Gastrointestinal Acidosis and Its Effect on *Escherichia coli* in Cattle

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Introduction

Escherichia coli is a normal inhabitant of the gastrointestinal (GI) tract of warm blooded animals, and most *E. coli* strains are harmless. However, gram-negative bacteria can release lipopolysaccharide complexes from their cell walls (including lipid A) when they lyse.[14] These endotoxins can cause fever, and even death, but only if the bacterium migrates from the gut to the blood. Some strains of *E. coli* produce an enterotoxin that resembles cholera toxin, and this protein causes acute diarrhea even if the bacterium never crosses the intestinal epithelium.[17]

In 1982, an *E. coli* strain designated as O157:H7 was isolated from the bloody feces of people that had consumed contaminated hamburgers, and these strains produced toxins that could diffuse into intestinal cells.[14,20] Genetic analyses of *E. coli* O157:H7 indicated that it carried two toxin genes that were homologous to the ones borne by *Shigella*.[17] *E. coli* O157:H7 has a powerful hemolysin and an intestinal adherence factor known as Intimin.[17] Humans that are infected with *E. coli* O157:H7 become acutely ill, but mature cattle are asymptomatic carriers.[1,9]

Ground beef has been a common source of *E. coli* O157:H7 infection, but fruits and vegetables have also caused outbreaks and illness.[1] Fruits and vegetables are often fertilized with cattle manure, and beef can be contaminated with fresh manure at slaughter. Cattle have been suspected as being a primary source of *E. coli* O157:H7.[9] However, it should be noted that other animals and wildlife can carry this bacterium.[1] Early work indicated that only small numbers of cattle carried *E. coli* O157:H7,[9] but sensitive detection methods that used immunomagnetic beads indicate that as 30% to 50% of the cattle may be infected.[15,16]

Hancock and his colleagues examined the effect of diet on *E. coli* O157:H7, but detection methods were insensitive and dietary correlations were either weak or inconsistent.[9,11] Recent work indicated that grain feeding, a practice common in the cattle industry, can alter the physiology and survival of *E. coli*, but a brief period of hay feeding was able to counteract this potentially dangerous effect.[4]

Human Gastric Stomach as a Barrier to *E. coli*

Food consumed by simple stomached animals is collected in the gastric stomach prior to intestinal digestion, and the low pH of this compartment is a barrier to some bacteria.[18] Stomach pH can be as high as 6.0 if a large amount of food has just been ingested, but the mean pH is 2.0.[26] Human gastric glands secrete approximately 3 liters of gastric juice per day, and this juice has an HCl content of approximately 0.17 N and a pH of approximately 0.9.[8] Food remains in the stomach until "the acidity of the gastric content reaches a relatively high value".[27] Residence time of food in the stomach is clearly not a constant, but the typical half residence time is 1.5 hours.[26]

Extreme Acid Resistance of *E. coli*

Poynter *et al*[19] noted that significant numbers of *E. coli* survived HCl at pH 2.5, and other workers noted that the survival of *E. coli* was even greater if the cells were first habituated at pH 5.0.[7] *Escherichia coli* cells that were grown in media containing carbohydrate were more acid-resistant than those grown without carbohydrate,[2] and recent work indicated that the effect of carbohydrate was mediated via an increase in fermentation acid concentration.[5]

The extreme acid resistance of *E. coli* was highly correlated with the concentration of undissociated volatile fatty acids in the growth medium, and this result indicated that pH itself was not the inducer.[5] If the extracellular pH was low, small amounts of fermentation acid could induce acid resistance, but extreme acid resistance was also observed at near neutral pH if the concentration was sufficiently high. *Escherichia coli* produces acetate and lactate at low pH values, but these acids differ greatly in their ability to induce extreme acid resis-

tance.[5] When *E. coli* cultures were grown anaerobically in a medium containing sodium acetate at pH of 7.0, cell survival after acid shock was nearly 10%, but the survival was only 0.1% if sodium lactate was added. Butyrate and propionate were nearly as effective as acetate in inducing extreme acid resistance, but formate and benzoate were 100- and 1000-fold less effective.

The extreme acid resistance of *E. coli* is also strongly influenced by other growth conditions. *E. coli* grown aerobically require at least 10-fold less VFA to induce extreme acid resistance than those grown anaerobically under conditions that would mimic the GI tract.[5] *E. coli* is often cultured in media with an excess of amino acids, and amino acids can also enhance the extreme acid resistance of *E. coli*.[13] These properties have confounded the extrapolation of *in vitro* results to *in vivo* situations.

E. coli O157:H7 grows better under acidic conditions than laboratory strains,[3] but it has the same pattern and degree of extreme acid resistance as non-pathogenic strains isolated from cattle.[4]

Effect of Grain Feeding on Acid Resistant *E. coli* in Cattle

It has long been recognized that rapid grain fermentation can decrease rumen pH,[25] but grain can also pass through the rumen to the colon and cause "hind gut" acidosis.[21] Cattle fed hay had VFA concentrations in the rumen and colon that were less than 70 and 30 mM, respectively, but both compartments had a near neutral pH. When cattle were fed 90% grain, ruminal VFA concentrations increased from 70 to 85 mM, but this increase only caused a modest decline in ruminal pH. Grain-feeding had a much greater impact on colonic fermentation, VFA increased 3-fold, and colonic pH decreased from 7.4 to 5.3 .

Cattle that were fed hay had approximately 10^9 and 10^8 anaerobic bacteria per g in the rumen and colon, respectively, and *E. coli* counts were less than 10^5 and 10^4 per g, respectively. When grain was added to the diet, the total anaerobic count of the rumen increased less than 1 log, 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.

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*.[4] 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. 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).

When cattle were switched from 90% grain to hay, however, there was an almost immediate decrease in the total and acid-resistant *E. coli* count.[4] After only 5 days, acid-resistant *E. coli* were less than 10 viable cells per gram colonic digesta, and these results indicated that a diet shift from grain to hay might be another method of combating *E. coli* O157:H7.

Criticisms and Controversy

The idea that diet shifts might be a practical and effective method of combating *E. coli* in cattle was rebuffed by Hancock and his colleagues,[10] and a variety of hypothetical criticisms were widely distributed: (i) "*E. coli* that contaminate beef typically originated from the hide, hooves or the equipment used in slaughter and processing rather than directly from the colon," (ii) "the induced acid resistance of *E. coli* contaminating beef is likely to be unrelated to the pH of its ancestral colonic environment," (iii) "abrupt feed change immediately prior to slaughter could have unexpected deleterious effects," and (iv) "acid resistance induced by exposure to weak acid may not influence the virulence of this pathogen [*E. coli* O157:H7]."

Hovde *et al*[12] then examined the effect of a grain to hay diet shift on *E. coli* and *E. coli* O157:H7 in cattle. They observed a decrease in colonic pH (7.2 to 5.5) when cattle were switched from hay to grain. However, the reported difference in acid-resistant *E. coli* number was small (< 1 log), and they indicated that diet would not affect the acid-resistance of *E. coli* O157:H7. Because cattle fed hay and administered with very large doses of *E. coli* O157:H7 (grown aerobically in Luria Broth) shed this bacterium longer than cattle fed grain, they concluded that that "feeding cattle hay may increase human infections with *E. coli* O157:H7."

Corroborations and Confirmations

Criticisms raised by Hancock *et al*[10] were not readily supported by cited references,[22,23] and it should be noted that other workers have provided corroboration and confirmation of the idea that hay feeding is a mechanism of counteracting *E. coli* acid resistance and *E. coli* O157:H7 shedding. When Scott *et al*[24] fed various grain sources to beef cattle, colonic pH values were greater than 6.4, but these diets were supplemented with limestone, a buffer that is known to increase colonic pH. Cattle fed large amounts of grain had only 1 log more colonic *E. coli* than cattle fed hay, but the cattle fed large amounts of grain had 2.63 logs more acid-resistant *E. coli* than cattle fed hay. When their cattle were switched from grain-based diets to hay for 7 days, acid-resistant

E. coli decreased from 10,000 to 20 viable cells per gram in 7 days, and they concluded that "this study confirms Diez-Gonzalez[4] report that feeding hay for a short duration can reduce acid-resistant *E. coli* populations."

Keen *et al*[15] 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 maintained 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 *in vitro* cultural conditions, inoculum size or the ability of the laboratory inoculum to re-initiate growth in the colon.

Keen *et al*[6] recently surveyed *E. coli* O157:H7 or O157:non-motile (EHEC O157) in feces and on hides within lots of cattle presented for slaughter at meat processing plants in the Midwestern United States and correlated these results with the frequency of carcass contamination. Because fecal and hide prevalence were significantly correlated with carcass contamination ($p = 0.001$), these authors concluded that there was indeed a need for the control of *E. coli* O157 in live cattle.

Cost of Hay *versus* Grain

Keen *et al*[15] noted that cattle on grain-based diets gained weight at a rate of 1 lb per day whereas cattle that were switched to hay lost weight at a rate of 0.25 lb per day. Based on a body weight difference of 1.25 lb per day, total weight difference would have been 8.75 lb after 7 days of hay feeding. If the live weight price were $0.62 per lb, the weight difference would have been equivalent to $5.42 per animal. Because cattle typically go to slaughter at 1200 lb, the overall cost would be 0.7%

Future Research Needs

Hay feeding is one method for preventing colonic grain fermentation, but it might also be possible to decrease acid-resistant *E. coli* by enhancing ruminal fermentation. Grain processing is already a common practice in the feed industry, and increases in total tract digestibility can completely off-set 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.

"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: (i) form a ruminal mat that entraps grain and reduces the rate of grain passage to the lower gut, (ii) act as a ruminal buffer to increase pH, and (iii) pull water and conceivably other buffers into the lower gut. If fiber is a key factor regulating the numbers of acid-resistant *E. coli* in cattle, it is conceivable that by-product 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* [24] than those not supplemented with limestone[4] 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 GI tract to the colon, but sodium bicarbonate has little if any effect on colonic pH.

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