

Grain-Feeding and the Dissemination of Acid-Resistant *Escherichia coli* From Cattle

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Introduction

Foods can be cooked or irradiated to kill bacteria, but there are approximately 30 million food-borne illnesses each year in the United States. *Escherichia coli* is a normal inhabitant of the gastrointestinal tract, some strains (e.g. O157:H7) produce toxins and are pathogenic. Hamburger has been frequently contaminated with pathogenic *E. coli*, and cattle are a natural reservoir.

The ability of bacteria to act as a food-borne pathogen depends on their capacity to survive the low pH of the gastric stomach and colonize the intestinal tract of man. Pathogenic and non-pathogenic *E. coli* cultures only develop extreme acid resistance when they are grown at mildly acidic pH. If *E. coli* is grown at neutral pH, it is acid-sensitive and killed by the low pH of gastric juice.

Since World War II, fattening beef cattle in the United States have been fed large amounts of grain (starch) and very little hay, but the impact of grain feeding on acid-resistant *E. coli* had not been examined. Many forms of starch pass through the pregastric stomach (rumen) to the intestines, and cattle are deficient in the starch-degrading enzyme, amylase. Starch can be fermented in the colon, and starch fermentation in the colon produces volatile fatty acids that decrease pH.

Materials and Methods

Colonic digesta from cattle fed hay, grass and varying amounts of rolled corn were diluted 10-fold with sterile anaerobic water, and the pH was measured. Coliforms were enumerated by serial dilution in lauryl sulfate. *E. coli* was determined by lactose fermentation, gas production, indole production, the methyl red reaction, Voges-Proskauer test and citrate fermentation. Acid shock was performed by diluting digesta samples 100-fold into Luria broth that had been adjusted to pH 2.0. After 1.0 h at pH 2.0, viable cell numbers were determined by serially diluting into lauryl sulfate broth.

Cattle were fed medium quality timothy hay (14% crude protein, 40% neutral detergent fiber) and a grain

mixture (89% rolled (cracked) corn and 11% soybean meal) every 2 h (10 kg dry matter/d). The diets were 0, 45 and 90% grain with the remainder being hay. Samples obtained from the rumen as well as the colon were centrifuged, and fermentation acids were analyzed by high-pressure liquid chromatography. Total count of anaerobic bacteria was determined by serially diluting in a nonselective medium. *E. coli* strains were obtained from sorbitol MacConkey's plates.

Results and Discussion

A survey of 61 cattle indicated that grain could increase total and acid-resistant *E. coli* numbers. Cattle fed either hay or pasture had a colonic pH > than 7.0, the total *E. coli* count was only 20,000 cells per g, and virtually all of these bacteria were killed by acid shock. Moderate amounts of grain (60%) did not cause a decrease in pH, but the total *E. coli* were 6,300,000 per g. Some of the *E. coli* were killed by acid shock, but acid-resistant were > 25,000 per g. When animals were fed > 80% grain, the pH was lower, and acid-resistant *E. coli* were 250,000 per g.

When cattle were fed increasing amounts of grain, the volatile fatty acids in the rumen did not increase significantly, but the concentration in the colon increased approximately 4-fold. Under these conditions, ruminal pH remained essentially constant, but the pH of the colon decreased when the volatile fatty acids accumulated. Grain had little impact on the numbers of anaerobic bacteria in the rumen, but the in colonic count increased 1000-fold. Cattle fed hay had less than 10^5 colonic coliforms, but those fed 90% grain had approximately 10^8 coliforms per g digesta. Only a small fraction of the ruminal coliforms were *E. coli*, but virtually all of the colonic coliforms were identified as *E. coli*. Cattle fed hay had a low concentration of volatile fatty acids in their colons, and acid shock killed more than 99.99% of the *E. coli*. When diets were supplemented with either 45 or 90% grain, acids accumulated, colonic pH declined, and a much larger percentage of the *E. coli* survived acid shock.

The idea that grain, by promoting acid production in the colon, was regulating acid resistance in vivo was corroborated by in vitro experiments. When *E. coli* strains isolated from cattle were grown in the laboratory with a high concentration of glucose, acetic acid accumulated in the medium, pH declined, and the cell survival after acid shock was high. If the glucose concentration of the medium was low, little acid was produced, and cell survival was extremely low. Cattle adapted to a 90% grain diet had an acid resistant *E. coli* count $>10^6$ viable cells per g digesta. Upon change to a hay diet, the viable cell number immediately declined, and after 5 days acid resistant *E. coli* were nearly 10^6 lower.

Strains isolated from cattle fed forage or grain, and *E. coli* O157:H7 behaved similarly, and this result indicated that grain-feeding was inducing acid resistance rather than selecting a different population of *E. coli*. None of our *E. coli* isolates (n = 155) tested positive for O157:H7 antigens. The absence of *E. coli* O157:H7 in our cattle is not surprising. The

percentage of O157:H7 positive animals in herds directly linked to outbreaks was less than 2%.

Conclusions

The finding that grain-feeding increased the number and acid resistance of *E. coli* in cattle could have implications for food safety. Not all *E. coli* are pathogenic, but at least some cattle will harbor pathogenic strains. Because acid resistance is a factor in the dissemination of *E. coli* from cattle to humans, it is reasonable to suggest that the induction of acid resistance could increase the risk of foodborne illness. Our studies indicated that the time needed to decrease *E. coli* numbers was relatively short. Grain-feeding is a practice that promotes cattle production, and it is unlikely that cattle will ever be fed diets consisting only of hay. However, our studies indicate that cattle could be given hay for a brief period immediately before slaughter to reduce the risk of food borne *E. coli* infection.