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Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly-weaned pigs

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Sources of viscous soluble fibre, such as barley and oats, have often been included in the weaning diet of the pig to accelerate development of the large intestine. Inclusion of a non-fermentable, viscous compound, sodium carboxymethylcellulose (CMC), in a low-fibre weaning diet was tested to assess the influence of digesta viscosity on the gut in the absence of increased fermentation. Two CMC sources, of low and high viscosity, were added to cooked rice-based diet at 40 g/kg total diet. A third control rice diet did not contain any CMC. Diets were fed for 13 d following weaning at 3 weeks of age. Addition of CMC to the diet significantly increased the intestinal viscosity of digesta within the small ($P<0{\cdot}001$) and large ($P<0{\cdot}05$) intestine. No simple association was found between increases in intestinal viscosity and effects on intestinal morphology and whole-body growth. The average empty-body-weight gain and the small intestinal villus height increased with low-viscosity CMC, but decreased with the high-viscosity CMC group. The full large intestinal weight increased in all pigs fed CMC. Dietary CMC (both low- and high-viscosity) increased the percentage moisture of digesta and faeces, and was associated with increased faecal shedding of enterotoxigenic haemolytic *Escherichia coli*. Feed ingredients in weaning diets that excessively increase the viscosity of the intestinal digesta may be detrimental to pig health and production.

Intestinal adaptation: Viscosity: Carboxymethylcellulose: *Escherichia coli*: Pig

There is increasing evidence that viscous dietary soluble NSP can interfere with nutrient digestion and absorption in single-stomached animals, although consequences differ between species. In poultry, addition of cereals containing high amounts of viscous soluble NSP to the diet depresses nutrient utilisation within the small intestine, reduces weight gain and can increase the incidence of wet, sticky droppings (Choct & Annison, 1992). The anti-nutritive effects of viscous soluble NSP in poultry have been linked primarily to increased viscosity of digesta within the small intestine. Increased intestinal viscosity is caused by the presence of soluble NSP, and the association with anti-nutritive effects is supported by the removal of detrimental effects through addition of exogenous enzymes that reduce viscosity by partial hydrolysis of the soluble NSP (Burnett, 1966; Campbell *et al.* 1989; Bedford & Classen, 1992; Choct & Annison, 1992; Choct *et al.* 1996).

A similar interference with nutrient absorption has been noted in pigs fed synthetic viscous NSP such as guar gum (Rainbird *et al.* 1984; Rainbird, 1986; Rainbird & Low, 1986*b*; Ellis *et al.* 1991, 1995; Ehrlein & Stockmann, 1998*a*,*b*). Increasing intestinal viscosity in pigs can decrease absorption of glucose (Rainbird *et al.* 1984) and other nutrients (Ehrlein & Stockmann, 1998*b*) as well as altering the force and frequency of peristaltic contractions (Cherbut *et al.* 1990). Although these studies were carried out in grower pigs (weighing about 40 kg) used as biomedical models, there is evidence that viscous soluble dietary NSP also may have detrimental effects in the small intestine of weaner pigs (McDonald *et al.* 1999). Increased intestinal viscosity is more likely to have an impact on weaner pigs than older pigs, as the physical capacity and therefore the dilution of viscosity by intestinal secretions is less in newly-weaned pigs.

The anti-nutritive effects of soluble NSP in poultry feeds also can be partially alleviated by feeding antibiotics, especially when the soluble NSP are from rye, which suggests that gastrointestinal microflora play a major role in

Abbreviations: CMC, carboxymethylcellulose; PWC, post-weaning colibacillosis; VFA, volatile fatty acid.

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the anti-nutritional influence (MacAuliffe & McGinnis, 1971; Misir & Marquardt, 1978; Antonious & Marquardt, 1982). Recent studies have shown a complex interaction among the microflora of the gut, intestinal viscosity and dietary soluble NSP, where both increased microbial fermentation (Choct *et al.* 1996) and increased microbial numbers in the small intestine (Smits *et al.* 1998) appear to be either the result of, or a contributing factor to, the deleterious effects caused by elevated intestinal viscosity. Langhout *et al.* (2000) demonstrated that microbial fermentation, intestinal viscosity and associated anti-nutritional effects were higher in conventionally raised as opposed to germ-free chickens when fed a synthetic viscous compound.

The present authors are not aware of any quantitative studies examining the effect of viscosity on the intestinal microflora of pigs. However, it has been noted that increased viscosity slows transit time of digesta through the upper small intestine of pigs (Cherbut *et al.* 1990), which would provide more time for microbial pathogens to proliferate. Increased intestinal proliferation of pathogenic *Escherichia coli* has been seen with addition of either guar gum (McDonald *et al.* 1999) or pearl barley (McDonald *et al.* 2000) to the diet of weaner pigs experimentally infected with enterotoxigenic haemolytic *E. coli*. These two types of soluble NSP were both highly fermentable and viscous in nature, which raises the question of whether fermentability, viscosity or a combination of both is likely to influence the porcine small intestinal microflora. Post-weaning colibacillosis (PWC) is the most common intestinal disorder of pigs in the immediate post-weaning period, and is associated with proliferation of enterotoxigenic haemolytic *E. coli* in the small intestine (Gyles, 1993).

The aim of the present study was to record the effects of increased intestinal viscosity on the intestinal tract in weaner pigs in isolation from the influence of increased fermentation. This was achieved by feeding weaner pigs a low-fibre rice-based experimental diet supplemented with carboxymethylcellulose (CMC), a water-soluble synthetic viscous polysaccharide resistant to microbial fermentation. Piglet growth and faecal excretion of haemolytic *E. coli* were monitored over a 13 d period before the pigs were slaughtered and carcass and gastrointestinal variables measured.

Materials and methods

Diets

Three experimental diets based on cooked white rice were used (presented in Table 1). The rice-without-CMC diet comprised mainly cooked medium-grain white rice (Sunwhite Calrose®; Ricegrowers Cooperative, Leeton, NSW, Australia), balanced for weaner nutritional requirements with an animal protein supplement. The other two diets contained cooked white rice plus an animal protein supplement with the addition of either a low-viscosity CMC (rice + low-viscosity CMC; 50–200 mPa·s for a solution of 40 g/l at 25°C, Sigma Aldrich C-5678; Sigma Chemical Co., St. Louis, MO, USA) or a medium viscosity CMC (referred to as rice + high-viscosity CMC in this trial; 400–800 mPa·s for a solution of 20 g/l at 25°C, Sigma Aldrich C-4888; Sigma Chemical Co.) respectively. The inclusion of CMC was 40 g/kg air-dry diet. Minor adjustments were made in amounts of ingredients in diets rice + low-viscosity CMC and rice + high-viscosity CMC to ensure

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

	Dietary group		
Ingredient	Rice	Rice + low-viscosity CMC	Rice + high-viscosity CMC
White rice	699·4	661·8	661·8
L-Threonine	0·6	0·6	0·6
Choline chloride	0·4	0·4	0·4
Vitamin+mineral premix*	1·5	1·5	1·5
Blood meal	25·3	25·1	25·1
Meat and bone meal	3·6	3·6	3·6
Fish meal	151·7	150·5	150·5
Skimmed-milk powder	79·4	78·7	78·7
Dicalcium phosphate	17·9	17·8	17·8
Low-viscosity CMC	–	40·0	–
Medium-viscosity CMC	–	–	40·0
Celite 545®†	20·0	20·0	20·0
Calculated analysis			
DE (MJ/kg)	14·9	14·4	14·4
Lysine	12	12	12
Chemical analysis (%)			
Crude protein	19·8	19·2	19·2
Crude fibre	0·4	0·4	0·4

CMC, carboxymethylcellulose; DE, digestible energy.

* Provided the following nutrients (mg/kg air-dry diet): retinyl acetate 3·44, cholecalciferol 0·065, α-tocophenyl 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.

† Celite 545 (Aldrich Chemical Company, Milwaukee, USA, cat. no. 41,993-1).

energy and protein content were as close as possible to the rice diet without CMC.

Animals

Thirty-two female pigs were weaned at 19–21 d of age. Eight pigs were killed on the day of weaning for measurement of intestinal organ and carcass weight. Twenty-four pigs were transported to the Medina Research Station, Perth, Western Australia, where they were allocated to one of the three dietary groups such that the average weight was similar per group. Pigs were housed in pairs within elevated pens, and fed *ad libitum* for 13 d after weaning. Daily individual body weights and pen voluntary food intake records were kept, and faecal swabs were cultured for presence of haemolytic *E. coli* on days 8 to 12 post-weaning. One pig from the rice + high-viscosity CMC group was excluded as it failed to eat and lost body condition. At the end of the 13 d period, the pigs were killed 1·5 h after feeding. Pigs were fed at staggered intervals for 5 d beforehand so they would be accustomed to their feeding time. Ethical approval was obtained for this study from the Animal Experimentation Ethics Committee (98MD18).

Collection of intestinal samples

Pigs were fed their morning meal on the day of sampling. Live weight was recorded for each pig immediately before administering a single intramuscular injection of ketamine (20 mg/kg, 100 mg/ml; Ketamil, Ilium; Troy Laboratories Pty. Ltd., Smithfield, NSW, Australia) combined with xylazine (2·2 mg/kg, 20 mg/ml; Xylazil-20, Ilium) to induce general anaesthesia. Once the pig was satisfactorily anaesthetised, a lethal dose of sodium barbiturate solution was injected into the heart. Cervical dislocation and exsanguination followed, reducing the amount of blood present as a potential contaminant during the collection of samples. Faecal samples were collected and the gastrointestinal tract was removed and divided into four segments (stomach, small intestine, caecum and colon), which were tied off with string before separation. The full weight of each segment was weighed. The small intestine was stripped free of its mesentery, laid out on a table and divided into three sections of equal length using the same procedure.

Keyhole incisions were made and separate sterile swabs placed in the digesta and wiped along the mucosal surface of the duodenum, ileum, caecum and rectum before rolling onto blood agar plates (50 g defibrinated sheep blood/l, trypticase soyabean agar, 40 g/l; Becton Dickinson and Co., Cockeysville, MD, USA). All plates were kept at room temperature until they were streaked out for single colonies and placed in a hot room at 37°C to incubate overnight in air. Digesta samples were collected into jars from the first and third sections of the small intestine, and a section of the small intestinal wall was collected from the mid-point of the middle section and placed into formalin (100 ml/l) for subsequent processing and histological measurement.

The colon was also divided into the proximal and distal colon. Proximal colonic digesta were collected from the section of colon closest to the caecum, while distal colonic digesta were taken from the apex of the spiral colon. The pH of digesta was measured immediately after removal of digesta from the intestine by inserting the electrode of a portable pH meter (Schindengen pH Boy-2; Schindengen Electric MFG, Tokyo, Japan) into the collected sample. Samples were kept on ice until transferred to a freezer at −20°C within 2 h. Each intestinal segment was then washed and blotted dry and an empty segment weight measured.

Histological sections were prepared and intestinal morphology recorded using an image analyser (Optimas; a.e.s., Victoria Park, Western Australia, Australia), as previously described (McDonald *et al.* 1999). In addition to measurements of small intestinal villus height and crypt depth, the total cross-sectional width of the underlying muscle layers was recorded, and the villus height:crypt depth ratio was calculated.

Microbiological assessment

Following overnight incubation, blood agar plates were assessed for the presence of β-haemolytic colonies with a morphology characteristic of *E. coli*. To allow a quantitative comparison between groups, a visual estimation was made of the proportion of viable bacterial colonies that were β-haemolytic colonies characteristic of *E. coli*. This estimation was expressed as % total bacterial population on the plate. Biochemical and serological tests were conducted on representative colony-forming units from each pig to confirm identification. Isolates of *E. coli* from each pig positive for *E. coli* were transferred to sterile nutrient agar slopes and sent to the National *E. coli* Laboratory at the Department of Natural Resources and Environment Agriculture, Bendigo, Victoria, Australia, for serotyping by slide co-agglutination (Hampson *et al.* 1993).

Analyses

The % DM of the digesta and faecal samples was determined by weighing each sample, oven-drying for 48 h at 105°C, then reweighing. To determine volatile fatty acid (VFA) concentration (C_2:C_6), thawed digesta samples from the ileum, caecum, proximal colon and distal colon were diluted either 1:1 (w/v) (ileal digesta) or 1:2 (w/v) (caecal and colonic digesta) with 3·3 M-phosphoric acid, mixed, centrifuged and the supernatant fraction analysed chromatographically. The supernatant fraction (0·1 ml) was added to 1 ml internal standard solution containing valeric acid before processing on a capillary GC. A working standard and a control (distilled water) were included in each assay run, where the working standard contained acetic acid (60 mM), propionic acid (20 mM), isobutyric acid (6·67 mM), butyric acid (20 mM), isovaleric acid (10 mM), valeric acid (10 mM), and caproic acid (4 mM). The Hewlett Packard 5890A capillary GC (Agilent Technologies, Forrest Hill, Victoria, Australia) was maintained at injector and detector FID settings of 260°C and 265°C respectively, an initial and final oven temperature of 120°C and 240°C, a carrier gas flow rate of 5 ml/min and a split-flow rate of 70 ml/min. The Hewlett Packard Chemstation integration system was used to calculate the VFA concentration from the area of the peaks.

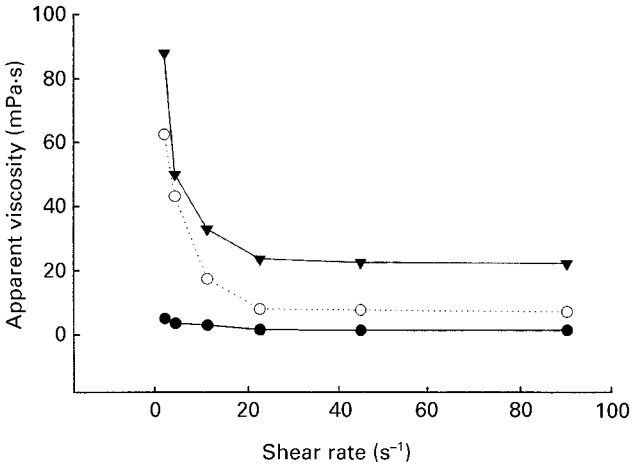

Fig. 1. Effect of shear rate on apparent viscosity of cooked rice-based diets at 25°C containing no carboxymethylcellulose (●) (*n* 8), low-viscosity carboxymethylcellulose (○) (*n* 8), or high-viscosity carboxymethylcellulose (▼) (*n* 7), where carboxymethylcellulose is included at 40 g/kg diet. For details of diets and procedures, see Table 1 and pp. 488–490.

Viscosity

Digesta samples taken from the large intestine and from pigs fed the rice diet were very dry and it was difficult to obtain more than 0·1 ml supernatant fraction from the samples by simply centrifuging the digesta sample. Accordingly, in this study, all intestinal samples were diluted 1:1 with distilled water to enable comparison between dietary groups and between sites along the intestine. This also made measurement of small intestinal viscosities easier as digesta was a very thick paste in pigs fed the diet with the highest viscosity CMC. Within 30 min of collection, digesta samples were diluted 1:1 with distilled water, mixed on a vortex, and centrifuged at 12 000 ***g*** for 8 min (Sigma benchtop centrifuge 1–15; Quantum Scientific Pty Ltd, Milton, Queensland, Australia). The supernatant fraction (0·5 ml) was placed in a Brookfield LVDV-II+ cone-plate rotational viscometer (CP40; Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) and the viscosity of all samples was measured at a shear rate of 60 s^{-1}. The viscosity value was recorded as an apparent viscosity.

The diets and digesta containing CMC behaved as a non-Newtonian fluid and displayed shear-thinning behaviour (see Fig. 1), where the viscosity reading is dependent on the shear rate of measurement. To minimise the effects of shear thinning and thixotropy on the viscometer reading, one reading was taken at 60 s^{-1} immediately after the reading stabilised, in a maximum time of 60 s/sample. The shear rate of 60 s^{-1} allowed the most accurate viscometer reading for the range of viscosities being measured and allowed comparison between sites in the small and large intestine. The flow behaviour of CMC diets and digesta fitted a power law relationship that is typical of a weak gel.

Presentation of data and statistical analyses

The pool of VFA was calculated by multiplying the concentration of VFA within an intestinal segment by the weight of the contents. In the large intestine, the proximal and distal colonic digesta weights were averaged for this calculation. In the small intestine the digesta weight of the last segment was estimated as one third of that from the whole small intestine, as the small intestine was divided equally into three sections. This weight was an under-estimation, as there tended to be more digesta in the last third compared to the other two thirds of the small intestine. The amount of VFA produced on a DM basis was calculated by dividing the concentration of VFA by the % DM of the same digesta.

Statistical analyses were conducted using Statview for

Table 2. Average voluntary food consumption, whole-body and intestinal growth of pigs fed cooked rice-based diets with or without the addition of 40 g carboxymethylcellulose (CMC)/kg diet for 13 d following weaning*

(Mean values for eight pigs per rice and rice + low viscosity CMC groups, mean values for seven pigs per rice + high viscosity CMC group)

	Dietary treatment				
	Rice	Rice + low-viscosity CMC	Rice + high-viscosity CMC	SED	Statistical significance of effect: *P*
Daily food consumption					
DM intake (g/d per pig	218	235	221	25	NS
DE intake (MJ/d per pig)	3·25	3·39	3·19	0·37	NS
Daily growth rates (g/d per pig)					
Live-weight gain	290·8^{a}	393·2^{b}	325·0^{ab}	63·4	0·046
Carcass gain†	101·5^{ab}	120·0^{a}	54·0^{b}	41·0	0·054
Full organ weights (g)‡					
Small intestine	461^{a}	579^{b}	589^{b}	78	0·03
Large intestine	186^{a}	312^{b}	294^{b}	36	<0·0001
Empty organ weights (g)					
Small intestine	420	455	447	14	NS
Large intestine	109	116	127	21	NS

DE, digestible energy.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 488–490.
† Live weight minus weight of full gastrointestinal tract.
‡ Organ weights measured 13 d after weaning.

Table 3. The effect of dietary carboxymethylcellulose (CMC) (40 g/kg) added to a cooked rice-based diet on the presence of diarrhoea in pigs after weaning*†

Diet	Day 7‡	Day 8‡	Day 9‡	Day 10‡
Rice	0/8[a]	1/8[a]	0/8[a]	0/8[a]
Rice+low-viscosity CMC	5/8[b]	3/8[b]	4/8[b]	4/8[b]
Rice+high-viscosity CMC	7/7[b]	7/7[b]	7/7[b]	5/7[b]
P value	<0·05	<0·05	<0·05	<0·05

[a,b]Mean values within a column with unlike superscript letters were significantly different: $P<0·05$.
* For details of diets and procedures, see Table 1 and pp. 488–490.
† Expressed as number of pigs with diarrhoea as a proportion of total number of pigs in the group.
‡ Day number is the number of days after weaning (weaning is day 1).

Windows (version 5.0; SAS Institute Corporation, Cary, NC, USA). One-way ANOVA determined significant differences between treatment groups and the mean values were compared using Fisher's least-significant difference test. Fisher's exact test was used to determine the effect of diet on daily counts of diarrhoea (Table 3). Carcass weight was defined as the live-body weight minus the weight of the full intestinal tract, heart, lungs, spleen and kidneys. The average daily carcass weight as a proportion of the weight of the eight pigs slaughtered on the day of weaning (86·14 % live weight) was used to calculate weaning carcass weights (Noblet & Etienne, 1987). The unit of replication was the individual pig for all measurements of body weight, internal measurements and microbiological data, and for food intake the unit of replication was each pen.

Results

Whole-body and intestinal growth and food intake

There was no difference in the average voluntary DM intake between pigs fed the rice diet, the rice + low-viscosity CMC or the rice + high-viscosity CMC diet (Table 2). Increasing the viscosity of the weaning diet resulted in an increase in live-weight gain ($P<0·05$) (Table 2). Higher intestinal viscosity was also associated with a greater full weight of the intestines ($P<0·05$), which accounted for the greater values for live-weight growth in pigs fed the viscous diets compared with the non-viscous diet. Once the effect of diet on intestinal weights was removed by subtracting the weight of the abdominal viscera, the carcass gain of pigs fed rice + low-viscosity CMC and rice + high-viscosity CMC was considerably reduced. The addition of the highest viscosity CMC depressed carcass growth relative to pigs fed the rice diet without CMC, but the pigs fed the lower viscosity CMC still had greater carcass gain than those fed the rice diet without CMC. Small intestinal full weights were greatest for pigs fed rice + high-viscosity CMC due to the greater volume of digesta in these pigs.

Faecal consistency

Pigs fed the viscous diets began to display loose faeces at about 4–5 d post-weaning. By the seventh day after weaning, five out of eight of the pigs fed rice + low-viscosity CMC and all of the pigs fed rice + high-viscosity CMC had diarrhoea (Table 3), which corresponded with a decrease in measured faecal % DM (Fig. 2). Pigs fed rice without CMC remained healthy and only one pig displayed diarrhoea for 1 d during the trial. When % DM along the length of the intestinal tract was examined after slaughter, there was a significant reduction in % DM of digesta due to addition of CMC (Fig. 3), which was most divergent at the distal end of the tract. The average % DM in the large intestine on all diets was significantly greater than that of the small intestine.

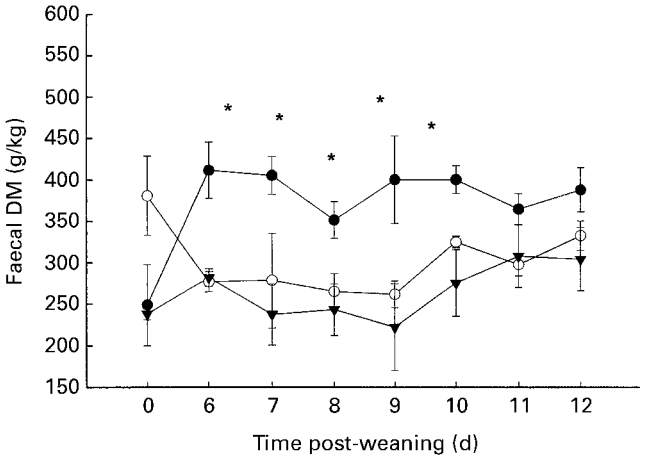

Fig. 2. Daily faecal DM (g/kg) in pigs fed diets containing no carboxymethylcellulose (●) (*n* 8), low-viscosity carboxymethylcellulose (○) (*n* 8), or high-viscosity carboxymethylcellulose (▼) (*n* 7), where carboxymethylcellulose was included at 40 g/kg diet. For details of diets and procedures, see Table 1 and pp. 488–490. Values are means with standard deviations represented by vertical bars. Mean values were significantly different from those of the control group: * $P<0·05$.

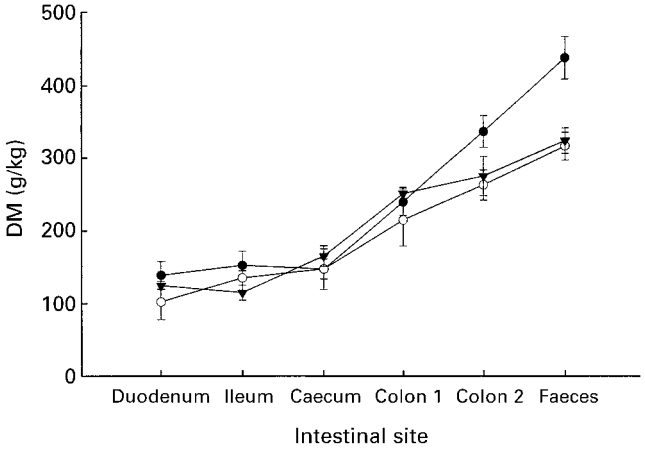

Fig. 3. Digesta DM (g/kg) along the intestinal tract of 34-d-old pigs fed diets containing no carboxymethylcellulose (●) (*n* 8), low-viscosity carboxymethylcellulose (○) (*n* 8), or high-viscosity carboxymethylcellulose (▼) (*n* 7), where carboxymethylcellulose was included at 40 g/kg diet. For details of diets and procedures, see Table 1 and pp. 488–490. Values are means with standard deviations represented by vertical bars. Colon 1 and 2 are the proximal and distal colon sites respectively. The value for DM (g/kg) differed significantly among diets ($P=0·0008$) and intestinal sites ($P<0·0001$).

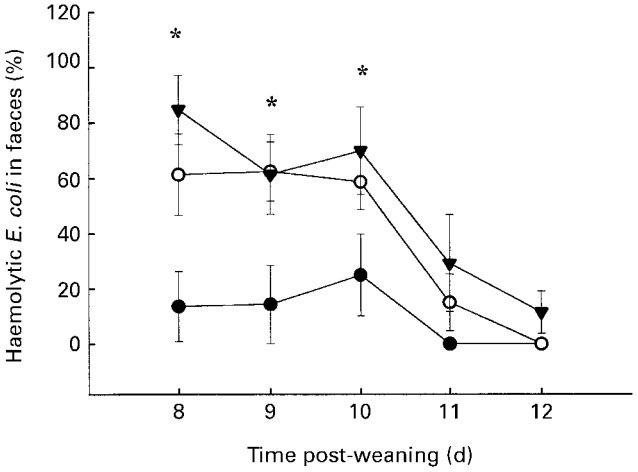

Fig. 4. Daily faecal shedding of haemolytic *E. coli* in pigs after weaning on three rice-based diets containing no carboxymethylcellulose (●) (*n* 8), low-viscosity carboxymethylcellulose (○) (*n* 8), or high-viscosity carboxymethylcellulose (▼) (*n* 8), where carboxymethylcellulose was included at 40 g/kg diet. Colonisation is expressed as % bacteria cultured from faeces that were haemolytic *E. coli*. For details of diets and procedures, see Table 1 and pp. 488–490. Values are means with standard deviations represented by vertical bars. Mean values were significantly different from those of the control group: * $P<0{\cdot}05$.

Enterotoxigenic E. coli

Both groups receiving rice + CMC shed significantly more haemolytic *E. coli* ($P=0{\cdot}011$, Fig. 4) in their faeces daily than pigs fed rice without CMC, and tended to have a greater intestinal colonisation of haemolytic *E. coli* at slaughter (Fig. 5). The serotype of *E. coli* shed from these naturally infected pigs in each case was identified as O149;K91;K88.

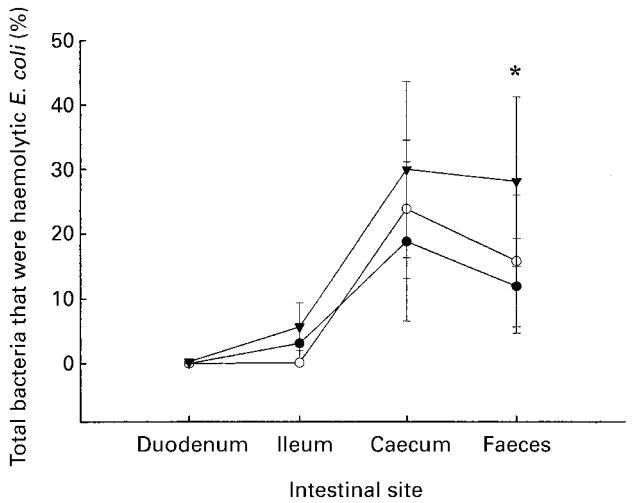

Fig. 5. Colonisation of the intestinal tract of 34-d-old pigs fed diets containing no carboxymethylcellulose (●) (*n* 8), low-viscosity carboxymethylcellulose (○) (*n* 8), or high-viscosity carboxymethylcellulose (▼) (*n* 7), with haemolytic *E. coli*. Colonisation is expressed as % bacteria cultured from faeces that were haemolytic *E. coli*. For details of diets and procedures, see Table 1 and pp. 488–490. Values are means with standard deviations represented by vertical bars. Mean values were significantly different from those of the control group: * $P<0{\cdot}05$.

Intestinal viscosity

Apparent intestinal viscosity values are recorded in Table 4. Addition of CMC to the diet significantly increased the apparent viscosity of the digesta supernatant fraction relative to the rice diet in the duodenum ($P<0{\cdot}001$), ileum ($P<0{\cdot}001$) and in the caecum ($P<0{\cdot}05$), in a manner reflecting the viscosity of CMC used. Within the two groups of pigs fed the diets containing CMC, the magnitude of apparent viscosity generally decreased from the proximal to distal end of the intestinal tract, although the reduction in viscosity between sites was not statistically significant (results not shown). In the pigs fed the rice diet without CMC, the intestinal apparent viscosity value was relatively constant in the duodenum, ileum and caecum but almost doubled in the proximal colon. This difference was not statistically significant, probably associated with the large variation between pigs. The contents of the proximal colon were very dry and even after dilution it was difficult to collect enough clear supernatant fraction to obtain a steady viscosity measurement in the pigs fed the rice-without-CMC diet. There was no statistically significant difference in the apparent viscosity of intestinal contents when measured at 4, 25, 37°C, after a period of freezing at −20°C or when retested 6 h after the first measurement.

Morphology of the small intestine

The height of the small intestinal villi and the width of the muscle layers underlying the intestinal villi were greatest in pigs fed the low-viscosity CMC and smallest in those fed the high-viscosity CMC (Table 4), with the values for the pigs fed the rice diet without CMC falling between these values. Addition of either low- or high-viscosity CMC to the rice diet was associated with an increase in the crypt depth ($P=0{\cdot}003$). The villus height:crypt depth ratio was significantly reduced ($P<0{\cdot}001$) in pigs fed the high-viscosity CMC diet as a result of low villus height values. The ratio was identical in the pigs fed rice without CMC and those fed rice + low-viscosity CMC (1·73).

Intestinal fermentation

There was no effect of addition of dietary CMC on the concentration of total VFA in the ileum or caecum (Table 5). VFA concentrations were lower in the colon of pigs fed diets including CMC compared with those fed the diet without CMC, confirming that CMC was not significantly contributing to fermentation within the tract. Values for pH also differed significantly in the proximal ($P=0{\cdot}035$) and distal ($P<0{\cdot}0001$) colon, but not more proximally in the tract, with the pH values being lower in pigs fed the diets containing CMC than in pigs fed rice without CMC (Table 4). The average total pool of VFA in the caecum and colon collectively was significantly increased by the addition of CMC ($P<0{\cdot}05$), with the mean values being 5·77 (SE 0·6) mmol/pig, 9·40 (SE 1·2) mmol/pig and 8·11 (SE 0·9) mmol/pig for pigs fed the rice diet without CMC, the

Table 4. Intestinal morphology and mean apparent intestinal viscosity of pigs fed a cooked rice-based diet with or without the inclusion of 40 g carboxymethylcellulose (CMC)/kg diet for 13 d following weaning*

(Mean values for eight pigs per rice and rice + low viscosity CMC groups, mean values for seven pigs per rice + high viscosity CMC group)

	Dietary treatment				
	Rice	Rice + low-viscosity CMC	Rice + high-viscosity CMC	SED	Statistical significance of effect: *P*
Apparent intestinal viscosity (mPa·s)					
Duodenum	1·42[a]	6·14[b]	8·93[b]	2·63	0·0007
Ileum	1·41[a]	6·04[b]	7·91[b]	2·20	0·0006
Caecum	1·67[a]	4·09[ab]	6·18[b]	2·43	0·0277
Poximal colon	2·48	4·33	5·20	2·08	NS
Small intestinal villi measurements (μm)					
Villus height	409[a]	451[b]	363[c]	106	<0·0001
Crypt depth	249[a]	273[b]	271[b]	41	0·0003
Villus height:crypt depth	1·73[a]	1·73[a]	1·39[b]	0·55	0·0002
Muscle thickness	289[a]	312[b]	238[c]	51	<0·0001

[a,b]Mean values within a row with unlike superscript letters were significantly different. ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 488–490.

rice + low-viscosity CMC or rice + high-viscosity CMC respectively.

Discussion

Viscosity

There are inherent difficulties in measuring the viscosity of the chyme along the intestinal tract that deserve mention before discussing the influence of viscosity on the gut. Viscosity is often measured on the liquid portion of digesta as separated by centrifugation (Blackburn & Johnson, 1981; Bedford & Classen, 1992; Gallaher *et al.* 1993; Choct *et al.* 1996; Smits *et al.* 1997), although a few studies have measured the viscosity of the whole digesta (Cameron-Smith *et al.* 1994; Larsen *et al.* 1994; Ellis *et al.* 1995). In the upper regions of the intestinal tract where there is greater water content, the magnitude of viscosity is most likely to be driven by components in the liquid fraction. In the large intestine there is relatively less fluid and the solid particles play an important role in generating viscosity (Morris, 1992; McRorie *et al.* 1998, 2000). The supernatant fraction apparent viscosity in this trial therefore has been viewed as an estimate of whole digesta viscosity, to be used for purposes of comparison between diets rather than as an absolute quantification.

Dilution of digesta, such as by intestinal secretions, reduces the apparent viscosity of the sample. Dilution *ex*

Table 5. Mean volatile fatty acid (VFA) concentrations and pH values in the intestinal tract of pigs fed cooked rice-based diets with or without the inclusion of 40 g carboxymethylcellulose (CMC)/kg diet for 13 d following weaning*

(Mean values for eight pigs per rice and rice + low viscosity CMC groups, mean values for seven pigs per rice + high viscosity CMC group)

	Dietary treatment				
	Rice	Rice + low-viscosity CMC	Rice + high-viscosity CMC	SED	Statistical significance of effect: *P*
Total VFA (mmol/kg wet digesta)					
Ileum	20·2	18·5	17·4	8·7	NS
Caecum	94·7	89·6	89·0	15·2	NS
Proximal colon	93·0[a]	52·2[b]	50·8[b]	18·7	0·002
Distal colon	65·9[a]	41·6[b]	31·4[b]	11·6	0·0004
Total VFA (mmol/kg digesta DM)					
Ileum	211·9	140·8	161·0	88·2	NS
Caecum	734·9	550·9	571·4	252·0	NS
Proximal colon	402·7	220·5	186·2	105·0	NS
Distal colon	204·9	162·7	129·9	61·1	NS
pH values					
Duodenum	6·19	5·80	5·74	0·36	NS
Ileum	6·67	6·86	6·71	0·39	NS
Caecum	6·33	6·27	6·26	0·14	NS
Proximal colon	6·57[a]	6·25[b]	6·34[ab]	0·19	0·035
Distal colon	7·08[a]	6·15[b]	6·22[b]	0·14	<0·0001
Faeces	7·06[a]	6·19[b]	6·22[b]	0·18	<0·0001

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and pp. 488–490.

vivo underestimated the apparent viscosity of the samples containing CMC compared with those without CMC due to the weak gel behaviour of CMC, although the apparent viscosities of the diluted solutions containing CMC remained higher than those without CMC at all shear rates (Fig. 1, Table 4). As the small intestine has been the main site sampled for viscosity measurements, the need for dilution does not seem to have been a commonly recorded problem, although some authors have diluted gastric and small intestinal samples (Cameron-Smith *et al.* 1994) or caecal samples (Topping *et al.* 1988) with water in a 1:1 (w/v) ratio or up to a designated volume (Ikegami *et al.* 1990) to enable measurement of viscosity. For the purposes of the present study, the measurement of viscosity was required to establish that CMC was creating a significantly different viscosity within the intestinal lumen. To this end, the apparent viscosity (that recorded directly by a viscometer) indicated this was successful.

The dietary composition, the extent of hydration of the dietary NSP and the time of measurement of viscosity after feeding all influence the value obtained for measurement of intestinal viscosity. All pigs in this study were killed 1·5 h after feeding to minimise timing complications and to target the maximal viscosity, based on the fact that viscosity of gastric digesta was highest 30–60 min post-feeding for CMC (Rainbird & Low, 1986*a*) and guar gum (Ellis *et al.* 1995) in grower pigs. The composition of the non-NSP components of the diet such as starch may also potentially enhance or decrease the viscosity (Rainbird, 1986).

Whole-body and intestinal development

Inclusion of the synthetic non-fermentable compound CMC to the diet of weaner pigs induced significant changes in intestinal structure within 13 d after weaning. The full small and large intestines increased in weight in response to the addition of CMC to the diet. The major proportion of the increase in weight was due to the increased weight of the digesta, indicated by the fact that the empty organ weights were not significantly different between dietary groups. Larsen *et al.* (1994) also found the empty intestinal weights of rats fed CMC tended to be non-significantly heavier with increasing viscosity of CMC in the diet. The increase in empty and full intestinal weights tends to be more significant when the dietary NSP are both viscous and fermentable, such as guar gum or pectin (Ikegami *et al.* 1990; Pluske *et al.* 1996; McDonald *et al.* 1999). However, it is now clear that viscosity alone can induce major increases in empty organ weights when the diet is fed for a longer period than in this study (Elsenhans & Caspary, 2000). It is possible that the empty intestinal weights in the weaner pigs may have increased had the animals been given a longer adaptation period.

Although the average daily voluntary food intakes were similar, pigs fed the diets with CMC had significantly greater digesta weights than those pigs fed the unsupplemented rice diet. Gel-forming substances such as CMC have the ability to hold water and swell, which would increase the weight of digesta that contained CMC. An increase in both the intestinal water content and water intake has been noted upon feeding of viscous polysaccharides in poultry (van der Klis *et al.* 1993; Choct *et al.* 1996; Smits *et al.* 1998). The presence of viscous polysaccharides in the lumen of the small intestine can also increase endogenous secretions such as pancreatic enzymes and mucus (Ikegami *et al.* 1990), and reduce water absorption (van der Klis *et al.* 1993), all of which would increase the water content of the digesta. In addition, a decrease in the rate of passage of digesta through the jejunum and ileum has been noted in poultry fed CMC (van der Klis *et al.* 1993) and rats fed guar gum (Brown *et al.* 1988), and may have caused pooling of digesta in the small intestine. This would have further increased the digesta weight measured at a point in time.

The difference between carcass and whole-body growth rates of pigs fed the viscous diets compared with the rice diet highlights the importance of recognising that a significant proportion of the total growth in pigs fed a fibrous diet (when compared to low- or non-fibrous diets) may be due to growth of the gut. This is especially relevant for weaner pigs as they preferentially develop their gastrointestinal tract to cope with the new weaning diet before muscle accretion begins in earnest (Cranwell & Moughan, 1989). There also appears to have been an effect of degree of viscosity on growth of the carcass, where the low-viscosity CMC was associated with slightly improved gain, and the higher viscosity CMC was associated with reduced carcass gain.

It is surprising that the pigs fed rice + low-viscosity CMC gained weight relative to pigs fed rice without CMC, as viscous compounds have been shown to hinder nutrient and energy absorption in the small intestine of pigs (Rainbird *et al.* 1984; Ehrlein & Stockmann, 1998*a*). Addition of the low-viscosity CMC to the rice diet increased the small intestinal villus height and crypt depth without altering the shape of the finger-like villi. This is considered beneficial, as longer villi contain a greater number of mature cells capable of absorbing nutrients, and villus height correlates positively with empty body-weight gain and DM intake (Pluske, 1993). Together with a slightly higher food intake, this may explain the greater growth in pigs fed low-viscosity CMC.

The villus height and villus height:crypt depth ratio of pigs fed rice + high viscosity CMC, however, was significantly reduced, suggesting a reduced overall capacity for digestion and absorption of nutrients compared to the pigs on the other two diets. Reduction of villous height has recently been demonstrated in chickens fed viscous highly methylated citrus pectin (Langhout *et al.* 2000), a diet that is also associated with depression of growth rate (Langhout, 1998). It is possible that a small increase in intestinal viscosity may be beneficial up to a threshold, beyond which value the viscosity becomes detrimental to the assimilation of nutrients through the gut, and therefore to the growth of the animal.

The thickness of the muscle layer underlying the small intestinal epithelium followed a similar dietary trend as the villous height, being greater upon addition of low-viscosity CMC to the rice diet and reduced when the high-viscosity CMC was included. An increase in muscle thickness would logically be expected with greater bulk and weight of digesta, as a means of coping with the requirements of peristalsis. It was therefore expected that the highest

viscosity CMC would induce the greatest increase in muscle thickness, but this was not the case.

Intestinal fermentation

The choice of CMC for the present study was based on the premise that the compound would not be fermented to any significant extent, allowing an examination of the effects of viscosity independent of potential effects of fermentation. The lack of significant fermentation of CMC was confirmed in pigs fed CMC by measurements of VFA, which give an indication of the rate of VFA production, and which were either not higher (in the ileum and caecum) or were significantly lower (in the colon) than in pigs fed the diet not containing CMC, on a wet basis (mmol/kg wet digesta).

The pH values in the large intestine were unexpectedly lower in pigs fed rice + CMC diets compared with the plain rice diet, which normally indicates an increase in microbial fermentation and coincides with increased end product formation of VFA (Pluske *et al.* 1996). A lower ileal pH in animals fed CMC has been noted in chickens fed CMC (van der Klis *et al.* 1993; Smits *et al.* 1998) and could be related to a change in the relative populations of microbes. Alternatively, the increased unstirred water layer induced by the presence of CMC in the intestinal tract may interfere with the exchange of HCO_3^- and H^+ ions across the epithelium, affecting the intralumen pH (van der Klis *et al.* 1993).

Although the rate of production of VFA/kg wet digesta was greatest in pigs fed rice without CMC, the total pool of VFA in the caecum and colon was significantly higher in those pigs fed rice + CMC due to the large volume of digesta. Low concentrations of VFA occurring simultaneously with large pools of VFA have also been noticed when feeding CMC to rats (Wyatt *et al.* 1988), and were attributed to the large amount of digesta in the organ examined. A greater accumulation of VFA in the caecum and colon at any one time would have induced a more acidic pH value, adding another possible explanation for the lower pH values found.

The fact that CMC was still able to exert a water-holding capacity in the distal colon infers that there was no significant breakdown of CMC by fermentation (Tomlin *et al.* 1986), and this is supported by work in rats where little CMC was broken down at the distal colon (Wyatt *et al.* 1988).

Changes in intestinal microflora and diarrhoea

The microbe of interest in the current study was a strain of haemolytic *E. coli*. The present study did not involve any experimental infection with *E. coli* and was originally intended as a preliminary trial for such an experimental infection trial. Unexpectedly, the pigs in this study succumbed to a natural infection of PWC caused by the intestinal proliferation of enterotoxigenic *E. coli* serotype O149;K91;K88. The influence of dietary CMC on the faecal shedding of this strain of *E. coli* and on faecal % DM was significant. PWC is a difficult disease to reproduce experimentally, requiring pigs to be susceptible to the disease (e.g. possessing intestinal receptors for enterotoxigenic *E. coli*) and requiring a certain level of stress (e.g. cold stress, crowding, fighting) on top of the stress of weaning before disease will occur experimentally. The pigs in this study were kept in a very clean environment at optimal ambient temperature and minimal stress levels. All pigs were housed in one large room with pigs fed rice without CMC less than 0·5 m away from those fed rice + CMC, enabling easy transmission of infection from one group to another. Nevertheless, only the pigs fed rice + CMC developed PWC.

It is common for haemolytic *E. coli* to appear in increased numbers in the faeces of pigs in the first week after weaning (Hinton *et al.* 1985; Jensen, 1998). This happens in both healthy and diarrhoeic pigs, although the numbers excreted increase dramatically in those with diarrhoea (Gyles, 1993). There was a clear, statistically significant increase ($P<0·05$) in the proportion of haemolytic *E. coli* shed in the groups fed rice + CMC in the present study, and this coincided with the appearance of sloppy faeces that were significantly wetter than those from pigs fed rice without CMC. The pigs fed rice + high-viscosity CMC had the worst diarrhoea and the highest levels of haemolytic *E. coli* shed in their faeces. The small intestinal villi of these pigs also were atrophied, a common feature seen in PWC (Kenworthy *et al.* 1967). It is unknown whether the shorter villi in this group were a result of, or a precipitating factor for, the development of intestinal disease.

The addition of viscous polysaccharides that are also fermentable, such as guar gum, to the weaner diet can exacerbate PWC (Mcdonald *et al.* 1999). Other studies testing non-viscous and fermentable dietary fibres have shown that they have an ability to alleviate PWC (Palmer & Hulland, 1965; Bertschinger & Eggenberger, 1978; Thomlinson & Lawrence, 1981), lending weight to the contention that intestinal viscosity has an influence on this disease. Some studies have tested diets containing fibres that are commonly viscous and fermentable (e.g. oats), but extrapolation of results is difficult because the intestinal viscosity of these diets and intestinal samples was not measured (Okai *et al.* 1976; Ball & Aherne, 1982; Etheridge *et al.* 1984).

Altered microbial activity has been noted in other species in association with increasing small intestinal viscosity (Choct *et al.* 1996: Smits *et al.* 1998; Langhout, 1998; Langhout *et al.* 2000). For example, the numbers of *E. coli* and total anaerobic counts in the ileum of chickens increased significantly with the addition of citrus pectin (Langhout, 1998), whilst total microbial counts (aerobic and anaerobic) increased in the duodena and jejuna of chickens fed diets containing CMC (Smits *et al.* 1998). When CMC was included in diets offered to rats there was no increase in the density of bacteria in the caecal or colonic contents, but the bacterial populations changed significantly, with the aerobic bacteria, in particular *E. coli*, being more numerous in the large intestine (Wyatt *et al.* 1988).

Smits *et al.* (1997, 1998) proposed that reduced absorption of nutrients allowed the secondary increase in microbial numbers in the ileum. Viscous polysaccharides are thought to drag digesta to the distal part of the intestines before digestion is complete, providing the substrate for microbial proliferation. Germ-free chicks have both higher pH and lower viscosity in their intestines than conventionally

reared chicks (Langhout *et al.* 2000), suggesting that the presence of microbes and an increase in viscosity are interrelated.

The presence of CMC in the digesta appears to provide an environment that favours establishment and growth of bacteria, especially *E. coli*, as well as providing osmotic drag that accelerates development of clinical diarrhoea (van der Klis *et al.* 1993). Bacteria possess an array of mechanisms for establishing colonisation within the gut. *E. coli* possess fimbriae that attach to the brush border of the small intestinal villi, but also allow the bacteria to attach to the mucus lining the intestinal tract (Conway, 1994). CMC adheres to and thickens porcine mucin (Rossi *et al.* 1996), and may also alter its composition, which in this case may have enhanced the ability of haemolytic *E. coli* to bind to the mucus lining the intestinal villi.

The presence of CMC in digesta of chicks reduces net Na^+ and water absorption and increases osmolality (Van der Klis *et al.* 1993). This would increase digesta water content, and may explain how feeding CMC at levels of about 10 % to rats results in sloppy faeces, diarrhoea and a faster total transit time (Gohl & Gohl, 1977; Johnson & Gee, 1986; Wyatt *et al.* 1988). Conversely, addition of CMC to oral rehydration solutions offered to healthy rats and those with chronic osmotic diarrhoea or malnutrition has also been positively correlated with increased intestinal water and Na^+ absorption (Go *et al.* 1994). Subsequent work revealed a complex interaction between concentration, chemical composition of CMC and effect on water and Na^+ absorption, where only low concentrations of particular types of CMC are capable of enhancing intestinal absorption (Wapnir *et al.* 1997).

The interplay between viscosity, fermentation and microflora is extremely complex and is undoubtedly altered by the type of fermentative or viscous compounds involved, amongst many other factors. In the present study the addition of CMC to the weaning diet significantly increased intestinal viscosity, altered the structure of the intestine and provided a microenvironment that favoured the proliferation of pathogenic *E. coli*. These findings have implications for the use of feed ingredients in weaner diets that may significantly increase the intestinal viscosity, such as those containing high levels of soluble NSP. Finally, the inclusion of CMC in weaner diets represents a novel method of inducing experimental PWC without requiring experimental inoculation of *E. coli*.

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