



DDGS and E.Coli in Cattle Diets: A Two-Part Study

December 2012

By:

J. P. Jaderborg, D. M. Paulus, R. Fink, F. Diez-Gonzalez, G. I. Crawford, and A. DiCostanzo
University of Minnesota, Saint Paul

Jim Drouillard, Ph.D.
Professor of Beef Cattle Nutrition and Management
Department of Animal Sciences & Industry
Kansas State University

A special thanks to our funding partners: Minnesota Corn Research & Promotion Council, Minnesota Soybean Research & Promotion Council, U.S. Department of Energy

Contents

Impact of Distillers Grains and Glycerin on Presence of E.Coli in Cattle	3
Summary	3
Introduction	4
Materials and Methods.....	5
Results and Discussion	6
Literature Cited	7
Intervention Strategies for Reduction of Food-borne Pathogens in Cattle Fed Ethanol Byproducts	14
PROJECT OBJECTIVES	Error! Bookmark not defined.
BRIEF DESCRIPTION OF RESEARCH	Error! Bookmark not defined.
EXPERIMENTAL PROCEDURES.....	Error! Bookmark not defined.
RESULTS and DISCUSSION.....	21
LITERATURE CITED	32

Impact of Distillers Grains and Glycerin on Presence of *E. coli* in Cattle

J. P. Jaderborg, D. M. Paulus, R. Fink, F. Diez-Gonzalez, G. I. Crawford, and A. DiCostanzo
University of Minnesota, Saint Paul

SUMMARY

Two experiments were designed to determine the effect of feeding distillers' grains (DGS) and glycerin on the fecal shedding, colonization and prevalence of *Escherichia coli* O157:H7 (ECO157) in cattle. The specific objectives of this study were to: 1) Assess the impact of feeding dry rolled corn (DRC) or steam flaked corn (SFC) and glycerin (GLY) in the presence of DGS on the fecal shedding and colonization of artificially inoculated calves (Experiment 1); 2) Determine the effects of feeding DGS on the ECO157 prevalence in naturally infected beef cattle (Experiment 2) fed SFC; 3) Assess the impact of feeding glycerin with or without DGS on ECO157 prevalence in naturally infected beef cattle (Experiment 2). In experiment 1, 28 Holstein steer calves (initial BW 353 ± 40 lb) were assigned randomly to one of four dietary treatment resulting from a 2 x 2 factorial arrangement (DRC vs SFC and GLY vs no GLY) of treatments in a completely randomized design. Diets contained (DM basis): 35% modified distillers grains with solubles, 8% grass hay, 9% supplement, and either 0 or 10% soy glycerin, with the balance of the diet made up of either SFC or DRC. Soy glycerin replaced corn in the 10% GLY treatments. Cattle were inoculated with a dose of 10¹¹ CFU per calf with a cocktail of four ECO157 strains resistant to two specific antibiotics (nalidixic acid and rifampin) at the start of the 20-d experiment. Individual fecal samples from each animal were collected three times weekly over the course of the experiment. No significant differences ($P \geq 0.05$) in ECO157 concentrations were found between treatments. Results from this experiment indicate that neither soy glycerin inclusion nor corn processing method stimulated the shedding of artificially inoculated of ECO157. In Experiment 2, 48 crossbred yearling cattle (21 steers and 27 heifers) averaging 838 lb initial BW were blocked by sex and allotted to one of 48 individual feed bunks. Nine animals were removed from the study; one for health reasons, and the others were confirmed outliers resulting from feed stealing. Cattle were fed one of four dietary treatments resulting from the 2 × 2 factorial arrangement of DGS at 0% or 35% of diet DM (resulting DGS concentrations were 40% of diet DM) and GLY at 0% or 10% of diet DM in SFC (36.5 lb/bu flake density) and grass hay (10% of diet DM) diets: 1) DGS and no GLY, 2) no DGS and no GLY, 3) DGS and GLY, 4) no DGS and GLY. Cattle for this study were selected from a larger population so the number of ECO157 positive for each dietary treatment group was 50%. After 14 d on feed, number of cattle testing ECO157 shedding ceased. Dry matter intake was greater ($P < 0.003$) for cattle fed diets containing DGS than for those fed diets without DGS (22.0 vs. 18.6 lb/d). Carcass-adjusted ADG (3.11 ± 0.53 lb) was not affected ($P > 0.10$) by feeding either coproduct. A

tendency ($P = 0.06$) for greater carcass-adjusted F:G (6.15 vs. 7.25) was observed for cattle consuming diets without DGS (analyzed as gain-to-feed). Carcass-adjusted F:G was similar ($P > 0.10$) for cattle fed GLY and those fed no GLY (6.84 vs. 6.57). Hot carcass weight (816 ± 62 lb), ribeye area (12.8 ± 1.0 sq in), 12th rib fat depth (0.551 ± 0.146 in), yield grade (2.74 ± 0.64) and marbling score (430 ± 60) were not ($P > 0.10$) affected by dietary treatment. However, KPH was greater ($P < 0.01$) for cattle fed DGS diets than for those fed diets without DGS (2.71% vs. 2.42%). Iterated ME values of diets containing DGS were 13% lower ($P < 0.05$) than those without DGS. Neither corn distillers grains, nor soy glycerin, in steam-flaked corn-based diets supported shedding of *E. coli* O157:H7. At the inclusion levels in this study, soy glycerin had a similar energy value and DGS a lesser energy value than SFC.

INTRODUCTION

***Escherichia coli* O157:H7 (ECO157) is common foodborne pathogen that is naturally present in the digestive tract of cattle. It can contaminate beef during cattle harvest. Concern with ECO157 has led to a great deal of investigation into what causes an increase or decrease in ECO157 prevalence in cattle. Diet, environment, and production practices have all been considered and examined for their effect on ECO157 prevalence in cattle.**

Results from previous studies indicate that there is a link between corn processing method and ECO157 prevalence in a beef cattle herd. Feeding steam flaked corn (**SFC**) resulted in a greater amount of ECO157 fecal shedding by beef cattle than when feeding dry rolled corn (**DRC**) (Depenbusch et al., 2008). Scientists have discovered that the hindgut of cattle is hospitable for ECO157. Thus, changes to the hindgut environment will have a greater impact on ECO157 prevalence than changes to the rumen environment. More processed corn, such as SFC, is mostly digested in the rumen; thereby, limiting concentration of starch reaching the small intestine. Lower starch concentrations in the small and large intestine are thought to lead to greater pH and lower VFA concentrations; conditions that support ECO157 growth. This theory appears to apply to corn distillers grains (**DGS**) also. Percentage of cattle positive for ECO157 in fecal or hide samples was greater for those fed 40% wet DGS than those fed 0% wet DGS (Wells et al., 2009). Whether soy glycerin (**GLY**), a coproduct that is mainly digested in the rumen, impacts ECO157 shedding is not known.

Increased production of both DGS and GLY over the last ten years coupled with recent spikes in corn grain prices led to greater incorporation of these coproducts in finishing diets. U.S. biodiesel production has grown rapidly to over 750 million gallons in 2008. This led to production of 564 million pounds of GLY allowing feedlot owners access to a potentially inexpensive feedstuff that is needed during times when corn prices increase. Glycerin's liquid form gives versatility to this coproduct while helping maintain diet integrity. In a recent experiment, feeding glycerin at 2% DM of diet improved gain and feed efficiency in feedlot heifers (Parsons et al., 2009). In a similar study Van Cleef et al. (2011) reported no effect on ADG from feeding GLY up to 15% diet DM, but a linear decrease in DMI with a concomitant improvement in gain efficiency was observed. However, while glycerin inclusion has been studied in SFC diets; there have been no studies evaluating this coproduct when fed in DGS. Therefore, our objectives were to: 1) Assess the impact of feeding dry rolled corn DRC or steam

flaked corn SFC and glycerin GLY in the presence of DGS on the fecal shedding and colonization of artificially inoculated calves (Experiment 1); 2) Determine the effects of feeding DGS on the ECO157 prevalence in naturally infected beef cattle (Experiment 2) fed SFC; 3) Assess the impact of feeding glycerin with or without DGS on ECO157 prevalence in naturally infected beef cattle (Experiment 2).

MATERIALS AND METHODS

Experiment 1. This experiment was conducted at the University of Minnesota Rosemount Research and Outreach Center feedlot at UMore Park in Rosemount, MN. Twenty-four Holstein steers (initial BW 353 ± 40 lb) were arranged randomly into one of four treatments with a 2 x 2 factorial arrangement of treatments. Cattle were housed in groups in an isolation facility with no physical contact between cattle groups and no contact with the outside environment.

Treatment diets contained (DM basis): 35% modified distillers grains with solubles (47% moisture; Western Wisconsin Energy, Boyceville, WI), 8% grass hay, 9% supplement, and either 0 or 10% soy glycerin, with the balance of the diet made up of either SFC (36.5 lb/bu) or DRC. Soy glycerin replaced corn in the 10% soy glycerin treatments. Cattle were fed *ad libitum* once daily at 0900 and were acclimated to the treatments diets prior to the start of the experiment.

On d 0 of the experiment cattle were fecal sampled. Following fecal sampling, the cattle were inoculated orally with a dose of 10^{11} CFU/calf cocktail of four *E. coli* O157:H7 strains resistant to two specific antibiotics (nalidixic acid and rifampin). Cattle were fecal sampled three times weekly beginning on d 1 of the experiment. In total the experiment lasted 20 d. Fecal samples were examined for concentrations of *E. coli* O157:H7 determined through enumeration on selective medium and reported in log CFU/g. Testing was sensitive for samples with *E. coli* O157:H7 concentrations greater than 1.0 log CFU/g.

Data were analyzed with the Proc Mixed procedure of SAS 9.2 with an autoregressive covariance structure. Fixed effects included treatment and day. Statistical significance was declared with *P*-values < 0.05, and trends were discussed with *P*-values between 0.05 and 0.10. Samples with *E. coli* O157:H7 concentrations less than 1.0 log CFU/g were reported as 1.0 log CFU/g for analytical procedures.

Experiment 2. This experiment was conducted at the University of Minnesota Rosemount Research and Outreach Center feedlot at UMore Park in Rosemount, MN. Forty-eight Charolais-cross, 21 steer and 27 heifer calves (initial BW 838 lb) were blocked by sex and assigned randomly to bunks in a Calan gate individual feeding system. The Calan gate system consisted of 48 bunks (22 linear in bunk space per animal) in four pens (12 bunks/pen). Nine animals were removed from the study; one for health reasons, and the others were confirmed outliers resulting from feed stealing. Dietary treatments were formulated to contain either modified DGS (Western Wisconsin Energy, Boyceville, WI) at 0% or 35% of dietary DM and glycerin at 0%

or 10% of diet DM in SFC (36.5 lb/bu flake density) and grass hay (10% of diet DM) diets: 1) DGS and no glycerin, 2) no DGS and no glycerin, 3) DGS and glycerin, 4) no DGS and glycerin. Diets contained 16.5% CP, 0.67 Mcal/lb NEg DM, 0.75% Ca, 0.47% P, and from 0.17% to 0.25% S (Table 1). Resulting DGS inclusion averaged 40% as its DM content was greater than anticipated. Treatments were assigned randomly within pen, for a total of 12 replications per treatment. Cattle were fed ad-libitum once daily at 0800 and feed refusals were collected daily prior to feeding. A bunk score of 0 (scale 0, slick, to 4, full) for two consecutive days elicited a 0.5-lb DM increase. Dry matter intake was calculated from DM offered and refusal collections.

Cattle were implanted on d 56 with a Revalor 200 (200 mg of trenbolone acetate and 20 mg of estradiol). Cattle were weighed every 28 d, and initial body weight was taken after a 16-h period without access to feed and water. Cattle were harvested in two harvest groups on d 124 and d 173 when determined to have 0.5 in of backfat at the 12th rib. All cattle were harvested at PM Beef in Windom, MN and carcass data were collected by University and USDA personnel. Carcass data collection included dressing percentage, HCW, marbling score, 12th rib fat thickness, ribeye area, kidney, pelvic, and heart fat (KPH), USDA yield grade, and USDA quality grade. Dressing percentage was calculated as the sum of the HCW from each block divided by the cumulative live BW of each block as measured at the packing plant prior to harvest.

Based on visual behavior observation after feed was delivered, the presence of outlier DMI data generated by cattle that stole or had feed stolen was tested using the Regression procedure of SAS. The Regression procedure led to the discovery of eight outliers which were removed from the study. Data were then analyzed using Mixed procedure of SAS with animal being experimental unit. Fixed effects included DGS and soy glycerin concentrations, with interaction affect between the two. Individual animal was the random effect. Effects were considered significant when *P* values were less than or equal to 0.05 and were considered trends when *P* values were between 0.05 and 0.10. Iterated ME values of the diet were calculated using iterative procedures of the NRC (2000) using DMI and ADG.

RESULTS AND DISCUSSION

Experiment 1. Results are displayed in Figure 1. Initial *E. coli* O157:H7 concentrations were less than 1.0 log CFU/g for all treatments. After inoculation, *E. coli* O157:H7 concentrations spiked to 3.6 log CFU/g or higher for all treatments. After d 1 *E. coli* O157:H7 concentrations decreased for the rest of the study. On the final d of the experiment *E. coli* O157:H7 concentrations were less than 1.0 log CFU/g for all treatments. No significant differences (*P* = 0.998) were observed between treatments throughout the entire experiment. However, *E. coli* O157:H7 concentrations varied (*P* ≤ 0.005) significantly between days with the highest *E. coli* O157:H7 concentrations occurring on d 1 for all treatments.

Based on the results of this study, it is clear that the dietary inclusion of processed corn or soy glycerin in feedlot diets has no effect on the shedding of *E. coli* O157:H7 in steers. Additionally, it is clear that the dietary ingredients utilized in this experiment do not contribute to the survival of *E. coli* O157:H7 in the gastrointestinal tract of feedlot steers. In fact, the ingredients

utilized in this study may in fact lead to a reduction in *E. coli* O157:H7 concentrations in the gastrointestinal tract of feedlot steers.

Overall the results presented in this study are contradictory to previous studies that have found that specific forms of processed corn can have an impact on the survival of *E. coli* O157:H7 in the gastrointestinal tract of feedlot steers. This suggests that environmental factors may play a larger role in *E. coli* O157:H7 survival than diet. However, more research is needed to determine what other factors contribute to *E. coli* O157:H7 survival in cattle.

Experiment 2. Treatment means for initial and final BW, ADG, DMI, and feed:gain are shown in (Table 2). Feed-to-gain ratio was analyzed as G:F. The interactive effect between DGS and soy glycerin was not significant ($P > 0.10$). Dry matter intake was greater ($P < 0.003$) for cattle fed diets containing DGS than for those fed diets without DGS (22.0 vs. 18.6 lb/d; Table 2). Final BW was not significant ($P < 0.10$) among treatments. Carcass-adjusted ADG (3.11 ± 0.53 lb) was not affected ($P > 0.10$) by feeding either coproduct. A tendency ($P = 0.07$) for greater carcass-adjusted F:G (6.15 vs. 7.25) was observed for cattle consuming diets without DGS. Carcass-adjusted F:G was similar ($P > 0.10$) for cattle fed glycerin and those fed no glycerin (6.84 vs. 6.57).

Hot carcass weight (816 ± 62 lb), ribeye area (12.8 ± 1.0 sq in), 12th rib fat depth (0.551 ± 0.146 in), yield grade (2.74 ± 0.64) and marbling score (430 ± 60) were not ($P > 0.10$) affected by dietary treatment (Table 3). However, KPH was greater ($P < 0.01$) for cattle fed DGS diets than for those fed diets without DGS (2.71% vs. 2.42%).

Iterated ME values of diets containing DGS were 13% lower ($P < 0.05$) than those without DGS (Figure 1). At the inclusion levels in this study, soy glycerin had a similar energy value and DGS a lesser energy value than SFC. With these results the economics of what purchasing soy glycerin compared to SFC in your area should be considered.

LITERATURE CITED

- Depenbusch, B. E., T. G. Nagaraja, J. M. Sargeant, J. S. Drouillard, E. R. Loe, and M. E. Corrigan. 2008. Influence of processed grains on fecal pH, starch concentration, and shedding of *Escherichia coli* O157 in feedlot cattle. *J. Anim. Sci.* 86:632-639.
- Fox, J.T., B. E. Depenbusch, J. S. Drouillard and T. G. Nagaraja. 2007. Dry-rolled or steam-flaked grain-based diets and fecal shedding of *Escherichia coli* O157 in feedlot cattle. *J. Anim. Sci.* 85:1207-1212.
- NRC. 2000. Nutrient Requirements of Beef Cattle (7th Rev. Ed.). National Academy Press. Washington, D.C.
- Parsons, G. L., M. K. Shelor, and J. S. Drouillard. 2009. Performance and carcass traits of finishing heifers fed crude glycerin. *J. Anim. Sci.* 87:653-657.

van Cleef, E. H. C. B., S. Uwituze, C. L. Van Bibber, K. A. Miller, C. C. Aperce, K. L. Blaine, J. J. Higgins, and J. S. Drouillard. 2011. Effects of crude glycerin in byproducts diets on performance and carcass characteristics of feedlot cattle. *J. Anim. Sci.* 89(E-Suppl. 1): 124(Abstr.).

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. Freetly, 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.

Table 1. Ingredient composition and nutrient analysis (% DM-basis) of finishing diets.

	Treatment ¹			
	DGS Y GLY N	DGS N GLY N	DGS Y GLY Y	DGS N GLY Y
Steam Flaked Corn ²	44.5	78.8	35.3	67.1
Modified DGS ³	41.0	0.0	40.7	0.0
Soy Glycerin	0.0	0.0	9.9	11.0
Geass Hay	10.6	11.7	10.3	11.6
Liquid mineral	3.6	4.0	3.6	3.9
Liquid protein	0.0	5.3	0.0	6.1
Myco CURB ⁴	0.2	0.2	0.2	0.2
DM, %	69.8	80.7	71.4	78.1
CP, %	17.6	13.8	16.4	13.7
ADF, %	15.2	9.4	11.8	11.7
Ca, %	0.74	0.77	0.89	0.82
P, %	0.47	0.35	0.48	0.39
S, %	0.25	0.13	0.22	0.17

¹ Treatment diets included: DGS Y GLY N, containing 40% distillers grains and 0% soy glycerin, DGS N GLY N, containing 0% distillers grains and 0% soy glycerin, DGS Y GLY Y, containing 40% distillers grains and 10% soy glycerin, DGS N GLY Y, containing 0% distillers grains and 10% soy glycerin.

² Flake density = 36.5 lb/bu.

³ Modified distillers grains with solubles (48% DM; Western Wisconsin Energy, Boyceville, WI).

⁴ Mold inhibitor (Kemin Industries, Inc., Des Moines, IA).

Table 2. Main effects of feeding distillers grains or glycerin on feedlot performance

	DGS¹				Glycerin²			
	No	Yes	SEM³	P-Value	No	Yes	SEM³	P-Value
Initial BW, lb	851	817	18	0.21	831	838	18	0.77
DMI, lb/d	18.6	22.0	0.7	< 0.01	21.0	19.6	0.7	0.16
ADG, lb	3.13	3.07	0.11	0.73	3.19	3.01	0.11	0.28
Feed:Gain	6.15	7.25	0.33	0.07	6.57	6.84	0.33	0.28
Final BW, lb	1,293	1,268	14	0.42	1,286	1,275	21	0.74

¹ DGS treatment diets included: No, containing 0% distillers grains, Yes, containing 40% distillers grains.

² Glycerin treatment diets included: No, containing 0% soy glycerin, and Yes, containing 10% soy glycerin.

³ Standard error of least square means.

Table 3. Main effects of feeding distillers grains or glycerin on carcass traits

	<u>DGS¹</u>				<u>Glycerin²</u>			
	No	Yes	SEM ³	P-Value	No	Yes	SEM ³	P-Value
HCW, lb	822	807	13	0.43	818	812	14	0.74
12th rib back fat, in	0.54	0.56	0.03	0.63	0.56	0.55	0.03	0.87
Ribeye area, sq. in	12.89	12.73	0.24	0.62	12.76	12.86	0.23	0.75
KPH ⁴	2.4	3.0	0.1	0.01	2.5	2.6	0.1	0.42
YG ⁵	2.6	2.8	0.1	0.22	2.7	2.6	0.1	0.57
Marbling score ⁶	424	434	14	0.63	435	424	20	0.62

¹ DGS treatment diets included: No, containing 0% distillers grains, Yes, containing 40% wet distillers grains.

² Glycerin treatment diets included: No, containing 0% soy glycerin, and Yes, containing 10% soy glycerin.

³ Standard error of least square means.

⁴ Kidney, pelvic, and heart fat.

⁵ USDA yield grade.

⁶ where 400 = small⁰⁰, 500 = modest⁰⁰.

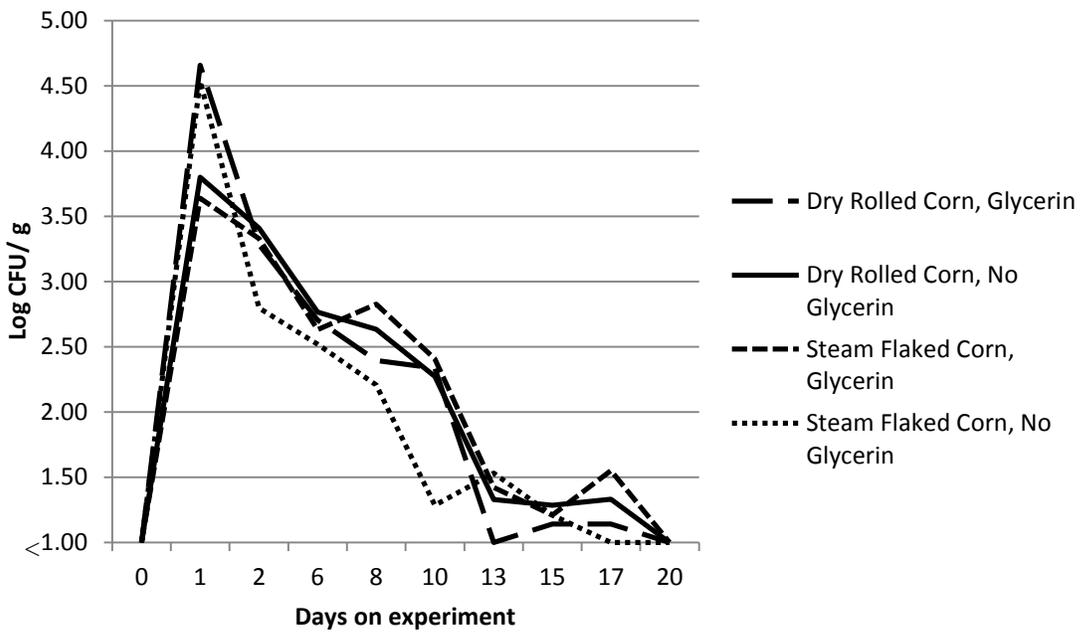


Figure 1. Effect of soy glycerin, steam flaked corn, and dry rolled corn on *Escherichia coli* O157:H7 concentrations in fecal samples.

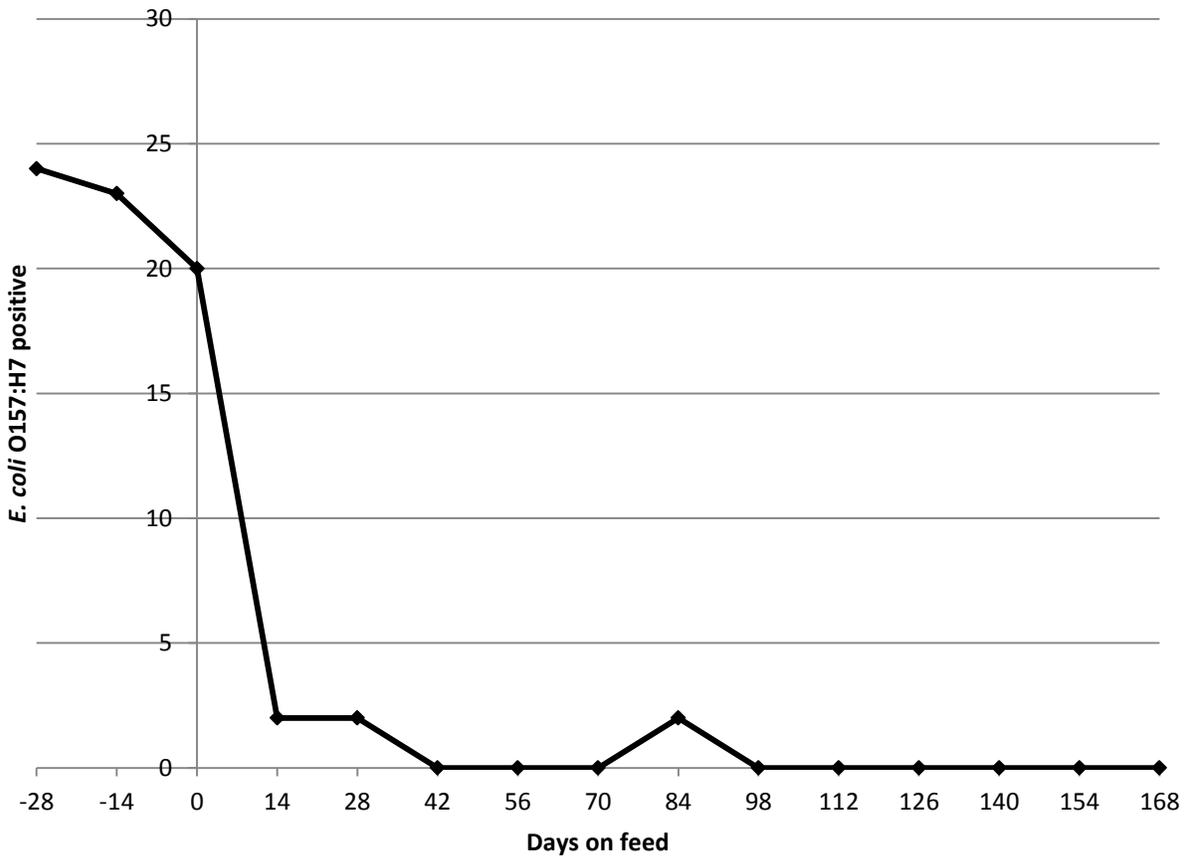


Figure 2. Prevalence of *Escherichia coli* 0157:H7 in fecal samples of all steers in Experiment 2 (treatment effects were not significant $P > 0.05$).

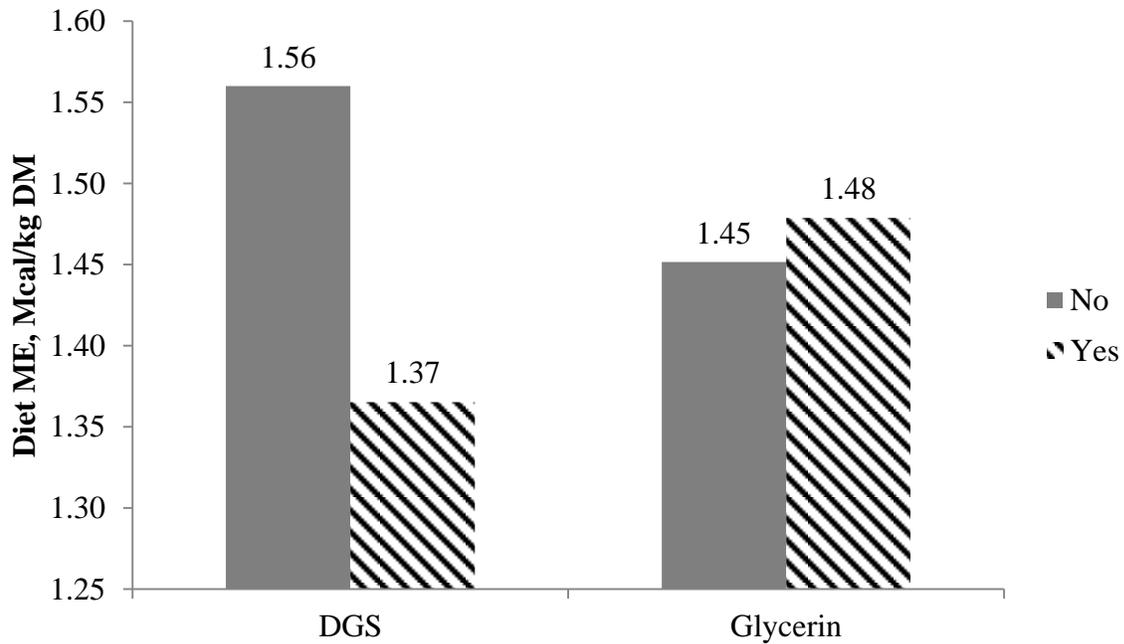


Figure 3. Iterated ME values of diets containing DGS were 13% lower ($P < 0.05$) than those without DGS. At the inclusion levels in this study, soy glycerin had a similar energy value and DGS a lesser energy value than SFC. DGS treatment diets included: No, containing 0% modified distillers grains, Yes, containing 40% modified distillers grains. Glycerin treatment diets included: No, containing 0% soy glycerin, and Yes, containing 10% soy glycerin.

Intervention Strategies for Reduction of Food-borne Pathogens in Cattle Fed Ethanol Byproducts

Prepared by:

Jim Drouillard, Ph.D.

Professor of Beef Cattle Nutrition and Management

Department of Animal Sciences & Industry

Kansas State University

Manhattan, Kansas 66506-5606

Phone. (785) 532-1204

E-mail: jdrouill@k-state.edu

PROJECT OBJECTIVES

Cattle are an important reservoir of two major food-borne pathogens: *Escherichia coli* O157:H7 and *Salmonella*. Both organisms colonize the lower gastrointestinal tract of cattle and are shed in the feces. Characterization of factors affecting *E. coli* O157 and *Salmonella* persistence and shedding patterns in cattle is important to develop intervention strategies and improve the safety of food. The role of diet on fecal shedding of *E. coli* O157 in cattle has been studied (Buchko et al., 2000; Callaway et al., 2003; Berg et al., 2004; Van Baale et al., 2004). Distillers grains, a byproduct derived during the production of ethanol from grains, particularly corn, are commonly used in ruminant diets (Ham et al., 1994; Lodge et al., 1997; Kleinschmitt et al., 2006). The byproduct is comprised principally of the bran (fiber), protein, and germ (lipid) fractions of the corn and is an excellent source of energy and protein for feedlot and dairy cattle (Spiehs et al., 2002). We have conducted preliminary studies in the past year that indicated a surprising association between distillers feed and prevalence of *E. coli* O157 and *Salmonella*.

We conducted studies aimed at identifying possible intervention strategies to reduce pathogen shedding in cattle fed distillers grains. Specifically, our objectives were to:

1. Evaluate the effects of grain processing (steam flaking versus dry rolling) on prevalence of *E. coli* O157 in cattle fed distillers grains.
2. Feed crude glycerin as a possible method for reducing prevalence of *E. coli* O157.
3. Utilize common feed additives as a possible means of reducing pathogen levels shed in feces of cattle fed distillers grains.

BRIEF DESCRIPTION OF RESEARCH

Trial 1: Grain processing as a preharvest intervention strategy.

We previously conducted studies that revealed large differences in prevalence of *E. coli* O157 shedding due to the method of grain processing. Essentially, less rigorously processed grain, such as dry-rolled grain, appeared to afford some protection against *E. coli* shedding compared to more extensively processed grain (i.e., steam flaked). We have speculated that these differences may be attributable to differences in energy substrate that appears in the hind gut. Flaked grains are extensively digested within the rumen, whereas rolled grains result in far greater starch supply to the hindgut. Increasing starch in the hindgut presumably creates an environment that is conducive to growth of bacteria that can effectively outcompete *E. coli* O157. We hoped to exploit this by substituting a portion of flaked corn with dry-rolled corn in an effort to increase starch supply to the hindgut.

Trial 2. Glycerin as an intervention strategy to control *E. coli* O157.

We have completed a series of experiments with crude glycerin, a coproduct derived from the biodiesel industry, to assess its value as a feed for cattle. In our early laboratory experiments, we observed that low levels of glycerin can have a profound effect on fermentation by rumen bacteria. Furthermore, these changes in fermentation manifested as rather large improvements in efficiency of feedlot cattle, particularly when glycerin was included in the diet at a low level (2%). We have hypothesized that this is the result of changes in the microbial population within the gut. Furthermore, we are aware of at least one laboratory experiment in which glycerin inhibited growth of *Salmonella*, which is another gram negative bacteria (like *E. coli*) that colonizes the gastrointestinal tract. Consequently, we believed it might be useful to evaluate the effects of feeding glycerin on populations of pathogenic *E. coli* in cattle.

Trial 3. Reducing *E. coli* O157 shedding in cattle with feed additive compounds

The gut ecosystem is highly complex, consisting of a myriad of bacterial species that compete for space and nutrients. The various species of microbes that exist within the gut are in a continuous state of flux. As one population proliferates, there must be a compensatory decline in another competing population. Conditions within the gut that favor the proliferation of one species logically will reduce the prevalence of another organism. Feed additives that alter populations of gut bacteria can have both positive and negative consequences for colonization by pathogenic, food-borne pathogens. This study was aimed at examining three feed additive products that are known to influence fermentation, and thus bacterial populations: Urea, Ractopamine, and Monensin.

EXPERIMENTAL PROCEDURES

Trial 1:

Thirty nine Holstein steer calves were used for this study. The average initial body weight was 213 ± 20 kg. During the pre-inoculation phase, cattle were assigned randomly to 4 outdoor pens. Each pen received one of the four experimental diets consisting of a steam-flaked corn (SFC) base diet with 0 or 25% of dry-rolled corn (DRC) and 0 or 25% dried distillers grains with soluble (DDGS, dry basis). Gradual transition, from high roughage diets to high concentrate diets, was accomplished by feeding a series of step-up diets over a 3-week period; after which, the calves received final diets containing approximately 6% alfalfa hay, as a roughage source, for 52 days. The detailed composition for the four final diets is given in Table 1.

At the end of the pre-inoculation phase, fecal sampling was achieved by rectal palpation to determine the prevalence of the wild-type of *E. coli* O157:H7. One gram of the fecal sample was enriched in 9 mL of Gram Negative broth (Difco, BD, Sparks, MD) with cefixime (0.05 mg/L), cefsulodin (10 mg/L), and vancomycin (8 mg/L; GNccv) for 6 h at 37°C. Immunomagnetic separation (IMS; Dynal, Inc., New Hyde Park, NY) was performed and samples were plated onto sorbitol-MacConkey agar (Difco, BD, Sparks, MD) with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L; ct-SMAC). Sorbitol-negative colonies were picked and tested for indole production and O157 antigen latex agglutination. Five of the steers were found positive for wild-type *E. coli* O157 and were excluded from the study. The repartition of the 31 animals, identified as negative carrier of *E. coli* O157, was as follows: 7 steers from the 25% DRC and 0% DDGS treatment and 8 animals from each of the other treatments. Selected calves were transferred to individual pens in a biosafety level-2 facility. Pens were separated by solid walls to prevent any physical contact between adjacent animals and were equipped with their own water supply and feed bunk. Calves were fed *ad libitum* once daily around 9:30am.

Composition of diets for Trial 1, dry matter basis

Ingredient, % dry matter	0% Dry-rolled Corn		25% Dry-rolled Corn	
	0% DDGS	25% DDGS	0% DDGS	25% DDGS
Steam-flaked corn	82.1	58.2	56.8	33.1
Dried distillers grains	-	25.4	-	25.3
Dry-rolled corn	-	-	25.5	25.3
Alfalfa hay	5.9	5.9	5.9	5.8
Corn steep liquor	6.5	6.4	6.4	6.4
Supplement ¹	5.5	4.2	5.5	4.1

¹Formulated to provide 0.1 mg Co, 10 mg of Cu, 0.5 mg of I, 50 mg of Mn, 0.25 mg of Se, 50 mg of Zn and 2200 IU vitamin A per kg of diet DM, and provided 30 g/ton monensin and 9 g/ton tylosin.

Five strains of *E. coli* O157:H7 were grown on Blood agar (BAP; Remel, Lenexa, KS) overnight at 37°C. A single colony was selected from each strain, inoculated into 10 mL of tryptic soy broth (TSB; Difco, BD, Sparks, MD) and incubated overnight at 37°C. Then, 1 mL of the resulting culture was transferred to 100 mL of TSB and again incubated at 37°C. After 7 hours, all strains were pooled together. The mixture was diluted ten-fold with buffered peptone water and spread onto sorbitol-MacConkey agar (Difco, BD, Sparks, MD) plates with cefixime (0.05 mg/L), potassium tellurite (2.5 mg/L) and nalidixic acid (50 µg/L; ctn-SMAC).

Three days after relocation, the calves were orally inoculated, via a gastric tube, with 5 mL of the 5-strain mixture of Shiga-toxin producing Nal^R *E. coli* O157:H7 mixed with 200 mL of 1% sterile skim milk. The final concentration of the inoculate was 3.2×10^9 colony-forming units (CFU) of Nal^R *E. coli* O157:H7.

After the oral challenge with Nal^R *E. coli* O157:H7, fecal samples were collected three times a week (Monday, Wednesday and Friday) over a 40-day period. To prevent cross contamination gloves were changed between pens and foot baths with bleach were used. Feces were collected in sterile bags, by rectal palpation, and transported immediately to the laboratory. Approximately 1 g of fecal sample was added to a 9 mL of GNccv. One milliliter of the culture was then diluted ten-fold in buffered peptone water. A 100-µL aliquot of the resulting dilution was directly plated, in triplicate, onto ctn-SMAC and incubated overnight at 37°C. The remaining of the GNccv broth was enriched for 6 h at 37°C, subsequently; 1 mL was removed and reinoculated into a fresh 9 mL of GNccv for a second enrichment. After 16 to 18 h of incubation at 37°C, 100 µL was plated on ctn-SMAC and incubated overnight at 37°C. Sorbitol negative colonies from the direct and enriched ctn-SMAC plates were counted and colonies were tested for indole production and O157 antigen.

At the end of the 40-day period, all calves were euthanized and submitted for post mortem examination. Animals were euthanized on a 3-day period by group with homogenous repartition of the treatments. Contents from the rumen, cecum, colon, and rectum were collected and the concentrations of Nal^R *E. coli* O157 were determined as described above. The rectum was, additionally, excised and the mucosa rinsed with water to remove visible fecal material. The proximal area (3 to 5 cm from the recto-anal junction) was swabbed with a foam-tipped applicator (RAMS; VWR International, Buffalo Grove, IL, USA). Swabs were immediately placed in 3 mL of GNccv broth, transported to the laboratory, vortexed and processed for detection of Nal^R *E. coli* O157 by direct plating or enrichment technique, as previously described. Swabs were only used for a positive (direct plating or enrichment) or negative type response.

Fecal concentrations of Nal^R *E. coli* O157:H7 were log₁₀ transformed for the need of the analysis. Lowest detectable concentration per gram of wet feces was considered (approximately 10¹ CFU/g), when only direct plating was positive for Nal^R *E. coli* O157:H7.

Effects of treatments, sampling days and the interaction treatment × sampling day, on the concentrations of Nal^R *E. coli* O157:H7 in the serial fecal collections (days 2 through 40), were analyzed using the Proc MIXED procedure in SAS (Version 9.1, SAS Institute, Cary, NC, USA). The model included DDGS level, DRC level, sampling day, and all possible interactions as fixed effects and animal as a random effect.

The generalized linear models procedure was used to assess differences in NaI^R *E. coli* O157:H7 concentration in gut contents collected at necropsy. The model included DDGS level, DRC level and the interaction between DDGS and DRC.

The prevalence of NaI^R *E. coli* O157:H7 (positive by direct plating or by enrichment) in recto-anal mucosal swab samples (RAMS) and gastrointestinal tract samples were analyzed using the GLIMMIX procedure of SAS, whereas the prevalence of NaI^R *E. coli* O157:H7 in fecal samples was analyzed by using a GENMOD procedure of SAS.

TRIAL 2:

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. We formulated the diets to be isonitrogenous.

Composition of diets for Trial 2.

Ingredients, % of DM	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
Dry matter	43.3	43.7	44.2
Organic matter	93.4	93.2	93.7
Crude protein	13.0	12.5	12.1
NDF	36.2	34.7	33.2
Calcium	0.9	0.9	0.9

¹Formulated to provide 0.1 mg Co, 10 mg of Cu, 0.5 mg of I, 50 mg of Mn, 0.25 mg of Se, 50 mg of Zn and 2200 IU vitamin A, per kg of diet DM.

²Provided 300 mg of monensin per animal daily in a ground corn carrier.

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

TRIAL 3:

Seven hundred and twenty crossbred beef steers (initial BW = 433 \pm 23.1 kg) were used in a randomized complete block design with a 2 x 3 x 2 factorial treatment arrangement. Factors were monensin level (33 or 44 mg/kg DM), level of supplemental urea (0, 0.35, or 0.70% of DM), and ractopamine (0 or 200 mg/steer daily, fed during the last 42 d before harvest). The basal diet consisted of steam-flaked corn with 30% wet sorghum DG. Steers were allowed *ad libitum* access to alfalfa hay and water from arrival until processing at the start of the adaptation phase. On day 0 of the adaptation period, steers were weighed, vaccinated, stratified by weight into two blocks and then randomly assigned to pens. Forty-eight dirt-floor pens (10.4 x 26.8 m) containing 15 steers each were randomly assigned to 1 of 12 treatment combinations. Two transition diets and a final diet were fed over a 21 day step-up period. The amount of feed offered to the pen was determined at approximately 7:00 a.m., and the daily rations for each pen was delivered at approximately 8:00 a.m. and 2:00 p.m. each day. Cattle were fed amounts sufficient to result in traces of residual feed at the next feeding and fresh water was available at all times.

The three feed additive manipulations were not started at the same time point of the study, and therefore, not all sample events occurred in steers fed diets with all treatments. Monensin was included in the transition and final diets, while urea supplementation was initiated at the end of the adaptation phase and after the first sampling event. Ractopamine administration began after five sampling events, and therefore, the last four samples were from steers fed diets that included all three treatments.

Ingredient and chemical composition of the diets for Trial 3.

Item	Percentage, DM basis
<i>Ingredient</i>	
Steam-flaked corn	58.1, 57.7, or 57.3
Wet sorghum distillers grain	30.0
Wheat straw	7.0
Premix ¹	2.38
Limestone	1.80
Urea	0, 0.35, or 0.70
Supplement ²	0.71
<i>Nutrient Composition</i>	
Dry matter, %	57.5
CP, % DM	14.2, 15.4, or 16.5
NDF, % DM	19.2

¹Feed additive premix provided 33 or 44 mg of monensin sodium (Elanco Animal Health, Greenfield, IN) and 11 mg tylosin (Elanco Animal Health, Greenfield, IN) per kg of feed.

²Supplement contained 2,650 IU vitamin A; 10 mg Cu; 0.5 mg I; 0.15 mg Co; 0.25 mg Se; 50 mg Zn; and 50 mg Mn per kg of diet DM; Ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN) included in the diet at 0 or 200 mg per animal daily the last 42 d before harvest.

Fecal samples (10/pen) were collected, July through November, from the surface of each pen approximately every 2 wk for 14 wk (n = 9). Samples (approximately 100 g) were collected from freshly defecated fecal pats using a plastic spoon, and care was taken to avoid ground contamination or repeat sampling from the same steer on the same sampling. The spoon with feces was placed into a Whirl-pack bag (Nasco, Ft. Atkinson, WI), and once samples were collected, they were placed in a cooler with ice packs and delivered to the Kansas State University College of Veterinary Medicine Pre-harvest Food Safety Laboratory for processing.

Fecal samples were cultured for *E. coli* O157:H7 as previously described (Greenquist et al., 2005). Briefly, samples were kneaded for 30 sec and approximately 1 g of feces was placed in tubes containing 9 mL Gram-negative (**GN**) broth (Becton Dickinson, Franklin Lakes, NJ) with cefixime (0.05 mg/L), cefsulodin (10 mg/L), and vancomycin (8 mg/L; **GNccv**). The GNccv broth was then incubated

for 6 h at 37°C. After enrichment, immunomagnetic bead separation (**IMS**) was performed, followed by plating onto sorbitol-MacConkey agar with cefixime (0.5 mg/L) and potassium tellurite (2.5 mg/L; **CT-SMAC**). Plates were incubated overnight at 37°C, and up to six sorbitol negative colonies were streaked onto blood agar plates. Colonies were tested for indole production and latex agglutination for the O157 antigen, and further characterized by multiplex PCR to identify *eae* (intimin), *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *hly* (hemolysin), *rfbE* (O157 antigen) and *fliC* (flagella) genes (Bai et al., 2010). Personnel conducting sampling and laboratory analyses were blinded to the treatment assignments.

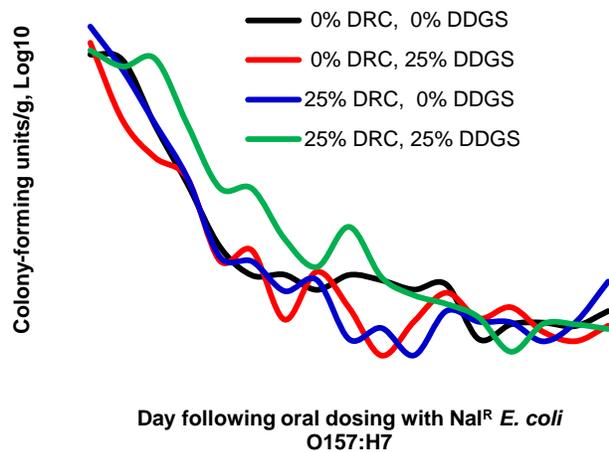
Descriptive statistics on prevalence outcomes were assessed prior to multivariable analysis. A repeated measures negative binomial generalized linear model (PROC GENMOD, SAS v. 9.2, Cary, NC) with a log links was used to analyze data. The negative binomial function was chosen, as it best represented data having a high number of zero prevalence samples and few samples with very high prevalence. It was verified to be the correct function by examining the dispersion parameter point estimate (α). Because the 95% confidence interval did not include 0, the negative binomial model was deemed appropriate (Heeringa et al., 2010). Pen was considered the experimental unit with the count of positive samples as the outcome. This was offset by the natural log of the number of samples processed for each pen. Pen over time was included as a repeated (random) effect to account for the lack of independence among samples from the same pen over sampling days. Score statistics for the generalized estimating equations were used to assess significance of sampling day, monensin, urea, ractopamine, and their interactions. Because urea and ractopamine were not fed throughout the trial, the data were analyzed in three phases. Results were considered significant at $P < 0.05$ and manual backward selection was used to remove variables that were not significant.

RESULTS and DISCUSSION

Trial 1:

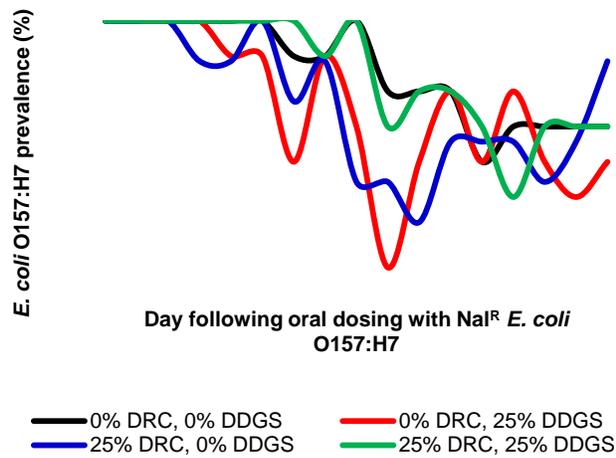
Figure 1 represents the concentration of NaI^R *E. coli* O157:H7 in feces of steers fed diet with 0 or 25% DDGS and 0 or 25% DRC following oral dosing with 3.2×10^9 CFU of the 5-strain mixture. Two days after inoculation, the concentration of NaI^R *E. coli* O157:H7 for all treatments was approximately 1×10^5 CFU/g of feces. Over the 40-day period, the concentration of NaI^R *E. coli* O157:H7 gradually decreased. The last measures fell between 1 and 2 log CFU/g of feces. There were no significant differences between diets ($P > 0.2$), however a day of sampling effect was observed ($P < 0.01$).

Figure 1: Concentration of *Nal^R E. coli* O157:H7 in fecal samples of steers, fed finishing diets with 0 or 25% dried distillers grains with solubles and 0 or 25% dry-rolled corn, following oral dosing with 3.2×10^9 colony-forming units of a 5-strain mix of *Nal^R E. coli* O157:H7. Diet effect, $P > 0.20$; Sampling day effect, $P < 0.01$; SEM=0.29.



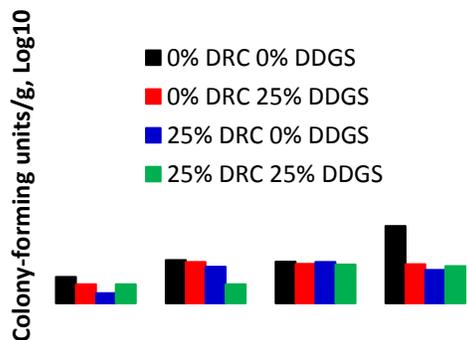
A comparison of the prevalence of culture positive for *Nal^R E. coli* O157:H7 in fecal samples among the different diets over the whole period of the experiment (Figure 2) revealed an effect of sampling day ($P < 0.0001$), a treatment interaction DRC*DDGS ($P < 0.01$), and a sampling day*DRC*DDG interaction ($P = 0.05$). The individual analysis of every sampling day revealed no DRC or DDG effect, but an interaction DRC*DDG on days 16, 21, 23 and, 26 ($P < 0.05$). The prevalence of culture positive samples was lower in steers receiving 25% DRC - 0% DDG and 0% DRG - 25% DDG.

Figure 2: Prevalence of culture positive for *Nal^R E. coli* O157:H7 in fecal samples from steers fed finishing diets with 0 or 25% dried distillers grains with soluble and 0 or 25% dry-rolled corn following oral dosing with 3.2×10^9 colony-forming units of a 5-strain mix of *Nal^R E. coli* O157:H7. Sampling day effect, $P < 0.0001$; DRC effect, $P > 0.90$; DDGS effect, $P > 0.70$; Diet interaction DRC*DDGS, $P < 0.01$ (Interaction DRC*DDG, $P < 0.05$ for sampling days 16, 19, 21, 23 and 26); Sampling day*DRC*DDG interaction, $P = 0.05$.



Forty days after the inoculation of the steers with 3.2×10^9 CFU of a 5-strain mixture of Nal^R *E. coli* O157:H7, the experiment was terminated. The concentration of *E. coli* O157:H7 at the various locations in the gastrointestinal tract of the steers (Figure 3) did not differ significantly ($P > 0.3$). DDG, DRC, or the interaction of both did not have an effect on the shedding of *E. coli* O157:H7 in the rumen, cecum, colon, and rectum of the steers.

Figure 3: Concentration of Nal^R *E. coli* O157:H7, at necropsy, in various locations of the gastrointestinal tract of steers fed finishing diets with 0 or 25% dried distillers grains with soluble and 0 or 25% dry-rolled corn following oral dosing with 3.2×10^9 colony-forming units of a 5-strain mix of Nal^R *E. coli* O157:H7. Location effect, $P > 0.3$; DRC effect, $P > 0.10$; DDGS effect, $P > 0.10$; interaction DRC*DDGS, $P > 0.10$; and location*DRC*DDGS interaction, $P > 0.10$.



The analysis of the prevalence of culture positive samples for *E. coli* O157:H7 at the various location of the gastrointestinal tract (Table 1) did not show any significant difference among treatments.

Table 1: Prevalence of Nal^R *E. coli* O157:H7 positive samples, at necropsy, in various location of the gastrointestinal tract of steers fed finishing diets with 0 or 25% dried distillers grains with soluble and 0 or 25% dry-rolled corn following oral dosing with 3.2×10^9 colony-forming units of a 5-strain mix of Nal^R *E. coli* O157:H7

	0% Dry-rolled Corn		25% Dry-rolled Corn		SEM
	0% DDGS	25% DDGS	0% DDGS	25% DDGS	
Cecum (%)	37.5	37.5	42.9	25.0	0.82
Colon (%)	37.5	37.5	42.9	50.0	0.71
RAMS (%)	37.5	25.0	42.9	62.5	0.73
Rectum (%)	62.5	37.5	28.6	50.0	0.71
Rumen (%)	37.5	25.0	14.3	25.0	0.82

*Effect of DDGS, $P > 0.90$; Effect of Dry-rolled Corn (DRC); $P > 0.97$; Interaction, DDG*DRC, $P > 0.504$.

E. coli is naturally present in the gastrointestinal tract and feces of cattle, but at low prevalence and concentration. After the dosing of the animal with Nal^R *E. coli* O157:H7, we observed a decrease of the *E. coli* concentration throughout the experiment which was not influenced by any of the diets. Our results correlate with previous findings showing that, following dosing, *E. coli* O157:H7 transit through the intestinal tract and is excreted by the animals allowing only low doses to persist over time in the gastrointestinal tract.

Our first objective was to compare fecal shedding and gastrointestinal tract colonization by *E. coli* O157:H7 in cattle fed steam-flaked corn (SFC) with or without DDGS. Based on previous publications, we hypothesized that feeding DDGS would increase the *E. coli* O157:H7 shedding in feces, as well as its colonization within the intestinal tract. By the end of the 40-day experimental period, there were no significant differences in the concentration of *E. coli* O157:H7 between animals receiving DDGS and the one receiving only SFC. Neither the prevalence of *E. coli* O157:H7 positive samples, nor the concentration of the bacteria in the positive samples, were affected by the presence or absence of DDGS in the diets. These observations concur with the absence of statistical differences in the colonization rate and prevalence of *E. coli* O157:H7 in the gastrointestinal tract between the two groups of animals. There was no relationship between the use of DDGS in the diet and the level of *E. coli* O157:H7 shedding in cattle in this study. Our second objective was to evaluate the addition of dry-rolled corn in the diet, as a pre-harvest strategy. Previous studies showed that change in grain processing can influence the shedding of *E. coli* O157:H7 in cattle (9, 16). By dry-rolling the corn, we thought that the starch content reaching the hindgut will be increased, inducing an increase of fermentative activity and acid production, producing less favorable condition of growth and shedding

for *E. coli* O157:H7. However, our results revealed no significant differences in the prevalence of *E. coli* O157:H7 in feces or in the gastrointestinal tract between animals receiving, or not, DRC. The concentrations of *E. coli* O157:H7 in positive samples were not different either. Once again, our results were in opposition with previous findings and our hypothesis.

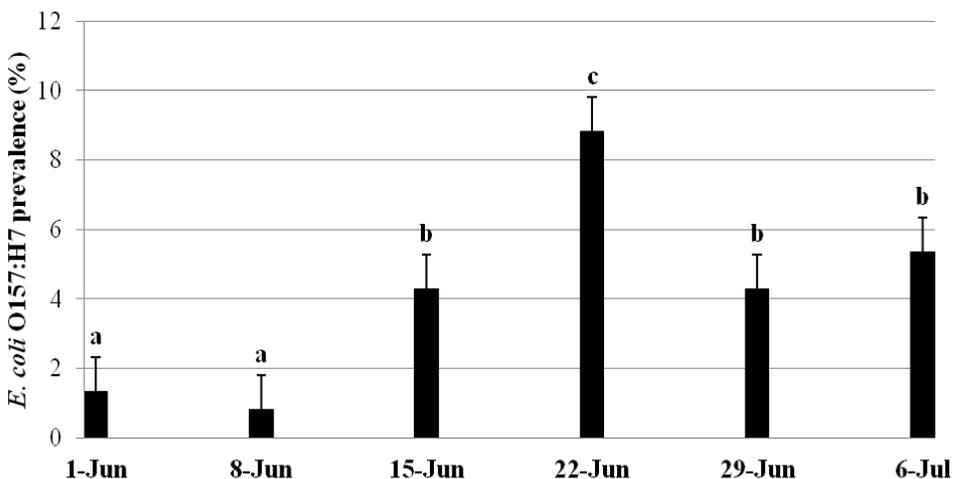
The influence of diet type and diet processing on *E. coli* O157:H7 shedding has been broadly studied and results have not always been consistent. The transient occurrence of *E. coli* O157:H7 and its low prevalence are possible explanations for this inconsistency, as well as many other factors, such as age, seasons and, animal stress. With so many variation sources, accurate results are challenging to obtain and require a large number of replications and animals.

In conclusion, shedding of *E. coli* O157:H7, under the conditions of our Trial 1, was not influenced by the presence of DDGS in the diet. The use of DRC as a preharvest intervention to reduce the carriage of *E. coli* O157:H7 in cattle was not effective.

Trial 2.

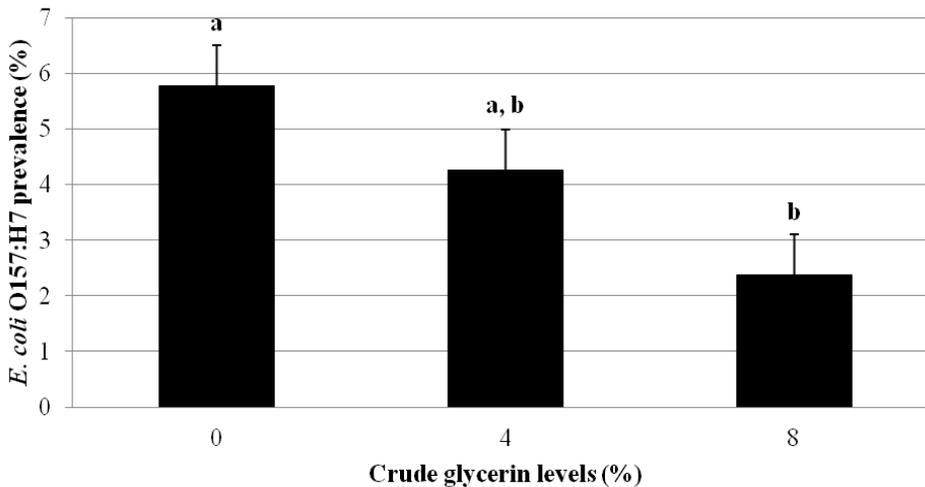
The crude glycerin used in this experiment contained 81.5% glycerol. After statistical analysis, we concluded that there was no interaction between sampling date and the levels of crude glycerin ($P>0.2$). There was, however, an effect of sampling date ($P<0.01$), as shown in Figure 4. For the two first weeks, percentages of samples that tested positive for *E. coli* O157:H7 were 1.3 and 0.8% respectively. Prevalence increased to peak of 8.8% during the 4th week, and then stabilized at around 4.3-5.8% in the last two weeks of the experiment.

Figure 4: *E. coli* O157:H7 prevalence in growing cattle fed crude glycerin



We also observed an effect of glycerin inclusion levels ($P < 0.01$, Figure 5). 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 0% glycerin was different from that of cattle fed 8% glycerin ($P < 0.05$), and tended to be different from cattle fed 4% glycerin ($P = 0.06$). 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.

Figure 5: Effect of crude glycerin levels fed to growing cattle on *E. coli* O157:H7 prevalence



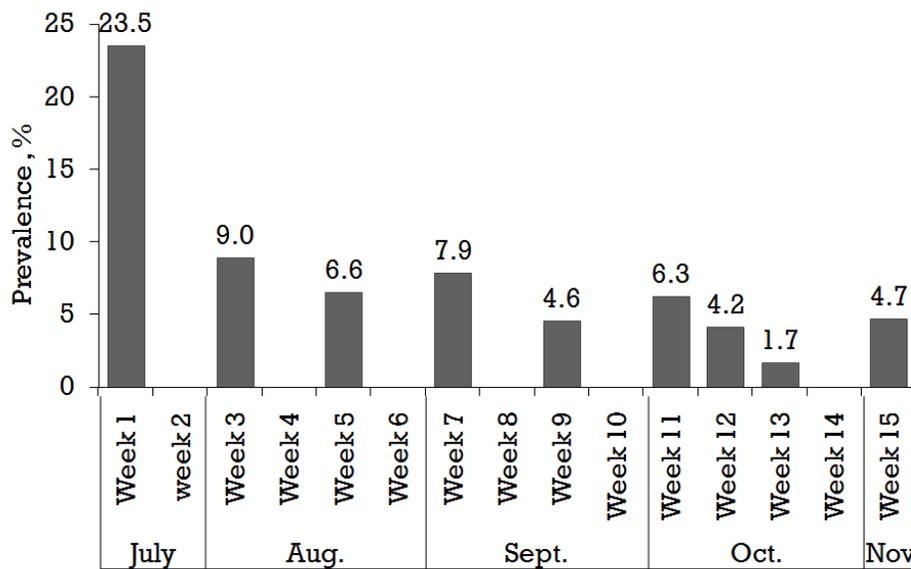
Our goal in this study was to determine whether or not glycerin could be added to cattle diets to decrease shedding of food-borne pathogens. Our results demonstrated that with increasing levels of crude glycerin the prevalence decreased. We find this observation to be of interest, as glycerin is a secondary metabolite of yeast and normally is found in significant quantities in distillers grain. This suggests that that glycerin is not the component within DDGS responsible for the increase in *E. coli* O157:H7, and that glycerin may actually be used as a means to decrease fecal prevalence of this pathogen in cattle.

TRIAL 3:

Overall, fecal prevalence of *E. coli* O157:H7 was 7.6% (327 of 4,300 fecal samples), ranging from 23.6% on the first collection (July) to 1.6% on the eighth collection day (October; Figure 6). The multiplex PCR analyses revealed that all isolates ($N = 327$) were positive for *eae*, *stx2*, *hly*, *rfbE* and *fliC*, and only 14% of the isolates (47/327) were positive for *stx1*. There were no two- or three-way interactions observed between monensin, urea and ractopamine treatment; however, there was a

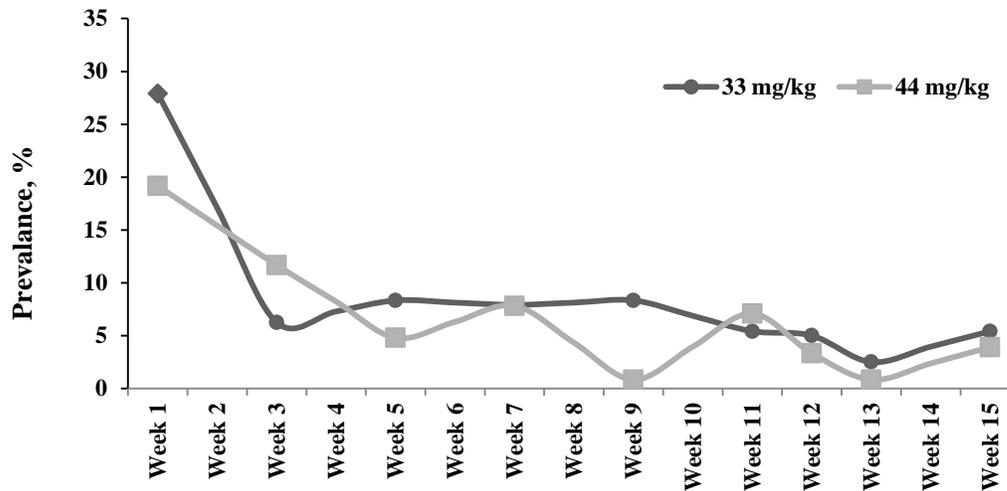
tendency for interaction between monensin and sampling week ($P = 0.06$), indicating that *E. coli* O157:H7 prevalence between monensin treatments tended to vary by sampling week. Fecal shedding of *E. coli* O157:H7 in cattle is influenced by a number of factors, including diets and dietary additives fed to the animal (Callaway et al., 2009; Jacob et al., 2009). It is believed that dietary ingredients that flow into the hindgut and alter the microbial population and fermentation products are likely to have an impact persistence and fecal shedding of *E. coli* O157:H7 (Jacob et al., 2009).

Figure 6. Overall fecal prevalence of *Escherichia coli* O157:H7 in cattle fed steam-flaked corn grain based diet supplemented with 30% wet distillers grains ($n = 48$ pens; SEM = 0.7)



Cattle fed monensin at 44 mg/kg of feed had lower ($P = 0.05$) *E. coli* O157:H7 prevalence than cattle fed 33 mg/kg (4.3 vs 6.8%, Figure 7). Previous studies generally examined monensin at 33 mg/kg and compared it to a control group that lacked monensin in the diet. Van Baale et al., (2004) in a challenge study ($n = 12$) fed at a slightly higher dose of 39.6 mg/kg, found no difference in fecal *E. coli* O157 over a 63-d study period, but observed an initial decrease (first 5 d) in fecal concentration of *E. coli* O157 in monensin-fed cattle on a grain diet, but not in forage-fed animals. McAllister et al. (2006) observed no difference in fecal shedding of *E. coli* O157 (66 vs. 71%) when cattle ($n = 32$) were fed monensin at 0 or 33 mg/kg in the diet. Jacob et al., (2008) in a natural prevalence study found no differences ($P = 0.8$) in fecal *E. coli* O157:H7 between cattle fed diets with 0 and 33 mg/kg monensin. Although monensin is not generally inhibitory to gram negative bacteria, some are susceptible to high concentrations (Nagaraja and Taylor, 1987). *In vitro* studies have shown that monensin at concentrations equal to (Bach et al., 2002) or higher (Van Baale et al., 2004) than concentrations expected in the rumen when 33 mg/kg of monensin is fed did not affect the growth of *E. coli* O157:H7. Because a substantial proportion of monensin passes through the intestinal tract intact, it is likely that the reduction in fecal shedding of *E. coli* O157:H7 observed with the higher dose of monensin is because of changes in the microbial populations and fermentation products in the hindgut. The effects of monensin on hindgut fermentation, such as increased propionic acid, are similar to that described in the rumen (Yokoyama et al., 1985; Marounek et al., 1990).

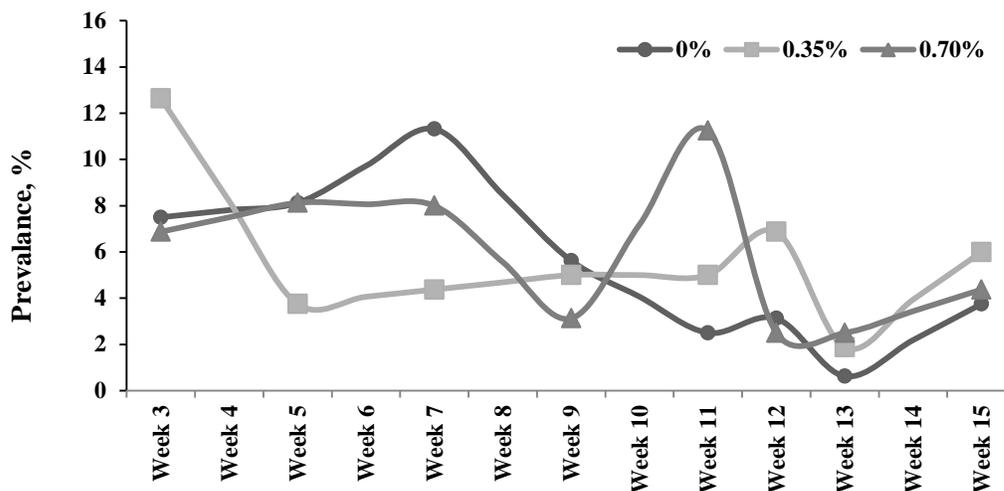
Figure 7. Fecal prevalence of *Escherichia coli* O157:H7 in steers fed steam-flaked corn grain-based diet with 30% wet sorghum distillers grains and supplemented with monensin at 33 or 44 mg/kg of feed (n



= 24 pens/treatment; SEM = 1.2).

Supplemental urea at 0.35 or 0.7 % had no effect ($P = 0.87$) on fecal shedding of *E. coli* O157:H7. Fecal prevalence of *E. coli* O157:H7 were 5.3, 5.7, and 5.9 % for groups fed 0, 0.35 and 0.7% urea, respectively (Figure 8). Distillers grains, which contain little starch (< 5% in DG vs. >70% in corn, Belyea et al., 2004) and approximately three times higher lipid, protein, and fiber contents than corn grain (Spiehs et al., 2002), have been shown to increase *E. coli* O157:H7 prevalence in cattle (Jacob et al., 2008; Wells et al., 2009). Increased flow of lipid, protein and fiber to the hindgut are likely to alter ecology of the hindgut, which may have an impact on persistence of *E. coli* O157:H7. Increased flow of lipid into the hindgut may have a negative effect on *E. coli* O157:H7 because of the antibacterial effects of the released free fatty acids (Annamalai et al., 2004). It is possible that lipid may get digested and absorbed from the small intestine and not flow in sufficient amounts into the hindgut to have a negative effect. Dietary fiber is known to alter the physiology and stimulate bacterial growth in the human colon (Cummings and Stephan, 1980). Possibly, increased flow of fiber into the hindgut stimulates mucus production, which may be stimulatory to *E. coli* O157:H7 growth (Fox et al., 2009). Because DG contain less degradable protein (Klopfenstein et al., 2008), ruminal availability of nitrogen may be limiting, which may hinder fiber (Firkins et al., 1986) or starch (Fron et al., 1996) digestion in the rumen. Provision of supplemental ruminal ammonia as urea may enhance ruminal fermentation and decrease flow of fiber to the hindgut. Firkins et al. (1986) demonstrated that diets containing DG had less ruminal NDF digestion compared to a diet containing dry corn gluten feed, but the total tract NDF digestion was greater for DG diet suggesting greater hindgut fermentation. Based on *in vitro* and *in vivo* studies, Fron et al. (1996) demonstrated that inclusion of condensed distillers byproducts altered microbial activities (increased ruminal populations of starch and lactate utilizing bacteria) and increased the rate of fermentation of lactic acid, however, ruminal pH was unaffected. Varying levels of urea, which could potentially improve ruminal fiber digestion, did not affect fecal prevalence of *E. coli* O157:H7 in this study. It is possible that ammonia was not a limiting factor, hence urea supplementation did not have an effect fecal shedding of *E. coli* O157:H7. Increased availability and degradation of protein in the hindgut and therefore, increased ammonia production and elevated pH may favor persistence of *E. coli* O157:H7.

Figure 8. Fecal prevalence of *Escherichia coli* O157:H7 in steers fed steam-flaked corn grain-based diet with 30% wet sorghum distillers grains and supplemented with urea at 0, 0.35, or 0.70 % of the diet (n



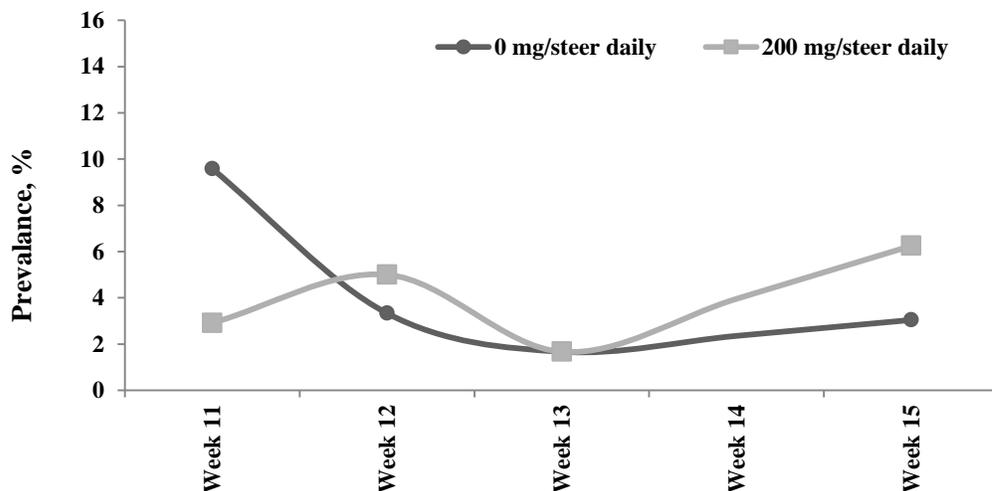
= 16 pens/treatment SEM = 1.2).

The inclusion of ractopamine at 0 or 200 mg/head/d had no effect ($P = 0.89$) on fecal prevalence of *E. coli* O157:H7 (4.4 vs 4.0 %; Figure 9). However, the power to detect an effect was low due to low *E. coli* O157:H7 prevalence in the final phase of the study. The mean fecal prevalence in the last four samples (collected in the month of October) was 4.2% compared to a mean of 10.3% in the first five samples (collected in July, August, and September). The reduction in prevalence in October is likely because of seasonal influence on fecal shedding of *E. coli* O157:H7 (Edrington et al., 2006a; Fernández et al., 2009). Ractopamine, a beta-agonist, is used to accelerate gain and improve efficiency in cattle during the final 4 to 6 wk of the finishing phase (Schroeder et al., 2003; Laudert et al., 2004). Ractopamine may potentially interact with the bacterial quorum-sensing systems (Sperandio et al., 2003) to affect *E. coli* O157:H7 prevalence. Studies have shown that the neuroendocrine environment of the gastrointestinal tract, particularly norepinephrine released by the enteric nervous system, has influence on bacterial growth, and in certain pathogens, including *E. coli* O157:H7, expression of virulence factors (Lyte et al., 1997). Edrington et al. (2006b) conducted a study with a small number of cattle (n=20) that were administered 0 or 20 mg of ractopamine daily for 28 d and observed a reduction in fecal shedding ($P = 0.006$) with ractopamine (51.2 vs 41.8%, for 0 and 20 mg ractopamine, respectively). In another study (Edrington et al., 2006b) with 1,800 feedlot heifers, in three replicates, ractopamine fed at 200 mg/animal daily for 28 d lowered ($P = 0.05$) *E. coli* O157:H7 prevalence (8.5 vs 13.8%) in feces compared to control heifers fed no ractopamine. However, there were no significant differences in two of the three replicates. Recently, Edrington et al., (2009) used 504 feedlot heifers in

a 2 x 3 factorial design, with ractopamine included at 0 or 200 mg/heifer daily for 14, 28, or 42 d. Fecal samples were obtained at day 0 and then at days 14, 28, or 42 corresponding to each treatment period. There were no significant differences in prevalence among treatments.

Figure 9. Fecal prevalence of *Escherichia coli* O157:H7 in steers fed steam-flaked corn grain-based diet with 30% wet sorghum distillers grains and supplemented daily with ractopamine at 0 or 200 mg/steer (n = 24 pens/treatment; SEM = 1.2).

In conclusion, results of trial 3 suggest inclusion of supplemental urea in the diet had no effect on fecal shedding of *E. coli* O157:H7 in cattle. Our premise in testing the effect of supplemental urea was the potential effects of altered ruminal and possibly hindgut fermentation on *E. coli* O157:H7. Because we did not monitor ruminal or hindgut fermentation changes, we have no evidence that fermentation was altered by feeding supplemental urea. Similarly, the low prevalence of *E. coli* O157:H7 during ractopamine feeding precluded us from assessing the impact of ractopamine. The reduction in fecal shedding of *E. coli* O157:H7 associated with feeding the high dose of monensin is interesting and somewhat surprising considering that prior studies (although with lower doses) had shown no effect. Therefore, additional research is needed to confirm the reduction in fecal shedding of *E. coli* O157:H7 in cattle fed 44 mg/kg monensin.



LITERATURE CITED

- Annamalai, T., M. K. M. Nair, P. Marek, P. Vasudevan, D. Schreiber, R. Knight, T. Hoagland, and K. Venkitanarayanan. 2004. *In vitro* inactivation of *Escherichia coli* O157:H7 in bovine rumen fluid by caprylic acid. *J. Food Prot.*, 67:884-888.
- Bach, S. J., T. McAllister, D. Veria, and R. Holley. 2002. Effect of monensin on survival and growth of *Escherichia coli* O157:H7 *in vitro*. *Can. Vet. J.* 43:718-719.
- Bai, J., X. Shi, and T. G. Nagaraja. 2010. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. *J. Microbiol. Meth.* 82:85-89.
- Belyea, R. L., K. D. Rausch, and M. E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. *Bioresource Technol.* 94:293-298.
- Berg, J., T. McAllister, S. Bach, R. Stilborn, D. Hancock, and J. LeJune. 2004. *Escherichia coli* O157:H7 Excretion by Commercial Feedlot Cattle Fed Either Barley- or Corn-Based Finishing Diets. *J. Food Prot.* 67:666-671.
- Buchko, S. J., R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira. 2000. The Effect of Different Grain Diets on Fecal Shedding of *Escherichia coli* O157:H7 by Steers. *J. Food Prot.* 63:1467-1474.
- Callaway, T. R., M. A. Carr, T. S. Edrington, R. Anderson, and D. Nisbet. 2009. Diet, *Escherichia coli* O157:H7, and cattle: A review after 10 years. *Curr. Iss. Mol. Biol.* 11:67-79.
- Callaway, T. R., R. O. Elder, J. E. Keen, R. C. Anderson, and D. J. Nisbet. 2003. Forage Feeding to Reduce Preharvest *Escherichia coli* Populations in Cattle, a Review. *J. Dairy Sci.* 86:852-860.
- Cummings, J. H., and A. M. Stephen. 1980. The role of dietary fibre in the human colon. *Can. Med. Assoc. J.* 123:1109-1114.

- Dewell, G. A., J. R. Ransom, R. D. Dewell, K. McCurdy, I. A. Gardner, A. E. Hill, J. N. Sofos, K. E. Belk, G. C. Smith, and M. D. Salman. 2005. Prevalence of and Risk Factors for *Escherichia coli* O157 in Market-Ready Beef Cattle from 12 U.S. Feedlots. *Foodborne Pathog Dis.* 2:70-76.
- Edrington, T. S., T. R. Callaway, S. E. Ives, M. J. Engler, M. L. Loooper, R. C. Anderson, and D. J. Nisbet. 2006a. Seasonal shedding of *Escherichia coli* O157:H7 in ruminants: A new hypothesis. *Foodborne Path. Dis.* 3:413-421.
- Edrington, T.S., T. R. Callaway, S. E. Ives, M. J. Engler, T. Welsh, D. Hallford, K. Genovese, R. A. Anderson, and D. J. Nisbet. 2006b. Effect of ractopamine HCL supplementation on fecal shedding of *Escherichia coli* O157:H7 and salmonella in feedlot cattle. *Curr. Microbiol.* 53:340-345.
- Edrington, T. S., R. L. Farrow, G. H. Loneragan, S. E. Ives, M. J. Engler, J. J. Wagner, M. J. Corbin, W. J. Platter, D. Yates, J. P. Hutcheson, R. A. Zinn, T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2009. Influence of α -agonists (ractopamine HCl and zilpaterol HCl) on fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle. *J. Food Prot.* 72:2587-2591.
- Fernández, D., E. M. Rodríguez, G. H. Arroyo, N. L. Padola, and A. E. Parma. 2009. Seasonal variation of Shiga toxin-encoding genes (*stx*) and detection of *E. coli* O157 in dairy cattle from Argentina. *J. Appl. Microbiol.* 106:1260-1267.
- Firkins, J. L., L. L. Berger, N. R. Merchen, and G. C. Fahey, Jr. 1986. Effects of forage particle size, level of feed intake and supplemental protein degradability on microbial protein synthesis and site of nutrient digestion in steers. *J. Anim. Sci.* 62:1081-1094.
- Fox, J. T., J. S. Drouillard, X. Shi and T. G. Nagaraja. 2009. Effects of mucin and its carbohydrate constituents on *Escherichia coli* O157 growth in batch culture fermentations with ruminal or fecal microbial inoculums. *J. Anim. Sci.* 87:1304-1313.
- Fron, M., H. Madeira, C. Richards, and M. Morrison. 1996. The impact of feeding condensed distillers byproducts on rumen microbiology and metabolism. *Anim. Feed Sci. Technol.* 61:235-245.

- Greenquist, M. A., J. S. Drouillard, J. M. Sargeant, B. E. Depenbusch, X. Shi, K. F. Lechtenberg, and T. G. Nagaraja. 2005. Comparison of Rectoanal Mucosal Swab Cultures and Fecal Cultures for Determining Prevalence of *Escherichia coli* O157:H7 in Feedlot Cattle. *Appl. Environ. Microbiol.* 71:6431-6433.
- Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain, and R. P. Huffman. 1994. Wet Corn Distillers Byproducts Compared with Dried Corn Distillers Grains with Solubles as a Source of Protein and Energy for Ruminants. *J. Anim. Sci.* 72:3246-3257.
- Heeringa, S., B. West, and P. Berglund. 2010. *Applied survey data analysis*. Chapman & Hall/CRC., Boca Raton, FL.
- Jacob, M. E., T. R. Callaway, and T. G. Nagaraja. 2009. Dietary interactions and interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. *Foodborne Path. Dis.* 6:785-792.
- 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.
- Kleinschmit, D. H., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of various sources of corn dried grains plus solubles for lactating dairy cattle. *J. Dairy Sci.*, 89:4784-4794.
- Klopfenstein T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-invited review: use of distillers by-products in the beef cattle feeding industry. *J. Anim. Sci.* 86:1223–1231.
- Laudert, S. B., G. L. Vogel, A. L. Schroeder, W. J. Platter, and M. T. Van Koevinger. 2004. The Effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx Exchange No. 4. Elanco Animal Health, Greenfield, IN.
- Lodge, S. L., R. A. Stock, T. J. Klopfenstein, D. H. Shain, and D. W. Herold. 1997. Evaluation of corn and sorghum distillers byproducts. *J. Anim. Sci.*, 75:37-43.

- Lyte, M., A. K. Erickson, B. P. Arulanandam, C. D. Frank, M. A. Crawford, and D. H. Francis. 1997. Norepinephrine induced growth and expression of virulence associated factors in enterotoxigenic and enterohemorrhagic strains of *Escherichia coli*. *Adv. Exp. Med. Biol.*, 412:331-339.
- Marounek, M., O. Petr, and L. Machanova. 1990. Effect of monensin on *in vitro* fermentation of maize starch by hindgut contents of cattle. *J. Agric. Sci. Camb.* 115:389-92.
- McAllister, T. A., S. J. Bach, K. Stanford, and T. R. Callaway. 2006. Shedding of *Escherichia coli* O157:H7 by cattle fed diets containing monensin or tylosin. *J. Food Prot.* 69:2075-2083.
- NADA 095-735, Freedom of Information Summary, Supplemental New Animal Drug Applications. 2006. <http://www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/FOIADrugSummaries/ucm064848.pdf> Accessed on 12/16/2009.
- Nagaraja, T. G. and M. B. Taylor. 1987. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. *Appl. Environ. Microbiol.* 53:1620-1625
- Naylor, S. W., J. C. Low, T. E. Besser, A. Mahajan, G. J. Gunn, M. C. Pearce, I. J. McKendrick, D. G. E. Smith, and D. L. Gally. 2003. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. *Infect. Immun.* 71:1505-1512.
- Rangel, J. M., P. H. Sparling, C. Crowe, P. M. Griffin, and D. L. Swerdlow. 2005. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* 11:603-609.
- Schroeder, A. L., D. M. Polser, S. B. Laudert, and G. J. Vogel. 2003. The effect of Optaflexx on growth performance and carcass traits of steers. Optaflexx Exchange No. 1. Elanco Anim. Health, Greenfield, IN.
- Sperandio, V., A. G. Torres, B. Jarvis, J. P. Nataro, and J. B. Kaper. 2003. Bacteria-host communication: The language of hormones. *Proc. Nat. Acad. Sci. U. S. A.* 100:8951-8956.

- Spiehs, M. J., M. H. Whitney, and G. C. Shurson. 2002. Nutrient database from distillers dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J. Anim. Sci.* 80:2639-2645.
- Van Baale, M. J., J. M. Sargeant, D. P. Gnad, B. M. DeBey, K. F. Lechtenberg, and T. G. Nagaraja. 2004. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. *Appl. Environ. Microbiol.* 70:5336-5342.
- Walker, C., X. Shi, M. Sanderson, J. Sargeant, and T. G. Nagaraja. 2010. Prevalence of *Escherichia coli* O157:H7 in gut contents of beef cattle at slaughter. *Foodborne Path. Dis.* 7:249-255.
- 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. Freetly, 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.
- Yokoyama, M. T., K. A. Johnson, P. S. Dickerson, and W. G. Bergen. 1985. Effect of dietary monensin on the cecal fermentation of steers. *J. Anim. Sci.* 61:469 (Abstr.).