The Effect of Dietary Protein, Fiber, and Digesta Passage Rate on the Duration and Concentration of *Escherichia coli* O157:H7 Shed by Cattle

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Abstract

Infection with *E. coli* O157:H7 is the most common cause of human hemorrhagic colitis in North America. Cattle are a reservoir for this human pathogen and individual animals are sporadically and transiently colonized. We hypothesize that pre-harvest dietary management may reduce the risk of *E. coli* O157-positive animals entering our food chain. The effect of dietary energy, fiber, and digesta passage rates on the duration and concentration of fecal *E. coli* O157 was determined. Sixteen healthy Charlais x Hereford or Holstein heifers were acclimated to one of four diets: a finishing diet (high in energy and low in fiber), a growing diet (low in energy and high in fiber), chopped alfalfa (small particle size), or long alfalfa (large particle size). A single oral dose of 1010 colony forming-units of *E. coli* O157 was administered to each animal and fecal samples were cultured for the bacteria by selective-enrichment twice a week for the duration of the study. No significant difference in the shedding of O157 by animals on the finishing or growing diet was observed. However, withholding feed of either type, for 24 hours, did increase the concentration of the bacteria in the fecal material of about half of the animals. A significant correlation (p value=0.008) was observed between digesta passage rate and the duration of fecal *E. coli* O157. Animals with slower passage rates of 2 to 3 % per hour cleared *E. coli* O157 from their intestinal tract in 10 days or less compared to animals with digesta passage rates of 4 to 5 % per hour who shed the bacteria for more than 69 days. These results show that cattle diet influences the duration and concentration of fecal *E. coli* O157:H7.

Introduction

Escherichia coli O157:H7, a member of the enterohemorrhagic *E. coli* (EHEC), has been implicated as the most common causative agent of hemorrhagic colitis (HC) in the United States, since 1982.[15,21,34,36] Approximately 2-15% of HC cases progress to serious non-intestinal sequelae as the hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP).[4,15,40] HUS, characterized by thrombocytopenia and microangiopathic anemia, is the most common cause of acute renal failure in children, and has a mortality rate of 5-10%.[4,15,16,36] TTP shares many clinical manifestations with HUS but primarily affects the elderly and has a mortality rate near 50%.[4,36,40]

E. coli O157:H7 infections occur worldwide. A recent outbreak in Japan, caused by contaminated radish sprouts, affected more than 10,000 school children and resulted in 13 deaths.[31,37,40] An outbreak in Scotland, linked to contaminated beef and gravy, affected more than 400 individuals and resulted in 16 deaths.[40] Most human EHEC infection results from the ingestion of contaminated bovine food products such as undercooked ground beef or unpasteurized dairy products.[4,15,32] In addition, contaminated water, fresh vegetables, and unpasteurized apple juice have also been implicated as sources of infection and in some of these cases the source of contamination is known to be bovine feces.[2,6,23,36,38] Economic impact of contaminated bovine products has been enormous and includes product recalls and caused the closure of one processing plant.[7]

Ruminants are reservoirs for *E. coli* O157:H7. We and others have suggested that preharvest management of ruminants may decrease the number of *E. coli*

O157:H7-positive animals that enter processing plants and thereby minimize the contaminated products that enter the food chain.[18,26,33] This paper describes the results of experiments to analyze the roles of dietary energy, fiber, and digesta passage rate in the shedding of *Escherichia coli* O157:H7 by experimentally inoculated cattle.

Materials and Methods

Experimental animals

Eight healthy 8-month old Charolais x Hereford heifers and eight healthy 6-month old Holstein steers were identified by ear tags and housed without contact between animals in concrete stalls on wood-chip bedding. The animals were fed once per day and had water *ad libitum*, unless noted otherwise.

Bacteria

E. coli O157:H7 strain ATCC 43894 (American Type and Culture, Rockville, MD), which produces Shiga toxin type 1 and type 2, was the inoculum. The bacteria were grown in Luria-Bertani (LB) broth at 37°C with aeration, to a cell density of 10^9 CFU/ml. The number of viable bacteria was confirmed by spread plate technique. Each animal received 10^{10} CFU of *E. coli* O157:H7 via a gastric tube placed directly into the rumen.

Diets

The animals were divided into four groups each on a different dietary regime. Two of the four diets compared a high-energy/low fiber finishing diet (F) with a low-energy/high fiber growing diet (G). The other two diets were selected to influence digesta passage rate while providing identical nutritional quality. For this purpose, the two diets differed only in physical form and were composed of alfalfa hay in long form (L) or alfalfa hay that was finely chopped (C) to a size that passed through a 0.8 cm pore screen. Animals were acclimated to a diet for a minimum of 3 weeks before inoculation. The animals receiving the F or G diet had feed and water withheld for 24 hours on day 17 post-inoculation. Digesta passage rates were determined for animals fed the L or C diet (see below: Determination of digesta passage rates).

Chemical analyses of feed

Samples of F or G feeds were dried at 60°C, ground and passed through a 1-mm screen. The samples were analyzed, using standard techniques for dry matter (DM),[1] crude protein (CP),[1] neutral detergent fiber (NDF),[24,41] and acid detergent fiber (ADF).[25] The samples were also incubated *in vitro* as described by Terry *et al.*[39] to determine dry matter degradability (IVDMD). Core samples of 10 bales of alfalfa hay were obtained and composited using a 46-cm long probe with a 2-cm inside diameter. The alfalfa was analyzed for DM, CP, ADF, and NDF as described above.

Determination of digesta passage rates

Prior to inoculation, digesta passage rates of animals fed L or C were determined using ytterbium (Yb) in the form of $Yb(NO_3)*5H_2O$, using a previously described technique.[12] Briefly, 1.5 g of Yb was dissolved in 100 ml distilled water and the solution was sprayed on to 100 g of L or C, dried under ambient conditions, and was fed to animals on the respective diets. Fecal samples were collected from each animal at 8, 16, 24, 32, 40, 48, 60, 72, 84, and 96 hours, following dosage with Yb. Fecal samples were dried at 55°C for 96 hours, ground, and passed through a 1-mm screen. Yb was extracted from each sample as described by Williams *et al.*[44] and analyzed by plasma emission spectroscopy. Fecal-Yb concentrations were transformed to natural logarithm values and digesta passage rates were calculated as the declining slopes of these values against time, post-Yb dose, and expressed as a percentage digesta passage per hour.

Fecal culture

Fecal samples were cultured every 3-4 days to monitor shedding of *E. coli* O157:H7 except for experiments involving withholding of feed and water when fecal samples were cultured every day for 72 hours post-feed withholding. Fecal samples were obtained by aseptic rectal palpation and cultured for *E. coli* O157:H7 as previously described.[9] Briefly, fecal samples were transported to the laboratory in sterile 50-ml conical tubes (VWR Scientific, San Francisco, California). Ten grams of feces were transferred into 50 ml Trypticase soy broth (BBL/Becton-Dickinson) supplemented with cefixime (50 µg/liter; Lederle Laboratories, Pearl River, NY), potassium tellurite (2.5 mg/liter; Sigma Chemical Company, St. Louis, MO), and vancomycin (40 mg/liter; Sigma) (TSB-CTV). Serial dilutions of each sample were prepared in sterile saline, both before and after 18-hour aerated incubation of the cultures at 37°C. Dilutions made prior to incubation, the non-enrichment cultures (NE), were spread plated on sorbitol MacConkey medium (SMAC) (Difco) containing 100 µg/ml 4-methylumbelliferyl-β-D-glucuronide (SMAC-MUG) (Biosynth Ag Biochemica and Synthetica; Skokie, IL). Dilutions made after incubation, the selective-enrichment cultures (E), were spread plated on SMAC-MUG supplemented with cefixime (50(µg/liter) and potassium tellurite (2.5 mg/liter) (SMAC-CTM). Colonies that did not utilize MUG or ferment sorbitol were confirmed as *E. coli* O157:H7 serologically, using a latex agglutination test (ProLab Diagnostics; Roundrock, TX).

Statistical analyses

Several analyses were performed to assess the statistical significance of the duration and concentration of *E. coli* O157:H7 shed by cattle while fed one of the diets in this study. The first method used standard survival time analyses with the outcome of either time to first culture-negative status (by enrichment culture) or time until the second consecutive culture-negative status (by enrichment culture). A second set of analyses was conducted on two variables: the proportion of time a given animal was culture-positive, and the mean value (by non-enrichment culture) of the concentration of *E. coli* O157:H7 in the feces at the time the animal was culture-positive (by enrichment culture). These two variables attempt to measure the amount of time an animal was culture-positive, and the concentration of bacteria shed (CFU/g). Analyses of variance was conducted on these two additional variables to test for diet differences. The third set of analyses examined digesta passage rate data and the number of days each animal was culture-positive, as measured by last day culture-positive. Graphs were examined, and correlation coefficients were computed to assess the relationship between digesta passage rate and number of days culture-positive, the day of first negative culture status and the first day at which an animal was culture-negative at two consecutive samplings. All analyses were conducted with the SAS (statistical analysis software) package.

Results

All animals remained healthy for the duration of the experiment. The animals, regardless of their diet, shed *E. coli* O157:H7 24 hours after inoculation. The concentration of *E. coli* O157:H7 shed by each animal gradually decreased with time, until they became culture-negative for the bacteria. Most of the animals eating F or G had stopped shedding the organism 33 days post-inoculation; most animals eating L or C had stopped shedding the organism 59 days post-inoculation.

Diet composition

The L and C diets were composed of alfalfa hay and differed only in their physical form. The ingredients of the F and G diets were typical of cattle growing and cattle finishing rations and are listed in Table 1. The chemical analysis of each diet are shown in Table 2. As expected, the F diet was higher in protein and digestible energy, and lower in fiber than the G diet. ADF and NDF values are negative indicators of digestible energy, and both values were lower for the F diet than for the G diet. In addition, high fiber values are associated with lower IVDMD values and were lower for the G than the F diets, respectively. The L diet and C diets were identical in chemical composition and were typical for alfalfa hay (Table 2).

Table 1. Components of the finishing and growing diets.

Ingredient	Finishing (High Energy) Ration-DMB (%)[a]	Growing (Low Energy) Ration-DMB (%)
Grass Hay	5.00	19.90
Alfalfa-Grass Silage	7.29	48.60
Barley	62.13	12.00
Corn	19.33	12.00
Limestone	0.71	0.80
Dical	0.29	0.40
Tm Salt[b]	0.50	0.50
SBM[c]	4.75	5.80

[a] Dry matter basis
[b] Trace mineralized salt
[c] Soybean meal

Table 2. Clinical analyses of feeds.

	Alfalfa Hay	Finishing	Growing
Dry Matter	89.8	74.1	36.5
IVDMD[a]	ND[b]	83.1	62.7
Neutral Detergent Fiber	41.1	25.1	41.8
Acid Detergent Fiber	27.0	9.6	26.4
Crude Protein	15.1	15.2	14.2

[a] In vitro dry matter disappearance
[b] Not done

All values in the table are a percentage.

Effect of dietary protein, fiber, and digestible energy on *E. coli* O157:H7 shedding. The results of fecal cultures from each animal fed the G or F diet are shown in Figure 1. Animals on either diet shed *E. coli* O157 similarly, for an average of about 5 days, at concentrations that could be detected without selective-enrichment culture. When selective-enrichment culture was used to detect the bacteria in fecal samples, animals fed the F diet were culture-positive slightly longer, for an average near 23 days, when compared with animals fed the G diet, that were culture-positive for an average near 17 days. In addition, the animal that shed the bacteria for the full duration of the study (46 days) was fed the F diet. However, the trend suggesting that the F diet prolonged shedding of *E. coli* O157:H7 was not statistically significant. The Wilcoxon test for censored data gave P values of 0.21 and 0.78, for the survival analyses on time to first culture-negative and second consecutive culture-negative, respectively. This test failed to find a difference in the pattern of *E. coli* O157:H7 shed by animals eating F or G. The analyses of variance on the proportion of time culture-positive and the mean CFU/g value while culture-positive, gave P values of 0.40 and 0.77, respectively. Taken in concert, these tests, although they have low power due to the small sample size, suggest that the G or F diets did not differentially affect the shedding of *E. coli* O157:H7, by experimentally inoculated cattle.

Effect of withholding food and water

Food and water were withheld for 24 hours from animals eating F or G and animals were monitored for

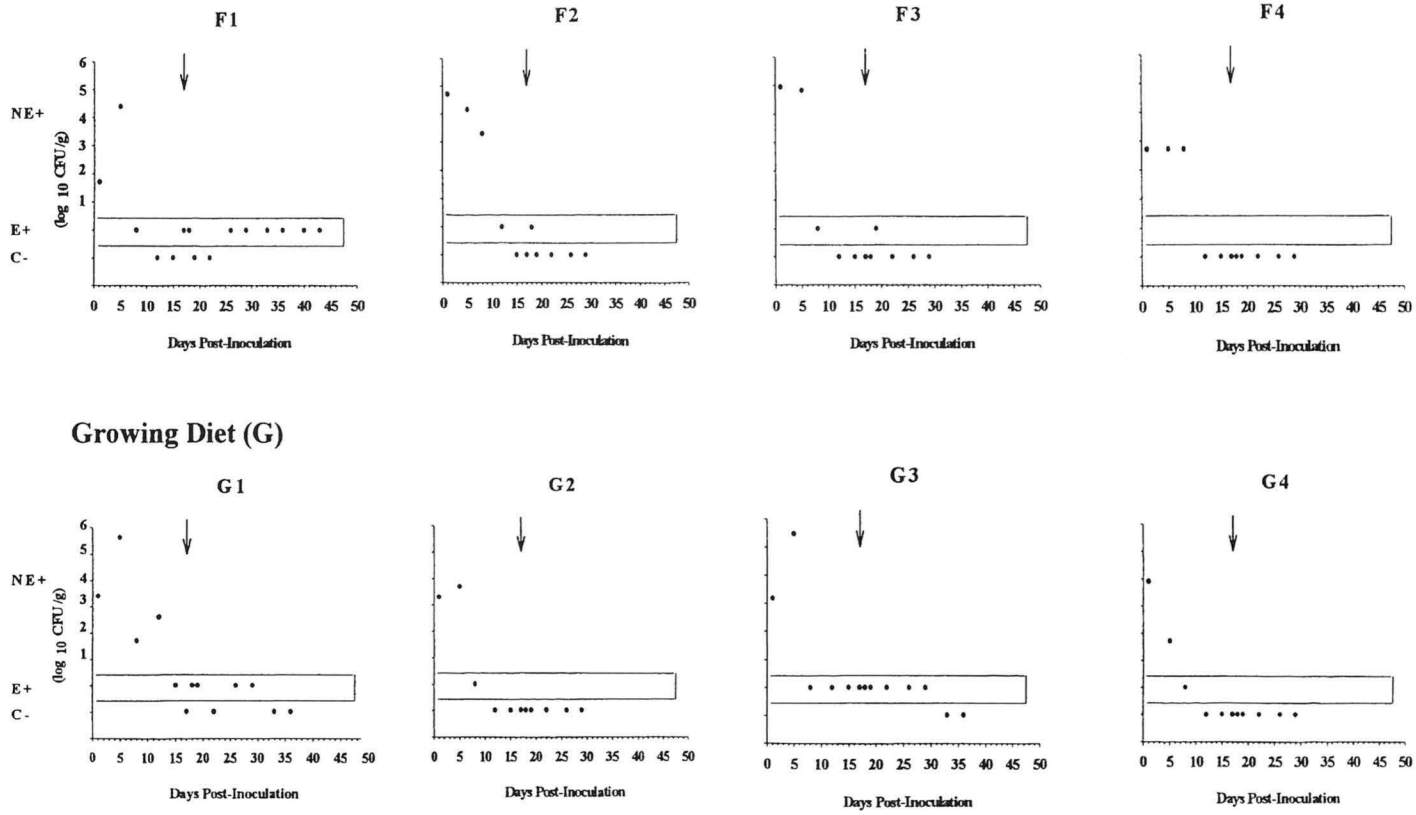

Figure 1. The effect of dietary protein and fiber on the shedding of *E. coli* O157:H7. Graphs show the concentration of fecal *E. coli* O157:H7 from each animal versus time post-inoculation. Top row graphs represent animals fed a finishing diet; bottom graphs represent animals fed a growing diet. Numbers at the top of each graph indicate animal ear tag designation. NE+, non-enrichment culture positive; E+, selective-enrichment culture positive; C-, culture negative. Data within boxes indicate cultures positive only by selective-enrichment culture.

E. coli O157:H7 (Table 3). This abrupt dietary disturbance was imposed at a time when most of the cattle had very recently become culture-negative for the bacteria. At the time feed and water were withheld, 2 of 8 animals remained culture-positive for the bacteria and after withholding feed and water, 5 of 8 animals were culture-positive. Three animals were culture-positive after the dietary disturbance that had been culture-negative previous to the treatment.

Effect of passage rate on E. coli O157:H7 shedding

The *E. coli* O157:H7 culture status of each animal during the experiment is shown graphically in Figure 2. Although the L and C diets were chosen to affect different digesta passage rates, we did not observe this correlation. For example, the slowest rate (2.76%/hr) and one of the fastest rates, (4.84 %/hr), were both animals fed L (Table 4). Despite our inability to affect particular digesta movement based on the particle size of a diet, we did see a range of significantly different digesta passage rates among the eight steers. There was a strong correlation between digesta passage rate and the duration animals were culture-positive for *E. coli* O157:H7 (Table 4). The relationship between digesta passage rate and the last day an animal was positive-culture indicated a positive linear trend by both the Pearson (P = 0.03) and Spearman (P = 0.008) correla-

Table 3. Effect of withholding feed and water on the shedding of *E. coli* O157:H7.

Animal #	Last Day Culture +[a]	Before Withholding Feed[b]	After Withholding Feed[c]
Finishing			
1	47	+	+
2	18	-	+
6	19	-	+
8	8	-	-
Growing			
3	29	-	+
4	8	-	-
5	29	+	+
7	8	-	-

[a] *E. coli* O157:H7 culture statistics determined by selective enrichment culture.
[b] Culture statuses before withholding feed and water for 24 hours.
[c] Culture statuses after withholding feed and water for 24 hours.

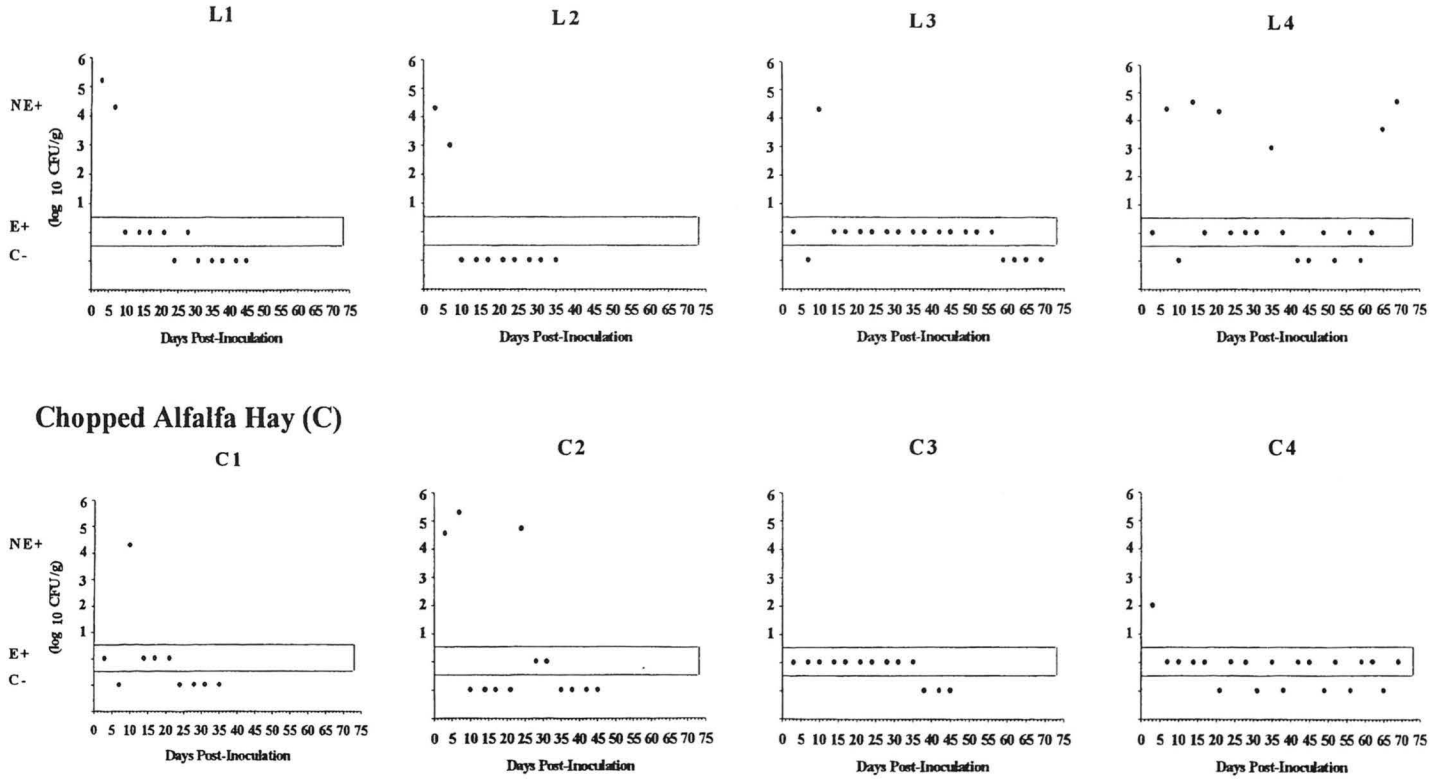

Figure 2. The effect of feed particle size on the shedding of *E. coli* O157:H7. Graphs show the concentration of fecal *E. coli* O157:H7 from each animal versus time post-inoculation. Top row graphs represent animals fed long hay; bottom graphs represent animals fed chopped hay. Numbers at the top of each graph indicate animal ear tag designation. NE+, non-enrichment culture positive; E+, selective-enrichment culture positive; C-, culture negative. Data within boxes indicate cultures positive only by selective-enrichment culture.

Table 4. Duration of E. coli O157:H7 shedding and digesta passage rates.

Diet	Animal #	Digesta Passage Rate (%/hour)	*E. coli* O157:H7 Culture Status (Days Post-Inoculation) 3 7 10 14 17 21 24 28 31 35 38 42 45 49 52 56 59 62 65 69
L[a]	2	2.76	+ + - - - - - - - -
L	1	2.97	+ + + + + + - + - - - - -
C[b]	4	4.09	+ + - - - - + + + - - - -
C	5	4.63	+ + + + + + + + + + - - -
C	3	4.71	+ - + + + + - - - -
L	7	4.73	+ + + + + + + + + + + + + + + + - - - -
L	8	4.84	+ + - + + + + + + + + - - + - + - + + +
C	6	5.25	+ + + + + - + + - + - + + - + - + + - +

[a] Long alfalfa hay
[b] Chopped alfalfa hay

tion coefficients. Animals with the fastest digesta passage rates (approximately 5%/hour), shed the bacteria for the longest duration and were culture-positive 69 days post-inoculation. The animal with the slowest digesta passage rate (2.8%/hour) shed the bacteria for the shortest time (about one week) and was consistently culture-negative from day 10-post inoculation.

Discussion

The most significant finding of this study was that cattle digesta passage rate correlated with the duration animals were culture-positive for *E. coli* O157:H7 . Animals with the fastest digesta passage rates shed the organism for the longest time. In addition, a dietary interruption, in the form of withholding feed and water for 24 hours, tended to increase the duration and concentration of fecal *E. coli* O157:H7. Finally, our results suggested that a high energy/low fiber finishing-type diet did not affect the duration or concentration of *E. coli* O157:H7 in the feces of cattle, when compared with a lower energy/high fiber growing-type diet.

It is well established that healthy, asymptomatic, dairy and beef cattle and sheep are reservoirs for *E. coli* O157:H7 and other EHEC (13,15,27,28). *E. coli* O157:H7 occurs with a prevalence in cattle of 0.15 to10.0 % and a herd prevalence of 8.3 to 100%.[8,11,17,19,30,46] The organism transiently colonizes cattle and the duration that individual animals shed *E. coli* O157:H7 is brief, averaging near 30 days and ranging from 8 to 46 days.[3,35,43] Similar *E. coli* O157:H7 colonization kinetics have been demonstrated in naturally infected and experimentally inoculated sheep.[27,28] The incidence of *E. coli* O157:H7 culture-positive cattle and sheep increase in the warmer months.[17-19,28] Studies by Hancock *et al* and Chapman *et al*, show the highest prevalence of *E. coli* O157:H7 in cattle occurs between May and October, with a signifi-

cant peak in prevalence between April and July .[17,19,30] This seasonal incidence parallels the seasonal incidence of human infections.[22,43]

Little is known about the factors that cause cattle or sheep to become colonized with EHEC or the factors that affect clearance of the organism from the ruminant gastrointestinal tract (GIT). Pre-harvest control of diet may reduce the risk of *E. coli* O157:H7-positive animals entering the food chain. The link between digesta passage rate, diet, and duration and concentration of *E. coli* O157:H7 shed by ruminants is not understood. We were unable to predict digesta passage rates, in this study, based on diet alone. The parameters that likely influence the rate of digesta flow include diet, water intake, the GIT microbiotia, and the genetic background of the animal. It was clear, however, that animals with fast digesta passage rates shed *E. coli* O157:H7 significantly longer than animals with slower digesta passage rates. Animals with digesta passage rates near 5%/hr shed the bacteria for more than three months compared to animals with digesta passage rates of 2 to 3 %/hr that shed the bacteria for one to four weeks. The rate of digesta movement through the GIT may influence the biochemistry and/or the complex rumen microbiotia and affect the ability of *E. coli* O157:H7 to compete in this environment.

Rassmussen *et al*, and others, speculates that GIT volatile fatty acids (VFA) concentrations and pH, influenced by dietary conditions, control *E. coli* O157:H7-colonization of ruminants.[33,42,45] *In vitro* studies demonstrate that, *E. coli* O157:H7 survives better in rumen fluid collected from fasted animals, due to its low volatile fatty acids (VFA) concentrations and high pH. These finding support our observation that withholding feed tended to increase GIT *E. coli* O157:H7. In addition, feed withdrawal during transit, has been associated with the presence of *E. coli* O157:H7-positive animals at slaughter. Bulaga *et al*, identified *E. coli* O157:H7-positive sheep only in a group that traveled the longest to a livestock market.[40] Likewise, the prevalence of *E. coli* O157:H7 culture-positive lambs to be the highest among animals that had traveled (without feed or water) for>18 hrs to a slaughterhouse.[40] Hancock *et al* found a higher *E. coli* O157:H7 prevalence among feedlot cattle that had been recently shipped, when compared to cattle that had been in the feedlot for many months.[20] Also, calves fasted for 48 hrs prior to *E. coli* O157:H7 inoculation shed the bacteria longer than non-fasted calves.[10]

The diet of cattle influences rumen VFA concentration. Recently, Zitnan *et al* demonstrated that the total rumen VFA concentration decreases during the transition from winter-feeding to pasture grazing (47). This decline persists for 4 weeks before rumen adaptation occurs and the VFAs return to their original concentration. During the period when lower VFA concentrations are observed in the rumen, a decrease in the anaerobic microflora and an increase in the microaerophilic microflora occur. *In vivo* studies, with sheep and cattle, indicate that diets of increased fiber content and/or withholding feed, which reduce total GIT VFA concentration, induce the proliferation of non-pathogenic *E. coli* and *Salmonella*.[5,14] We demonstrated previously that experimentally inoculated sheep fed a low energy/high fiber grass-hay diet shed *E. coli* O157:H7 about twice as long as sheep fed a high energy/low fiber diet of corn and alfalfa pellets.[29] The observations that the F and G diets did not influence the culture status of cattle do not support these earlier findings and the trend, seen in this study, that the high quality finishing diet provided the most favorable conditions for proliferation and persistence of GIT *E. coli* O157:H7 sharply contrast with our previous work. These differences may be because the F and G diets did not adequately mimic the dietary conditions tested in sheep or influenced other unknown physiological factors. Also, we did not measure rumen VFA concentrations, or digesta passage rates among cattle fed the F or G diets and do not know if these parameters influenced the results. It is likely that we did not find statistically significant differences between the F and G fed animals because of the small number of animals in the study and the trend that we observed may be misleading.

The mechanism by which digesta flow influences *E. coli* O157:H7 survival in the ruminant GIT will be the subject of future investigations. It may be that rapid digesta movement lowers the VFA concentration and provides a more hospitable environment for this human pathogen. An important issue to be addressed is whether culture-positive animals with rapid digesta passage rates can be cleared of their infection by pharmacological manipulations that slow digesta flow. Investigations in this area are ongoing in our laboratory.

Acknowledgments

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References

1.AOAC. 1990. Association of Official Analytical Chemists. AOAC, Washington, DC. 2.Banatvala, N., A.R. Magnano, M.L. Cartter, T.J. Barrett, W.F. Bibb, L.L. Vasile, P. Mshar, M.A. Lambert-Fair, J.H. Green, N.H. Bean, and R.V. Tauxe. 1996. Meat Grinders and Molecu-

lar Epidemiology: Two Supermarket Outbreaks of *Escherichia coli* O157:H7 Infection. *J. Infect. Dis.* 173:480-483. 3.Besser, T.E., D.D. Hancock, L.C. Pritchett, E.M. McRae, D.H. Rice, and P.I. Tarr. 1997. Duration of Detection of Fecal Excretion of *Escherichia coli* O157:H7 in Cattle. *J. Infect. Dis.* 175:726-729. 4.Boyce, T.G., D.L. Swerdlow, and P.M. Griffin. 1995. Current concepts - [i*Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. *N. Engl. J. Med.* 333:364-368. 5.Brownlie, L.E., and F.H. Grau. 1967. Effect of food intake on growth and survival of Salmonella and *Escherichia coli* in the bovine rumen. *J. Gen. Microbiol.* 46:125-134. 6.CDC. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. *Morbid. Mortal. Weekly Rep.* 44:157-160. 7.CDC. 1997. *Escherichia coli* O157:H7 infections associated with eating a nationally distributed commercial brand of frozen ground beef patties and burgers—Colorado, 1997. *Morbid. Mortal. Weekly Rep.* 46(33):777-778. 8.Chapman, P.A., C.A. Siddons, D.J. Wright, P. Norman, J. Fox, and E. Crick. 1993. Cattle as a possible source of verocytotoxin-producing *Escherichia coli* 0157 infections in man. *Epidemiol. Infect.* 111:439-447. 9.Chapman, P.A., C.A. Sidons, P.M. Zadik, and L. Jewes. 1991. An improved selective medium for the isolation of *Escherichia coli* 0157. *J. Med. Microbiol.* 35:107-110. 10.Cray, J., WC, and H.W. Moon. 1995. Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 61:1586-1590. 11.Faith, N.G., J.A. Shere, R. Brosch, K.W. Arnold, S.E. Ansay, M.S. Lee, J.B. Luchansky, and C.W. Kaspar. 1996. Prevalence and clonal nature of *Escherichia coli* O157:H7 on dairy farms in Wisconsin. *Appl. Environ. Microbiol.* 62:1519-1525. 12.Fassel, V.A. 1978. Quantitative elemental analyses by plasma emission spectrophotometry. *Science.* 202:183. 13.Fowler, R.G., G.E. Degnen, and E.C. Cox. 1974. Mutational specificity of a conditional *Escherichia coli* mutator, mutD5. Mol. Gen. Genet. 133:179-191. 14.Grau, F.H., L.E. Brownlie, and M.G. Smith. 1969. Effects of food intake on numbers of *Salmonella* and *Escherichia coli* in rumen and faeces of sheep. *J. Appl. Bacteriol.* 32:112-117. 15.Griffin, P.M. 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, p. 739-761. In M.F. Blaser, P.D. Smith, J.I. Ravdin, H.B. Greenberg, and R.L. Guerrant (ed.), Infections of the Gastrointestinal Tract. Raven Press, Ltd., New York. 16.Griffin, P.M., and R.V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13:60-98. 17.Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I. Tarr, D.H. Rice, and M.G. Paros. 1994. The prevalence of Escherichia coli O157:H7 in dairy and beef cattle in Washington State. Journal of Epidemiology and Infection. 113:199-207. 18.Hancock, D.D., T.E. Besser, and D.H. Rice. 1993. Program Abstr., abstr.. 19.Hancock, D.D., T.E. Besser, D.H. Rice, D.E. Herriott, and P.I. Tarr. 1997. A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. *Epidemiol. Infect.* 118:193-195. 20.Hancock, D.D., D.H. Rice, L.A. Thomas, D.A. Dargatz, and T.E. Besser. 1997. Epidemiology of *Escherichia coli* O157 in feedlot cattle. *J. Food Prot.* 60:462-465. 21.Hermiston, M.L., M.H. Wong, and J.I. Gordon. 1996. Forced expression of E-cadherin in the mouse intestinal epithelium slows cell migration and provides evidence for nonautonomous regulation of cell fate in a self-renewing system. Genes & Development. 10:985-996. 22.Keene, W.E., K. Hedberg, D.E. Herriott, D.D. Hancock, R.W. McKay, T.J. Barrett, and D.W. Fleming. 1997. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. J. Infect. Dis. 176(3):815-818. 23.Keene, W.E., E. Sazie, J. Kok, D.H. Rice, D.D. Hancock, V.K. Balan, T. Zhao, and M.P. Doyle. 1997. An Outbreak of *Escherichia coli* O157:H7 Infections Traced To Jerky Made from Deer Meat. *JAMA.* 277:1229-1231. 24.Komarek, A.R. 1993. A filter bag procedure for improved efficiency of fiber analysis. *J. Dairy Sci.* 76:250. 25.Komarek, A.R. 1993. Improved efficiency of ADF analysis using a filter bag procedure. *J. Anim. Sci.* 71:284. 26.Kudva, I.T., P.G. Hatfield, and C.J. Hovde. 1995. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. Appl. Environ. *Microbiol.* 61:1363-1370. 27.Kudva, I.T., P.G. Hatfield, and C.J. Hovde. 1996. Characterization of *Escherichia coli* O157:H7 and other Shiga toxin producing-*E. coli* isolated from sheep. J. Clin. Microbiol. 35:892-899. 28.Kudva, I.T., P.G. Hatfield, and C.J. Hovde. 1996. Escherichia coli O157:H7 in microbial flora of sheep. *J. Clin. Microbiol.* 34:431-433. 29.Kudva, I.T., C.W. Hunt, C.J. Williams, U.M. Nance, and C.J. Hovde. 1997. Evaluation of Dietary Influences on *Escherichia coli* O157:H7 shedding by Sheep. Appl. Environ. Microbiol. 63:3878-3886. 30.Mechie, S.C., P.A. Chapman, and C.A. Siddons. 1997. A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. Epidemiol. Infect. 118:17-25. 31.Nathan, R. 1997. American seeds suspected in Japanese food poisoning epidemic. *Nat Med.* 3(7):705-706. 32.Persson, B., and P. Argos. 1994. Prediction of Transmembrane Segments in Proteins Utilising Multiple Sequence Alignments. *J. Mol. Biol.* 237:182-192. 33.Rasmussen, M.A., W.C. Cray, T.A. Casey, and S.C. Whipp. 1993. Rumen contents as a reservoir of enterohemorrhagic Escherichia coli. FEMS *Microbiol. Lett.* 114:79-84. 34.Riley, L.W., R.S. Remis, S. Helgerson, H.B. McGee, J. Wells, B. Davis, R.J. Hebert, E.S. Olcott, L. Johnson, N. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308:681-685. 35.Sanderson, M.W., J.M. Gay, T.E. Besser, D.D. Hancock, L.K. Fox, and C.C. Gay. 1995. Sensitivity of bacteriologic culture for detection of *Escherichia coli* O157:H7 in bovine feces. *Clin. Microbiol. Rev.* 999:999. 36.Su, C.Y., and L.J. Brandt. 1995. *Escherichia coli* 0157:H7 infection in humans. *Ann. Intern. Med.* 123:698-714. 37.Swinbanks, D. 1996. Outbreak of *E. coli* infection in Japan renews concerns. Nature. 382:290. 38.Tarr, P.I. 1995. Escherichia coli O157:H7: Clinical, diagnostic, and epidemiological aspects of human infection. Clinical Infectious Diseases. 20:1-10. 39.Terry, R.A., J.M.A. Tilley, and G.E. Outen. 1969. Effect of pH on cellulose digestion under in vitro conditions. *J. Sci. Food Agric.* 20:317. 40.USDA/APHIS/VS. 1997. An Update: *Escherichia coli* O157:H7 in Humans and Cattle. Report from Centers for Epidemiology and Animal Health. :1-28. 41.Van Soest, P.J., J.B. Robertson, and B.A. Lews. 1991. Methods of dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583. 42.Wallace, R.J., M.L. Falconer, and P.K. Bhargava. 1989. Toxicity of volatile fatty acids at rumen pH prevents enrichment of *Escherichia coli* by sorbitol in rumen contents. *Curr. Microbiol.* 19:277-281. 43.Wells, J.G., L.D. Shipman, K.D. Greene, E.G. Sowers, J.H. Green, D.N. Cameron, F.P. Downes, M.L. Martin, P.M. Griffin, S.M. Ostroff, M.E. Potter, R.V. Tauxe, and I.K. Wachsmuth. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. *J. Clin. Microbiol.* 29:985-989. 44.Williams, C.H., D.J. David, and O. Iismaa. 1962. The determination of chromic oxide in faecal samples by atomic absorption spectrophotometry. *J. Agri. Sci.* 59:381. 45.Wolin, M.J. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. *Appl. Microbiol.* 17:83-87. 46.Zhao, T., M.P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. *Appl. Environ. Microbiol.* 61:1290-1293. 47.Zitnan, R., A. Sommer, J. Gallo, A. Laukov'a, A. Bomba, and J. Venglovsk'y. 1994. Volatile fatty acid concentrations, enzyme activities and microflora in the rumen contents of heifers during transition to pasture. Arch. *Tierernahr.* 46:51-60.