Using diet to control gastrointestinal infections – a practical alternative to antibiotics?

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Summary

Several potentially adverse affects arising from the use of antibiotics to prevent and/or control infections in animals have been identified. Consequently, to reduce antimicrobial use in animals, alternative non-antimicrobial approaches are being sought. This paper summarises experiments undertaken in our laboratory where we have used certain dietary manipulations to reduce the susceptibility of pigs to a number of specific enteric bacterial infections. These conditions include intestinal spirochaete infections of the large intestine (swine dysentery and intestinal spirochaetosis), post-weaning infections of the small intestine with enterotoxigenic strains of *Escherichia coli*, and ulceration of the stomach associated with spiral bacteria including *Helicobacter* spp. and *Campylobacter* spp. In all three regions of the gastrointestinal tract it has been possible to reduce the susceptibility of pigs to the various specific infections by feeding specific diets. No single simple and universal means of reducing susceptibility to pathogenic bacteria has been identified, but generally diets that are easily digestible in the upper part of the tract, and are low in rapidly fermentable fibre (eg diets based on cooked white rice), confer some protection against experimental disease. The porcine and human gastrointestinal tracts have many physical similarities, hence the pig could be considered a model for a similar dietary approach to reducing human susceptibility to selected intestinal bacterial infections.

Introduction

In response to increasing pressures to find non-antimicrobial methods to control enteric bacterial infections in pigs, our group has been investigating whether an element of control of selected pathogens can be achieved by the optimal selection and appropriate manipulation of the diet fed to pigs. For us this has been a somewhat unusual approach to animal nutrition, as well as to microbial disease. Studies conducted to date have focused on three distinct areas of the gastrointestinal tract – the stomach, the small intestine and the large intestine – in each case attempting to control very different specific bacterial pathogens that exert their prime pathological effects at these diverse sites. The purpose of this paper is to outline the results achieved to date, and where it seems appropriate to attempt to compare and extrapolate the results obtained to enteric bacterial pathogens that infect human beings. The specific pathogens that infect pigs and humans are not identical, nor are the gastrointestinal tracts of the two species completely the same, but nevertheless the pig may serve as a useful general model of human intestinal infection. The practicalities and economics of using diet to control intestinal infections are different in pigs and humans, and many of the financial constraints on dietary control in pigs may not apply to humans, particularly for individuals in affluent western countries. The specific pig diseases studied are described here in retrograde fashion, starting from the large intestine and working proximally to the stomach, where our most recent work has concentrated.

Specific pig diseases

The large intestine: Swine dysentery

Swine dysentery (SD) is a severe colitis with characteristic mucohaemorrhagic diarrhoea, and occurs especially in young grower pigs. It has no counterpart in human beings, and the only other species that is naturally affected is the Rhea (*Rhea americanus).* The disease follows infection with the anaerobic intestinal spirochaete *Brachyspira (Serpulina) hyodysenteriae*, a bacterium which only colonises the large intestine (1). Studies in gnotobiotic pigs have indicated that the spirochaete requires the presence of other anaerobic bacterial species before it will colonise and/or produce lesions (2). One field study found that the expression of SD was reduced when pigs were transferred from a diet based on maize to one where the maize was ensiled (3). The presence of short chain fatty acids (SCFA) combined with a low pH value in the ensiled maize was thought to be bacteriocidal for the spirochaete. This scenario is somewhat similar to the situation with *Clostridium difficile* infections in the human large bowel, where a range of dietary fibre types have been tested for their ability to inhibit the organism through SCFA produced by the resident microbiota (4).

These considerations led us to compare pigs fed on a typical Western Australian pig diet based on wheat and lupins (rapidly fermented in the large intestine) with those on an experimental diet based on cooked white rice and animal protein that contained little fermentable substrate (5). As predicted, indices of fermentation (reduced pH values, increased SCFA, increased large intestinal weight) were greater in the wheat and lupin-fed pigs. Unexpectedly, when subgroups of the animals on the two diets were experimentally infected with *B. hyodysenteriae* the wheat-fed pigs all developed SD, whilst none of the rice-fed pigs developed disease. Although it was suspected that the reduced fermentation with rice was the protective influence, an experiment was undertaken in which four groups of pigs were fed all combinations of rice or wheat as the cereal grain, and lupins or animal protein as the protein source (5). Again the protective effect was limited to the rice and animal protein diet, where there was reduced fermentation in the large intestine compared to the other three diet combinations (Table 1).

We then examined the susceptibility of 15 groups of pigs to experimental SD. These animals were fed a range of different cereal grains, either heat processed or not (6). Full protection from SD again was obtained with cooked rice, but partial protection (fewer diseased pigs) occurred when steam-flaked maize or sorghum was used as the cereal grains, together with animal protein. Overall, it was apparent that diets containing < 1.5% soluble non-starch polysaccharide (sNSP) and <10% resistant starch (RS) resulted in limited fermentation in the large intestine, and in relative protection from experimental SD.

Table 1. Pooled results showing faecal shedding of spirochaetes and the incidence of swine dysentery in pigs fed wheat or rice-based diets following oral challenge with *B. hyodysenteriae* (adapted from 5).

	Diet Type[1]			
	RA	RL	WA	WL
No. of pigs challenged	16	6	6	16
No. of pigs shedding *B. hyodysenteriae* in faeces	3	6	5	13
Mean duration (days) of shedding in faeces	4.6	5.4	5.6	8.5
No. pigs developing SD	0	5	3	10
Incidence of disease, %	0	83.3	60	62.5

[1]Diets: RA: 77% cooked white rice + 18% animal protein; RL: 64% cooked white rice + 13% animal protein + 15% dehulled Australian sweet lupins; WA: 75% wheat + 17% animal protein; WL: 62% wheat + 11.5% animal protein + 15% dehulled Australian sweet lupins.

To confirm the role of dietary sNSP and RS in both large intestinal fermentation and susceptibility to SD, an experiment was conducted in which five groups of pigs were all fed the cooked rice diet. One group was supplemented with oat chaff as a source of insoluble NSP (slowly fermented), one with guar gum as a source of rapidly fermentable sNSP, one with retrograde maize starch as a source of RS, and one group with both guar gum and retrograde maize starch (7). Consistent with the hypothesis that rapid fermentation in the large intestine is required for colonisation and development of SD, only pigs fed the non-supplemented cooked rice, or rice plus oat chaff remained protected after experimental challenge (7).

The results from these experiments demonstrate that the situation with SD is different from that assumed to occur with many other bacterial pathogens that colonise the large intestine. Bacterial fermentation is actually required for colonisation, and there is no evidence for the normal microbiota providing "colonisation resistance" against the pathogen. In this situation the addition of prebiotic fermentable substrate might well exacerbate SD rather than help control it.

Preliminary attempts to use dietary enzymes to increase digestion of dietary carbohydrate in the small intestine and hence limit fermentation in the large intestine have met with limited success (8). Similarly the influence of diet on the normal large intestinal microbiota of the pig, and how this relates to protection from SD is not particularly clear, and requires further study (8,9).

The large intestine: Intestinal spirochaetosis

Intestinal spirochaetosis (IS) is of greater comparative interest than SD since the causal spirochaete *Brachyspira (Serpulina) pilosicoli* not only infects pigs (10), but also human beings (11,12) and other species including chickens (13) and dogs (14). In pigs IS is associated with a chronic intermittent diarrhoea and reduced growth rates in weaner and grower animals, and this can be induced with strains isolated from humans, as well as with strains from pigs (15). The spirochaete colonises the large intestine of around 30% of human beings in developing countries, and is present at a similar prevalence in Australia in male homosexuals, HIV patients and in Aboriginal children in remote communities (11,16,17). The spirochaete is closely related to *B. hyodysenteriae,* and again only colonises the large intestine. In view of the similar genetic background and habitat of *B. pilosicoli* and *B. hyodysenteriae,* we attempted to determine whether the cooked rice-based diet also protected experimentally infected pigs from IS (18). In this experiment, control pigs on a wheat and lupin diet started to shed spirochaetes in their faeces a mean of 3 d after challenge, and shedding continued for a mean of 16 d. In contrast, pigs fed the cooked rice diet did not start shedding for a mean of 10 d, and had a duration of shedding of five days. Both these differences were significant ($p>0.001$). In this case the rice based diet did not prevent colonisation by *B. pilosicoli,* but did significantly delay its onset and shorten its duration.

One of the peculiarities noted with the rice-based diet was the sparseness and dryness of the large intestinal contents and faeces compared to conventional pig diets. It seemed possible that such physical changes in the digesta might by themselves provide a sub-optimal environment for the spirochaetes to colonise and proliferate. To test this possibility, we added 4% carboxymethyl cellulose (CMC) to the diet of a group of pigs fed the rice-based diet, then challenged them with *B. pilosicoli* (19). Addition of CMC significantly increased the viscosity of the caecal and colonic contents, as measured using a cone-plate rotational viscometer, and was associated with significantly increased colonisation with *B. pilosicoli* and more diarrhoea (Table 2) – essentially to levels normally seen with pigs fed wheat-based diets (18). The viscosity and hydration of the

large intestinal contents therefore may be important in facilitating colonisation by the pathogenic spirochaetes. Again, the presence of dietary components such as sNSP are likely to increase the viscosity of the digesta and perhaps enhance colonisation by selected bacterial pathogens. The results suggest that feeding human IS patients with diets that are low in sNSP (eg cooked rice) could be a useful adjunct to antimicrobial therapy.

Table 2. The effect of addition of 4% carboymethyl cellulose (CMC) to a rice-based diet on proliferation of *B. pilosicoli* in pigs experimentally infected with *B. pilosicoli* and killed 22 days later (adapted from 19).

	Rice (n=6)	Rice + CMC (n=6)	sem	P-value
Caecal viscosity (mPa•s)	2.13	4.50	1.26	0.013
Colonic viscosity (mPa•s)	1.78	5.98	1.86	0.007
No. diarrhoeic pigs at end of trial	0	4	-	0.061
Mean number of tested days that faecal swabs were positive for *B. pilosicoli* (total possible =9)	1.50	4.17	1.19	0.003

The small intestine: Post-weaning colibacillosis

Post-weaning colibacillosis (PWC) is a common problem in the pig industry, associated with an overgrowth of enterotoxigenic strains of *Escherichia coli* (ETEC) in the small intestine immediately after weaning. This overgrowth results in a severe secretory diarrhoea, and surviving pigs can have significant checks in growth rate. Unlike most humans, pigs are usually weaned abruptly at 3-4 weeks of age. Besides the abrupt change to a solid diet at weaning, there are numerous other dietary influences on PWC (20). In recent work we have compared growth and disease outcomes in pigs fed standard weaner diets compared to diets based on cooked white rice (21). The rice-based diet encouraged increased carcase weight, and pigs on this diet had fewer ETEC in their small intestinal content at slaughter one week after experimental challenge compared to pigs fed a wheat-based diet (21). When guar gum was added to the rice-based diet as a source of sNSP, a significantly increased number of ETEC were found in the small intestine one week after challenge compared to the basal rice diet (22). When 4% CMC was added to the rice-based diet, this significantly increased the viscosity in the small and large intestine, and again resulted in significantly greater shedding of ETEC in the faeces (23), and significantly higher ETEC numbers in the small and large intestines five d after weaning (19; Table 3). Presumably the increased viscosity of the intestinal contents led to local stasis, which, together with the presence of trapped undigested carbohydrate from the cooked rice as a readily-utilized substrate, encouraged proliferation of the ETEC strains.

Table 3. The effect of addition of 4% carboxymethyl cellulose (CMC) to a cooked rice-based diet on mean intestinal viscosity and proliferation of enterotoxigenic *E. coli* (ETEC) in pigs killed 7 d post-weaning (adapted from 19).

	Rice (n=6)	Rice + 4% CMC (n=6)	sem	P-value
Duodenal viscosity (mPa•s)	1.19	6.58	1.35	0.0001
Ileal viscosity (mPa•s)	1.52	7.23	2.39	0.0020
ETEC in jejunum (log_{10})	1.49	5.79	1.43	0.0004
Colonic viscosity (mPa•s)	1.26	2.80	1.24	0.0584
ETEC in colon (log_{10})	2.54	8.22	1.70	0.0002

Taken together, these results indicate that feeding a diet based on cooked white rice is beneficial in reducing proliferation of ETEC, and that diets or dietary ingredients that increase viscosity of the intestinal contents tend to favour proliferation of ETEC. These protective effects of cooked rice are distinct from additional effects of rice extracts used as rehydration therapy (24), and as a

means of inhibiting specific ETEC-induced secretory mechanisms via alterations to cyclic AMP concentrations in the small intestinal crypt epithelium (25). Again the pig could be a useful model for studying specific dietary influences on secretory diarrhoea in the small intestine.

The stomach: Stomach ulceration and erosions

Pigs frequently develop ulcers in the *pars oesophagea* of the stomach – a unique area of stratified squamous epithelium that extends from the oesophagus around its opening into the first part of the pig's stomach. It is debated how serious a problem this is in relation to pig production, but complications such as perforations and haemorrhage can occur, and are a major cause of mortality in young breeding pigs. This condition has long been known to be linked to a variety of dietary inputs – for example feeding wheat predisposes to ulcers, as does fine grinding of the diet (26). More recently, it has been claimed that spiral bacteria – most notably *Helicobacter heilmannii* – are associated with development of the lesions (27). This parallels the situation in human gastritis and ulceration, where *Helicobacter pylori* is recognised as the main causative agent.

Whilst investigating the condition in pigs, we developed a model where weaner pigs fed a diet based on finely ground wheat developed ulcers in the fundic region. If this finely ground wheat was extruded, significantly fewer pigs developed ulcers (28). Examination of biopsy material from these pigs using a series of polymerase chain reaction assays (PCR) demonstrated that although there was no relationship with *Helicobacter heilmannii*, there was a significant association between the absence of ulcers and the presence of *Campylobacter* spp. (28). These unexpected results demonstrated links between diet, ulcers and changes in the stomach microflora (Table 4). In a parallel study using PCR to analyse stomach samples from commercial adult pigs at slaughter, again there was a highly significant association between lack of ulcers and the presence of *Campylobacter* spp., and no positive link was found between ulcers and colonisation by *Helicobacter heilmannii* (29). Although it is uncertain how these results relate to human gastritis and stomach ulceration, links between diet and human peptic ulceration have been made (30). Future work will focus on whether dietary substrate or other factors are involved in altering the microflora of the porcine stomach, and determining whether *Campylobacter* spp. have a protective role or if they are normal flora whose presence reflects a healthy stomach environment.

Table 4. Occurrence of ulcers and colonisation by *Helicobacter* spp. and *Campylobacter* spp. in weaner pigs fed diets containing finely-ground wheat, or the same wheat after extrusion (adapted from 28).

Diet	No. of pigs	Ulcers	*Helicobacter* spp.	*Campylobacter* spp.
Wheat (raw)	1	-	+	+
Wheat	1	+	+	+
Wheat	2	+	-	+
Wheat	4	+	-	-
Extruded wheat	1	+	-	+
Extruded wheat	2	-	+	+
Extruded wheat	5	-	-	+

Conclusions

This paper has briefly outlined situations in the stomach, small intestine and large intestine of pigs where infections with specific and distinct bacterial pathogens can be influenced by the diet fed to the animals. Although no one protective diet was identified, diets that contain little

fermentable substrate (sNSP and RS), and in particular cooked white rice, appeared to be beneficial in a number of cases. Dietary manipulation of other intestinal infections in the pig are being studied, most notably carriage of *Salmonella* serovars. Lessons learnt from these studies may have application in human infections, with the pig appearing to be a potentially useful model in which to study interactions between diet and selected pathogenic enteric bacteria.

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