ABSTRACT

*Escherichia coli* O157:H7 is a pathogenic bacterium that causes acute illness in humans, but mature cattle are not affected. *E. coli* O157:H7 can enter the human food supply from cattle via fecal contamination of beef carcasses at slaughter. Previous attempts to correlate the incidence of *E. coli* O157:H7 with specific diets or feeding management practices gave few statistically significant or consistent findings. However, recent work indicates that cattle diets may be changed to decrease fermentation acid accumulation in the colon. When fermentation acids accumulate in the colon and pH decreases, the numbers of acid-resistant *E. coli* increase; acid-resistant *E. coli* are more likely to survive the gastric stomach of humans. When cattle were fed hay for a brief period (<7 d), acid-resistant *E. coli* numbers declined dramatically. Other workers have shown that brief periods of hay feeding can also decrease the number of cattle shedding *E. coli* O157:H7, and a similar trend was observed if cattle were taken off feed and exposed to simulated transport. These observations indicate that cattle feeding management practices may be manipulated to decrease the risk of foodborne illness from *E. coli*, but further work will be needed to confirm these effects.

(Key words: *Escherichia coli*, acid-resistant, hay, grain)

INTRODUCTION

In recent years, “*E. coli* O157:H7” has become part of our modern vernacular, and stories about this bacterium regularly appear in newspapers, magazines, radio and television. To the average American, *Escherichia coli* O157:H7 is another modern day scourge, a potentially fatal bacterium that can be avoided, but not completely dismissed, a pathogen that can be transmitted by drinking water, the supermarket, the restaurant, the household kitchen, the backyard barbecue, and even public swimming pools. Mature cattle are usually unaffected by *E. coli* O157:H7, and cattle can serve as asymptomatic carriers (1). Because ground beef has been, and continues to be a common source of *E. coli* O157:H7 infection (13), it has long been suspected that cattle are a primary reservoir (1). Beef carcasses, fruits, vegetables, and ground water have all been contaminated with *E. coli* and *E. coli* O157:H7 (Figure 1).

WHAT ARE *E. COLI*?

*E. coli* are facultative, anaerobic bacteria that are found in the gastrointestinal tract of mammals (42). *E. coli* is never a predominant gut bacterium, but it can account for as many as 1% of colonic bacteria (17). Most strains of *E. coli* are harmless commensal organisms, but any *E. coli* can cause disease if it penetrates the gut mucosa and enters the blood stream (42). When *E. coli* reaches the blood and lyses, it releases an endotoxin that causes fever and even death (42). Endotoxin is a normal part of their bacterial cell wall (chiefly lipid A of the lipopolysaccharide). Some strains of *E. coli* produce an enterotoxin that resembles cholera toxin, and this protein causes acute diarrhea even if the bacteria never cross the intestinal epithelium. Some *E. coli* can attach tightly to the gut mucosa, but the attachment process was poorly understood until recently. *E. coli* O157:H7 attaches tightly to the gut mucosal via a protein known as intimin (44), and this strain also releases highly virulent toxins (Shiga toxins) into animal cells (75).

HOW DANGEROUS IS *E. COLI* O157:H7?

*E. coli* O157:H7 causes approximately 40,000 infections and 250 deaths each year in the United States (1). The infective dose of most foodborne pathogens is greater than 10,000 cells but the infective dose of *E. coli* O157:H7 can be as low as 10 cells (1, 69). *E. coli* O157:H7 causes bloody diarrhea and severe stomach cramping that can last for as long as 7 d (75). Antibiotic therapy is ineffective and in some cases seems to have made the illness worse (58). If *E. coli* O157:H7 toxins are circulated by the blood, the kidneys can fail and kidney dialysis is needed (75). The recovery period from
Escherichia coli from cattle to man. When cattle are fed large amounts of grain, the acid-resistance of *E. coli* can increase, and acid-resistant *E. coli* are more apt to survive the low pH of the human gastric stomach. *E. coli* O157:H7 infections is relatively long, and the kidney damage can be permanent.

**WHAT ARE THE SOURCES OF *E. COLI* O157:H7?**

*E. coli* O157:H7 was first isolated from the feces of patients that had consumed contaminated hamburger (63), and hamburger is still a source of this bacterium. Outbreaks of *E. coli* O157:H7 have been linked to a variety of foods including vegetables, fruit, and fruit juices (8), and drinking water supplies have also been contaminated with *E. coli* O157:H7 (1). The idea that cattle are a reservoir is supported by the observation that carcasses can be contaminated with feces at slaughter, and cattle manure is often used as a fertilizer. However, it should be realized that *E. coli* O157:H7 has been isolated from other livestock and wild animals (31). Human-to-human transmission is also possible, and this type of transmission explains cases related to swimming pools (75).

**HOW HAS *E. COLI* O157:H7 BEEN COMBATTED?**

Slaughterhouses, food processing plants, supermarkets, and kitchens can all be sanitized to decrease the risk of fecal contamination and cross-contamination of one food by another (8). When cattle are killed, the rectum is commonly tied-off to reduce the chance of fecal contamination, but fecal material from the hooves and the hide is still a problem. Carcasses can also be washed with water or vinegar solutions to rinse away fecal material, but some *E. coli* are not easily removed (26).

Cooking has been and continues to be a cost-effective and reliable method of combating foodborne *E. coli* O157:H7 (8). Commercial restaurants have made great strides in making sure that foods are adequately cooked and handled properly, but the backyard barbecue and the household kitchen can also be a source of *E. coli* O157:H7 infection (37). The popular press and government agencies have increased their education efforts on this front, and the average American is now more aware. Ground beef should be thoroughly cooked and foods served raw (e.g., salads and fruits) should be isolated from meats and other potential sources of *E. coli* O157:H7.

Some foods cannot be cooked, but virtually all foods can be irradiated to destroy foodborne pathogens and *E. coli* O157:H7 (2). However, initial equipment investments can be beyond the reach of small food processors and meat packers, and some consumer groups have opposed irradiation on the grounds that old and nutritionally compromised food might be marketed. Many foodborne pathogens can be killed by ultraviolet light, and this technology has been used to combat *E. coli* O157:H7. Fruit juices can be pumped between layers of glass and treated with ultraviolet light, but the penetration of ultraviolet light into other foods is still a problem (R. Worobo, 1998, personal communication).

**WHERE DID *E. COLI* O157:H7 COME FROM?**

*Shigella*, a bacterium closely related to *E. coli*, produces intestinal inflammation and bloody diarrhea, but *Shigella* is not normally found in the gastrointestinal tract at high numbers (42). In the 1970s, *E. coli* infections similar to those produced by *Shigella* were reported, but these strains only produced intestinal inflammation (42). In the early 1980s, an *E. coli* strain designated as O157:H7 was isolated from the bloody feces of people who had consumed contaminated hamburger (63), and this strain produced two toxins that were homologous to the ones carried by *Shigella* (57). Because temperate phages with the Shiga-toxin genes were isolated from *E. coli* O157:H7, phage is suspected as the route of transmission from *Shigella* to *E. coli* (75). *E. coli* O157:H7 has a hemolysin that enhances its virulence, and the gene encoding this protein is located on a large plasmid (57). The intestinal adherence...
factor of *E. coli* O157:H7, intimin, is encoded by a chromosomal gene (57). The hemolysin and intimin genes have some homology to previously identified proteins from enteric bacteria, but the exact origins are not known (44).

The epidemiology of *E. coli* O157:H7 in humans is complicated by a large number of potential influences (e.g., sanitation, cooking methods, type of food consumed, and water quality). Infection rates have been highest in the United States, Canada, and the United Kingdom, but these statistics may be at least partially explained by more effective diagnosis (27, 73). Very high rates of hemolytic uremic syndrome were reported for Argentina, and this incidence was explained by the high meat consumption of very young children (52). A large outbreak was reported in Japan, and these cases were linked to contaminated alfalfa sprouts that were imported from the United States (55).

*E. coli* O157:H7 was first isolated from cattle in Canada (7), but the bacterium appears to be ubiquitous. Western countries have conducted the largest surveys, but *E. coli* O157:H7 has been isolated from cattle in Africa, Latin America, and Australia (77). Wildlife in the United States also have *E. coli* O157:H7, but these animals were exposed to cattle manure (1). The epidemiology of *E. coli* O157:H7 in cattle is not yet complete, but there appear to be no discrete geographical boundaries.

**HOW IS *E. COLI* O157:H7 IDENTIFIED?**

The identification of *E. coli* O157:H7 has been hampered by different methods of detection as well as by genetic variation. Early work with *E. coli* O157:H7 indicated that it was sorbitol negative, and this defect was explained by a lack of β-glucuronidase activity (62). The ratio of β-glucuronidase and β-galactosidase can be easily assayed with chromogenic and fluorescent substrates, but sorbitol-positive *E. coli* O157:H7 strains have been found (29). More definitive identifications have used antibodies against the O157 or H7 antigens (62), but Shiga-positive *E. coli* with different antigens have also been detected (5). Some workers have also used vero cell lysis to diagnose *E. coli* O157:H7 (62).

The detection of *E. coli* O157:H7 in fecal material is confounded by the density of other colonic bacteria and the presence of undigested feed materials. Some researchers have used antibiotics (e.g., ceftaxime) and tellurite to inhibit the growth of other bacteria, and glucuronidase activity on sorbitol MacConkey’s agar plates is then assayed with a fluorescent dye (16, 31, 32, 33, 34, 35, 48, 49). White (sorbitol-negative) colonies that lack fluorescence are presumptively identified as *E. coli* O157:H7. This traditional identification of *E. coli* O157:H7, however, does not account for cells that are attached to solid surfaces not based on the idea that all *E. coli* O157:H7 are sorbitol negative (11).

Because *E. coli* O157:H7 is often present in cattle feces at relatively low numbers (<10^5 cells/g of feces), traditional identification techniques can give false negative results (11). In the 1990s, English workers used immunomagnetic beads coated with an antibody against the O157 antigen to extract *E. coli* O157:H7 from feces, and this method was as much as 100-fold more sensitive than traditional methods (11, 12, 13, 54). Because immunomagnetic beads serve as a capture mechanism for *E. coli* O157:H7, it is possible to use large samples of fecal material to improve the reliability of the overall determination (R. O. Elder, J. E. Keen, and T. J. Klopfenstein, 1999, personal communication).

**HOW MANY CATTLE CARRY *E. COLI* O157:H7?**

Shiga toxin-producing *E. coli* were isolated from cattle in the 1980s (7). Epidemiological studies indicated that the percentage of O157:H7 positive cattle was only 0 to 3% (16, 36), and correlations of diet and the incidence of *E. coli* O157:H7 were in most cases weak or inconsistent (16, 23, 31, 34, 36, 48). However, enumerations using immunomagnetic beads indicate that 30% or more of the cattle can carry *E. coli* O157:H7 (12, 13, 54), and at least three studies indicate that feeding management can play a key role in determining the properties of *E. coli* in cattle (17, 46, 68).

Because initial reports indicated that relatively few cattle were carriers, inoculation has often been used as an experimental protocol (7, 14, 15, 39, 83). Recently Hovde et al. (39) used large oral doses of *E. coli* O157:H7 to examine the effect of diet on shedding, and they concluded that “feeding cattle hay may increase human infections with *E. coli* O157:H7.” However, it should be noted that these latter authors grew their inocula aerobically in Luria-Bertani broth at neutral pH. When the inoculated cattle were fed hay or grass, the pH gradient from the broth to the colon was negligible, and the cattle shed *E. coli* O157:H7 as long as 76 d. If cattle were fed grain, the pH gradient from the broth to the colon was >1.5 units, and the cattle shed *E. coli* O157:H7 for only 2 to 4 d.

Hancock et al. (35) recently hypothesized that diet shifts (e.g., grain to hay) would promote *E. coli* O157:H7 shedding, but they cited the studies of Kudva et al. (48, 49). In the first study (48), experimentally inoculated sheep were switched from alfalfa hay pellets to a sagebrush and bunch grass mixture or to kochia weeds. Some *E. coli*-negative animals became positive, but statistical significance was not reported. In the second study (49), inoculated sheep were switched from a 50:50
alfalfa and corn ration to very poor quality grass hay or starved completely. E. coli O157:H7 were detected longer and in greater numbers, but most statistical tests “were not significant.”

Keen et al. (46) recently studied the effect of a grain to hay diet shift on the prevalence of E. coli O157:H7 in cattle, and they used natural carriers rather than artificially inoculated animals. When the beef cattle (n = 200 animals) were fed rations rich in grain and immunomagnetic beads were used in the determination method, 53% of the animals were E. coli O157:H7 positive. Fifty two percent of the cattle that were main-
tained on grain continued to shed E. coli O157:H7, but only 18% of the cattle that were switched to hay were E. coli O157:H7 positive (P < 0.05). It should be noted that these estimates were not biased by the ability of the laboratory inoculum to reinitiate growth in the colon.

IS THE HUMAN STOMACH A BARRIER?

Food consumed by simple stomached animals is first deposited in the gastric stomach where it is subjected to HCl and pepsin (Figure 1). The gastric stomach is a hostile environment to bacteria (4, 60), and Waterman and Small (81) hypothesized that gastric acidity was a “first line of defense against foodborne pathogens.” This conclusion supported the observation that “the ability of pathogens to resist pH corresponds to their infective dose” (81), and the finding that humans given oral doses of sodium bicarbonate were more susceptible to Vibrio cholerae than patients not given bicarbonate (10). Few other studies have been conducted with humans, but Freter et al. (20) noted that significant numbers of E. coli were able to survive the gastric stomach of mice and colonize the intestine.

The human stomach secretes approximately 3 L of gastric juice per day, and the HCl content of this juice is approximately 0.17 N (pH 0.9) (24). The secretion of gastric juice is stimulated by hunger (appetite) and inhibited by the passage of food from the stomach to the intestines (24). The pH of the human gastric stomach can be as high as 6.0 if a large meal has been ingested, but mean stomach pH is typically 2.0 (76). The stomach is not a simple flow through system, and the passage of digesta is regulated by the opening and closing of the pyloric valve (22). The pylorus does not open until “the acidity of the gastric contents reaches a relatively high value” (82). Stomach residence times as short as 1 h have been reported, but the mean residence time is typically 1.5 h (76). Ruminants also have a gastric stomach (abomasum), but the pH of the abomasum is higher (2.5 to 3.0) (3).

CAN E. COLI BECOME ACID-RESISTANT?

Fermentation acids are used as a food preservative (43), but many lactic acid bacteria tolerate fermentation acids even if the pH is acidic (66). The ability of lactic bacteria to grow at acidic pH in the presence of fermentation acids is related to their ability to decrease intracellular pH (65). If the bacteria maintain a near neutral intracellular pH, the pH gradient across the cell membrane increases. Membrane permeable acids diffuse across the cell membrane and dissociate in the more alkaline interior. Intracellular fermentation anion accumulation is a logarithmic function of the pH gradient, but lactic acid bacteria have evolved mechanisms of decreasing intracellular pH (66). When lactic acid bacteria let their intracellular pH decline, potentially toxic accumulations of fermentation anion are avoided, but this strategy is only advantageous if the metabolic scheme is acid-resistant (66).

Continuous culture studies indicated that E. coli O157:H7 could grow at mildly acidic pH even if fermentation acids were present, and under these conditions the intracellular pH declined (18). When intracellular pH declined, pyruvate formate lyase was inhibited and the fermentation switched from acetate, formate, and ethanol to lactate, but the cultures washed out if the intracellular pH was less than 6.1. Streptococci and lactobacilli can grow when the intracellular pH is less than 5.5, and these bacteria can grow at a lower external pH than E. coli (45, 66).

Lactic acid bacteria grow fairly well at mildly acidic pH, but they do not survive extremely acidic conditions that mimic the human gastric stomach (59). Naturally occurring E. coli do not grow as well at low pH values, but they can survive low pH and reinitiate growth if the pH is again favorable (50, 51). The term “acid resistance” has been used interchangeably to describe growth at moderately acidic pH and survival after acid shock at very low pH, and this lack of specificity has sometimes created confusion (19). Given these complications, Lin et al. (50) indicated that “extreme acid resistance” would be a more useful term to describe viability after acute mineral acid (HCl) shock.

In the 1980s, Poynter et al. (61) noted that the survival of E. coli at pH 2.5 could be enhanced if the cultures were grown at pH 5.0 rather than neutral pH values, and this observation indicated that extreme acid resistance might be induced by low pH (25). Escherichia coli cells that were grown in media containing carbohydrate were more acid-resistant than those grown without carbohydrate, and it appeared that induction might be mediated by fermentation acids (9). Guilfoyle and Hershfield (28) reported that butyrate
and propionate could promote the extreme acid resistance of *E. coli*, even if the pH was neutral.

Recent work indicated that the extreme acid resistance of *E. coli* was highly correlated with the concentration of undissociated VFA in the growth medium, and this result indicated that pH itself was not the inducer (19). Only small amounts of fermentation acid were needed to induce acid resistance when the extracellular pH was low, but extreme acid resistance (pH 2.0, 6 h) was also observed at neutral pH, if the concentration was sufficiently high (Figure 2). Acetate, propionate, and butyrate gave a high degree of extreme acid resistance (approximately 10% survival), but formate, lactate, and benzoate were less effective (<0.1% survival). These later results indicated that the induction had some specificity.

Slonczewski et al. (70) indicated that membrane permeable acids were inducing acid-regulated genes of *E. coli* via a reduction in intracellular pH, but our work indicated that extreme acid resistance was not operating in a similar fashion (19). Undissociated VFA decreased the intracellular pH of *E. coli*, but the synthetic uncoupler carbonyl-cyanide m-chlorophenylhydrazone (CCCP) did not induce extreme acid resistance, even though it also decreased intracellular pH (19). Given the observation that a combination of acetate and CCCP was no more effective than acetate alone, it appears that undissociated VFA are needed to trigger the induction (19).

Most scientists have measured the acid resistance of *E. coli* after growth under aerobic conditions (4, 50, 72), but the gastrointestinal tract of animals and man is a highly reduced, anaerobic environment. The impact of oxygen and reducing agents on the extreme acid resistance is manifested by the amount of volatile acid needed to promote cell survival (19). *Escherichia coli* cultures that were grown aerobically needed more VFA to induce extreme acid resistance than those grown anaerobically, and the addition of cysteine, a reducing agent, to anaerobic cultures increased the volatile fatty acid requirement even more (19). These latter results indicate that redox state is involved in the induction of extreme acid resistance in *E. coli*.

The extreme acid resistance of *E. coli* O157:H7 is a feature that is most advantageous at pH values that mimic the gastric stomach (pH 2.0). At pH 2.0, the difference between induced and uninduced cells is approximately 3 logs, but this difference declines to 2.0 logs at pH 2.5 and is less than 1 log at pH 3.0 (unpublished results). Extrapolation of data from laboratory cultures to in vivo situations appears to be confounded by the richness of laboratory media and the ability of amino nitrogen sources to enhance extreme acid resistance (51). When fecal material was obtained from cattle fed hay, virtually all of the *E. coli* were killed by a 1 h, pH 2.0 acid shock, but *E. coli* strains isolated from these same cattle that were grown in the laboratory did not die until the acid shock was extended to 6 h (17).

Genes encoding the extreme acid resistance of *E. coli* have not been precisely defined. However, in the 1940s, Gale and Epps (21) reported that the AA decarboxylases of *E. coli* were induced by growth at low pH, and recent work indicated that mutants defective in arginine and glutamate decarboxylase were more sensitive to extreme acid shock (51). Arginine decarboxylation would yield agmatine, a membrane impermeable amine that could act as a base to prevent a decline in intracellular pH (30). In *E. coli*, the sigma factor, rpoS, is triggered by many stresses, and Schellhorn and Stones (67) reported that acetate could induce *rpoS*. The importance of *rpoS* in the extreme acid resistance of *E. coli* was strengthened by the observation that an *E. coli* mutant defective in *rpoS* was more sensitive to acute acid shock than wild-type (19, 72). *RpoS* has often been described as a “stationary” phase sigma factor, but it should be noted that *rpoS* is actually synthesized in late exponential phase as soon as the growth rate decreases (30).

Waterman and Small (81) indicated that enteric pathogens inoculated onto solid food sources are more resistant to acid-shock than those grown in liquid cul-

![Figure 2](image)

**Figure 2.** The relationship between extracellular undissociated acetate and the survival of wild-type *Escherichia coli* 0157:H7 after acid shock (pH 2, 6 h). The triangles show an experiment in which pH was 7.0 and acetate concentration changed. The squares show an experiment in which acetate concentration was 3 mM and pH changed with HCl. The circles show an experiment in which glucose concentration was varied and *E. coli* O157:H7 produced acetate and decreased pH. Figures redrawn from the data of Diez-Gonzalez et al. (19).

ture, but this work: 1) focused on Salmonella rather than E. coli, 2) employed cultures that were grown aerobically rather than anaerobically, and 3) used acid shocks that had a pH of 2.5 or greater. Sometimes the food caused an increase in pH, and in these cases the survival also increased.

**CAN CATTLE DIETS BE MANIPULATED TO CHANGE E. COLI?**

Cattle evolved as grazing herbivores (40), and ruminal microorganisms can ferment fibrous materials not digested by mammalian enzymes. However, cellulose digestion is not usually very rapid or complete. When the plant cell wall matures and becomes lignified, the rate and extent of fiber digestion decreases (80). Cereal grains ferment at a faster rate than fiber, and grain can be an extremely valuable supplement for cattle production. Cattle supplemented with grain grow faster and more efficiently than those fed only grass or hay (47), and grain is often a cheaper energy source for fattening beef cattle.

It has long been recognized that the rapid grain fermentation can decrease rumen pH (71), but grain can also pass through the rumen to colon and cause “hind gut” acidosis (64). The passage of grain to the colon is influenced by feed intake, the particle size of the grain, and processing methods that increase ruminal fermentation (79). The starch granules of corn are surrounded with a protein matrix (zein) that is relatively resistant to ruminal fermentation (53), and large amounts of raw corn can pass through the rumen to the colon (64).

Cattle fed hay had VFA concentrations in the rumen and colon less than 70 and 30 mM, respectively (Figure 3a), and both compartments had a near neutral pH (Figure 3b). When cattle were fed 90% grain, ruminal VFA concentrations increased from 70 to 85 mM (Figure 3a), but this increase only caused a modest decline in ruminal pH (Figure 3b). Grain-feeding had a much greater impact on colonic fermentation, VFA increased 3-fold (Figure 3a), and colonic pH decreased from 7.4 to 5.3 (Figure 3b). Hovde et al. (39) observed a similar decrease in colonic pH (7.2 to 5.5) when cattle were switched from hay to grain, but Scott et al. (68) reported that their grain-fed cattle had colonic pH values that were greater than 6.4. These latter diets, however, were supplemented with limestone, a buffer that is known to increase colonic pH (74).

Cattle that were fed hay had approximately $10^9$ and $10^8$ anaerobic bacteria per g in the rumen and colon, respectively (Figure 4a), and E. coli counts were less than $10^5$ and $10^4$ per g, respectively (Figure 4b). When grain was added to the diet, the total anaerobic count of the rumen increased less than 1 log (Figure 4a), but grain had a much greater impact on bacterial counts in the colon. The total anaerobic count increased 2.5 logs and the E. coli count was approximately 3 logs higher (Figure 4b). Scott et al. (68) noted that the cattle fed large amounts of grain had only 1 log more colonic E. coli than cattle fed hay, but the total anaerobic counts and VFA concentrations (an index of grain fermentation) were not reported.

When cattle were fed hay, virtually all of the E. coli in colonic digesta were killed by an acid shock that mimicked gastric stomach of humans (pH 2.0, 1 h), but cattle fed 90% grain had large numbers of acid-resistant
E. coli (17). The survival of E. coli after acid shock (pH 2.0, 1 h) was highly correlated with the undissociated VFA concentration of the colonic digesta (Figure 5). When the undissociated VFA concentration was 0.1 mM (hay diet), the survival was only 0.01%, but the survival was approximately 10% when the undissociated VFA concentration was greater than 10 mM (90% grain diet).

Scott et al. (68) noted that the cattle fed large amounts of grain had only 2.63 logs more acid-resistant E. coli than cattle fed hay, but their colonic pH values were more than 1.0 unit higher. Lactating dairy cattle that were fed moderate amounts of grain (60%) and limestone had similar numbers (4.4 vs. 3.99 logs) of acid-resistant E. coli as those reported by Scott et al. (68), and these dairy cattle also had a near neutral colonic pH (17). These results support the idea that colonic pH, by altering the concentration of undissociated VFA, may play a role in regulating the acid-resistance of E. coli.

Hovde et al. (39) reported that cattle fed diets rich in grain had more acid-resistant E. coli than cattle fed only hay. However, the difference was small (<1 log), and they indicated that diet would not affect the acid-resistance E. coli O157:H7. However, Hovde et al. (39) stated that “differences in methods of acid-exposure or bacterial enumeration” could have confounded a direct comparison of their work to that reported by Diez-Gonzalez et al. (17). If colonic digesta are not diluted 1 to 100, the pH of the acid shock solution can increase, and the difference between acid-resistant and acid-sensitive E. coli is noticeably less. See section entitled “Does E. coli become Acid-Resistant” above.

**ADAPTATION OR SELECTION?**

E. coli strains isolated from cattle fed hay or grain became highly acid-resistant when they were grown in the laboratory in a medium containing high concentrations of glucose (high acetate production) and these same strains became acid sensitive if they were grown in a medium containing low concentrations of glucose.

Figure 4. The effect of grain-feeding (% of diet DM) on total anaerobes (a), and the percentage of coliforms that were identified as Escherichia coli (b). The bars indicate standard deviations of the mean (three animals, four sampling days). Figures redrawn from the data of Diez-Gonzalez et al. (17).

Figure 5. The correlation between the undissociated VFA of colonic digesta and the survival of Escherichia coli from colonic digesta after an acid shock (pH 2, 1 h). Figure redrawn from the data of Diez-Gonzalez et al. (17).
The survival of colonic Escherichia coli isolates (hay vs. grain) and E. coli O157:H7 after acid shock (pH 2.0, Luria broth, 6 h). When the cultures (shaded columns) were cultivated overnight in broth containing large amounts of glucose (10 mg/ml) the final pH was 4.8. Cultures (open columns) with small amounts of glucose (0.5 mg/ml) produced less acid and the final pH was 6.8. The bars indicate standard deviations of the mean (10 strains, two replicates per strain). Figure redrawn from the data of Diez-Gonzalez et al. (17).

These results indicated the extreme acid resistance of colonic E. coli from cattle is an inducible trait present in existing strains rather than selection of strains that are always acid-resistant. E. coli O157:H7 had the same pattern of induction, and this result indicates that E. coli O157:H7 regulates acid-resistance in a similar fashion.

**HOW LONG DOES THE DIET SHIFT TAKE?**

Cattle fed large amounts of grain had more acid-resistant E. coli than cattle fed hay, but the time needed to reverse this trend was relatively short (17). When cattle were switched from 90% grain to hay, there was an almost immediate decrease in the total (Figure 7a) and acid-resistant E. coli count (Figure 7b), and after only 5 d, acid-resistant E. coli were less than 10 viable cells per gram colonic digesta (Figure 7b). Other workers have corroborated that short-term dietary shifts could play an important role in decreasing the risk of foodborne infection. When Scott et al. (68) switched cattle from 90% grain to hay on day zero (a), Part (b) shows the numbers of E. coli that were able to survive acid shock (pH 2.0, Luria broth, 1 h). The bars indicate standard deviations of the mean (three animals, one replicate per animal, two independent experiments). The dotted lines show the detection limit of our enumerations. Figures redrawn from the data of Diez-Gonzalez et al. (17).

(46) noted that a similar diet shift (grain to hay for 7 d) caused a large decrease (53 to 18%) in the number of cattle that were E. coli O157:H7 positive.

**COST OF HAY VERSUS GRAIN?**

Keen et al. (46) noted that cattle on grain-based diets continued to gain weight at a rate of 1 lb per day (0.45 kg/d), whereas cattle that were switched to the hay lost

![Figure 6](image)

![Figure 7](image)
weight at a rate of 0.25 lb per day. Based on a BW difference of 1.25 lb per day (0.56 kg/d), total weight difference would have been 8.75 lb (4.0 kg). If the animals went to slaughter at 1200 lb (545 kg), and the live weight price was $0.62 per lb ($0.28 per kg), the total cost would be $5.42 or a total increase of 0.7%.

**IS HAY THE ONLY WAY?**

Hay feeding is one method of preventing colonic grain fermentation, but grains can be treated to enhance ruminal fermentation. When cereal gains are heated, the protein coating of the starch granules rupture, and the starch becomes more accessible to ruminal bacteria. If the grain is digested in the rumen, less will reach the colon (47). Grain processing is already a common practice in the feed industry, and increases in total tract digestibility can completely offset the cost of the processing. In some areas of the United States grain is stored as a high moisture fermented feed, and this type of starch is also more ruminally digestible.

However, it should be noted that “grain-dependent increases” in acid-resistant *E. coli* were only observed if hay was deleted from the diet, and it is conceivable that hay could be having an impact on the colonic environment that is independent from starch fermentation per se. Fiber can: 1) form a ruminal mat that entraps grain and reduces the rate of grain passage to the lower gut, 2) act as a ruminal buffer to increase pH, and 3) pull water and conceivably other buffers into the lower gut (78). If fiber is the key factor regulating the numbers of acid-resistant *E. coli* in cattle, it is conceivable that byproduct feeds rich in fiber (e.g., soy and cotton seed hulls) could be substituted for hay. These byproduct feeds can be handled like grain and would not create the feeding problems that hay would.

The observation that cattle fed limestone had fewer acid resistant *E. coli* (68) than those not supplemented with limestone (17) supports the idea that colonic pH is important, but further work is clearly needed to see if grain-dependent increases in acid-resistant *E. coli* can be routinely offset by buffers. Limestone and magnesium oxide are relatively insoluble compounds that can pass through the gastrointestinal tract to the colon, but sodium bicarbonate has little if any effect on colonic pH (74).

The effect of hay on *E. coli* O157:H7 shedding is not as easily explained. Because *E. coli* O157:H7 grows better at neutral than acidic pH, and volatile fatty acids are more detrimental when the pH is acidic (18), it is unlikely that the hay-dependent changes in the colon pH would have a negative impact. However, the gastrointestinal tract is a highly competitive environment that is inhabited by an array of different bacteria (56).

If increases in colonic pH promoted the growth of non-pathogenic *E. coli* and other colonic bacteria to a greater extent than *E. coli* O157:H7, the shedding rate of *E. coli* O157:H7 would, in theory, decrease. Recent work by Zhao et al. (83) indicated that calves previously inoculated with nonpathogenic, colicin-producing *E. coli* were less likely to shed *E. coli* O157:H7 than those given only *E. coli* O157:H7.

**FOOD SAFETY?**

Hovde and her colleagues (35, 39) rebuffed the potential impact of acid-resistance on food safety with the hypothesis that *E. coli* “very likely replicate outside the bovine colon . . . before they are ingested by humans.” This argument is contradicted by a variety of observations: 1) cattle often have fresh fecal material on their feet and hides prior to slaughter, 2) fresh fecal material is more easily dislodged from the carcass than dried fecal material and 3) the carcass is quickly refrigerated to prevent subsequent bacterial growth. To test this hypothesis, we mixed fecal material from cows fed hay or grain with hamburger, incubated the samples in the refrigerator (5°C), and measured total and acid-resistant *E. coli* (survived pH 2.0, 1 h shock) on each day for 7 d. Results indicated that: 1) total *E. coli* counts did not increase significantly, 2) acid-resistant *E. coli* was *never* detected in hamburger samples that had been contaminated with feces from cattle fed hay, and 3) acid-resistant *E. coli* persisted in the hamburger that had been contaminated with feces from cattle fed 90% grain (unpublished results).

Keen et al. (46) recently reported that brief starvation and simulated shipping could decrease the percentage of cattle shedding *E. coli* O157:H7 (46). This is very good news for the cattle industry. If starvation, by increasing the pH of the colon decreases the acid-resistance of *E. coli* O157:H7, the news would be even better. However, it should also be stressed that the manipulation of cattle diets to prevent grain-dependent increases in acid-resistant *E. coli* and *E. coli* O157:H7 shedding is only one factor in the total risk assessment. It would not reduce the importance of other food safety practices (e.g., sanitation, refrigeration, cooking, and irradiation).

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