Effects of pectin on fermentation characteristics, carbohydrate utilization, and microbial community composition in the gastrointestinal tract of weaning pigs

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Scope: We aimed to investigate the effects of three different soluble pectins on the digestion of other consumed carbohydrates, and the consequent alterations of microbiota composition and SCFA levels in the intestine of pigs.

Methods and results: Piglets were fed a low-methyl esterified pectin enriched diet (LMP), a high-methyl esterified pectin enriched diet (HMP), a hydrothermal treated soybean meal enriched diet (aSBM) or a control diet (CONT). LMP significantly decreased the ileal digestibility of starch resulting in more starch fermentation in the proximal colon. In the ileum, low-methyl esterified pectin present was more efficiently fermented by the microbiota than high-methyl esterified pectin present which was mainly fermented by the microbiota in the proximal colon. Treated soybean meal was mainly fermented in the proximal colon and shifted the fermentation of cereal dietary fiber to more distal parts, resulting in high SCFA levels in the mid colon. LMP, HMP, and aSBM decreased the relative abundance of the genus Lactobacillus and increased that of Prevotella in the colon.

Conclusion: The LMP, HMP, and aSBM, differently affected the digestion processes compared to the control diet and shaped the colonic microbiota from a Lactobacillus-dominating flora to a Prevotella-dominating community, with potential health-promoting effects.

Keywords: Autoclave soybean meal / Dietary fiber / Digestibility / Fermentation / Microbiota composition

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1 Introduction

Dietary fibers (DFs) are reported to reduce the risk for obesity, type 2 diabetes mellitus, cardiovascular disease, colon cancer, and to improve immunity by modulating the gut microbiota composition [1]. Understanding the correlations...
between DFs, intestinal microbiota, and bacterial metabolites could lead to an improved control of microbiota composition by DFs intake targeted at health improvement and disease prevention [2].

Pectins are important water-soluble DFs present in the cell walls of fruits, vegetables, and legumes. In addition, they are used as ingredients in composite foods. The most familiar and predominant structural element in pectin is formed by the “smooth” homogalacturonan (HG) regions, composed predominantly of a homopolymer of partially methyl esterified (1-4-linked α-D-galacturonic acid (GalA) units [3]. A second well-characterized element of pectin is “hairy” rhamnogalacturonan I (RG-I). It is composed of the repeating dianhydrohexitol [-2]-α-L-Rhap-(1-4)α-D-GalpA-[-1] as backbone and is decorated with side chains of arabinan, galactan, and arabinoxylan at the O-4 position of the rhamnose residues [3]. Health effects of pectin are dependent on the variations in the chemical composition [3].

Pectins are fermented in the intestine by the gut microbiota. Pectins are beneficial for certain microbiota (e.g. Bacteroides) and contribute to the production of microbial short chain fatty acids (SCFAs) acetate, propionate, and butyrate in the intestine [4], which are assumed to contribute to the health benefits. The effects of pectins on the colonic microbiota are, however, dependent on the structure of the pectins [5, 6]. Citrus pectins with low degree of methyl esterification (DM) are fermented faster in the large intestine of rats and consequently result in a higher production of SCFAs than high DM pectins [7]. Both low and high DM citrus pectins, good fiber substrates for acetate production [8], increased the abundance of Bacteroides, but showed no bifidogenic effect during both in vivo [6] and in vitro fermentation [5]. In contrast, soy pectin with both HG and high arabinogalactan containing RG-I regions had strong bifidogenic and lactogenic effects which are not observed with citrus pectins [5, 9]. Fermentation of soy pectin in vitro results in a relatively high proportion of propionate instead of acetate as observed with citrus pectins [8].

Next to the HG and RG-I composition and DM, the solubility of pectins may play a role in their health effects. Soluble DFs are usually considered to be more fermentable than insoluble ones, and high levels of soluble DFs programmed microbiota in vitro to ferment soluble DFs effectively [10]. Introducing soluble DFs, such as inulin, into the diet was associated with changes in microbiota composition and SCFA production, resulting in protection of mice against increased adiposity [11]. Soluble pectic fibers represent only 27% of the total DFs in soybean meal [12], which have been reported to correlate with visceral fat mass reduction and production of SCFAs, particularly butyrate, in rats (unpublished data). Previous studies revealed that hydrothermal treatment can solubilize DFs from maize [13] and sugar beet pulp [14]. The intake of soluble pectins is usually accompanied by the consumption of other DFs present in the diets. We observed that citrus and soy pectin shift the fermentation site of DFs throughout the colon and consequently change microbiota composition and SCFA pattern in rats [7].

In the present study, we studied the effect of different pectin compositions on fermentation characteristics, carbohydrate utilization, and microbiota composition in the gastrointestinal tract of weaning pigs. Weaning pigs are relevant models for infant development of intestinal and metabolic health [15]. We hypothesized that (i) supplementation of pectin affects not only the fermentation of other DFs in the large intestine, but also the utilization of starch and protein in the small intestine; (ii) the effects of pectin supplementation on utilization patterns of DFs present in the diets are structure dependent. To prove these hypotheses, high-and low-methyl esterified pectins, and a more complex pectin, solubilized from soybean meal by autoclaving, were added to piglet diets. The degradation of pectins and other DFs in the ileum and different parts of the large intestine of weaning piglets was monitored. Next, the consequent effects on microbiota composition and SCFAs were evaluated.

## 2 Materials and methods

### 2.1 Animals and diets

A basic diet, termed CONT was formulated as shown in Supporting Information Table 1. Low-methyl esterified citrus pectin (Classic CU-L 020/13, Herbstreith & Fox, Neuenburg, Germany) and high-methyl esterified citrus pectin (Classic CU-L 021/13, Herbstreith & Fox) were kindly supplied by Prof. Hans-Ulrich Endress and were used to replace 3% (w/w) of the basic diet, respectively. The low-methyl esterified pectin enriched diet and high-methyl esterified pectin enriched diet were denoted LMP and HMP, respectively. Autoclaved SBM (120°C, 60 min, material : water = 30 : 70; DSM biotechnology center, Delft, The Netherlands) was used to replace 7% (w/w) untreated soybean meal present in the diet and the diet was named aSBM. TiO₂ was added to the diets as a marker, to a concentration of 0.25% (w/w).

The protocol of animal use was reviewed and approved by Animal Ethics Committee of Ghent University (no. 522008). Sixteen piglets (Dutch Landrace × Large White) with similar body weight (mean ± SEM, 6.1 ± 0.02 kg) at weaning (age of 3 weeks) were randomly allotted to the four diets with four piglets per diet for 28 days. Piglets were housed ad libitum in metabolic cages. Cages were placed in an environmentally controlled room with ambient temperature maintained at 22°C.

### 2.2 Sample collection and preparation

Diets and fecal samples from each piglet were collected at days 14 (d14) and 28 (d28). At d28 two piglets were randomly selected from each group, anesthetized and euthanized after collection of feces (Fig. 1). Digesta were collected from distal
ileum, proximal colon (pCol), mid colon (mCol), and distal colon (dCol) and immediately stored at −80°C.

Organic acids and microbiota composition were measured in thawed feces and digesta. For all other analyses, samples were freeze-dried and milled using a ball-milling apparatus (MM2000, Retsch, Haan, Germany). Total nonstarch polysaccharides (NSP) in milled diets were analyzed as described elsewhere [16]. The digestibility of nutrients in the feces and digesta was calculated using the TiO₂ concentration of feed, digesta, and feces.

2.3 Chemical analysis

Feces and digesta were analyzed for dry matter by drying to a constant weight at 103°C [17]. Protein content (N × 6.25) was determined using a Thermo Quest NA 2100 Nitrogen analyzer (Interscience, Breda, The Netherlands). Constituent monosaccharide composition of feces, digesta, and diets were measured after acid hydrolysis as described elsewhere [17]. Low molecular weight (LMW) sugars, including fructose, sucrose, raffinose, and stachyose, were analyzed using high performance anion exchange chromatography (HPAEC) [18]. Total starch content was analyzed using the starch kit from Megazyme (Bray, Ireland). SCFAs were analyzed using gas chromatography (GC) with 2-ethylbutyric acid as the internal standard [7]. Lactate and succinate were analyzed using high performance liquid chromatography (HPLC) [7]. TiO₂ contents in feeds, feces, and digesta were analyzed as described elsewhere [19].

2.4 DNA extraction, MiSeq sequencing, and data processing

Total bacterial DNA was extracted from 250 mg of digesta (d28) and feces (d14) of piglets as previously described [7]. For 16S ribosomal RNA (rRNA) gene-based microbial composition profiling, barcoded amplicons from the V1-V2 region of 16S rRNA genes were generated by a 2-step PCR protocol, and sequencing of the amplicons was performed on the Illumina MiSeq platform as described before [7]. Raw Illumina fastq files were demultiplexed, quality filtered, and analyzed using QIIME 1.8.0, as described previously [20], while using the Silva 111 reference database.

2.5 Statistical analysis

The results were analyzed using the Graphpad Prism program (La Jolla, CA, USA). The parametric distribution of data was confirmed using the Kolmogorov–Smirnov test. Results are expressed as mean ± SEM. Statistical comparisons were performed using paired t-test for parametrically distributed data. Where nonparametric distribution could be demonstrated, we applied Mann–Whitney test. p < 0.05 was considered as statistically significant (*p < 0.05, **p < 0.01).

To relate changes in total bacterial community composition to environmental variables, redundancy analysis (RDA) was used as implemented in the CANOCO 5 [21]. Environmental variables were considered to significantly affect microbial composition with values p < 0.05.

3 Results

3.1 Characteristics of diets

All piglets used in this study were healthy throughout the duration of the experiment. The basic diet CONT was mainly composed of starch (44.5%, w/w), protein (17.6%, w/w), NSP (13.8%, w/w), 5.3% (w/w) of fat, and 1.8% (w/w) of LMW-sugars (Table 1). The starch was mainly derived from cereals present in the diets, while the NSP originated from cereals and soybean, which constituted 69% (w/w) and 17% (w/w) in the diet, respectively (Supporting Information Table 1). The constituent monosaccharides in the NSP of CONT were glucose (33 mol%), xylose (29 mol%), arabinose (17 mol%), galactose (9 mol%), uronic acid (7 mol%), and mannose (5 mol%) (Table 1). Compared to CONT, higher molar proportions of uronic acid were detected in LMP (19 mol%) and HMP (20 mol%), since 3% (w/w) of commercial citrus pectins have been added to the diets. Although the molar proportions of constituent monosaccharides were similar between aSBM and CONT, more soluble soy pectin was present in aSBM due to the solubilization of pectin from SBM during hydrothermal treatment (Supporting Information Fig. 1).

3.2 Ileal, colonic, and fecal digestibility of carbohydrates

The apparent digestibilities of dry matter, nutrients, and selected NSP monosaccharides in intestinal digesta are shown
Table 1. Constituent monosaccharide composition of total non-starch polysaccharides (NSP), protein, and starch contents in the experimental diets (g/100 g dry weight)

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>LMP</th>
<th>HMP</th>
<th>aSBM</th>
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<tr>
<td>Fat</td>
<td>5.3</td>
<td>5.2</td>
<td>5.3</td>
<td>5.5</td>
</tr>
<tr>
<td>Protein</td>
<td>17.6</td>
<td>16.7</td>
<td>16.8</td>
<td>18.4</td>
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<tr>
<td>Starch</td>
<td>44.5</td>
<td>42.8</td>
<td>41.9</td>
<td>44.0</td>
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<tr>
<td>LMW sugars(^a)</td>
<td>1.80</td>
<td>1.82</td>
<td>1.78</td>
<td>1.67</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.12</td>
<td>0.15</td>
<td>0.16</td>
<td>0.28</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.68</td>
<td>0.64</td>
<td>0.65</td>
<td>0.52</td>
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<tr>
<td>Raffinose</td>
<td>0.25</td>
<td>0.27</td>
<td>0.24</td>
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<tr>
<td>Stachyose</td>
<td>0.75</td>
<td>0.76</td>
<td>0.73</td>
<td>0.67</td>
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<tr>
<td>NSP</td>
<td>13.8</td>
<td>16.3</td>
<td>16.2</td>
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Molar proportion (mol%)

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<th>HMP</th>
<th>aSBM</th>
</tr>
</thead>
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<tr>
<td>Glucose</td>
<td>33</td>
<td>28</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>Xylose</td>
<td>29</td>
<td>27</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>Arabinose</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Uronic acid</td>
<td>7</td>
<td>19</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Galactose</td>
<td>9</td>
<td>8</td>
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<td>10</td>
</tr>
<tr>
<td>Mannose</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Arabinose/xylose</td>
<td>0.57</td>
<td>0.56</td>
<td>0.58</td>
<td>0.60</td>
</tr>
</tbody>
</table>

\(^a\) LMW: low molecular weight.

in Fig. 2. Dry matter, protein, and starch were digested to a lower degree in all feces at d14 and d28 from pigs fed with pectin supplemented diets compared to controls. Although the diet did not significantly influence the digestibility of NSP, the diet significantly \((p < 0.01)\) affected the digestibility of three constituent monosaccharides of NSP, being glucose, uronic acid, and galactose.

The protein digestibility was lower for LMP- and HMP-fed pigs than for CONT-fed pigs throughout the ileum and large intestine, whereas it was higher for aSBM-fed pigs than for CONT-fed pigs. The digestibility of starch in the ileum was much lower for LMP-fed pigs (89.0%) than for CONT-fed pigs (95.2%), while this difference became smaller in the large intestine. The ileal digestibility of NSP was 21.4% for CONT-fed pigs, and it was 9.6, 17.5, and 12.6% for LMP-fed pigs, HMP-fed pigs, and aSBM-fed pigs, respectively (Fig. 2). In the large intestine, lowest digestibility of NSP was observed for aSBM-fed pigs, followed by those for LMP- and HMP-fed pigs. The ileal digestibility of glucose present in NSP was lower for LMP-fed pigs than for pigs fed with the other three diets. The colonic utilization of glucose, xylose, arabinose, and galactose present in NSP was comparable for
Table 2. Concentration of lactate, succinate and short chain fatty acids (SCFAs), and the proportions of acetate, propionate, butyrate, isobutyrate, and isovalerate in digesta from piglets fed the experimental diets with and without addition of pectin sources

<table>
<thead>
<tr>
<th></th>
<th>Ileum CON</th>
<th>CON</th>
<th>LMP</th>
<th>HMP</th>
<th>aSBM</th>
<th>CON</th>
<th>LMP</th>
<th>HMP</th>
<th>aSBM</th>
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<th>aSBM</th>
<th>CON</th>
<th>LMP</th>
<th>HMP</th>
<th>aSBM</th>
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<tr>
<td>Concentration (µmol/g dry matter)</td>
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<tr>
<td>Lactate</td>
<td>323</td>
<td>131</td>
<td>102</td>
<td>82</td>
<td>6</td>
<td>12</td>
<td>3</td>
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<td>64</td>
<td>12</td>
<td>5</td>
<td>4</td>
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</tr>
<tr>
<td>Succinate</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>10</td>
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<td>10</td>
<td>5</td>
<td>6</td>
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<tr>
<td>Total SCFAs</td>
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<td>60</td>
<td>97</td>
<td>188</td>
<td>1252</td>
<td>1286</td>
<td>913</td>
<td>1074</td>
<td>790</td>
<td>966</td>
<td>1003</td>
<td>915</td>
<td>629</td>
<td>835</td>
<td>627</td>
<td>688</td>
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<tr>
<td>Acetate</td>
<td>130</td>
<td>44</td>
<td>87</td>
<td>157</td>
<td>652</td>
<td>729</td>
<td>492</td>
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<td>559</td>
<td>507</td>
<td>327</td>
<td>418</td>
<td>345</td>
<td>393</td>
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<tr>
<td>Propionate</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>327</td>
<td>290</td>
<td>238</td>
<td>255</td>
<td>179</td>
<td>226</td>
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<td>237</td>
<td>120</td>
<td>198</td>
<td>149</td>
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<tr>
<td>Butyrate</td>
<td>14</td>
<td>1</td>
<td>5</td>
<td>27</td>
<td>242</td>
<td>249</td>
<td>173</td>
<td>162</td>
<td>156</td>
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<td>116</td>
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<td>113</td>
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<td>0</td>
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<td>10</td>
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<td>2</td>
<td>25</td>
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<td>Molar proportions of SCFAs (mol%)</td>
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<td>26</td>
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<td>9</td>
<td>2</td>
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<td>1</td>
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</table>

3.3 Organic acid contents in feces and digesta

Supplementation of soluble pectins increased the lactate content in the feces at d14 (Supporting Information Table 2). In contrast, the concentration of lactate in feces at d28 was decreased for LMP- and HMP-fed pigs compared to CONT-fed pigs. The lactate content in the feces was significantly higher for aSBM-fed pigs than for CONT-fed pigs at d14 and d28. Overall, the SCFA patterns (concentrations and proportions) in the pig feces were not significantly affected by the diet and intervention time.

In the digesta, the highest contents of lactate and lowest contents of total SCFAs were found in the ileum for all groups (Table 2). The content of lactate was lower in the digesta for LMP- and HMP-fed pigs than for CONT-fed pigs at all four sites of the intestine. The aSBM diet decreased lactate concentration in the ileum, but increased lactate concentration along the entire colon (pCol, mCol, and dCol). Lower contents of total SCFAs, acetate, and butyrate in the ileum were observed for LMP- and HMP-fed pigs than for CONT-fed pigs. The concentrations of total SCFAs, acetate, and butyrate in the digesta from all sites of the large intestine were higher for LMP-fed pigs than for CONT-fed pigs. Compared to CONT, HMP resulted in lower concentrations of all individual SCFAs in pCol, but higher concentrations of acetate, propionate and butyrate in mCol. In contrast, aSBM resulted in higher levels of total SCFAs and acetate compared to controls in the colon. Lower contents of branched SCFAs (iso-butyrate and iso-valerate) were observed in the large intestine of LMP-, HMP-, and aSBM-fed pigs than of CONT-fed pigs.

3.4 Microbiota composition

Simpson/Shannon diversity analyses showed that pectin supplementation tended to increase alpha diversity of intestinal microbiota (Supporting Information Fig. 2), although the effects were not significant. To assess the impact of different pectins on the microbiota composition, we performed nonparametric multidimensional scaling (NMDS) based on Bray-Curtis distances calculated from genus-level (Supporting Information Fig. 3). The fecal samples from LMP-fed pigs at d14 clustered distinctly from those in CONT-, HMP-, and aSBM-fed pigs. No segregation between fecal samples from HMP-, aSBM-, and CONT-fed pigs was observed. For the digesta samples collected at d28, all ileal samples significantly clustered separately from colonic digesta. The colonic samples from LMP- and HMP-fed pigs were separated from those from CONT- and aSBM-fed pigs. The ileal samples from aSBM-fed pigs clustered separately from those of LMP-, HMP-, and CONT-fed pigs.

OTU assignment of all sequences retained after quality filtering showed that ten phyla were detected across all treatment groups. In the CONT groups, the most abundant phylum detected in feces, ileal, and colonic digesta was Firmicutes, followed by Bacteroidetes, phyla Verrucomicrobia, and Proteobacteria. The pectin supplemented diets led to a higher relative abundance of Bacteroidetes, whereas that of Firmicutes was lower in the colon and feces, but not in the ileum (Supporting Information Fig. 4).

Data analysis on genus level showed a total of 47 genera were detected. This number was higher in soluble pectin enriched diet-fed pigs than in CONT-fed pigs. In the fecal samples collected at d14, pectin enriched diets exhibited a...
Figure 3. Differential modulation of microbiota composition by pectins. Bacterial community at the genus level in feces at day 14 (A) and in digesta at day 28 (B). Correlation triplots based on a redundancy analysis (RDA) depicting the relationship between microbiota composition in feces at day 14 and colonic digesta at day 28 and the differences induced by pectins (C); These variables explain 55.5% of total variation, and 67.8% of that variation was explained by the first two canonical axes.

decrease in the relative abundance of the genus Lactobacillus and an increase in the relative abundance of Prevotella and an unclassified genus assigned to the family Ruminococcaceae (Fig. 3A, Supporting Information Table 3). With respect to these predominant taxa, the biggest change was observed for LMP-fed pigs. The relative abundance of unclassified microbes in the family S24-7 decreased in the feces of pigs fed with pectin supplemented diets for 2 weeks. The relative abundance of Megasphaera increased when animals received HMP and aSBM for 14 days. The LMP- and HMP-fed pigs had a higher relative abundance of unclassified microbes within the family Lachnospiraceae in their feces, while the aSBM tended to lead to a decreased relative abundance of this group.

We measured the relative abundance of different genera in different parts of the intestine at d28. Pectin supplementation made only a small impact on the microbiota composition in ileal digesta for LMP- and HMP-fed pigs, but had a strong effect on the ileal microbiota composition for aSBM-fed pigs, as compared to the CONT group (Fig. 3B). LMP and HMP decreased the relative abundance of the genus Lactobacillus and increased that of Prevotella in the colon of pigs. Similar changes in Lactobacillus and Prevotella were observed for aSBM-fed pigs, although the changes were not as big as in the LMP- and HMP-fed pigs. The increase in the relative abundance of genus Dialister in the colonic digesta was specific for the LMP-fed pigs, but was not observed for the HMP- and aSBM-fed pigs. The increase in relative abundance of unclassified microbes within the family Prevotellaceae occurred in the colon of the LMP- and HMP-fed pigs, but not for the aSBM group.

4 Discussion

4.1 Effects of soluble pectins on digestion processes in small intestine

The ileal digestibility of starch was significantly decreased by the presence of low-methyl esterified pectin in LMP. The soluble DFs, particularly pectin could have increased the viscosity of digesta in the upper part of small intestine and thereby hinder the access of digestive enzymes to protein and starch [22]. Starch and protein escaping from digestion due to the
presence of the soluble DFs end up in the ileum and large intestine, together with DFs.

A partial utilization of soluble pectins was found in the ileum of pigs. In agreement to our data, fermentation of DFs has been reported in the ileum of pigs fed with diets based on cereals and soybean meal [23]. Low-methyl esterified pectin was fermented more efficiently than high-methyl esterified pectin in the ileum of pigs. Similar results were observed in the cecum of rats fed with the citrus pectin enriched diets [7]. Although the supplementation of pectin in the diets was only 3% (w/w), this addition represented a 20% increase in NSP content. After passing through the small intestine, the major part of starch and protein are digested and absorbed. The presence of supplemented pectins involved in fermentation slightly modulated the microbiota towards more pectin fermentation in the ileum. The changes of microbial community resulted in lower concentrations of SCFAs and lactate in the ileum of LMP- and HMP-fed pigs compared to CONT-fed pigs. In contrast, the microbiota composition in the ileum of aSBM-fed pigs was influenced more profoundly. This could be attributed to the heterogeneous structure of soluble soy pectin available for different populations within microbiota.

4.2 Effects of soluble pectins on fermentation patterns in the large intestine

Pectin delayed the digestion of DFs to more distal parts of the intestine as illustrated by the fact that the total DFs in pectin supplemented groups were fermented more slowly, and the fermentation site of DFs was shifted to more distal regions of colon where fermentation was increased compared to DFs of the control. This competitive response could take place within microbial populations being able to use multiple substrates, or between different species that can only utilize certain types of DFs, present within a DF mixture [24]. In the proximal colon of LMP-fed pigs, on average 6% of the starch ingested was fermented, which is much higher than that for CONT-fed pigs (2%) and accounted for 30% of the total carbohydrates fermented in this section of the large intestine (Fig. 4). The proximal colonic microbiota adapted to the starch enriched substrates and consequently delayed the fermentation of cereal DFs (e.g. arabinoxylans) in LMP-fed pigs (Fig. 2). Resistant starch has been reported to be preferably utilized above other cereal DFs by the microbiota in the large intestine of pigs [16]. Although the fermentation of cereal DFs in the proximal colon of LMP-fed pigs

Figure 4. The proportions and contents (g/kg diet intake) of digested carbohydrates in different parts of the intestine of pigs fed the experimental diets with and without addition of pectin sources. Supplemented citrus pectins were not included in the NSP.
was delayed, the fermentation of starch contributed to higher levels of SCFAs. Because the high-methyl esterified pectin was less efficiently fermented by the microbiota than the low-methyl esterified pectin in the ileum of pigs, higher amounts of high-methyl esterified pectin were available for microbial fermentation in the proximal colon of HMP-fed pigs. The microbiota in the proximal colon of HMP-fed pigs adapted to the high-methyl esterified pectin enriched substrate. As a result, the fermentation of cereal DFs present in HMP was shifted from the proximal colon to more distal regions, and the microbially produced SCFA levels were lower in the proximal colon of HMP-fed pigs compared to CONT-fed pigs. Similar effects of pectin on the fermentation site of cereal arabinoxylan have been found in the large intestine of rats [7]. Although low-methyl esterified pectin is reported to be more easily degradable than high-methyl esterified pectin by the microbial enzymes [6], the net digestibility of low-methyl esterified pectin was lower than that of high-methyl esterified pectin in the proximal colon (Fig. 4). This could be explained by the utilization of a high amount of starch in the proximal colon of pigs, which delayed the fermentation of low-methyl esterified pectin. Consequently, 10% and 4% of the low-methyl esterified pectin ingested was fermented in the mid colon and distal colon, respectively. In contrast, due to the adaptation of microbiota to the available substrate and the consequent changes of microbial community, high-methyl esterified pectin was efficiently utilized in the proximal colon, resulting in the absence of high-methyl esterified pectin in the mid colon and distal colon (Fig. 4). As a result, the microbiota present utilized the resistant starch from the HMP diet. For the same reasoning, the fermentation of soluble soy pectin in aSBM was also shifted to more distal parts of the colon by the resistant starch. Due to the higher levels of DFs in the distal parts of colon (Fig. 4), the microbiota composition was modulated to favor the degradation of DFs rather than protein. Consequently, branched SCFAs (iso-butyrate and iso-valerate) levels were lower in the groups with pectin supplementation than in the control group, which has been suggested to benefit colon health [25].

4.3 Microbial composition

In the present study, Bacteroidetes and Firmicutes were the predominant phyla, regardless of the sample origin and diet provided, representing approximately 95% of the total sequences (Supporting Information Fig. 2). The predominance of Bacteroidetes and Firmicutes has been previously reported in rats [7], pigs [26], and humans [27]. We found that supplementation of pectins resulted in a higher relative abundance of member of the Bacteroidetes in colonic digesta and feces. This effect may be of particular interest, as a previous study has shown that a Bacteroidetes-rich microbiota correlated with a reduced risk of obesity in humans [28].

The relative abundances of genera *Prevotella* and *Dialister* in the large intestine were significantly increased after supplement of low-methyl esterified pectin. This increase in relative abundance of these two genera had been previously reported in pigs fed with high resistant starch diets [29]. In the same study it was shown that the relative abundance of the genus *Lactobacillus* being predominant in controls, was significantly decreased by the presence of resistant starch [29].

We observed a similar shift in relative abundance of genera *Lactobacillus* to *Prevotella* in the large intestine of HMP and aSBM-fed pigs, although the amount of utilized starch by microbiota was much lower for HMP- and aSBM-fed pigs than for LMP-fed pigs. *Prevotella* plays a role in enhancement of glucose metabolism, potentially by promoting increased glycogen storage [30]. The increased level of *Prevotella* is associated with consumption of a dietary fiber-rich diet [31, 32], which has been linked to vegetarianism in Western populations [31]. The association with a plant-rich diet has suggested that *Prevotella* is a beneficial microbe. The relative abundance of *Megasphaera*, which has been reported to feed on organic acids particularly lactate [33], was significantly increased by HMP and aSBM. We did not detect any *Bifidobacterium* in digesta from pigs, in line with previous reports [34]. In contrast to the human intestinal tract, the relative amount of *Bifidobacterium* present in the intestine of pigs is much lower, amounting to less than 1% of total bacteria or being even undetectable [34].

In addition, high-methyl esterified pectin, and arabinan and galactan present in soy pectin could also significantly increase *Prevotella* species as reported before [5]. Nonetheless, *Prevotella* is still one of the predominant genera in the more distal parts of the large intestine where most of the resistant starch and pectin have already disappeared, thereby leaving cereal DFs as the main substrate for the microbiota. Cereal xylans and cellulose have been suggested to be the substrates for *Prevotella* growth in humans and ruminants [35]. In contrast, in the large intestine of CONT-fed pigs, where arabinoxylans were the main substrates for the microbiota, the relative abundance of *Prevotella* was quite low, suggesting that *Prevotella* preferably utilizes pectin and/or resistant starch.

4.4 Link between microbiota composition, SCFAs, and fiber digestibility

To relate changes in microbiota composition to the different diets, the SCFAs and the digestibility of carbohydrates, the relative abundance of colonic microbiota at genus level was subjected to multivariate redundancy analysis (RDA) (Fig. 3C). Fecal samples collected at d14 were clearly clustered separately from colonic digesta collected at d28 for LMP-, HMP-, and aSBM-fed pigs. In addition, the 2 weeks pectin intervention altered the fermentation patterns in fecal samples, although the RDA analysis did not show very clear separation between the four groups at d14. The supplemented soluble pectins have quick influence on the microbiota metabolism of DFs rather than changing the microbial
community rapidly. Nonetheless, after longer-time intervention (d28), colonic digesta from LMP- and HMP- and aSBM-fed pigs clustered separately from the samples obtained from CONT-fed pigs. This might be explained by the molar proportion of butyrate and digestibility of carbohydrates, which showed to have a significant impact (Monte Carlo Permutation test, $p < 0.05$) on the observed variation in microbiota composition in the three groups with soluble pectin supplementation. In the plot, *Prevotella*, *Dialister*, and unclassified microbes in the family Ruminococcaceae correlated with the LMP diet. In contrast, the CONT diet strongly correlated with the relative abundance of the genus *Lactobacillus* and the proportion of acetate. *Prevotella* in the colonic digesta of LMP- and HMP-fed pigs correlated with the production of butyrate and propionate. The digestibilities of NSP and the individual neutral monosaccharides correlated with the production of acetate and the relative abundance of the genus *Lactobacillus*. In contrast, the digestibility of uronic acid correlated with the production of butyrate and genera *Prevotella*, *Dialister*, and unclassified microbes in the family Ruminococcaceae. Our findings support the hypothesis that the consumption of citrus pectin and soluble soy pectin could shape the microbiota community to a healthier pattern. As a result, it could play a role in health, feed conversion efficiency of farm animals, and improved body weight management in humans.

In conclusion, we have demonstrated that the supplementation of citrus pectins and the heat-treated, soluble soy pectin shifted the fermentation site of the DFs to more distal parts of the pig large intestine. These soluble pectins shape the colonic microbiota from a *Lactobacillus* dominated microbiota to *Prevotella*-dominating communities, depending on the actual pectin structure and origin. This study provides strong supports for future human studies using pectin supplementation to counteract the consequences of a typical unhealthy Western diet.

All authors participated in the design of the study. L.T. analyzed samples, performed data analysis, and interpretation, and wrote the manuscript. H.Sc. and H.G. contributed to data interpretation and the manuscript. G.B. carried out the pig experiment, contributed to data interpretation and the manuscript. M.B. pre-treated the soybean meal, contributed to data interpretation and the manuscript. K.B. and H.Sm. analyzed the microbiota composition, contributed to data interpretation and the manuscript. A.S., E.B. and P.V. contributed to discussion on results and the manuscript.

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5 References


