Effects of mucin and its carbohydrate constituents on *Escherichia coli* O157 growth in batch culture fermentations with ruminal or fecal microbial inoculum1

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ABSTRACT: In cattle, *Escherichia coli* O157 generally persists in the large intestine more often than in the rumen. In contrast to the rumen, the large intestine is lined by an epithelial membrane that secretes mucus. We hypothesize that substrates contained in intestinal mucus may constitute a source of energy that is preferentially used by *E. coli* O157. Therefore, our objective was to test the effects of mucin and its carbohydrate constituents on in vitro growth of *E. coli* O157 in ruminal or fecal microbial fermentations. Ruminal contents and feces were collected from a ruminally cannulated donor steer fed a corn grain-based finishing diet. Ruminal contents were strained through 2 layers of cheesecloth and incubated at 39°C for 1 h; the floating hay mat was removed with a vacuum suction; and the remaining material was utilized as rumen microbial inoculum. Feces were suspended in physiologic saline to increase fluidity, blended, and strained through 2 layers of cheesecloth. The resulting fluid was utilized as fecal microbial inoculum. Fermentations (50 mL) were performed in serum bottles with a 2:1 mineral buffer to microbial inoculum ratio. Substrates (mucin, fucose, galactose, mannose, gluconic acid, galacturonic acid, glucuronic acid, galactosamine, and glucosamine) were added at 10 mg/mL. A mixture of 5 strains of nalidixic acid-resistant (NalR) *E. coli* O157 strains was added to each fermentation and concentrations were determined after 0, 6, 12, and 24 h of incubation. In ruminal fermentations, fucose, mannose, glucuronic acid, galacturonic acid, glucosamine, galactosamine, and mucin had no effect on NalR *E. coli* O157 concentration compared with the control (no substrate added) fermentation. At 24 h of fermentation, the mean concentration of NalR *E. coli* O157 in fermentations with galactose was less than the control. However, including gluconic acid as substrate increased NalR *E. coli* O157 concentration at 24 h. In fecal fermentations, mannose, galactose, gluconic acid, glucuronic acid, galacturonic acid, glucosamine, and mucin increased NalR *E. coli* O157 growth compared with control at 24 h, whereas galactosamine and fucose did not. Gluconic acid was the most stimulatory substrate, increasing NalR *E. coli* O157 by more than 1.0 log in ruminal fermentations and 2.0 log in fecal fermentations. In summary, availability of mucous constituents, particularly gluconic acid, may explain the greater prevalence of *E. coli* O157 in the large intestine compared with the rumen of the digestive tract.

Key words: carbohydrate constituent, *Escherichia coli* O157, growth, in vitro fermentation, mucus

INTRODUCTION

*Escherichia coli* O157:H7 causes food borne illness that generally is characterized by abdominal pain and hemorrhagic colitis, and in children and the elderly it can cause a more severe form of the disease, called hemolytic uremic syndrome (Rangel et al., 2005). Cattle are a major reservoir of *E. coli* O157, and the organism is more prevalent in digesta of the large intestine (cecum, colon, and rectum) compared with that of the rumen (Grauke et al., 2002; Naylor et al., 2003; Van Baale et al., 2004). The conditions in the large intestine, in contrast to the rumen, may be more hospitable to the survival and growth of *E. coli* O157 (Fox et al., 2007). The large intestine may provide nutrient substrates that provide a selective advantage for growth of *E. coli* O157. Components of gastrointestinal mucus (fucose, galactose, mannose, N-acetylgalactosamine, N-
acetylglucosamine, galacturonic acid, glucuronic acid, and gluconic acid) are known to perpetuate colonization of *E. coli* in the mouse intestine and stimulate in vitro growth of *E. coli* (Peekhaus and Conway, 1998; Montagne et al., 2000; Chang et al., 2004). Therefore, it has been suggested that components of mucus may serve as substrates or growth factors for *E. coli* O157 (Miranda et al., 2004).

In vitro fermentations with ruminal fluid or fecal inoculum have been used to assess gut microbial activities and digestibility of specific substrates (El Shaer et al., 1987; Mould et al., 2005; Varadyova et al., 2005). Ruminal fluid inoculum also has been used to evaluate factors that impact in vitro growth and survival of *E. coli* O157 (Diez-Gonzalez and Russell, 1997; Bach et al., 2003; Edrington et al., 2003; Annamalai et al., 2004; Edrington et al., 2006). Our objectives were to conduct in vitro ruminal or fecal microbial fermentations to determine whether mucin or carbohydrate constituents of mucus influence *E. coli* O157 growth and assess how fermentation pH and VFA concentrations may be related to *E. coli* O157 concentrations.

**MATERIALS AND METHODS**

Animal management and handling procedures for this study were approved by the Kansas State University Institutional Animal Care and Use Committee.

*Escherichia coli* O157 Inoculum Preparation

A mixture of 5 strains (01–2-1863, 01–2-7443, 01–2-10004, 01–2-10530, and 01–2-12329) of *E. coli* O157 was used for in vitro fermentation studies. These strains were isolated from feedlot pen fecal samples (Sargeant et al., 2003). The strains were made resistant to nalidixic acid (50 µg/mL; *NalR*) in the laboratory and stored in protective beads (CryoCare, Key Scientific Products, Round Rock, TX) at −80°C. The major virulence genes (*eae, fliC, stx1, stx2*) and the genetic relatedness of the 5 isolates were determined by PCR (Gannon et al., 1997; Fagan et al., 1999) and pulsed-field gel electrophoresis (PFGE, Sargeant et al., 2006), respectively. Strains were considered distinct types based on differences in more than 2 bands (<95% Dice similarity; Sargeant et al., 2006). To prepare the inoculum for the in vitro fermentation experiments, isolates of each strain from protective beads were streaked onto blood agar and incubated for approximately 16 h at 37°C. An individual colony of each strain was picked and inoculated into a separate bottle containing 100 mL of tryptic soy broth (Becton Dickinson and Co., Sparks, MD). Bottles were vortexed and incubated at 37°C for 18 h. After incubation, 1-mL aliquots from each culture were pooled and vortexed to obtain the 5-strain cocktail of *NalR* *E. coli* O157 for in vitro fermentations. The batch culture fermentations with ruminal or fecal microbial inoculum were performed 3 times with ruminal fluid and feces collected and prepared for each replication.

**Preparation of Ruminal and Fecal Microbial Inocula**

Ruminal fluid and feces were collected from a ruminally cannulated steer fed a high-grain diet consisting primarily of steam-flaked corn and alfalfa hay. The steer was housed in a pen (36 m²) in the university Beef Cattle Research Center. Ruminal fluid was strained through 2 layers of cheesecloth to remove large feed particles and placed into a flask and capped with a butyl rubber stopper fitted with a Bunsen valve. Feces were collected from the rectum from the same steer and placed into a Whirl-Pak bag (Nasco, Ft. Atkinson, WI). Ruminal fluid and feces were then transported (approximately 5 km) to the Preharvest Food Safety Laboratory. Upon arrival, ruminal fluid was incubated for 1 h at 39°C and then the floating hay mat/foam fraction was removed by vacuum suction. Because the DM content of feces was greater than that of ruminal fluid, Ringer’s solution was added to the feces (6.0 mL/g) to prepare a fecal suspension (Mould et al., 2005). The resulting fecal slurry was then blended in a Waring blender for 1 min under a stream of oxygen-free CO₂ gas (Hunstig, 1966), strained through 2 layers of cheesecloth to remove large particles, and used as the fecal microbial inoculum.

**In Vitro Fermentations**

Batch culture fermentations were set up in 60-mL serum bottles (Wheaton Science Products, Millville, NJ) capped with butyl rubber stoppers fitted with Bunsen valves. Each bottle contained 50 mL of fermentation mixture composed of 33 mL of McDougal’s buffer (McDougal, 1948) and 17 mL of the ruminal fluid or fecal microbial inoculum. The buffer and the microbial inoculum were added under a stream of oxygen-free CO₂ gas to create and maintain an anaerobic environment within the bottles. Bottles were numbered 1 to 40, and treatments were assigned in a completely randomized design with a 10 × 2 factorial treatment arrangement. Factor 1 was the type of substrate used, and factor 2 was the inoculum (ruminal or fecal microbial inoculum). Substrates (all from Sigma-Aldrich, St. Louis, MO) evaluated were added at 10 mg/mL and consisted of control (no substrate), gluconic acid, glucuronic acid, galacturonic acid, fucose, galactose, mannose, galactosamine, glucosamine, and porcine stomach mucin. The 5-strain mixture of *NalR* *E. coli* O157, prepared as described above, was diluted 1,000-fold in buffered peptone water (Sigma-Aldrich), and 100 µL was inoculated into each fermentation. Serum bottles were incubated in an orbital shaking incubator (Gallenkamp, Leicester, UK) set at 70 rpm and 39°C.

**Fermentation Sample Collection**

Samples were collected at 0, 6, 12, and 24 h to determine fermentation pH and concentrations of *NalR*.
E. coli O157 and VFA. At each sampling time, bottles were swirled by hand and stoppers removed to place bottles under the flow of O2-free CO2 during removal of sample. An aliquot (1 mL) of each fermentation sample was pipetted into a 96-well (2.0-mL well capacity) assay block (Corning Inc., Corning, NY) for enumeration of NalR E. coli O157. Another aliquot of each fermentation sample (4 mL) was placed into a vial, and pH of each sample was recorded immediately (ACCUMET model AR 10 pH meter, Fisher Scientific International, Pittsburgh, PA). The pH probe was placed in a 70% isopropyl alcohol solution for a minimum of 10 s and then rinsed in distilled water after each sample pH was measured. To each vial, 1 mL 25% metaphosphoric acid (Erwin et al., 1961) was pipetted. Vials were capped, inverted to mix, and frozen at −20°C for VFA analysis.

**Determination of NalR E. coli O157 Concentration**

Serial dilutions of each sample were made in a 96-well assay block by transferring 100 µL into 0.9 mL buffered peptone water. For 0-h samples, two 10-fold dilutions (10−1 and 10−2) were performed and 100 µL of the original sample (100), 10−1, and 10−2 dilutions were spread plated onto sorbitol MacConkey agar (Becton, Dickinson and Co.), supplemented with cefixime (50 ng/mL), potassium tellurite (2.5 µg/mL), and nalidixic acid (CT-SMACnal; 50 µg/mL). Six-hour samples were diluted to 10−4, and 100 µL of 10−1 through 10−4 dilutions were spread plated onto CT-SMACnal. Samples obtained at 12 and 24 h were diluted to 10−5, and 100 µL of 10−1 through 10−5 dilutions were spread plated onto CT-SMACnal. All dilutions were plated in triplicate and incubated at 37°C for 18 to 24 h. After incubation, sorbitol-negative colonies were counted to determine NalR E. coli O157 concentrations (cfu/mL) in fermentations (Van Baale et al., 2004), and colony counts were log (base 10) transformed for data analyses.

**VFA Analysis**

Concentrations of VFA in fermentation samples were determined using gas chromatography (Vanzant and Cochran, 1994). Only the major VFA (acetate, propionate, and butyrate) and their sum, reported as total VFA concentration, were used in the data analyses.

**Statistical Analysis**

Data from ruminal and fecal microbial fermentations were analyzed separately. The concentrations of log_{10} NalR E. coli O157, fermentation pH, and VFA concentrations were analyzed as repeated measures over time (variance components covariate structure) with the MIXED procedure (SAS Institute, Cary, NC) and included main effects of substrate and sampling (h), the interaction between substrate and hour, and the random effect of replication of experiment. Least squares means were used to determine the level of significance between control and substrate treatments. Pearson correlation coefficients between NalR E. coli O157 concentrations, pH values, and VFA concentrations were determined by modeling these as dependent variables with substrate as an independent variable using the PROC GLM, MANOVA/PRINTE option of SAS. Treatment was included when determining these correlation coefficients to assess correlations independent of substrate effects. Because of the large number of comparisons evaluated, a P < 0.01 level of significance was used to reduce type I error rate.

**RESULTS**

All 5 nalidixic acid adapted strains used in the study were positive for eae and fliC, and all but 1 strain were negative for stx1 and positive for stx2 genes (Figure 1). The stx1-positive strain (01–2-10530) was negative for the stx2 gene. The pulsed-field gel electrophoresis banding patterns indicated that all 5 strains were of distinct genetic types with <95% Dice similarity (Figure 1).

**Ruminal Fluid Fermentations**

Substrate, sampling hour, and the substrate × hour interaction were significant (P < 0.001) for NalR E. coli O157 concentration, fermentation pH, and VFA concentrations. Figures 2A, B, and C show concentrations of NalR E. coli O157 for control and fermentations of all substrates with ruminal microbial inoculum over the 24-h incubation period. The mean concentrations
of $\text{Nal}^R\text{E. coli O157}$ at 0 h (overall mean = 2.8 log$_{10}$ cfu/mL) were not different across all substrates ($P > 0.20$). In control (no substrate) fermentations with ruminal microbial inoculum, concentrations of $\text{Nal}^R\text{E. coli O157}$ at 0, 6, 12, and 24 h of fermentation were 2.9, 2.7, 3.0, and 3.4 ± 0.3 log$_{10}$ cfu/mL, respectively (sampling hour had a significant effect; $P < 0.01$). At 12 h of fermentation, the concentration of $\text{Nal}^R\text{E. coli O157}$ in the control was not different ($P > 0.05$) from fermentations with mucin or carbohydrate constituents of mucin (Table 1). At 24 h of fermentation, the concentration of $\text{Nal}^R\text{E. coli O157}$ in fermentations with galactose was less ($P < 0.001$) than that of the control, and the concentration in gluconic acid fermentation was greater ($P < 0.01$) than that of the control and all other substrates except glucuronic acid (Table 1). Addition of fucose, mannose, galactosamine, mucin, or glucosamine as substrate had no effect on the in vitro growth of $\text{E. coli O157}$ during fermentation for 24 h.

The pH in control fermentations with ruminal microbial inoculum at 0, 6, 12, and 24 h of incubation were 6.59, 7.03, 6.96, and 6.99 ± 0.09, respectively. Fermentation pH at 0 h was less ($P < 0.01$) in fermentations with galacturonic acid compared with all other substrates except glucosamine. Changes in fermentation pH were evident over the 24-h fermentation period and were contingent upon substrate (substrate × hour interaction, $P < 0.01$; Figure 2D, E, and F). Compared with the control (no substrate), fermentation pH at 12 and 24 h were less ($P < 0.01$) when fucose, galactose, mannose, galacturonic acid, or glucosamine were added as substrate (Table 1). Fermentation pH did not correlate ($P > 0.20$) with $\text{Nal}^R\text{E. coli O157}$ concentration in ruminal microbial fermentation (Table 2).

Figure 2. Least squares means of nalidixic acid-resistant $\text{Escherichia coli O157}$ concentrations (A, B, C) and pH (D, E, F) after 0, 6, 12, and 24 h of fermentation with ruminal microbial inoculum. Error bars represent SEM.
Total VFA (acetate + propionate + butyrate) concentrations at 0, 12, and 24 h were 26.3, 48.5, and 60.9 ± 5.7 mM, respectively, in ruminal microbial fermentations. Initial (0 h) total VFA concentrations were not different (P > 0.20) for all substrates and control fermentations. There were marked differences in concentrations of acetate, propionate, butyrate, and total VFA among substrates after 24 h of fermentation in ruminal microbial inoculum (Table 3). Acetate, butyrate, and total VFA concentrations at 24 h were greater for all substrate fermentations compared with the control, with the exception of galactosamine. In the case of propionate, concentrations for all substrates were greater than the control, except for galacturonic and galactosamine. Among VFA, only propionate concentration was positively correlated (P = 0.05) with NalR E. coli O157 concentrations at 12 h, but not at 24 h. No other significant correlations between VFA and NalR E. coli O157 concentrations were observed in fermentations with ruminal microbial inoculum (Table 2).

### Fecal Microbial Fermentation

In fecal microbial fermentations, substrate, sampling hour, and the substrate × hour interaction were significant (P < 0.001) for fermentation pH and VFA concentrations, but the substrate × hour interaction only tended (P = 0.06) to have an effect on NalR E. coli O157 concentration. However, there were significant substrate and hour effects (P < 0.001) on NalR E. coli O157 concentration. The concentrations of NalR E. coli O157 among substrates over the 24-h fermentation period in fecal microbial inoculum are shown in Figure 3. At 0, 6, 12, and 24 h, NalR E. coli O157 concentrations in control fermentations were 2.8, 3.3, 2.9, and 2.75 ± 0.6 log_{10} cfu/mL, respectively. At 12 h, the concentra-

### Table 1. Concentrations of nalidixic acid-resistant Escherichia coli O157 (log_{10} cfu/mL) and pH after 12 and 24 h of fermentation of mucin or its carbohydrate constituents with ruminal or fecal microbial inoculum

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ruminal microbial inoculum</th>
<th>Fecal microbial inoculum</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>12 h</td>
<td>24 h</td>
</tr>
<tr>
<td></td>
<td>E. coli O157 pH</td>
<td>E. coli O157 pH</td>
</tr>
<tr>
<td>None (control)</td>
<td>2.97&lt;sup&gt;ab&lt;/sup&gt; 6.96&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.35&lt;sup&gt;b&lt;/sup&gt; 6.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mucin</td>
<td>3.14&lt;sup&gt;ab&lt;/sup&gt; 6.91&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.24&lt;sup&gt;b&lt;/sup&gt; 6.77&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fucose</td>
<td>2.80&lt;sup&gt;ab&lt;/sup&gt; 6.70&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.39&lt;sup&gt;bc&lt;/sup&gt; 6.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactose</td>
<td>2.49&lt;sup&gt;a&lt;/sup&gt; 6.08&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.17&lt;sup&gt;b&lt;/sup&gt; 6.19&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mannose</td>
<td>3.16&lt;sup&gt;ab&lt;/sup&gt; 6.13&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.55&lt;sup&gt;bc&lt;/sup&gt; 6.19&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gluconic acid</td>
<td>3.60&lt;sup&gt;b&lt;/sup&gt; 6.75&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>4.39&lt;sup&gt;d&lt;/sup&gt; 6.86&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>2.43&lt;sup&gt;a&lt;/sup&gt; 6.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.72&lt;sup&gt;ab&lt;/sup&gt; 6.29&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>3.55&lt;sup&gt;b&lt;/sup&gt; 6.79&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.89&lt;sup&gt;cd&lt;/sup&gt; 6.77&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>3.11&lt;sup&gt;b&lt;/sup&gt; 6.83&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.72&lt;sup&gt;ab&lt;/sup&gt; 6.76&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>3.41&lt;sup&gt;b&lt;/sup&gt; 6.48&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.42&lt;sup&gt;bc&lt;/sup&gt; 6.43&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;bc&lt;/sup&gt; 0.09&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.26&lt;sup&gt;bc&lt;/sup&gt; 0.09&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-value (sub main effect)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>1</sup>n = 60.

### Table 2. Pearson correlation coefficients between nalidixic acid-resistant Escherichia coli O157 concentrations and pH or VFA concentrations after 12 and 24 h of fermentation with ruminal or fecal microbial inoculum

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ruminal microbial inoculum</th>
<th>Fecal microbial inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-h pH</td>
<td>E. coli O157 12 h</td>
<td>E. coli O157 24 h</td>
</tr>
<tr>
<td>24-h pH</td>
<td>E. coli O157</td>
<td></td>
</tr>
<tr>
<td>12-h acetate</td>
<td>0.17 0.15</td>
<td>-0.02 -0.02</td>
</tr>
<tr>
<td>24-h acetate</td>
<td>0.13 0.13</td>
<td>-0.01 -0.01</td>
</tr>
<tr>
<td>12-h propionate</td>
<td>0.27† 0.09</td>
<td>0.09 0.09</td>
</tr>
<tr>
<td>24-h propionate</td>
<td>0.19 0.19</td>
<td>-0.02 -0.02</td>
</tr>
<tr>
<td>12-h butyrate</td>
<td>-0.03 -0.05</td>
<td>0.12 0.12</td>
</tr>
<tr>
<td>24-h butyrate</td>
<td>-0.09 -0.23</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>12-h total VFA</td>
<td>0.22 0.01</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>24-h total VFA</td>
<td>0.16 -0.04</td>
<td>-0.04 -0.04</td>
</tr>
</tbody>
</table>

†, *, **Correlations significant at P < 0.10, 0.05, and 0.01 levels, respectively.
Table 3. Concentrations (mM) of acetate (Ace), propionate (Pro), butyrate (But) and their total after 24 h of fermentation with ruminal or fecal microbial inoculum

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ruminal microbial inoculum</th>
<th>Fecal microbial inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ace</td>
<td>Pro</td>
</tr>
<tr>
<td>None (control)</td>
<td>38.9</td>
<td>12.2</td>
</tr>
<tr>
<td>Mucin</td>
<td>72.7</td>
<td>24.7</td>
</tr>
<tr>
<td>Fucose</td>
<td>61.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Galactose</td>
<td>65.9</td>
<td>14.9</td>
</tr>
<tr>
<td>Mannose</td>
<td>59.5</td>
<td>33.4</td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td>49.5</td>
<td>38.6</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>82.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>43.9</td>
<td>11.9</td>
</tr>
<tr>
<td>Glucose</td>
<td>60.2</td>
<td>23.6</td>
</tr>
<tr>
<td>SEM</td>
<td>4.95</td>
<td>1.57</td>
</tr>
<tr>
<td>a–fWithin a column, means without a common superscript letter differ (P &lt; 0.01).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlations of 

\[ \text{Nal}^E \text{. coli O157} \] were greater (P < 0.01) for all substrates, except fucose, mannose, and galactosamine, than control (no substrate; Table 1). Fucose and galactosamine fermentations remained different (P > 0.05) from control fermentations even after 24 h with respect to \[ \text{Nal}^E \text{. coli O157} \] fermentations, whereas all other substrates yielded greater (P < 0.01) concentrations at the end of the fermentation period (Table 1) compared with controls. The concentrations of \[ \text{Nal}^E \text{. coli O157} \] in fermentations supplemented with gluconic acid were 1.5 and 2.0 log10 cfu/mL greater (P < 0.01) than control fermentations after 12 and 24 h, respectively.

In fecal microbial fermentations, pH values at 0, 6, 12, and 24 h were 6.64, 7.13, 7.04, and 7.08 ± 0.06, respectively, for fermentations without substrate (control). Interestingly, addition of galactosamine or galacturonic acid as the substrate resulted in less (P < 0.01) mean fermentation pH at 0 h compared with control (Figure 3). Fermentation pH was less in all substrates compared with control at 12 and 24 h (Table 1). Twelve-hour fermentation pH was positively correlated with 12 h fermentation pH, but 24-h total VFA concentration was not correlated (P > 0.20) with 24-h fermentation pH (data not shown).

**DISCUSSION**

The objective of this study was to evaluate whether mucin and its carbohydrate constituents may give \[ \text{E. coli O157} \] an advantage in the large intestine compared with the rumen of cattle. This may explain why the organism is able to establish and persist in the large intestine, but not in the rumen (Rasmussen et al., 1993; Brown et al., 1997; Gruene et al., 2002; Laven et al., 2003; Van Baale et al., 2004). Availability of dietary substrates in the large intestine for microbial fermentation is limited due to the vast microbial population in the rumen that depletes many of the rapidly fermentable sugars and nitrogen compounds in cattle diets. This reduction in substrates is advantageous to \[ \text{E. coli O157} \] because potentially inhibitory fermentative products are not produced in large quantities in the large intestine. Organic acids (common fermentative products) are known to reduce viability and increase acid resistance of \[ \text{E. coli O157} \] (Diez-Gonzales and Russell, 1997; Jordan et al., 1999; Shin et al., 2002).

The substrates evaluated in this study are components of mucus located in the small intestine (fucose, galactose, mannose, galactosamine, and glucosamine; Montagne et al., 2000) or cecum (gluconic acid, glucuronic acid, and galacturonic acid; Peekehaus and Conway, 1998). The D-isomers of gluconate, glucuronate, and galacturonate can be metabolized via the Entner-Duodoroff pathway of \[ \text{E. coli} \] (Peekehaus and Conway, 1998). Additionally, fucose, glucuronic acid, and galactose are components...
of colanic acid, an exopolysaccharide of *E. coli* O157, which enables the pathogen to survive under acidic conditions (Mao et al., 2006). Porcine stomach mucin was used in the study because bovine mucin is not commercially available. The monosaccharide composition of porcine mucin includes \(N\)-acetylgalactosamine, \(N\)-acetylglucosamine, galactose, fucose, and \(N\)-acetyl, or \(N\)-glycolly neuraminic acids (Allen, 1981). Carbohydrate constituents of mucin from the large intestine of cattle are likely to be similar to that of the mucin from the small intestine, which has been isolated and analyzed (Montagne et al., 2000). Mucin from the intestine of calves is similar to mucin from other animal species (Allen, 1981; Montagne et al., 2000).

To evaluate the effect of carbohydrate constituents of mucin, we utilized an in vitro batch culture fermentation system with an initial *E. coli* O157 concentration of about 3.0 \(\log_{10}\) cfu/mL. The fecal concentrations of *E. coli* O157 in cattle range from 0.6 to 7.0 \(\log_{10}\) cfu/g, but most animals shed around 1.0 to 2.0 \(\log_{10}\) cfu/g (Gyles, 2007). Though our initial *E. coli* O157 inoculum was 10-fold greater than fecal concentrations in most cattle, it is still biologically relevant given the range of concentrations seen in cattle. In vitro fermentations with ruminal fluid and fecal inocula have historically been used to assess gut microbial activities and interactions, and digestibility of specific substrates (El Shaer et al., 1987; Mould et al., 2005; Varadyova et al., 2005).

Ruminal fluid fermentation also has been used to evaluate factors that impact in vitro growth and survival of *E. coli* O157, such as ecological factors (Diez-Gonzalez and Russell, 1997), antimicrobials (Edrington et al.,

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**Figure 3.** Least squares means of nalidixic acid-resistant *Escherichia coli* O157 concentrations (A, B, C) and pH (D, E, F) after 0, 6, 12, and 24 h of fermentation with fecal microbial inoculum. Error bars represent SEM.
2003, 2006), probiotics (Bach et al., 2003), and other bactericidal agents (Annamalai et al., 2004). Chaucheyras-Durand et al. (2006) evaluated the growth of *E. coli* O157 in both ruminal fluid and fecal microbial fermentations and reported that ruminal fluid, because of the resident microbial population, was inhibitory to *E. coli* O157. Our intention was to simulate the microbiological aspects of ruminal digesta and feces and evaluate compounds that commonly are found in mucous secretions in the large intestine and assess differences in *NalR* *E. coli* O157 concentrations, pH, and VFA concentrations. Fecal microbial fermentation was used to represent the large intestine fermentation (Rumney and Rowland, 1992; Mould et al., 2005). Ideally, we would have liked to directly compare the growth of *NalR* *E. coli* O157 in ruminal and fecal microbial inoculum to answer this question, but because of differences in DM content and the types and concentrations of microbes and nutrients, it is not meaningful to evaluate both fermentations on an equal basis. Therefore, we did not statistically compare the 2 fermentation inocula, although they were from the same donor steer. The difference between the 2 fermentations is evident in the control fermentations based on the apparent difference in concentrations of fermentation products. The concentrations of acetate, propionate, butyrate, and total VFA were numerically less in fecal microbial fermentation than ruminal microbial fermentations, but were not statistically compared. The difference may be reflective of a smaller microbial population in feces compared with ruminal fluid. The addition of physiological saline to approximate the DM of the fecal microbial inoculum may have contributed to further reduction in the microbial population. However, addition of physiological saline to prepare a fecal suspension was needed to remove large particles and for the ease in setting up the fermentation. Even at *P*-values of <0.01, we observed many differences between substrates with respect to the outcomes evaluated. Some substrates did not elicit responses different than control, which suggests that they may not have been fermented by the microbial population.

Many of the compounds evaluated affected the growth of *NalR* *E. coli* O157 in ruminal or fecal microbial fermentations. Of the sugars (fucose, galactose, and mannose), mannose and fucose elicited moderate increase in *NalR* *E. coli* O157 concentration and decrease in fermentation pH compared with the control. However, in fermentations with ruminal microbial inoculum, galactose reduced *NalR* *E. coli* O157 concentration and pH below that of control, but other substrates, such as mannose, reduced pH in a similar manner without reducing *NalR* *E. coli* O157 concentrations. The decrease in *NalR* *E. coli* O157 in ruminal microbial fermentations containing galactose was not observed in fecal microbial fermentations; in fact, *NalR* *E. coli* O157 concentration was actually greater compared with the control. It has previously been observed that in continuous culture of ruminal contents, an inoculated population of *E. coli* O157 was not altered by fermentation pH, but competitive exclusion by other microbes caused a decrease in the population of *NalR* *E. coli* O157 (Thran et al., 2003). This could explain the difference in response of *NalR* *E. coli* O157 to galactose in ruminal and fecal microbial inoculum, because galactose is highly fermentable in the rumen by lactate-producing bacteria, which have very rapid growth rates (Dennis et al., 1981), thus limiting availability of the substrate in batch culture fermentation.

Galactosamine fermentation did not result in greater concentrations of *NalR* *E. coli* O157 compared with the control treatment, but fermentations with glucosamine had greater *NalR* *E. coli* O157 concentrations than control in 12- and 24-h samples with moderate decreases in pH. Mucin did not affect *NalR* *E. coli* O157 concentrations in ruminal microbial inoculum, but increased the concentration in fecal microbial inoculum. The difference in response of *NalR* *E. coli* O157 between fecal microbial and ruminal microbial inocula may be reflective of differences in mucinolytic activities. Because mucus is secreted in the large intestine and not in the rumen, the resident flora in the large intestine may contain greater numbers of bacteria capable of degrading mucin and releasing its carbohydrate constituents. The fact that mucin was less stimulatory than some of the individual components suggests that it may be related to the rate of mucinolytic activity or specific components of mucin may be responsible for the stimulation of *E. coli* O157.

Mucus serves as an important defense mechanism in the gastrointestinal tract to remove slow growing pathogens and support commensal bacteria (such as *E. coli*) of the gut (Deplancke and Gaskins, 2001). In germ-free rats, lipopolysaccharide of an experimental *E. coli* strain was able to alter mucin glycosylation patterns to allow more favorable conditions for the growth and attachment of the organism (Enss et al., 1996). Because *E. coli* O157 is nonpathogenic to cattle, it is conceivable that it may alter mucin chemistry to increase specific carbohydrate constituents, thus allowing it to thrive in the large intestine.

Few substrates elicited significant responses in ruminal microbial inoculum, but gluconic acid increased the 24-h concentration of *NalR* *E. coli* O157 by 1.0 log_{10} cfu/mL in ruminal microbial inoculum and 2.0 log_{10} cfu/mL in fecal microbial inoculum compared with control. Again, the increase may be reflective of the ability of *E. coli* O157 to utilize gluconic acid as an energy source. Given the ability of *E. coli* to utilize gluconic acid via the Entner-Duodoroff pathway (Peekehaus and Conway, 1998), it is possible that this compound may serve as a nutrient for *E. coli* O157 in the large intestine or other sites of the gastrointestinal tract where mucus is secreted. Additionally, this compound also is fermented by *Lactobacillus* sp. (Tsukahara et al., 2002), which may provide an explanation for the efficacy of *Lactobacillus*-based direct-fed microbials for reducing *E. coli* O157 prevalence in cattle or inhibiting *E. coli* O157 in vitro (Brashears et al., 2003; Chaucheyras-Durand et al.,...
The uronic acids (galacturonic and glucuronic acids) increased $NaI^{+} E. coli O157$ concentrations in fecal microbial inoculum, but galacturonic acid reduced pH more than glucuronic acid. However, galacturonic acid decreased $NaI^{+} E. coli O157$ concentrations in ruminal microbial fermentations. This decrease may be because initial pH was less in fermentations with galacturonic acid compared with other substrates and control; however, this was true for both inocula. The difference in response of $NaI^{+} E. coli O157$ to uronic acids in different inocula may suggest that substrate effects are indirect and actually are a function of how other microbes respond to the substrate.

Almost all substrates had greater VFA concentrations than control after 24 h of fermentation. This was expected because the control fermentation did not have substrate and the initial inoculum was diluted with buffer, saline, or both; and therefore had relatively low concentrations of endogenous nutrients. In fecal microbial inoculum, acetate and total VFA concentrations were negatively correlated with $NaI^{+} E. coli O157$ concentrations. Because acetate is the predominant VFA, it is likely that the correlation between total VFA and $NaI^{+} E. coli O157$ was the result of the correlation between acetate and $NaI^{+} E. coli O157$, especially because the acetate correlations are stronger. These correlations suggest that elevated acetate concentrations may inhibit or kill $E. coli O157$, but these correlations were not observed in ruminal microbial inoculum. It has been documented that $E. coli O157$ has cellular mechanisms to prevent the toxic accumulation of acetate within the bacterial cell (Diez-Gonzalez and Russell, 1997). The correlations may also be explained by competitive exclusion of $E. coli O157$ by acetate-producing bacteria present in fecal microbial inoculum (Wolfe, 2005). It is difficult to assess if the substrates evaluated in our study that increase $NaI^{+} E. coli O157$ concentrations have direct stimulatory effects on $E. coli O157$, indirectly affect the organism by altering or inhibiting other microflora, or both.

For most of the substrates evaluated, the effect of fermentation on $NaI^{+} E. coli O157$ concentration was numerically greater in fecal microbial inoculum compared with ruminal microbial inoculum, although we did not compare the 2 fermentations statistically. The total number of bacteria was likely greater for the rumen, and few statistical differences were evident in fermentations with ruminal microbial inoculum, suggesting that ruminal microorganisms may degrade these components and reduce their availability to $E. coli O157$.

Many of the substrates we evaluated stimulated $NaI^{+} E. coli O157$, particularly in fecal microbial fermentation. Gluconic acid stimulated $NaI^{+} E. coli O157$ in ruminal microbial inoculum and had the greatest response of any substrates in fecal microbial inoculum. These results suggest that gluconic acid may serve as a unique substrate available to promote growth of $E. coli O157$ in the large intestine of beef cattle.

**LITERATURE CITED**


Fox, J. T., B. E. Depenbusch, J. S. Drouillard, and T. G. Nagaraja. 2007. Dry-rolled or steam-flaked grain-based diets and fecal...


