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Effect of Cattle Diet on *Escherichia coli* O157:H7 Acid Resistance

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The duration of shedding of *Escherichia coli* O157 isolates by hay-fed and grain-fed steers experimentally inoculated with *E. coli* O157:H7 was compared, as well as the acid resistance of the bacteria. The hay-fed animals shed *E. coli* O157 longer than the grain-fed animals, and irrespective of diet, these bacteria were equally acid resistant. Feeding cattle hay may increase human infections with *E. coli* O157:H7.

Preharvest cattle management significantly impacts public health. Cattle transiently harbor *Escherichia coli* O157:H7 in their gastrointestinal tracts, and many human infections result from ingestion of contaminated bovine food products (8). *E. coli* O157:H7 contamination, which has caused product recalls and plant closures (3), has an enormous economic impact. Human infections with *E. coli* O157:H7 result in hemorrhagic colitis that can progress to hemolytic-uremic syndrome, a life-threatening sequela that is the most common cause of acute renal failure in children (8). Diez-Gonzalez et al. recently reported that cattle fed grain diets have large numbers of acid-resistant total generic *E. coli* organisms in their feces, while cattle fed hay diets do not (4). They suggest that feeding cattle hay diets would reduce the risk of food-borne *E. coli* O157:H7 infections for humans (4). In view of the apparent differences between the published results of this laboratory (11, 12), and the conclusions of Diez-Gonzales et al. (4) and in view of the consequences this issue has for public health, we compared the durations for which hay-fed and grain-fed cattle were culture positive for *E. coli* O157:H7 and assessed the acid resistance of fecal *E. coli* O157:H7 from both groups of cattle.

Eight healthy 1- to 2-year-old Holstein steers were fed different diets in a modified crossover experimental design. The animals were fed once per day, had access to water ad libitum, and were housed separately in concrete stalls with cedar-chip bedding. The effects of four diets were assessed: typical finishing diets containing 82 to 90% grain (either 62.1% barley–19.3% corn or 90% corn, with the balance being forage), medium-quality 100% alfalfa hay, and low-quality 100% timothy grass hay. As expected, the grain diets were higher in energy and lower in fiber than the hay diets, as determined by standard techniques (data not shown) (1, 9, 10, 14). After 3 weeks of adaptation to a particular diet, cattle were inoculated with *E. coli* O157:H7 which had been grown in Luria-Bertani broth at 37°C with aeration to a cell density of 10^9 CFU/ml. Each animal received 10^{10} CFU of *E. coli* O157:H7 via a gastric tube placed directly into the rumen. Fresh fecal samples were obtained by rectal palpation every 3 to 4 days and were cultured by a highly sensitive technique to monitor shedding of *E. coli* O157, as previously described (11, 12).

Hay-fed (grass or alfalfa) cattle shed fecal *E. coli* O157:H7 longer than grain-fed cattle (Fig. 1). All animals were culture positive for *E. coli* O157:H7 24 h after inoculation. All animals remained healthy, and the concentration of fecal *E. coli* O157:H7 gradually decreased, until each animal became culture negative for the bacteria. Regardless of their diet, animals shed similar titers of fecal *E. coli* O157:H7 that ranged from 7.1×10^6 CFU/g of feces to levels detectable only by selective enrichment culture ($<10^2$ CFU/g of feces). The average duration grain-fed cattle were culture positive was 4 days. In contrast, cattle fed either alfalfa or grass hay diets shed fecal *E. coli* O157:H7 for longer times, averaging 39 and 42 days, respectively. The difference between the durations that grain-fed and hay-fed (grass or alfalfa) cattle were culture positive was highly significant (paired t test; $P = 0.0004$).

Although these cattle shed *E. coli* O157:H7 longer when they were fed hay than when they were fed grain, Diez-Gonzalez et al. (4) predicted that the pathogens from hay-fed animals would be killed by an acid shock similar to the conditions of the human stomach and would therefore pose less of a disease risk. Central to their argument is the extrapolation that *E. coli* O157:H7 is similar to the generic nonpathogenic *E. coli* strains they analyzed. To determine if *E. coli* O157:H7 from the colonic digesta of grain-fed ruminants is more acid resistant than *E. coli* O157:H7 from the colonic digesta of hay-fed ruminants, we compared the acid resistance of *E. coli* O157:H7 shed by the cattle on these two diets.

The acid resistance of *E. coli* O157:H7 was unaffected by the diets (Table 1). Eight Holstein steers were put on a rotation between diets of either grain or hay. When they had adapted to a diet, animals were inoculated with *E. coli* O157:H7. Fresh fecal samples were collected by rectal palpation on three separate days during the first week postinoculation, and the acid resistance of *E. coli* O157:H7 was assessed. To prevent bacterial growth outside the colonic conditions, only fecal samples containing *E. coli* O157:H7 in concentrations high enough to detect without selective enrichment culture ($>10^2$ CFU/g of feces) were tested. *E. coli* O157:H7 and total coliform titers in each sample were compared before and after acid shock and were expressed as percent survival. To mimic exposure to stomach acid, conditions similar to those employed by Diez-Gonzalez et al. (4) were used, except that we modified a non-enrichment culture procedure (12). Fecal samples were suspended in Trypticase soy broth, pH 2.0, incubated 1 h at 37°C, and neutralized by dilution. Bacteria were enumerated by plate count. Although there was variation in *E. coli* O157:H7 percent survival among the isolates obtained from the animals, there

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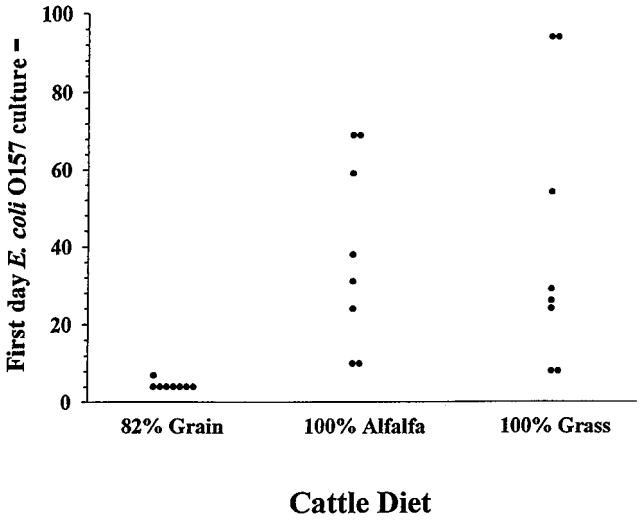

FIG. 1. Effect of diet on the duration for which *E. coli* O157:H7 is shed from cattle. Shown is the first day that each of the cattle tested culture negative for *E. coli* O157:H7 while being fed grain or hay (alfalfa or grass). Each dot represents an individual animal. The inoculum was *E. coli* O157:H7 strain ATCC 43894 (American Type Culture Collection, Manassas, Va.). Animals were considered culture negative for the bacteria after three consecutive culture-negative analyses, spanning 10 days.

was consistently less than 1 log of *E. coli* O157:H7 death, and the difference between these two groups was insignificant ($P = 0.77$) (Table 1). In fact, *E. coli* O157:H7 isolates from two hay-fed animals (animals 2 and 3) were completely resistant to acid shock under the conditions used. The same analyses on two subsequent days were similar and did not show significant differences in *E. coli* O157:H7 acid resistance ($P = 0.17$ and 0.69; data not shown). Like Diez-Gonzalez et al. (4), we determined that nonpathogenic coliforms from hay-fed cattle were more sensitive to acid shock than the coliforms from grain-fed animals ($P = 0.0024$). The mean percents coliform survival from three sampling days were 50% for hay-fed animals and 86% for grain-fed animals. However, this difference was small compared to the >3-$\log_{10}$-fold difference reported by Diez-Gonzalez et al. (4); it may be due to the differences in the methods of acid exposure or bacterial enumeration.

TABLE 1. Acid resistance of *E. coli* O157:H7 from grain-fed and hay-fed cattle

Animal no.	% *E. coli* survival in cattle fed[a]:	
	Grain	Hay
1	75	29
2	34	100
3	12	100
4	10	15
5	82	23
6	22	29
7	34	19
8	23	20
Mean	37	42

[a] The grain diet contained 90% corn–10% alfalfa silage. The hay diet contained 100% timothy. The average pH of the colonic digesta from animals fed grain was 5.5, and the average pH of the colonic digesta from animals fed hay was 7.2; both are consistent with the digesta pH values reported in reference 4. Percents survival were calculated from cultures on differential sorbitol MacConkey agar supplemented with 4-methylumbelliferyl-β-D-glucuronide (100 mg/liter) before and after acid shock at pH 2.0 for 1 h at 37°C. Presumptive *E. coli* O157:H7 isolates were confirmed serologically. The inoculum was *E. coli* O157:H7 strain ATCC 43895 (American Type Culture Collection, Manassas, Va.). All animals were tested on three separate days; data from one typical sampling day are shown.

Despite the fact that acidic contents of the human stomach provide some defense against pathogens and that acid-resistant *E. coli* O157:H7 isolates are more likely to survive passage through the acidic conditions of the stomach, the significance of acid-resistant *E. coli* O157:H7 in bovine feces is questionable. For example, humans rarely, if ever, ingest *E. coli* O157:H7 directly from the colonic digesta of cattle. The bacteria very likely replicate outside the bovine colon on the hides of the animals, in manure, in low-nutrient environments, or in contaminated food or water before they are ingested by humans (5, 13). In addition, Waterman and Small (15) recently showed that *E. coli* O157:H7 and other enteric pathogens may be protected from killing under extreme acidic conditions when they are on solid food surfaces. Bacteria inoculated onto the surface of ground beef survived acid shock conditions that a pure culture of the same organism did not (15). Because the *E. coli* O157:H7 ingested by humans is likely to have replicated outside the bovine colon and is likely to be on the surface of food when ingested, we compared the acid resistance of *E. coli* O157:H7 from the hay-fed cattle with that from the grain-fed cattle under similar conditions (15).

E. coli O157:H7 isolates from grain- or hay-fed cattle were acid resistant when they were placed on the surface of ground beef. Isolates from cattle feces were minimally subcultured in the laboratory (at the original isolation from feces and once before inoculation onto the ground beef) before testing. Isolates were placed on the surface of the ground beef, the inoculated ground beef was suspended in Luria-Bertani broth adjusted to pH 2.0 for 1 h at 37°C, and the percents survival were calculated following the acid shock procedure in reference 15, except that the pH used in this study was 2.0. Percentages greater than 100 resulted from the recovery of higher *E. coli* O157:H7 titers after pH 2.0 treatment than after pH 7.0 treatment. The percents survival for *E. coli* O157:H7 strain ATCC 43894 from grain- and hay-fed cattle were 84 and 110, respectively. For *E. coli* ATCC 43895 from grain- and hay-fed cattle, the percentages were 133 and 100, respectively. Data are the averages of three separate experiments (standard error = 10%). Interestingly, even *E. coli* O157:H7 ATCC 43894, which had previously been reported to be acid sensitive (2), was acid resistant under these conditions.

Proper preharvest cattle management has the potential to significantly reduce the risk of *E. coli* O157:H7 human disease (14). This laboratory has previously shown that hay diets and abrupt dietary changes extend the duration for which culture-positive sheep shed *E. coli* O157:H7 (11, 12). Similarly, the results of this study confirm that cattle shed *E. coli* O157:H7 longer when they are fed hay than when they are fed grain. Although this diet effect is clear in experimentally inoculated animals, there has been no epidemiological report showing a correlation between the incidence of *E. coli* O157:H7 culture-positive cattle and their diet (6, 7). Perhaps grain-fed animals are in more heavily contaminated environments or in environments that promote transmission of infection so that differences in incidence are masked by more frequent reinfection. Also, the breed, age, or number of animals analyzed could influence any inoculation-type study. Since we show here that the acid resistance of *E. coli* O157:H7 is not affected by the diet of cattle, we caution against preharvest management that includes an abrupt dietary change from grain to hay. Rather than reduce the risk, this change may increase the likelihood that *E. coli* O157:H7 culture-positive cattle will enter our food chain.

More extensive scientific information about the relationship between the diet of cattle and *E. coli* O157:H7 is needed before management changes are advocated.

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