Human infections with *Escherichia coli* O157:H7 cause hemorrhagic colitis that can progress to a life-threatening sequelae. The most common mode of disease transmission is ingestion of contaminated bovine food products, and it is well established that *E. coli* O157:H7 is a transient member of the bovine microbiota. However, the conditions that induce acquisition and subsequent clearance of this bacterium from the ruminant gastrointestinal tract (GIT) are not understood. Evidence that the rates of epithelial cell proliferation in the lower GIT of cattle are associated with the duration animals remained *E. coli* O157:H7 culture positive is presented. Cattle with slower rates of intestinal cell proliferation in the cecum and the distal colon were culture positive significantly longer than cohort cattle with faster cell proliferation rates. Cell death rates (apoptotic indices) between the short- and long-term culture-positive animals were not different. Typical grain-based finishing diets and forage-based growing diets did not effect GIT cell proliferation or the duration animals remained *E. coli* O157:H7 culture positive. To identify a dietary intervention that would effect GIT cell proliferation, we used sheep as a model ruminant. A fasting-refeeding regime that increased the rate of GIT cell proliferation was developed. The fasting-refeeding protocol was used in cattle to test the hypothesis that feeding interventions that increase the rate of GIT cell proliferation induce the clearance of *E. coli* O157:H7 from the bovine GIT.

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and apoptosis in the intestinal tract, (iii) developed a fasting-refeeding regime to manipulate ruminant GIT cell proliferation, and (iv) tested the fasting-refeeding regime in cattle experimentally inoculated with \textit{E. coli} O157:H7.

**MATERIALS AND METHODS**

**Experimental animals.** Healthy 9- to 12-month-old Charolais × Hereford heifers and 1-year-old Holstein steers were identified by ear tags and housed without contact between animals in concrete stalls on wood-chip bedding. Healthy 1-year-old Suffolk ewes were obtained from the University of Idaho sheep herd and housed by treatment group in concrete stalls without bedding and without contact between groups.

**Bacteria.** The inoculum was \textit{E. coli} O157:H7 strain ATCC 43894 (American Type Culture Collection, Manassas, Va.). The bacteria were grown in Luria-Bertani 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 10^9 CFU of \textit{E. coli} O157:H7 via a gastric tube placed directly into the rumen.

**Rations and housing.** Animals were fed daily and had water ad libitum. Cattle were fed a typical grain-based finishing diet (referred to throughout as grain), a typical forage-based growing diet (referred to throughout as forage), or alfalfa hay. The grain diet was composed of 5% grass hay, 7.29% alfalfa silage, 62% barley, and 19.33% corn. The forage diet was composed of 19.9% grass hay, 48.6% alfalfa silage, 12% barley, and 12% corn. The remaining contents of both diets were similar and contained soybean meal, ground limestone, dicalcium phosphate, and trace mineralized salt. Cattle were adapted to a diet for a minimum of 3 days before inoculation with \textit{E. coli} O157:H7.

Ewes were divided into three groups and adapted to a diet of alfalfa hay for 3 weeks. One group of three ewes had feed withheld for 48 h prior to sacrifice (referred to throughout as fasted). A second group of three ewes had feed withheld for 48 h and was refed the alfalfa hay for 4 h prior to sacrifice (referred to throughout as fasted-refed). A third group of three ewes was fed consistently for all days prior to sacrifice (referred to throughout as consistently fed). Cattle were fed twice daily (referred to as continuously fed) or had feed withheld for 24 h before being injected with the challenge dose (referred to as fasted-refed). Cattle were fed twice daily and ad libitum access to water was resumed (referred to as fasted-refed).

**Chemical analyses of feeds.** Samples of the grain or forage feeds were dried at 60°C and ground to pass through a 1-mm screen. The samples were analyzed, using standard techniques for dry matter, crude protein, neutral detergent fiber, and acid detergent fiber (1, 30, 31, 45). The samples were also incubated in vitro as described by Terry et al. (43) to determine dry matter degradability.

**Fecal culture.** Cattle were cultured as indicated in Table 5 to monitor fecal \textit{E. coli} O157:H7. Fecal samples of 10 g were obtained by aseptic rectal palpation and cultured for \textit{E. coli} O157:H7 by both nonenrichment and enrichment culture protocols. The samples were cultured in cecal contents from animals that were typed for 

**Quantification of apoptotic cells.** As the rate of cell turnover in the intestine is affected by both cell proliferation and cell death, we quantified apoptotic cells in the distal and proximal colon regions of the cattle. The 3′ labeling of apoptotic cell DNA was performed using an ApoTag in situ apoptosis detection kit (Oncor, Gaithersburg, Md.) according to the manufacturer's instructions. Tissues were counterstained with methyl green. The number of apoptotic cells per crypt was recorded for 20 randomly selected complete crypts per animal in the proximal and distal colon segments. Sections used for measurement of apoptotic cells were from the same tissue blocks used for PCNA measurements.

**RESULTS**

All animals remained healthy for the duration of the experiment. The cattle that were inoculated with \textit{E. coli} O157:H7 were all culture positive for the bacteria 24 h after inoculation. Fecal \textit{E. coli} O157:H7 titers decreased with time until all animals became culture negative.

**The grain diet was higher in energy and lower in fiber than the forage diet.** The ingredients of the grain and forage diets were typical of cattle grain-based finishing and forage-based growing rations. Both diets are considered high-quality rations, and average weight gains were 0.9 kg/day with the forage diet and 1.4 kg/day with the grain diet. The chemical analyses of grain and forage, respectively, were as follows: dry matter, 74.1 and 63.5%; in vitro dry matter degradability, 83.1 and 62.7%; neutral detergent fiber, 25.1 and 41.8%; acid detergent fiber, 9.6 and 26.4%; and crude protein, 15.2 and 14.2%. As expected, the grain diet, which was higher in grain content, was higher in protein and digestible energy and lower in fiber than the forage diet. Acid and neutral detergent fiber values are negative indicators of digestible energy, and both values were lower for the grain diet than for the forage diet. In addition, fiber concentration is inversely related to in vitro dry-matter degradability values, and dietary fiber was lower for forage than for grain.

**The grain and forage diet did not affect the duration or concentrations of fecal \textit{E. coli} O157:H7.** Heifers fed the forage or grain diet were inoculated with \textit{E. coli} O157:H7, and fecal samples were cultured for the bacteria (Fig. 1). Animals on either diet were similarly \textit{E. coli} O157:H7 positive by nonenrichment culture for an average of 5 days, after which enrichment culture was required to detect the bacterium in fecal samples. Although we observed a wide variation in the duration that individuals remained culture positive for the bacteria, there was no difference between the groups fed grain or forage (Fig. 1). The Wilcoxon test for censored data gave a P value of
0.78 for a diet effect on the time-to-culture-negative status. This test failed to detect a difference in the pattern of E. coli O157:H7 culture status in animals eating grain or forage. Also, the ANOVA of the mean CFU/gram value (data not shown), while culture positive at titers detected by nonenrichment, had a P value of 0.77.

**The grain and forage diet did not affect cell proliferation or apoptosis indices.** There was no difference between the grain and forage diet groups in the number of PCNA-labeled cells or the proliferation index for any segment of the intestine (all P values were >0.3) (data not shown). Split-plot ANOVA did not detect a significant diet effect for any of the cell proliferation indices (all P values were >0.4) (data not shown). There was no effect of diet on the distribution of labeled cells in the crypts or in the number of apoptotic cells per crypt in any location (all P values were >0.3) (data not shown). However, crypt height was significantly higher (P = 0.03) in the distal colon of animals fed the forage diet (65.7 ± 2.3 cells) compared with animals fed the grain diet (57.4 ± 1.7 cells).

**Short-term E. coli O157 culture-positive status was associated with high indices of intestinal cell proliferation.** To evaluate GIT cell proliferation with E. coli O157:H7 culture status, we categorized the eight heifers fed the grain or forage diets according to the duration they harbored the bacteria (Table 1). The mean duration cattle were culture positive for E. coli O157:H7 was 23 days. Three animals (animals 1, 3, and 5) were culture positive for longer than 29 days postinoculation (above the mean) and were designated as the long-term culture-positive group (Fig. 1). Five animals (animals 2, 4, 6, 7, and 8) were culture positive for 8 to 19 days (below the mean) and were designated as the short-term culture-positive group (Fig. 1).

The cell proliferation indices in the short-term and long-term groups at each GIT location are summarized in Table 1. The short-term culture-positive-positive group had higher indices of intestinal cell proliferation than the long-term culture-positive group. The greatest difference in the number of PCNA-labeled cells between the long-term and short-term groups was observed in the cecum (P = 0.016) and the distal colon (P = 0.049) (Table 1). Over all GIT locations, the number of PCNA-labeled cells tended to be higher in the short-term group than in the long-term group (effect of culture-positive duration by split-plot ANOVA: P = 0.02). There was no difference (all P values were >0.31) in mean crypt heights between the short-term and long-term groups at any GIT location. The proliferation index, which takes into account the number of labeled cells, and the total crypt height was higher in the short-term group than in the long-term group (effect of culture-positive duration by split-plot ANOVA: P = 0.043). Again, the differences in proliferation index between groups were greatest in the cecum (P = 0.027). Interestingly, animal 1 (Fig. 1) was culture positive for 69 days, 39 days longer than the other animals in this study, and also had the lowest GIT cell proliferation indices. The values for number of labeled cells/crypt in this animal were all well below the mean: ileum, 21.5; cecum, 0.8; proximal colon, 5.1; central colon, 2.3; and distal colon, 7.8. Although the crypt heights at all GIT locations in animal 1 were similar to those for the other animals, the cell proliferation indices were well below the mean: ileum, 18.9%; cecum, 0.9%; proximal colon, 6.8%; central colon, 2.2%; and distal colon, 6.6%.

Therefore, short-term culture-positive animals had a significantly higher overall rate of cell proliferation in the GIT than did long-term culture-positive animals. This was most pronounced in the cecum and distal colon. The GIT location affected all indices. For example, the ileum had the highest number of labeled cells and labeling index of all GIT locations (P < 0.0001). Crypt height was higher in the ileum and distal

### Table 1. Cattle intestinal cell proliferation and persistence of E. coli O157:H7

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration culture positive for E. coli O157:H7</th>
<th>Mean ± SE results:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long-term</td>
<td>All locations</td>
</tr>
<tr>
<td>Labeled cells (no. of cells/crypt)</td>
<td></td>
<td>Ileum</td>
</tr>
<tr>
<td>Long-term</td>
<td>27.5 ± 3.7</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>Short-term</td>
<td>29.1 ± 3.0</td>
<td>10.9 ± 1.6</td>
</tr>
<tr>
<td>Crypt height (no. of cells/crypt column)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term</td>
<td>68.7 ± 7.6</td>
<td>40.0 ± 2.3</td>
</tr>
<tr>
<td>Short-term</td>
<td>65.1 ± 2.3</td>
<td>41.8 ± 1.6</td>
</tr>
<tr>
<td>Proliferation index (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term</td>
<td>20.3 ± 2.9</td>
<td>3.9 ± 1.9</td>
</tr>
<tr>
<td>Short-term</td>
<td>22.3 ± 2.0</td>
<td>13.2 ± 2.2</td>
</tr>
</tbody>
</table>

* Labeled cells are the number of PCNA-labeled cells per crypt.
* Crypt height is the total number of cells in each crypt column.
* The proliferation index is the percentage of PCNA-labeled cells calculated as follows: [labeled cells/(2 × crypt height)] × 100.
* Long-term, animals that remained E. coli O157:H7 culture positive for 29 to 69 days (n = 3); short-term, animals that remained E. coli O157:H7 culture positive for 8 to 19 days (n = 5).
* Labeled cells were fewer in the long-term than in the short-term group in the cecum (P = 0.016) and distal colon (P = 0.027) (t test).
* Significant effect of E. coli O157:H7 culture-positive duration over all locations (P = 0.043, split-plot ANOVA).

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**FIG. 1.** Effect of diet on the duration cattle remained culture positive for E. coli O157:H7. Heifers were adapted to a typical forage-based growing diet (open bars) or grain-based finishing diet (shaded bars) and inoculated with a single oral dose of E. coli O157:H7. Each bar represents a single animal. The vertical line separates the animals into short-term (<19 days) (left side) and long-term (>29 days) (right side) E. coli O157:H7 culture-positive groups.
colon than in the other locations ($P < 0.0001$). However, these location effects had no interaction effects with the duration animals were *E. coli* O157:H7 culture positive.

There were no differences (all $P$ values were $>0.16$) between short- and long-term culture-positive animals in the distribution of labeled cells throughout individual crypts at any location (data not shown). In other words, the percentages of total labeled cells in crypts were similar in each location for both groups. In all locations, approximately 60 to 70% of the total number of labeled cells occurred in the lower third of the crypts. There was also no difference (all $P$ values were $>0.39$) in the number of apoptotic cells in crypts in the proximal or distal colon between short-term and long-term culture-positive groups (data not shown).

**Intestinal cell proliferation indices were increased in fasted-refed sheep.** To identify a dietary intervention that would effect GIT cell proliferation we used sheep as a model ruminant. Sheep that were fasted-refed had a significantly higher number of PCNA-labeled cells per crypt in the distal colon ($62.9 \pm 9.2$) than did sheep that were either fasted ($26.3 \pm 8.5$) or continuously fed ($33.6 \pm 6.1$) ($P = 0.0395$ by one-way ANOVA). There was no difference (all $P$ values were $>0.28$) between groups in the number of PCNA-labeled cells in the ileum, cecum, central colon, or proximal colon. However, there was a difference among groups in the proliferation indices of the distal colon. Sheep that were fasted and refed had a higher labeling index ($53.9 \pm 6.0$) than sheep that were either fasted ($23.5 \pm 7.3$) or continuously fed ($25.6 \pm 5.3$) ($P = 0.017$). A similar trend occurred in the proximal and central colon, but differences did not reach statistical significance. Split-plot ANOVA was used to assess this dietary intervention effect over all GIT locations. The fasted-refed sheep had significantly higher proliferation indices over all GIT locations than either fasted or continuously fed sheep ($P = 0.024$). The proliferation index was significantly higher in the cecum and proximal colon than in the central colon ($P = 0.0014$). There were no significant differences among groups or locations in the crypt height (all $P$ values were $>0.24$). Interactions between diet and GIT location effects were not significant.

**Fasted-refed cattle were *E. coli* O157:H7 culture positive for a shorter duration than continuously fed cattle.** The effect of fasting and fasting-refeeding dietary manipulation on the duration that cattle remained *E. coli* O157:H7 culture positive was tested (Table 3). All animals were fecal O157 culture negative before receiving an oral dose of *E. coli* O157:H7, and all animals were fecal O157 culture positive 24 h after the dose. The number of O157 culture-positive animals in the continuously fed group declined steadily with time. This steady decline was not observed in the fasting-refeeding treatment group. Prior to the fast, six of the eight animals in this treatment group were culture negative. After a 24-h fast, three animals that were O157 culture negative prior to fasting (Table 3, day 17 post-*E. coli* O157:H7 dose) tested O157 culture positive (Table 3, day 18). Also, 24 and 72 h after feeding was resumed, the number of culture-positive animals declined rapidly so that only one of the eight steers in that group remained O157 culture positive on day 21. This finding is in contrast to the animals in the continuously fed group, where five of eight animals remained O157 culture positive at the same time (Table 3, day 21: chi-square test, $P = 0.04$; Fisher exact test, $P = 0.059$).

**DISCUSSION**

The most significant finding of the present study was that the rates of cell proliferation in the GIT of cattle were associated with dietary intervention. The most significant finding of the present study was that the rates of cell proliferation in the GIT of cattle were associated with dietary intervention.
with the duration animals remained *E. coli* O157:H7 culture positive. Cattle with slower rates of intestinal cell proliferation in the cecum and the distal colon were culture positive significantly longer than cohort cattle with faster cell proliferation rates. Also, we identified a fasting-refeeding regime that increased the rate of GIT cell proliferation in sheep, and we tested this dietary intervention in O157 culture-positive cattle.

Little is known about the relationship between *E. coli* O157:H7 and the ruminant intestinal mucosal surface. A perplexing issue has been the inability to identify a GIT site of bacterial colonization. Although experimentally induced enterocolitis in neonatal calves results in intimately adhered *E. coli* O157:H7 in the ileum, cecum, colon, and rectum (14, 16), these findings do not reflect *E. coli* O157:H7 colonization patterns seen in healthy cattle populations. In calves at 1 to 2 weeks after oral inoculation, *E. coli* O157:H7 has been isolated from tissue and digesta of the rumen, reticulum, abomasum, jejunum, ileum, cecum, and colon (12). Similar analyses still later (13 to 28 days) after oral inoculation isolated *E. coli* O157:H7 only from the rumen, omasum, or colon (6), suggesting that the O157:H7 serotype, as with other *E. coli*, is best adapted to the lower GIT. No study in healthy adult cattle or sheep has found histologic or immunohistochemical evidence that *E. coli* O157:H7 adheres intimately to the mucosa (15).

It has been suggested that *E. coli* O157:H7 persists in the lumen as the source of fecal shedding (6). However, it is likely that the bacteria restricted to the lumen would be flushed from the system by digesta passage in a few days. The fact that *E. coli* O157:H7 can persist in the colonic digesta of some animals for many months suggests that the bacteria associate with the mucosa in some way. The lack of evidence for this association may be a reflection of the extremely low numbers of *E. coli* O157:H7 in the ruminant GIT. The anatomy of the lower GIT mucosa is comprised of invaginated surfaces covered with crypts. We measured the lower GIT crypts of sheep and cattle to average between 40 and 89 epithelial cells long and to create cavities of approximately 400 to 900 μm deep (data not shown). The crypts contain microbial flora and may provide a physical niche that sequesters replicating *E. coli* O157:H7 and prevents complete clearance of the bacteria by digesta movement. Alternatively, *E. coli* O157:H7 may be on the mucosal surface, in association with cells at the top of the crypts. In either scenario, persistence of the bacteria in the GIT may be dependent on the balance between bacterial replication and the rate of epithelial cell proliferation and thus migration and sloughing of epithelial cells from the top of the crypts.

Among the inoculated heifers in this study, a widely varying persistence of *E. coli* O157:H7 occurred, and animals were grouped as short-term (≤19 days) and long-term (≥29 days) culture-positive animals. An association was found between increased epithelial cell proliferation in the lower GIT and the more rapid clearance of *E. coli* O157:H7. Increased cell division in the absence of increased crypt height (total number of cells) suggests that rates of cell death or cell sloughing into the lumen may be increased. Differences in the cell death rates (apoptotic indices) between the short- and long-term culture-positive animals were not detected, but the method used only detects apoptotic cells that have not been sloughed into the lumen. The mechanism by which increased intestinal cell proliferation is associated with the clearance of *E. coli* O157:H7 is beyond the scope of this investigation. However, it may be that this bacterium is associated with the colonic crypts so that increased proliferation and sloughing of crypt epithelial cells may physically remove the niche more rapidly than bacterial replication takes place.

Previous studies comparing grossly different grain and forage diets show significant dietary effect on the duration cattle were *E. coli* O157:H7 culture positive (27). However, the duration cattle were *E. coli* O157:H7 culture positive was not affected by the nutritious forage and grain diets used in this study. In addition, the grain and forage diets did not affect the cattle GIT cell proliferation. Although the concentrations and proportions of acetate and propionate or the pH of ruminal and colonic digesta were not measured, the grain and forage diets are different enough to have affected these parameters. We must conclude, however, that the differences were not great enough to affect GIT cell proliferation or the duration of culture-positive status. In monogastric animals, changes in the short-chain fatty acid concentrations have been suggested as a mechanism by which fermentable fibers in the diet lead to an increase in intestinal cell proliferation (41). Unfortunately, a consistent correlation between diet quality (fiber) and intestinal cell proliferation has not been observed in studies of monogastrics. For example, rats fed cellulose, a poorly fermentable fiber, had higher luminal pH and lower short-chain fatty acids concentrations in the cecum and proximal and distal colon than rats fed fermentable fiber sources (pectin or oat bran) (48). A positive correlation between short-chain fatty acids and proliferation index was observed in the cecum but not in the distal colon (indices for the proximal colon were not reported) (48). In another study, the addition of wheat bran, but not pectin, to the diet of rats increased the short-chain fatty acid concentration in the cecum, whereas both fibers increased short-chain fatty acid concentrations in the proximal colon but had no effect in the distal colon (36). Increased crypt height occurred in the cecum of pectin-fed rats and in the distal colon of both pectin- and wheat bran-supplemented rats, illustrating the lack of a consistent correlation between short-chain fatty acids and cell proliferation (36). Malville-Shipan and Fleming (38) reported that neither cereal short-chain fatty acid concentrations nor the proliferation index in the cecum and proximal or distal colon were altered by the addition of wheat bran to the diets of rats when the energy intake was equivalent. The high-fiber diet did significantly lower pH of the luminal contents of the cecum and distal colon. Various fibers fed to miniature pigs significantly altered cereal short-chain fatty acid concentrations and pH, but these did not correlate with changes in cell proliferation in either the cecum or distal colon (18). The importance of other dietary variables, including total energy and nutrient intake on cell proliferation, has been emphasized (38).

To further investigate the relationship between intestinal cell proliferation and clearance of *E. coli* O157:H7, intestinal cell proliferation will need to be manipulated in a predictable manner. We have established the use of sheep previously as a model animal to enhance investigations of the relationship between cattle and *E. coli* O157:H7 (32–34). Here we used sheep as a model ruminant to test whether lower gastrointestinal cell proliferation could be manipulated using fasting-refeeding. Increased intestinal cell proliferation following fasting-refeeding in rats and mice has been reported by numerous investigators (8, 9, 11, 21, 26, 37, 39). Peak intestinal cell proliferation in these animals occurs at between 16 and 24 h and slowly returns to normal with refeeding (8, 11, 21). The timing of GIT cell proliferation response after fasting-refeeding is unknown in ruminants. Here we show in sheep a decrease with fasting and an increase 24 h after refeeding in GIT cell proliferation. We predict that the return to baseline GIT cell proliferation in ruminants would be similar or longer than in monogastrics due to the retention of digesta in the rumen. Fleming et al. (18) suggest that effects of short-chain fatty acids on intestinal proliferation may be observed only when baseline
concentrations of short-chain fatty acids are very low, such as following prolonged fasting. Sakata and Tamate (42) reported that rapid intraruminal infusion of sodium n-butyrate to adult male sheep resulted in an increase in ruminal cell proliferation, while a slow administration of butyrate had no effect on rumi- nal cell proliferation. Gaflı et al. (19) reported similar results. Only cell proliferation in the ovine rumen was evaluated in these studies. Our observation that cell proliferation in the ovine lower GIT is responsive to dietary manipulation complements this earlier work, and the fact that the greatest cell proliferation difference occurred in the distal colon is in agree- ment with observations in monogastric animals. For example, Butler et al. (9) showed that changes in cell proliferation with fasting-refeeding were greater in the large intestine than in the small intestine in rats.

We tested our hypothesis that a fasting-refeeding dietary manipulation would affect the duration animals were O157 culture positive in a predicted manner in Holstein steers. Feed and water were withheld for 24 h, conditions similar to those cattle may experience before processing. We began the fasting- refeeding regime when at least half of the cattle had become culture negative for O157 so that we could detect increases in the culture-positive status if they occurred with fasting. By this time postinoculation, animals were positive only by selective culture negative for O157 so that we could detect increases in O157 cell proliferation if they occurred with fasting. By this time postinoculation, animals were positive only by selective enrichment culture so that enumeration of O157 organisms/g of feces was not possible. We cannot explain the marked differ- ence between the number of O157 culture-positive cattle in the two groups just before fasting (Table 3, 17 days after the E. coli O157:H7 dose); however, the influence of dietary inter- vention is clear. The number of culture-positive animals, among eight total, increased from two to five after this fast (Table 3, days 17 and 18). The finding that withholding feed increases the number of O157 culture-positive animals has been observed by many other investigators (6, 7, 12, 20). This phenomenon has been noted both in animals experimentally dosed with E. coli O157:H7 and in the prevalence of naturally O157 culture-positive sheep and cattle that have traveled the farthest to the market or feedlot (25, 44). As in most previous studies, whether or not E. coli O157:H7 present in the GIT is induced to proliferate to detectable numbers or if animals become more susceptible to reinfections with the bacteria from the environment was not determined. The decrease in the number of O157 culture-positive animals we observed 72 h after feeding was resumed coincides with predicted increases in lower GIT cell proliferation. We did not measure a time course of GIT cell proliferation in these steers; to do so would have required that animals were sacrificed at each time point. We caution against adoption of a fasting-refeeding dietary intervention until this hypothesis is tested further in larger numbers of cattle and with a variety of O157 strains. Elucida- tion of the mechanism(s) that clear E. coli O157:H7 from the ruminant GIT may lead to the development of preharvest interventions that reduce culture-positive animals from enter- ing our food chain.

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