Effect of Galactan on Selected Microbial Populations and pH and Volatile Fatty Acids in the Ileum of the Weanling Pig$^{1,2}$


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ABSTRACT: Studies were conducted to determine the effect of galactan on the colonization of $E. coli$ and lactobacilli and ileal pH and volatile fatty acid production in the digestive tract of the weanling pig. In each of two replicate trials, eight 21-d-old nursing pigs were cannulated in the terminal ileum. After a 7-d recovery period, the pigs were weaned and randomly assigned to two test diets: 1) a corn-soybean meal-based control diet and 2) a similar diet containing 1% galactan. On d 1 after weaning, all pigs were orally subjected to K88+ $E. coli$ (2 $\times$ 10$^9$ colony forming units). Ileal digesta samples were collected on d 0, 2, 4, 6, 8, and 10 after weaning and assayed for total $E. coli$, K88+ $E. coli$, lactobacilli, pH, and VFA. At the end of the trials, the pigs were killed and digesta samples were collected from the stomach, duodenum, cecum, and colon. Assays similar to those performed on the digesta samples collected from ileal cannulas were performed. Pigs fed 1% galactan had lower ($P < .10$) ileal pH, lower ($P < .05$) total $E. coli$ on d 6 and 8, and lower ($P < .05$) K88+ $E. coli$ concentrations in the ileum than pigs fed the control diet. There were no differences in ADG or gain:feed ratio between diets. The VFA concentrations were not different in the ileum between diets. The VFA were higher ($P < .10$) on d 0 than on any other day of the study. Acetate and isobutyrate concentrations were lower ($P < .10$) in the cecum in pigs fed 1% galactan. Galactan included in the feed on a 1% DM basis decreased pH and $E. coli$ in the small intestine of newly weaned pigs.

Key Words: Escherichia coli, Pigs, Volatile Fatty Acids, Ileum, Galactans

Introduction

Management of the weaned pig presents one of the most significant challenges to swine producers. The stress of weaning and movement to another environment increases the potential for disease, poor feed intake, and nutritional disorders in the young pig (Kohler and Moon, 1984).

Escherichia coli are often associated with postweaning secretory diarrhea (Hampson et al., 1985). The physiological events that lead to $E. coli$ colonization in the small intestine are not well understood. Certain serogroups of $E. coli$, including those exhibiting K88, K99, and 987P antigens, possess proteinaceous filaments (fimbria), which enable the bacteria to adhere to the epithelial lining of the small intestine. The importance of adherence to colonization and pathogenic characteristics of $E. coli$ has been well demonstrated (Arbuckle, 1970; Bertschinger et al., 1972; Wilson and Hohmann, 1974).

Glycoproteins in porcine colostrum have been shown to inhibit hemagglutinating activity of K88 adhesins, and this inhibiting ability is lost when the glycoproteins are treated to remove the 0-D-galactose terminal residues (Gibbons et al., 1975). More complex oligosaccharides such as stachyose ($\alpha$-D-galactosyl-$\alpha$-D-galactosyl-$\alpha$-D-galactosyl-$\beta$-D-fructose) and galactan ($\alpha$ polymer of D- and L-galactose) have shown some ability to inhibit adherence of K88 fimbria in vitro (Sellwood, 1980).

The objectives of this study were 1) to determine the ability of galactan to inhibit colonization by $E. coli$ in the small intestine of the weanling pig and 2) to quantify changes that occur in microbial populations, pH, and volatile fatty acid concentrations in the gastrointestinal tract immediately after weaning and upon challenge by a pathogenic strain of $E. coli$. 

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Table 1. Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil</td>
<td>30.75</td>
</tr>
<tr>
<td>Soybean meal (48% CP)</td>
<td>30.75</td>
</tr>
<tr>
<td>Dried whey</td>
<td>10.00</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1.00</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.30</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>.90</td>
</tr>
</tbody>
</table>
| Vitamin premix
  a               | .25     |
| Trace mineral premix
  b              | .05     |
| Selenium premix
  c               | .10     |
| Total                         | 100.00  |

*Ingredients (amount/kilogram of diet): vitamin A, 6,098.4 IU; vitamin D₃, 606.4 IU; vitamin E, 23.1 IU; menadione, 1.6 mg; vitamin B₁₂, .03 mg; riboflavin, 6.1 mg; d-pantothenic acid, 22.4 mg; and niacin, 34.9 mg.

Materials and Methods

Experimental Design

In each of two replicate trials, eight 21-d-old crossbred (Landrace, Yorkshire, Hampshire) gilts weighing approximately 8 kg were surgically fitted with T-cannulas in the terminal ileum according to the procedures approved by the Purdue Animal Care and Use Committee. The cannulas were made from Delrin 600 (DuPont Chemical, Wilmington, DE) and were similar in design to that reported by Walker et al. (1986). After a 7-d recovery period, the pigs were weaned, moved to individual pens, and assigned to two dietary treatments with four pigs per treatment. Littermates were divided equally between treatments. A total of four litters were equally represented between two dietary treatments. The treatments were 1) a control consisting of a nonpelleted corn-soybean meal-based diet containing 21% CP protein and 10% whey that met NRC requirements (Table 1) and 2) a similar treatment in which galactan (Sigma, St. Louis, MO) was included at 1% of the diet, replacing an equivalent weight of corn on a DM basis. No medications were included in the diets and pigs were allowed ad libitum access to feed. All pigs were challenged by oral injection of 2 x 10⁹ colony forming units (cfu) of E. coli serogroup 0157:K88:H13 (E. coli Reference Center, University Park, PA) on d 1 after weaning to ensure exposure to an adhesive strain. Room temperature was maintained at 26°C for the duration of the study.

Sampling and Analysis

Ileal digesta samples were collected on d 0, 2, 4, 6, 8, and 10 after weaning by attaching a balloon to the open cannula at 0830 on each sampling day. When approximately 10 g of digesta had accumulated, balloons were removed, and the samples were assayed immediately for specific microbial concentrations and pH. Pigs were weighed on each sampling day at the time of sampling. Any signs of scouring or changes in the overall health of each animal were noted and recorded.

Volatile fatty acid determinations were conducted using gas chromatographic methods described by Playne (1985). In this procedure 8 g of ileal contents was mixed with 2 mL of 25% metaphosphoric acid and incubated at room temperature for 30 min. The samples were centrifuged at 12,100 x g at 4°C for 10 min in a Beckman model J-21C centrifuge with a JA-20 rotor (Beckman Instruments, Palo Alto, CA). The supernatants were drawn off by pipette and frozen for a minimum of 24 h before being passed through filters with .45-μm pores. Three microliters of the filtered samples was injected into a Varian model 3700 gas chromatograph (Varian, Walnut Creek, CA) for determination of propionate, acetate, butyrate, valerate, isobutyrate, and isovalerate concentrations.

For E. coli determinations, 1-g aliquots of digesta samples were serially diluted in PBS. Aliquots of the serial dilutions were dispensed into Bacto MacConkey (DIFCO Laboratories, Detroit, MI) agar and incubated at 37°C for 24 h. Total lactobacilli were determined by dispensing aliquots of serial dilutions onto Bacto Rogosa (DIFCO) agar and incubating at 37°C for 48 h. After their respective incubation periods, colonies were visually counted using a Quebec Darkfield Colony Counter model 3325 (Baxter, McGaw Park, IL) and recorded. Pink to red colonies, indicating lactose fermentation, were counted for the determination of total E. coli concentrations.

The K88+ E. coli concentrations were determined using an immunoaffinity magnetic particle assay as described by Lund et al. (1988). In this procedure, 5 mg of Dynabead M-280 paramagnetic particles (Dynal, Oslo, Norway) was coated with 20 μg of ELISA monoclonal K88 antiserum derived from mouse ascites fluid (E. coli Reference Center) and mixed with 1 mL of serial dilutions from each sample. Samples were incubated for 10 min with gentle shaking at room temperature and placed in a magnetic particle concentrator MPC-1 (Dynal) for 2 min to isolate the particles and any bound K88+ E. coli. The remaining solution containing unbound bacteria was drawn off by pipette. The particles were washed five times in 5 mL of PBS plus 1% (by volume) fetal bovine serum albumin (FBSA). After the wash procedure, the particles were resuspended in PBS-FBSA to the original sample volume of 1 mL. One hundred microliters of the magnetic particles with adhering bacteria was dispensed onto Bacto MacConkey agar and incubated at 37°C for 24 h for numerical determinations.

On d 14 of each experiment, the pigs were euthanatized by i.m. injection of 20 mg of succinylcholine chloride (Burroughs Wellcome, Research Triangle
Figure 1. Effect of 1% galactan on ileal pH of pigs challenged with K88+ E. coli. n = 8 for each treatment. SE = .20. Diet effect; P = .10; day effect, P = .45; diet x day effect, P = .65.

Pigs fed diets containing 1% galactan had lower (P < .10) ileal pH throughout the study (Figure 1). Total E. coli concentrations were numerically lower (P > .10) in pigs fed 1% galactan (Figure 2). There was a significant (P < .10) interaction of diet x day for total E. coli. Escherichia coli concentrations were lower (P < .05) in pigs fed 1% galactan on d 6 and 8 after weaning as determined by t-test. K88+ E. coli concentrations were lower (P < .05) in pigs fed 1% galactan (Figure 3), although most of the effect seemed to occur on d 10. There were no differences (P > .10) in the proportion of K88+ to total E. coli between treatments (Figure 4). There were no differences (P > .10) in lactobacilli concentrations between diets at any time during the study (data not shown). A numerical (P > .10) increase in gain as well as a numerical (P > .10) increase in gain:feed ratio was noted in pigs fed 1% galactan (Table 2). Ileal VFA concentrations were not different between diets (data not shown).

Results

Pigs fed diets containing 1% galactan had lower (P < .10) ileal pH throughout the study (Figure 1). Total E. coli concentrations were numerically lower (P > .10) in pigs fed 1% galactan (Figure 2). There was a significant (P < .10) interaction of diet x day for total E. coli. Escherichia coli concentrations were lower (P < .05) in pigs fed 1% galactan on d 6 and 8 after weaning as determined by t-test. K88+ E. coli concentra-
Figure 4. Effect of 1% galactan on percentage of K88+ E. coli in the ileal digesta of pigs challenged with K88+ E. coli. Expressed as K88+/total E. coli. n = 8 for each treatment. SE = 11.8. Diet effect, P = .26; day effect, P = .001; diet × day effect, P = .12.

not shown). There was a significant (P < .10) interaction of diet × day for acetate concentration but no differences were found on any one day between diets as determined by t-test. Although a diet × day interaction was noted for acetate, data for all VFA were pooled over both diets (Table 3) to demonstrate the decline in VFA concentrations that occurred after weaning. Total VFA concentration was greater (P < .001) on the day of weaning than at any time within the 2-wk period after weaning.

At the end of the trial, production of acetate and isobutyrate was lower (P < .10) in the cecum of pigs fed 1% galactan (data not shown). There were no differences in VFA concentrations at other locations between diets. Although a significant diet × location effect was noted for acetate and isobutyrate, data have been pooled over treatments (Table 4) to demonstrate the location effect on VFA. Concentrations of individual and total VFA were lowest (P < .10) in the duodenum among all locations. Total VFA concentration was greatest (P < .10) in the colon, followed by the stomach, and duodenum.

Most pigs exhibited loose to watery stools within 2 d after the challenge. No differences could be detected between diets in either the frequency or severity of the scouring. In general, scouring lasted 1 to 4 d and coincided with a loss of weight by the affected pig. All pigs demonstrated more normal fecal material and positive weight gains by the end of the study.

Discussion

Administration of antibiotics on a subclinical level has been employed to minimize the potential for enteric disorders and therapeutic levels are sometimes used to correct an outbreak of malabsorptive or secretory diarrhea due to specific bacterial agents. Repeated use of drugs in the production of meat animals, however, is a concern. The encouragement of drug-resistant strains of bacteria by continual use of antibiotics, drug residues in meat products as a result of improper use, and other factors may cause the industry to reevaluate the use of feed antibiotics. An alternative for controlling deleterious enteric bacteria would be to regulate the environment and(or) the commensal microflora of the gastrointestinal tract through dietary factors.

Nutritional factors have been shown to affect intestinal E. coli concentrations. These factors include intake of vitamins (Jorgensen and Wegger, 1979; Van Vleet, 1980), organic acids (Kerchgessner and Roth, 1976; Sutton et al., 1989), fiber content (Smith and Halls, 1968; Dunne, 1975), and volatile fatty acids (Prohaszka and Lukas, 1984).

Treatments that affect the pH of intestinal contents may also affect enteric bacteria populations, including E. coli. Low pH (5.0 vs 7.0) reduced protein synthesis and internal pH in E. coli compared with Salmonella typhimurium (Hickey and Hirshfield, 1990). Expression of adhesive filaments is also dependent on the physiological environment surrounding the E. coli cell (Jacobs and De Graaf, 1985) and thus may be dependent on nutritional factors, other microbial species, and their fermentation products.

Recent work in cell sorting techniques (Lea et al., 1985; Lund et al., 1988) have led to the development of techniques that allow the investigator to separate

<table>
<thead>
<tr>
<th>Table 2. Effect of 1% galactan on pig performancea,b</th>
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<tr>
<td>Item</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Gain, kg</td>
</tr>
<tr>
<td>Feed intake, kg</td>
</tr>
<tr>
<td>Avg daily gain, kg</td>
</tr>
<tr>
<td>Gain/feed</td>
</tr>
</tbody>
</table>

a n = 8 for each treatment.

b Feed intake and gain data are for 42-d-old pigs for the 14-d period after weaning.
cells based on their immunoadherence characteristics while still maintaining the viability of the bacterial cell. This allows for more direct enumeration and proportional determinations of specific serogroups in a given population. Although enumeration of adhesive bacteria in digesta samples may not directly indicate the degree of colonization on the intestinal mucosa, this analysis may allow the comparison of treatment effects on the expression of adhesive filaments and the potential for colonization by certain serogroups. In addition, the sloughing of mucosal cells and bacteria in response to E. coli colonization as well as the presence of adhesive bacteria that have been displaced by the flow of digesta would be detected by this immunoadherence method, resulting in a preliminary indication of colonization on the mucosa. Histological determinations to detect attached E. coli directly will be required to assess adequately the effect of galactan on E. coli adherence.

Earlier research in our laboratory (Mathew, 1991) indicated that a significant decrease in lactobacilli concentrations occurred within 2 d after weaning in the small intestine of the young pig. This was often accompanied by an increase in total E. coli concentrations. It was surprising to find that the proportion of K88+ to total E. coli was lowest on d 2 of the experiment, because it might be assumed that the increase in total E. coli that occurs at this time is due to an increase in adhesive strains, such as K88+. It is possible that other serogroups are also involved in attachment and proliferation and thus increase the total population in the early postweaning period. In addition, the change from a milk diet to a dry feed often leads to reduced feed intake during the first few days after weaning. This would lead to a decreased flow of digesta through the small intestine. It was found in these studies that the pH of the small intestine often increases within the first few days postweaning, possibly due to overbuffering by pancreatic and other secretions until normal intake resumes. Both decreased digesta flow and increased pH may allow several serogroups of E. coli to colonize the anterior small intestine, thus lowering the K88+ to total E. coli ratio within the first 2 d after weaning.

In general, pigs fed both diets refused dry feed for a period of 24 to 36 h after weaning, although pigs fed the control diet had numerically higher intake during the entire study. Lower concentrations of E. coli in

<table>
<thead>
<tr>
<th>Location</th>
<th>A</th>
<th>P</th>
<th>IB</th>
<th>B</th>
<th>IV</th>
<th>V</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>9.54f</td>
<td>7.19e</td>
<td>.31f</td>
<td>5.12f</td>
<td>.22g</td>
<td>1.96f</td>
<td>24.24e</td>
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<tr>
<td>D</td>
<td>96b</td>
<td>28a</td>
<td>.27f</td>
<td>.19b</td>
<td>.16a</td>
<td>.09g</td>
<td>1.90b</td>
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<tr>
<td>C</td>
<td>71.68e</td>
<td>59.17e</td>
<td>.49f</td>
<td>25.67e</td>
<td>.62g</td>
<td>.53e</td>
<td>162.99e</td>
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<tr>
<td>L</td>
<td>61.01f</td>
<td>37.24f</td>
<td>.49f</td>
<td>16.20e</td>
<td>1.80e</td>
<td>3.97e</td>
<td>121.71f</td>
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<tr>
<td>SE</td>
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<td>2.07</td>
<td>.08</td>
<td>1.54</td>
<td>.09</td>
<td>.59</td>
<td>4.94</td>
</tr>
</tbody>
</table>

*Expressed in millimoles per liter.

Table 4. Volatile fatty acid (VFA) concentrations in weanling pigs at various locations in the digestive tract.*

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**Table 3. Volatile fatty acid (VFA) concentrations in pigs on various days after weaning.**

<table>
<thead>
<tr>
<th>Day</th>
<th>A</th>
<th>P</th>
<th>IB</th>
<th>B</th>
<th>IV</th>
<th>V</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.26d</td>
<td>4.07e</td>
<td>.36</td>
<td>5.39d</td>
<td>.74d</td>
<td>.24de</td>
<td>43.06d</td>
</tr>
<tr>
<td>2</td>
<td>14.00e</td>
<td>4.46e</td>
<td>.42</td>
<td>1.38e</td>
<td>.36e</td>
<td>.05e</td>
<td>20.72e</td>
</tr>
<tr>
<td>4</td>
<td>10.00e</td>
<td>3.81e</td>
<td>.34</td>
<td>.63e</td>
<td>.23e</td>
<td>.15de</td>
<td>16.15e</td>
</tr>
<tr>
<td>6</td>
<td>8.19e</td>
<td>4.72e</td>
<td>.46</td>
<td>.79e</td>
<td>.19e</td>
<td>.17de</td>
<td>14.53e</td>
</tr>
<tr>
<td>8</td>
<td>11.07e</td>
<td>9.32e</td>
<td>.41</td>
<td>1.47e</td>
<td>.31e</td>
<td>.3§d</td>
<td>23.00e</td>
</tr>
<tr>
<td>10</td>
<td>10.40e</td>
<td>5.11e</td>
<td>.30</td>
<td>1.04e</td>
<td>.25e</td>
<td>.25de</td>
<td>17.37e</td>
</tr>
<tr>
<td>SE</td>
<td>2.30</td>
<td>1.08</td>
<td>.07</td>
<td>.51</td>
<td>.10</td>
<td>.07</td>
<td>3.64</td>
</tr>
</tbody>
</table>

*aExpressed in millimoles per liter.

*bData pooled over both diets. n = 16 for each day.

cA = acetate, P = propionate, B = butyrate, IB = isobutyrate, V = valerate, IV = isovalerate.

d,eMeans in same column with different superscripts are significantly different at P < .10.
pigs fed galactan were noted after d 4 when more normal intake patterns had resumed for all pigs. It is unlikely that galactan affected any changes that occurred in microbial concentrations before d 2. Samples taken after d 2, with the exception of those from galactan-fed pigs taken 10 d after weaning, indicate that as digesta flow increased, the proportion of K88+ to total E. coli increased. This may support the view that adhesive strains of E. coli have an advantage under conditions of more rapid digesta flow.

The fact that K88+ E. coli were noted in nearly all pigs before the challenge is further evidence that pathogenic E. coli are ubiquitous in swine populations (Nielsen, 1986), commonly existing in small numbers without causing disease symptoms. Pigs were challenged in this study in an attempt to ensure that all animals had a significant and equal exposure to a K88+ strain after weaning; however, it was not the intent of this research to induce clinical symptoms of severe or fatal scouring or edema disease.

Digesta samples taken from cannulated animals do not allow a direct measurement of gut volume or the ability to delineate changes due to dilution effects or flow rates. However, because a close association of bacteria with the intestinal mucosa seems necessary for the induction of E. coli-related digestive disorders (Bertshinger et al., 1972; Nielsen, 1986), comparisons of concentrations of E. coli per unit of digesta may be superior to quantifying total numbers of bacteria within the intestine in terms of the risk of infection. Concentration measurements would seem to give a better indication of the number of bacteria in the digesta that are in contact with the mucosal wall and therefore comparisons could be made between animals regardless of their respective gut size or digesta flow rates.

The VFA concentrations in all pigs were greatest on d 0 and fell rapidly by d 2. Although the decrease of most VFA after weaning might be attributed to a dilution effect, the initial decrease occurred during the period of low intake after weaning. Additionally, concentrations of E. coli, propionate, and isobutyrate increased or remained constant during this time, contrary to what would be expected for a dilution effect. As pigs consumed higher levels of feed during the latter part of the study, total VFA concentrations remained nearly constant at the lower, postweaning levels. It seems that even after the pigs are consuming higher levels of a starter diet containing 10% whey, VFA concentrations in the ileum do not return to levels observed when pigs are nursing.

The VFA data indicate that the cecum and colon exhibit the greatest fermentation activity. It was observed that concentrations of VFA in the stomach were higher than duodenal concentrations. The VFA data indicate that fermentation does continue to occur in the stomach even as it becomes more acidic as the pig develops. This finding is in agreement with earlier studies by Friend et al. (1963) and Keys and DeBarthe (1974). Rapid absorption of VFA in the small intestine may explain the lower concentrations there.

Although a numerical difference in performance of pigs was noted between diets, it was not found to be statistically significant. The effects of cannulation as well as the large variation in intake, growth, and efficiency in the pig soon after weaning may prohibit speculation of the effects of galactan on performance in this study. Long-term feeding trials with larger numbers of pigs will be necessary to assess performance effects.

It is still uncertain how compounds such as galactan influence microbial populations in the intestine. Galactan may affect E. coli concentrations indirectly by altering microbial fermentation, lowering pH of intestinal contents, and causing a decline in bacteria such as E. coli that are more sensitive to low pH. In vitro studies in our laboratory using immunoaffinity magnetic particles and additional cannulation studies (Mathew, 1991) indicated that a pH in the range of 7.5 stimulates expression of K88+ fimbria and that these high pH levels can sometimes occur in the ileum of the pig shortly after weaning. These effects may be partly responsible for the enhanced ability of E. coli to colonize the small intestine after weaning.

Implications

If nutritional factors could be identified that maintain a gastrointestinal environment resistant to adverse bacterial agents, losses due to postweaning digestive disorders resulting from bacteria could be minimized and reliance on feed antibiotics could be reduced. The results of this research indicate that the inclusion of complex carbohydrates such as galactan in starter diets may alter the digestive physiology and the microbial populations in the weanling pig to confer such resistant qualities.

Literature Cited


Gibbons, R. A., G. W. Jones, and R. Sollwood. 1975. An attempt to identify the intestinal receptor for the K88 adhesin by means of


