Polydextrose Enrichment of Infant Formula Demonstrates Prebiotic Characteristics by Altering Intestinal Microbiota, Organic Acid Concentrations, and Cytokine Expression in Suckling Piglets

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Abstract

Oligosaccharides, the 3rd-most abundant component in human milk, are virtually absent from infant formulas and from the cow milk on which most are based. In breast-fed infants, human milk oligosaccharides (HMO) act as both receptor analogs, interfering with pathogen adhesion, and as prebiotics, stimulating the growth of certain commensal bacteria (e.g. bifidobacteria) and supporting the innate immunity. To further align the functional properties of infant formula with those of human milk, polydextrose (PDX) is proposed as a substitute for HMO. To determine the prebiotic functionality of PDX, 1-d-old pigs were fed a cow milk-based formula supplemented with increasing concentrations of PDX (0, 1.7, 4.3, 8.5, or 17 g/L) for 18 d (n = 13). Additional reference groups included pigs sampled at d 0 and sow-reared pigs sampled at d 18 (n = 12). Ileal Lactobacilli CFU, but not Bifidobacteria, increased linearly with increasing PDX (P = 0.02). The propionic acid concentration in digesta linearly increased with the PDX level (P = 0.045) and lactic acid increased linearly by 5-fold with increasing PDX (P = 0.001). Accordingly, digesta pH decreased linearly (P < 0.05) as PDX increased, with a maximal reduction approaching 0.5 pH units in pigs fed 17 g/L. Expression of TNFα, IL-1β, and IL-8 showed a negative quadratic pattern in response to PDX supplementation, declining at intermediate concentrations and rebounding at higher concentrations of PDX. In summary, PDX enrichment of infant formula resulted in a prebiotic effect by increasing ileal lactobacilli and propionic and lactic acid concentrations and decreasing pH with associated alterations in ileal cytokine expression. J. Nutr. 141: 2139–2145, 2011.

Introduction

Human milk is widely considered the optimum food for meeting the nutritional needs of infants, but by 2 mo of age, the majority of infants in North America have received some quantity of infant formula (1). Though iron-fortified infant formulas are the most appropriate nutritional substitutes, their composition does not fully duplicate that of human milk, particularly with regards to bioactive components such as HMO that perform functional roles beyond basic nutrition, such as innate immune support. To more closely approximate both nutritional and functional properties of human milk, efforts are under way to identify novel ingredients with similar bioactive properties. To that end, PDX is thought to function similarly to HMO and is proposed for addition to a new generation of pediatric nutritional products.

Oligosaccharides constitute the third most abundant component in human milk after lactose and lipids, ranging in concentration from 5 to 10 g/L in milk (2). Oligosaccharides are virtually absent from cow milk (<0.08 g/L) and most infant formula, which may account in part for the difference in GI microbiota reported among breast-fed and formula-fed infants (2–4). Bifidobacteria, dominant organisms among the microbiota of breast-fed infants, are less prevalent in the gut of formula-fed infants where they compete with higher levels of other bacterial groups (e.g., Bacteroides sp.) (5). Bifidobacteria are considered beneficial commensal bacteria, because they help maintain healthy mucosal surfaces in the GI tract (6) and have the capacity to inhibit pathogenic bacteria, populations of which also differ among breast-fed and formula-fed infants. In a study of over 1000 infants, fecal counts of Clostridium

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2 Diet composition and PCR primers are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
3 Abbreviations used: GI, gastrointestinal; HMO, human milk oligosaccharide; IEL, intraepithelial lymphocyte; MPO, myeloperoxidase; NEC, necrotizing enterocolitis; NB, newborn; PDX, polydextrose; SR, sow-reared.
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difficile were significantly higher for formula-fed infants. C. difficile colonization has been associated with diseases such as NEC due to immaturity of the intestine (7,8). Interestingly, C. difficile also was higher in preterm infants than in term infants (9). Establishing lactic acid bacteria and bifidobacteria commensals early in the life of formula-fed infants may competitively exclude potentially pathogenic bacteria like C. difficile. The presence of fermentable oligosaccharides (e.g., HMO, prebiotics) that are not digested by the host aid in the establishment of commensal bacteria that are able to capture energy from the carbohydrates that would otherwise escape the human digestive process (10).

Approximately 200 molecular species of oligosaccharides have been identified in human milk and are synthesized from D-glucose, D-galactose, D-N-acetylglucosamine, L-fucose, and D-N-acetylneuraminic acid (sialic acid) monomers. In contrast, bovine milk oligosaccharide composition is much simpler, because the 10 molecular species that have been identified consist largely of sialic acid linked with lactose (2,4,11,12). Similarly, only 29 molecular species have been identified among porcine milk oligosaccharides, of which over 50% are sialylated (13). Due to the complexity of HMO and the lack of commercial sources, they are not presently used to supplement infant formulas. Instead, supplementation with alternative prebiotic oligosaccharides is expected to provide some of the functional properties of HMO, such as supporting intestinal commensal bacteria, softening stools, and effects desired intestinal immunomodulation.

Prebiotic supplementation of pediatric nutritional products is associated with increased levels of lactic acid bacteria and bifidobacteria, decreased diarrhea, improved allergy symptoms, and decreased rates of infection in infants and children (14–18). Decreased incidence of disease may be related to changes in immune regulation through cytokine secretion. Although changes to the immune system have been demonstrated, the exact mechanism of immunomodulation remains unknown. Commensal bacteria fed as isolated strains also can have varying effects ranging from antiinflammatory to proinflammatory (19). The mechanisms through which commensal bacteria modulate the host immune system are poorly understood, rendering the prediction of specific immune-related effects challenging, especially for newer prebiotics like PDX.

PDX is an indigestible, selectively fermented carbohydrate that is a candidate prebiotic. First developed as a bulking agent for foods, PDX’s structure of randomly bonded glucose polymer with some sorbitol end groups allows for uses that include both food and prebiotic applications. Though the random structure of PDX contains numerous glycosidic bonds, those in the β (1→6) configuration predominate, rendering the material resistant to mammalian digestive enzymes and allowing it to reach the large intestine, stimulating fermentation by the commensal microbiota. Both the indigestible nature and selective fermentation of PDX support its utilization as a prebiotic, which was recently redefined as “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the GI microbiota that confers benefits upon host well-being and health,” but currently evidence is too sparse to define PDX as a prebiotic (20,21). Our research was designed to investigate the prebiotic nature of PDX by determining changes in intestinal microflora and the health status of neonatal pigs supplemented with various levels of PDX.

The supplementation of PDX with up to 17 g/L of PDX was previously shown to be safe by our laboratory, supporting normal piglet growth and development (22). Research into the efficacy of PDX supplementation is ongoing. The objective of this study was to determine the enteric responses of incremental dietary PDX in infant formula, including effects on the intestinal immune responses using a piglet model.

Methods

Pigs and study design. Full-term pigs were vaginally delivered at the North Carolina State University Swine Education Unit and allowed colostrum for 24 h. Reference SR piglets remained with their respective sow at the Swine Education Unit. After colostrum consumption, treatment pigs were transferred to the Laboratory of Developmental Nutrition at North Carolina State University. A baseline reference group of NB pigs was sampled after colostrum consumption. Treatment pigs were housed in individual pens in an environmentally controlled room (32°C) programmed to deliver a light/dark cycle of 16/8 h, respectively. For the first 24 h, pigs were trained to suckle from bottles using the control diet. Pigs were fed at ~60% ad libitum with fresh diet offered 3 times daily for 18 d to achieve growth rates similar to sow-fed pups (22). The experiment was run in 2 replicates. The first replicate included 6 pigs/treatment and the second included 7, yielding a total of 13 pigs/treatment. All pig procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Diet. Pigs were randomly allotted to 1 of 5 dietary treatments or 2 reference groups (n = 13 pigs/treatment, initial weight 1.71 ± 0.27 kg). A basal diet patterned after term human infant formula was prepared to meet the nutrient requirements of neonatal pigs (49). The ingredients of the dry basal diet amended to provide 1 of 4 levels of PDX (1.7, 4.3, 8.5, and 17.0 g/L) (Supplemental Table 1; Litesse Two, Danisco) (22). The form of PDX had a pH of 3.9 and a reduced citric acid content compared with the original Litesse formulation. The oligosaccharides of porcine milk contain at least 29 distinct molecular species (compared to ~200 identified in human milk) of which 50% are sialylated (13). Although the quantitative content of oligosaccharides in sow milk is unknown, piglets consuming sow milk were considered a positive reference group. Dry ingredients were mixed and reconstituted with water, fat, and the dietary intervention level of PDX and then homogenized and refrigerated. Commercially, PDX and other prebiotics are directly included in both dry and liquid preparations of supplemented infant formulas, so our diets were created to mimic this supplementation pattern at multiple dietary levels. Calculated total dietary fiber for the basal diet was 1.05% and for the PDX diets were: 1.06% (1.7 g/L PDX), 1.08% (4.3 g/L PDX), 1.10% (8.5 g/L PDX), and 1.15% (17.0 g/L PDX). A dietary fat blend, including DHA and arachidonic acid, was added to the dry basal diet at 26% of the diet. Excluding the SR reference group, the mean total energy intakes of the treatment groups did not differ (P = 0.15) and ranged from 365 to 399 ± 11 kcal/kg body weight.

Sampling. Piglets were allowed free access to feed on the day of killing. Piglets were killed using American Veterinary Medical Association-approved electrocution followed by exsanguination. The intestine was then removed. The first meter proximal to the ileocecal junction was removed. The first proximal 0.5 m was rinsed with cold PBS, mucosa collected, immediately flash frozen in liquid nitrogen, and later stored at −80°C. The distal 0.5 m was collected for digesta and histology measurements. At the center section of the distal sample, ~2 cm of tissue was excised and placed in neutral buffered formalin for fixing. The proximal colon and cecum digesta were collected for pH measurement, which was performed at the time of sampling. After pH measurement, a cecal digesta subsample was frozen on dry ice and an additional subsample was collected and placed on ice for bacterial enumeration. Colon digesta was collected only for pH measurement.

Ileal histology. Ileal histology was performed as previously described (23). To enumerate the IEL, 5 well-defined villi were identified. The IEL and epithelia cells were counted on each of these villi.

Ileal mucosal enzyme assays. To determine maturation of the ileum, maltase and lactase activities were assessed in the formula-fed and SR
piglets. Ileal lactase and maltase activities were analyzed based on modifications for a 96-well plate assay (24). To determine neutrophil infiltration in the ileum, MPO activity was determined with modifications for a 96-well plate (25). Substrate concentration was calculated based on the Beer’s-Lambert equation with the molar absorption coefficient of tetramethylbenzidine (Sigma no. 860336) being $3.9 \times 10^{6} \text{ L \cdot mol}^{-1} \cdot \text{cm}^{-1}$. One unit of specific MPO activity was defined as that degrading 1 nmol of tetramethylbenzidine/min. Total protein was determined (26) using a commercially available bicinchoninic acid kit (Pierce no. 23225).

**Cecal SCFA concentration.** SCFA concentrations in cecal digesta from the first replicate were determined using a GC method (27) using a Varian CP 3380/3800 with a NUKOL Fused Silica Capillary Column ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ µm}$).

**Lactic acid concentration.** Lactic acid concentrations in cecal digesta from the second replicate were determined GC (28). Methylated is-lactate was detected on a Hewlett Packard Series II 5890 Gas Chromatograph with HP 6890 Series Injector and an HP Innowax column ($30 \text{ m long with 0.32-mm i.d.}$).

**Cecal bacterial concentration.** Serial dilutions of cecal digesta were plated for lactobacilli and bifidobacteria concentrations on Difco Rogosaagar and Difco Differential Closstridium Agar. Plates were placed in Bio-Bags to generate an anaerobic environment and then incubated for 48 h at 37°C. After 48 h, colonies were counted from 2 plates for each bacteria sampling per pig.

**RNA isolation and real-time RT-PCR.** Total RNA was isolated and treated with DNase I (Qiagen) from ileal mucosal scrapings using a commercially available kit (Qiagen). Purity was assessed by determining the ratio of the absorbance at 260 and 280 and the 18S and 28S ribosomal bands visualized on an agarose gel. Total RNA (1 µg) was reverse-transcribed using a commercially available cDNA synthesis kit (iScript Select, BioRad Laboratories). Real-time PCR detection of mRNA was conducted using the SYBR Green assay. Primer sequences are listed in Supplemental Table 2. Amplification was carried out in a total volume of 25 µL containing 1× iQ SYBR Green Supermix (BioRad Laboratories), forward and reverse primers (400 nmol/L each), and 100 ng of the reverse-transcribed cDNA. The PCR program consisted of an initial 5-min denaturation step at 95°C followed by 39 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s (BioRad Laboratories). At the end of the PCR, melt curve analysis was conducted to validate the specificity of the primers. Both no-template and no-reverse-transcribed-RNA controls were used with every assay and all determinations were performed in at least duplicates. External cDNA standards were constructed by cloning the corresponding RT-PCR product into a PCR TOPO vector (Invitrogen) and the resulting plasmids were sequenced at Operon MWG Biotech. Amplification was carried out in a 96-well plate for each bacteria sampling per pig.

**Digesta pH changes.** Both cecal and colon digesta pH decreased linearly with increasing PDX concentration (Table 2). The digesta pH of piglets fed 0 g/L PDX did not differ from that of the NB pigs but was higher than that of the SR piglets (P < 0.001). Feeding 4.3 g/L PDX or more decreased the pH to the level of the SR reference group. For colon digesta, piglets fed 8.5 and 17 g/L PDX did not have significantly different pH values compared to the SR piglets. As reported in our previous study, ileal digesta pH also declined linearly from 6.63 to 6.47 with increasing PDX ($P_{\text{linear}} = 0.021$) (22). Overall, as PDX increased, the pH of the digesta decreased to that in SR piglets (Table 2).

**Digesta metabolites.** The pH of digesta in the cecum and colon decreased with increasing PDX, suggesting that fermentation of the material by gut microbes formed organic acids as end-products. To determine if the pH changes of digesta were due to bacteria, lactic acid and SCFA were measured in the cecum digesta. Total SCFA did not change linearly with increasing PDX ($P > 0.05$; Table 3). Both propionic ($P < 0.05$) and lactic ($P = 0.001$) acids increased linearly with increasing PDX. Piglets fed 8.5 and 17 g/L PDX had lactic acid concentrations that exceeded 0 g/L PDX-fed pigs by >5-fold and a linear increase of lactic acid with PDX supplementation was observed ($P = 0.02$; $P_{\text{linear}} < 0.01$; SEM = 0.81). The reference SR piglets contained 3.17 µmol/g

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**TABLE 1** Ileal morphology and enzyme activity in piglets fed various concentrations of PDX in formula and in SR piglets

<table>
<thead>
<tr>
<th>Variable</th>
<th>SR</th>
<th>0</th>
<th>1.7</th>
<th>4.3</th>
<th>8.5</th>
<th>17</th>
<th>SEM</th>
<th>$P$</th>
<th>Orthogonal $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Linear</td>
</tr>
<tr>
<td>Maltase, U/g protein</td>
<td>18</td>
<td>21</td>
<td>21</td>
<td>24</td>
<td>22</td>
<td>23</td>
<td>2.29</td>
<td>0.44</td>
<td>0.89</td>
</tr>
<tr>
<td>Lactase, U/g protein</td>
<td>36</td>
<td>28</td>
<td>14</td>
<td>40</td>
<td>19</td>
<td>12</td>
<td>18</td>
<td>0.62</td>
<td>0.47</td>
</tr>
<tr>
<td>Villus height, µm</td>
<td>557</td>
<td>445</td>
<td>457</td>
<td>480</td>
<td>447</td>
<td>483</td>
<td>31</td>
<td>0.10</td>
<td>0.35</td>
</tr>
<tr>
<td>Villus width, µm</td>
<td>155</td>
<td>159</td>
<td>156</td>
<td>149</td>
<td>152</td>
<td>147</td>
<td>6.51</td>
<td>0.71</td>
<td>0.11</td>
</tr>
<tr>
<td>Crypt depth, µm</td>
<td>90a</td>
<td>159b</td>
<td>156b</td>
<td>154b</td>
<td>121c</td>
<td>145d</td>
<td>12</td>
<td>0.001</td>
<td>0.11</td>
</tr>
<tr>
<td>IEL, n/100 enterocytes</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>0.98</td>
<td>0.39</td>
<td>0.88</td>
</tr>
<tr>
<td>MPO, U/g protein</td>
<td>37</td>
<td>46</td>
<td>33</td>
<td>39</td>
<td>47</td>
<td>46</td>
<td>11.3</td>
<td>0.66</td>
<td>0.73</td>
</tr>
</tbody>
</table>

a Values are least-square means, $n = 12–13$. Means in a row with superscripts without a common letter differ, $P < 0.05$. IEL, intraepithelial lymphocyte; MPO, myeloperoxidase; PDX, polydextrose; SR, sow reared.
lactic acid, which was similar to that of the piglets fed 8.5 and 17 g/L PDX. The SR pigs had higher levels of lactic acid compared to the 0 g/L PDX-fed piglets \( (P = 0.036) \) and tended to have a higher concentration than those fed 1.7 or 4.5 g/L PDX \( (P < 0.10) \).

**Cecal bacterial contents.** To further confirm the hypothesis that pH changes were due to lactic acid produced by intestinal bacteria, cecal lactobacillus and bifidobacteria were enumerated (Table 4). Cecal lactobacilli concentrations increased linearly with increasing dietary PDX concentration \( (P = 0.01) \). The number of cecal bifidobacteria did not differ among the groups \( (P = 0.93) \).

**Ileal mRNA expression.** Message abundance of the IgG receptor (FcRn; NM_214197.2) was compared between the 0- and 17-g/L PDX piglets only. The copy number/100 ng cDNA was 5170 and 6510 (SEM = 540) for piglets fed 0 and 17 g/L PDX, respectively. There was a trend for increased FeRn expression with piglets fed 17 g/L PDX compared to 0 g/L PDX fed pigs \( (P = 0.10) \). The inflammatory cytokines TNFα \( (P\text{-quadratic} = 0.09) \), IL-8 \( (P\text{-quadratic} = 0.06) \), and IL-1β \( (P\text{-quadratic} = 0.05) \) had similar trends for expression, with the lowest expression observed in piglets fed 4.3 and 8.5 g/L PDX (Fig. 1). Cytokine expression did not differ between the groups fed 0 and 17 g/L PDX. There were no significant quadratic trends for expression of the proinflammatory cytokines but there were for TNFα and IL-8 \( (P = 0.09, P = 0.06) \). The message abundance of the antiinflammatory cytokine IL-10 also tended to be lower in piglets fed 4.3 and 8.5 g/L PDX than in the 0 g/L PDX-fed pigs \( (P = 0.02) \). Feeding 1.7 and 17 g/L PDX increased the IL-10 message level compared to the 0 g/L PDX-fed pigs \( (P = 0.015) \).

**Discussion**

The purpose of this study was to examine potential prebiotic characteristics of PDX in an infant pig model. The FAO defines a prebiotic “as a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota” \( (30) \). To determine if PDX meets the FAO definition, gut development, the metabolic response of the GI microbiota, and the impact of supplementation on select markers of mucosal immunity were evaluated in suckling swine fed PDX. Given the importance of gut and immune development to healthy infants and the scarcity of validated, noninvasive markers, piglets were selected as an appropriate neonatal model due their anatomical and functional similarities with human infants. Previously, our laboratory observed that formula inclusion levels of PDX up to 17 g/L were safe as determined in our pig model \( (22) \). In the present study, indices of ileal health \( (MPO \text{ activity and IEL numbers}) \) were not affected by formula feeding \( \text{compared to SR pigs} \) or by PDX supplementation, indicating that piglets fed formula had similar ileal health status as the SR reference group. Maturation of the ileum, as indicated by maltase and lactase activity, did not differ among formula-fed and sow-fed groups. Furthermore, villi height and width did not differ. Formula-fed pigs were healthy and maturing properly.

PDX supplementation changed the cecal and colonic environment by increasing lactobacilli, the most likely source of the increased lactic acid and SCFA and decreased luminal pH that was observed \( (31) \). Both cecal and colonic pH decreased linearly with increased PDX \( (P < 0.001) \). Piglets fed 8.5 and 17 g/L PDX had similar cecal and colonic pH as the SR reference group. The decrease in cecal and colonic pH is consistent with the fermentation pattern of PDX in weaned pigs \( (32) \). Decreased luminal pH due to increased organic acid concentration was frequently reported with feeding of nondigestible carbohydrates \( (33–35) \). In this study, the decrease in pH accompanied an increase in total lactobacilli in the cecum and increased lactic acid. *Lactobacillus* strains from porcine mucosa produce high levels of lactic acid relative to other organic acids in the absence of excess fermentable carbohydrates \( (36) \). Our findings demonstrate not only

**Table 2** Ceca pH in piglets fed various concentrations of PDX in formula, in SR piglets, and in 1-d-old piglets \(^1\)**

<table>
<thead>
<tr>
<th>Reference groups</th>
<th>0</th>
<th>1.7</th>
<th>4.3</th>
<th>8.5</th>
<th>17</th>
<th>SEM</th>
<th>Orthogonal P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum</td>
<td>6.04(^{bc})</td>
<td>6.32(^{b})</td>
<td>6.46(^{b})</td>
<td>6.43(^{b})</td>
<td>6.24(^{ab})</td>
<td>6.12(^{d})</td>
<td>5.84(^{b})</td>
</tr>
<tr>
<td>Colon</td>
<td>5.93(^{bc})</td>
<td>6.58(^{b})</td>
<td>6.41(^{b})</td>
<td>6.47(^{b})</td>
<td>6.31(^{bc})</td>
<td>6.13(^{d})</td>
<td>5.85(^{b})</td>
</tr>
</tbody>
</table>

\(^1\) Values are least-square means, \( n = 12–13 \). Means in a row without a common letter differ, \( P < 0.05 \). NB, newborn; PDX, polydextrose; SR, sow reared.

**Table 3** Cecal organic acid concentrations in piglets fed various concentrations of PDX in formula \(^1\)

<table>
<thead>
<tr>
<th>PDX, g/L</th>
<th>0</th>
<th>1.7</th>
<th>4.3</th>
<th>8.5</th>
<th>17</th>
<th>SEM</th>
<th>Orthogonal P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>53.3</td>
<td>56.6</td>
<td>68.6</td>
<td>64.9</td>
<td>58.3</td>
<td>8.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Propionic</td>
<td>11.6</td>
<td>11.6</td>
<td>16.3</td>
<td>15.9</td>
<td>16.6</td>
<td>2.2</td>
<td>0.24</td>
</tr>
<tr>
<td>Isobutyric</td>
<td>1.02</td>
<td>1.02</td>
<td>3.29</td>
<td>0.57</td>
<td>1.82</td>
<td>1.13</td>
<td>0.55</td>
</tr>
<tr>
<td>Butyric</td>
<td>7.38</td>
<td>7.72</td>
<td>7.38</td>
<td>8.63</td>
<td>8.29</td>
<td>1.36</td>
<td>0.93</td>
</tr>
<tr>
<td>Isovaleric</td>
<td>1.17</td>
<td>1.57</td>
<td>1.86</td>
<td>2.15</td>
<td>2.35</td>
<td>1.08</td>
<td>0.94</td>
</tr>
<tr>
<td>Valeric</td>
<td>2.15</td>
<td>1.57</td>
<td>1.57</td>
<td>2.55</td>
<td>2.45</td>
<td>0.69</td>
<td>0.69</td>
</tr>
<tr>
<td>SCFA total</td>
<td>77.4</td>
<td>80.8</td>
<td>97.5</td>
<td>95.1</td>
<td>90.5</td>
<td>11.3</td>
<td>0.65</td>
</tr>
<tr>
<td>Lactic</td>
<td>0.63(^{b})</td>
<td>1.12(^{b})</td>
<td>0.69(^{b})</td>
<td>3.52(^{a})</td>
<td>3.83(^{a})</td>
<td>0.81</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^1\) Values are least-square means, \( n = 6–7 \). Means in a row with superscripts without a common letter differ, \( P < 0.05 \). PDX, polydextrose.
increases in lactic acid but also propionic acid in response to PDX supplementation. Comparable stimulation of lactic acid has not been uniformly observed, particularly in studies involving in vitro models (36,37). Typically, in in vitro studies a substrate like PDX is introduced and acid production measured during 24 h, an interval that may not allow the bacterial community sufficient time to fully respond and metabolize the material. Such studies have shown that the fermentation of PDX takes longer than other less complex prebiotics (36,37). Similar to our results, others have reported decreased luminal pH in response to increased lactic acid in weaning piglets fed a diet supplemented with PDX (32). Our study findings indicate the intestine acclimated to PDX after 18 d of feeding with increased lactobacilli, lactic acid, and SCFA.

Although SCFA have been reported to increase with prebiotic feeding (33,34), the neonatal environment may be more conducive to changes in lactic acid, the main product of homofermentation. This is supported by the observation that breast-fed infants have decreased stool pH due to increased lactic acid, not SCFA (38). In infants, while the intestine is still maturing, increases in SCFA have been implicated in the pathology of NEC (39,40). In a study examining the effect of high levels of organic acids on intestinal injuries in a rat model, lactic acid (at 150 and 300 mmol/L) did not cause gross or microscopic colonic lesions, whereas even the low levels of butyrate (150 mmol/L) and acetic acid (150 mmol/L) did, even when pH was held constant for the treatments (41). In a piglet NEC model, piglets fed lactose had fewer incidences of NEC and significantly higher colonic levels of lactic acid but lower levels of butyric acid (39). In infants with NEC, SCFA cause mucosal injury and, in the case of butyrate, decrease the recovery from injury due to blocking trefoil factor, which is important to the maintenance and repair of the intestinal mucosal barrier function (42). A potential benefit of feeding PDX, compared to other prebiotics such as fructooligosaccharide and inulin, is that it takes longer to achieve maximum rate of gas production and therefore is more slowly fermented with lactic acid being a primary product (37,43). Establishing a lactic acid-producing bacterial population early in life may promote maintenance and repair of the mucosal barrier function. As an example, when 300 mmol/L of butyric acid was administered luminally in rats, trefoil factor expression decreased after 1 h and remained decreased after 24 h (42). In comparison, administration of lactic acid at the same concentration caused a decrease in trefoil factor expression after 1 h, but expression continued to increase at 3, 6, and 24 h until its expression was 90% of that of the control (42). Lactic acid may protect infants from pathogens by decreasing pH without interfering with barrier function while it is undergoing maturation.

In general, ileal cytokine expression tended to decrease with increasing dietary PDX, except at the highest supplementation level (17 g/L) where cytokine expression rebounded. This trend occurred for both proinflammatory and antiinflammatory cytokines. The other tested variables did not follow this pattern and therefore cannot be adequately explained based on the available data. One possibility is that at the highest PDX supplementation level the concentration of Lactobacillus and/or lactic acid reached a threshold value sufficient to induce increased cytokine expression. Prior to reaching that threshold value, lower cytokine expression could result from decreased pathogen load, although this aspect was not specifically evaluated. Lactic acid, the predominant end product of Lactobacillus fermentation, acts as an antimicrobial by inhibiting the growth of pH-sensitive, gram-negative bacteria, which can include pathogenic species such as E. coli O157:H7 (44). Isolated Lactobacillus strains from

### TABLE 4

<table>
<thead>
<tr>
<th>PDX, g/L</th>
<th>Orthogonal P</th>
<th>SEM</th>
<th>P</th>
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</thead>
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<tr>
<td><strong>Linear</strong></td>
<td><strong>Quadratic</strong></td>
<td><strong>Linear</strong></td>
<td><strong>Quadratic</strong></td>
</tr>
<tr>
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<td>4.3</td>
<td>8.5</td>
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<tr>
<td>Lactobacilli</td>
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</tr>
<tr>
<td>Bifidobacteria</td>
<td></td>
<td>72</td>
<td>37</td>
</tr>
</tbody>
</table>

1 Values represent least-square means, n = 12–13. PDX, polydextrose; SR, sow reared.

### FIGURE 1

Effects of formula PDX concentration on TNFα (A), IL-8 (B), IL-1β (C), and IL-10 (D) mRNA expression in the ileal mucosa of piglets fed various concentrations of PDX in formula for 18 d. Values are mean ± SEM, n = 13. Labeled means for a variable without a common letter differ, P < 0.05. PDX, polydextrose.
porcine ileal mucosa have been shown to inhibit a range of pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, and *Clostridium perfringens* (36). Although not evaluated in this study, we hypothesize that at the lower, physiologic levels of PDX supplementation, increases in *Lactobacillus*-associated lactic acid and other antimicrobials were able to competitively exclude pathogenic bacteria, decreasing the need for inflammatory response-driven cytokine production. In young pigs, a lack of overall intestinal microbial diversity, but increased lactobacilli, is associated with decreases in expression of IFN-inducible genes and antigen presentation MHC class I genes [including the chemokines CCL2, CCL8 (IL-8), CCL28, CCR1, and CXCR4] (45). Similarly, in the current study, increased lactobacilli and decreased cytokine expression occurred in response to PDX supplementation, while all piglets remained healthy.

In contrast to the cytokine data, expression of the neonatal FcRn increased in piglets fed 17 g/L PDX compared to the 0 g/L PDX piglets. To our knowledge, changes in FcRn expression when feeding prebiotics have not been previously reported. Albumin and IgG are the 2 ligands for FcRn (46,47). FcRn has several functions, which include: extension of IgG half-life, intestinal delivery of IgG, and translocation of IgG and albumin from one cell compartment to another (46,47). The translocation of FcRn to the intestinal apical membrane and binding of IgG are pH dependent (47,48). Although not fully confirmed by this study, it appears that changes in pH may affect the expression and not just function of FcRn. Further work is needed to confirm this observation.

PDX supplementation in infant formula mimics some of the functional properties of HMO. The results from the current study demonstrate the ability of PDX-supplemented formula to reduce ileal IL-1 expression (P = 0.015), whereas the cytokines TNFα and IL-8 tended to decrease at low levels but returned to levels similar to that of control pigs with high level supplementation (P-quadratic = 0.09; P-quadratic = 0.06, respectively). The reduced cytokine expression in pigs fed low levels of PDX was accompanied by increased cecal lactobacilli and lactic acid concentrations. Lactic acid can inhibit pH-sensitive pathogenic bacteria while not compromising the neonatal intestinal barrier function compared to other organic acids, which may be contributing to this finding (41,42). In conclusion, PDX potentially modulates the intestinal immune system by increasing luminal lactobacilli and lactic acid.

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Literature Cited


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