British Journal of Nutrition (2020), 124, 567–576
doi:10.1017/S0007114520001385

Ileal and hindgut fermentation in the growing pig fed a human-type diet

Anna M. E. Hoogeveen[1,2], Paul J. Moughan[2], Edward S. de Haas[2], Paul Blatchford[3], Warren C. McNabb[2] and Carlos A. Montoya[2,4]*

[1]*School of Food and Advanced Technology, Massey University, Palmerston North 4442, New Zealand*
[2]*Riddet Institute, Massey University, Palmerston North 4442, New Zealand*
[3]*The New Zealand Institute for Plant and Food Research Limited, Palmerston North 4442, New Zealand*
[4]*Grasslands Research Centre, AgResearch Limited, Palmerston North 4442, New Zealand*

(Submitted 6 November 2019 – Final revision received 26 March 2020 – Accepted 30 March 2020 – First published online 21 April 2020)

Abstract

Dietary fibre fermentation in humans and monogastric animals is considered to occur in the hindgut, but it may also occur in the lower small intestine. This study aimed to compare ileal and hindgut fermentation in the growing pig fed a human-type diet using a combined *in vivo/in vitro* methodology. Five pigs (23 (SD 1·6) kg body weight) were fed a human-type diet. On day 15, pigs were euthanised. Digesta from terminal jejunum and terminal ileum were collected as substrates for fermentation. Ileal and caecal digesta were collected for preparing microbial inocula. Terminal jejunal digesta were fermented *in vitro* with a pooled ileal digesta inoculum for 2 h, whereas terminal ileal digesta were fermented *in vitro* with a pooled caecal digesta inoculum for 24 h. The ileal organic matter fermentability (28 %) was not different from hindgut fermentation (35 %). However, the organic matter fermented was 66 % greater for ileal fermentation than hindgut fermentation ($P = 0{\cdot}04$). Total numbers of bacteria in ileal and caecal digesta did not differ ($P = 0{\cdot}09$). Differences ($P < 0{\cdot}05$) were observed in the taxonomic composition. For instance, ileal digesta contained 32-fold greater number of the genus *Enterococcus*, whereas caecal digesta had a 227-fold greater number of the genus *Ruminococcus*. Acetate synthesis and iso-valerate synthesis were greater ($P < 0{\cdot}05$) for ileal fermentation than hindgut fermentation, but propionate, butyrate and valerate synthesis was lower. SCFA were absorbed in the gastrointestinal tract location where they were synthesised. In conclusion, a quantitatively important degree of fermentation occurs in the ileum of the growing pig fed a human-type diet.

Key words: **Ileal fermentation: Hindgut fermentation: SCFA: Microbiota: Human-type diets: Pig models**

Gastrointestinal tract (GIT) microbial fermentation is an important process in humans and monogastric animals. During fermentation, dietary fibre and non-dietary material, such as mucin, are degraded by the GIT microbiota. The end products of fermentation are mainly SCFA, organic acids, such as lactate, and gasses like CO_2, CH_4 and H_2[1]. The SCFA have beneficial health effects for the host, both locally within the GIT (e.g. as an energy source for epithelial cells)[2] and systemically (e.g. regulation of glucose homoeostasis)[3]. A prevailing paradigm is that fermentation in humans and monogastric animals occurs predominantly in the hindgut with little fermentation in the foregut[1].

There is a considerable number of microbes present in the foregut of the human and growing pig[4–7]. These microbes may ferment dietary fibre, and several studies have reported significant disappearance (i.e. fermentability) of dietary fibre at the end of the small intestine in both human ileostomates and pigs[8–11]. For example, 13 % of the NSP in potato[8] and 15–46 % of the dietary pectin[9] disappeared in the foregut of human ileostomates. Results of several experiments also indicate low ileal digestibility values for dietary fibre in human ileostomates and ileal-cannulated pigs[11–13]. Montoya *et al.*[14] discussed that non-dietary gut materials may interfere with dietary fibre determination in ileal digesta and thus lead to underestimation of dietary fibre ileal digestibility. For instance, an estimate of the ileal digestibility of soluble fibre in kiwifruit increased 50 % units when it was corrected for interfering non-dietary materials[15]. Consequently, dietary fibre fermentation in the foregut may be greater than is commonly believed.

The greater number and more diverse population of microbes in the ileum[4] coupled with a longer transit time of digesta in the lower small intestine indicate that fermentation in the foregut may occur mainly within the ileum. Moreover, and based on functional genome analysis, the human ileal microbiota appears to be able to take up and metabolise simple carbohydrates (i.e. low-molecular-weight carbohydrates such as oligosaccharides) rapidly[5]. This is important as transit time in the ileum is considerably shorter than in the hindgut[16]. In

Abbreviations: GIT, gastrointestinal tract; OM, organic matter.

* **Corresponding author:** Carlos A. Montoya, email carlos.montoya@agresearch.co.nz

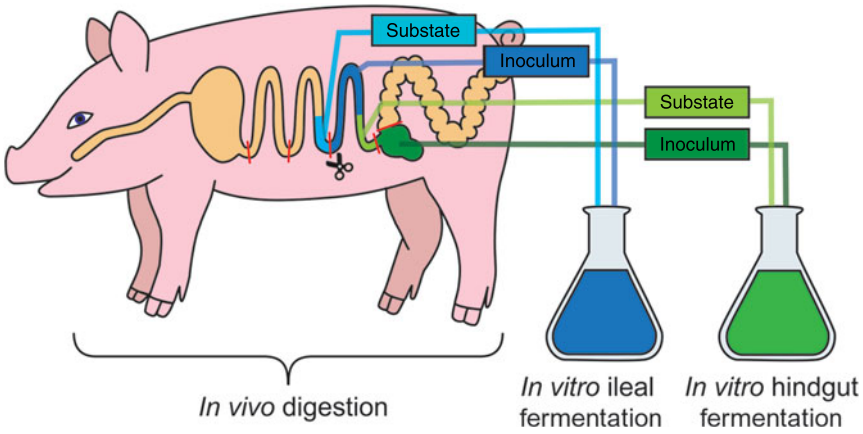

Fig. 1. Schematic overview of the *in vivo*/*in vitro* ileal and hindgut fermentation methodology.

the growing pig, the adenylate energy charge in the last third of the small intestine was similar to that of the caecum, despite the lower number of anaerobic bacteria[17]. These observations indicate the potential for a quantitatively significant amount of fermentation occurring in the small intestine of humans and pigs. However, there are no reported studies that have been able to quantify the ileal fermentation due to the lack of a methodology to do so. It is hypothesised that ileal fermentation is as important as hindgut fermentation in terms of organic matter (OM) disappearance (i.e. fermentability) and synthesis of SCFA despite difference in microbial population.

In this study, growing pigs were fed a human-type diet and ileal and hindgut OM fermentations were determined in the same animal using optimised *in vivo*/*in vitro* fermentation assays[18,19]. This is the first study able to quantify OM fermentability, OM fermented, synthesis and absorption of SCFA in the ileum. The quantitative importance of ileal fermentation was then assessed by comparing the ileal fermentation parameters with those obtained in the hindgut fermentation. The growing pig was used as an animal model for the adult human foregut digestion[20,21], as collection of digesta in different locations of the small intestine is difficult and invasive in humans.

Materials and methods

In vivo *assay*

Dietary treatment. A high-fibre diet comprising foods commonly consumed by humans[22] was formulated to meet the nutrient requirements of the growing pig (National Research Council[23]) (online Supplementary Table S1). Titanium dioxide (TiO_2) was added to the diet as an indigestible marker.

Animals housing and experimental design. Ethics approval for the animal trial was obtained from the Massey University Animal Ethics Committee (Palmerston North, New Zealand). The animal housing and experimental design were as described in detail by Montoya *et al.*[18]. Briefly, five 9-week-old entire male pigs (Hampshire × (Landrace × Large white), 23 (SD 1·6) kg body weight) were housed individually in metabolism pens (1·5 × 0·5 m) in a room maintained at 24 (SD 2·4)°C with a 10 h light–14 h dark cycle. Pigs received the experimental diet for 14 d, with a gradual adaptation from commercial diet to human-type diet during the first 3 d. The daily ration was 100 g DM/kg metabolic body weight ($BW^{0·75}$) per d and given as two equal meals at 08.00 and 16.00 hours. Pigs had free access to water during the study. Pigs were monitored during feeding. After feeding, cages were thoroughly washed and toys were provided to the pigs. On day 15, pigs were fed half their daily ration as one meal and euthanised 5 h postprandial by intracardial injection of sodium pentobarbitone (0·3 ml Pentobarb 300 per kg body weight; Provet). The small intestine was dissected out immediately and ligated into three equal parts. Digesta from the last 50 cm of the second (approximate terminal jejunum) and last (approximate terminal ileum) thirds of the small intestine were collected and used as substrates for the *in vitro* ileal and hindgut fermentation, respectively (Fig. 1). Digesta from the remaining final third (i.e. last third minus terminal ileum) of the small intestine were collected along with caecal digesta for preparing microbial inocula, for the *in vitro* ileal and hindgut fermentation, respectively. All digesta were collected in plastic bags containing carbon dioxide before being stored in insulated containers at 4°C to minimise bacterial activity during the time required to weigh the fresh digesta substrates. Representative samples of terminal jejunal digesta, terminal ileal digesta and faeces were collected in Eppendorf tubes and stored at −20°C for determination of concentration of SCFA. For the microbial analysis, aliquots from the ileal (i.e. last third minus last 50 cm) and caecal digesta were collected in Eppendorf tubes and stored at −80°C. Terminal jejunal and terminal ileal digesta and faeces were also collected, stored at −20°C, freeze-dried and finely ground for the determination of DM, OM and TiO_2.

In vitro *fermentation assays*

A combined *in vivo*/*in vitro* methodology was used based on the growing pig. The pig provided both the substrate (terminal jejunal and terminal ileal digesta) entering each of the fermentation sections (ileum and hindgut) and the microbial inocula (ileal and caecal digesta) for the ileal and hindgut fermentation, respectively[18]. The substrate and inoculum of each fermentation

section are then fermented *in vitro* to be able to determine OM fermentability and synthesis of SCFA. The *in vivo* and *in vitro* results were then combined to predict amounts of OM fermented and synthesis and absorption of SCFA.

The combined *in vivo/in vitro* ileal and hindgut fermentation assays were optimised for different parameters such as incubation time, amount of digesta and pH medium as reported elsewhere[18,19]. For example, there was no effect of the incubation time (1–7 h) on the ileal OM fermentability using the *in vivo/in vitro* methodology[18].

Ileal fermentation. The *in vitro* ileal fermentation was performed according to Montoya *et al.*[18]. The inoculum was prepared by pooling ileal digesta (digesta from the final third of small intestine minus the last 50 cm) from all pigs and mixing with a sterilised anaerobic 0·1M phosphate buffer saline (PBS) solution (4·1 mM L-cysteine, pH 7). The ratio digesta:PBS was 0·22:1, w/v. Bottles contained either 1 g of fresh substrate (i.e. terminal jejunal digesta for each of the five pigs) suspended in 5 ml of PBS or PBS alone (blanks). These bottles were then inoculated with 5 ml of the ileal inoculum. A total of six bottles per pig were used. The ileal fermentation was conducted anaerobically at 37°C for 2 h.

Hindgut fermentation. The *in vitro* hindgut fermentation was performed according to Coles *et al.*[19]. The inoculum was prepared by pooling caecal digesta from all pigs and mixing them with a sterilised anaerobic 0·1M PBS solution (4·1 mM L-cysteine, pH 7). The ratio digesta:PBS was 0·33:1, w/v. Bottles contained either 1 g of fresh substrate (i.e. terminal ileal digesta for each of the five pigs) suspended in 5 ml of PBS or PBS alone (blanks). These bottles were then inoculated with 5 ml of the caecal inoculum. A total of six bottles per pig were used. Hindgut fermentation was conducted anaerobically at 37°C for 24 h.

After ileal and hindgut fermentation, the contents of three bottles were analysed to determine the concentration of SCFA. The remaining three bottles were autoclaved (121°C for 20 min) to inactivate the bacteria and remove fermentation products prior to OM determination. The values of the three bottles for concentration of SCFA and OM determination were averaged per pig. Thus, the number of replicates was five for both ileal and hindgut fermentation.

Chemical analysis

The diet and substrate materials were analysed in duplicate for DM, ash, OM (DM – ash), TiO_2[24], starch (Kit AA/AMG; Megazyme), crude protein (N × 6·25; using a LECO elemental analyser)[25] and lipids (by Soxhlet extraction using petroleum diethyl ether)[25]. The diet was also analysed for gross energy (using a LECO AC-350 Automatic Calorimeter) and soluble and insoluble dietary fibre[26]. DM, ash and OM contents were also determined on material remaining after the *in vitro* fermentation. The concentration of SCFA was determined in the terminal jejunal digesta, terminal ileal digesta and faeces, and in the samples after *in vitro* fermentation, as described previously[27] with iso-caproic acid as an internal standard.

Microbial analysis

DNA extraction. DNA was extracted from ileal and caecal digesta (0·25 g) using the DNeasy Powersoil kit (QIAGEN), with alterations described by Healey *et al.*[28]. Prior to extraction, the sample was homogenised in bead tubes (0·1 mm and 0·5 mm mix in bead solution) using a FastPrep-24 5 G instrument (MP Biomedicals) at 5·5 m/s for three 60 s cycles with 5 min rest on ice in between. Extracted DNA was quantified and quality-checked on a Qubit fluorometer (Invitrogen) and QIAxpert spectrophotometer (QIAGEN), respectively.

Quantitative PCR. *Escherichia coli* (Nissle) was used as a representative bacterium for the total bacteria and was grown in tryptic soya broth (Oxoid) at 37°C aerobically. Cell density was determined using a haemocytometer (Neubauer), and the culture was concentrated as required to $1{\cdot}0 \times 10^9$ cells/ml. DNA was then extracted as described above. A standard curve was constructed using 1:10 dilutions of the extracted standard DNA. Samples and standards were run in triplicate by absolute quantification on the Light Cycler 480 real-time PCR instrument (Roche). SYBR Green I Master Mix (Roche) detection chemistry was used to detect double-stranded DNA amplification. The total reaction volume was 20 µl, consisting of 10 µl SyBr Green I Master mix, 4 µl forward primer (2·5 µM), 4 µl reverse primer (2·5 µM) and 2 µl DNA template or sterile water (blank). Each quantitative PCR run included one activation cycle (95°C, 5 min), 32–40 run cycles (including denaturation (95°C, 30 s), annealing (60°C, 60 s) and extension (72°C, 60 s)), and one melt curve cycle (60–95°C at 0·1°C/s with continuous fluorescence acquisition) followed by a cooling cycle (40°C). The melt curve cycle enabled the differentiation between target product and non-specific double-stranded products such as primer-dimers. The universal primers used were forward (5′- TCCTACGG GAGGCAGCAGT) and reverse (5′ – GGACTACCAGGGTATCTA ATCCTGTT)[29].

16S ribosomal RNA gene sequencing and bioinformatics. Purified DNA from each sample was sent to the Massey Genome Service (Massey University). The samples underwent library preparation as previously described[30] using primers that amplified the V3–V4 hypervariable region of the 16S rRNA gene, 16SF_V3 (5′ – AATGATACGGCGACCACCGAGATCTACAC-index-TATGGTAATTGGCCTACGGGAGGCAGCAG) and 16SR_V4 (5′ – CAAGCAGAAGACGGCATACGAGAT-index-AGTCAGTCAGC CGGACTACHVGGGTWTCTAAT). The library was pooled at equal concentrations and run on one lane of an Illumina MiSeq instrument using 2 × 250 bp paired-end chemistry. Quantitative Insights Into Microbial Ecology software version 1.8.0 was used to analyse the Illumina MiSeq sequencing data[31]. To assemble the forward and reverse reads into a continuous sequence, PANDASeq was used with parameters of at least 40 bp overlap, a minimum of 350 bp length and a maximum of 500 bp length. Chimeras were filtered from the sequences and the reads clustered into operational taxonomic units (OTU) based on a 97 % identity threshold using USEARCH (-cluster_-fast command with default parameters)[32,33]. Sequence alignment was carried out using PyNAST with reference to the Greengenes database (version 13_8)[34]. The resultant OTU table was denoised by

removing taxa with fewer than five total sequences across all samples. Alpha rarefaction was calculated using the Faith's Phylogenetic Diversity metric[35] to a rarefaction depth of 10 000 sequences. Beta diversity was determined using Euclidean distances as input to generate principal coordinate plots. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States was used to predict the functional profiling of ileal and caecal microbial communities.

Calculations

Due to different amounts of DM entering the ileum and hindgut, data were normalised for dietary DM intake based on the ratio of the indigestible marker in the diet and digesta (i.e. data were expressed per kg diet DM intake). Normalising of parameters allowed comparing gut locations, as measures were expressed in the same unit. The calculations used for determining *in vitro* OM fermentability[27] and *in vivo/in vitro* fermented OM for either ileal or hindgut fermentation were as follows:

$$\begin{aligned}\text{OM fermentability}_{\textit{in vitro}}\,(\%) = (&\text{OM}_{\text{before } \textit{in vitro} \text{ fermentation}} \\ &- (\text{OM}_{\text{after } \textit{in vitro} \text{ fermentation}} \\ &- ((\text{OM}_{\text{blank initial}} + \text{OM}_{\text{blank final}})/2))) \\ &/\ \text{OM}_{\text{before } \textit{in vitro} \text{ fermentation}} \times 100\end{aligned}$$

$$\begin{aligned}&\text{Fermented OM}_{\textit{in vivo/in vitro}}\,(\text{g/kg diet DM intake}) \\ &\quad = \text{OM fermentability}_{\textit{in vitro}} / 100 \\ &\qquad \times \left(\text{TiO}_{2-\text{diet}} / \text{TiO}_{2-\text{terminal jejunal or terminal ileal digesta}}\right)\end{aligned}$$

where $\text{OM}_{\text{blank initial}}$ and $\text{OM}_{\text{blank final}}$ are the amount of OM in the blanks prior (initial) and after (final) *in vitro* fermentation, respectively. *In vitro* ileal and hindgut fermentation had their own blanks. $\text{TiO}_{2\text{-diet}}$ and $\text{TiO}_{2\text{-terminal jejunal or terminal ileal digesta}}$ are the TiO_2 concentrations (g/kg DM) in the diet and digesta, respectively. The $\text{TiO}_{2\text{-terminal jejunal digesta}}$ was used for the *in vitro* ileal fermentation, whereas $\text{TiO}_{2\text{-terminal ileal digesta}}$ was used for the *in vitro* hindgut fermentation.

The normalised total number of bacteria and archaea and number per phyla/genus, and the predicted metabolic activity in ileal and caecal digesta were calculated as follows:

$$\begin{aligned}&\text{Normalised total number of bacteria and archaea}_{\text{ileal or caecal digesta}} \\ &\quad (\text{16S rRNA gene copy number/kg diet DM intake}) \\ &= \text{number of bacteria and archaea}_{\text{ileal or caecal digesta}} \\ &\quad (\text{16S rRNA gene copy number/kg DM}) \\ &\times \left(\text{TiO}_{2-\text{diet}}/\text{TiO}_{2-\text{terminal ileal or caecal digesta}}\right)\end{aligned}$$

$$\begin{aligned}&\text{Normalised number of bacteria or} \\ &\quad \text{archaea per phylum/genus}_{\text{ileal or caecal digesta}} \\ &\quad (\text{16S rRNA gene copy number/kg diet DM intake}) \\ &= \text{normalised total number of} \\ &\quad \text{bacteria and archaea}_{\text{ileal or caecal digesta}} \\ &\quad (\text{16S rRNA gene copy number/kg diet DM intake}) \\ &\times \text{relative abundance}_{\text{phylum/genus}}(\%) / 100\end{aligned}$$

$$\begin{aligned}&\text{Normalised predicted metabolic activity}_{\text{ileal and caecal digesta}} \\ &\quad (\text{activity/kg diet DM intake}) = \text{relative activity/kg DM} \\ &\times \left(\text{TiO}_{2-\text{diet}}/\text{TiO}_{2-\text{terminal ileal or caecal digesta}}\right)\end{aligned}$$

The synthesis of SCFA during *in vitro* ileal and hindgut fermentation, estimated *in vivo/in vitro* synthesis of SCFA (i.e. estimated ileal or hindgut synthesis based on the amount of DM entering either the ileum or hindgut per kg DM diet intake), normalised concentration of SCFA in terminal jejunal digesta, terminal ileal digesta and faeces and estimated *in vivo/in vitro* disappearance (absorption) of SCFA in ileum and hindgut were determined as described previously[27], using the following equations:

$$\begin{aligned}&\text{Synthesis of SCFA}_{\text{ileal or hindgut } \textit{in vitro}} \\ &\qquad (\text{mmol/kg substrate DM incubated}) \\ &\quad = (\text{SCFA}_{\text{after } \textit{in vitro} \text{ fermentation}}(\text{mmol/kg DM}) \\ &\quad - \text{SCFA}_{\text{jejunum or terminal ileum digesta}}(\text{mmol/kg DM}) \\ &\quad - ((\text{SCFA}_{\text{blank initial}} + \text{SCFA}_{\text{blank final}})/2))\end{aligned}$$

$$\begin{aligned}&\text{Estimated synthesis of SCFA}_{\text{ileal or hindgut } \textit{in vivo/in vitro}} \\ &\quad (\text{mmol/kg diet DM intake}) \\ &= \text{synthesis of SCFA}_{\text{ileal or hindgut } \textit{in vitro}} \\ &\quad (\text{mmol/kg substrate DM incubated}) \\ &\times \left(\text{TiO}_{2-\text{diet}}/\text{TiO}_{2-\text{terminal jejunum or terminal ileal digesta}}\right)\end{aligned}$$

$$\begin{aligned}&\text{Normalised concentration} \\ &\quad \text{of SCFA}_{\text{terminal jejunum, terminal ileum digesta or faces}} \\ &\quad (\text{mmol/kg diet DM intake}) \\ &= \text{SCFA}_{\text{terminal jejunum, terminal ileum digesta or faces}}(\text{mmol/kg DM}) \\ &\times \left(\text{TiO}_{2-\text{diet}}/\text{TiO}_{2-\text{terminal jejunal or terminal ileal digesta or faces}}\right)\end{aligned}$$

$$\begin{aligned}&\text{Estimated disappearance} \\ &\quad \text{of SCFA}(\text{mmol/kg diet DM intake})_{\text{ileal or hindgut } \textit{in vivo}} \\ &= \text{Normalised concentration of} \\ &\quad \text{SCFA}_{\text{terminal jejunum or terminal ileum digesta}}(\text{mmol/kg diet DM intake}) \\ &+ \text{estimated synthesis of SCFA}_{\text{ileal or hindgut } \textit{in vivo/in vitro}} \\ &\quad (\text{mmol/kg diet DM intake}) - \text{Normalised concentration of} \\ &\quad \text{SCFA}_{\text{termina lileum digesta or faces}}(\text{mmol/kg diet DM intake})\end{aligned}$$

where $\text{SCFA}_{\text{blank initial}}$ and $\text{SCFA}_{\text{blank final}}$ are the SCFA (mmol/kg DM) in the blanks prior (initial) and after (final) *in vitro* fermentation, respectively. *In vitro* ileal and hindgut fermentation had their own blanks. $\text{SCFA}_{\text{jejunum or terminal ileum digesta}}$ are the SCFA (mmol/kg DM) in fresh terminal jejunal (ileal fermentation) or terminal ileal (hindgut fermentation) digesta, which represents the SCFA present in the digesta prior to being fermented.

The calculation used to determine the normalised nutrient content was as follows:

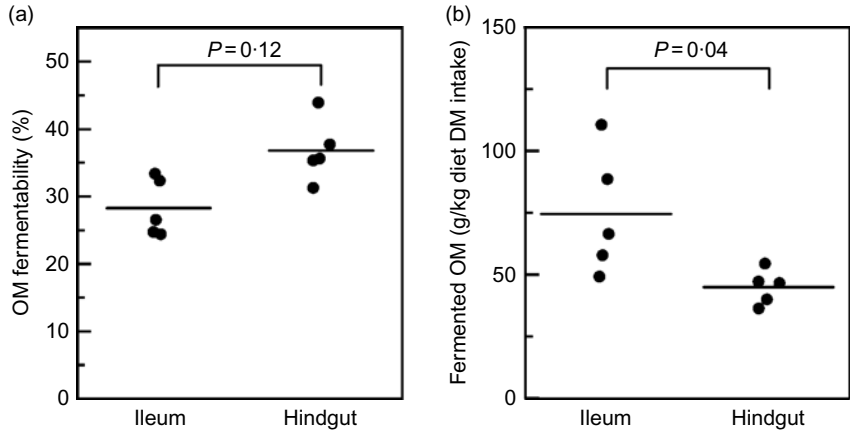

Fig. 2. Ileal and hindgut *in vitro* organic matter (OM) fermentability values (a) and *in vivo/in vitro* fermented OM (b) of pigs fed a human-type diet (n 5). The line for each gastrointestinal tract location represents the mean value.

$$\text{Normalised nutrient content}_{\text{terminal jejunal or terminal ileal digesta}}\ (\text{g/kg diet DM intake}) = \text{nutrient concentration}_{\text{terminal jejunal or terminal ileal digesta}}\ (\text{g/kg DM}) \times \left(\text{TiO}_{2-\text{diet}}/\text{TiO}_{2-\text{terminal jejunum or terminal ileal digesta}}\right)$$

Statistical analysis

For this study, a sample size of five replicates was required to detect a statistical difference (5 %) between GIT locations, with a power >80 % at a two-tailed 5 % significance level, based on variance (SD 2·4 %) and means reported in previous studies[7,11,36].

The statistical analyses were performed in SAS version 9.4 (SAS Institute). The difference between ileal and hindgut fermentation within each pig for the OM fermentability, fermented OM, estimated synthesis of SCFA, estimated disappearance of SCFA, the normalised number of bacteria and archaea (total, phyla and genus) and the normalised predicted metabolic activity was tested using a paired t test. The normal distribution of the difference for the t test was evaluated with the use of the Output Delivery System graphics and univariate procedure of SAS. Probability values of $P < 0{\cdot}05$ were considered statistically significant, and a trend when $0{\cdot}05 < P < 0{\cdot}10$.

The non-parametric two-sample t test (Monte Carlo permutation) from Quantitative Insights Into Microbial Ecology was used to determine alpha diversity significance. The Euclidean distance Principal Coordinates Analysis groupings were tested for significant separation using the adonis test (9999 permutations).

Results

With the exception of a pig that had loose stools during the first experimental days, all pigs were healthy.

Ileal and hindgut organic matter fermentability were not different, but the quantity of ileal fermented organic matter was greater than the organic matter fermented in the hindgut

There was no difference ($P = 0{\cdot}12$) between ileal and hindgut *in vitro* OM fermentability, 28 (SEM 1·9) and 35 (SEM 2·1) %, respectively (Fig. 2). However, there was 66 % more fermented OM in the ileum than in the hindgut ($P = 0{\cdot}04$).

Ileal and caecal microbiota have different taxonomic composition, diversity and predicted metabolic activity

The normalised total number of bacteria and archaea in the caecum tended to be greater than in the ileum ($P = 0{\cdot}09$; Table 1). Ileal digesta contained greater ($P < 0{\cdot}05$) numbers of the family Micrococcaceae (42-fold greater), and the genera *Enterococcus* (32-fold greater) and *Leuconostoc* (55-fold greater). In contrast, caecal digesta had greater ($P < 0{\cdot}05$) numbers of the class Clostridiales (57-fold greater), the families Coriobacteriaceae (91-fold greater), Lachnospiraceae (443-fold greater), Tenericutes (24-fold greater) and Ruminococcaceae (227-fold greater), and the genera *Methanosphaera* (15-fold greater), *Blautia* (65-fold greater), *Coprococcus* (17-fold greater), and *Ruminococcus* (82-fold greater). Some bacteria observed in considerable numbers in the caecal digesta (e.g. Bacteriodales, *Prevotella*, *Dorea*, *Lachnospira*, *Roseburia*, Erysipelotrichaceae, *Treponema* and *TM7-3_F16*) were not detected in ileal digesta. Similar conclusions can be drawn from the relative abundance data (non-normalised data) (online Supplementary Figs. S1 and S2). The alpha diversity tended to differ between the ileal and caecal microbiota ($P = 0{\cdot}09$; Fig. 3). Based on the normalised data, the microbiota in the caecal digesta resembled a more closely related community than the microbiota in the ileal digesta (Fig. 4). The differences in the ileal and caecal microbiota composition are reflected in differences in their predicted metabolic activity according to Phylogenetic Investigation of Communities by Reconstruction of Unobserved States analysis of pathways related to carbohydrate and protein metabolism (online Supplementary Table S2). For example, the caecal predicted metabolic activity for pyruvate metabolism was 1·2-fold greater ($P = 0{\cdot}02$) than the ileal pyruvate metabolism.

Estimated synthesis of SCFA and disappearance differs between ileal and hindgut fermentation

The estimated synthesis of acetate and iso-valerate was 6·1- and 1·3-fold greater ($P < 0{\cdot}05$), respectively, during ileal fermentation

Table 1. Taxonomic composition (×10^9 16S ribosomal RNA gene copy number/kg diet DM intake) in ileal and caecal digesta of pigs fed a human-type diet (*n* 5)*
(Mean values with their standard errors)

		Gastrointestinal tract location				
		Ileum		Caecum		
Phylum	Genus	Mean	SEM	Mean	SEM	*P*
Total number of bacteria and archaea		828	268	1946	203	0·09
Actinobacteria		30·4	0·81	32·0	0·54	0·81
	Actinomycetales†	5·13	3·14	0·08	0·08	‡
	Micrococcaceae†	32·5	11·1	0·78	0·38	0·05
	Coriobacteriaceae†	0·22	0·13	20·4	1·27	0·00
	Collinsella	2·14	2·07	10·2	4·63	0·19
Bacteroidetes		0·10	0·10	270	66·0	‡
	Bacteroidales†	0·05	0·05	87·6	19·7	‡
	Prevotella	ND		175	63·7	‡
Euryarchaeota		1·17	0·80	15·8	3·07	0·01
	Methanosphaera	1·04	0·83	15·3	2·81	0·01
Firmicutes		784	258	1513	149	0·18
	Enterococcus	37·0	12·1	1·15	0·16	0·04
	Lactobacillus	10·6	6·75	4·48	1·33	0·34
	Leuconostoc	81·6	29·0	1·48	1·11	0·05
	Streptococcus	509	160	299	56·9	0·27
	Turicibacter	0·77	0·37	15·6	0·77	0·13
	Clostridiales†	3·46	2·28	197	22·6	0·00
	Christensenellaceae†	ND		0·45	10·18	‡
	Clostridiaceae†	30·7	11·6	49·7	14·9	0·63
	Lachnospiraceae†	0·18	0·13	79·7	12·9	0·00
	Blautia	0·41	0·34	26·8	4·33	0·00
	Coprococcus	1·85	1·71	30·9	5·94	0·01
	Dorea	0·55	0·51	22·3	7·98	‡
	Lachnospira	0·00	0·00	40·1	14·0	‡
	Roseburia	0·02	0·02	14·7	10·6	‡
	Ruminococcaceae†	1·25	1·01	284	32·3	0·00
	Ruminococcus	3·68	3·20	302	63·1	0·01
	Mogibacteriaceae†	0·19	0·05	1·60	0·78	0·21
	Erysipelotrichaceae†	0·18	0·17	34·3	12·2	‡
Proteobacteria		7·34	2·70	4·04	1·24	0·42
	Enterobacteriaceae†	4·94	1·69	1·99	0·61	0·86
Spirochaetes		ND		47·7	25·5	‡
	Treponema	ND		47·3	25·3	‡
Tenericutes		0·60	0·53	14·4	3·84	0·02
TM7		0·11	0·10	17·0	9·75	‡
	TM7-3_F16	ND		17·0	9·75	‡
Unassigned		9·08	3·65	24·6	6·21	0·15

ND, not detected.
* Only bacteria phyla/genera with >1 % abundance in at least one of the samples are reported.
† Bacteria could only be classified as far as class, order or family level.
‡ Statistical analysis was only conducted when bacteria were detected in a minimum of three pigs.

compared with hindgut fermentation (Fig. 5). In contrast, the estimated synthesis of propionate, butyrate and valerate was greater (4·6-, 8·3- and 4·5-fold, respectively; $P < 0·05$) during hindgut fermentation compared with ileal fermentation. Similar trends were observed for the *in vitro* synthesis of SCFA (online Supplemenatry Table S3). Based on the estimated disappearance data (Fig. 6), most SCFA disappeared in the GIT location where they were synthesised and the statistical differences were as for the synthesis of SCFA values.

Discussion

The ileal fermentation results confirm earlier observations in pigs[10,11,18] and establishes that such fermentation is observed

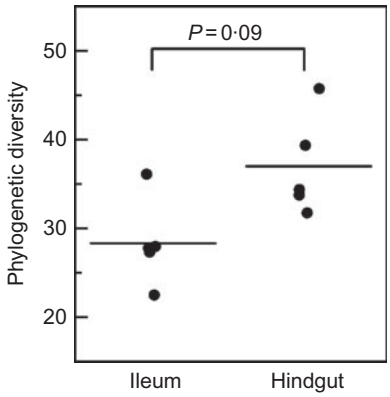

Fig. 3. Alpha diversity numbers showing Faith's phylogenetic diversity of microbial communities in ileal and caecal digesta of pigs fed a human-type diet (*n* 5) based on the normalised number of bacteria and archaea. The line for each gastrointestinal tract location represents the mean value.

for a human-type diet. Indeed, one of the main results of this study was that the amount of fermented OM was 1·5-fold greater in the ileum compared with the hindgut, which is explained by a greater amount of OM entering the ileum than the hindgut (246 *v.* 141 g OM/kg diet DM intake) (online Supplementary Table S4). A greater amount of acetate and iso-valerate were synthesised during ileal fermentation compared with hindgut fermentation, but for hindgut fermentation, the synthesis of butyrate, propionate and valerate was greater. The present results are the first to demonstrate that SCFA disappeared in the same GIT location where they were synthesised.

The normalised total number of bacteria and archaea (16S rRNA gene copy number/kg diet DM intake) in the caecal digesta ($19·5 \times 10^{11}$) tended to be greater than in the ileal digesta ($8·3 \times 10^{11}$). Rowan *et al.*[37] reported that, when taking the dietary DM into account, concentration of DNA (i.e. microbial marker) in fresh ileal digesta and faeces of pigs fed a human-type diet was not different. Recently, Montoya *et al.*[7] reported two times greater normalised total number of bacteria (per kg diet DM intake) in ileal digesta compared with faeces of pigs fed diets containing kiwifruit as the sole dietary fibre source. When the concentration data are normalised for diet DM intake, different conclusions are drawn and the potential role of the ileal microbiota in fermenting undigested material is highlighted. In the pig ileal digesta, the predominant bacterial genus was *Streptococcus*, which is also the predominant bacterial genus in ileal effluent from human ileostomates[5]. *Streptococcus* is well adapted to the ileum because it has the ability to rapidly ferment simple carbohydrates (i.e. mono-, di- and oligosaccharides), which is important in the ileum as the retention time is shorter than in the hindgut[5,16]. A greater number of *Streptococcus* was observed in the ileal digesta of the pigs fed the human-type diet compared with caecal digesta. This may be related to a tending ($P = 0·07$) towards a greater amount of starch, a rapidly fermentable carbohydrate, entering the ileum (31 g/kg diet DM intake) compared with the hindgut (14 g/kg diet DM intake) (online Supplementary Table S4). In caecal digesta, the main bacteria present belonged to the class Clostridiales, the family Ruminococcaceae and the genera *Ruminococcus*, *Streptococcus* and *Prevotella*. Both *Clostridium* sp. and

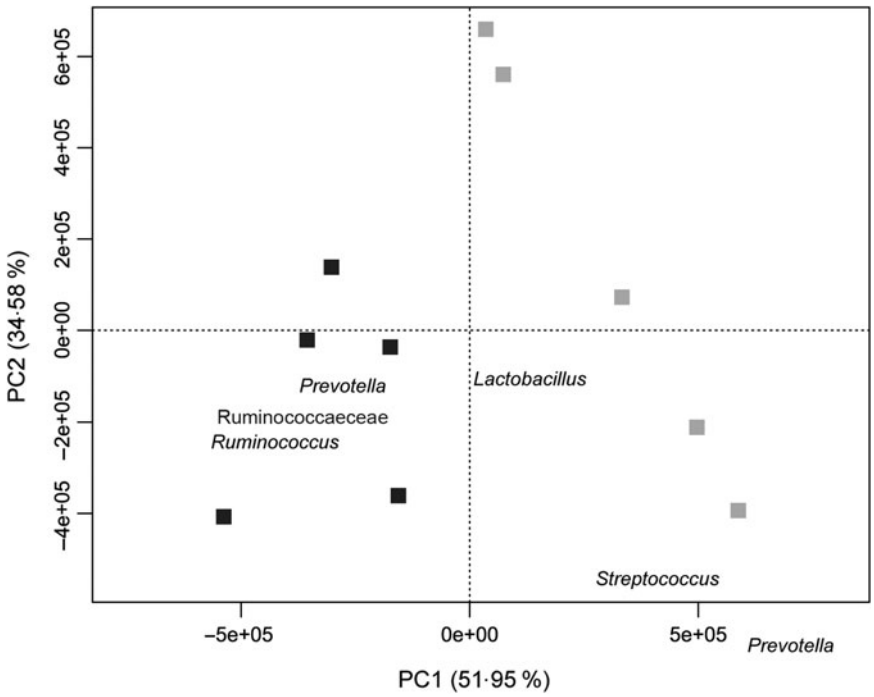

Fig. 4. Euclidean principal coordinates (PC) analysis of the distances of normalised relative abundance data (16S ribosomal RNA gene copy number/kg diet DM intake) in caecal (■) and ileal digesta (■) for pigs fed a human-type diet (n 5). Groupings exhibited a significant difference ($P = 0{\cdot}01$, $R^2 = 0{\cdot}43$) as determined by the non-parametric adonis test (9999 permutations). Most prevalent taxa responsible for variation in the plot are displayed.

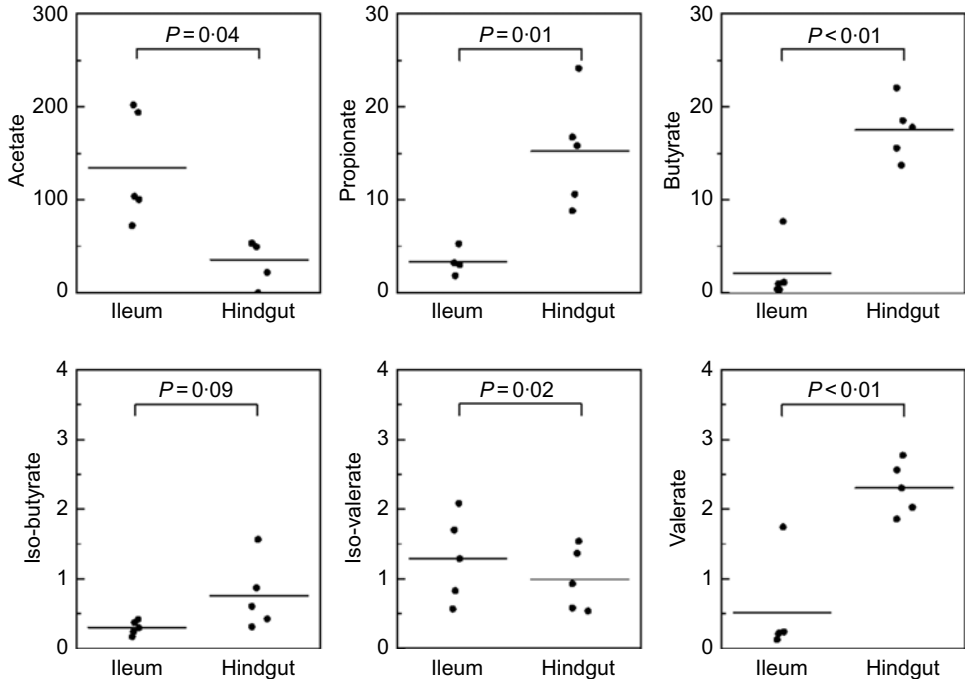

Fig. 5. Estimated synthesis of SCFA (mmol/kg diet DM intake) in the ileum and hindgut of pigs fed a human-type diet (n 5). The line for each gastrointestinal tract location represents the mean value.

Ruminococcus sp. have the ability to ferment cellulose[38], a fibre that needs a longer time to ferment. Cellulose is expected to be a main component of the insoluble dietary fibre fraction of the human-type diet (online Supplementary Table S1). The predicted metabolic activity related to carbohydrate and protein metabolism demonstrated that the ileal and caecal microbiota of the pigs fed the human-type diet had different metabolic activity profiles. The overall ileal predicted metabolic activity was 84 % of the overall caecal predicted metabolic activity. Despite the differences in predicted metabolic activity, similar degrees

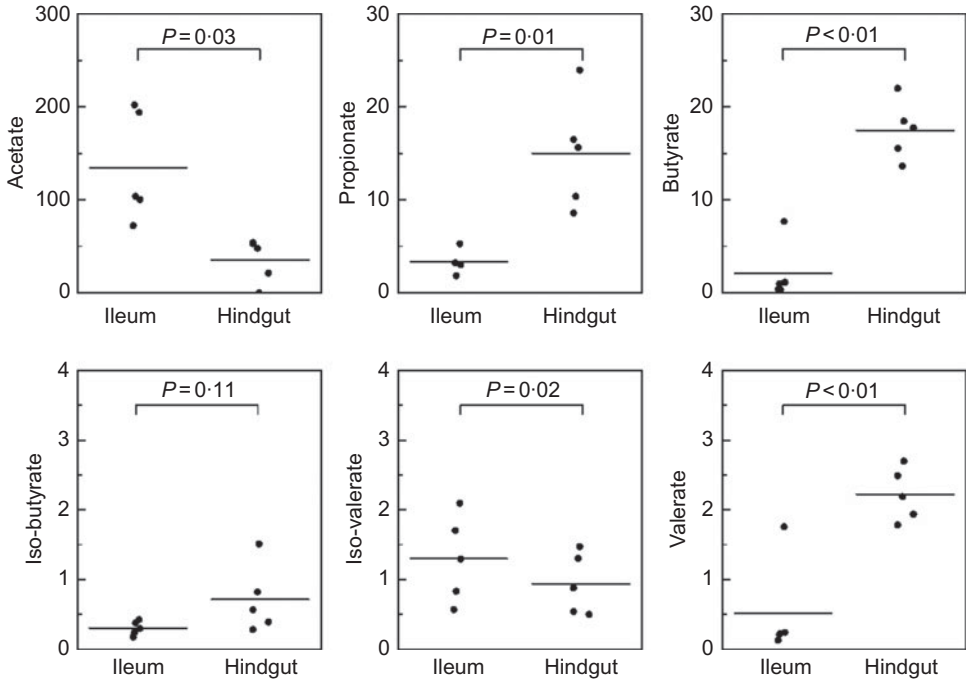

Fig. 6. Estimated disappearance of SCFA (mmol/kg diet DM intake) in the ileum and hindgut of pigs fed a human-type diet (*n* 5). The line for each gastrointestinal tract location represents the mean value.

of OM fermentability were observed during ileal and hindgut fermentation. The tendency towards greater microbial diversity in caecal digesta compared with ileal digesta coincides with a longer transit time in the hindgut, which gives bacteria a greater opportunity to grow, and for cross-feeding to occur[39]. The differences in the microbial community indicate that the ileal microbial community has evolved aligned to ferment rapidly fermentable substrates in accordance with the faster transit time, whereas the hindgut microbial community has evolved to ferment more slowly fermentable substrates in line with the slower transit time. The co-existence of these microbial communities may result in more efficient and effective fermentation of diets as humans eat diets that are complex in nature and composition.

During ileal fermentation, acetate was the main SCFA synthesised, whereas butyrate and propionate were synthesised in greater amounts during hindgut fermentation. Similar trends were reported for concentrations of these SCFA in the ileal and caecal digesta of adult humans suffering sudden death[40]. Concentration data need to be interpreted carefully, however, as concentration of SCFA represents only the amount of SCFA that has not been absorbed at the time of collection. Synthesis data are more meaningful. The differences seen here for synthesis of SCFA related to the ileal and hindgut fermentation may be related to several factors: (i) the incubation time of ileal fermentation (2 h) compared with hindgut fermentation (24 h), (ii) the microbial composition and (iii) the amount and chemical composition of the substrate available. A longer fermentation time may be one of the factors explaining the greater butyric acid synthesis. Longer fermentation time allows cross-feeding to occur whereby SCFA like acetate can be converted into other SCFA, like butyrate[41–43]. This would lower the concentration of acetate while increasing the concentration of butyrate. Another factor explaining the greater hindgut butyrate synthesis is higher numbers of butyrate-synthesising bacteria reported in caecal digesta. Similarly, the greater caecal propionate synthesis may be related to higher numbers of propionate-synthesising bacteria such as *Prevotella*[44] reported in the caecal digesta. The tendency to a lower phylogenetic diversity in the ileum compared with the caecum may explain the greater concentration of acetate observed after ileal fermentation compared with hindgut fermentation. Almost all GIT bacteria have the ability to synthesise acetate, whereas butyrate and propionate synthesis pathways are highly conserved in a limited number of GIT bacteria[45]. The amount of crude protein entering the ileum was 2-fold greater than the amount entering the hindgut (online Supplementary Table S4), which may explain the greater iso-valerate synthesis during ileal fermentation since iso-valerate is a product of protein fermentation[46]. The SCFA synthesised during ileal fermentation are expected to have similar effects on the ileal microbiota as has been reported for the caecal microbiota. For example, the synthesis of SCFA reduces the pH and promotes the growth of different bacteria, like *Roseburia*[43].

Based on the estimated disappearance of SCFA, the SCFA were absorbed and/or metabolised in the same GIT location as they were synthesised. Previously, human studies have shown that SCFA can be absorbed in both the ileum and hindgut[47,48]. These results indicate that SCFA synthesised in the ileum may have a local effect at the ileal epithelium and/or be absorbed to serve systemically in the host. For example, human ileal epithelial cells contain NEFA receptors, which, upon binding with SCFA, can stimulate the production of satiety hormones, such as peptide YY and glucagon-like peptide-1[49], which then increase ileal motility[50]. Dietary intervention may be a strategy to modulate the ileal synthesis of SCFA and therefore the delivery

of SCFA both locally and systemically. Further studies to investigate the influence of diet on ileal microbiota, their fermentation capacity and synthesis of SCFA, and how ileal fermentation affects the host are warranted.

To compare estimated ileal and hindgut fermentation in the present work, a combined *in vivo/in vitro* methodology was used. Limitations and advantages of this methodology have been described previously[18]. One of the limitations of this *in vivo/in vitro* methodology is that *in vivo* absorption and fermentation of dietary and non-dietary nutrients occur simultaneously in the ileum. During the *in vitro* fermentation, this absorption is not simulated. The presently described study involves only one human-type diet, and the work needs to be extended to more diverse diets and sources of dietary fibre. That the human-type diet was highly fermentable in the ileum, however, demonstrates that ileal fermentability may be a hitherto largely underrecognised yet important characteristic of foods and diets consumed by humans. Some studies have reported important ileal digestibility values of dietary fibre in human ileostomates[8,9]. Both the ileal and hindgut fermentation of foods need to be better understood. To apply the combined *in vivo/in vitro* methodology in humans, however, requires an animal model to allow for the sampling of terminal jejunal digesta and ileal digesta. A faecal inoculum sourced from adult humans can replace the caecal inoculum as used here for determining hindgut fermentation[19]. The growing pig is a valid animal model for the adult human for the foregut digestion of food[20,51], and it can be expected that pig terminal ileal digesta samples can be used to provide a suitable substrate for the hindgut fermentation methodology (faecal inoculum). The question remains, however, as to whether pig ileal digesta provide a suitable inoculum for a fermentation assay related to human ileal fermentation. There may be differences in the microbiota between the two species, and controlled comparisons in this respect are needed. The second question is whether the species differences in the microbiota composition if they do occur have a significant effect on fermentation. As demonstrated in this experiment, despite differences between ileal and hindgut microbial composition, OM fermentability was not different between the two sections of the intestinal tract. The pig-based *in vivo/in vitro* ileal fermentation model needs to be fully validated, as does its extension to evaluating human foods.

In conclusion, results of this experiment demonstrate that a diet consisting of foods commonly consumed by humans was well fermented in the ileum of the growing pig. Indeed, the amount of fermented OM was greater during ileal than hindgut fermentation. The ileal fermentation synthesised important amount of SCFA (mainly acetic acid), which are absorbed or metabolised in the ileum. Given similarities in the foregut of humans and pigs, such fermentation may also occur in humans, but the suitability of the growing pig as an animal model for the adult human to provide inocula and substrate for the fermentation methodology remains to be assessed.

Acknowledgements

We acknowledge Patrick Morel for assisting with the Animal Ethics application.

This study was funded by the Centre of Research Excellence Fund from the Tertiary Education Commission and the New Zealand Ministry of Education

C. A. M. and P. J. M. designed the study. C. A. M. and E. S. H. conducted the *in vivo/in vitro* fermentation experiments. P. B. conducted the microbial analysis and analysed the microbial data. A. M. E. H. analysed data, performed statistical analysis and wrote the paper. C. A. M., P. J. M., P. B. and W. C. M. critically reviewed the manuscript. All authors have read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520001385

References

1. Cummings JH & Englyst HN (1987) Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* **45**, 1243–1255.
2. Roediger WE (1980) Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* **21**, 793–798.
3. Morrison DJ & Preston T (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200.
4. Wang M, Ahrne S, Jeppsson B, *et al.* (2005) Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *FEMS Microbiol Ecol* **54**, 219–231.
5. Zoetendal EG, Raes J, van den Bogert B, *et al.* (2012) The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J* **6**, 1415–1426.
6. Metzler-Zebeli BU, Hooda S, Pieper R, *et al.* (2010) Nonstarch polysaccharides modulate bacterial microbiota, pathways for butyrate production, and abundance of pathogenic *Escherichia coli* in the pig gastrointestinal tract. *Appl Environ Microbiol* **76**, 3692–3701.
7. Montoya CA, Henare SJ, Zhu P *et al.* (2019) Adaptation over time of intestinal fermentation in the growing pig is influenced by the amount of kiwifruit consumed. *Br J Nutr* **121**, 601–614.
8. Englyst HN & Cummings JH (1987) Digestion of polysaccharides of potato in the small intestine of man. *Am J Clin Nutr* **45**, 423–431.
9. Holloway WD, Tasman-Jones C & Maher K (1983) Pectin digestion in humans. *Am J Clin Nutr* **37**, 253–255.
10. Jaworski NW & Stein HH (2017) Disappearance of nutrients and energy in the stomach and small intestine, cecum, and colon of pigs fed corn-soybean meal diets containing distillers dried grains with solubles, wheat middlings, or soybean hulls. *J Anim Sci* **95**, 727–739.
11. Bach Knudsen KE & Canibe N (2000) Breakdown of plant carbohydrates in the digestive tract of pigs fed on wheat- or oat-based rolls. *J Sci Food Agric* **80**, 1253–1261.
12. Lien KA, McBurney MI, Beyde BI, *et al.* (1996) Ileal recovery of nutrients and mucin in humans fed total enteral formulas supplemented with soy fiber. *Am J Clin Nutr* **63**, 584–595.
13. Englyst HN & Cummings JH (1986) Digestion of the carbohydrates of banana (*Musa paradisiaca sapientum*) in the human small intestine. *Am J Clin Nutr* **44**, 42–50.
14. Montoya CA, Henare SJ, Rutherfurd SM, *et al.* (2016) Potential misinterpretation of the nutritional value of dietary fiber:

correcting fiber digestibility values for nondietary gut-interfering material. *Nutr Rev* **74**, 517–533.
15. Montoya CA, Rutherfurd SM & Moughan PJ (2015) Nondietary gut materials interfere with the determination of dietary fiber digestibility in growing pigs when using the Prosky method. *J Nutr* **145**, 1966–1972.
16. Graff J, Brinch K & Madsen JL (2001) Gastrointestinal mean transit times in young and middle-aged healthy subjects. *Clin Physiol* **21**, 253–259.
17. Jensen BB & Jørgensen H (1994) Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. *Appl Environ Microbiol* **60**, 1897–1904.
18. Montoya CA, de Haas ES & Moughan PJ (2018) Development of an *in vivo* and *in vitro* ileal fermentation method in a growing pig model. *J Nutr* **148**, 298–305.
19. Coles LT, Moughan PJ, Awati A, *et al.* (2013) Optimisation of inoculum concentration and incubation duration for an *in vitro* hindgut dry matter digestibility assay. *Food Chem* **136**, 624–631.
20. Food and Agriculture Organization (2013) Dietary protein quality evaluation in human nutrition. *FAO Food Nutr Pap* **92**, 1–66.
21. Deglaire A & Moughan PJ (2012) Animal models for determining amino acid digestibility in humans – a review. *Br J Nutr* **108**, Suppl. 2, S273–S281.
22. Baer DJ, Rumpler WV, Miles CW, *et al.* (1997) Dietary fiber decreases the metabolizable energy content and nutrient digestibility of mixed diets fed to humans. *J Nutr* **127**, 579–586.
23. National Research Council (1998) *Nutrient Requirements of Swine*, 10th rev. ed. Washington, DC: The National Academies Press.
24. Short FJ, Gorton P, Wiseman J, *et al.* (1996) Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Anim Feed Sci Technol* **59**, 215–221.
25. AOAC (2012) *Official Method of Analysis*, 19th ed. Rockville, MD: AOAC International.
26. Prosky L, Asp NG, Schweizer TF, *et al.* (1988) Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *J Assoc Off Anal Chem* **71**, 1017–1023.
27. Montoya CA, Rutherfurd SM & Moughan PJ (2016) Kiwifruit 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. *Br J Nutr* **115**, 1317–1324.
28. Healey G, Murphy R, Butts C, *et al.* (2017) Variability in gut microbiota response to an inulin-type fructan prebiotic within an *in vitro* three-stage continuous colonic model system. *Bioact Carbohydr Diet Fibre* **11**, 26–37.
29. Nadkarni MA, Martin FE, Jacques NA, *et al.* (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology* **148**, 257–266.
30. Kozich JJ, Westcott SL, Baxter NT, *et al.* (2013) Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl Environ Microbiol* **79**, 5112–5120.
31. Caporaso JG, Kuczynski J, Stombaugh J, *et al.* (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**, 335–336.
32. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461.
33. Edgar RC, Haas BJ, Clemente JC, *et al.* (2011) UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194–2200.
34. DeSantis TZ, Hugenholtz P, Larsen N, *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* **72**, 5069–5072.
35. Faith DP (1992) Conservation evaluation and phylogenetic diversity. *Biol Conserv* **61**, 1–10.
36. Awati A, Williams BA, Bosch MW, *et al.* (2006) Dietary carbohydrates with different rates of fermentation affect fermentation end-product profiles in different sites of gastro-intestinal tract of weaning piglet. *Anim Sci* **82**, 837–843.
37. Rowan AM, Moughan PJ & Wilson MN (1992) The flows of deoxyribonucleic acid and diaminopimelic acid and the digestibility of dietary fibre components at the terminal ileum, as indicators of microbial activity in the upper digestive tract of ileostomised pigs. *Anim Feed Sci Technol* **36**, 129–141.
38. Wedekind KJ, Mansfield HR & Montgomery L (1988) Enumeration and isolation of cellulolytic and hemicellulolytic bacteria from human feces. *Appl Environ Microbiol* **54**, 1530–1535.
39. Falony G, Joossens M, Vieira-Silva S, *et al.* (2016) Population-level analysis of gut microbiome variation. *Science* **352**, 560–564.
40. Cummings JH, Pomare EW, Branch WJ, *et al.* (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227.
41. Boets E, Gomand SV, Deroover L, *et al.* (2017) Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. *J Physiol* **595**, 541.
42. Barcenilla A, Pryde SE, Martin JC, *et al.* (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol* **66**, 1654–1661.
43. Walker AW, Duncan SH, Carol McWilliam Leitch E *et al.* (2005) pH and peptide supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial communities from the human colon. *Appl Environ Microbiol* **71**, 3692–3700.
44. Long W, Zhang C, Liu S, *et al.* (2017) Fiber-utilizing capacity varies in Prevotella- versus Bacteroides-dominated gut microbiota. *Sci Rep* **7**, 2594.
45. Louis P & Flint HJ (2017) Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol* **19**, 29–41.
46. Macfarlane GT, Gibson GR & Cummings JH (1992) Comparison of fermentation reactions in different regions of the human colon. *J Appl Bacteriol* **72**, 57–64.
47. Schmitt MG, Soergel KH, Wood CM, *et al.* (1977) Absorption of short-chain fatty acids from the human ileum. *Am J Dig Dis* **22**, 340–347.
48. McNeil NI, Cummings JH & James WP (1978) Short chain fatty acid absorption by the human large intestine. *Gut* **19**, 819–822.
49. Kaji I, Karaki S-i, Tanaka R, *et al.* (2011) Density distribution of free fatty acid receptor 2 (FFA2)-expressing and GLP-1-producing enteroendocrine L cells in human and rat lower intestine, and increased cell numbers after ingestion of fructo-oligosaccharide. *J Mol Histol* **42**, 27–38.
50. Kamath PS, Phillips SF & Zinsmeister AR (1988) Short-chain fatty acids stimulate ileal motility in humans. *Gastroenterology* **95**, 1496–1502.
51. Miller ER & Ullrey DE (1987) The pig as a model for human nutrition. *Annu Rev Nutr* **7**, 361–382.