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Evaluation of Dietary Influences on *Escherichia coli* O157:H7 Shedding by Sheep

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The effect of diet, an abrupt diet change, and fasting on the shedding of *Escherichia coli* O157:H7 was investigated with experimentally inoculated sheep as a ruminant model. Sheep were fed a grass hay diet (G), which was low in protein and digestible energy and high in fiber, or a mixture of corn and pelleted alfalfa (C), which was high in protein and digestible energy and low in fiber. After a single oral inoculation of *E. coli* O157:H7, all the animals shed fecal *E. coli* O157:H7. However, sheep that were fed G shed the bacterium almost twice as long as, and in larger numbers than, did sheep that were fed C. The number of culture-positive animals increased after the diet was abruptly changed from C to G and decreased with the opposite change (G to C). A 24-h fast did not influence *E. coli* O157:H7 shedding. Horizontal transmission of infection between animals occurred. Recent shedding of *E. coli* O157:H7 did not affect recolonization with *E. coli* O157:H7. The findings presented in this study indicate that preharvest control of diet may reduce the risk of *E. coli* O157:H7-positive animals entering the food chain.

Escherichia coli O157:H7 is the prototype of enterohemorrhagic *E. coli* and has been the cause of most outbreaks of hemorrhagic colitis in the United States since 1982 (1, 22). About 5 to 10% of the hemorrhagic colitis cases progress to the hemolytic uremic syndrome, characterized by hemolytic anemia, thrombocytopenia, and renal failure. Hemolytic uremic syndrome is a serious sequela with a mortality rate of 3 to 5% (1, 11).

The pathogenesis of enterohemorrhagic *E. coli* infections is associated with bacterial production of one or both Shiga toxins (type 1 [Stx1] or 2 [Stx2]) and probably the formation of attaching-effacing lesions in the intestinal mucosa similar to those that occur in animal models of infection (10). Most human infections with *E. coli* O157:H7 are caused by the consumption of contaminated and improperly cooked beef, unpasteurized milk, or fecally contaminated vegetables, water, or apple cider (22). In addition, direct transmissions from animals or humans have been reported, although these instances are rarer (22). Recent surveys have established ruminant animals as reservoirs for this human pathogen (3, 13, 18, 19).

We (17) and others (14, 21) have suggested that preharvest dietary management may play a role in the incidence of *E. coli* O157:H7-positive ruminants. In this study, we investigated the effects of dietary fiber and nutrients on the shedding of *E. coli* O157:H7 by sheep. Our goals were to study the effects of (i) different diets (grass hay [G] versus a mixture of corn and pelleted alfalfa [C]), (ii) an abrupt diet change, and (iii) fasting. In addition, we assessed the effect of recent colonization with *E. coli* O157:H7 on our ability to reestablish colonization with the same serotype.

MATERIALS AND METHODS

Experimental animals. Healthy 1-year-old ewes of the Columbia or Panama breed were used in the experiments. The ewes were housed in groups (Table 1), without contact between groups, in raised, grated-floor pens without bedding. The animals had water ad libitum and were fed twice daily, unless indicated otherwise. They were identified by ear tags with letter/number combinations, with the letter(s) indicating the diet and the number referring to the individual animal (Table 1).

Bacterial strains and inocula. *E. coli* O157:H7 strain ATCC 43894 (American Type Culture Collection, Rockville, Md.), sensitive to nalidixic acid, or a spontaneous nalidixic acid-resistant mutant of a bovine *E. coli* O157:H7 strain (Nal^r; provided by T. E. Besser, Washington State University, Pullman, Wash.) was used. Both strains produce Stx1 and Stx2. Within a group of copenned animals, the strains were alternatively administered, with odd-numbered animals receiving ATCC 43894 and even-numbered animals receiving the Nal^r strain (Table 1).

The inoculum was prepared by culturing each *E. coli* O157:H7 strain in separate flasks of Luria-Bertani (LB) broth. The cultures were grown at 37°C with aeration until the culture densities reached 10^8 CFU of *E. coli* O157:H7/ml (absorbance at 600 nm ≅ 4.0). Then the cells were harvested by centrifugation and resuspended at 10^9 CFU/ml in sterile saline. Viable cell counts were estimated by spread plate culture of triplicate serial dilutions on LB agar. Sterile 10-ml syringes (BBL/Becton Dickinson, Detroit, Mich.) were used to administer 10^{10} CFU of *E. coli* O157:H7 to each ewe in a single oral infusion.

Dietary differences and abrupt dietary change. Two feeds were used in this study: a relatively low-nutrient and high-fiber feed consisting of 100% grass hay (G) and a relatively high-nutrient and low-fiber feed consisting of 50% corn and 50% pelleted alfalfa (C). No supplements were included in either feed. The animals were acclimated to their pen mates, housing, and diet for approximately 3 weeks before inoculation. The effect of the G or C diet on the quantity and duration of *E. coli* O157:H7 shedding was measured for 17 days. During this time, animals in both groups I and II were fed G and animals in both groups III and IV were fed C. Ewes in groups I and III had their diets abruptly changed on day 20 postinoculation (Table 1). The change from C to G was immediate. The change from G to C was accomplished by stepwise increases in C over five consecutive days. The feed was changed to 50% C-50% G for 2 days, 75% C-25% G for the next 2 days, and 100% C thereafter. The gradual change from G to the high-protein and low-fiber C was required to curtail sudden increases in the gastrointestinal tract anaerobic flora that can lead to intoxication and death of an animal (4, 25). Fecal samples were cultured prior to inoculation (predose) and from days 2 through 41, at 3- or 4-day intervals (postinoculation).

Feed withdrawal. The effect of withholding feed and water for 24 h was tested on seven ewes that had recently shed *E. coli* O157:H7 and became culture negative 24 to 38 days postinoculation. These animals were penned in two separate groups. The first group of four animals were fed G (G1, CG2, CG4, and CG6); the second group of three animals were fed C (GC1, GC2, and GC5). These rations were maintained until day 48 postinoculation, when feed and water were withheld for 24 h. Fecal samples were cultured for *E. coli* O157:H7 before and after feed withdrawal, on days 48 and 49 postinoculation, respectively.

Reinoculation with *E. coli* O157:H7. The effect of recent colonization with *E. coli* O157:H7 on the ability to recolonize animals with identical or similar *E. coli* O157:H7 strains was tested with 11 ewes that had stopped shedding *E. coli* O157:H7 between days 10 and 13 postinoculation. The ewes were placed in two

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TABLE 1. Experimental animals, diets, and *E. coli* O157:H7 inoculum strains used in this study

Group[a]	Diet	Sheep	Inoculum strain[b]
I	G to C	GC1	43894
		GC2	Nal^r
		GC3	43894
		GC4	Nal^r
		GC5	43894
		GC6	Nal^r
		GC7	43894
II	G	G1	43894
		G2	Nal^r
		G3	43894
		G4	Nal^r
		G5	43894
		G6	Nal^r
		G7	43894
III	C to G	CG1	43894
		CG2	Nal^r
		CG3	43894
		CG4	Nal^r
		CG5	43894
		CG6	Nal^r
		CG7	43894
IV	C	C1	43894
		C2	Nal^r
		C3	43894
		C4	Nal^r
		C5	43894
		C6	Nal^r

[a] Sheep in groups I and III were fed G or C, respectively, until day 20 postinoculation, and then their diets were abruptly changed to C or G, respectively. Sheep in groups II and IV were fed one diet (G or C, respectively) throughout the study.

[b] 43894, ATCC 43894; Nal^r, nalidixic acid-resistant strain.

isolated groups. The first group of five animals were fed G (G3, G5, CG1, CG3, and CG5), and the second group of six animals were fed C (C1, C3, C4, C5, C6, and GC6). On day 61 after the primary inoculation, each ewe, regardless of the first inoculation strain received, was given a single oral infusion of 10^{10} CFU of ATCC 43894. Weekly fecal samples were cultured for the bacterium after this secondary inoculation for 42 days.

Feces and water culture. Fecal samples (10 g) were collected aseptically by rectal palpation and cultured for *E. coli* O157:H7 by previously described nonenrichment and selective-enrichment protocols (17). Briefly, the feces were transported to the laboratory in ice-cold, sterile Trypticase soy broth (BBL/Becton Dickinson) supplemented with cefixime at 50 μg/liter (Lederle Laboratories, Pearl River, N.Y.; provided by D. D. Hancock, Washington State University), potassium tellurite (2.5 mg/liter; Sigma Chemical Co., St. Louis, Mo.) and vancomycin (40 mg/liter; Sigma) (TSB-CTV). In the laboratory, appropriate serial dilutions, ranging from neat to 10^{10}, of each sample were prepared in sterile saline (0.15 M NaCl) both before and after overnight incubation under aeration at 37°C. Dilutions prepared before the incubation were spread plated on sorbitol MacConkey agar with 4-methylumbelliferyl-β-D-glucuronide (100 mg/liter; Biosynth Ag Biochemica and Synthetica, Skokie, Ill.) (SMAC-MUG). Dilutions prepared after the overnight incubation were spread plated on SMAC supplemented with cefixime (50 μg/liter), potassium tellurite (2.5 mg/liter), and MUG (100 mg/liter) (SMAC-CTM). By either method, colonies that did not ferment sorbitol and did not utilize MUG were confirmed to be *E. coli* O157 serologically. Similarly, 10-ml trough water samples were tested for the presence of *E. coli* O157:H7 by the two methods.

Strain differentiation. To differentiate the ATCC 43894 and Nal^r strains of *E. coli* O157:H7, at least 20 *E. coli* O157:H7 colonies, isolated from each positive fecal sample, were simultaneously subcultured on LB agar with or without nalidixic acid (20 μg/ml; Sigma). Growth patterns were analyzed to determine the presence of each strain in a fecal sample. Assuming that a random sample of the *E. coli* is obtained in a 10-g fecal sample, the probabilities of detecting both strains, if present, are 88 and 65% if the prevalences of the second strain are 10 and 5%, respectively.

Chemical analysis of feed and feces. The G and C feeds and individual fecal samples were analyzed by using the standard criteria of fiber, pH, and protein content to establish differences. The pH of liquefied fecal samples was determined with a pH meter. Samples of feces or feed were analyzed, by previously described methods, for Kjeldahl nitrogen (6) and neutral and acid detergent fiber (NDF and ADF) (8, 16). To acquire the appropriate volume for triplicate analysis, two or three fecal samples, collected from individual animals between days 21 and 41 postinoculation, were pooled and dried in a force-draft oven at 55°C for 48 h. Dried samples were ground in a hammer mill to pass through a 1-mm screen before testing. The samples were also incubated in strained ruminal fluid to determine dry-matter (DM) degradability. The one-stage Tilley and Terry incubation procedure (20) was used with 15 ml of ruminal fluid and 35 ml of McDougall's buffer solution per 0.5 g of sample. Incubations took place in 100-ml polyethylene tubes in a shaking water bath maintained at 39°C. Following incubation, the tubes were centrifuged at 3,000 × *g* for 15 min and the supernatants were removed by aspiration. The residues were dried at 55°C for 96 h, and the in vitro DM degradability (IVDMD) was calculated as the percentage of DM which disappeared during incubation.

Statistical analyses. For statistical analyses, graphs were generated with the S Plus statistical package and data were analyzed with the SAS statistical package.

RESULTS

The sheep remained healthy throughout the study. All preinoculation fecal samples were negative for *E. coli* O157:H7. Regardless of their diet, after inoculation all 27 animals initially shed *E. coli* O157:H7 in amounts detectable without enrichment culture. There were no differences in apparent colonization, duration of shedding, or quantity of *E. coli* O157:H7 organisms shed with regard to the initial inoculum strains. For most animals, the number of *E. coli* O157:H7 organisms in fecal samples declined steadily over time until detection in fecal samples required selective-enrichment culture, and by day 41, most animals stopped shedding the bacteria.

Diet composition. G was lower in protein and digestible energy and higher in fiber than C. The protein contents of G and C were 4.69 and 12.82%, respectively. NDF and ADF values, which are negative indicators of digestible energy, were 57.97 and 38.20%, respectively, for G and 31.03 and 22.09%, respectively, for C. High-fiber values are associated with lower IVDMD values. The IVDMDs were 45.94% for G and 71.98% for C.

Effect of diet on the shedding of *E. coli* O157:H7 by sheep. Sheep that were fed G shed the bacterium (as detected by selective-enrichment culture) almost twice as long as sheep that were fed C, as shown in Fig. 1 and 2 and Table 2. (The feeds differed significantly in nutrient and fiber content [see above].) The average duration over which G-fed sheep shed *E. coli* O157:H7 at levels detectable without selective-enrichment culture was approximately 2 weeks, in contrast to C-fed sheep, for which the organism was detectable without enrichment for only about 1 week (Fig. 1). When the number of *E. coli* O157:H7 organisms being shed in the feces could not be detected by direct plating, animals in both groups continued to shed the bacteria, at levels detectable only by selective-enrichment culture, for about 1 week. Selective-enrichment culture of feces also demonstrated the trend that animals being fed G shed *E. coli* O157:H7 for a longer duration than did animals fed C. At 17 days postinoculation, 11 of the 14 G-fed sheep were culture positive for the bacterium compared with only 3 of the 13 C-fed sheep (Table 2). Among the animals for which the diet was not changed (groups II and IV), 50% of the G-fed animals stopped shedding the bacterium between days 21 and 24 postinoculation while 50% of the C-fed animals stopped shedding the bacterium 13 days postinoculation (Fig. 2; Table 2).

Survival analyses were used to assess the effect of diet on differences in *E. coli* O157:H7 shedding as detected by selective-enrichment methods (Fig. 3 and 4). Two end points were examined, the first being the first time that an animal was

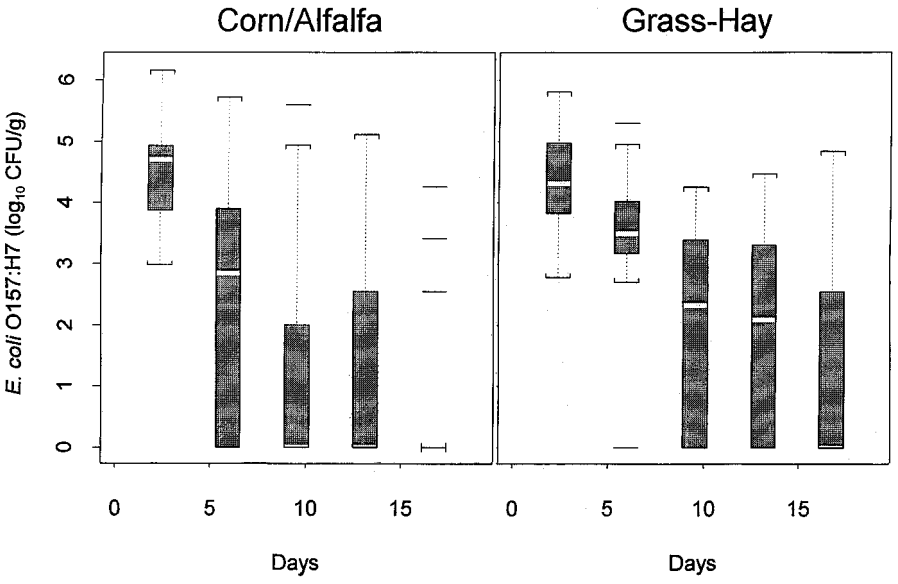

FIG. 1. Concentration of fecal *E. coli* O157:H7 from sheep fed C or G during the first 17 days postinoculation. The plots show the concentration of *E. coli* O157:H7 per gram of feces obtained by nonenrichment culture on each sampling day. The plots represent $\log_{10}$ (CFU/g + 1), so that the log of a zero reading is defined as 0. The plots depict the distribution of data, with the white band representing the median and the shaded box representing the middle 50% of the values. The lines extending from each plot and ending in a bracket represent data that fell outside the middle 50% (the interquartile range) but were within 1.5 times the interquartile range from the adjacent quartile. A horizontal line outside the brackets shows an individual datum point that falls outside the bracketed range.

culture negative and the second being the first time that an animal was culture negative on two consecutive sampling days. All four groups of animals were used, but in groups I and III (in which the diet was changed), data were only used through day 17. The resulting *P* values (Wilcoxon test for censored data) for the "first time an animal was culture negative" and "two consecutive culture-negative samples" events were 0.002 and 0.025, respectively, indicating that the animals that were fed C shed *E. coli* O157:H7 for a shorter duration than did the animals that were fed G. The *P* values must be interpreted cautiously because horizontal transmission of strains occurred (see below), which violates the assumption of independence of animals that is used in the Wilcoxon test. However, whenever animals showed evidence of horizontal transmission, it was typically within the first 10 days postinoculation and only rarely was the transmitted strain detected in feces more than once (Fig. 3 and 4). For these reasons, a cautious interpretation of the Wilcoxon test is appropriate and lends support to the theory that C leads to faster clearance of the bacterium than does G.

Effect of an abrupt dietary change on the shedding of *E. coli* O157:H7 by sheep. We compared the effect of abruptly changing the diet (from G to C or from C to G) with that of no dietary change. The animals in groups II and IV had no dietary alterations and were fed their original diet throughout the study. With one exception, the number of positive animals among these two groups steadily declined and the concentration of *E. coli* O157:H7 in fecal samples, as determined by both nonenrichment (Fig. 1) and enrichment (Fig. 2) cultures, steadily declined over time. The single exception to this pattern was seen with animal G1, which was culture negative on day 21 but culture positive on days 26, 31, and 34. In contrast, we observed more fluctuation in the number of culture-positive animals among the animals that experienced an abrupt diet change (Fig. 2). Two animals, CG2 and CG4, were culture negative and became culture positive within 24 h of the dietary change. Also, when the reverse dietary change (G to C) was imposed, two previously *E. coli* O157:H7-positive animals (GC3 and GC4) became culture negative. After the dietary change, there were more culture-positive animals in groups I and III (dietary change) than in groups II and IV (no dietary change) (Fig. 2). Tests were conducted separately comparing these results on days 21, 24, and 27 postinoculation and also longitudinally combining the three days. In these analyses, all conducted by Fisher's exact test, the lowest *P* value was obtained for day 24 ($P = 0.046$). Other tests were not significant at a *P* value of 0.05. The caveats regarding the independence of each animal, discussed above, also apply to this analysis. Interestingly, regardless of the dietary regimen of the group, one animal in each group shed *E. coli* O157:H7 at every sampling for the 41 days of the study: animals G4, GC7, C2, and CG7. These animals were identical, in every way that we measured, to animals in their respective group that shed the bacterium for much shorter durations (6 to 13 days).

Comparison of the selective-enrichment and nonenrichment protocols. All fecal samples were cultured for *E. coli* O157:H7 by both the selective-enrichment and nonenrichment protocols. Fewer *E. coli* O157:H7-positive fecal samples were iden-

TABLE 2. Effect of G and C on *E. coli* O157:H7 shedding during the first 17 days postinoculation

Sampling day	No. of *E. coli* O157:H7-positive animals fed:	
	G (groups I and II) (14)[a]	C (groups III and IV) (13)[a]
Predose	0	0
2	14	13
6	14	13
10	14	11
13	14	9
17	11	3

[a] The number in parentheses indicates the total number of sheep in the groups.

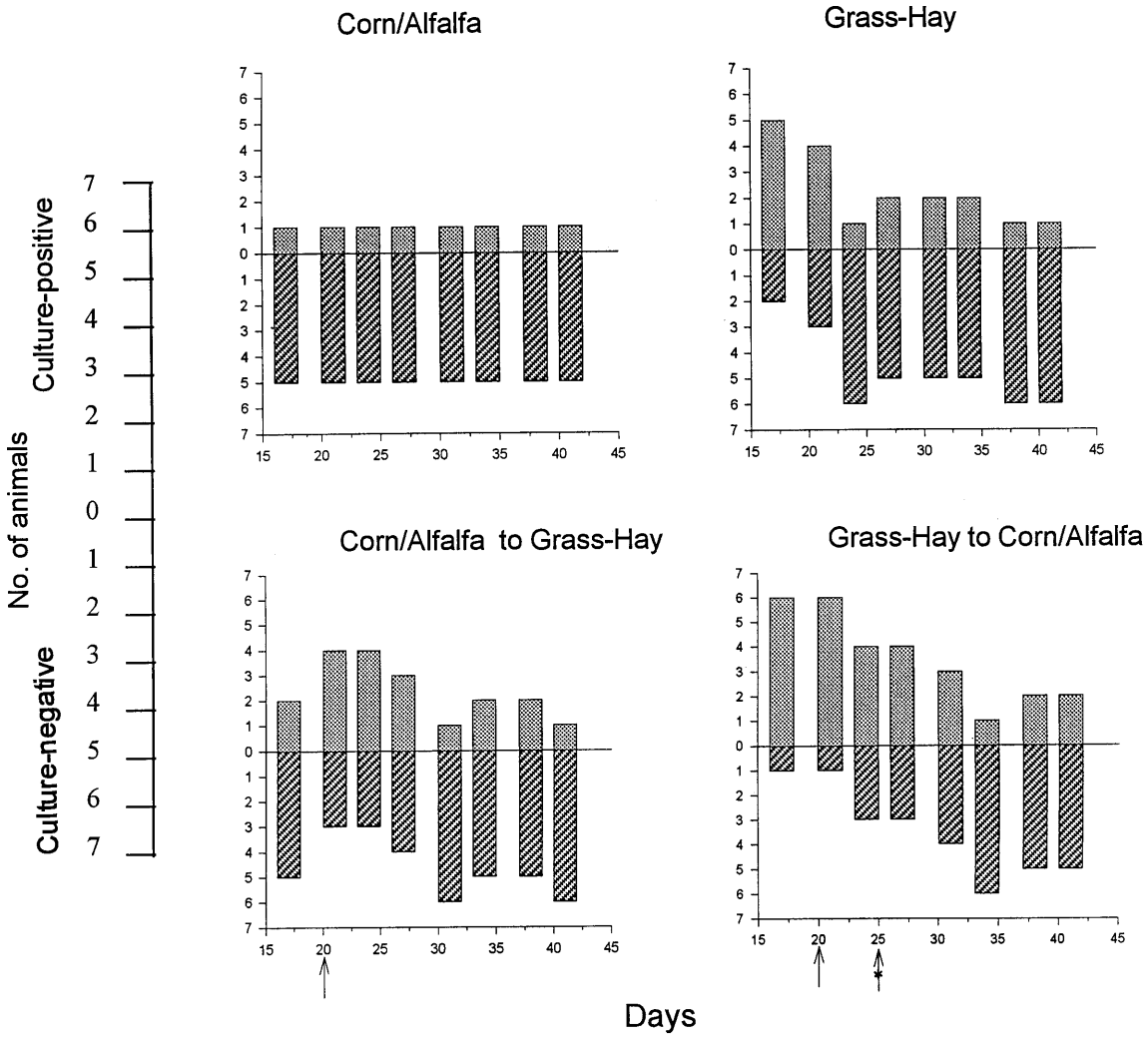

FIG. 2. Number of *E. coli* O157:H7-positive (stippled bars) and -negative (hatched bars) sheep identified by selective-enrichment culture of feces between days 17 and 41 postinoculation. The top two graphs represent the sheep that were fed C or G without dietary change. The bottom two graphs represent the sheep that experienced a dietary change on day 20 (vertical arrow). The diet change from C to G was immediate, and the change from G to C was gradual and was completed on day 25 (asterisk).

tified by nonenrichment than by selective enrichment (data not shown). For instance, *E. coli* O157:H7 was detected in the feces of group II ewes (GC1 to GC7) by both protocols on days 2 and 6 postinoculation. However, by day 21 postinoculation, only one fecal sample was *E. coli* O157:H7 positive by nonenrichment culture but six samples were positive by selective-enrichment culture. As expected, the ability to detect *E. coli* O157:H7 in fecal samples by the nonenrichment culture was directly linked to the concentration of the bacterium. *E. coli* O157:H7 concentrations near 10^2 CFU/g were detected by the nonenrichment culture, but lower concentrations required selective-enrichment culture for detection.

Horizontal transmission of strains between pen mates. The two O157 strains were shed simultaneously in the same fecal sample by 10 of the 27 (37%) animals tested. Among these, eight animals shed the two strains simultaneously at only one sampling time and the remaining two shed both strains at two sampling times (Fig. 3 and 4). Most of the detected horizontal transmission occurred early in the study; in seven animals it occurred only on or before day 10 postinoculation. All but two of the 20 O157 colonies tested for nalidixic acid resistance were of the original inoculum strain, and in no case did the transmitted strain persist or become the dominant colonizing strain. The strain acquired by horizontal transmission was detected only by selective-enrichment culture and never by nonenrichment culture, indicating its low concentration. Of the 324 fecal samples analyzed, only 12 (3.7%) were positive for both the *E. coli* O157:H7 strains simultaneously. Considering that we were not able to detect the acquisition by an animal of its original inoculum strain from the environment, a more accurate estimate of the incidence of two strains being present in a single fecal sample would double that measured, to 7.4% ± 1.9% (standard error). The two strains were probably not different in their ability to be passed between animals, because we did not measure any differences between infectious doses, patterns of presence in fecal samples, or laboratory survival of the two strains (data not shown). Both ATCC 43894 and the Nal^r strain were isolated from the drinking-trough water samples but only by selective-enrichment culture.

Fecal chemical composition. Fecal sample results for the variables pH, NDF, ADF, IVDMD, and protein content were analyzed by a two-factor analysis of variance model, with the

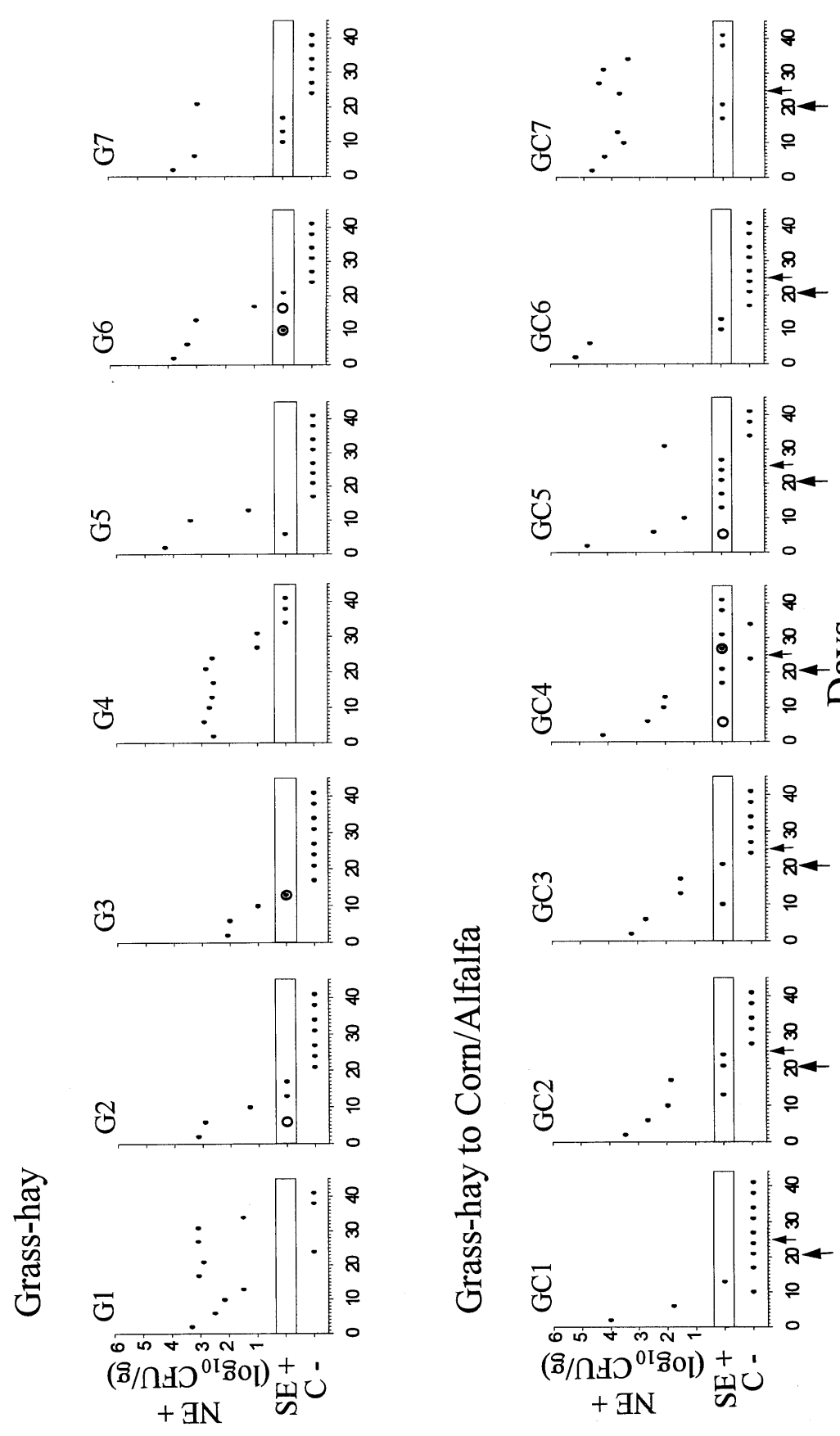

FIG. 3. Effect of a G-to-C change on *E. coli* O157:H7 shedding by sheep. Each graph represents the concentration of *E. coli* O157:H7 recovered from an individual animal over time. The animals are indicated by the letter-number designation at the top left corner of each graph. The top row represents animals that were fed G without a dietary change. The bottom row represents animals that were fed G and then C. The arrows indicate the day of dietary change. NE+, nonenrichment culture positive; SE+, selective-enrichment culture positive; C−, culture negative. Data within the SE+ box were culture positive only by selective enrichment. Open circles indicate that both the ATCC 43894 and the Nal' *E. coli* O157:H7 strains were recovered from that fecal sample by selective enrichment.

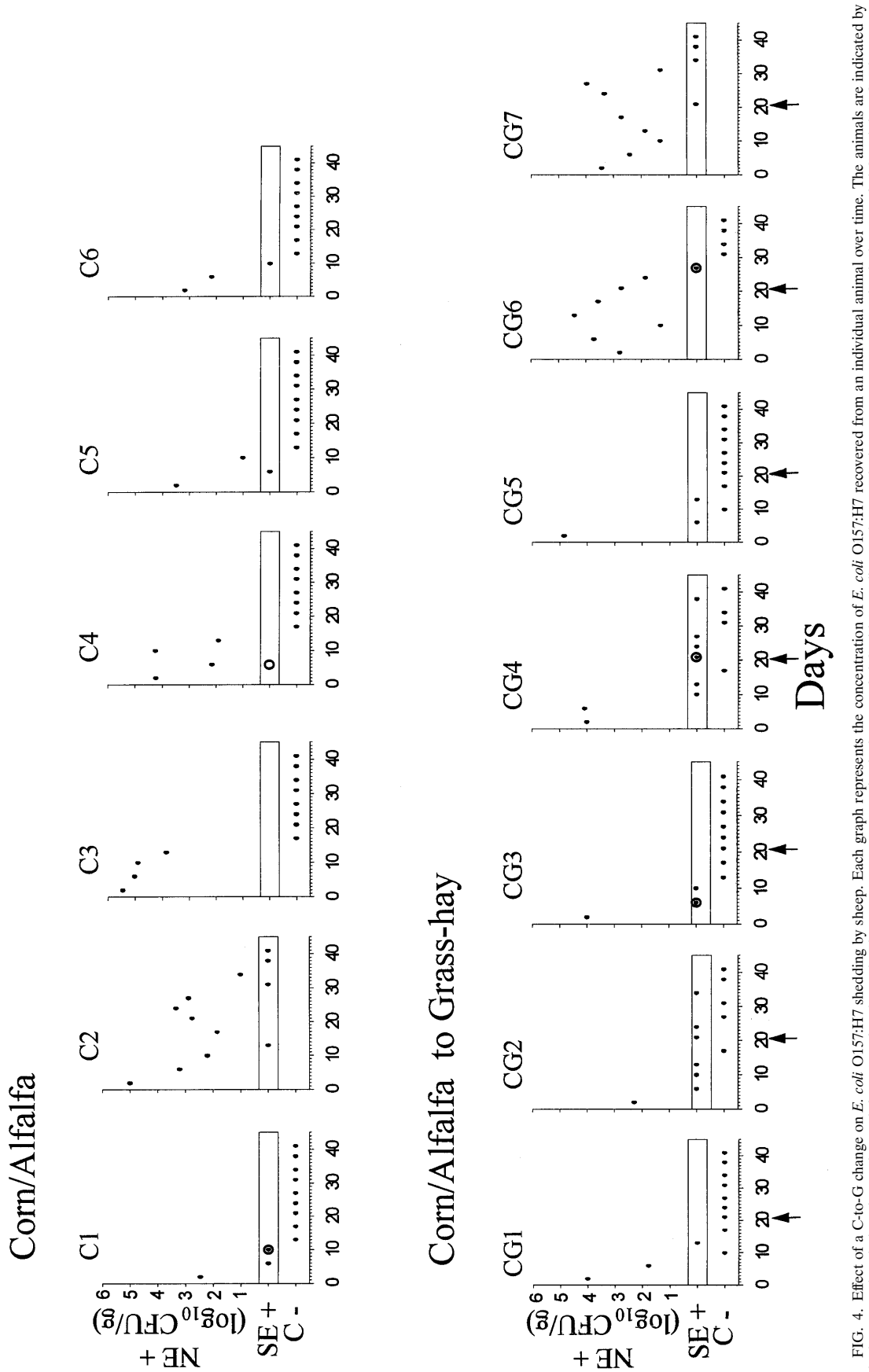

FIG. 4. Effect of a C-to-G change on *E. coli* O157:H7 shedding by sheep. Each graph represents the concentration of *E. coli* O157:H7 recovered from an individual animal over time. The animals are indicated by the letter-number designation at the top left corner of each graph. The top row represents animals that were fed C without a dietary change. The bottom row represents animals that were fed C and then G. The arrows indicate the day of dietary change. NE+, nonenrichment culture positive; SE+, selective-enrichment culture positive; C−, culture negative. Data within the SE+ box were culture positive only by selective enrichment. Open circles indicate that both the ATCC 43894 and the Nal^r *E. coli* O157:H7 strains were recovered from that fecal sample by selective enrichment.

TABLE 3. Duration of *E. coli* O157:H7 shedding by sheep after the primary and secondary inoculations

Sheep	Duration (days) of *E. coli* O157:H7 shedding after:	
	Primary inoculation	Secondary inoculation
CG1	13	7
CG3	10	7
CG5	13	14
G3	13	14
G5	13	7
GC6	13	14
C1	10	14
C3	13	7
C4	13	28
C5	10	14
C6	10	21

first factor being the diet for the first stage of the experiment (G or C) and the second factor being the diet for the second stage of the experiment (again G or C). The average pH of the feces for all animals, regardless of their diet or if their diet had abruptly changed, did not differ and ranged from 7.44 to 7.61. For the NDF measurement, a significant interaction between the two factors ($P = 0.0054$) was found. The highest fecal NDF content was observed for group II (G1 to G7) ewes (61.13% [mean]), while the lowest fecal NDF content was observed for group III (CG1 to CG7) ewes (56.27%). The fecal NDF content was intermediate between these measurements for groups I and IV and was not significantly different from the values for group III. The fecal concentration of ADF was significantly lower (34.10%) for ewes fed G than for ewes fed C (40.10%) during sampling ($P < 0.0001$). Ewes fed C throughout the study had feces with higher IVDMD (mean IVDMD = 19.0%) than did ewes fed the other three diet regimens (13.1%) (all pairwise P values were <0.005). Animals fed G during sampling had a lower fecal protein content (mean protein content = 6.76%) than did animals fed C (13.63%) ($P < 0.0001$).

Effect of withholding feed and water on the shedding of *E. coli* O157:H7 by sheep. Feed and water were withheld from a group of seven ewes, among which one animal was *E. coli* O157:H7 culture positive and six were *E. coli* O157:H7 culture negative. All the animals were culture negative 24 h after the dietary stress.

Ability of *E. coli* O157:H7 to recolonize sheep that had recently shed *E. coli* O157:H7. Of the 11 ewes selected for the recolonization study, all except animal G5 were *E. coli* O157:H7 negative on the day of the second inoculation. Fecal samples from ewe G5 gave a positive result for the Nal^r strain by nonenrichment culture at approximately 5×10^2 CFU/g. At 24 h after the secondary inoculation, all fecal samples were *E. coli* O157:H7 positive by nonenrichment culture and the animals continued to shed the bacterium for various durations (Table 3). Interestingly, animal G5 shed only ATCC 43894 for 21 days after the secondary inoculation and the Nal^r strain was not detected in any fecal sample. Similar to the primary inoculation, animals initially shed the bacterium at concentrations detectable by nonenrichment culture and over time progressed to shed levels of the bacterium that were culturable only by the selective-enrichment technique. For example, by day 21 after the secondary inoculation, two animals were positive by selective-enrichment culture and none were positive by nonenrichment culture (data not shown). Statistical analysis confirmed that there were no differences in the duration of shedding or concentration of *E. coli* O157:H7 organisms shed by animals after the primary and secondary inoculations. In addition, no differences after the secondary inoculation were seen between animals that had previously shed the same (ATCC 43894) or similar (Nal^r) strains of *E. coli* O157:H7. We believe that horizontal transmission from the environment occurred in three animals during this study, although we could not demonstrate it by strain difference. Animals G5, GC6, and C4 had one culture-positive fecal sample on days 21, 35, and 35 after the secondary inoculation, respectively, after they had been culture negative.

DISCUSSION

The results presented here support three conclusions about the relationship between *E. coli* O157:H7 and culture-positive ruminants: (i) diet influences the concentration of *E. coli* O157:H7 organisms shed and the duration of shedding, (ii) an abrupt diet change can induce an increase in the number of *E. coli* O157:H7 culture-positive animals, and (iii) recent colonization with this bacterium does not prevent recolonization.

We used sheep as an economical and easy-to-handle ruminant model that can be experimentally inoculated with *E. coli* O157:H7 (17). Sheep, like cattle, are naturally colonized by *E. coli* O157:H7 in a transient and seasonal manner and by other enterohemorrhagic *E. coli* strains and other Stx-producing *E. coli* strains (18, 19).

To determine if diet, abrupt diet change, or withholding of feed and water had an effect on fecal *E. coli* O157:H7, the sheep were fed a grass-hay diet (G), which was low in protein and digestible energy and high in fiber, or a mixture of corn and pelleted alfalfa (C), which was high in protein and digestible energy and low in fiber. The choice of these diets was influenced by our previous study, in which sheep were on a sagebrush bunch-grass range or fed pelleted alfalfa, diets less extreme than G and C (17). In that study, culture-positive animals on the sagebrush bunch-grass range (higher in fiber and lower in nutrients) shed the bacteria longer than animals being fed a lower-fiber, more nutritious diet of pelleted alfalfa (average durations, 8 to 15 days and 2 to 6 days, respectively). The results in the present study parallel these previous results.

Diet quality differences resulted in distinctive patterns of *E. coli* O157:H7 shedding between sheep. Ewes that were fed G shed *E. coli* O157:H7 for the longest duration. Many earlier studies have established an association between the ruminal and other gastrointestinal tract volatile fatty acid concentration (VFA) and pH and the dietary fiber and nutrient quality (2, 4, 9, 23). High-fiber low-nutrient feeds decrease the VFA and increase the pH, while low-fiber and high-nutrient feeds have the opposite effect (4, 12, 25). Although we did not measure gastrointestinal VFA or pH, our analysis of the diets suggests that C generated high VFA and low pH, a less hospitable environment for *E. coli*, while G generated low VFA and high pH, which was more permissive for the growth and survival of *E. coli* (23). These conditions, which would be expected to influence *E. coli* in general, may have had similar effects on *E. coli* O157:H7.

We hypothesized that if diet influences fecal *E. coli* O157:H7, an abrupt change to a diet that creates a more hospitable gastrointestinal tract environment for *E. coli* O157:H7 should induce increased concentrations of the bacteria in the feces of culture-positive animals and vice versa. The two diet changes seemed to have opposite effects on fecal *E. coli* O157:H7. Although caution must be used when drawing conclusions based on so few animals, we observed the following trends: increased shedding of *E. coli* O157:H7 when the diet was abruptly changed from C to G and decreased shedding with the

opposite change, G to C. Interestingly, when all animals that experienced a dietary change were compared to all animals that did not, there was a significantly larger number of culture-positive ewes 4 days after the dietary change (day 24 postinoculation). Thus, both disruptions in the diet regimen (C to G and G to C) may have altered the gastrointestinal tract environment to induce proliferation of *E. coli* O157:H7.

Fecal composition was measured to draw associations between components in the feces and the diet fed, as well as the shedding of *E. coli* O157:H7. Ewes fed C had a greater fecal ADF content and IVDMD than did ewes fed G. A greater fecal IVDMD for C-fed ewes would suggest that a greater proportion of fermentable constituents of the diet escaped ruminal and hindgut fermentation and arrived in the feces. The high starch content of C may have disrupted ruminal fiber digestion, which would explain the higher fecal ADF and IVDMD of the C-fed ewes. The detrimental effects that starch from grains commonly has on ruminal fiber digestion have been reported (15). Also, it is interesting that G-fed ewes shed *E. coli* O157:H7 in feces longer than did C-fed ewes, with feces characterized by low fiber, protein, and IVDMD. It remains unclear whether differences in shedding of the bacterium, associated with differences in diet, are produced by changes in fecal composition or by changes in digesta passage kinetics present in the gastrointestinal tract. In addition, the complexity of the ruminant digestive system and its flora cannot be overlooked, and diet composition may not be the immediate influence on the shedding of *E. coli* O157:H7.

Because the sheep within a group were penned together in close contact and with common water troughs, we predicted that horizontal transmission of *E. coli* O157:H7 between pen mates would occur. This mode of infection has been reported previously between experimentally dosed and nondosed sheep (17). In support of the idea that ruminants can acquire an infection from the environment, Faith et al., in a recent survey, showed that *E. coli* O157:H7 isolates from some culture-positive cattle on the same farm had identical restriction endonuclease digestion profiles (7). In addition, they suggested that one possible source of the *E. coli* O157:H7 on one farm was the drinking water used by the animals (7). Horizontal transmission, in our study, was assessed by using only two marked strains of *E. coli* O157:H7, so that we were able to measure approximately half of the occurrences. As might be expected, horizontal transmission was most frequently seen when most animals were culture positive and the fecal concentrations of *E. coli* O157:H7 were high (day 6 postinoculation). Although we did not culture environmental surfaces, we did isolate both *E. coli* O157:H7 strains from the drinking water, and so water may have been a vehicle for dissemination of *E. coli* O157:H7 among pen mates.

Similar to the effects of a lower-quality feed, fasting decreases the gastrointestinal tract VFA, and we predicted that it would increase fecal *E. coli* O157:H7 counts in culture-positive animals (21, 24). In this study, we did not see an effect on fecal *E. coli* O157:H7 counts after withholding feed and water for 24 h. It may be that when feed and water were withheld from the animals in this study, most of the animals were no longer colonized with *E. coli* O157 and therefore increases in the fecal concentration could not occur. There may be an undefined difference in gastrointestinal colonization that precludes our seeing an effect from fasting. A few animals in this study had wide variation in the duration of fecal shedding (6 to 41 days) that is similar to the previously reported variation in shedding among cattle experimentally inoculated with *E. coli* O157:H7 (5).

The susceptibility of previously *E. coli* O157:H7 culture-positive ruminants to reinoculation was first reported by Cray and Moon (5). In that study, which was done with calves, it was shown that previous infection did not prevent reinfection with the same strain of *E. coli* O157:H7 (5). Likewise, in our study, previous exposure to *E. coli* O157:H7 did not affect the susceptibility of the animals to either strain, at least with high-dose (10^{10} CFU) inocula. Upon secondary inoculation, all animals were colonized by *E. coli* O157:H7 and shed the organism for durations equal to those seen after the primary inoculation. This finding correlates with our earlier report of the isolation of naturally occurring *E. coli* O157:H7 organisms, of similar or different fingerprint types, from sheep at sampling times 60 to 365 days apart (18, 19).

The findings presented here indicate that preharvest control of the diet may reduce the risk of *E. coli* O157:H7-positive animals entering our food chain. Animals on a high-quality feed, which do not experience a dietary disturbance, may have a lower incidence of *E. coli* O157:H7 shedding and therefore would be less likely to result in contaminated products. Experiments are under way in our laboratories to confirm that the effects we have demonstrated in sheep are reproducible in cattle.

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