British Journal of Nutrition (2002), **88**, 523–532
DOI: 10.1079/BJN2002694

Increasing the viscosity of the intestinal contents stimulates proliferation of enterotoxigenic *Escherichia coli* and *Brachyspira pilosicoli* in weaner pigs

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(Received 14 November 2001 – Revised 22 May 2002 – Accepted 18 June 2002)

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 ($P=0{\cdot}01$), caecum ($P=0{\cdot}0007$) and colon ($P=0{\cdot}0035$), without increasing indices of large intestinal fermentation. Pigs fed R + CMC developed a natural infection with enterotoxigenic *E. coli* after weaning and had more ($P<0{\cdot}0001$) diarrhoea than pigs fed R. Subsequent experimental infection of two groups of pigs with *B. pilosicoli* resulted in more ($P<0{\cdot}0001$) colonisation in pigs fed R + CMC than R. At this time, all pigs fed R + CMC had wetter ($P<0{\cdot}0001$) faeces than those fed R, irrespective of whether they were infected with *B. pilosicoli*, but infected pigs also had an increased ($P=0{\cdot}025$) 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 β-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 β-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.* 1996*b*;

Abbreviations: CMC, carboxymethylcellulose; PIS, porcine intestinal spirochaetosis; PWC, post-weaning colibacillosis; R, cooked rice-based diet; VFA, volatile fatty acid.

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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)

	Diet			
	R	R + CMC*	R1	R1 + CMC*
Ingredient				
White rice†	699·4	661·8	744·8	704·8
L-Threonine	0·6	0·6	0·7	0·7
L Lysine-HCl	0·00	0·00	0·05	0·05
Limestone	0·00	0·00	0·05	0·05
Choline chloride	0·4	0·4	0·5	0·5
Vitamin+mineral premix‡	1·5	1·5	3·0	3·0
Blood meal	25·3	25·1	37·0	37·0
Meat and bone meal	3·6	3·6	69·1	69·1
Fishmeal	151·7	150·5	90·1	90·1
Soyabean meal	0·00	0·00	4·00	4·00
Salt	0·00	0·00	0·40	0·40
Skimmed-milk powder	79·4	78·7	0·0	0·0
Dicalcium phosphate	17·9	17·8	9·7	9·7
Medium-viscosity CMC	0·00	4·00	0·00	4·00
Celite 545®§	20·0	20·0	0·0	0·0
Calculated and chemical analysis				
DE (MJ/kg)	14·9	14·4	15·01	15·01
Lysine (g/kg)	12	12	111	111
Crude protein (N × 6·25) (g/kg)	205·4	206·1	207·5	201·5

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-tocopheryl acetate 20, menadione 4·4, riboflavin 4, pyridoxine 1·6, cyanocobalamin 0·02, pantothenic acid 14, nicotinic 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 were fed R1 for 3 weeks, then transferred to diet R1 + CMC for 2 weeks. They were not inoculated with *B. pilosicoli*, nor killed. The second experiment aimed to test whether increased viscosity of the intestinal contents stimulated proliferation of haemolytic *E. coli* in pigs that had been weaned for several weeks. The experimental design is summarised in Table 2.

Detection of haemolytic Escherichia coli

Rectal swabs were taken daily from pigs in all groups for the first week after weaning to detect the presence of haemolytic *E. coli*. For the subsequent 2 weeks, pigs in groups 3–6 continued to have rectal swabs taken daily. Pigs in group 7 had swabs taken daily for 2 weeks after weaning and then for 2 weeks after their transfer to diet R1 + CMC. Similar quantities of faeces collected onto the rectal swabs from each pig were inoculated onto sheep blood (50 ml/l) agar plates (Columbia base; Oxoid, London, UK) and streaked out to obtain single colonies. The plates were incubated at 37°C overnight and then assessed for the presence of β-haemolytic colonies with a morphology characteristic of *E. coli*. Representative positive colonies were stored on nutrient agar slopes before being checked for serotype and common biochemical characteristics. Isolates of *E. coli* were sent to the National *E. coli* Laboratory at the Department of Natural Resources and Environment, (Bendigo, Victoria, Australia) for serotyping by slide co-agglutination (Hampson *et al.* 1993). A representative isolate of the single predominant serotype was sent to Professor C. L. Gyles at the Ontario Veterinary College (Guelph, Canada) for identification of toxin and adhesin genes by DNA probe and polymerase chain reaction analysis (Hammermueller *et al.* 1995).

Infection with Brachyspira pilosicoli

B. pilosicoli strain 95/1000 was revived from frozen stock cultures held at the Australian Reference Centre for Intestinal Spirochaetes at Murdoch University. The strain had originally been isolated from an Australian pig with PIS, and previously had been used to reproduce PIS in experimentally infected weaners (Trott *et al.* 1996a). The strain was grown to mid-log phase in Kunkle's pre-reduced anaerobic broth (Kunkle *et al.* 1986). Oral inoculation of 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 10^8 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 2. Experimental groups and their allocated treatments

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

R, R1, riced-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 5890 A 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 ($P<0{\cdot}01$) 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 ($P<0{\cdot}0001$) 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 ($P<0{\cdot}0001$) 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 ($P=0{\cdot}0052$; $y = 0{\cdot}004 + 0{\cdot}428x$, $R^2 0{\cdot}559$). 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

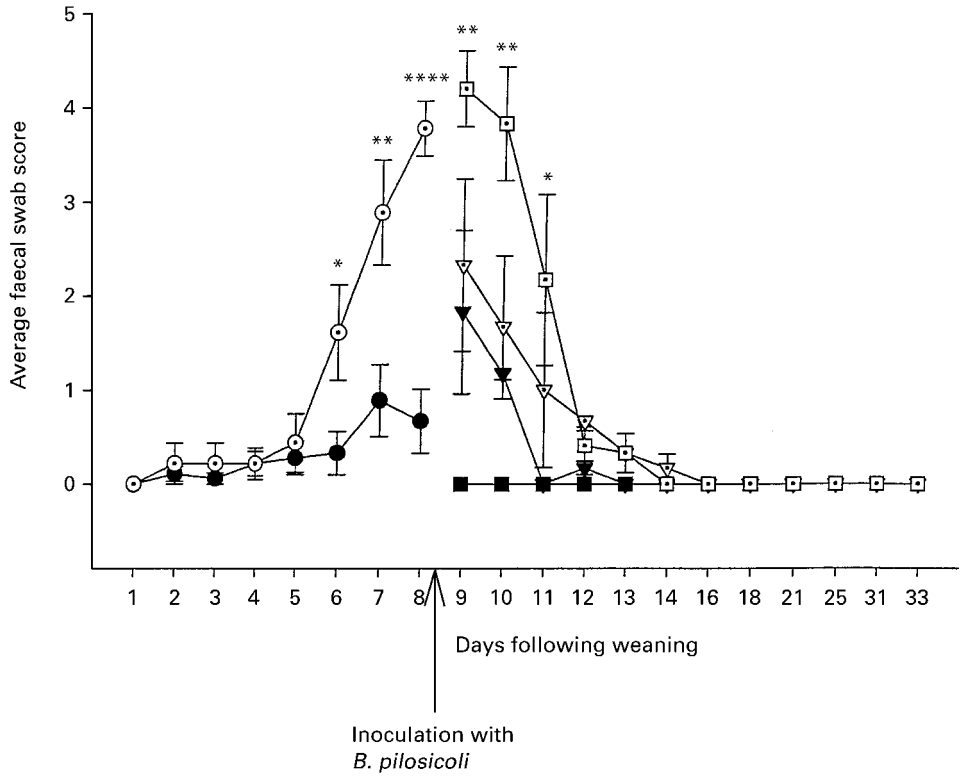

Fig. 1. Faecal shedding of haemolytic *Escherichia coli* over 4 weeks following weaning in pigs fed rice-based diets (R) with or without the addition of 40 g carboxymethylcellulose (CMC)/kg diet. ⊙, All pigs fed R; ●, all pigs fed R + CMC; ▼, group 3; ▽, group 4; ■, group 5; ⊡, group 6. For details of 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: *$P<0{\cdot}05$, **$P<0{\cdot}01$, ****$P<0{\cdot}0001$.

Table 3. 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 *Escherichia coli* in the intestines of pigs in groups 1 and 2, killed 8–9 days post-weaning*

(Mean values with their standard errors)

	Diet and group				
	R (group 1)		R + CMC (group 2)		Statistical significance of treatment: *P*
	Mean	SEM	Mean	SEM	
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
Haemolytic *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
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
Cumulative intestine§	2·3	0·6	15·3	1·3	<0·0001
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
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
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

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.
† Viable counts (colony forming units/g mucosal scraping).
‡ Swab scores range from 0 to 5 according to the number of quadrants in which haemolytic *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.

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 *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 Escherichia coli *after the first week post-weaning (groups 3–6)*

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

Effect of diet and experimental infection on growth, diarrhoea and shedding of Brachyspira pilosicoli

Pigs experimentally infected with *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 *B. pilosicoli* in both uninfected and *B. pilosicoli*-infected pigs (Table 4). Experimental infection with *B. pilosicoli* also resulted in an increase ($P=0·025$) in the number of diarrhoea days with both

Table 4. Effect of diet on shedding of haemolytic *Escherichia coli* and *Brachyspira pilosicoli*, and digesta viscosity, faecal % DM and volatile fatty acid concentrations (mmol/kg wet digesta) in intestinal contents from uninfected pigs (groups 3 and 4) and pigs infected with *B. pilosicoli* (groups 5 and 6)*

Diet. *B. pilosicoli* infection	Group 3 R No	Group 4 R + CMC No	Group 5 R Yes	Group 6 R + CMC Yes	SED	Statistical significance of effect: *P*		
						Diet	*B. pilosicoli* infection	Diet × *B. pilosicoli* infection
Diarrhoea post-infection (d)†	0·0	2·5	0·7	4·8	1·1	0·0001	0·025	0·1918
Intestinal sites positive for *B. pilosicoli*‡ (*n*)	0·0	0·0	1·3	1·2	0·4	0·7527	0·0001	0·753
Faeces positive for *B. pilosicoli*§ (d)	0·0	0·0	1·5	4·2	0·6	0·0001	0·0001	0·0001
Faeces positive for haemolytic *E. coli* post-infection‖ (d)	1·2	2·2	0·0	3·8	0·8	0·0001	0·6254	0·01
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	14·2	45·3	28·7	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 days 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 ($P<0{\cdot}0001$) 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 ($P=0{\cdot}06$), caecum ($P<0{\cdot}0001$) and colon ($P=0{\cdot}06$) 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 ($P=0{\cdot}06$). The DM content of digesta was unaffected by the addition of CMC to the diet (results not shown). The faeces were wetter ($P<0{\cdot}0001$) 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 ($P<0{\cdot}0001$), 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 (Cherbut *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.* 1996), and may also alter its composition. Consistent with this, it has been shown that feeding broiler chicks diets containing CMC increases total microbial counts in the duodenum and jejunum (Smits *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.

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