The present study was designed to evaluate the effect of increased viscosity of the intestinal digesta on proliferation of enterotoxigenic Escherichia coli and the intestinal spirochaete Brachyspira pilosicoli in weaned pigs. Pigs were fed an experimental diet based on cooked white rice (R), which was supplemented with carboxymethylcellulose (CMC; 40 g/kg diet) to increase digesta viscosity. Thirty-six piglets weaned at 21 d of age were divided into six groups, three of which were fed R and three R + CMC: Addition of CMC increased digesta viscosity in the ileum \((P=0.01)\), caecum \((P=0.0007)\) and colon \((P=0.0035)\), without increasing indices of large intestinal fermentation. Pigs fed R + CMC developed a natural infection with enterotoxigenic E. coli after weaning and had more \((P<0.0001)\) diarrhoea than pigs fed R. Subsequent experimental infection of two groups of pigs with B. pilosicoli resulted in more \((P<0.0001)\) colonisation in pigs fed R + CMC than R. At this time, all pigs fed R + CMC had wetter \((P<0.0001)\) faeces than those fed R, irrespective of whether they were infected with B. pilosicoli, but infected pigs also had an increased \((P=0.025)\) number of days with diarrhoea post-infection irrespective of diet. In pigs fed R + CMC, it was not clear to what extent the increased viscosity associated with CMC, or the concurrent infection with enterotoxigenic E. coli, was responsible for the increased proliferation of B. pilosicoli. In a second experiment, five pigs that were weaned onto an R diet were transferred onto R + CMC 3 weeks later. These pigs did not develop a natural infection with enterotoxigenic E. coli after the diet change, confirming the particular susceptibility of pigs to enterotoxigenic E. coli proliferation immediately post-weaning.

Digesta viscosity: Carboxymethylcellulose: Escherichia coli: Brachyspira pilosicoli: Weaner-pig diarrhoea

Post-weaning colibacillosis (PWC) is a diarrhoeal disease of newly weaned pigs resulting from the action of certain serotypes of typically enterotoxigenic \(\beta\)-haemolytic Escherichia coli that proliferate in the small intestine. The proliferation of E. coli strains and the development of PWC can be manipulated to an extent by altering the composition, form and amount of the weaner diet fed (Palmer & Hulland, 1965; Smith & Halls, 1968; Bertschinger & Eggenberger, 1978; Hampson, 1987). A recent study in which weaner pigs were fed cooked rice-based diets (R) found that increasing the viscosity of the intestinal contents by addition of non-fermentable carboxymethylcellulose (CMC) to the diet was associated with an increase in faecal shedding of enterotoxigenic \(\beta\)-haemolytic E. coli and an increased occurrence of PWC (McDonald et al. 2001). As these pigs were not killed during the period of faecal shedding, it was not documented whether the bacteria were proliferating in the small intestine, and hence contributing to the aetiology of the diarrhoea, or were confined to the large intestine from where they were being shed as a result of CMC-induced diarrhoea.

Porcine intestinal spirochaetosis (PIS) occurs in weaner and grower pigs and is characterised by diarrhoea and a mild typhlocolitis. The condition results from colonisation of the large intestine with the anaerobic spirochaete Brachyspira (Serpulina) pilosicoli (Trott et al. 1996b;
Hampson & Trott, 1999). Infection can result in obvious signs of diarrhoea and/or weight loss, but, as with the E. coli strains that cause PWC, sub-clinical colonisation by B. pilosicoli also may occur (Taylor & Trott, 1997; Hampson & Trott, 1999). Recent work suggests that, as with PWC, the development of PIS can be affected by diet (Hampson et al. 2000). In the latter study, feeding pigs a highly digestible diet based on cooked white rice significantly reduced colonisation by B. pilosicoli compared with pigs fed a wheat-based diet. Similar results have been seen in pigs fed these diets and experimentally infected with the related intestinal spirochaete B. hyodysenteriae, the agent of swine dysentery (Pluske et al. 1996; Siba et al. 1996; Pluske et al. 1998).

The current study had two aims: first, to confirm the effect of increasing viscosity of the intestinal contents on stimulating proliferation of enterotoxigenic E. coli in the small intestine of weaner pigs and second, to examine whether increased viscosity and/or associated prior proliferation of enterotoxigenic E. coli would increase the susceptibility of pigs to PIS following experimental exposure to B. pilosicoli.

**Materials and methods**

**Permission**

This study was conducted with the approval of the Murdoch University Animal Ethics Committee.

**Animals**

In the first experiment, thirty-six Landrace × Large White female pigs from a commercial piggery were weaned at 21 d of age, transported to Murdoch University, stratified into equal live-weight groups, and then randomly allocated to one of the six groups outlined later. In the second experiment, another five pigs of the same age and source were obtained and housed in a single group.

**Diets and feeding**

Two diets were offered to the pigs in the first experiment (Table 1). The R diet comprised mainly white rice, cooked in an autoclave at 121°C for 15 min (water–dry rice (2 : 1, v/v)) and was balanced for nutritional requirements with an animal protein supplement (blood meal, meat and bone meal, fishmeal), which was mixed in with the cooked rice immediately prior to feeding. On an air-dry basis, this diet contained 2·5 g soluble NSP and 5·0 g insoluble NSP/kg, and it was offered to pigs in groups 1, 3, and 5. The R+carboxymethylcellulose (CMC) diet, which was offered to pigs in groups 2, 4 and 6, contained the same cooked white rice–animal protein base, with minor adjustments in the amounts of ingredients made for the inclusion of medium-viscosity CMC (20 g/l at 25°C has viscosity 0·4–0·8 Pa s; Sigma Aldrich C-4888 Sigma Chemical Co., St Louis, MO, USA) at 40 g/kg air-dry diet. The CMC was 100% soluble NSP. The CMC was added to the diet immediately prior to feeding. All groups were offered the same quantity of diet each day.

**Table 1. Composition and analysis of experimental diets (g/kg air-dry diet)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>R</th>
<th>R + CMC*</th>
<th>R1</th>
<th>R1 + CMC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>White rice†</td>
<td>699·4</td>
<td>661·8</td>
<td>744·8</td>
<td>704·8</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0·6</td>
<td>0·6</td>
<td>0·7</td>
<td>0·7</td>
</tr>
<tr>
<td>Lysine-HCl</td>
<td>0·00</td>
<td>0·00</td>
<td>0·05</td>
<td>0·05</td>
</tr>
<tr>
<td>Limestone</td>
<td>0·00</td>
<td>0·00</td>
<td>0·05</td>
<td>0·05</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0·4</td>
<td>0·4</td>
<td>0·5</td>
<td>0·5</td>
</tr>
<tr>
<td>Vitamin+mineral premix‡</td>
<td>1·5</td>
<td>1·5</td>
<td>3·0</td>
<td>3·0</td>
</tr>
<tr>
<td>Blood meal</td>
<td>25·3</td>
<td>25·1</td>
<td>37·0</td>
<td>37·0</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>3·6</td>
<td>3·6</td>
<td>69·1</td>
<td>69·1</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>151·7</td>
<td>150·5</td>
<td>90·1</td>
<td>90·1</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>0·00</td>
<td>0·00</td>
<td>4·00</td>
<td>4·00</td>
</tr>
<tr>
<td>Salt</td>
<td>0·00</td>
<td>0·00</td>
<td>0·40</td>
<td>0·40</td>
</tr>
<tr>
<td>Skimmed-milk powder</td>
<td>79·4</td>
<td>78·7</td>
<td>0·0</td>
<td>0·0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>17·9</td>
<td>17·8</td>
<td>9·7</td>
<td>9·7</td>
</tr>
<tr>
<td>Medium-viscosity CMC</td>
<td>0·00</td>
<td>4·00</td>
<td>0·00</td>
<td>4·00</td>
</tr>
<tr>
<td>Celite 545§§</td>
<td>20·0</td>
<td>20·0</td>
<td>0·0</td>
<td>0·0</td>
</tr>
</tbody>
</table>

**Calculated and chemical analysis**

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>R + CMC*</th>
<th>R1</th>
<th>R1 + CMC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE (MJ/kg)</td>
<td>14·9</td>
<td>14·4</td>
<td>15·01</td>
<td>15·01</td>
</tr>
<tr>
<td>Lysine (g/kg)</td>
<td>12</td>
<td>12</td>
<td>111</td>
<td>111</td>
</tr>
<tr>
<td>Crude protein (N × 6·25) (g/kg)</td>
<td>205·4</td>
<td>206·1</td>
<td>207·5</td>
<td>201·5</td>
</tr>
</tbody>
</table>

R, R1, rice-based diets; CMC, carboxymethylcellulose; DE, digestible energy.

*40 g CMC/kg air-dry diet.

†Sunwhite calrose, medium-grain.

‡Provided the following nutrients (mg/kg air-dry diet): retinyl acetate 3-44, cholecalciferol 0-065, α-tocopherol acetate 20, menadione 4·4, riboflavin 4, pyridoxine 1·6, cyanocobalamin 0·02, pantothenic acid 1·4, niacin acid 20, Co 0·2, I 0·6, Fe 120, Mn 60, Zn 100, Cu 10, Se 0·13.

§Catalogue no. 41,993-1; Aldrich Chemical Company, Milwaukee, WI, USA.
such that it was just finished by all groups 24 h later at the time the next feed was due. Water was available ad libitum.

Pigs in the second experiment (group 7) were initially fed a cooked rice-based diet (R1) and later transferred to the same diet containing 40 g CMC/kg (R1 + CMC). Both diets were similar to those fed in the first experiment (Table 1).

Experimental design

The pigs were housed in groups of six, with each pig in the group representing the experimental unit in relation to colonisation by pathogenic bacteria. Group housing was used to facilitate transmission of the pathogenic bacteria within the group. Pigs in the first experiment were housed in three rooms in an isolation animal house. Each room contained two groups of pigs held in adjacent pens, which were raised above the ground and had wire-mesh sides that allowed contact between the animals. In each room, one group was fed R and one group R + CMC. All pigs in groups 1 and 2 (room 1) were killed on day 8 after weaning, to investigate effects of digesta viscosity on intestinal haemolytic E. coli and gut measurements. Pigs in groups 3 and 4 (room 2) were simply fed their assigned diet for 3 weeks after weaning, then killed. Pigs in groups 5 and 6 (room 3) were orally inoculated with B. pilosicoli strain 95/1000 on days 8–10 after weaning and killed 4 weeks after weaning. In the second experiment, the pigs in group 7 were held in room 2 and killed 4 weeks after weaning. Pigs in groups 5 and 6 with strain 95/1000 was timed to start once natural colonisation with haemolytic E. coli had become established. Rectal swabs were taken before inoculation to check for the presence of any resident pathogenic intestinal spirochaetes. Pigs were orally inoculated with 100 ml broth containing 10⁸ cells B. pilosicoli strain 95/1000 per ml on days 8, 9 and 10 post-weaning. Faecal swabs from inoculated pigs then were cultured every 3 d to check for spirochaete shedding.

Swabs were streaked onto selective trypticase soy agar plates that contained: defibrinated ovine blood (50 g/l), spectinomycin (400 g/ml), colistin (25 g/ml) and vancomycin (25 g/ml) (Jenkinson & Wingar, 1981). These were incubated anaerobically for up to 2 weeks at 37°C. Spirochaete isolates were identified visually according to their growth, haemolytic pattern and morphological characteristics under phase-contrast microscopy of wet mounts of culture. Growth from the primary plate was harvested and subjected to a polymerase chain reaction amplification

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group no.</th>
<th>Diet*</th>
<th>Infected with Brachyspira pilosicoli</th>
<th>Time of study period post-weaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>R</td>
<td>No</td>
<td>8–9 d</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>R + CMC</td>
<td>No</td>
<td>8–9 d</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>R</td>
<td>No</td>
<td>3 weeks</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>R + CMC</td>
<td>No</td>
<td>3 weeks</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>R</td>
<td>Yes</td>
<td>4 weeks</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>R + CMC</td>
<td>Yes</td>
<td>4 weeks</td>
</tr>
<tr>
<td>2</td>
<td>7†</td>
<td>R1, then R1 + CMC</td>
<td>No</td>
<td>5 weeks</td>
</tr>
</tbody>
</table>

R, R1, rice-based diets; CMC, carboxymethylcellulose.
* For details of diets, see p. 524.
† Group 7 used in the second experiment. These pigs were fed R1 for 3 weeks after weaning, then transferred to R1 + CMC for 2 weeks.
of a portion of the 16S rRNA gene of B. pilosicoli, as previously described (Atyeo et al. 1998).

**Body weights and faecal consistency**

Live body-weight measurements were recorded once per week throughout the experiment. Faeces were collected each day from all pigs following weaning to determine DM content. Pigs and faeces were examined daily throughout the experiment to determine faecal consistency scores and presence of diarrhoea. During the first week after weaning, faecal measurements were determined from pigs in all groups. After groups 1 and 2 were killed on days 8–9 post-weaning, the faecal measurements for the next 2 weeks were only taken from pigs in groups 3–6. For the pigs in group 7, faecal measurements were taken daily for 2 weeks after weaning and for 2 weeks after transfer from diet R1 to R1 + CMC.

**Post-mortem sampling procedures and measurements**

On the sampling day allocated for each group, feed was offered to all pigs within that group 1.5 h before the first pig was killed. Each pig was killed by sodium barbiturate overdose and exsanguination. The abdominal cavity was then opened, the gastrointestinal tract removed, the small intestine, caecum and colon tied off, and their full and empty weights recorded. The small intestine was stripped free of its mesentery, laid out on a table and divided into three sections of equal length.

Swabs were rolled in the intestinal contents and along the adjacent section of intestinal wall. Swabs from the small intestine were cultured for *E. coli*, and those from the large intestine for both *E. coli* and *B. pilosicoli* where appropriate. Additional mucosal scrapings and digesta from the mid-small intestine and proximal colon were taken from pigs in groups 1 and 2 and used to determine viable colony counts of haemolytic *E. coli* following serial dilution, as described previously (McDonald et al. 1999). Records were made of the appearance of any gross lesions in the large intestine in pigs exposed to *B. pilosicoli*.

Samples of digesta were collected from the duodenum, ileum, caecum and colon for analysis of viscosity, DM content, and volatile fatty acids (VFA). A portion of digesta was analysed immediately for viscosity and the remaining sample was frozen at −20°C for subsequent analysis. The time at which the pig was killed was recorded and this range was used to observe the effect of time on the viscosity values.

**Analyses**

To determine VFA concentration, digesta samples from the ileum, caecum, proximal colon and distal colon were thawed to 4°C and diluted either 1:1 (w/v) (ileal digesta) or 1:2 (w/v) (caecal and colonic digesta) with 3·3 M-phosphoric acid before mixing, centrifuging and analysing the supernatant fraction using a Hewlett Packard 5890A capillary GC (Agilent Technologies, Forrest Hill, Victoria, Australia). The method used for VFA analysis was a modification of the method of Pethick et al. (1981), and is described in more detail in our previous publication (McDonald et al. 2001).

To determine viscosity, digesta samples from the small and large intestine were diluted 1:1 (v/v) with distilled water within 30 min of collection, mixed, and then centrifuged at 12 000 g for 8 min (Sigma bench top centrifuge 1–15; Quantum Scientific Pty Ltd, Milton, Queensland, Australia). The viscosity of 0·5 ml supernatant fraction was measured at 25°C, applying a shear rate of 60 s⁻¹ in a Brookfield LVDV-II+ cone-plate (CP40) rotational viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) for all samples. This procedure has been shown to provide the most appropriate results for these types of samples (McDonald et al. 2001). Diets and digesta containing CMC exhibited shear-thinning behaviour.

DM content was determined for faecal samples. Of each thawed digesta sample, 1–3 g was weighed accurately and placed on a foil tray of known weight. The tray and sample were oven-dried for 48 h at 105°C, re-weighed and the % DM calculated.

**Escherichia coli swab scoring**

The sheep blood agar plates were given a swab score according to the number of streaked sections that contained viable haemolytic *E. coli*, where: 0, no growth; 1, haemolytic *E. coli* in 1st section; 2, haemolytic *E. coli* in 2nd section; 3, haemolytic *E. coli* in 3rd section; 4, haemolytic *E. coli* in 4th section; 5, *E. coli* in the 5th section (usually with all the bacteria on the plate being haemolytic *E. coli*).

**Faecal consistency score**

Faeces were scored from 1–5 depending on their consistency, using the following criteria: 0, very hard, often pellet-like faeces; 1, well-formed faeces, firm to cut; 2, formed faeces, soft to cut; 3, faeces falling out of shape upon contact with surfaces, sloppy; 4, pasty diarrhoea; 5, liquid diarrhoea. An average score per pen was then calculated.

**Statistical analyses and presentation of results**

Dietary effects on any given measurement in pigs of the same age and experimental treatment were determined by performing an unpaired *t* test or simple regression analysis using the software package StatView 5.0 for Windows (1998; SAS Institute Inc., Cary, NC, USA). Viable counts of haemolytic *E. coli* were transformed logarithmically before analysis. Significant effects were determined at the 5% level. When there was the added variable of experimental infection with *B. pilosicoli*, the effects of both diet and infection on the measurement were elicited by employing ANOVA with two independent variables. The % DM of faecal samples were pooled according to the diet the pigs were fed (i.e. within the same pen) and dietary effects were compared using unpaired *t* test on each day. The number of pigs in the analyses changed according to the number of pigs remaining in the experiment at the time.
Results

Diarrhoea and faecal shedding of haemolytic Escherichia coli in the first week after weaning (groups 1–6)

Diarrhoea was observed in all three pens of pigs fed R + CMC from day 5 after weaning. Individual pigs had sloppy faeces and dirty perineal regions. In contrast, all the pigs fed R continued to have firm, well-formed faeces and had no evidence of diarrhoea. On day 7 after weaning, faecal % DM was greater ($P < 0.01$) for the eighteen pigs fed R (40.3 (SEM 2.0) %) than for the eighteen pigs fed R + CMC (27.5 (SEM 1.3) %). The onset of diarrhoea on day 5 after weaning was followed on day 6 by an increase in faecal shedding of haemolytic E. coli in pigs fed R + CMC (Fig. 1). The total faecal haemolytic E. coli swab score (mean value of the sum faecal swab score of each pig in the dietary group) was greater ($P < 0.0001$) for the pigs fed R + CMC (9.4 (SEM 1.3)) than for the pigs fed R (2.6 (SEM 0.8)). Overall, in the first week after weaning, the eighteen pigs eating R + CMC had a greater ($P < 0.0001$) number of days on which they had diarrhoea (1.2 (SEM 0.2) d) than those eating R (0.1 (SEM 0.1) d).

Intestinal viscosity and colonisation by haemolytic Escherichia coli in pigs killed 8–9 d after weaning (groups 1 and 2)

Throughout the intestinal tract, the digesta was more viscous, and there was greater intestinal colonisation by haemolytic E. coli, in pigs in group 2 receiving diet R + CMC than in pigs in group 1 fed R. All these differences were significant (Table 3). By regression analysis, the magnitude of duodenal viscosity accounted for over 50 % of the variation in the number of haemolytic E. coli at that site as assessed by intestinal swab scores ($P=0.0052; y = 0.004 + 0.428x, R^2=0.559$). The serotype of E. coli isolated from all pigs that had positive cultures of haemolytic E. coli was O149:K91:K88 and a representative strain was shown to have genes for enterotoxins LT, STa and STb. This serotype was also the predominant serotype associated with PWC on the pigs’ farm of origin.

Body growth and VFA production in pigs killed 8–9 days after weaning (groups 1 and 2)

In the first week after weaning, the pigs on both diets had similar growth rates (Table 3). There was relatively little fermentation of CMC, as judged by low VFA concentrations and pool sizes in the large intestine.

Results from uninfected pigs in groups 3 and 4

Pigs in groups 3 and 4 were not experimentally infected with B. pilosicoli and were kept for 3 weeks following weaning. The faeces of pigs eating R + CMC remained wetter in the second and third week after weaning than

Fig. 1. Faecal shedding of haemolytic Escherichia coli over 4 weeks following weaning in pigs fed rice-based diets (R) with or without the addition of 40 g carboxymethylcellulose (CMC)/kg diet. ○, All pigs fed R; ●, all pigs fed R + CMC; ▼, group 3; ▼, group 4; ▼, group 5; ▼, group 6. For details of diet see Table 1, and for details of group numbers, swab scoring system and procedures see pp. 524–525. Values are means with standard errors shown by vertical bars. Mean values significantly different from those of other dietary groups: *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$. 

Digesta viscosity and bacteria
those eating R. At 19 d post-weaning, the faecal % DM of pigs eating R + CMC (30·51 (SEM 0·88) %) was less (\(P = 0·0034\)) than that of the pigs eating R (44·24 (SEM 2·03) %).

In these pigs, shedding of haemolytic \textit{E. coli} decreased gradually from day 8 after weaning onwards, and ceased by day 14. From day 8 until the end of the experiment, the pigs eating R + CMC had a greater (\(P = 0·0024\)) average number of days of diarrhoea (2·5 (SEM 0·62) d) than the pigs eating R (0 d). The effects of CMC on faecal consistency and fermentation were the same 3 weeks after weaning as seen in pigs 1 week after weaning.

Shedding of haemolytic \textit{Escherichia coli} after the first week post-weaning (groups 3–6)

Pigs in groups 3–6 continued to shed haemolytic \textit{E. coli} for a few days after day 8, the first day pigs in groups 5 and 6 were experimentally infected with \textit{B. pilosicoli} (Fig. 1). After inoculation with \textit{B. pilosicoli} on the 8th day after weaning, pigs in group 6 (R + CMC) had both haemolytic \textit{E. coli} and \textit{B. pilosicoli} in their intestinal tracts for up to 6 d, until day 14. During this period, these pigs shed more haemolytic \textit{E. coli} than did pigs not infected with \textit{B. pilosicoli} but eating the same diet (group 4), pigs not infected with \textit{B. pilosicoli} and fed diet R (group 3) and pigs infected with \textit{B. pilosicoli} and eating R (group 5). All these differences were significant on days 9–11 (Fig. 1). The number of days of diarrhea post-infection with \textit{B. pilosicoli} was also extended in those pigs colonised with both \textit{B. pilosicoli} and haemolytic \textit{E. coli}. The distribution of diarrhoea days was intermittent and extended beyond the time when faecal shedding of haemolytic \textit{E. coli} ceased.

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Diet and group} & \textbf{R (group 1)} & \textbf{R + CMC (group 2)} & \textbf{Statistical significance of treatment:} \(P\) \\
\hline
\textbf{Mean} & \textbf{SEM} & \textbf{Mean} & \textbf{SEM} & \\
\hline
\textbf{Viscosity (mPa·s)} & & & & \\
Duodenum & 1·19 & 0·09 & 6·58 & 0·7 & 0·0001 \\
Ileum & 1·52 & 0·2 & 7·23 & 1·3 & 0·0020 \\
Colon & 1·26 & 0·2 & 2·80 & 0·7 & 0·0584 \\
\hline
\textbf{Haemolytic \textit{E. coli} (log_{10})}† & & & & \\
Jejunum & 1·49 & 0·6 & 5·79 & 0·4 & 0·0004 \\
Colon & 2·54 & 0·9 & 8·22 & 0·2 & 0·0002 \\
\hline
\textbf{Swab score‡} & & & & \\
Duodenum & 0·3 & 0·21 & 3·0 & 0·6 & 0·0025 \\
Ileum & 0·7 & 0·6 & 4·2 & 0·2 & <0·0001 \\
Caecum & 1·2 & 0·4 & 4·5 & 0·2 & <0·0001 \\
Faeces & 0·2 & 0·2 & 3·7 & 0·4 & <0·0001 \\
\textbf{Cumulative intestine§} & & & & \\
Weight (kg) & & & & \\
At weaning & 5·26 & 0·3 & 5·19 & 0·2 & 0·838 \\
Day 7 & 5·83 & 0·2 & 5·62 & 0·4 & 0·3673 \\
\hline
\textbf{VFA} & & & & \\
Large intestine pool (mmol/pig) & 5·54 & 1·0 & 5·76 & 0·6 & 0·846 \\
Ileum (mmol/kg wet digesta) & 7·94 & 2·8 & 12·5 & 2·8 & 0·3821 \\
Caecum (mmol/kg wet digesta) & 91·6 & 6·9 & 67·0 & 5·6 & 0·020 \\
\hline
\textbf{Colon} & & & & \\
Proximal (mmol/kg wet digesta) & 93·5 & 5·4 & 59·1 & 3·9 & 0·0004 \\
Distal (mmol/kg wet digesta) & 75·9 & 8·0 & 39·7 & 4·6 & 0·0018 \\
\hline
\end{tabular}
\caption{The effect of addition of 40 g CMC/kg to a cooked rice-based diet (R) on whole-body and intestinal growth, volatile fatty acid production, digesta viscosity and proliferation of haemolytic \textit{Escherichia coli} in the intestines of pigs in groups 1 and 2, killed 8–9 days post-weaning* (Mean values with their standard errors)}
\end{table}

\* For details of diets, groups and procedures, see Tables 1 and 2 and pp. 524–525.
† Viable counts (colony forming units/g mucosal scraping).
‡ Swab scores range from 0 to 5 according to the number of quadrants in which haemolytic \textit{E. coli} was cultured on sheep blood agar plates (For details, see p. 526).
§ Mean sum of scores for all intestinal sites for each pig on a particular diet.

\begin{figure}
\caption{Effect of diet and experimental infection on growth, diarrhoea and shedding of \textit{Brachyspira pilosicoli} pigs}
Pigs experimentally infected with \textit{B. pilosicoli} (groups 5 and 6) continued to gain weight after inoculation. The daily weight gain of infected pigs over the entire experiment was not affected by the presence of CMC in the diet, with both groups of pigs gaining on average between 252 and 253 g/d.

Including CMC in the diet increased (\(P = 0·0001\)) the incidence of diarrhoea in the period following the time of inoculation with \textit{B. pilosicoli} in both uninfected and \textit{B. pilosicoli}-infected pigs (Table 4). Experimental infection with \textit{B. pilosicoli} also resulted in an increase (\(P = 0·025\)) in the number of diarrhoea days with both

\begin{table}
\centering
\begin{tabular}{|l|l|}
\hline
\textbf{R, rice-based diet; CMC, carboxymethylcellulose; VFA, volatile fatty acid.} & \\
\textbf{* For details of diets, groups and procedures, see Tables 1 and 2 and pp. 524–525.} & \\
\textbf{† Viable counts (colony forming units/g mucosal scraping).} & \\
\textbf{‡ Swab scores range from 0 to 5 according to the number of quadrants in which haemolytic \textit{E. coli} was cultured on sheep blood agar plates (For details, see p. 526).} & \\
\textbf{§ Mean sum of scores for all intestinal sites for each pig on a particular diet.} & \\
\end{tabular}
\caption{The effect of addition of 40 g CMC/kg to a cooked rice-based diet (R) on whole-body and intestinal growth, volatile fatty acid production, digesta viscosity and proliferation of haemolytic \textit{Escherichia coli} in the intestines of pigs in groups 1 and 2, killed 8–9 days post-weaning* (Mean values with their standard errors)}
\end{table}
Diarrhoea post-infection (d)† | 0·0 | 2·5 | 0·7 | 4·8 | 1·1 | 0·0001 | 0·025 | 0·1918
Intestinal sites positive for | 0·0 | 0·0 | 1·3 | 1·2 | 0·4 | 0·7527 | 0·0001 | 0·753
B. pilosicoli§ (n) | 0·0 | 0·0 | 1·5 | 4·2 | 0·6 | 0·0001 | 0·0001 | 0·0001
Faeces positive for | B. pilosicoli§ (d) | 1·2 | 2·2 | 0·0 | 3·8 | 0·8 | 0·0001 | 0·6254 | 0·01
Faeces positive for haemolytic E. coli post-infection† | Viscosity (mPa·s) | ileum | 1·34 | 3·43 | 2·20 | 13·9 | 0·8 | 0·06 | 0·1216 | 0·1853
caecum | 1·47 | 3·48 | 2·13 | 4·51 | 0·6 | 0·0001 | 0·06 | 0·6695
proximal colon | 1·44 | 3·58 | 1·78 | 5·98 | 1·9 | 0·0003 | 0·06 | 0·1515
Faecal DM (%) | 41·77 | 28·70 | 37·90 | 26·83 | 4·1 | 0·0001 | 0·2639 | 0·6645
VFA (mmol/kg) | ileum | 15·3 | 41·0 | 13·2 | 20·7 | 5·5 | 0·01 | 0·0001 | 0·0288
caecum | 124·4 | 78·1 | 133·9 | 94·9 | 18·5 | 0·0007 | 0·3445 | 0·7316
proximal colon | 91·9 | 59·2 | 112·5 | 82·9 | 16·3 | 0·0035 | 0·029 | 0·8738

R, rice-based diet; CMC, carboxymethylcellulose; VFA, volatile fatty acid.
† For details of diets, groups and procedures, see Tables 1 and 2 and pp. 524–525.
‡ Infection with B. pilosicoli on days 8–10 after weaning.
§ Sites tested were the caecum, colon and faeces, with a maximum number of sites possible being four.
maximum number of sites possible was nine, as this is the number of days that these variables were tested or recorded for.
No. of days after experimental infection with B. pilosicoli that swabs were taken for culture of haemolytic E. coli, which was a maximum of ten times.

Diets. No significant interaction between diet and B. pilosicoli was found.

Of the days that faeces were cultured for B. pilosicoli, experimentally infected pigs fed R + CMC intermittently shed B. pilosicoli for a greater (P<0·0001) number of days (mean value 4·2 d) than infected pigs fed R (mean value 1·5 d) (Table 4). None of the control pigs shed B. pilosicoli. None of the pigs eating R displayed diarrhoea after the first week post-weaning.

Post mortem measurements in pigs from groups 3–6

The presence of CMC increased the viscosity of the contents of the ileum (P=0·06), caecum (P<0·0001) and colon (P=0·06) in both B. pilosicoli -infected and uninfected pigs 3 weeks post-weaning (Table 4). The large intestinal viscosity tended to be greater in infected pigs compared with uninfected pigs fed the same diet (P=0·06). The DM content of digesta was unaffected by the addition of CMC to the diet (results not shown). The faeces were wetter (P<0·0001) at slaughter in pigs fed R + CMC than in those fed R, but the DM was not further decreased upon infection with B. pilosicoli at this time. Intestinal fermentation (VFA concentration) at slaughter was greater in the ileum (P<0·0001), but not in the caecum and proximal colon of B. pilosicoli-infected pigs than in the uninfected pigs. Both uninfected and experimentally infected pigs eating R + CMC had lower VFA concentrations in all areas of the intestinal tract than pigs fed R (Table 4).

When the intestines were examined at post mortem, all infected pigs had hyperaemia and mild lesions in the colon, with individual variation, but no obvious differences between dietary treatments. At this time, there was no statistically significant effect of addition of dietary CMC on the presence of B. pilosicoli in the caecum or colon, with both dietary groups having five of six experimentally infected individuals with large intestinal cultures positive for B. pilosicoli.

The two groups of pigs that were kept as uninfected controls (groups 3 and 4), and simply fed diet R or R + CMC, did not display any colonic lesions consistent with PIS, nor were any of their faecal or colonic cultures positive for B. pilosicoli.

Second experiment (group 7)

No faecal shedding of haemolytic E. coli was detected in any of the five pigs whose diet was changed from R1 to R1 + CMC, either whilst eating R after weaning or after the dietary change to R1 + CMC 3 weeks later.

Discussion

Consistent with our previous findings (McDonald et al. 2001), addition of CMC to an experimental highly digestible cooked rice-based weaner diet (40 g/kg) resulted in an increased viscosity of the small and large intestinal contents, in the absence of an increase in fermentation in the large intestine. Previously, other studies have shown that increasing the viscosity of diets for grower pigs results in corresponding increases in the viscosity of the small intestinal contents for several hours after feeding (Chebur et al. 1990; Ellis et al. 1995; Ehrlein & Haas-Deppe, 1998; Ehrlein & Stockmann, 1998).

Again, as in our previous experiment (McDonald et al.
2001), a significant association was found between consuming CMC and an increased faecal shedding of enterotoxigenic haemolytic E. coli in pigs in the week after weaning. All pigs eating the viscous diet developed PWC, with a decrease in faecal DM and frank diarrhoea during the first week after weaning. The pigs in adjacent pens, eating the non-viscous R diet, remained relatively unaffected. The decrease in faecal DM in all pigs fed R + CMC preceded the faecal shedding of haemolytic E. coli, suggesting that changes in the intestinal environment created by feeding CMC predisposed to proliferation of the E. coli strain, and hence to PWC.

Assessment of a sub-set of pigs killed at 8–9 days after weaning (groups 1 and 2) confirmed that, in association with the increased faecal shedding, there was an increase in colonisation and proliferation of haemolytic E. coli in both the small and large intestines in pigs eating R + CMC. Furthermore, the increased small intestinal colonisation was significantly associated with an increase in viscosity in the duodenum. This finding demonstrates that both the intestinal environment and proliferation of pathogens in the intestine can be manipulated by diet, and also describes a potential ‘natural’ experimental model of PWC whereby endogenous strains of haemolytic E. coli can be stimulated to proliferate and cause diarrhoea by the addition of CMC to a rice-based diet. Conversely, the R diet did not support proliferation of these micro-organisms, suggesting that diets that result in low viscosity in the intestinal tract may provide protection from enterotoxigenic E. coli.

Although there was a clear link between increased digesta viscosity and proliferation of E. coli, the mechanisms by which these may be related were not investigated. There are numerous ways in which viscosity may have influenced the proliferation of intestinal E. coli. One strong possibility is that there was stasis of the viscous digesta overlying the epithelium in the small intestine, trapping substrate and potentially pathogenic E. coli in a viscous matrix in which the bacteria could rapidly multiply. This matrix has been called the ‘unstirred layer’ (Johnson & Gee, 1981). CMC has been shown to adhere to and thicken porcine mucin (Rossi et al., 1998). The fact that in the current experiment there was more VFA in the ileum and less in the large intestine of pigs receiving CMC is also consistent with there being additional trapping and bacterial utilisation of substrate in the small intestine in these pigs. In addition to local stasis, changes may have occurred in cell surface receptors or in the composition of the mucus, which in turn favoured colonisation. Recently, Fernandez et al. (2000) showed that addition of xylanase to cereal-based diets reduced viscosity in the intestinal tract of broiler chicks, through its action on viscous-forming NSP in the diets. The change in viscosity was also associated with increased crypt-surface glycosylation of sialic acid residues, and changes in the amount of neutral and acid mucins in the tract. Furthermore, following experimental challenge with Campylobacter jejuni, birds receiving xylanase had less intestinal colonisation than birds not receiving xylanase.

It is generally assumed that PWC is confined to the period immediately after weaning because proliferation of the associated E. coli strains is stimulated by changes in diet, altered intestinal structure, and the reduced intestinal function that occur at this time (Hampson, 1994). To test this further, five pigs in group 7 which had been weaned onto the R1 diet, and which did not develop PWC, were transferred to R1 + CMC after 3 weeks. In this case, the change of diet and increased viscosity did not induce shedding of haemolytic E. coli. This result emphasises the particular susceptibility of the porcine small intestine to proliferation of these bacteria in the period immediate post-weaning.

Inoculation of pigs with B. pilosicoli was successful as a means of colonising the large intestines in both dietary groups, and led to an increase in the number of diarrhoea days in infected pigs. Feeding R + CMC compared with R, however, also led independently to more and earlier faecal shedding of B. pilosicoli, as well as to more diarrhoea in both infected and uninfected pigs. The infected pigs eating R + CMC already had sloppy faeces from the presence of CMC in the diet and from the prior enterotoxigenic E. coli infection. The dual colonisation with haemolytic E. coli and B. pilosicoli in the presence of CMC also was associated with increased shedding of haemolytic E. coli. From these observations it is not possible to dissect out the extent to which the activity of the enterotoxigenic E. coli or the increased digesta viscosity due to CMC in the diet contributed to the proliferation of B. pilosicoli. Both may contribute independently. Interestingly, in a recent experiment in layer hens we have found that addition of xylanase to a wheat-based diet resulted in significantly less caecal colonisation with the intestinal spirochaete B. intermedia (Hampson et al. 2002). This result tends to support the likelihood that increased digesta viscosity per se can increase colonisation of the large intestine with intestinal spirochaetes. To investigate this further, it will be necessary to experimentally infect older pigs fed R or R + CMC with B. pilosicoli, after their susceptibility to E. coli infections has diminished.

The viscosity of large intestinal contents is dramatically affected by % DM, and increases as the % DM increases (McRorie et al. 1998, 2000). In the current experiment, there was no significant difference in the % DM of caecal and colonic digesta of infected or uninfected pigs, although digesta of infected pigs fed the R + CMC diet tended to be wetter than those fed R. The increase in viscosity was therefore not a result of different water contents.

The number of days that faecal swabs were positive for B. pilosicoli in pigs fed R (mean 1.5 out of 9 d tested) was identical to that recorded in an earlier study feeding a very similar cooked rice diet to pigs experimentally infected with B. pilosicoli (Hampson et al. 2000). Feeding the R diet thus produced a consistent response to B. pilosicoli infection across experimental trials. In the same study, the mean number of B. pilosicoli-positive faecal shedding days for pigs fed a commercial diet based on wheat and
lupins was 5.3. In the current experiment, the mean number of *B. pilosicoli*-positive faecal shedding days for pigs fed R + CMC was 4.2, approaching that found in pigs fed the wheat–lupin-based diet used in the previous experiment (Hampson et al. 2000). This result suggests that the viscosity generated by CMC exerts similar properties to, or possibly some of the same properties of, the natural dietary fibre that is present in commercial pig diets.

Pigs with PIS were found to have a greater microbial fermentation in the large intestine compared with the healthy controls. In previous studies, increased large intestinal fermentation has been shown to predispose pigs to development of swine dysentery, a severe colonic infection caused by *B. hyodysenteriae*, an intestinal spirochaete which is similar to *B. pilosicoli* (Pluske et al. 1996). Hence the increased fermentation recorded here may have enhanced colonisation by *B. pilosicoli*. On the other hand, the presence of increased levels of VFA in the large intestine could have been the result of an inability to effectively absorb microbial end products due to the mucosa being damaged by the spirochaete.

Increased viscosity of the intestinal contents could influence the pathogenesis of PIS in a way similar to that proposed for PWC, for example as a result of the viscous digesta being retained for longer within the large intestine, and providing additional time and substrate for bacterial growth. Alternatively, it might have had an effect by increasing the thickness or altering the composition of the mucus overlying the epithelium and crypts of the large intestine. Recent studies suggest that some components of mucus may be chemotactic for *B. pilosicoli* (Witters & Duhamel, 1999). Similarly, *B. hyodysenteriae* is attracted to mucin by chemotaxis, and can move freely through the mucus layer to reach the epithelium (Milner & Sellwood, 1994). As a result of these similarities, it is possible that CMC also would exacerbate expression of swine dysentery.

In conclusion, increasing the viscosity of the intestinal contents in young weaned pigs encouraged proliferation of haemolytic *E. coli* in the small intestine, and subsequent colonisation by *B. pilosicoli* in the large intestine. These results were consistent with studies in broiler chicks where reductions in digesta viscosity resulted in reduced colonisation with *C. jejuni* (Fernandez et al. 2000). The precise mechanisms by which viscosity influences proliferation of enteropathogenic bacteria remain uncertain, and require further study. Nevertheless, it would appear that dietary and other interventions that result in reduced viscosity of the digesta could be beneficial to intestinal health. The bacterial species that have been influenced by viscosity are very different from each other, and colonise different parts of the tract. Strains of all these bacterial species can colonise human subjects, and the present study suggests that examination of diet and digesta viscosity should also be made in the context of human enteric bacterial infections.

Acknowledgements

The present study was supported by a grant from the former Australian Pig Research and Development Corporation (now Australian Pork Limited). D. E. H. (née McDonald) was in receipt of a postgraduate scholarship from the former Corporation. We thank Sophy Oxberry and Malcolm Boyce for technical assistance, Dr Bruce Mullan for formulating the diets, and Professor Carlton Gyles for assisting in characterisation of the *E. coli* toxin and adhesin genes.

References


Hampson DJ, Robertson ID, La T, Oxberry SL & Pethick DW (2000) Influences of diet and vaccination on colonisation of pigs by the intestinal spirochaete *Brachyspira (Serpulina) pilosicoli*. *Veterinary Microbiology* 73, 75–84.

Hampson DJ, Phillips ND & Pluske JR (2002) Dietary enzyme
and zinc bacitracin inhibit colonisation of layer hens by the intestinal spirochaete Brachyspira intermedia. Veterinary Microbiology 86, 351–360.


Siba PM, Pethick DW & Hampson DJ (1996) Pigs experimentally infected with Serpulina hydysenteriae can be protected from developing swine dysentery by feeding them a highly digestible diet. Epidemiology and Infection 116, 207–216.


Trott DJ, Huxtable CR & Hampson DJ (1996a) Experimental infection of newly weaned pigs with human and porcine strains of Serpulina pilosicoli. Infection and Immunity 64, 4648–4654.
