Influence of genotype and diet on steer performance, manure odor, and carriage of pathogenic and other fecal bacteria. II. Pathogenic and other fecal bacteria[1,2]

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ABSTRACT: This study assessed the influence of cattle genotype and diet on the carriage and shedding of zoonotic bacterial pathogens and levels of generic *Escherichia coli* in feces and ruminal contents of beef cattle during the growing and finishing periods. Fifty-one steers of varying proportions of Brahman and MARC III [0 (15), ¼ (20), ½ (7), and ¾ Brahman (9)] genotypes were divided among 8 pens, such that each breed type was represented in each pen. Four pens each were assigned to 1 of 2 diets [100% chopped bromegrass hay or a diet composed primarily of corn silage (87%)] that were individually fed for a 119-d growing period, at which time the steers were switched to the same high-concentrate, corn-based finishing diet and fed to a target weight of 560 kg. Feces or ruminal fluid were collected and analyzed at alternating intervals of 14 d or less. Generic *E. coli* concentrations in feces or ruminal fluid did not differ ($P > 0.10$) by genotype or by growing diet in the growing or finishing periods. However, the concentrations in both feces and ruminal fluid increased in all cattle when switched to the same high-corn diet in the finishing period. There was no effect ($P > 0.25$) of diet or genotype during either period on *E. coli* O157 shedding in feces. Forty-one percent of the steers were positive for *Campylobacter* spp. at least once during the study, and repeated isolations of *Campylobacter* spp. from the same steer were common. These repeated isolations from the same animals may be responsible for the apparent diet ($P = 0.05$) and genotype effects ($P = 0.02$) on *Campylobacter* in feces in the finishing period. Cells bearing *stx* genes were detected frequently in both feces (22.5%) and ruminal fluid (19.6%). The number of *stx*-positive fecal samples was greater ($P < 0.05$) for ½ Brahman steers (42.9%) than for ¼ Brahman (25.0%) or ¾ Brahman steers (22.2%), but were not different compared with MARC III steers (38.3%). The greater feed consumption of ½ Brahman and MARC III steers may have resulted in greater starch passage into the colon, accompanied by an increase in fecal bacterial populations, which may have further improved the ability to detect *stx* genes in these cattle. There was no correlation between either ADG or daily DMI and the number of positive samples of *E. coli* O157, *Campylobacter* spp., or *stx* genes, which agrees with our current understanding that these microorganisms occur commonly in, and with no measurable detriment to, healthy cattle.

Key words: beef cattle, *Bos indicus*, *Campylobacter*, *Escherichia coli* O157, forage, grain

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INTRODUCTION

Maximizing animal performance through improved genetics and nutrition has been a primary effort of animal production research. Recently, there have been concerns about the environmental effects of cattle production practices, which are focused primarily on nutrient and bacterial contamination and emissions issues associated with manure accumulation and handling. Cattle nutrition has been shown to be a valuable part of the solution to the problems of excess nitrogen and phosphorus excretion (Klopfenstein and Erickson, 2002; Satter et al., 2002). Whereas it is likely that modifying animal diets will play a role in minimizing the problems associated with fecal bacteria excretion and odor, there is no agreement yet on the effects of cattle diets on the prevalence and shedding of *Escherichia coli* O157 and other zoonotic pathogens (Bach et al., 2002; Callaway et al., 2003; Vanselow et al., 2005). Human waterborne disease caused by *E. coli* O157 and *Campylobacter je-*

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juni has been linked to water supply contamination with runoff containing bovine manure (O'Connor, 2002). Furthermore, foodborne illness caused by *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. is commonly associated with consumption of undercooked beef and raw milk (CDC, 1993, 2002, 2006). An objective of these experiments was to assess the influence of bovine genotype and diet on the prevalence of *E. coli* O157, *Salmonella* spp., and *Campylobacter* spp. in growing-finishing beef cattle, and the numbers of generic *E. coli* in ruminal contents and feces of these cattle. There is no information regarding differences in carriage of bacterial pathogens by *Bos taurus* vs. *Bos indicus* cattle. Among other differences, *Bos indicus* cattle are more resistant to some parasites than *Bos taurus* cattle (Frisch, 1976; Frisch et al., 2000). The influences of genotype and diet on steer performance and manure odor compound production are addressed in the companion articles (Ferrell et al., 2006; Miller et al., 2006).

MATERIALS AND METHODS

Animals, Feeding, and Sample Collection

All animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee.

Breeding, management, feeding, and the experimental diets of the 51 steers utilized for this study are described in detail in the accompanying article by Ferrell et al. (2006). The 4 genotypes examined were of varying proportions of Brahman and MARC III (*Bos taurus*) genotypes, with 15, 20, 7, and 9 animals of 0, ¼, ½, and ¾ Brahman, respectively. The MARC III cattle are a composite population composed of ¼ each of Hereford, Angus, Pinzgauer, and Red Poll. At 28 d after weaning, approximately equal numbers of each breed type were allotted among 8 pens, to become adjusted to using individual Calan-Broadbent electronic headgates (American Calan, Inc., Northwood, NH) over a 28-d period.

During the entire 56-d postweaning period, all steers were fed a 50:50 blend of the 2 experimental grower diets. One diet was 100% chopped bromegrass hay and the other diet was primarily corn silage (87%; Ferrell et al., 2006). At the end of the 56-d postweaning period, the steers were divided approximately equally between the 2 diets, with 4 pens assigned to each diet. Steers were individually fed 1 of these 2 diets ad libitum for a 119-d growing period, then all animals were gradually switched over a 2-wk interval to the same finishing diet, which was composed primarily of 70% ground corn and 24% corn silage (Ferrell et al., 2006). Steers were fed this diet during the finishing period until they reached a final target BW of 560 kg. Steers were weighed at the beginning and end of the growing period, and at 14-d intervals throughout the growing and finishing periods. Feces and ruminal fluid were collected for microbial analyses through d 196 of the study (d 77 of the finishing period), when the first steers had reached the target BW. These samples were collected before the daily feeding.

Fecal samples were collected directly from the rectum of each steer on d 0, 7, 28, 56, 84, 112, 126, 133, 154, and 196. A clean latex glove covered with a new, clean, shoulder-length glove was donned for collection of each sample. Ruminal fluid samples were collected by stomach tube and vacuum pump from each steer on d 14, 42, 70, 98, 140, and 182. A separate sanitized stomach tube and sterile polypropylene filter flask were used for collection of each ruminal fluid sample. All samples were transported to the laboratory for immediate processing.

Microbial Analyses

For isolation of *E. coli* O157, 5 g of feces or 5 mL of ruminal fluid was placed into a sterile filtered sample bag (Spiral Biotech, Norwood, MA), 45 mL of tryptic soy broth (**TSB**; Becton, Dickinson and Company, Sparks, MD) were added, and the bag contents were blended with a Stomacher 400 Circulator (200 rpm, 1 min; Seward Limited, London, UK). After the removal of a 1- to 2-mL aliquot to a sterile test tube for bacterial population determinations (described below), the remaining sample was enriched by incubation at 37°C for 7 h. After this incubation, an additional 1 mL of each enriched sample was measured into a labeled vial containing 1 mL of sterile TSB containing 0.5% yeast extract and 50% glycerol, which was frozen at –20°C for later *stx* gene PCR, as described below.

One-milliliter volumes of the nonselective enrichments were subjected to immunomagnetic separation and concentration as described by Berry and Miller (2005) using Dynabeads anti-*E. coli* O157 (Dynal Biotech ASA, Oslo, Norway). Fifty microliters of the concentrated bead suspensions was plated onto sorbitol MacConkey agar plates containing 0.05 μg of cefixime/mL and 2.5 μg of potassium tellurite/mL, and plates were incubated at 37°C for 20 to 22 h before examination for characteristic sorbitol-negative *E. coli* O157 colonies. Suspect colonies were tested for agglutination with *E. coli* O157 latex test reagents (Oxoid Ltd., Basingstoke, UK). Latex-agglutinating isolates were further confirmed as *E. coli* by biochemical testing (Hitchins et al., 1998) and as *E. coli* O157 by PCR screening for the genes stx_1, stx_2, *eaeA*, EHEC *hlyA*, and $rfbE_{O157}$ (Paton and Paton, 1998).

For isolation of *Campylobacter* spp., 1 swabful of feces (approximately 1 g) or 1 mL of ruminal fluid was placed into 13.5 mL of Bolton selective enrichment broth (Oxoid), mixed by inversion, and then incubated at 37°C for 4 h, followed by incubation at 42°C for 20 h. Twenty microliters of this enrichment was streaked onto Campy-Cefex agar plates (made according to Stern et al., 1992), which were incubated microaerobically at 42°C for 48 h in anaerobic jars containing CampyGen (Oxoid). Characteristic colonies were confirmed serolog-

ically using PanBio-Campy (jcl) latex agglutination reagents (PanBio, Inc., Columbia, MD). These presumptive *Campylobacter* spp. were further confirmed and speciated by hippurate hydrolysis testing and by PCR screening for genes specific for thermophilic *Campylobacter* spp., *Campylobacter jejuni*, or *Campylobacter coli* (Eyers et al., 1993; Fermér and Engvall, 1999; Cloak and Fratamico, 2002).

The experimental samples were examined for the presence of *Salmonella* spp. utilizing method 2 described in Davies et al. (2000), using 1 swabful of feces or 1 mL of ruminal fluid. Suspect colonies were tested for agglutination with *Salmonella* latex test reagents (Oxoid) and were further tested for typical reactions on triple sugar iron and lysine iron agar slants after incubation at 37°C for 24 h.

The retained fecal and ruminal fluid enrichments were examined by PCR for determination of the presence of the Shiga toxin genes stx_1 and stx_2. Cells were concentrated from 100 μL of the thawed samples by centrifugation (12,000 × *g*). Cell pellets were resuspended in 400 μL of sterile, distilled, deionized water, concentrated again, and resuspended in 100 μL of sterile, distilled, deionized water. The resulting suspensions were heated at 100°C for 15 min, then frozen at –20°C until used in PCR reactions. Two-microliter volumes of these cell lysates were used as template in 25-μL PCR reactions, using the reaction conditions and primers described by Paton and Paton (1998). Samples positive for *E. coli* O157 were presumed to be positive for *stx* genes. Samples were considered positive for *stx* genes if stx_1, stx_2, or both, were detected after gel electrophoresis.

For enumeration of the fecal bacterial populations, the reserved volumes of the initial 10-fold TSB dilutions of feces or ruminal fluid were serially diluted further in 2% buffered peptone water. For generic *E. coli* and coliforms, 1-mL volumes were plated onto Petrifilm E. coli/coliform count plates (3M Microbiology Products, St. Paul, MN), which were incubated at 37°C for 24 h before counting. For *Enterobacteriaceae*, 1-mL volumes were plated onto Petrifilm Enterobacteriaceae count plates (3M), which were incubated at 37°C for 24 h before enumeration.

The pH was determined from fecal samples diluted 1:6 in distilled, deionized water or from undiluted ruminal fluid samples, using a thin (6 mm), gel-filled pH electrode (Broadley James Corp, Irvine, CA).

Statistical Analyses

Populations of bacteria from duplicate plates were averaged and transformed to $\log_{10}$ colony-forming units per gram of feces or $\log_{10}$ colony-forming units per milliliter of ruminal fluid. For analyses of pathogen prevalence, fecal and ruminal fluid sample data were pooled and analyzed as a binomial distribution. The unit of observation was the individual steer. The model included the fixed effects of diet (bromegrass hay or corn silage), genotype (breed type), diet × genotype, day, genotype × day, and diet × day, with day as a repeated measure [with an AR(1) error structure], and the random effects of pen(diet) and steer × genotype × pen(diet). The MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) was used for analysis of the data. Differences were considered significant at $P < 0.05$ and a tendency was declared when the *P*-value ranged from $P = 0.05$ to $P < 0.10$. Posthoc mean separation was done using a modified Student's *t*-test.

RESULTS AND DISCUSSION

Effects of grain vs. forage diets on the prevalence, shedding, and duration of shedding of *E. coli* O157 or generic *E. coli* by cattle have been the topic of several studies, but there is no agreement on the role that diet may play in these occurrences (as recently reviewed by Bach et al., 2002; Callaway et al., 2003; Vanselow et al., 2005). Reasons for the contradictory conclusions of these various works are likely due all or in part to such differences as the diets examined (different grains and forages), the length of feeding or feed switches (including fasting), the age and type of cattle utilized (adult cattle and young calves; beef and dairy animals), the species utilized (cattle and sheep), and the use of naturally colonized cattle vs. experimentally inoculated cattle. One of our goals for the current study was to conduct a longer term, diet-effect study examining the relevant target population of naturally contaminated finishing beef calves throughout the growing and finishing periods. In addition to determining the influence of genotype and diet on the carriage and shedding of *E. coli* O157, the influences on *Salmonella* spp., *Campylobacter* spp., and other fecal bacteria were also assessed, because limited information is available regarding potential influence of cattle diet on the carriage and shedding on these zoonotic pathogens.

Generic *E. coli* populations in bovine feces did not differ ($P > 0.22$) by steer genotype in either the growing or finishing period (Figure 1). Furthermore, these populations did not differ ($P = 0.11$) by diet in the growing period, and tended to differ ($P = 0.10$) in the finishing period by diet fed in the growing period. However, these generic *E. coli* fecal populations were greater ($P < 0.001$) in the finishing period than in the growing period. In steers fed bromegrass hay and corn silage, mean *E. coli* concentrations during the growing period ranged from 4.99 to 5.56 and 5.21 to 5.94 $\log_{10}$ cfu/g of feces, respectively. These *E. coli* populations increased after the switch from either bromegrass hay or corn silage to the high-concentrate finishing diet, to levels of 6.73 and 6.67 $\log_{10}$ cfu/g of feces, respectively, by d 154. During the growing period, fecal pH was typically 0.5 to 0.6 units lower ($P = 0.002$) for steers fed the corn silage-based diet than for steers fed the bromegrass diet (Figure 1). Regardless of previous diet, fecal pH was lower ($P < 0.03$) after the switch to the high-concentrate finishing diet, ranging from pH 5.3 to 5.9, and no longer

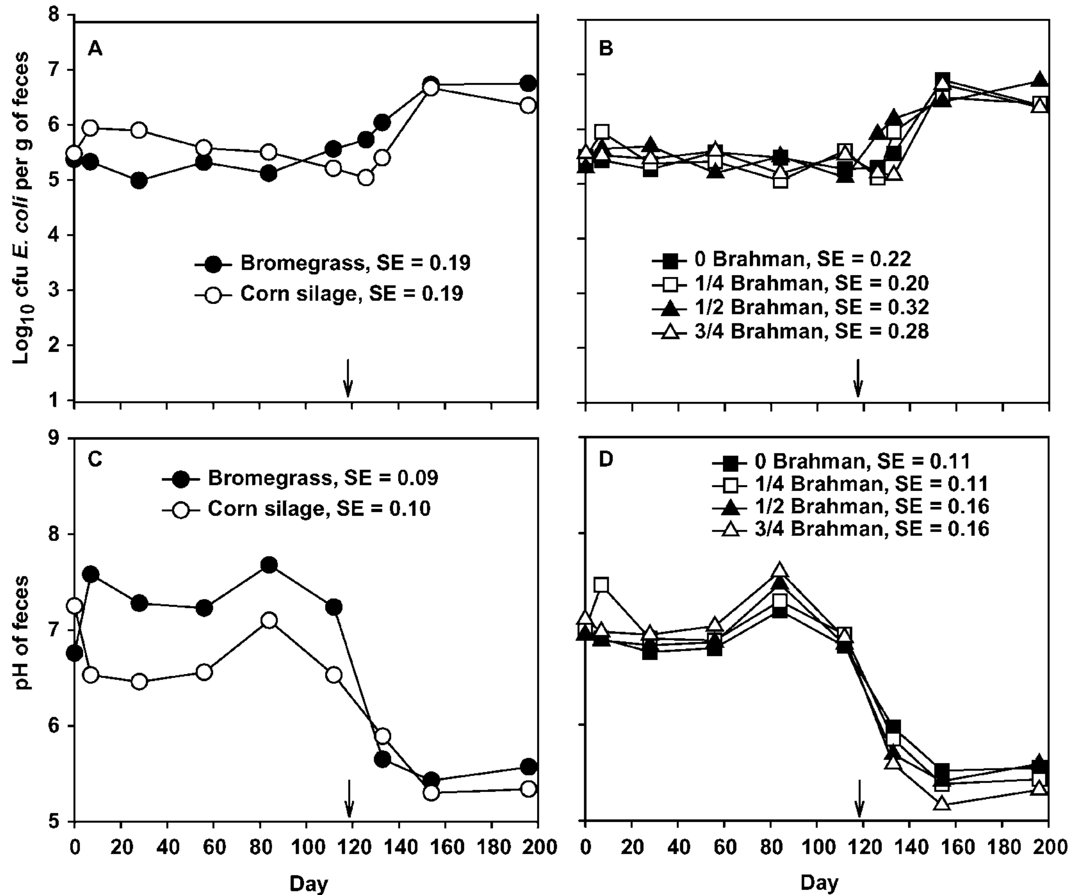

Figure 1. Concentrations of generic *Escherichia coli* in feces (A, B), and pH of feces (C, D) plotted by diet and by genotype (proportion Brahman). One diet was 100% chopped bromegrass hay and the other diet was 87% corn silage. Steers were switched to the same high-concentrate diet (70% corn and 24% corn silage) at 119 d (arrow). The SE noted are the SE of the least squares means.

differed (P = 0.67) between the 2 dietary treatments. These results are similar to those of others reporting greater fecal populations of generic *E. coli* and lower colonic feces pH in cattle fed greater grain diets (Diez-Gonzales et al., 1998; Jordan and McEwen, 1998; Scott et al., 2000), although others have not observed these effects on coliform or *E. coli* populations (Keen et al., 1999). Fecal pH did not differ ($P > 0.16$) by genotype in either the growing or finishing periods.

Similar to results observed for feces, generic *E. coli* concentrations in ruminal fluid did not differ ($P > 0.35$) by diet or by genotype in either period (Figure 2). In addition, *E. coli* populations increased ($P < 0.001$) when switched to the finishing diet by greater than 1.0 log to a mean level of 4.23 $\log_{10}$ cfu/mL of ruminal fluid, from a mean level of 2.93 $\log_{10}$ cfu/mL in the growing period. Unlike fecal pH, the pH of the ruminal fluid did not differ (P = 0.72) between steers fed bromegrass hay or corn silage in the growing period. Similar to fecal pH, however, ruminal fluid pH decreased ($P < 0.001$) after the switch to the finishing diet, from an average pH of 7.31 in the growing period to an average pH of 6.33 in the finishing period (Figure 2). Previous studies have reported that high-concentrate diets, as compared with high forage diets, can result in greater ruminal populations of *E. coli* and lower ruminal pH in cattle (Diez-Gonzales et al., 1998; Tkalcic et al., 2000). Ruminal fluid pH differed (P = 0.01) by genotype in the finishing period. On d 140 and 182, ruminal fluid pH of ½ Brahman steers was greater (6.62 and 6.46, respectively) than that of the MARC III steers (6.19 and 6.11, respectively), but did not differ from the intermediate ruminal fluid pH values of ¼ and ¾ Brahman steers (¼: 6.45 and 6.22; ¾: 6.37 and 6.19, respectively). To our knowledge, breed differences in ruminal pH have not been observed, and may be a reflection of increased turnover rate as a result of relatively high feed intake and reduced rumen size of the ½ Brahman steers (Ferrell and Jenkins, 1998; Ferrell et al., 2006).

In both feces and ruminal fluid, coliform and *Enterobacteriaceae* populations were comprised primarily of generic *E. coli*. As a result, coliform and *Enterobacteriaceae* population responses paralleled those of *E. coli* populations (data not shown).

Escherichia coli O157 was not frequently isolated. From a total of 816 fecal or ruminal fluid samples, *E. coli* O157 was recovered from 24 samples (2.9%), including 21 fecal samples and 3 ruminal fluid samples. How-

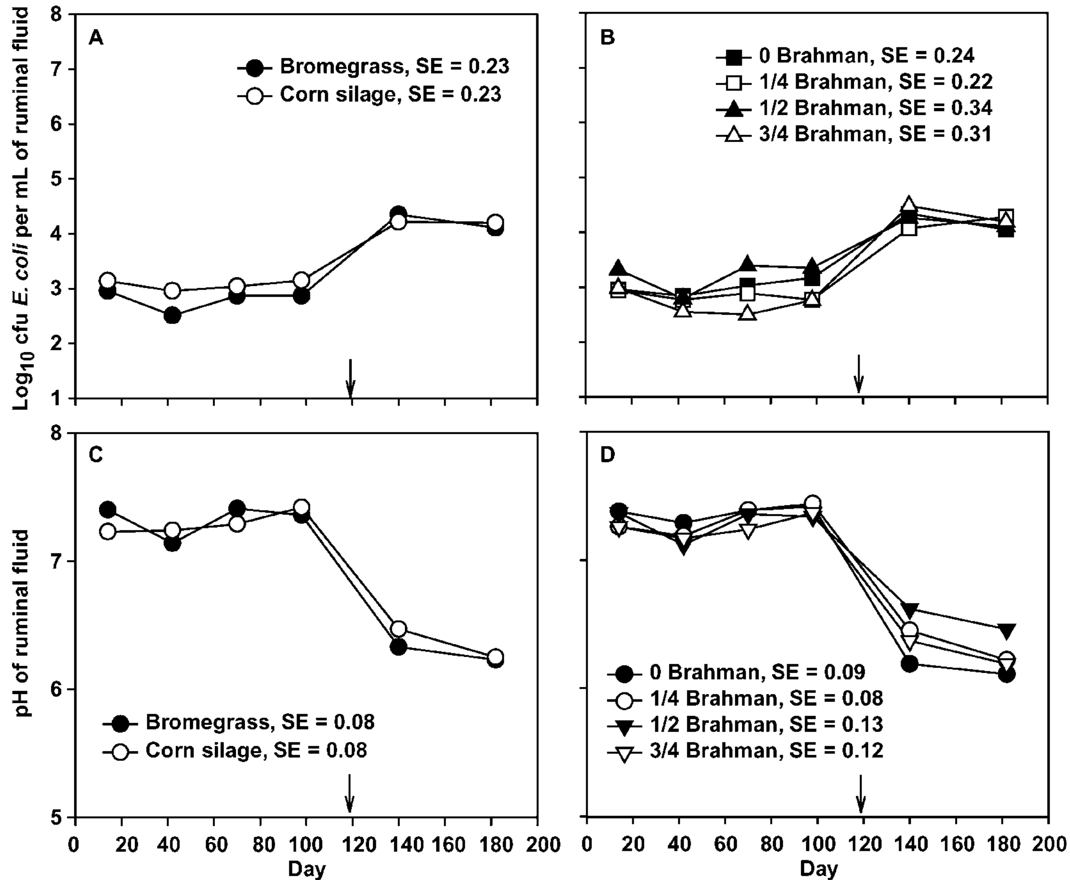

Figure 2. Concentrations of generic *Escherichia coli* in ruminal fluid (A, B), and pH of ruminal fluid (C, D) plotted by diet and by genotype (proportion Brahman). One diet was 100% chopped bromegrass hay and the other diet was 87% corn silage. Steers were switched to the same high-concentrate diet (70% corn and 24% corn silage) at 119 d (arrow). The SE noted are the SE of the least squares means.

ever, over the course of the entire study, 19 of the 51 steers (37%) were *E. coli* O157-positive at least once; *E. coli* O157 was isolated only once from 15 steers, twice from 3 steers, and 3 times from 1 steer. This is in agreement with previous reports that indicate that cattle tend to shed this pathogen intermittently or transiently, although prolonged shedding has been described (Robinson et al., 2004). There was no effect of diet fed during the growing period or genotype ($P \geq 0.25$) on the proportions of *E. coli* O157-positive fecal samples in either the growing or finishing periods (Table 1; because only 3 of the 306 ruminal fluid samples were *E. coli* O157-positive, these samples were not included in the analysis). The proportions of *E. coli* O157-positive fecal samples tended ($P = 0.09$) to be greater in the finishing period. Rather than a response to the switch to the finishing diet, however, this tendency is likely a reflection of the seasonal variation of shedding of this pathogen by cattle. Numerous studies have reported seasonal differences in prevalence of fecal shedding of this pathogen; typically, the lower prevalences are reported in the cooler winter months and the greater prevalences are reported in the warmer summer and early fall months (Van Donkersgoed et al., 1999; APHIS, 2001; Barkocy-Gallagher et al., 2003). The growing period of our study was initiated in early December, with the finishing period beginning in late March and ending in mid June. The isolation rates of *E. coli* O157 of 2.9% of fecal samples collected in the growing period and 5.9% of fecal samples collected in the finishing period are consistent with prevalence previously reported for these times of the year (Van Donkersgoed et al., 1999; APHIS, 2001; Barkocy-Gallagher et al., 2003), and our overall low isolation of this pathogen is likely due to conducting the study during this time of year.

Salmonella was isolated only once during the study, on d 14 in a ruminal fluid sample taken from a ¼ Brahman steer fed bromegrass hay. Previous studies have reported low frequencies of infection of *Salmonella* shedding by feedlot cattle in North America (Fedorka-Cray et al., 1998; Van Donkersgoed et al., 1999; Sorensen et al., 2002).

Campylobacter spp. were isolated from 47 fecal samples and 1 ruminal fluid sample over the course of the entire study (5.9% of 816 samples). Forty-four isolates were identified as *C. jejuni,* and 3 isolates were identified as *Campylobacter coli.* The 3 *C. coli*-positive fecal

Table 1. Prevalence of *Escherichia coli* O157, *Campylobacter* spp., and *stx* genes in feces and ruminal fluid of steers during the growing and finishing periods[1]

Pathogen species or genes (sample type)	Diet[2]		Proportion Brahman[3]				Probability[4]		
	Bromegrass	Corn silage	0	¼	½	¾	D	B	D × B
No. of animals	26	25	15	20	7	9			
Growing period									
E. coli O157 (feces)	1/156 (0.6)	8/150 (5.3)	2/90 (2.2)	4/120 (3.3)	2/42 (4.8)	1/54 (1.9)	0.25	0.84	0.35
Campylobacter spp. (feces)	21/156 (13.5)	10/150 (6.7)	12/90 (13.3)	6/120 (5.0)	8/42 (19.0)	5/54 (9.3)	0.06	0.12	0.23
stx genes[5] (feces)	22/156 (14.1)	30/150 (20.0)	18/90 (20.0)	19/120 (15.8)	7/42 (16.7)	8/54 (14.8)	0.36	0.90	0.77
stx genes (ruminal fluid)	7/104 (6.7)	30/100 (30.0)	11/60 (18.3)	13/80 (16.3)	7/28 (25.0)	6/36 (16.7)	0.29	0.79	0.80
Finishing period									
E. coli O157 (feces)	7/104 (6.7)	5/100 (5.0)	5/60 (8.3)	3/80 (3.8)	2/28 (7.1)	2/36 (5.6)	0.74	0.63	0.31
Campylobacter spp. (feces)	12/104 (11.5)	4/100 (4.0)	6/60 (10.0)	1/80 (1.3)	7/28 (25.0)	2/36 (5.6)	0.05	0.02	0.16
stx genes (feces)	35/104 (33.7)	28/100 (28.0)	23/60 (38.3)	20/80 (25.0)	12/28 (42.9)	8/36 (22.2)	0.31	0.01	0.25
stx genes (ruminal fluid)	14/52 (26.9)	9/50 (18.0)	5/30 (16.7)	11/40 (27.5)	4/14 (28.5)	3/18 (16.7)	0.54	0.51	0.37

[1]Number of positive samples/number of tested samples (percentage positive). Because of the low frequency of isolation of *E. coli* O157 and *Campylobacter* spp. from ruminal fluid (3 samples and 1 sample, respectively), these data are not shown.
[2]Diets were 100% chopped bromegrass hay or primarily corn silage (87%; Ferrell et al., 2006). These 2 diets were fed ad libitum for the 119-d growing period, when all animals were gradually switched to the same finishing diet, which was composed primarily of 70% ground corn and 24% corn silage.
[3]Steers were 0, ¼, ½, and ¾ Brahman, with the remaining proportion from MARC III (¼ each of Hereford, Angus, Pinzgauer, and Red Poll).
[4]Probability that the number of positive samples within the treatment group differed due to diet fed in the growing period (D), proportion Brahman (B), or the 2-way interaction (D × B).
[5]Samples were considered positive for *stx* genes if either stx_1, stx_2, or both were detected.

samples were obtained from 2 steers in the same pen; both steers were positive on d 0, and 1 steer was positive again on d 7. This is consistent with previous information reporting that of these 2 species, *C. jejuni* is more commonly found in cattle than is *C. coli* (Nielsen et al., 1997; Wesley et al., 2000; Bae et al., 2005).

Twenty-one of 51 steers (41%) yielded a *Campylobacter*-positive sample at least once during the study, and many of these animals were positive multiple times. *Campylobacter* was isolated once from 10 steers, twice from 4 steers, 3 times from 3 steers, 4 times from 1 steer, 5 times from 2 steers, and 7 times from 1 steer. These results corroborate those of Inglis et al. (2004), who found that a high percentage of Angus-cross steers repeatedly shed *Campylobacter* spp. over a 4-mo period. There was an effect of genotype ($P = 0.02$) on *Campylobacter* fecal shedding in the finishing period (Table 1). However, this observation should be interpreted with caution. Given the low prevalence of *Campylobacter*-positive samples, this genotype effect is likely due to the presence of "chronic shedders" within genotype groups, namely one ½ Brahman steer with 4 positive samples and 2 MARC III (0 Brahman) steers with 2 positive samples each during the finishing period. Further work would be needed to determine if chronic or protracted *Campylobacter* shedding is linked to breed type.

Similarly, additional research may be warranted to determine if cattle diet affects the duration of *Campylobacter* shedding. In both the growing and finishing periods, the proportion of *Campylobacter*-positive samples tended ($P = 0.06$ and $P = 0.05$, respectively) to be greater for steers fed bromegrass hay during the growing period (Table 1). Steers in pens 4, 6, and 8 were fed the bromegrass hay diet, and these 3 pens had the greatest numbers of *Campylobacter*-positive samples at 9, 13, and 9 positive samples, respectively, over the entire study. Each of these pens contained 1 or more steers that chronically shed this pathogen: pen 4 housed 1 steer with 4 positive samples and 1 steer with 5 positive samples; pen 6 housed 2 steers with 3 positive samples and 1 steer with 5 positive samples; and pen 8 housed 1 steer with 7 positive samples. (Pen 2 steers, also fed bromegrass hay, had 3 *Campylobacter*-positive samples, including 1 steer with 2 positive samples.) Whereas samples from these animals made up the majority of the *Campylobacter*-positive samples from these pens, the persistence of the pathogen within the pen due to chronic shedding would facilitate horizontal transmission to other steers within the pens. Thus, as with the genotype effect on *Campylobacter*-positive fecal samples in the finishing period, the observed diet effect may be due in part to steers providing multiple positive samples. However, it is also possible that the effect is a true diet effect, and that cattle fed bromegrass hay may shed *Campylobacter* spp. for protracted periods. Increased duration of shedding of *E. coli* O157:H7 by cattle fed forage diets compared with high-grain diets of corn or barley has been reported (Hovde et al., 1999; Van Baale et al., 2004). However, it is important

to note that repeat shedders of *Campylobacter* spp. also were present among steers fed the corn silage-based diet, including 4 animals with 2 positive samples each and 1 animal with 3 positive samples. In addition, many of the steers continued to shed after the switch to the greater concentrate finishing diet, suggesting a lack of "true" diet effect. Furthermore, the chronically shedding steers described by Inglis et al. (2004) were fed a high-concentrate diet based on steam rolled barley.

The prevalence of *Campylobacter*-positive fecal samples was not different ($P = 0.64$) in the growing and finishing periods. Although studied less thoroughly than *E. coli* O157:H7, seasonal changes in *Campylobacter* shedding by cattle, with peaks in prevalence occurring both in the spring and fall, have been described (Stanley et al., 1998). Overall, our sample isolation rate of *Campylobacter* spp. was somewhat lower than might be expected, given the reports of other studies in beef cattle. Using PCR detection, Inglis et al. (2004) found during their 4-mo study that 100 and 31.7% of beef steers shed *Campylobacter* spp. and *C. jejuni* at least once, respectively, and that 86% of the steers shed *Campylobacter* spp. 4 times or more. Using cultural isolation procedures, Bae et al. (2005) found 31.6 and 13.3% of feedlot cattle fecal samples positive for *C. jejuni* and *C. coli,* respectively. Stanley et al. (1998) isolated thermophilic *Campylobacter* spp. from the small intestine contents of 89.4% of beef cattle at slaughter. These differences in prevalence may be due to a number of reasons, in addition to the use of different detection methodologies (cultural vs. PCR-based), but it is clear that thermophilic *Campylobacter* spp. are widely distributed in cattle.

Feces and ruminal fluid sample enrichments were examined by PCR for the presence of stx_1 and stx_2 genes, as a means of estimating the incidence of Shiga toxin-producing *E. coli* (**STEC**), including non-O157:H7 STEC, and other Shiga toxin gene-bearing cells, in response to differences in steer genotype and the diet regimens. Whereas *E. coli* O157 is the STEC serotype most commonly associated with food- and waterborne disease, numerous other STEC serotypes, including O26, O55, O111, O145, O103, and O121, have also been linked to human illness (Nataro and Kaper, 1998; Bettelheim, 2003). Although it is clear that not all STEC are pathogenic, it is becoming apparent that STEC, and possibly other *stx*-bearing bacteria, are commonly present in the bovine gastrointestinal tract.

In contrast to either *E. coli* O157 or *Campylobacter* spp., *stx* genes were detected more frequently overall, and were also commonly detected in ruminal fluid (Table 1). Over the entire study, one or both *stx* genes were detected in 115 of 510 fecal samples (22.5%) and 60 of 306 (19.6%) ruminal fluid samples. Fifty of the 51 steers had a *stx*-positive fecal or ruminal fluid sample at least once. These results agree with those of other recent studies that have reported the frequent occurrence of STEC and *stx* genes in beef cattle. In an examination of beef cattle at slaughter, Barkocy-Gallagher et al. (2003) found that 34.3% of fecal samples were positive for *stx* genes by PCR. Of these fecal samples, 19.3 and 5.9% were positive for non-O157 STEC and *E. coli* O157, respectively. Cobbold et al. (2004) reported *stx* gene and STEC prevalences of 16 and 7.3%, respectively, in bovine feces from dairy, feedlot, and cow-calf operations. Shiga toxin-producing *E. coli* were recovered from 16.7% of fecal samples from Australian dairy cattle, including the serotypes of O157:H7 (1.9% of samples) and O26:H11 (1.7% of samples; Cobbold and Desmarchelier, 2000). As noted above, *stx* genes were detected in 60 of the 306 ruminal fluid samples, whereas *E. coli* O157 was detected in only 3 of these samples. Although *E. coli* O157:H7 has been isolated from ruminal contents of both naturally colonized and orally inoculated animals (Van Donkersgoed et al., 1999; Buchko et al., 2000), it is generally understood that when present, like generic *E. coli, E. coli* O157:H7 normally inhabits and proliferates in the bovine lower gastrointestinal tract (Buchko et al., 2000; Grauke et al., 2002; Van Baale et al., 2004). The presence of *E. coli* O157 in the rumen may reflect recent ingestion from food, water, or the environment. However, as we found, generic *E. coli* are typically present in the rumen, at reported concentrations ranging from 10^1 to 10^5 cfu/mL or per g of ruminal fluid (Diez-Gonzales et al., 1998; Jacobson et al., 2002). Thus, the high prevalence of *stx* genes in the rumen, as well as the feces, may simply be a reflection of the wide distribution of STEC or other stx-bearing bacteria in cattle and the production environment. Van Donkersgoed et al. (1999) detected Shiga toxins (verotoxins) more commonly than *E. coli* O157:H7 in ruminal fluid samples from cattle at slaughter, at 6.4 vs. 0.8%.

We note that multiple *stx*-positive samples (either feces or ruminal fluid) were typical of most of the steers. The majority of steers (58.8%) yielded *stx*-positive samples 3 or more times, including 1 steer with 9 positive samples and 3 steers with 8 positive samples each. Additional work would be needed to determine if this was due to chronic shedding, or if this is a reflection of the high prevalence of STEC or *stx* gene-bearing cells among cattle. As reviewed by Bettelheim (2003), there are no effective media or efficient methods for the differential isolation of general STEC, like those specifically available for *E. coli* O157:H7. Isolation of STEC generally has been accomplished by PCR screening of samples for *stx* genes, followed by colony blotting and hybridization procedures. However, recovery of STEC, including non-O157 STEC, from *stx* PCR-positive samples using these procedures can be quite low, with reported recoveries ranging from 38 to 84% (Arthur et al., 2002; Barkocy-Gallagher et al., 2003; Cobbold et al., 2004). For these reasons, we did not attempt the recovery and characterization of non-O157 Shiga toxin-producing cells from *stx*-positive samples. The recent introduction and continued development of additional specific immunological reagents and other useful products should contribute to our understanding of the epi-

demiology and ecology of STEC in cattle (Pearce et al., 2004).

There was no effect of diet or genotype ($P > 0.29$) on the presence of *stx* genes in feces or ruminal fluid in the growing period (Table 1). In the finishing period, there was no effect ($P > 0.31$) of growing period diet on *stx* genes in either feces or ruminal fluid. However, there was an effect of genotype ($P = 0.01$) on *stx*-positive fecal samples in the finishing period. The prevalence of *stx*-positive fecal samples was greater ($P < 0.008$) for ½ Brahman steers (42.9%) than for ¼ Brahman steers (25.0%) or ¾ Brahman steers (22.2%), but were not different ($P = 0.22$) from MARC III (0 Brahman) steers (38.3%). As seen for *Campylobacter,* there was a contribution to this effect from steers providing multiple *stx*-positive samples. Although these multiple samples might be due to chronic infection (which may be animal-specific or bacteria strain-specific), the repeated shedding might also be due to repeated colonization by different *stx*-bearing cells. Thus, it is uncertain if breed type influences either (1) the colonization of cattle by *stx*-bearing cells, or (2) the duration of colonization/shedding by these *stx*-bearing cells, or both. Additional work would be needed to confirm and clarify this effect. Regarding genotype effects on STEC shedding, Riley et al. (2003) noted a possible breed effect on the proportion of *E. coli* O157:H7-positive fecal samples in beef cows, although they qualified that this result may have been confounded by other variables.

Another plausible explanation for this genotype effect on *stx*-positive fecal samples may be related to genotype effects on steer performance. In the companion paper to this work, Ferrell et al. (2006) found that feed consumption (daily DM, CP, and ME intake) and ADG of ½ Brahman and MARC III steers were similar, and were greater than that of ¼ and ¾ Brahman steers, which is consistent with expected greater levels of heterosis for these traits in these animals. In the same fashion that consumption of high-concentrate diets by cattle can result in increased starch in the colon and a concomitant increase in fecal *E. coli* populations (Diez-Gonzales et al., 1998; Jordan and McEwen, 1998; Scott et al., 2000), the increased feed intake by ½ Brahman and MARC III steers may have further increased starch content in colon contents, thus increasing the numbers of *stx*-bearing cells, and thereby improving our ability to detect *stx* genes. This idea is supported by recent results of Gilbert et al. (2005), who found that fecal concentrations of enterohemorrhagic *E. coli* virulence genes, including stx_1, were greater in cattle fed a high grain diet compared with those fed diets of roughage or roughage plus 50% molasses. The effect of increased feed intake and any concomitant increased colonic starch is not reflected in the *E. coli* counts that we found in feces, which did not differ (growing period, $P = 0.97$; finishing period, $P = 0.22$) by genotype. However, *stx* genes do occur in species other than *E. coli* (Lindberg et al., 1998). The relative sensitivity of the PCR protocol has not been established and we used a crude DNA preparation method to provide template for the PCR. However, given the sensitivity of PCR, only subtle increases in the populations of the target cells containing the *stx* genes might be needed to improve the ability to detect these genes. Indeed, the greater prevalence of *E. coli* O157 in cattle fed high-grain diets that has been reported by some studies may be directly related to increases in their populations in the feces in response to the diet, which would improve the ability to detect them, should they be present.

The prevalence of *stx*-positive samples increased ($P = 0.001$) in the finishing period, primarily due to an increase ($P = 0.001$) in the prevalence of positive fecal samples. This may be an effect of the switch from the growing diets of bromegrass hay and corn silage to the high-concentrate finishing diet, but may also be due to seasonal variation. Seasonal differences in the prevalence of general STEC or *stx* genes in cattle have been reported, although this aspect has not been as well studied as it has for *E. coli* O157:H7. Cobbold et al. (2004) found that both STEC and *stx* gene prevalence were greater for fall than for winter, similar to *E. coli* O157:H7 prevalence. In beef cattle feces at slaughter, non-O157 STEC and *stx* genes were more prevalent in spring and fall than in summer and winter (Barkocy-Gallagher et al., 2003).

To ascertain if there was any association between pathogen carriage and measures of feed efficiency, we examined the relationships between the numbers of pathogen- or *stx* gene-positive samples obtained from each steer, and its ADG and daily DMI (Ferrell et al., 2006). There was poor correlation between the number of positive samples of either pathogen or of *stx* genes obtained from each steer, and the ADG or daily DMI of these steers (data not shown). This agrees with many previous observations that indicate that cattle generally are asymptomatic carriers of these pathogens (Stanley et al., 1998; Dean-Nystrom et al., 1999; Wesley et al., 2000).

In summary, for the diets examined, clear-cut effects of diet or genotype on the prevalence of carriage and shedding of *E. coli* O157 or *Campylobacter* spp. by cattle were not observed. Rather, this study highlighted additional potential confounding variables that can influence the interpretation of the results of long-term feeding studies, including seasonal variations of pathogen shedding, chronic shedding of pathogens, and horizontal transmission of pathogens among animals. In comparison to such zoonotic pathogens as *E. coli* O157 or *Salmonella,* relatively little is known about *Campylobacter* colonization of beef cattle. This work confirms recent reports of chronic shedding of this microorganism by cattle, and additional examination is planned to determine roles for chronic shedding and horizontal transmission in the maintenance of *Campylobacter* spp. in cattle. Our results also corroborate the common occurrence of *stx* gene-bearing cells in cattle. The greater feed consumption by the ½ Brahman and MARC III cattle may be responsible for the greater proportions of

stx gene-positive fecal samples in these steers during the finishing period. As was the case for generic *E. coli* populations in feces and ruminal fluid when cattle were switched to the high-concentrate diet, we speculate that *stx* gene-bearing cell levels increased in response to the greater starch load in the colon, thus improving the detection of these genes.

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