

Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis

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The purposes of the present study were to investigate the effects of feeding a cereal grain containing NSP on body growth and the intestinal microenvironment of recently weaned pigs, and to examine resultant associations with pathogenic *Escherichia coli* in the intestinal tract. In Expt 1, pearl barley, a grain rich in soluble NSP, was incorporated (250, 500 or 750 g/kg diet) into a low-fibre control diet based on cooked white rice and fed for 7–10 d following weaning. Consumption of pearl barley did not significantly alter piglet live-weight gain compared with the control cooked rice diet, but it accelerated large intestinal growth and fermentation, decreased ileal starch digestibility and increased intestinal viscosity. Expt 2 was conducted to determine whether these differences would favour proliferation of enterotoxigenic *E. coli*, the bacterium causing post-weaning colibacillosis (PWC). Three groups of pigs were weaned onto diets based on cooked white rice, rice with 500 g pearl barley/kg, or rice with 500 g pearl barley/kg supplemented with exogenous enzymes (Porzyme™ 8100; Danisco, Marlborough, Wilts., UK). Pigs were inoculated orally with haemolytic *E. coli* serovar O8;K87;K88 after weaning. Animals eating the pearl barley had increased viscosity of the intestinal contents, greater intestinal colonisation with the *E. coli* strain and more diarrhoea than pigs fed the rice-only diet. The enzymes did not reduce viscosity or protect from PWC. The results suggest that pearl barley alters the intestinal microenvironment and predisposes to PWC, whilst a low-viscosity, highly digestible diet based on cooked white rice is protective.

Pearl barley: Non-starch polysaccharides: Intestinal viscosity: Diarrhoea: *Escherichia coli*: Weaner pigs

NSP, a class of dietary fibre, can stimulate significant changes in intestinal fermentation and in the physico-chemical environment of the healthy porcine gut (Bach Knudsen *et al.* 1991; Bach Knudsen & Hansen, 1991; Jensen & Jorgensen, 1994). These changes in the intestinal environment can be modulated by the solubility, fermentability and viscosity of the ingested NSP, and these alterations may in turn modulate the progression or expression of infectious intestinal diseases (Bertschinger & Eggenberger, 1978; Bolduan *et al.* 1988; Pluske *et al.* 1996, 1997, 1998).

Post-weaning colibacillosis (PWC) is a common and important infectious diarrhoeal disease of pigs, usually occurring in the first 3–10 d after weaning (Hampson, 1994). The primary aetiological agents are predominantly β -haemolytic enterotoxigenic strains of *Escherichia coli* (ETEC) that proliferate in the intestinal tract after weaning. These strains adhere to villous enterocytes mainly in the anterior small intestine, where they release enterotoxins that cause fluid and electrolyte loss, leading to diarrhoea (Francis, 2002).

There is continuing debate as to whether NSP exerts a beneficial or detrimental influence on the development of

PWC (Bolduan *et al.* 1988). For example, fibre from the outer hull of barley is rich in insoluble NSP, and its consumption has been linked to a reduced severity of PWC (Smith & Halls, 1968). Conversely, feeding barley meal, the soluble NSP-rich component of barley, has been associated with an increased susceptibility to the development of PWC (Smith & Halls, 1968). Bertschinger & Eggenberger, (1978) noted that high levels of insoluble NSP in a diet with low protein and energy content reduced expression of PWC, whilst in more recent studies, weaner diets incorporating soluble and viscous NSP in the form of guar gum or carboxymethylcellulose (CMC) resulted in an exacerbation of experimental PWC (McDonald *et al.* 1999, 2001).

The relevance of the viscosity of the intestinal contents in relation to pig nutrition and gut function is still relatively unexplored. Viscosity is often measured in studies evaluating exogenous enzyme supplementation, as viscosity decreases when enzymes are effective. In turn, this observation suggests that a reduced viscosity of the intestinal contents is beneficial. In poultry, increased intestinal viscosity is associated with increased small intestinal fermentation, and predisposes to digestive disturbances

Abbreviations: CMC, carboxymethylcellulose; DE, digestible energy; ETEC, enterotoxigenic *Escherichia coli*; PWC, post-weaning colibacillosis; VFA, volatile fatty acid.

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(Choct & Annison, 1992). In the current study two hypotheses were proposed and tested: (1) that soluble, viscous NSP sources are capable of significantly altering the intestinal microenvironment in newly weaned pigs; (2) that dietary NSP can influence the pathogenesis and expression of PWC.

To test these hypotheses, the whole-body and intestinal influences of increasing levels of dietary NSP were investigated in healthy newly weaned pigs (Expt 1) and in an experimental model of PWC (Expt 2). Pearl barley was chosen as a naturally rich source of soluble NSP. This grain contains minimal insoluble NSP, due to the abrasion of the outer barley hull during the pearling process. The product after pearling primarily consists of starch, β -glucans and some protein (Bhatty, 1993). Cooked white rice, containing approximately 10 g total dietary NSP/kg, was chosen as a near 'fibre-free' base for the pearl barley diets, and to serve as a control low-fibre diet. Exogenous enzymes were added to a diet containing 500 g barley/kg in Expt 2 to hydrolyse the NSP and potentially counter some of its proposed adverse effects on PWC.

Experimental methods

General

The research was reviewed and approved by the Animal Ethics Committees of the Western Australian Department of Agriculture (Expt 1) and Murdoch University (Expt 2), in accordance with the NH & MRC/CSIRO/AAC Code of Practice for the Care and Use of Animals for Experimental Purposes.

In Expt 1, a cooked white-rice-based diet and the same diet with three different inclusion levels of pearl barley (250, 500 and 750 g/kg diet) were used (Table 1). The effects of feeding these four diets on pig growth and selected aspects of gut development were compared in the 10 d period following weaning. The pigs in Expt 1 were not experimentally infected with ETEC.

In Expt 2, the effects of diet on pigs that were experimentally infected with β -haemolytic ETEC after weaning were examined. The three diets were fed from the point of weaning until 9 d after weaning. They were based on cooked white rice with or without pearl barley included at 500 g/kg total diet, with exogenous enzyme added to one of the two diets containing 500 g pearl barley/kg to make the third diet.

Animals and feeding

In Expt 1, twenty-four Large White \times Landrace pigs obtained from a specific-pathogen-free piggery were weaned at 19–21 d of age and allocated to one of the four dietary treatments listed in Table 1. Group allocation was stratified so that the average body weight was equal across groups. Pigs were housed in pairs for 10 d following weaning, and were fed *ad libitum*.

In Expt 2, thirty-nine Large White \times Landrace pigs from the same source as in Expt 1 were weaned at 21 d of age, transported to Murdoch University and randomly allocated to the three diets (Table 1). Pigs in each dietary treatment were housed in two groups of six or seven per pen to

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

Ingredient	Incorporation of pearl barley (g/kg)			
	0	250	500	750
Cooked rice	702.5	497.0	275.0	0.0
Pearl barley	0.0	250.0	500.0	750.0
Rapeseed oil	0.0	0.0	12.5	30.0
L-Thr	0.6	0.5	0.7	0.4
Celite*	20.0	20.0	20.0	20.0
Choline chloride (600 g/kg)	0.4	0.4	0.4	0.4
Vitamin and minerals†	1.5	1.5	1.5	1.5
Bloodmeal (850 g/kg)	25.0	25.0	25.0	25.0
Meat- and bonemeal (500 g/kg)	3.6	26.6	13.2	5.3
Fishmeal (650 g/kg)	150.0	150.0	141.5	150.0
Skimmed-milk powder	78.7	20.0	10.0	10.0
Lys	0.0	0.0	0.6	0.0
Ca ₂ PO ₄	17.7	9.1	0.0	0.0
Calculated and chemical analyses				
DE (MJ/kg)	14.9	14.5	14.5	14.5
Lys (g/kg)	12.0	11.0	11.0	11.0
Crude protein (N \times 6.25) (g/kg)	199.7	196.1	225.0	221.0
Soluble NSP (g/kg)	2.5	14.0	25.3	36.5
Insoluble NSP (g/kg)	4.7	12.7	20.5	28.1

DE, digestible energy.

* Catalogue no. 41,993-1; Aldrich Chemical Company, Milwaukee, WI, USA.

† 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.

facilitate transmission of *E. coli*. All pigs were fed their respective diets on an *ad libitum* basis for 9 d after weaning.

The diet base for both experiments consisted of cooked medium-grain white rice (Sunwhite Calrose®; Ricegrowers Cooperative, Leeton, New South Wales, Australia) fortified with an animal protein supplement that contained fishmeal, bloodmeal and meat- and bonemeal. Pearl barley was hammer-milled through an 8 mm screen and incorporated into the animal-protein component of the feed at the appropriate inclusion level for the experimental group (see Table 1). The white rice was mixed at a rice:water ratio of 1:2 and cooked by autoclaving at 121°C for 15 min on a porous cycle. Cooked rice was mixed with the animal protein or animal protein plus pearl barley mix component immediately before feeding. A small amount of additional water was used to facilitate mixing. The diet containing 750 g barley/kg was fed dry. All the remaining diets were fed moist, due to the presence of cooked rice in varying amounts.

In Expt 2, the 500 g pearl barley/kg diet was duplicated, and Porzyme™ 8100 (Danisco, Marlborough, Wilts., UK) was added to one of these diets (1 g/kg). The Porzyme™ 8100 enzyme mix contained β -glucanase (250 units/g), xylanase (400 units/g) and α -amylase (1000 units/g).

Diets were formulated to meet weaner pig nutrient requirements (National Research Council, 1988) and to be as close as possible in digestible energy (DE), N and lysine content without changing ingredients that would alter the NSP level. Their compositions are shown in Table 1. The NSP content of the diets was determined at the Department of Animal Sciences, University of New

England, Armidale, Australia, using a method based on that described by Spiller (1993). There were minimal amounts of soluble NSP in the rice diet (4.0 g/kg), whereas the barley diets contained up to 36.5 g/kg soluble NSP. The insoluble NSP content of the rice diet was 4.7 g/kg, and this increased progressively to 28.1 g/kg in the diet containing 750 g pearl barley/kg. The diets also varied in crude protein ($N \times 6.25$) content by up to 28.9 g/kg.

Procedures and measurements

In Expt 1, individual daily live-weight measurements were made and rectal faeces collected on bacteriology swabs. The swabs were streaked onto blood agar plates (Columbia agar containing 50 ml defibrinated sheep blood/l), cultured overnight in air at 37°C, and the proportion of haemolytic coliform colonies recorded. In Expt 1, food intake for the pairs of piglets was recorded. In Expt 2, group food intake was recorded, and faeces were collected and pooled per pen for determination of DM content. At the end of the experimental period the pigs in both experiments were killed by intravenous injection with an overdose of sodium pentobarbitone. Their intestines were removed and divided into three small intestinal segments (25 ('duodenum'), 50 ('jejunum') and 75 ('ileum') % along the small intestine), and into caecum, proximal colon and distal colon. The entire small and large intestines were weighed full and empty. Indices of fermentation were measured in the duodenum (pH value of digesta), in the ileum, caecum and colon (pH values and volatile fatty acid (VFA) concentrations), and in the faeces (pH value only). The pH values were determined using a portable pH meter (Orion, Sydney, New South Wales, Australia). VFA analysis was conducted using a Hewlett Packard 5890 A capillary GC (Agilent Technologies, Forrest Hill, Victoria, Australia), as described previously (McDonald *et al.* 2001). The viscosity was measured in fresh contents collected from the duodenum and ileum. Digesta were diluted 1:1 (v/v) with distilled water, mixed and then centrifuged at 12 000 g for 8 min (Sigma benchtop centrifuge 1-15; Quantum Scientific Pty Ltd, Milton, Queensland, Australia). The viscosity of 0.5 ml supernatant fractions 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), as previously described (Hopwood *et al.* 2002). Starch digestibility was measured in digesta from the terminal ileum and in the faeces using the amyloglucosidase- α -amylase method employed in the Megazyme Total Starch Assay kit (Megazyme Australia Pty, Warriewood, New South Wales, Australia), as previously described (Durmic *et al.* 2002). The presence of haemolytic ETEC was determined by inserting bacteriology swabs into digesta and rubbing on the intestinal wall in the duodenum, ileum, caecum, and faeces within the rectum. The swabs were then plated onto 50 ml sheep blood/l agar and incubated at 37°C overnight in air. Methods used were as previously described (McDonald *et al.* 1999, 2001). Whole-body weights were measured at the beginning and end of the trial. Empty gut body-weight was calculated as the live body-weight minus the weight

of the digesta within the gastrointestinal tract, and was used to determine the extent of intestinal growth without being influenced by the weight of digesta.

In Expt 2, pigs were orally inoculated with ETEC, serovar O8;K87;K88, which had been recovered from a Western Australian pig with PWC, and which previously had been used to induce PWC (McDonald *et al.* 1999). The strain, and selected isolates recovered from piglets following Expt 2, were serotyped at the *E. coli* Reference Laboratory, Bendigo, Victoria, Australia. Each pig was inoculated orally with 5×10^{10} colony forming units of the *E. coli* strain at 48, 72 and 96 h post-weaning. In the initial 24 h after the first inoculation, three of the pigs refused to eat or drink and developed signs of endotoxaemia. These animals were removed from the study.

At the end of the experiment, pigs were killed as in Expt 1, and the gastrointestinal tract was exteriorised and divided into sections for measuring gut weights, pH values and VFA concentrations, as described for Expt 1. Starch digestibility was not determined. Intestinal colonisation and proliferation of haemolytic ETEC O8;K87;K88 were determined by culture of digesta and mucosal scraping in the jejunum and proximal colon, and by culture of swabs taken of the digesta at the other intestinal sites. Individual animal body weights were measured at the beginning and end of the trial, and faecal samples were collected daily and pooled according to group.

A visual score was attributed to the consistency of the pig's faeces each day. These were scored from 1–5, and an average per pen score was calculated: 0, very hard, often pellet-like faeces; 1, well-formed faeces firm to cut; 2, formed faeces but soft to cut; 3, faeces falling out of shape upon contact with surfaces, sloppy; 4, pasty diarrhoea; 5, liquid diarrhoea.

An estimation of ETEC present in the swabs was made from the growth on the plates. Viable bacterial colonies that were β -haemolytic and morphologically consistent with ETEC were counted and expressed as a percentage of the total bacterial population grown on the plate. This value was referred to as % ETEC from intestinal swabs. The plates were also scored according to the number of streaked sections that had viable haemolytic ETEC, where: 0, no growth; 1, haemolytic ETEC in first section; 2, haemolytic ETEC in second section; 3, haemolytic ETEC in third section, 4, haemolytic ETEC in fourth section; 5, haemolytic ETEC in fifth section, and all the bacteria cultured on the plate were haemolytic ETEC. This was referred to as the swab score.

Presentation of results and statistical analyses

The effect of diet on measurements in both experiments was determined by ANOVA, and mean values were compared using Fisher's least significant difference method. Bacterial counts in Expt 2 were transformed logarithmically before analysis by ANOVA. Statistical analyses were calculated using the computer package Statview 5.0 (SAS Institute, Inc., Cary, NC, USA). In Expt 1, the pigs were housed in pairs in order to encourage them to eat. An estimate of individual intake was made by assuming each piglet ate an equal amount of the total food consumed

by the pair. For these pigs, a simple linear regression analysis was carried out on two sets of variables, those being the relationship between DE intake and body growth, and the relationship between soluble NSP intake and viscosity of the small intestinal digesta (pooled viscosity values from duodenum and ileum).

Results

Expt 1

Pigs fed the rice-only diet regained their weaning live weight by day 3–4 on average, whereas pigs fed 750 g barley/kg diet took an average of 6 d to regain their initial weaning weight. Although the numerical difference in average weight gains among groups appeared large (Table 2), there was much in-group variation, and group mean values were not significantly ($P > 0.05$) different.

The DE intake appeared to be a dominant factor in determining the growth rate immediately after weaning, accounting for over half of the variation in whole-body growth ($r^2 = 0.572$; daily live-weight gain = $-94.82 + 7.756 \times$ total DE intake (MJ per pig); $P < 0.0001$). Total DE intake decreased slightly as levels of barley inclusion increased (Table 2).

There was no diarrhoea or faecal shedding of ETEC observed in the pigs in Expt 1. On a group average basis, faecal DM ranged from 220–320 g/kg on the day of weaning to 310–380 g/kg by 9 d after weaning, and were unaffected by diet ($P = 0.562$). No ETEC were cultured from intestinal swabs at post mortem, 10 d after weaning.

There was no statistical effect of diet on pH values in the small intestine (Table 2) or proximal colon ($P = 0.357$, results not shown), nor on small intestinal weights

($P = 0.846$, results not shown). However, the large intestines were progressively heavier and pH values became more acidic in the distal colon and faeces as dietary barley levels increased (Table 2).

The total large intestinal VFA pool was greatest in pigs fed 750 g barley/kg and least in those receiving no barley (Table 2). Considering individual sections of the large intestine, the highest VFA concentration was found in the caecum and proximal colon for the groups of pigs fed rice or 250 g pearl barley/kg, whilst when barley comprised ≥ 500 g/kg diet, the VFA concentration was greatest in the proximal and distal colon (Table 3).

The mean viscosity of combined intestinal contents collected from the duodenum and ileum was significantly altered according to the experimental diet that was fed (Table 2). Upon regression of individual total intake of soluble NSP over the experimental period with the mean viscosity of the small intestinal contents, there was a significant positive linear relationship (Fig. 1), where intake of dietary soluble NSP accounted for 30 % of the variation in intestinal viscosity (average viscosity = $1.246 + 0.133 \times$ soluble NSP intake (g DM/d); $r^2 = 0.298$; $P = 0.007$).

Starch digestibility in the ileum ($P = 0.007$) and faeces ($P = 0.096$) was reduced when the proportion of dietary barley increased (Table 2). There was much greater variation in digestibility measurements within the barley groups than within the rice-only group.

Expt 2

All the pigs in Expt 2 lost weight in the first 24 h after inoculation with ETEC. The average live-weight gain for the experimental period was negative for those pigs

Table 2. Live-weight gain, digestible energy intake and intestinal measurements of pigs fed rice-based diets containing different inclusion levels of pearl barley for 10 d following weaning in Expt 1* (Mean values for six pigs per group)

	Incorporation of pearl barley (g/kg)				SED	Statistical significance of effect: <i>P</i>
	0	250	500	750		
Daily live-weight gain (g/d)	142	84	94	83	38.46	0.243
Total DE intake (MJ per pig)	28.1	24.4	24.9	19.8	4.57	0.224
Full LI (% live weight)	2.7 ^a	3.3 ^{ab}	3.8 ^b	4.1 ^b	0.55	0.030
Empty LI (% EGBW)	1.47	1.53	1.71	1.89	0.18	0.086
pH						
Duodenal	5.67	5.89	5.7	5.81	0.27	0.755
Ileal	6.65 ^a	6.33 ^{ab}	6.63 ^b	6.07 ^b	0.29	0.090
Distal colonic	6.78 ^a	6.62 ^a	6.06 ^b	5.73 ^b	0.35	0.009
Faecal	6.90 ^a	6.88 ^a	6.47 ^b	6.38 ^b	0.25	0.040
Total pool VFA (mmol)	9.68	15.76	15.49	17.78	4.12	0.137
Viscosity (mpa·s)						
Duodenal	1.11 ^a	1.28 ^{ab}	1.53 ^b	1.57 ^b	0.20	0.035
Ileal	1.38	1.78	2.84	2.24	0.68	0.125
SI	1.22 ^a	1.53 ^{ac}	2.18 ^b	1.91 ^{bc}	0.34	0.015
Average faecal swab score†	0	0	0	0	–	–
Starch digestibility (%)						
Ileum	95.8 ^a	66.3 ^{bc}	77.5 ^{ac}	49.0 ^b	11.5	0.007
Faeces	100.0	99.6	99.1	98.4	0.70	0.096

DE, digestible energy; LI, large intestine; EGBW, empty gut body-weight (live weight – weight of digesta within gut); VFA, volatile fatty acid; SI, small intestine.

^{a,b,c} 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 p. 420.

† Score 0–5 refers to number of sections of culture plate that grew enterotoxigenic *Escherichia coli*: 0, no growth; 5 entire plate.

Table 3. Concentration of volatile fatty acids (mmol/kg wet digesta) in different sections of the intestinal tract in pigs 10 d after weaning in Expt 1*
(Mean values for six pigs per group)

	Incorporation of pearl barley (g/kg)				SED	Statistical significance of effect: <i>P</i>
	0	250	500	750		
Ileum	18.24	11.87	9.08	16.78	6.39	0.303
Caecum	93.26	134.56	88.91	89.87	35.30	0.355
Proximal colon	98.61	131.55	118.06	113.75	20.37	0.294
Distal colon	83.55	97.90	113.46	109.27	13.92	0.074

* For details of diets and procedures, see Table 1 and pp. 420–421.

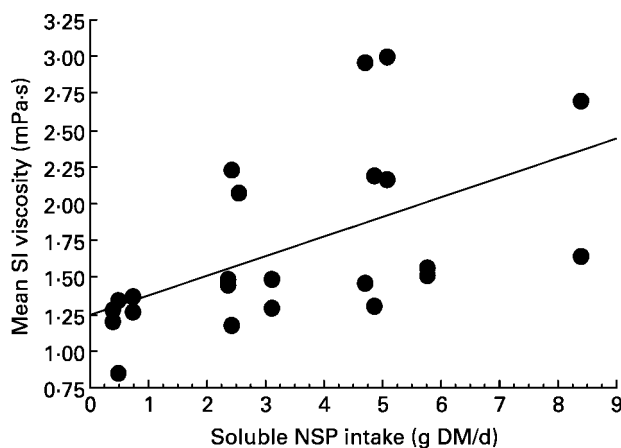


Fig. 1. Relationship between the intake of soluble NSP and viscosity of the pooled small intestinal content of newly weaned pigs fed a cooked rice-based diet with varying levels of pearl barley inclusion in Expt 1 (r^2 0.298, $P=0.007$). SI, small intestine. For details of diets and procedures, see Table 1 and pp. 420–421.

eating diets that contained pearl barley, and positive for those fed the rice-only diet (Table 4). The effects of NSP on the small and large intestine were similar to those in the pigs in Expt 1. Intestinal viscosity, large intestinal weights and acidity of the ileal digesta were greater in infected pigs fed 500 g pearl barley/kg compared with pigs not receiving barley (Table 4). Small intestinal weights remained unaffected by the type of diet fed (results not shown). There was a tendency ($P=0.09$) for pigs eating the rice-only diet to have a greater total pool of VFA within the gut than pigs fed the other two diets. For all infected pigs, the site of most active VFA concentration was the caecum (mmol/kg wet digesta: 0 g barley/kg, 103.3 (SD 6.9); 500 g barley/kg, 80.6 (SD 6.7); 500 g barley/kg + enzyme, 84.1 (SD 8.8)).

The pH values in the proximal small intestine were more alkaline in pigs eating the diets containing 500 g barley/kg than in pigs eating the rice-only diet (Table 4). This situation was reversed in the ileum. Average faecal pH values of infected pigs at the end of the experiment ranged between 7.13 and 6.85 units and were not altered by diet. There were very little digesta within the small intestine at the time of sampling.

The addition of enzyme to the 500 g pearl barley/kg diet was associated with a lighter large intestine compared with the pigs fed 500 g pearl barley/kg without enzyme, and a more alkaline content in the distal colon compared with

the group fed the rice-only diet. There were no other effects of enzyme addition on intestinal measurements compared with the other two groups.

No ETEC were isolated from faecal swabs taken before experimental inoculation, and there was no diarrhoea at this time. Within 1–2 d of experimental challenge, pigs shed ETEC in their faeces and developed diarrhoea. Diarrhoea and faecal shedding continued for 4–5 d, at which time the experiment ceased. Diarrhoea was intermittent, making assessment of diarrhoea in individual animals difficult in a pen situation. Consistency of collected faecal material ranged from normal, to poorly formed, to liquid.

Culture of mucosal scrapings taken at the end of the experiment revealed greater proliferation of ETEC within the small ($P=0.028$) and large ($P=0.006$) intestines of pigs consuming diets containing pearl barley than in those eating the rice-only diet (Table 5). In addition, the ETEC were more dominant within the microbiota of pigs eating pearl barley compared with that within pigs eating rice. At each of the intestinal sites swabbed there were more ETEC on the culture plates from pigs eating the barley diets compared with the pigs not receiving pearl barley.

The proportion of ETEC shed in the faeces was similar among groups, and did not reflect the group-related differences observed in the intestinal viable counts and cultures. The faecal DM, which were measured daily on faeces collected from under the pens, did not differ between dietary groups. The post-infection faecal consistency scores were different ($P=0.03$), with pigs receiving the rice-only diet having firmer and better-formed faeces than pigs fed either of the barley diets (Table 5).

Discussion

Expt 1

Whilst rice and pearl barley both have relatively high starch contents, pearl barley has more soluble NSP, particularly mixed linked β -glucans, as well as insoluble NSP. The resultant increased NSP content in the barley-containing diets had no apparent influence on average live-weight gain post-weaning. Most performance studies of newly weaned pigs eating diets rich in NSP also have reported no significant difference in whole-body growth rates (Dritz *et al.* 1994; Longland *et al.* 1994; Gill *et al.* 2000), provided that the diets are isonitrogenic and isoenergetic. The performance of the uninfected pigs immediately after

Table 4. Growth and intestinal measurements of weaner pigs killed 3–4 d after experimental infection with enterotoxigenic *Escherichia coli* in Expt 2*

(Mean values)

	Incorporation of pearl barley (g/kg)†			SED	Statistical significance of effect: <i>P</i>
	0	500	500 + enzyme‡		
Live-weight gain (g/d)	10.5	–7.8	–27.0	44.48	0.270
Full large intestine (% EGBW)	2.62 ^{ab}	3.22 ^a	2.35 ^b	0.66	0.034
Empty small intestine (% EGBW)	4.59	4.68	4.34	0.68	0.390
Empty large intestine (% EGBW)	1.17 ^a	1.43 ^b	1.13 ^a	0.22	0.012
pH					
Duodenal	5.42 ^a	6.14 ^b	5.98 ^b	0.51	0.024
Ileal	6.84 ^a	6.11 ^b	6.46 ^{ab}	0.55	0.045
Distal colonic	6.78 ^a	6.46 ^{ab}	6.94 ^b	0.32	0.016
Ileum + LI VFA pool (mmol)	7.9	5.0	6.4	2.50	0.090
Viscosity (mpa-s)					
Duodenal	1.78	2.05	2.64	0.81	0.374
Ileal	1.59 ^a	2.31 ^b	2.17 ^{ab}	0.50	0.022
SI	1.7	2.16	2.45	0.69	0.061

EGBW, empty gut body-weight (live weight – weight of digesta within gut); LI, large intestine; VFA, volatile fatty acid; SI, small intestine.

^{a,b,c} 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. 420–421.

† *n* 11, 13 and 12 for 0, 500 and 500 + enzyme groups respectively.

‡ Porzyme™ 8100; Danisco, Marlborough, Wilts., UK.

weaning in the current study depended on their intake of DE rather than the amount of pearl barley included in their diet. It was recognised that the individual intake was not accurately determined because the pigs were housed in pairs, and a mean intake value was assigned to each pig. Housing piglets in pairs was necessary to encourage their post-weaning intake, and because it was considered better for their welfare. A similar positive relationship between DE intake and piglet growth has been noted previously in studies conducted immediately after weaning (Campbell *et al.* 1975; Pluske, 1993).

Feeding piglets different levels of pearl barley influenced their intestinal development, as well as the physical

microenvironment in their intestinal lumen. The site of digestion was moved distally by the addition of pearl barley, associated with reduced starch digestion in the ileum, heavier large intestinal segments and greater, more distal fermentation. These findings are consistent with adaptive changes that have been documented in older pigs fed soluble or insoluble NSP (Durmick *et al.* 2002).

The increase in the viscosity of intestinal contents of pigs eating pearl barley was of particular interest. This increase was presumably associated with the increased soluble NSP content rather than the insoluble NSP content, since insoluble NSP has not previously been implicated as a cause of altered intestinal viscosity. In turn, increased

Table 5. Faecal DM, swab scores and proportion of β -haemolytic enterotoxigenic *Escherichia coli* (ETEC) cultured from intestinal swabs in weaner pigs experimentally infected with ETEC and fed different diets in Expt 2*

(Mean values)

	Incorporation of pearl barley (g/kg)			SED	Statistical significance of effect: <i>P</i>
	0	500	500 + enzyme†		
Viable CFU/g mucosal scraping (\log_{10})					
Mid-small intestine	0.98 ^a	4.14 ^b	3.48 ^b	2.03	0.028
Proximal colon	2.29 ^a	5.21 ^b	6.00 ^b	1.86	0.006
ETEC from intestinal swabs at post mortem (%)					
Duodenum	7.5	22.1	26.5	28.61	0.405
Ileum	11.0 ^a	47.6 ^b	21.4 ^{ab}	30.58	0.051
Caecum	16.5 ^a	53.2 ^b	53.0 ^b	28.53	0.023
Faeces	27.9	44.5	38.8	32.58	0.589
Mean daily faecal swab score post-infection‡	1.9	2.1	1.6	0.89	0.183
Faecal DM (g/kg)					
Average over 6 d post-weaning	304	295	299	33.0	0.890
Average post-infection	301	292	277	58.6	0.762
Mean faecal consistency score§					
Pre-infection	1.5	1.8	1.7	0.19	0.406
Post-infection	2.9 ^a	3.6 ^b	3.7 ^b	0.27	0.030

^{a,b,c} 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 p. 420–421.

† Porzyme™ 8100; Danisco, Marlborough, Wilts., UK.

‡ Score 0–5 refers to the number of sections of the culture plate that grew ETEC: 0, no growth; 5, entire plate.

§ Score 0–5 where: 0, very hard, pellet-like faeces; 5, liquid diarrhoea. CFU, colony forming units.

viscosity is likely to reflect quite pronounced changes in the intestinal microenvironment. For example, increased intestinal viscosity induced by adding viscous-forming CMC to rice-based diets has been associated with changes in the intestinal microflora of weaner pigs (McDonald *et al.* 2001; Hopwood *et al.* 2002). The slightly lower ileal viscosity in pigs receiving 750 g compared with 500 g pearl barley/kg was unexpected, although the values were still greater than in the pigs not receiving barley. Factors that may have influenced the magnitude of intestinal viscosity include food intake (lower in pigs fed 750 g barley/kg), the DM content of food and digesta (higher in pigs fed 750 g barley/kg), and the volume of endogenous secretions by the animal.

Immediately after weaning at 3 weeks of age, there is insufficient production of pancreatic enzymes required for starch digestion (Efrid *et al.* 1982); it generally takes 3 weeks after weaning for energy and protein digestibility to stabilise (Bedford *et al.* 1992). Despite this, starch digestibility at the ileum was close to 100% in pigs eating the rice-only diet, reflecting the highly digestible nature of the cooked cereal. Cooking the rice gelatinised the starch, allowing greater interaction of digestive enzymes with the increased surface area of the starch granules. Addition of uncooked pearl barley to comprise ≥ 250 g/kg diet, however, significantly depressed the digestion of starch within the small intestine. The resultant excess starch present within the lumen of the small intestine would provide a potential source of nutrient for micro-organisms present, including enteropathogens such as ETEC.

Expt 2

The experimental model of PWC used in Expt 2 was successful in that inoculated pigs showed intestinal colonisation with the haemolytic ETEC strain to which they were exposed, and many succumbed to diarrhoea and weight loss. The pigs fed the rice-only diet maintained their body weight, had faeces of more solid consistency and had less intestinal colonisation than the pigs eating pearl barley; thus, they appeared to withstand the experimental infection more effectively.

The main intestinal effects of dietary soluble NSP from pearl barley demonstrated in Expt 1 held true for the infected pigs in Expt 2. Addition of exogenous enzymes was successful in reducing the extent of the increased weight of the large intestine associated with eating barley. This suggests that the exogenous enzymes may have improved the digestibility of the starch in pearl barley, but this could not be confirmed as digestibility was not measured in Expt 2. Unexpectedly, the degree of fermentation (as shown by VFA concentrations) in the large intestine of the pigs eating 500 g barley/kg was less than that in pigs eating the rice-only diet. This resulted in similar pH values in the large intestine among the groups, and may have contributed to a lack of an enzyme effect on colonic pH values. The pigs receiving pearl barley had a greater incidence of diarrhoea, and this would have resulted in an increased rate of digesta passage

through the gut, which may have resulted in less opportunity for microbial fermentation to occur.

The large intestine has a role in preventing loss of water in the face of small intestinal diarrhoea (Argenzio *et al.* 1984; van Beers-Schreurs *et al.* 1998), and there is evidence that this ability is compromised in the first week following weaning (van Beers-Schreurs, 1996). As suggested by Bolduan *et al.* (1988), accelerating the development of the large intestine should aid in preventing diarrhoea, through a number of mechanisms. Increased fermentation increases VFA production, which enhances Na^+ and water absorption (Crump *et al.* 1980) and creates an acidic environment that is said to be detrimental to the rapid growth of *E. coli* (Diez-Gonzalez *et al.* 1998). The latter effect was not found in the present study, despite the fact that the pearl barley stimulated fermentative and physical development of the large intestine.

It is clear that the diets containing pearl barley were associated with an altered intestinal microenvironment and increased proliferation of ETEC compared with the rice-only diet. Soluble NSP is highly fermentable, viscous, delays glucose absorption, increases digesta bulk and has a high water-holding capacity (Bedford & Schultze, 1998). Any or all of these features may have contributed to the increased proliferation of ETEC in pigs receiving barley. The results of the current experiment are consistent with those of a previous study, which found increased proliferation of ETEC and more diarrhoea in pigs fed a rice-based diet containing guar gum, also a soluble viscous NSP, compared with pigs fed cooked rice without guar gum (McDonald *et al.* 1999).

Recently, the role of increased viscosity of the intestinal contents in facilitating PWC has been investigated by including CMC in the weaner diet. CMC is a synthetic compound that increases the viscosity of the intestinal contents without altering fermentation. Pigs consuming CMC had increased numbers of naturally acquired ETEC in the intestinal tract in the first week post-weaning (Hopwood *et al.* 2002). A positive relationship between increasing dietary NSP content and occurrence of diarrhoea has also been noted in two other important bacterial diseases of pigs, swine dysentery and porcine intestinal spirochaetosis (Pluske *et al.* 1996, 1998; Siba *et al.* 1996; Durmic *et al.* 1998; Hampson *et al.* 2000; Hopwood *et al.* 2002).

Whilst the majority of the NSP introduced with the pearl barley was soluble NSP, there was about 20 g insoluble NSP/kg in the 500 g barley/kg diet. Previous experiments have suggested that dietary insoluble NSP may decrease the expression of PWC (Bertschinger & Eggenberger, 1978). Although the dietary effects observed in the current experiment were consistent with those arising from the soluble NSP, it is not possible to dissect the contributory effects of insoluble NSP from the soluble NSP. Insoluble NSP is considered to depress ileal digestibility of nutrients, increase large intestinal microbial fermentation, especially distally, increase faecal bulk and faecal water content, and reduce gut transit time, but has not been reported to cause any increase in digesta viscosity. As feeding pearl barley had no effect on faecal water content, this also suggests that its insoluble NSP content had little influence on the faecal water content.

Another potentially confounding dietary factor was the 2.8% greater crude protein ($N \times 6.25$) content of the 500 g barley/kg diet compared with the rice-only diet. Although extreme differences in dietary protein content may have an influence on PWC (Bertschinger & Eggenberger, 1978; Prohaszka & Baron, 1980), all diets in the current experiments had a high protein content, which was certainly greater than the 150 g dietary protein/kg level suggested by Bolduan *et al.* (1988) as being the threshold below which PWC is inhibited. Nevertheless, the increased protein content and proportion of plant-derived protein in the pearl barley diets must be considered as a possible contributing factor to the increased proliferation of ETEC.

The combination of addition of soluble NSP and resultant increased viscosity of the intestinal contents appeared to encourage bacterial growth. A similar phenomenon has been identified in poultry, and is alleviated by the actions of exogenous enzymes. In the current experiment, enzyme addition failed to decrease the intestinal viscosity. This was not completely unforeseen, as, for various reasons, such enzymes produce less consistent results in pigs than in poultry (Partridge, 1993; Dierick & Decuypere, 1994, 1996; Bedford & Schultze, 1998). Consistent with a lack of influence on viscosity, enzyme supplementation in the current experiment tended to maintain or increase the proliferation of ETEC rather than to decrease it. A possible explanation for this may be that the enzyme succeeded in partially degrading the NSP, thereby decreasing its molecular mass, but also increasing the availability of sugars that acted as substrates supporting the growth of ETEC.

One of the most important means to maintain the small intestine relatively free of bacteria is by maintaining a fast digesta transit time. Soluble fibres, especially viscous ones, can delay the time taken for digesta to reach the caecum (Cherbut *et al.* 1990). There also is some suggestion that they displace nutrients more distally within the small intestine before absorption (Brown *et al.* 1988). The rate of passage in the small intestine is not as rapid distally, where there are larger numbers of microbes present. A prolonged transit time could allow establishment of pathogens that would otherwise have been flushed through to the large intestine, especially if there was both extra time and increased substrate present in the distal small intestine. The increased acidity of the ileal digesta would suggest that increased microbial fermentation occurred in the ileum of pigs fed the barley diets compared with those fed the rice-only diet.

There are many possible reasons why establishment and proliferation of ETEC may have been facilitated in the more viscous intestinal environment provided by the diet containing pearl barley. Interactions between dietary soluble NSP, insoluble NSP, starch and intestinal pathophysiology are complex and require further elucidation. However, consistent with previous observations (McDonald *et al.* 1999, 2001), a highly digestible rice-based diet low in soluble NSP minimised the occurrence of PWC, and maintained positive growth when piglets were showing signs of PWC. Diets based on cooked white rice may be particularly useful for newly weaned pigs, and their use may reduce the need for chemotherapeutic intervention in piggeries where PWC is endemic.

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