Kiwiﬁruit ﬁbre level inﬂuences the predicted production and absorption of SCFA in the hindgut of growing pigs using a combined in vivo–in vitro digestion methodology

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Abstract

Combined in vivo (ileal cannulated pig) and in vitro (faecal inoculum-based fermentation) digestion methodologies were used to predict the production and absorption of SCFA in the hindgut of growing pigs. Ileal and faecal samples were collected from animals (n 7) fed diets containing either 25 or 50 g/kg DM of kiwiﬁruit ﬁbre from added kiwiﬁruit for 14 d. Ileal and faecal SCFA concentrations normalised for food DM intake (DMI) and nutrient digestibility were determined. Ileal digesta were collected and fermented for 38 h using a fresh pig faecal inoculum to predict SCFA production. The predicted hindgut SCFA production along with the determined ileal and faecal SCFA were then used to predict SCFA absorption in the hindgut and total tract organic matter digestibility. The determined ileal and faecal SCFA concentrations (e.g. 8·5 and 4·4 mmol/kg DMI, respectively, for acetic acid for the low-ﬁbre diet) represented only 0·2–3·2 % of the predicted hindgut SCFA production (e.g. 270 mmol/kg DMI for acetic acid). Predicted production and absorption of acetic, butyric and propionic acids were the highest for the high-ﬁbre diet (P < 0·05), but these inter-diet differences were not observed for the ileal and faecal SCFA concentrations (P > 0·05). In conclusion, determined ileal and faecal SCFA concentrations represent only a small fraction of total SCFA production, and may therefore be misleading in relation to the effect of diets on SCFA production and absorption. Considerable quantities of SCFA are produced and absorbed in the hindgut of the pig by the fermentation of kiwiﬁruit.

Key words: In vivo digestion: In vitro fermentation: SCFA: Absorption: Growing pigs: Kiwiﬁruit dietary ﬁbre

Food not only undergoes digestion by the mammalian gastrointestinal tract (GIT) digestive enzymes but can also be subjected to fermentation by the resident microbial population, and both dietary and endogenous (derived from the body) organic materials can be fermented(1–3). The degree of fermentation depends on the diet and the region of the GIT, with greater fermentation occurring in the hindgut compared with the small intestine(4–6). The main end products of microbial fermentation in the GIT are the SCFA. SCFA have been associated with a number of beneﬁcial effects in the GIT, including intestinal tissue proliferation, enhanced absorption of minerals and water, modulation of GIT contractility, increased numbers of beneﬁcial bacteria and reduced numbers of pathogenic bacteria. In addition, SCFA are the main energy source for epithelial cells(1,5–8). Given the importance of SCFA, information about their production and absorption in the hindgut is a key consideration.

A number of studies in both humans and farm animals have determined SCFA concentrations in different regions of the GIT and also in the faeces, and these have been used to draw conclusions regarding the production of SCFA in the hindgut(2,9,10). The latter approach has limitations, however, as SCFA concentrations are not a measure of SCFA production but rather are the net result of SCFA production and absorption(11–13). Seemingly, only approximately 5 % of the SCFA produced in the hindgut are excreted in the faeces(7,13). The appearance of SCFA in the portal vein and in exhaled breath have also been used(12–16) as measures related to production. However, these methods do not account for the utilisation of SCFA within the intestinal epithelium (portal blood method) or the body in general (exhaled breath). Another approach is to collect ileal digesta for a given diet and use this as a substrate for in vitro fermentation with a faecal inoculum (to simulate the action of the microbes in the hindgut)(17–21) in order to obtain an estimate of SCFA production based on the amount of DM entering the large intestine and the measure of fermentability. The latter approach has been used to estimate the available energy content of selected foods for humans(21). Several groups have also used this approach to estimate the SCFA production in the hindgut(17,18,22,23), but very few studies(18,22) have determined SCFA absorption.

The overall aim of this study was to use a combined in vitro–in vitro digestion methodology(17,18,21,22) to predict both the

Abbreviations: DMI, DM intake; GIT, gastrointestinal tract; OM, organic matter.

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hindgut production and the absorption of SCFA in growing pigs (an animal model for the digestion of food in humans) fed diets containing kiwifruit as the only source of dietary fibre. Kiwifruit was selected as it is a well-characterised source of dietary fibre for humans, containing pectins, hemicelluloses and cellulose. In contrast to previous studies, the hindgut absorption of SCFA also includes the SCFA present in terminal ileal digesta, thereby taking into account the SCFA entering the hindgut as a result of fermentation in the small intestine.

Methods

Dietary treatments

Two diets containing 133 and 266 g of green kiwifruit (Actinidia deliciosa cv. Hayward) DM/kg diet DM, as the only dietary fibre source (25 or 50 g fibre/kg DM, respectively), were formulated to meet the nutrient requirements of growing pig as prescribed by the National Research Council (25 or 50 g DM/kg of metabolic body weight (BW)). The daily ration was calculated as 90 g of DM/kg of metabolic body weight per d and was fed to the pigs as two equal meals provided at 09.00 and 16.00 hours. Titanium dioxide was included as an indigestible marker in the diets. Kiwifruit were added to the semi-synthetic experimental diets and were freshly peeled and crushed just before each meal. Fresh water was provided ad libitum.

<table>
<thead>
<tr>
<th>Table 1. Ingredients and determined nutrient compositions of the experimental diets</th>
<th>Diet fibre concentration (g/kg)</th>
<th>25</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient (g/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiwifruit DM†</td>
<td>133</td>
<td>266</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>157</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>70</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3</td>
<td>3</td>
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</tr>
<tr>
<td>Calcium carbonate</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Wheat starch</td>
<td>559</td>
<td>506</td>
<td></td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nutrient (g/kg DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude lipid</td>
<td>171</td>
<td>162</td>
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</tr>
<tr>
<td>Total lipid</td>
<td>57</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>33</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Soluble dietary fibre</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Insoluble dietary fibre</td>
<td>21</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>28</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Gross energy (MJ/kg DM)</td>
<td>18.5</td>
<td>18.8</td>
<td></td>
</tr>
</tbody>
</table>

† Freshly peeled and crushed kiwifruit was added to the diets just before feeding.

In vivo assay

Animals and housing. Ethics approval for the animal trial was obtained from the Animal Ethics Committee, Massey University. A total of fourteen entire male pigs (PIC Camborough 46 × PICboar 356L) (41.4 (sd 2.98) kg mean body weight) were housed individually in pens (1.5 × 1.5 m) in a room maintained at 21 (sd 2) °C with a 10 h light–14 h dark cycle. Pigs were surgically modified by the implantation of a simple T cannula at the terminal ileum as previously described.

Experimental design. Pigs were randomly allocated to the two kiwifruit-containing diets (seven pigs/diet), which were fed to the animals for 16 d. Faecal samples were collected directly on the 14th and 15th d following anal stimulation, and were immediately frozen at −20 °C for determining total tract digestibility of organic matter (OM) and at −80 °C for determining faecal SCFA concentrations. Ileal digesta were collected into a plastic bag attached to the cannula. Digesta were collected from each pig on the 15th and 16th day and over a 6-h period each day starting from 1 h after feeding. Plastic bags were changed every 30 min and the digesta were frozen immediately to minimise further digestion. From the first batch of fresh digesta, a subsample was collected into a sealed Eppendorf tube for SCFA determination in order to reduce the risk of volatilisation. The ileal digesta samples were used to determine the apparent ileal digestibility of OM, ileal SCFA concentrations and also to provide a substrate for the in vitro fermentation assay. The faecal samples were collected before collecting the ileal digesta samples in order to avoid interference with the hindgut flow of DM.

In vitro fermentation assay

Fermentation of the ileal digesta was carried out using a pig faecal inoculum as previously described. Fresh faeces samples were collected from five healthy pigs (fed a commercial grower diet) into isolated containers flushed with CO2 at 37 °C. In brief, the pig faecal inoculum was prepared by homogenising the faeces with 0.1 M-phosphate buffer at pH 7 (1:5, w/v) and filtering the homogenate. Inoculum (5 ml) was added to a McCartney bottle containing 5 ml phosphate buffer either alone (blank incubation) or containing 100 mg of finely ground freeze-dried ileal digesta sample collected from the pigs fed the experimental diet. There were four replicate bottles per blank or ileal digesta sample; one bottle was used to determine the SCFA after fermentation and the other three bottles were used to determine the OM fermentability. All the bottles were flushed with CO2, capped and incubated in the pig inoculum for 38 h at 37 °C. After incubation, the SCFA concentrations in the material of one of the replicate bottles were determined after thoroughly mixing the contents of the bottle and transferring an aliquot (1 ml) to an Eppendorf tube, which was then centrifuged at 14 000 rpm for 15 min at 4 °C. The supernatant (500 μl) was transferred to another Eppendorf tube and frozen at −20 °C before analysis for SCFA. The other three bottles were placed in an autoclave (121 °C for 20 min) to completely inactivate the bacteria and to remove the end products of OM.
fermentation (e.g. SCFA). The DM of the unfermented residue was determined in the remaining three bottles by drying them in an oven at 60°C until they reached a constant weight.

Chemical analysis

The diets, ileal digesta and faeces were analysed in duplicate for DM, OM, ash and titanium dioxide. DM, OM and ash were determined using standard procedures, and titanium dioxide was determined according to the method of Short et al. The diets were also analysed for crude protein, gross energy, diethyl ether extract and soluble, insoluble and total dietary fibre contents. The dried contents of the in vitro fermentation media from the three bottles were analysed for OM.

SCFAs were determined in the faecal and ileal digesta samples and in the supernatants obtained from the in vitro fermentation. Thawed faecal samples were prepared for analysis by mixing with Milli-Q water (Millipore) (4°C) (1:3, w/v). Thawed ileal digesta and the prepared faecal samples were then centrifuged at 14 000 g for 15 min at 4°C. The supernatants were collected and analysed in duplicate for acetic, butyric, propionic and valeric acids, using a Shimadzu GC2010 Gas Chromatograph System fitted with a Zebron ZB-FFAP column (30 m × 0.32 mm) (Phenomenex) as described previously.

Calculations and statistical analysis

In vivo assay. The apparent ileal and faecal digestibilities and the hindgut fermentability were calculated as follows:

Apparent digestibility (%) = \(1 - \left(\frac{(OM_{f-I}/OM_{b}) \times (TD/T_{f-I})}{100}\right)\)

Hindgut fermentability in vitro (%) = \(1 - \left(\frac{(OM_{f}/OM_{b}) \times (TI/T_{f})}{100}\right)\)

where TD and TI are the titanium dioxide contents (g/kg DM) in the diet and in the faeces or ileal digesta, respectively; OMb and OMf are the contents of OM (g/kg DM) in the diet and in the faeces or ileal digesta, respectively.

The concentrations of SCFA in the terminal ileal digesta and in the faeces (normalised for the food DM intake (DMI)) were calculated using the following equation:

Normalised SCFA concentration (mmol/kg DMI) = SCFA concentration (mmol/kg DM) \(\times\) \(\left(\frac{TD}{TI}\right)\).

In vitro fermentation assay. Predicted hindgut fermentability of OM and SCFA produced by fermentation were obtained after in vitro fermentation of the ileal digesta with the faecal inoculum and calculated using the following equations:

Hindgut fermentability in vitro (%) = \(\left(\frac{OM_{b} - (OM_{f} - (OM_{b}\text{ blank initial} + OM_{b}\text{ blank final}) / 2))}{OM_{b} \times 100}\right)\)

SCFA produced by fermentation (mmol/kg DM incubated)

\(=\) \(\left(\frac{SCFA_{\text{sample}} - \left((SCFA_{\text{blank initial}} + SCFA_{\text{blank final}}) / 2\right))}{\text{sample weight (g DM)} \times 1000}\right)\)

where OMb and OMf are the OM (mg) of the ileal digesta either before or after in vitro fermentation. OMblank initial, OMblank final, SCFAbank initial and SCFAbank final are the OM (mg) and the SCFA (mmol) in the blank bottle (which contained inoculum but no ileal digesta) before (initial) and after (final) in vitro fermentation, respectively.

Predicted total tract digestibility, SCFA production and absorption in the hindgut. The predicted apparent total tract digestibility (PADf\text{inal}) of OM and the SCFA production and absorption in the hindgut were calculated based on combining results for in vivo ileal digesta flows (ileal cannulated pig) with in vitro concentrations (hindgut fermentation). The in vivo values represented digestion in the upper gut, and the in vitro data represented fermentation in the hindgut. PADf\text{inal} of OM and predicted hindgut SCFA production were calculated as follows:

\(\text{PADf}_{\text{inal}}(\%) = \left(\frac{OM_{p} - (\left((100 - \text{Hindgut OM fermentability}_{\text{in vitro}})/100\right) \times \text{ileal OM flow})}{OM_{p} \times 100}\right)\)

Predicted hindgut SCFA production (mmol/kg DMI)

\(=\) SCFA produced by fermentation (mmol/kg ileal digesta DM incubated) \(\times\) ileal DM flow (kg DM/kg DMI),

where OMp (g/kg DM) is the OM content in the diet, and ileal OM flow (g/kg DMI) is the ileal flow of OM. Hindgut OM fermentability in vitro (%) was determined using the in vitro fermentation assay.

The amounts of SCFA entering the hindgut (i.e. ileal normalised SCFA concentration) and the amounts produced in the hindgut (i.e. in vitro predicted hindgut SCFA production) were used to predict the amounts of SCFA absorbed in the hindgut based on the following equation:

Amount of SCFA absorbed from hindgut (mmol/kg DMI)

\(=\) in vitro predicted hindgut SCFA production (mmol/kg DMI)

+ ileal SCFA concentration (mmol/kg DMI)

– faecal SCFA concentration (mmol/kg DMI),

Extent of SCFA absorbed in the hindgut (%)

\(=\) \(\left(\frac{\text{amount of SCFA absorbed from the hindgut (mmol/kg DM intake)} / \text{in vitro predicted hindgut SCFA production (mmol/kg DMI)}}{\text{+ ileal SCFA concentration (mmol/kg DMI)}}\right)\) \times 100.

Statistical analyses were performed using SAS (version 9.3, 2011; SAS Institute Inc.). A two-independent samples t test procedure was performed to test the effect of dietary kiwifruit fibre (25 and 50 g/kg DM) on both the in vivo and in vitro data.
The same procedure was used to compare the determined and predicted digestibilities and fermentabilities for each kiwifruit diet. A paired t test procedure was used to compare the ileal and faecal SCFA concentrations. The normal distribution for the t test was evaluated using the ODS graphics procedure of SAS. When the variances were unequal, the P value reported was obtained using the Satterthwaite separate variance t test.

Results

The diet containing the highest concentration of kiwifruit fibre had a 1.7-fold greater content of total dietary fibre than the lower-fibre diet (Table 1). This difference, however, was reduced to only 1.2-fold in the ileal digesta (345 and 399 g/kg DM ileal digesta for pigs fed diets containing 25 and 50 g fibre/kg DM, respectively).

Determined ileal and faecal concentrations of SCFA (in vivo)

There was no difference (P > 0.05) for either the ileal or the faecal concentrations of the SCFA between the two fibre-containing diets (Table 2). Similarly, there was no difference between the ileal and faecal SCFA concentrations within each diet (P > 0.05), with the exception of acetic acid for the diet containing 50 g/kg of fibre. For this diet, the ileal concentration of acetic acid was 2.54-fold higher than the faecal concentration (P < 0.05).

Table 2. Ileal and faecal concentrations of SCFA for ileal cannulated pigs fed diets containing different concentrations of fibre (Mean values with their pooled standard errors; n 7 per group)

<table>
<thead>
<tr>
<th>Diet fibre concentration (g/kg DM)</th>
<th>25</th>
<th>50</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determined ileal SCFA concentrations (mmol/kg DM intake) (in vivo)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>8.51</td>
<td>14.68</td>
<td>3.120</td>
<td>0.097</td>
</tr>
<tr>
<td>Butyric</td>
<td>1.09</td>
<td>1.11</td>
<td>0.428</td>
<td>0.956</td>
</tr>
<tr>
<td>Propionic</td>
<td>2.07</td>
<td>2.40</td>
<td>0.943</td>
<td>0.730</td>
</tr>
<tr>
<td>Valeric</td>
<td>0.08</td>
<td>0.17</td>
<td>0.063</td>
<td>0.195</td>
</tr>
<tr>
<td>Determined faecal SCFA concentrations (mmol/kg DM intake) (in vivo)†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td>4.35</td>
<td>5.77</td>
<td>1.735</td>
<td>0.431</td>
</tr>
<tr>
<td>Butyric</td>
<td>0.58</td>
<td>0.79</td>
<td>0.370</td>
<td>0.586</td>
</tr>
<tr>
<td>Propionic</td>
<td>0.83</td>
<td>1.36</td>
<td>0.590</td>
<td>0.392</td>
</tr>
<tr>
<td>Valeric</td>
<td>0.21</td>
<td>0.29</td>
<td>0.127</td>
<td>0.545</td>
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<tr>
<td>Statistical analysis for ileal v. faecal SCFA concentrations</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Acetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.061</td>
<td>0.015</td>
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<td></td>
</tr>
<tr>
<td>SEM</td>
<td>1.809</td>
<td>2.652</td>
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</tr>
<tr>
<td>Butyric</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td>0.535</td>
<td>0.346</td>
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<tr>
<td>SEM</td>
<td>0.742</td>
<td>0.738</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionic</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td>0.981</td>
<td>0.729</td>
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<tr>
<td>SEM</td>
<td>0.617</td>
<td>0.359</td>
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</tr>
<tr>
<td>Valeric</td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td>0.112</td>
<td>0.109</td>
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</tr>
<tr>
<td>SEM</td>
<td>0.168</td>
<td>0.089</td>
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</tbody>
</table>

* The ileal concentrations of SCFA were determined directly from the frozen samples of ileal digesta collected from the pigs.
† Faecal concentrations were determined directly from the frozen samples of faeces collected from the pigs.

Production of SCFA after in vitro fermentation and predicted hindgut SCFA production (in vivo–in vitro)

The SCFA produced by in vitro fermentation were similar for all the SCFA between the two fibre-containing diets (P > 0.05; Table 3). In contrast, the predicted hindgut production of acetic, butyric and propionic acids was higher (P < 0.05) for the diet containing 50 g/kg of fibre compared with the diet containing 25 g/kg of fibre (1.7-, 1.9- and 1.5-fold higher, respectively; Table 3). There was no difference (P > 0.05) between diets in the case of valeric acid.

Predicted hindgut SCFA absorption (in vivo–in vitro)

The predicted amounts of acetic, butyric and propionic acids absorbed from the hindgut were higher for the diet containing the highest fibre concentration (1.8-, 1.9- and 1.5-fold higher, respectively; P < 0.05; Table 3). There was no difference (P > 0.05) between diets for valeric acid. The predicted apparent absorption of the SCFA in the hindgut was similar between the two fibre-containing diets (P < 0.05; Table 3), and near complete.

Determined (in vivo) and predicted (in vivo–in vitro) digestibilities

There was an effect of dietary fibre concentration on the determined apparent ileal and total tract digestibilities (P < 0.05; Table 4). The diet containing the highest concentration of fibre had a lower determined digestibility of OM (3.1 % unit
25 g/kg of fibre (9 % units), whereas there were no differences for the diet containing 50 g/kg of fibre (9 % units).}

Table 3. Predicted production and absorption of SCFA in the pig hindgut (\textit{in vivo–in vitro} assay) for diets containing different concentrations of fibre

\begin{tabular}{lccc}
\hline
Diet fibre concentration (g/kg DM) & & & \\
\hline
& 25 & 50 & SEM & \\
Hindgut SCFA produced by fermentation (mmol/kg DM incubated) (\textit{in vitro} methodology)* & & & \\
Acetic & 3315 & 3970† & 352-0 & 0-086 \\
Butyric & 502 & 633 & 108-0 & 0-247 \\
Propionic & 1488 & 1440 & 137-2 & 0-841 \\
Valeric & 512 & 403 & 117-4 & 0-372 \\
\hline
Predicted hindgut SCFA production (mmol/kg DM intake) (\textit{in vivo–in vitro} methodology)‡ & & & \\
Acetic & 270 & 465 & 61-8 & 0-008 \\
Butyric & 42 & 80 & 15-4 & 0-031 \\
Propionic & 119 & 180 & 24-6 & 0-029 \\
Valeric & 41 & 52 & 12-9 & 0-432 \\
\hline
Predicted SCFA absorbed from the hindgut (mmol/kg DM intake) (\textit{in vivo–in vitro} methodology)§ & & & \\
Acetic & 276 & 482 & 61-4 & 0-006 \\
Butyric & 42 & 80 & 15-3 & 0-030 \\
Propionic & 120 & 181 & 24-3 & 0-027 \\
Valeric & 41 & 52 & 12-8 & 0-423 \\
\hline
Predicted apparent SCFA absorption in the hindgut (%) (\textit{in vivo–in vitro} methodology)‖ & & & \\
Acetic & 99-7 & 99-9 & 0-07 & 0-276 \\
Butyric & 99-7 & 99-9 & 0-11 & 0-208 \\
Propionic & 99-9 & 99-9 & 0-06 & 0-548 \\
Valeric & 99-9 & 99-9 & 0-03 & 0-765 \\
\hline
\end{tabular}

* The hindgut production of SCFA in pigs was determined after \textit{in vitro} fermentation of the ileal digesta collected from pigs fed the experimental diets with a pig faecal inoculum for 38 h at 37°C.
† n 6, an outlier was removed from the statistical analysis based on the output of SAS.
‡ The predicted hindgut production of SCFA in pigs was estimated based on the SCFA produced after \textit{in vitro} incubation of pig ileal digesta with a pig faecal inoculum corrected for the ileal flow of DM.
§ The SCFA absorption in the pig hindgut was obtained after summing the SCFA entering (ileal concentrations) and produced (predicted based on an \textit{in vitro} assay) in the hindgut, and then subtracting the excreted SCFA (faecal concentrations).
‖ The apparent absorption in the pig hindgut was predicted based on the ratio between the predicted amount of SCFA absorbed from the hindgut and the sum of SCFA entering (ileal concentrations) and produced (predicted based on an \textit{in vitro} assay) in the hindgut.

Table 4. Determined and predicted ileal and total tract apparent digestibilities of organic matter for ileal cannulated pigs fed diets containing different concentrations of fibre

\begin{tabular}{lccc}
\hline
Diet fibre concentration (g/kg DM) & & & \\
\hline
& 25 & 50 & SEM & \\
Determined ileal digestibility (%) (\textit{in vivo})* & 93-7 & 90-6† & 0-51 & <0-001 \\
Determined total tract digestibility (%) (\textit{in vivo})‡ & 96-8 & 94-4 & 0-42 & <0-001 \\
Predicted total tract digestibility (%) (\textit{in vitro–in vitro} methodology)§ & 96-2 & 94-1 & 0-30 & <0-001 \\
Statistical analysis for determined vs predicted total tract digestibility & & & \\
P & 0-034 & 0-594 & \\
SEM & 0-25 & 0-45 & \\
\hline
\end{tabular}

* Ileal digestibility was determined in pigs fed the experimental diets.
† n 6, an outlier was removed from the statistical analysis.
‡ In \textit{vivo} total tract digestibility was determined directly in pigs.
§ The predicted total tract digestibility in pigs was estimated based on the ileal organic matter digestibility determined in the pig and the predicted hindgut fermentability of organic matter in the pig hindgut based on an \textit{in vitro} fermentation assay using a pig faecal inoculum.

\begin{tabular}{l}
\textbf{Determined (\textit{in vivo}) and predicted (\textit{in vivo–in vitro}) hindgut fermentabilities} \\
\end{tabular}

There was no effect of dietary fibre concentration on either the determined or predicted hindgut fermentability of OM (P > 0-05; Table 4). The determined hindgut fermentability of OM differed (P < 0-05) from its predicted counterpart for the diet containing 25 g/kg of fibre (9 % units), whereas there were no differences for the diet containing 50 g/kg of fibre (P > 0-05).
Predicted production and absorption of SCFA in the hindgut

Owing to the difficulty in investigating the production and absorption of SCFA in the GIT directly, studies investigating SCFA production in humans and farm animals focus mainly on the faecal concentrations of SCFA. The latter approach, however, has limitations because faecal SCFA concentrations reflect the overall net production and absorption of SCFA in the GIT, but provide no information about the production or the absorption of SCFA per se. Other studies have been carried out to determine SCFA absorption by quantifying the appearance of SCFA in portal blood and in exhaled breath. However, significant amounts of SCFA absorbed from the GIT are utilised by the epithelial cells, and therefore may not appear in the bloodstream; thus, the latter approaches are also limited. In the present study, a combined in vivo–in vitro methodology was used to predict the production and absorption of SCFA in the hindgut using diets containing different concentrations of kiwifruit as a model dietary fibre. The latter approach used the growing pig as a model to derive estimates of upper tract digestion and an in vitro fermentation assay, where pig ileal digesta were incubated with a pig faecal inoculum, to model hindgut fermentation. By combining the two (in vivo and in vitro) sets of data, the production and absorption of SCFA in the hindgut can be predicted. In contrast to previous studies, the predicted amount of SCFA absorbed from the hindgut also considered the SCFA entering the hindgut (based on determined ileal concentrations) (Fig. 1). The latter explains the higher values of SCFA absorbed than that produced in the hindgut. It is important to note that the present combined in vivo–in vitro approach does not account for the SCFA produced and absorbed in the upper GIT (in Fig. 1), but the contribution of the stomach and small intestine to SCFA production and absorption across the entire GIT is expected to be relatively low. In addition, this approach does not consider the SCFA potentially produced by the fermentation of endogenous material entering the large intestine directly from colonic tissues.

The similar content of total dietary fibre in the ileal digesta of both kiwifruit fibre diets explains the similar hindgut SCFA concentrations after in vitro fermentation. The predicted production of total SCFA in the hindgut of pigs fed diets containing 25 and 50 g/kg DM of kiwifruit fibre was 472 and 777 mmol/kg DMI, respectively. Christensen et al. also, using a combined in vivo–in vitro approach, reported similar predicted production of total SCFA in the hindgut of ileal cannulated pigs to the values reported in our study with wheat flour, wheat bran and oat bran as fibre sources (369–850 mmol/kg DMI). The wider range in the predicted hindgut production of SCFA in the study of Christensen et al. may be explained by a higher and wider range in dietary fibre content of the diets (63–110 g/kg DM of dietary fibre) and the type of fibre used.

In the present study, the normalised faecal concentrations of SCFA represented only 0.5–1.6% of the predicted hindgut SCFA production when examined across both fibre-containing diets.
and all SCFA. Such low values are not surprising, as faecal SCFA output is the net result of SCFA production and absorption in the hindgut, and therefore is a measure of the unabsorbed SCFA rather than SCFA production per se.

It is noteworthy that based on faecal SCFA concentrations it can be concluded that dietary fibre concentration had no effect on SCFA production and absorption. However, based on the combined in vivo–in vitro approach, it is apparent, perhaps not unexpectedly, that higher dietary fibre concentrations led to a greater production of SCFA in the simulated hindgut fermentation. Clearly, using faecal SCFA concentrations to describe SCFA production in the hindgut can be misleading, and the present study supports the findings of McNeil et al.,113, Cummings & Macfarlane12 and Topping & Clifton13, all of whom have cautioned against the use of faecal SCFA flows and concentrations for describing SCFA production in the hindgut.

Dietary fibre comprises a mixture of complex NSP (e.g. hemicellulose and cellulose), lignin, resistant starch, oligosaccharides and resistant maltodextrins that differ among foods.34 The relative amounts of SCFA produced by fermentation are influenced by the type of dietary fibre being fermented5,12,35, and it is not appropriate to extrapolate the results of the present study to other fibre sources. However, using the approach described in the present study, it is possible to predict the production and absorption of SCFA in the hindgut of pigs after consumption of fibre sources other than kiwifruit and such studies are warranted. This approach can also be applied to humans by collecting ileal digesta from the growing pig (an accepted animal model for upper gut digestion in humans), which can be fermented in vitro with a human faecal inoculum.20,21 The combined in vivo–in vitro methodology can also be extended to other species of animals (e.g. poultry).

The predicted extent of SCFA absorption in the hindgut, based on the amounts of SCFA entering the hindgut (estimated from the SCFA in the ileal digesta) and the SCFA produced in the hindgut, was high (mean absorption across all SCFA and dietary treatments was 99–8 %) as has been reported previously (95–99 %)15,13. Based on the SCFA content of the ileal digesta, it would appear that as much as 3 % of the SCFA absorbed from the hindgut was derived from fermentation in the small intestine. Given that it is likely that a high proportion of the SCFA produced in the small intestine was also absorbed in the small intestine, it would appear that microbial fermentation in the small intestine may be more quantitatively significant than is often recognised. Indeed, a recent study has shown that the corrected upper gut fermentation of the soluble fraction of kiwifruit fibre was 80 % (27).

Predicted total tract digestibility and hindgut fermentability

When the fibre-containing diets were compared, both the predicted and determined hindgut fermentabilities of OM showed the same trend in results (i.e. no difference in hindgut fermentability between the two fibre-containing diets). Similarly, both the predicted and determined values for total tract digestibility of OM led to the same conclusions (i.e. the diet containing the lowest fibre concentration had lower feed tract digestibility). In addition, and in general, the predicted and determined total tract digestibilities of OM were very similar quantitatively, supporting the combined in vivo–in vitro approach.21 Although in the present study the combined in vivo–in vitro method was used to predict the total tract digestibility of OM, this technique can also be used to predict the digestibility of other compounds (e.g. NSP).21,22

In conclusion, the combined in vivo–in vitro methodology is a useful approach for predicting the production and absorption of SCFA in the hindgut. The ileal and faecal concentrations of SCFA per se do not reflect the SCFA production and absorption in the hindgut and may lead to misleading conclusions. Considerable quantities of SCFA are produced and absorbed in the hindgut when pigs are fed diets enriched with kiwifruit.

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References