain-based diets with and without glycerin might have been significant with higher rates of inclusion, as a linear effect of glycerin was observed in our first study. The absence of an effect of glycerin in cattle fed diets containing byproducts tempers the potential to use glycerin as a mitigating agent. Comparison of 2% glycerin grain-based diet and the 2% glycerin byproduct-based diet underscore potentially important differences in fiber content, 11.8 and 28.8% respectively, and fat content, 2.5 and 6.6%, respectively.

It is conceivable that glycerin effects are different for microflora from an animal fed a typical corn-based diet compared to cattle fed diets with greater concentrations of fiber and fat. Finally, in our finishing experiment, we did not observe any difference in shedding of *E. coli* O157 in animals fed 0 or 15% WDG with soybean hulls, which differs from previous findings (Dewell et al., 2005; Wells et al., 2009; Jacob et al., 2010), and might be explained by the variability in nutrients content between various distiller's grains (Kleinschmit et al., 2007).

Glycerol content of the byproduct used in previous studies have rarely been reported, it is possible that the presence of 1.09% of glycerin in the initial byproduct-based diet used in this experiment might have affected the prevalence of *E. coli* O157, inhibiting its increase. The subsequent addition of 2% glycerin in the diet may not have been sufficient to lead to a significant difference in *E. coli* O157 prevalence, but did induce a numerical decrease. Taken together that glycerin was not responsible for increased prevalence of *E. coli* O157 in animal fed distiller's grains and that WDG did not have an effect on foodborne pathogen prevalence, it is probable that the compound allegedly benefiting *E. coli* O157 was absent or in too low concentration in the WDG used in this study. It is also possible that WDG level of inclusion in our study (15%) were too low to significantly impact *E. coli* O157 prevalence. Studies testing increasing level of WDGS inclusion often showed no significant difference in prevalence between control group and animals receiving less than 40% WDGS (Klopfenstein et al., 2009; Jacob et al., 2010; Wells et al., 2011).

In conclusion, glycerin inclusion ranging from 2 to 8% decreased the shedding of *E. coli* O157 in feces of growing heifers, and tended to do so in finishing heifers. Based on our results, we can conclude that glycerin is unlikely to be the component of distiller's grains responsible for greater shedding of *E. coli* O157 in feedlot cattle. Glycerin could indeed be a means of mitigating prevalence of the foodborne pathogen in feces, though its utility for this purpose may be influenced by composition of the basal diet, and explanations for this difference are still lacking. Given the increasing availability of glycerin from biodiesel production and the need for preharvest intervention to control foodborne pathogen shedding in feedlot cattle, glycerin may prove useful as a potential candidate to alleviate foodborne pathogen shedding in cattle fed grain-based diets.

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