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Capacity of the Bovine Intestinal Mucus and Its Components to Support *Escherichia coli* O157:H7 Growth[1]

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Introduction

Escherichia coli O157:H7 contamination of human food products is a major concern for the beef industry. The pathogens responsible for outbreaks often originate from cattle, and *E. coli* O157:H7 can thrive in healthy cattle. To control contamination in the food chain, it is essential to understand how this pathogen is able to grow and compete with other bacteria in the gastrointestinal tracts of cattle.

Previous studies have shown that bovine intestinal mucus supports bacterial colonization and can selectively influence makeup of the bacterial population. Intestinal mucus is made of mucins, which are gel-forming glycoproteins. Mucin molecules contain sialic acid that must be removed by neuraminidase enzyme to allow for complete degradation of mucin. *E. coli* O157:H7 lacks neuraminidase and should have little ability to degrade the complex mucin molecules. Our objective was to evaluate bovine intestinal mucus and its components in terms of their capacity to support *E. coli* O157:H7 growth in the presence or absence of feces and to understand the roles various enzymes play in this process.

Experimental Procedures

Intestinal tissues from freshly harvested cattle were collected and transported to our laboratory in chilled saline. Sections of the ileum and colon were washed with buffer solution, and mucus was harvested by gently scraping the epithelium. We prepared a mix of five selected strains of Shiga toxin-producing *E. coli* O157:H7 resistant to nalidixic acid (Nal^R) and added the mix to a buffer or a similar amount of fecal inoculums collected from the rectum of a steer fed a high-grain diet.

Subsequently, we added harvested intestinal mucus or individual mucus components to the culture to assess which components were most capable of supporting Nal^R *E. coli* O157:H7 growth. Intestinal mucus was added at a concentration of 10 mg/mL. Single components of mucus (galactose, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, mannose, L-alpha-phosphatidylserine, sialic acid, and N-acetyl-D-glucosamine) were added at the same concentration, except for L-alpha-phosphatidylserine, which was added at 1 mg/mL. Initial concentrations of *E. coli* O157:H7 and fecal bacteria in the cultures were 10^3 and 10^4 CFU/mL, respectively.

We also evaluated the impact of adding enzymes and enzyme inhibitors associated with mucus degradation on *E. coli* O157:H7 growth. Proteases, endoglycosidases, sialidases, or lipases were added to the batches at a concentration of one unit per milliliter. Beta-galactosidase inhibitor was added at a final concentration of 200 μM, and protease inhibitor was added at either 0.25 or 2.5 mL/g of *E. coli* O157.

[1] Funding for this project was provided by the Beef Checkoff.

After 0, 6, 8, and 12 hours of anaerobic incubation at 104°F on a laboratory shaker, we plated 100 µL of culture at different dilutions on agar selective for Nal^R *E. coli* O157:H7. After incubating the plates for 24 hours at 98°F, we counted the Nal^R *E coli* O157:H7 colonies and established the Nal^R *E coli* O157:H7 growth within the different batches (expressed in Log_{10} of CFU/mL).

Results and Discussion

There were no significant differences ($P>0.10$) in *E. coli* O157:H7 growth between mucus derived from the large intestine (colon) and small intestine (ileum). The bacteria increased from 10^3 CFU/mL of culture at time zero to 10^7 to 10^8 CFU/mL at hour 8. There was an overall time effect on bacteria growth but no significant difference between hour 8 and 12, which drove us to use hour 8 as a point of comparison. Presence or absence of fecal inoculums in the culture affected ($P<0.01$) *E. coli* O157:H7 growth. The final concentration of bacteria decreased from 10^7 to 10^5 CFU/mL, which is likely due to competition for nutrients with the fecal bacteria.

Figure 1 depicts *E. coli* O157:H7 growth after 8 hours of anaerobic incubation without feces but with whole mucus or selected components of mucous as substrates. With the exception of L-alpha-phosphatidylserine, almost all of the mucus components tested increased growth of the bacteria compared with the batch containing only buffer ($P<0.05$). However, mucus originating from the large and small intestines supported greater growth than the individual mucus fractions ($P<0.05$). Of the individual mucus components evaluated, only gluconic acid resulted in growth similar to that achieved with whole intestinal mucus. These observations suggest *E. coli* O157:H7 may need a combination of components to ensure optimal growth or that the bacteria are utilizing a key element present in the mucus that we did not evaluate in this experiment.

In our attempt to analyze the stimulatory effect of mucus-degrading enzymes on growth of Shiga toxin-producing *E. coli* O157:H7, we found no significant difference in growth of cultures treated with enzymes or protease inhibitors compared with untreated batches ($P>0.05$). Conversely, as illustrated in Figure 2, addition of beta-galactosidase enzyme inhibitor increased the growth of Nal^R *E. coli* O157:H7 cultured with either small or large intestinal mucus ($P<0.05$). The increase of growth, instead of the expected inhibition, could be due to the bacteria's inability to use the inhibitor as a source of protein. However, the amount of inhibitor added to the culture was very small and, therefore, seems an unlikely explanation. It is equally possible that mucus galactosides that have not been enzymatically degraded are more stimulatory to *E. coli* O157:H7 growth. Additional controlled experiments are needed to further investigate the increase of growth induced by the beta-galactosidase inhibitors.

Figure 3 illustrates *E. coli* O157:H7 growth in response to small intestinal mucus or sialic acid substrates in the presence or absence of a fecal inoculum. *E. coli* O157 growth was significantly lower in sialic acid than in mucus ($P<0.01$), indicating the pathogen has limited capacity to use sialic acid as a substrate for growth. When fecal inoculum was added to the culture with small intestinal mucus, there was a significant decrease in *E. coli* growth compared with the same culture without feces. This again suggests the bacteria are competing for nutrients. However, *E. coli* O157:H7 growth increased when fecal inoculum was added to the culture containing sialic acid. Bacteria present in feces

may have the ability to degrade sialic acid, thus allowing *E. coli* O157:H7 to use the intermediates or end products of the degradation as a substrate for growth.

Cattle fed distillers grains have been shown to have an increase in *E. coli* O157 shedding, and distillers grains contain a substantial proportion of yeast, which has a high sialic acid content (3% of dry weight). It is possible that sialic acid or other glycoprotein constituents of distillers grains are the active components that stimulate proliferation of *E. coli* O157:H7 in cattle fed distillers grains.

Implications

This study offers insight regarding the potential of intestinal mucus and its components to promote *E. coli* O157:H7 growth in cattle. Further investigations are needed to establish whether one of these components could inhibit, or at least regulate, the proliferation of important foodborne pathogens.

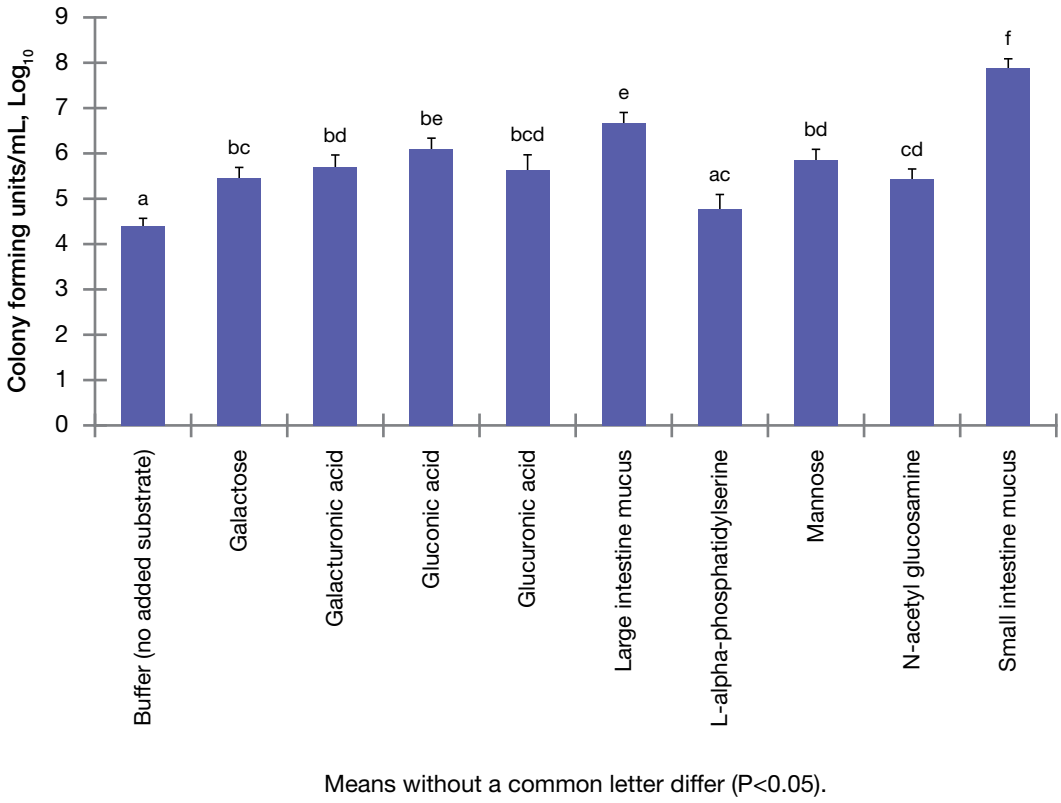

Figure 1. Growth of nalidixic acid-resistant *Escherichia coli* O157:H7 on small intestinal mucus, large intestinal mucus, or components of mucus after 8 hours of incubation. Cultures were inoculated with 10^3 CFU/mL of nalidixic acid-resistant *E. coli* O157:H7 prior to incubation.

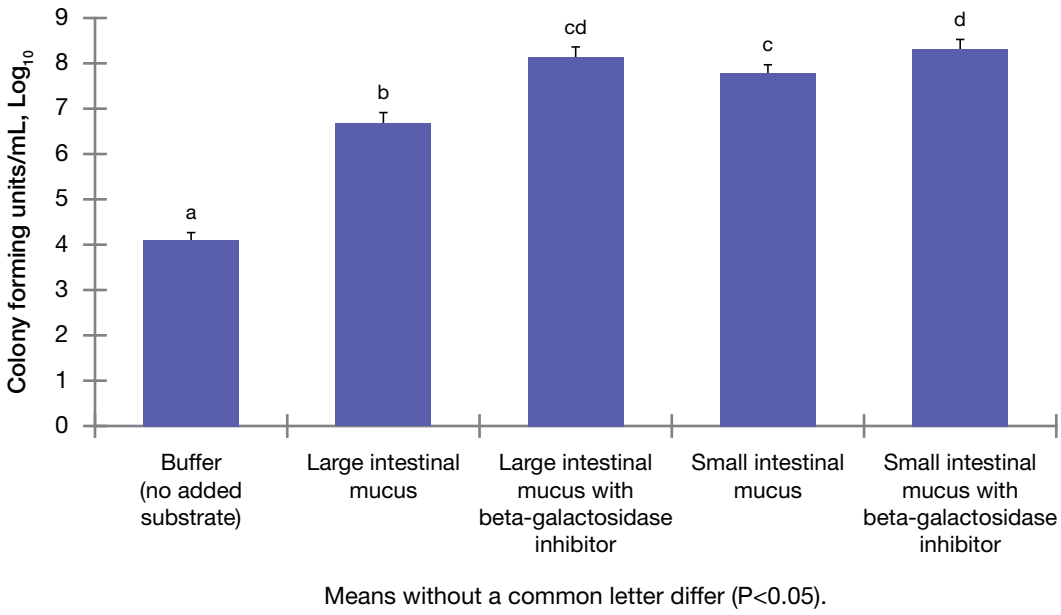

Figure 2. Growth of nalidixic acid-resistant *Escherichia coli* O157:H7 with small or large intestinal mucus in the presence and absence of beta-galactosidase enzyme inhibitor.

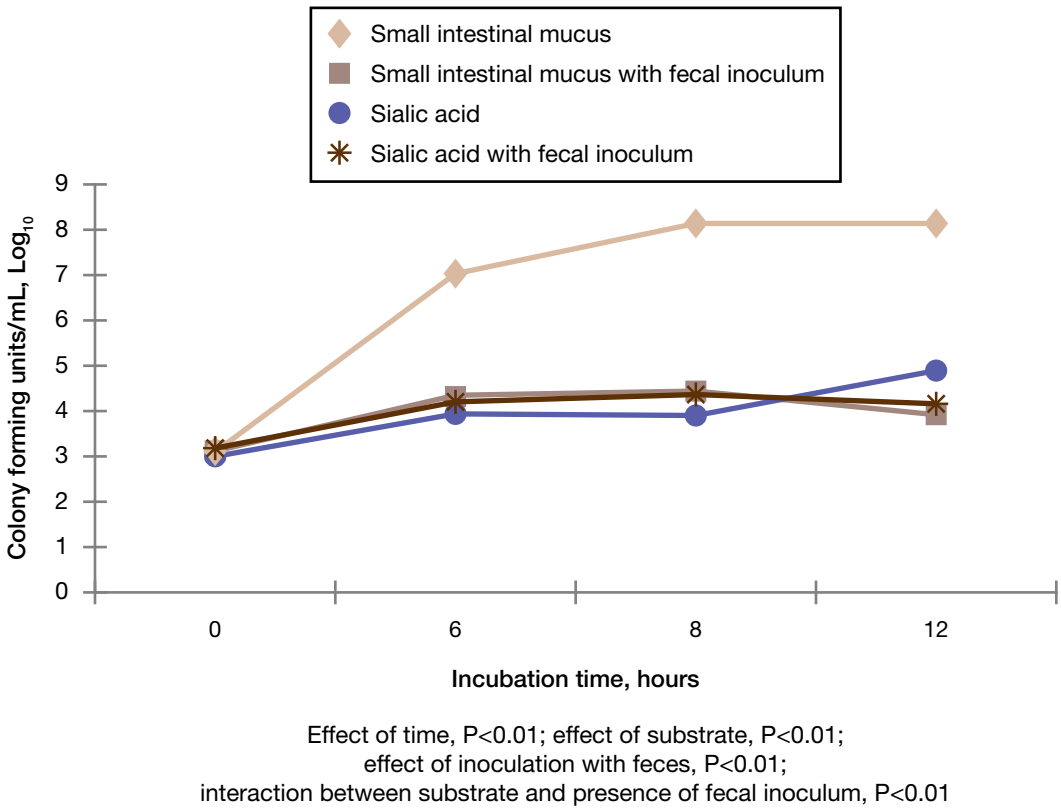

Figure 3. Growth of nalidixic acid-resistant *Escherichia coli* O157:H7 in response to small intestinal mucus or sialic acid as substrate in the presence and absence of a fecal inoculum.