

Nutritional influences on some major enteric bacterial diseases of pigs

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There are several enteric bacterial diseases and conditions of pigs that require control to prevent overt disease, to reduce morbidity and mortality, and to improve the efficiency of production. Traditionally, veterinarians, feed manufacturers and producers have relied upon antibiotics and minerals (for example, ZnO, CuSO₄) in diets for a large part of this control. However, recent trends, particularly in Europe, are to reduce antimicrobial use and seek alternative or replacement strategies for controlling enteric bacterial diseases. The majority of these strategies rely on 'nutrition', taken in its broadest sense, to reduce the susceptibility of pigs to these diseases. Evidence to date suggests that specific dietary interventions, for example feeding very highly-digestible diets based on cooked white rice, can reduce the proliferation of a number of specific enteric bacterial infections, such as post-weaning colibacillosis. No simple and universal way to reduce susceptibility to pathogens in the gastrointestinal tract has been identified, and the underlying basis for many of the reported positive effects of 'nutrition' on controlling enteric infections lacks robust, scientific understanding. This makes it difficult to recommend dietary guidelines to prevent or reduce enteric bacterial diseases. Furthermore, some diseases, such as porcine intestinal spirochaetosis caused by *Brachyspira pilosicoli*, are sometimes associated with other pathogens (co-infections). In such cases, each pathogen might have different nutrient requirements, ecological niches and patterns of metabolism for which a variety of dietary interventions are needed to ameliorate the disease. Greater understanding of how 'nutrition' influences gut epithelial biology and immunobiology, and their interactions with both commensal and pathogenic bacteria, holds promise as a means of tackling enteric disease without antimicrobial agents. In addition, it is important to consider the overall system (i.e. management, housing, welfare) of pig production in the context of controlling enteric bacterial diseases.

Disease: Bacteria: Gastrointestinal tract: Pigs

Abbreviations: CMC, carboxymethylcellulose; CP, crude protein; FLF, fermented liquid feed; NDO, non-digestible oligosaccharides; PIS, porcine intestinal spirochaetosis; PPE, porcine proliferative enteropathies; ppm, parts per million; PWC, post-weaning colibacillosis; RS, resistant starch; VFA, volatile fatty acid; SD, swine dysentery; ST, heat-stable toxin.

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Introduction

A variety of commensal and pathogenic bacterial species colonise the gastrointestinal tract of the pig. The pathogenic bacteria can cause clinical disease, morbidity and mortality, and are a major source of economic loss to the pig industry worldwide. A number of distinct genera and species of bacteria are involved, and each of these pathogens tends to inhabit a different region of the gastrointestinal tract and is generally associated with a different age or class of pig. For example, post-weaning colibacillosis (PWC) caused by *Escherichia coli* specifically affects the small intestine in the first 3–10 d after weaning. The major form of control of these enteric infections is antimicrobials, which are provided to treat overt disease, to provide prophylaxis in situations where a disease (or diseases) is (are) liable to occur, and to improve growth rates in the absence of disease (the ‘growth-promoting’ effect of antibiotics) (Hampson *et al.* 2001).

In some countries though, particularly in Europe, there have been widespread bans (both legislative and voluntary) placed on the use of antimicrobials in pig diets for growth promotion. This is largely because of rising fears regarding the implications for both animal and human health (i.e. food safety) of continued use of antimicrobials in the intensive livestock industries, although other influences such as the establishment and/or protection of export markets for pork also play a role. The prolonged use of antimicrobials most likely selects for the survival of resistant bacterial species or strains, and genes encoding this resistance can be transferred to other formerly susceptible bacteria. A number of bacterial pathogens of pigs are showing resistance to a range of antimicrobial drugs (Barton, 1999). Not only is this reducing the number of antimicrobials available to the industry to control bacterial infections, but this resistance also poses risks to human health. For example, the transfer of resistant zoonotic pathogens such as *Salmonella typhimurium* and *Campylobacter jejuni* from pigs to man has been reported, and the direct or indirect transfer of resistance genes from the porcine gastrointestinal microflora to human bacterial strains also poses considerable risks (Barton, 2000; Hampson *et al.* 2001). Concerns about these issues are leading to reduced availability of antimicrobial agents for use in the pig industry. Consequently, it is important to develop means both of controlling bacterial infections and promoting growth in pigs without recourse to the use of antimicrobials and certain minerals, such as ZnO and CuSO₄. In this regard, ‘nutrition’, in its broadest sense, has attracted enormous interest as a means of ameliorating enteric infections in pigs, and the search for antimicrobial alternatives or replacements will continue as pressure for the complete ban of growth-promoting antimicrobials in the pig industry continues to mount.

The purpose of the present review is to assimilate past and current knowledge pertaining to the use of nutrition, in its broadest sense, and its roles in causing and modulating major enteric bacterial diseases in pigs. The review will first describe the ecology of the gastrointestinal tract in relation to the genera and species present and their interactions with the host. This is followed by a discussion of the major enteric diseases in pigs that cause production and economic losses, and how the use of nutrition is involved in both the aetiology of the disease and in its modulation. Although important associations between gut immune function and disease are recognised, the gut-associated lymphoid tissue and its development will not specifically be reviewed here. The reader is directed towards several excellent recent reviews in this area by Kelly *et al.* (1994), Deplancke & Gaskins (2001), Gaskins (2001), Kelly & King (2001) and Stokes *et al.* (2001).

The ecology of the gastrointestinal tract

What comprises the normal microflora?

The porcine intestinal microflora is established within 48 h after birth via ingestion of maternal faeces, and involves complex successional changes until dense, stable populations colonise the

gastrointestinal tract (Mackie *et al.* 1999). The microbiota is characterised by its high population density, extensive diversity, and complexity of interactions throughout the gastrointestinal tract. A distinction between indigenous (autochthonous) and non-indigenous (allochthonous) bacteria is required for an ecological understanding of colonisation, succession and mechanisms of host interactions. Autochthonous (indigenous) bacteria are those that have co-evolved with the host and colonise all habitats and niches in the gastrointestinal tract, whereas allochthonous (non-indigenous) bacteria may pass through specific microhabitats, being derived from food, water or another gut habitat, and do not colonise the tract (Dubos *et al.* 1965). Colonisation describes the process by which a population of bacteria in the gastrointestinal tract becomes stable in size over time without the need for periodic reintroduction (Gaskins, 2001). These bacteria colonise at a rate that equals or exceeds their rate of washout or elimination from an intestinal habitat. Pathogens can be autochthonous or allochthonous, and generally cause disease when the gut ecosystem is disturbed in some manner.

The stomach and proximal small intestine (duodenum) contain relatively low numbers of bacteria (10^3 – 10^5 bacteria/g or ml of contents) due to low pH and/or rapid digesta flow. Whilst the piglet is still suckling, the dominant bacteria within the stomach and small intestine tend to be *Lactobacillus* spp. and *Streptococcus* spp. (Jensen, 1998). In the proximal small intestine, digesta flow rate and the rate of bacterial washout exceeds the maximal growth rates of most bacterial species, and the bacteria that are present typically adhere to the mucus or epithelial cell surface (Gaskins, 2001). In contrast, the distal small intestine harbours a more diverse and numerically greater (10^8 bacteria/g or ml of contents) population of bacteria. The large intestine is the major site of microbial colonisation because of the high residence time of the digesta. The luminal contents of the colon support in excess of 400 different bacterial species with numbers as high as 10^{10} and 10^{11} culturable bacteria/g (wet weight) of digesta (King & Kelly, 2001). Characterisation of the intestinal microbiota has been conducted using anaerobe culturing techniques, and numerous studies show that the major bacterial groups isolated from the pig intestine are *Streptococcus*, *Lactobacillus*, *Prevotella*, *Selenomonas*, *Mitsuokella*, *Megasphaera*, *Clostridia*, *Eubacteria*, *Bacteroides*, *Fusobacteria*, *Acidodaminococci*, and the *Enterobacteria* (Salanitro *et al.* 1977; Allison *et al.* 1979; Russell, 1979; Robinson *et al.* 1981; Moore *et al.* 1987; Jensen, 2001) (Table 1). The hindgut flora is considered both diverse and stable, with the many species and strains appearing to coexist without one or few ever becoming dominant. Further information pertaining to the composition of the microbiota can be found in reviews by Stewart (1997), Mackie *et al.* (1999), Gaskins (2001), Jensen (2001) and Leser *et al.* (2002).

In addition to a proximal to distal gradient in bacterial numbers in the gastrointestinal tract, Gaskins (2001) described radial distributions of microbes within each segment of the gut. The four microhabitats for the commensal flora include: the intestinal lumen; the unstirred mucus layer, or layer that covers the mucosal epithelium; the deep mucus layer found in the crypts; the surface of the intestinal epithelial cells. The diversity of the hindgut microflora and that of the distal region of the small intestine reflects in part the types of nutrient substrates found in these regions. The diversity of bacterial populations within a particular ecosystem is directly related to the number and composition of limiting nutrients, since each limiting nutrient will support the one bacterial species or strain that is most efficient in utilising it (Gaskins, 2001). Moreover, the stability of these bacterial populations will also be influenced by the inhibitory actions of a number of compounds such as volatile fatty acids, H_2S , deconjugated bile salts, NH_3 and bacteriocins (Gaskins, 2001). In this regard, it is likely that certain nutrients and their associated physicochemical effects play a major role in maintaining the balance of the microflora in these parts of the gastrointestinal tract, and subsequently in determining whether a pathogenic bacteria proliferates to cause overt expression of disease.

Table 1. The predominant bacteria cultivated from the small and large intestines of the pig (total isolates 1679) (from Jensen, 2001)

Isolate number	Proportion of isolates (%)				Similarity to known bacterial species
	Total	SI*	Caecum*	Colon*	
1. <i>Escherichia coli</i>	22.0	49.0	9.6	5.5	<i>E. coli</i> (99.8 %)
2. <i>Streptococcus alactolyticus</i>	13.5	18.2	10.7	8.4	<i>S. alactolyticus</i>
3. <i>Prevotella</i> sp. 1	10.0	0.0	17.0	1.0	UB adhesin 94 (98.1 %)†
4. <i>Streptococcus hyointestinalis</i>	9.5	11.8	8.1	6.3	<i>S. hyointestinalis</i>
5. <i>Prevotella</i> sp. 1a	2.9	0.0	6.3	4.8	<i>P. oulora</i> (92.4 %)†
6. <i>Lactobacillus acidophilus</i> and <i>jonsonii</i>	2.2	0.0	3.2	3.6	<i>L. jonsonii</i> (99.7 %)
7. <i>Lactobacillus</i> sp. 2	2.0	0.3	1.5	2.2	<i>L. vitulinus</i> (93.9 %)†
8. <i>Selenomonas</i> sp. 1	1.9	0.0	2.3	3.6	<i>S. ruminantum</i> (94.7 %)†
9. <i>Mitsuokella</i> sp.	1.7	0.7	1.9	2.7	<i>M. multiacidicus</i> (97.8 %)
10. <i>Megasphera</i> sp.	1.7	0.0	1.3	3.9	<i>M. elsdenii</i> (93.7 %)†
11. <i>Acidaminococcus fermentans</i>	1.5	0.3	4.2	1.5	<i>A. fermentans</i> (99.4 %)
12. <i>Clostridium perfringens</i>	1.5	2.7	1.5	0.4	<i>C. perfringens</i> (99.7 %)
13. <i>Eubacterium</i> sp.	1.1	0.0	1.3	1.4	Butyrate-producing bacteria (96.6 %)†
14. <i>Prevotella</i> sp. 13	1.1	0.0	0.0	1.3	Not known (<97 %)†
15. <i>Bacteroides</i> sp. 1	1.0	0.2	2.7	0.0	<i>B. vulgatus</i> (99.5 %)
16. <i>Megasphera elsdenii</i>	1.0	0.0	2.5	0.4	Did not survive freezing
17. <i>Fusobacterium mortiferum</i>	1.0	2.9	0.0	0.0	<i>F. mortiferum</i> (99.9 %)
18. <i>Eubacterium</i> sp. B	0.4	0.0	1.0	0.9	UB adhesin 420 (95.6 %)†

SI, small intestine; UB, uncultured bacteria.

*In the small intestine there were 579 isolates, in the caecum 529 isolates and in the colon 571 isolates recovered. Rows and columns do not add up to 100 % because here is shown only the predominant isolates that have been identified; there are > 600 isolates at current estimations, hence the numbers do not equal 100 %.

† Isolates could not be assigned to any known species.

Current studies in microbial ecology in the pig

Concerns associated with the loss of growth-promoting antibiotics and minerals such as ZnO and CuSO₄ in the pig industry have caused renewed interest in studying the microbial ecology of the gastrointestinal tract. Not only has this area received little scientific attention in the pig, but it has also raised many questions concerning the composition, structure and stability of ecosystems in the gut as well as the activity and function of individual inhabitants. Increased understanding of these associations is pivotal to determining both the efficacy of antimicrobial 'alternatives' or 'replacements', and developing new methods to control enteric diseases. Nevertheless, current knowledge of microbial diversity and ecology is based largely on anaerobic culture techniques that, as stated by Gaskins (2001), are limited by three major factors: culturing can only be performed on those organisms for which nutritional and growth requirements are known; there is a lack of a phylogenetically based classification scheme; there is unavoidable bias introduced by culture-based enumeration and characterisation techniques because of different survival rates *in vitro*.

Modern molecular techniques based on, for example, comparative sequence analysis of small subunit ribosomal RNA (16S rRNA) molecules, can be used to provide molecular characterisation (Simpson *et al.* 1999; Tannock, 1999), while at the same time providing a classification system that predicts natural evolutionary relationships (Pace, 1997). A recent study of microbial diversity in the mucosal layer of the pig colon with molecular analysis compared with culture-based methods highlights the problems mentioned earlier. Pryde *et al.* (1999) demonstrated that *Streptococci* spp. and *Lactobacilli* spp. comprised the majority of isolates recovered (54 %) from the colon wall by culturing; however these groups accounted for only

33 % of the sequence variation for the same sample from random cloning. In addition, 59 % of randomly cloned sequences showed less than 95 % similarity to database entries or sequences from cultivated organisms. Data presented in Table 1 by Jensen (2001) similarly demonstrate the shortcomings of traditional culturing techniques, since numerous bacterial species present in the intestines belong to unknown species. As suggested by Jensen (2001), despite the majority of the larger bacterial populations being represented using culture techniques, it is likely that small populations performing key ecological functions may escape detection.

There is a dearth of studies investigating the bacterial ecology of the pig's gastrointestinal tract, especially using molecular techniques. Simpson *et al.* (1999) used denaturant gradient gel electrophoresis to measure changes in bacterial populations in the gastrointestinal tract of the pig between 18 d and 6 months of age. These workers detected differences in populations between different gut compartments and specific locations within each compartment. Similarly, Simpson *et al.* (2000) studied the diversity and stability of the faecal bacterial microbiota in weanling pigs after introduction of an exogenous *Lactobacillus reuteri* strain (MM53). The use of such techniques, in which individual bacterial species can be identified, is a potentially powerful tool for monitoring changes in the microbiota of individual pigs in relation to nutritional changes and disease. In this regard, Leser *et al.* (2002) have recently compiled a library of 4270 cloned 16S rDNA sequences representing 375 phylotypes from the ileum, caecum or the colon of pigs aged 12–18 weeks. The design of specific oligonucleotide probes to characterise the phylotypes will facilitate the analysis of many samples to characterise the responses of the intestinal bacterial community to interventions such as antibiotic replacements, probiotics, diets and so on.

Interactions between the luminal bacteria and the gut epithelium

In conjunction with the renewed interest in identifying and quantifying the microbial ecology of the pig's gastrointestinal tract, a resurgence of interest has occurred in examining the mechanisms of action and extent of 'cross talk' between the enteric bacteria and the host (for a recent review, see King & Kelly, 2001). The chemistry and distribution of bacterial binding sites on gut mucosal surfaces play important roles in determining host and tissue susceptibility and in triggering host responses, especially in young animals (Kelly & King, 2001). Individual mucin carbohydrates have the capacity either to repel or bind to microbial surface adhesins. Enteric bacterial strains that cause diarrhoea, for example, are generally classified according to their lectins (fimbria), which constitute proteinaceous appendages that protrude from the bacterial surface and recognise sugar moieties of glycoproteins and/or glycolipids on epithelial surfaces. The synthesis of these appendages (pili) and the production of enterotoxins are key virulence factors that enable enteric pathogens to colonise the intestines (Kelly & King, 2001). Some evidence suggests that dietary proteolytic treatment of the glycoprotein receptors can prevent attachment of enterotoxigenic *E. coli*. For example, Mynott *et al.* (1996) and Chandler & Mynott (1998) reported that feeding bromelain, a proteolytic extract from pineapple stems, significantly reduced K88 (+) attachment in the small intestine commensurate with decreased diarrhoea and improved weight gain in piglets.

Protection of the epithelium against the microbes lies in the capacity of mucin carbohydrates, particularly in the small intestine, to either repel or bind microbial adhesins (Belley *et al.* 1999). The ability to bind to mucin carbohydrates enables some bacterial groups, both commensal and pathogenic, to colonise the mucus layer. Gaskins (2001) commented that bacteria living in the mucus layer prevent the attachment of pathogenic microbes by occupying avail-

able binding sites, although further evidence *in vivo* to support this claim is required. This is likely to be an active area of future research as new techniques are developed to examine the mucus layer without disrupting its integrity.

The nature of the diet, the microbial flora, and interactions between them influence the composition and functional characteristics of intestinal mucins (Sharma *et al.* 1997). It is likely that many enteric bacteria produce mucolytic or glycosidic enzymes that alter the chemical nature of the mucins, and an ability to degrade mucus has been documented in both commensal bacteria and pathogens (for example, see Deplancke & Gaskins, 2001; Kelly & King, 2001). The process of mucolysis (Deplancke & Gaskins, 2001; Gaskins, 2001) would compromise epithelial barrier function, leading to possible bacterial translocation into the lamina propria and subsequent recruitment of inflammatory cells. This can be perceived as being a logical defensive response by the host, but this response uses energy that would otherwise be directed towards carcass gain and may also predispose to disease. A recent study showed that inclusion of galactose in the diet of weanling pigs modified the mucin (glycoprotein) composition compared with a control diet (Pestova *et al.* 2000), leading these authors to surmise that this dietary change might have limited the microbial degradation of mucins.

The mucus-secreting goblet cells play a crucial role in intestinal homeostasis, especially with regard to how enteric pathogens cause disease and what factors (for example, dietary, environmental) trigger these events. Changes in both the number of goblet cells and the chemical composition of the mucus layer occur under conditions of intestinal 'insults', such as weaning (Dunsford *et al.* 1991), dietary change (Brunsgaard, 1998), and total parenteral nutrition (Ganessunker *et al.* 1999). For example, Ganessunker *et al.* (1999) compared goblet cell and immune-related parameters in neonatal piglets fed a milk replacer intravenously via total parenteral nutrition with those fed the same diet enterally. Total goblet cell numbers in the jejunum and ileum and sulfomucin-positive goblet cells within ileal villi were increased in total parenteral nutrition-fed pigs *v.* enterally-fed pigs. Furthermore, goblet cell and mucin subtype alterations were correlated to local expansion of T-lymphocyte populations.

At present the taxonomy and distribution of bacterial groups, both commensal and pathogenic, which preferentially reside within the intestinal mucus layer must be better defined to ascertain the role of 'normal' gut bacteria in mucogenesis and mucolysis. It is generally thought that a bacterial consortium comprising several genera provide the necessary enzymes for mucolysis and perform mucin degradation *in vivo*. Deplancke & Gaskins (2001) commented that microbial populations residing in mucus are poorly (if at all) characterised for the pig. As goblet cells are a key component of epithelial defence, then a greater understanding of mechanisms regulating mucin production and degradation by bacteria is necessary.

Major enteric bacterial diseases of the pig

In the future, increasing attention will be directed towards finding practical and cost-effective solutions to replace growth-promoting antimicrobials in diets for pigs. One solution is to find dietary means of ameliorating intestinal diseases. Some of these are already well established in the pig industry, for example the routine inclusion of pharmacological levels of ZnO and/or CuSO₄ in diets. The following discussion reviews some of the major enteric bacterial diseases of pigs in relation to aspects concerning nutrition. It will attempt to identify areas and opportunities whereby nutrition may predispose pigs to enteric disease, and can be used prophylactically or therapeutically to ameliorate disease, in the absence of antimicrobial agents.

Post-weaning colibacillosis

Pathogenesis of post-weaning colibacillosis. PWC is a disease of the small intestine. Although digesta flows relatively quickly through the small intestine, the pathogenic *E. coli* that proliferate in the condition possess fimbriae, or pili, that attach to the enterocytes lining the small intestinal villi or to the mucus covering the villi. Attachment prevents the bacteria from being flushed through to the large intestine. After colonising the small intestine, enterotoxigenic *E. coli* provoke hypersecretory diarrhoea through the release of specific enterotoxins. Secretion of chloride ions, sodium ions, bicarbonate ions, and water into the lumen is induced by the actions of a heat-labile toxin binding irreversibly to the mucosal cells and activating the adenylyl cyclase–cyclic AMP system (Argenzio, 1992). A second heat-stable toxin (ST), with subtypes STa and STb, inhibits the absorption of sodium and chloride ions from the lumen into the epithelial cell via the guanyl cyclase–cyclic GMP system.

PWC is a major cause of mortality and morbidity worldwide. Immunity to one strain of pathogenic *E. coli* does not necessarily protect from others, and successive strains can pass through herds. Colonisation of the small intestine and diarrhoea usually last between 4 and 14 d, with the strains being spread between animals primarily by the faecal–oral route, and also by aerosols (Bertschinger, 1999). Research has shown that most *E. coli* associated with PWC are enterotoxin-producing, β -haemolytic strains. The presence of particular serotypes has also been correlated with haemolytic *E. coli* causing clinical disease, in particular types O8, O138, O139, O141, O147, O149, and O157 (Hampson, 1994). Numerous reviews of post-weaning *E. coli* diarrhoea (or PWC) have been presented previously (Hampson, 1987, 1994; Bertschinger, 1999). It is common for haemolytic *E. coli* to appear in the faeces of pigs in increased numbers in the first week after weaning in both healthy and diarrhoeic pigs, although the numbers of *E. coli* in diarrhoeic pigs is higher (Kenworthy & Crabb, 1963; Hampson *et al.* 1985). Pigs displaying PWC harbour massive numbers of haemolytic *E. coli* in the jejunum, whilst there is minimal change in numbers of other bacteria (Smith & Jones, 1963).

Predisposing factors for post-weaning colibacillosis. Despite haemolytic enterotoxigenic *E. coli* being identified as the primary infectious agent in this disease, there is abundant evidence to suggest that other factors are necessary for PWC to occur (for example, see Madec *et al.* 1998, 2000; Jones *et al.* 2001). In the small intestine, *E. coli* fimbriae attach to glycoprotein receptors expressed in the brush border of cells lining the intestinal villi. The most common fimbriae associated with *E. coli* causing PWC is K88, renamed as F4. The receptor for F4 disappears a few weeks after weaning, offering only a brief window of opportunity for this pathogen to attach and proliferate. Some pigs do not possess the receptors for the *E. coli*, and some have receptors that are only weakly adhesive (Hampson, 1994).

The act of weaning is an essential precipitating factor for PWC, regardless of the age at weaning. All of the factors involved with weaning create an environment suitable for the proliferation of *E. coli* in the small intestine. Slower gut transit time and gut stasis immediately after weaning allow bacteria the opportunity to attach and time to multiply. Numerous studies (for a review, see Hampson, 1987) suggest that the form of the feed (for example, liquid *v.* dry) influences a pig's susceptibility to PWC, with a liquid feed fed at regular intervals after weaning being beneficial in reducing diarrhoea (Lecce *et al.* 1983). Consequently, it has been reported that restricting the amount of feed given to pigs reduces the incidence of PWC (for example, Rantzer *et al.* 1996), presumably because undigested food particles in the lumen of the small intestine supply less substrate for bacterial growth. In contrast, Madec *et al.* (1998) reported

that low feed intake (< 1000 g) in the first week after weaning placed piglets at a greater risk of PWC than intake in excess of this amount. In addition, the change from sows' milk to solid feed results in the loss of any passive protection provided by the milk.

An inability of piglets to adequately thermoregulate, combined with sub-standard weaning accommodation, may result in cold stress. This alters intestinal motility and is thought to be an important factor in the pathogenesis of PWC (Wathes *et al.* 1989). Social stresses from mixing, fighting and crowding trigger cortisol release, most likely increasing transit time (via the sympathetic nervous system) and depressing the immune response to bacterial infection. Moving to a new pen environment causes increased antigenic exposure to microbes residing in fresh or dry faecal matter. The presence of other pathogens such as rotavirus in the environment increases the likelihood and severity of disease occurring (Lecce *et al.* 1983), and poorer hygiene will result in a greater antigenic load delivered to the small intestine because of faecal–oral cycling (Madec *et al.* 1998).

Influence of carbohydrate ('fibre') sources on post-weaning colibacillosis. Numerous reports dating back to the 1960s and 1970s showed that addition of insoluble fibre sources such as the husks from cereals could reduce the excretion of haemolytic *E. coli* and the incidence of PWC. For example, Smith & Halls (1968) found that barley hulls fed *ad libitum*, but not pearl-barley meal, prevented disease in weaner pigs inoculated with *E. coli*. Those fed barley meal remained susceptible to PWC. The barley fibre used in this trial was the outer hull of barley, collected in the making of pearled barley. Barley hulls contain a considerable amount of insoluble NSP and lower levels of soluble NSP (Bach Knudsen, 1997). Barley meal, on the other hand, would have a higher proportion of soluble NSP. Diets used in trials by Bertschinger *et al.* (1978, 1979) that were associated with reduced *E. coli* proliferation and diarrhoea were high in crude fibre (10–17 %) and low in nutrients, particularly crude protein (CP).

Relatively little subsequent research has been conducted in this area, although reports of 'complex-' v. 'simple-' density diets (for example, Ball & Aherne, 1982) continued to show the effects of diet on diarrhoea after weaning. However, concerns related to the use of antimicrobials in diets after weaning has caused a sharp refocus on alternative control strategies. Research on PWC at Murdoch University has examined interrelationships between different sources of NSP and proliferation of *E. coli* in weaner pigs. Increased proliferation of pathogenic *E. coli* in both the small and large intestines has been seen with addition of either guar gum (McDonald *et al.* 1999) or pearl barley (McDonald *et al.* 2001b) to the diet of 21 d old weaner pigs. For example, McDonald *et al.* (2001b) assessed the effect of adding a soluble NSP source (pearl barley) to a cooked white rice-based diet on the performance, gastrointestinal physiology and intestinal proliferation of enterotoxigenic *E. coli* in weaned pigs experimentally inoculated with *E. coli* O8; K87; K88. Pigs were infected at 48, 72 and 96 h after weaning, and were allowed *ad libitum* access to their feed. Pigs were euthanased 7–9 d after weaning. Pigs fed the rice-based diet grew faster, had a greater empty body-weight gain, and had a reduced large-intestinal weight (expressed as a proportion of empty body weight) than pigs fed the rice-based diet supplemented with pearl barley (Table 2). Lower concentrations of volatile fatty acids and a lower pH of digesta in the large intestine indicated greater fermentative activity in pigs fed the pearl barley-based diet. Pigs offered the rice-based diet showed a smaller reduction in empty body-weight gain associated with *E. coli* infection, and showed significantly reduced numbers of haemolytic *E. coli* in the jejunum and the colon than their counterparts fed the diet containing cooked white rice plus pearl barley (Table 2).

The addition of pearl barley to the rice-based diet altered the physicochemical properties in

Table 2. Performance, large-intestinal weights, volatile fatty acid (VFA) levels and ileal digesta viscosity in non-infected and infected pigs fed either a rice-based diet or one containing pearl barley (from McDonald *et al.* 2001b)

	Non-infected pigs		Infected pigs		SEM	<i>P</i> value	
	Rice†	Barley‡	Rice†	Barley‡		Diet	Disease
Carcass gain (g/d)	74	26	-28	-56	36.3	*	***
Large intestine (% live weight)	2.7	3.8	2.6	3.2	0.62	**	NS
Distal colon VFA (mm)	84	114	60	78	20.4	**	**
Distal colon pH	6.8	6.1	6.8	6.5	0.37	**	NS
<i>Escherichia coli</i> in jejunum§	0	0	0.9	4.2	2.44	*	
<i>E. coli</i> in colon§	0	0	3.2	6.2	1.89	**	
Viscosity in ileum (cP)	2.1	2.8	1.6	2.3	1.13	*	*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† Rice-based diet (g/kg diet): cooked white rice, 702; dietary soluble NSP, 4; animal protein sources, 197.

‡ Pearl barley-based diet (g/kg diet): pearl barley, 500; dietary soluble NSP, 25; rice, 275; animal protein sources, 200.

§ Expressed as \log_{10} colony-forming units of haemolytic *E. coli*/g mucosal scraping.

the intestines, increased the viscosity, and altered the site of microbial fermentation. The energy expended in adapting the intestinal tract for digestion of NSP caused a depression in carcass growth, and this was exacerbated by PWC. These data suggest that the presence of soluble NSP in weaner diets is detrimental for piglet growth and stimulates proliferation of *E. coli* in the small intestine. It also indicates that there are benefits in feeding a highly-digestible rice-based diet to weaners, although it is not at present known what mechanism(s) promote this protection. Besides cooked rice reducing *E. coli* numbers, work by Mathews *et al.* (1999) suggests that components in boiled rice also inhibit electrolyte secretions in the small intestine, and hence reduce the magnitude of secretory diarrhoea. Further understanding is needed, because highly-digestible rice-based diets may prove to be a viable alternative to the use of growth-promoting antimicrobials currently used in the control of diarrhoea after weaning.

The two types of soluble NSP used by McDonald *et al.* (1999, 2001b) were highly fermentable and viscous in nature, which raises the question as to what extent fermentability, viscosity or combinations of both are likely to influence the small-intestinal microbiota. To investigate further the potential detrimental effects of increased intestinal viscosity in weaner pigs on proliferation of enterotoxigenic haemolytic *E. coli*, McDonald *et al.* (2001a) fed experimental diets supplemented with two sources of carboxymethylcellulose (CMC) to 21-d old weaned pigs for 10 d. CMC is a water-soluble synthetic viscous polysaccharide resistant to microbial fermentation. The pigs were then euthanased and the effects of two types of CMC, either low-viscosity (50–200 cP *in vitro*) or high-viscosity (400–800 cP *in vitro*), on gastrointestinal development, growth performance, faecal DM and proliferation of haemolytic *E. coli* were monitored.

Dietary CMC increased the viscosity along the entire lumen of the small intestine and in the caecum, and resulted in increased intestinal weights. Pigs fed the rice-based diet remained healthy, whereas those fed either low- or high-viscosity CMC developed diarrhoea within 7 d of weaning, and this continued until they were euthanased on day 10. Pigs fed the low- or high-viscosity CMC diets shed more haemolytic *E. coli* (O141; K88) daily than pigs fed the rice-only-based diet (Table 3).

The presence of CMC might provide a favourable luminal environment for the establishment and growth of bacteria, especially *E. coli*. This bacterium possesses pili that allow it to

Table 3. The effect of dietary carboxymethylcellulose (CMC; 40 g/kg) added to a cooked rice-based diet on the presence of post-weaning colibacillosis in pigs after weaning (after McDonald *et al.* 2001a)
(Proportion of pigs in groups with diarrhoea)

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
<i>P</i> value	< 0.05	< 0.05	< 0.05	< 0.05

^{a,b} Values within a column with unlike superscripts were significantly different ($P < 0.05$).

*Day number is the number of days after weaning (weaning is day 1).

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). Of particular interest is the fact that CMC adheres to and thickens porcine mucin (Rossi *et al.* 1996) and may also alter its composition. These events may enhance the ability of haemolytic *E. coli* to bind to the mucus lining the intestinal villi and cause diarrhoea.

Altered microbial activity has also been noted in other species in association with increased viscosity of the small intestinal contents (for example, Choct *et al.* 1996; Smits *et al.* 1998; Langhout *et al.* 2000). For example, *E. coli* numbers 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 duodenum and jejunum of chickens fed diets containing CMC (Smits *et al.* 1998). Wyatt *et al.* (1988) found that the addition of CMC in diets for rats failed to increase the density of bacteria in the caecal or colonic contents, but the bacterial populations changed significantly such that the aerobic bacteria, in particular *E. coli*, were more numerous in the large intestine.

Oligosaccharides and control of post-weaning colibacillosis. Some oligosaccharides, such as inulin and oligofructose, have been proposed as 'prebiotics' because of their potential to selectively stimulate growth of *Bifidobacterium* spp. within the human large intestine, suppress proliferation of potential pathogens (Gibson & Roberfroid, 1995) and modulate a variety of human enteric conditions and diseases (Steer *et al.* 2000). Prebiotics are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacteria in the colon, and hence improve host health' (Gibson & Roberfroid, 1995).

Arising from work conducted in human subjects, and coupled to the withdrawal of growth-promoting antimicrobials, there has been interest in the use of selected oligosaccharides (non-digestible oligosaccharides; NDO) as prebiotics in the weaner pig. Since 1990, a considerable amount of work has been conducted in this field in the pig, but generally with equivocal results (Chesson & Stewart, 2001). An exhaustive investigation into the effect of NDO in young pig diets on the microflora, fermentation characteristics and digestibility of nutrients was conducted in a doctoral thesis by Houdijk (1998). In essence, Houdijk (1998) reported no biologically significant effects on any indices measured in response to the NDO used (fructo-oligosaccharides and transgalacto-oligosaccharides). The effectiveness of prebiotics such as NDO will depend largely on the environments (for example, dirty, clean) under which pigs are kept, and may be masked by the pre-caecal digestion of such compounds. In addition, Mikkelsen & Jensen (2001) reported that the populations of *Bifidobacterium* spp. in the gastrointestinal tract of pigs aged 2–4 weeks constituted less than 1 % of the total bacterial population. It is difficult to

imagine how even a 100 % increase in numbers caused by feeding NDO could have a marked effect on intestinal health. Nevertheless, McDonald (2001), using weaned piglets naturally colonised by haemolytic *E. coli*, and Rossi *et al.* (2001), using isolated jejunal loops, both reported decreased proliferation of *E. coli* in response to inulin added to the diet.

Dietary oligosaccharides might also be generated in the gastrointestinal tract itself from polysaccharides that are more complex, and exert effects on gut function and bacterial dynamics. As discussed earlier, however, observing beneficial effects on amelioration of bacterial diseases might be compounded by the lack of any objective measure of microbial status of the gastrointestinal tract, and so it is often difficult to predict the response of the host's gut microflora to any additive, such as an oligosaccharide. In this regard, Reid & Hillman (1999) proposed that an assessment of a pig's capacity to resist pathogens could be based on the faecal ratio of lactobacilli : coliforms in the gut contents, since lactic-acid bacteria are known to inhibit the growth of enterotoxigenic *E. coli* (Hillman *et al.* 1995). Although it could be argued that faecal populations and numbers do not represent those present in the small intestine, where *E. coli* attaches and causes disease, the lactobacilli:coliforms ratio suggests that a larger population of lactic-acid bacteria relative to coliforms provides some indication that enhanced numbers of those strains that are capable of inhibiting coliforms, including pathogens, might be present (Reid & Hillman, 1999).

Resistant starch and post-weaning colibacillosis. Some recent studies in pigs have investigated the use of 'resistant starch' (RS; see below for definition) as a means of extending large-intestinal fermentation, such that the microflora (for example, *Bacteroides* spp.) do not use proteins as an energy source with the production of metabolites (for example, cadaverine, putrescine) that have been associated with diarrhoea after weaning (Aumaitre *et al.* 1995). The hydrolysis of starch granules by α -amylase in the small intestine is not always complete. Starch that enters the caecum and colon is termed RS (Annison & Topping, 1994; Baghurst *et al.* 1996). There are three main forms: (i) starch granules bound in the middle of a large food particle that are not physically accessible to digestive enzymes (RS1); (ii) starch granules resistant to degradation due to the crystalline structure within starch, such as that found in potato (RS2); (iii) some starches when heated and then cooled can reform (retrograde) in a type of crystalline structure that inherently resists digestion (RS3). Once in the large intestine, RS is degraded by the microflora, especially in the caecum (Pluske *et al.* 1998).

Reid & Hillman (1999) found that differences existed in both protein degradation and bacterial counts in the colon of weaner pigs when different starches were fed. *In vitro* studies conducted previously by these authors (Reid *et al.* 1998) indicated that retrogradation of the amylose in starch caused resistance to bacterial degradation, an effect suggested to be the result of amylopectin 'coating' by the amylose. Reid *et al.* (1996) also showed that certain intestinal bacteria from pigs, such as *Clostridium butyricum*, might prefer to ferment the amylopectin fraction of starch rather than the amylose fraction, such that a starch lower in amylose would leave the amylopectin more accessible to the microflora. In addition, the α -1,-4,1-6 structure of amylopectin should reduce the rate of fermentation, and hence extend fermentation along the intestinal tract. Reid & Hillman (1999) found that protein fermentation in the mid- to distal colon was best reduced by inclusion of retrograded waxy maize, which also increased the lactobacilli : coliform ratio in these segments.

Liquid feeding and post-weaning colibacillosis. Considerable interest has arisen in the use of liquid feeding of pigs in the past 10 years. Liquid feeding has been used as a way not only of

enhancing feed intake and growth rate, but also as a means of manipulating the microflora, both in the fermentation tank and the gastrointestinal tract, with the object of ameliorating enteric diseases (Brooks *et al.* 1996, 2001; Jensen, 1998; Geary *et al.* 1999; Mikkelsen & Jensen, 2000). Fermented liquid feed (FLF) is characterised by high numbers of lactic acid bacteria, high numbers of yeast, a low pH (< 4.0), and a high concentration of lactic acid (132–244 mM) (only the undissociated form of lactic acid is bactericidal or bacteriostatic; Russell & Diez-Gonzalez, 1998), and typically results in reduced numbers of coliform bacteria in the feed, provided fermentation conditions are correct. Lactic acid has antibacterial effects on *E. coli* and *Salmonella* species (Nout *et al.* 1989), and lactobacilli can inhibit adhesion of *E. coli* to the intestines (Hillman *et al.* 1994). For example, Beal *et al.* (2001) demonstrated that fermenting liquid feed at 37°C was an effective way of eliminating potentially pathogenic species such as *E. coli*, although at 20°C the antimicrobial effects of FLF were very much less apparent. This was possibly due to the expression of heat-shock proteins that enabled *E. coli* to withstand the antimicrobial effects of lactic acid (Phadtare *et al.* 1999). There were marked differences between *E. coli* strains in their ability to withstand the antimicrobial effects of FLF, suggesting that the effects of feeding FLF on pathogenic populations in the gastrointestinal tract might also be variable.

Nevertheless, FLF has been shown to alter populations of the microbiota in the intestines and influence volatile fatty acid (VFA) levels (Jensen, 1998; Jensen & Mikkelsen, 1998; Mikkelsen & Jensen, 2000; Moran *et al.* 2001). For example, Moran *et al.* (2001) showed that no coliform bacteria (< 3.0 log₁₀ colony forming units/ml) were detectable in the terminal ileum of pigs fed FLF, and reduced concentrations were found in the large intestine, in comparison to pigs fed dry feed or non-fermented liquid feed (Table 4). Moreover, the use of pre-fermentation (steeping) of the feed in water as a means of hydrolysing the soluble NSP before feeding is likely to reduce the viscosity induced by intact soluble NSP in cereals. This is because endogenous glycosidases in the grain have already begun the process of polysaccharide breakdown.

Use of exogenous enzymes and post-weaning colibacillosis. The use of in-feed enzymes for enhanced production and control of certain enteric conditions in chickens (for example, sticky droppings) is commonplace. In contrast, the generalised use of enzymes in pig diets is less common (Partridge, 2001), although they could offer potential for the amelioration of some enteric conditions and diseases. Unfortunately, there are few studies investigating the influence of supplementary enzymes on reducing enteric bacterial infections in pigs. It is difficult to imagine a direct effect of an exogenous enzyme *per se* on the gastrointestinal microflora; however enzymes might act in other ways.

There is recent interest in the use of supplementary enzymes to create oligosaccharides *in situ* from the hydrolysis of branched-chain NSP, such as arabinoxylans and xyloglucans, which might then influence the composition of the microbial flora. This has been coined the 'pre-pro-biosis' concept (Partridge & Tucker, 2000). Certainly, Austin *et al.* (1999) reported the presence of a number of oligosaccharide configurations when a single cloned endo-1,4-xylanase was used with different UK wheat cultivars, although the effects of these oligosaccharides *in vivo* were not investigated. Xylanase addition to broiler chicken diets resulted in a relative increase in *Bifidobacteria* spp. and *Bacteroides* spp., a response that might be expected due to an increase in xylo-oligosaccharides. The proportion of *Lactobacillus* spp., however, which might also be expected to increase in response to more xylo-oligosaccharides present, remained unchanged (Bedford & Apajalahti, 2001). More recently, Fernandez *et al.* (2000)

Table 4. Microbial counts (\log_{10} colony-forming units per ml) and pH of the digesta in the terminal ileum, caecum and colon of pigs fed different diets after weaning at 23 d of age (after Moran *et al.* 2001)

Site along gastrointestinal tract	Diet type			
	FLF	NFLF	DF	S
Ileum				
pH	6.1	6.4	6.3	5.9
Lactobacilli	8.8 ^a	7.0 ^a	< 3.0	7.3 ^a
Coliforms	< 3.0	8.1 ^{ab}	8.5 ^a	6.0 ^b
Caecum				
pH	6.0	6.0	5.8	6.1
Lactobacilli	8.5 ^a	8.1 ^a	5.5 ^b	7.3 ^a
Coliforms	5.5 ^a	7.4 ^{ab}	8.4 ^b	7.5 ^{ab}
Colon				
pH	6.2 ^a	6.0 ^a	5.9 ^a	6.6 ^b
Lactobacilli	8.6 ^a	7.9 ^a	5.0 ^b	8.0 ^a
Coliforms	5.6 ^a	8.1 ^a	8.6 ^b	7.3 ^a

FLF, fermented liquid feed; NFLF, non-fermented liquid feed; DF, dry feed; S, sows' milk.

^{a,b} Values within a row with unlike superscript letters were significantly different ($P < 0.05$).

reported that broiler chickens fed a wheat-based diet supplemented with xylanase (Avizyme 1300[®]; Danisco Animal Nutrition, Marlborough, Wiltshire, UK) showed reduced viscosity and less *Campylobacter jejuni* in the caeca following experimental infection. Interestingly, this was associated with an increased number of neutral and sulfated mucins in goblet cells. Furthermore, Hampson *et al.* (2002) reported that 22-week-old laying hens experimentally infected with a virulent strain of *Brachyspira intermedia* and fed Avizyme 1302[®] (Danisco Animal Nutrition) in a wheat-based diet showed reduced proliferation of this spirochaete in the caeca.

In relation to PWC, Inbarr & Ogle (1988) and Partridge & Tucker (2000) reported a reduction in the frequency and severity of diarrhoea in weaned piglets fed diets supplemented with glycosidases. Chesson & Stewart (2001), however, cautioned about the precise cause of the diarrhoea seen in such studies, and reinforced the need for microbiological assessment to examine the effect of, for example, oligosaccharides derived *in situ*. This is because the diarrhoea seen after weaning can be osmotic in nature rather than being of bacterial origin. Future studies in this area require alignment of dietary work and age of the pig with assessments of microbial populations to truly study the effects of enzymes and oligosaccharides on enteric bacterial diseases.

In addition, the response of the microflora to enzyme addition probably depends on the initial microbial status of the pigs, which in turn depends on the form and digestibility of the diet and the extent of the microfloral challenge it evokes. Since enzymes are likely to change substrate flow into the small and large intestines, the subsequent responses will vary according to the populations present at time of administration and their reaction to such changes (Bedford & Apajalahti, 2001). It is not surprising that given the huge range of microfloral conditions likely to exist between studies, the responses to enzyme use, rather than being absolute, are a continuum or a population of responses varying from detrimental to highly positive. Nevertheless, many of the factors governing the extent of enzyme responses are most likely similar to those influencing responses to antimicrobials. For example, the response to antimicrobials in the diet depends to a large degree on the conditions animals are kept under. It is likely that any positive responses to enzymes will be dictated not only by the status of the microflora in the pig's

gastrointestinal tract, but also by environmental, management and dietary factors such as cereal type and quality, and processing (Bedford & Apajalahti, 2001).

Influence of protein sources on post-weaning colibacillosis. Certain components of weanling pig feeds, such as soyabean meal, have been implicated in causing intestinal mucosal damage and intestinal fluid accumulation in weaner pigs. When fed in creep feeds, it has been suggested that soya protein may act as a primer for hypersensitivity reactions after weaning that, in turn, predispose pigs to PWC (Miller *et al.* 1984). Evidence to support this notion is equivocal (Hampson, 1994; Dréau & Lallès, 1999). The source of protein used has received some attention with regard to PWC, although again the evidence is confusing. Hampson (1987) reviewed the literature at the time, and reported research from several groups implicating the protein content and protein composition of the diet in the aetiology of PWC. For example, Prohászka & Baron (1980) reported increased numbers of haemolytic *E. coli* in the small intestines of pigs that were fed 210 g CP/kg as opposed to 130 g CP/kg. Although a level of 130 g CP/kg would never be fed commercially, these data suggest that it might be possible to limit the extent of PWC by formulating diets with lower amino acid (protein) levels, or by using more digestible protein ingredients. Changing from skimmed milk powder to soyabean and maize increased the severity of diarrhoea and appearance of enterotoxigenic *E. coli* in 3-week-old pigs (Shimizu & Terashima, 1982), although Pouteaux *et al.* (1982) found no difference in diarrhoea or bacterial populations when comparing buttermilk powder, soyabean meal and pea protein concentrate. Interestingly, Kiers *et al.* (2001) recently reported that processed soyabean products, including mould-fermented soyabean (tempe), reduced the fluid and electrolyte loss (particularly sodium and chloride) in jejunal segments in the presence of enterotoxigenic *E. coli*.

In the case of the newly-weaned pig, typical starter diets contain 220–250 g CP/kg, and apparent digestibility of this protein at the terminal ileum is 75–85 %. This results in a considerable amount of 'escape' (resistant) protein entering the large bowel. For instance, a 7 kg pig eating 300 g/d of a 220 g CP/kg diet might be 'losing' 33–55 g protein daily to its hindgut. Protein is fermented rapidly by the microbiota with the production of diamines (for example, putrescine, cadaverine, tryptamine) and gases (for example, NH₃) that have been implicated in the clinical expression of PWC (Aumaitre *et al.* 1995). Little attention, however, has been given to the digestion and metabolism of resistant protein by intestinal bacteria of pigs, and consequently knowledge of the proteolytic activities of gut bacteria in the pig is lacking. It is recognised, however, that a number of bacterial groups including *Bacteroides*, *Clostridium*, *Enterobacterium*, *Lactobacillus* and *Streptococcus* possess the ability to produce diamines, such as putrescine, cadaverine, histamine and tyramine, via decarboxylation of amino acids (for example, tyrosine, tryptophan, lysine) and breakdown of polyamines (Gaskins, 2001). Diamines have been implicated in the aetiology of PWC. In the 3-week-old weaned pig, for example, Porter & Kenworthy (1969) observed that increased urinary heterocyclic amine excretion was associated with diarrhoea after weaning, with putrescine and cadaverine levels being particularly high. Porter & Kenworthy (1969) commented that it is not likely to be the absolute amount of these amines produced, but their site of production, that might predispose to PWC. These workers found that the small intestine was the main site of amine production in severely diarrhoeic pigs, whereas in clinically unaffected pigs there was only a low level of amine production in the small intestine. Nollet *et al.* (1999a,b) reported protective effects against neonatal and PWC in piglets by feeding bovine plasma powder; however when the powder was included in the diet at 90 g/kg, diarrhoea attributable to biogenic amines was found.

Aumaitre *et al.* (1995) showed that the activity of major gut proteases (trypsin, chy-

motrypsin) were stimulated after weaning by an increase in the level of CP in the weaning diet of up to, but not exceeding, 200 g/kg. This partially helps to explain data showing a decrease in the apparent ileal digestibility of N in pigs fed diets containing more than 225 g CP/kg (Li *et al.* 1993). If this occurred, then the undigested protein would move distally and may be decarboxylated to amines that, in turn, could predispose the young pig to diarrhoea. In contrast to this notion, other authors (for example, Armstrong & Cline, 1976; Pouteaux *et al.* 1982; Etheridge *et al.* 1984) failed to find any association between dietary protein source and the incidence of diarrhoea after weaning. Diets containing a large number of protein sources may increase the severity of diarrhoea compared with diets with fewer sources of protein (Etheridge *et al.* 1984). In addition, there are other possible reasons for these discrepancies between studies. Very few (if any) studies have used controlled infections, and most of the research has confounded protein type with protein level. Further investigations using controlled infection studies and defined protein sources are needed to separate these causative factors to assess their effects on intestinal health.

The bacterial products of protein fermentation are more likely to be produced in response to an acid environment, a process itself generated by rapid fermentation of soluble NSP (and possibly starch). An interaction might exist, therefore, between resistant protein and NSP in the aetiology of PWC. In this regard, Bolduan *et al.* (1988) and Aumaitre *et al.* (1995) commented that the appropriate addition of insoluble NSP sources might ameliorate PWC. Bolduan *et al.* (1988), for example, presented evidence showing that the production of diamines in the colon reduced linearly with an increase in the crude fibre content of a weaner feed (Fig. 1). Based on this work, it is possible that appropriate (slowly or moderately fermentable) NSP sources, such as wheat bran or beet pulp, may reduce PWC at a given dietary protein concentration. These substrates will also promote the physiological and functional development of the hindgut of the young pig. In turn, a shift towards acid fermentation based on these NSP could decrease the formation in the colon of diamines that have been implicated in the aetiology of PWC. This notion, however, needs to be considered in relation to the work described earlier (see p. 340) showing that feeding a very digestible diet based on cooked white rice is also protective against PWC. This is because the levels of acid formed in the hindgut of rice-fed pigs are markedly lower than those observed when sources of NSP are fed. Clearly, further research is required to identify appropriate cereal, fibre and protein sources and their interaction(s) on the pathogenesis of PWC, in addition to practices such as pre-fermentation and liquid feeding as have been discussed previously. Identification of such interactions and practices will assist in design of nutritional programmes that will reduce reliance on the use of growth-promoting antimicrobials in pig diets.

Swine dysentery

In many countries swine dysentery (SD) is one of the most economically important endemic bacterial diseases of swine. The disease is a mucohaemorrhagic colitis in grower (and sometimes weaner) pigs affecting the caecum, colon and rectum. SD is caused by the anaerobic spirochaete *Brachyspira (Serpulina) hyodysenteriae* (Harris *et al.* 1999). Clinical manifestations vary greatly, and include both mild and sub-clinical disease. In typical cases, infected pigs initially show a slight depression and reduced feed intake. They develop diarrhoea, which is grey to black and sometimes watery but is more often soft and porridge-like. This diarrhoea progresses to consist of mucus plugs, fibrin, epithelial cell casts, and flecks of fresh blood. Affected animals have faecal staining of the hindquarters, become dehydrated and appear

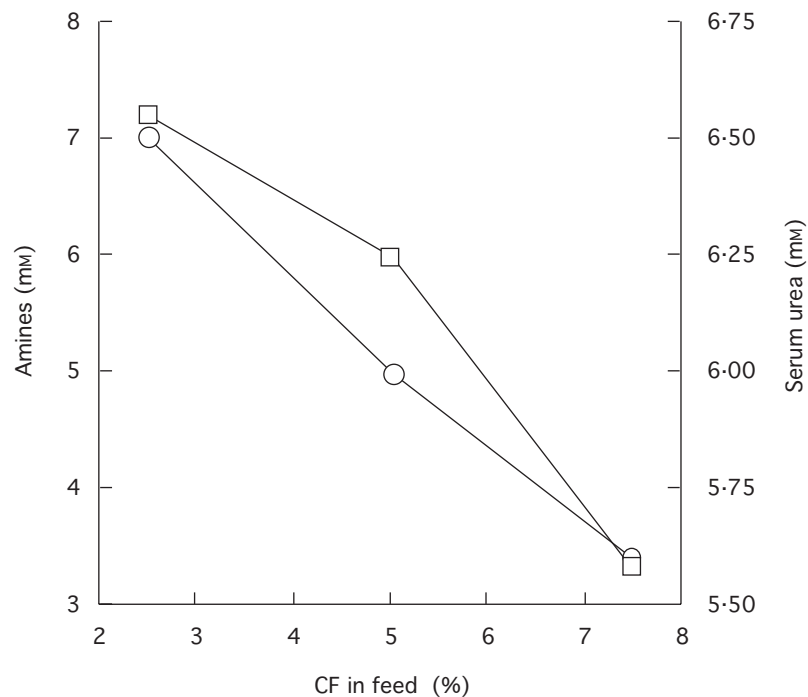


Fig. 1. Relationship between the dietary crude fibre (CF) content of the diet (achieved by adding wheat bran) fed to weaner pigs and the concentrations of diamines (□, cadaverine, putrescine, histamine and tryptamine) and serum urea (○) (from Bolduan *et al.* 1988).

gaunt, with a tucked-in abdomen and an arched back. If left untreated, around 10 % of affected pigs can die within 5 d of first showing clinical signs (Hampson & Trott, 1995).

The precise pathogenesis of SD is not well understood, though it is recognised that the disease does not always express itself clinically in pig herds despite the presence of the bacterium (Hampson *et al.* 1992). For example, a herd survey conducted in the state of Western Australia showed that 33 % of herds were serologically positive for *B. hyodysenteriae*, yet little clinical disease was present (Mhoma *et al.* 1992). Many factors have been implicated in the aetiology of SD (for reviews, see Hampson & Trott, 1995; Harris *et al.* 1999), however it is evident that nutrition may modulate the expression of the disease. The first report of such an influence was a retrospective study by Prohászka & Lukács (1984), who found that a diet based on maize silage that lowered pH and increased VFA concentrations in the large intestine was apparently bactericidal to *B. hyodysenteriae* and reduced clinical expression of the disease (Table 5). These authors attributed the intensity of the antibacterial effect of the diet to the lower base value, and hence greater acidity (lower pH) present in the large intestine.

Siba *et al.* (1996) attempted to replicate this work with pigs (25–30 kg live weight) that were experimentally inoculated with a virulent strain of *B. hyodysenteriae* after being fed diets designed to either promote or limit fermentation in the large intestine. In contrast to the expectations based on the work of Prohászka & Lukács (1984), Siba *et al.* (1996) found that a diet based on cooked white rice and (predominately) animal protein ingredients (for example, bloodmeal, meat and bone meal) reduced the degree of hindgut fermentation and reduced both the proliferation of *B. hyodysenteriae* and clinical expression of the disease. A diet based on

Table 5. A comparison of physiological variables between pigs free from swine dysentery (SD) and pigs clinically affected with the spirochaete. A total of thirty clinically healthy pigs were sampled from each group (from Prohászka & Lukács, 1984) (Mean values and standard deviations)

	Pigs free of SD*		Pigs clinically affected with SD†		P value
	Mean	SD	Mean	SD	
Distal colon pH	6.0	0.3	6.8	0.4	< 0.001
<i>Escherichia coli</i> counts (log ₁₀ /g)	5.2	1.0	7.8	1.4	< 0.001
Volatile fatty acids (mmol/kg)	120	15	138	18	NS
C ₂ :C ₃ :C ₄	62:29:9		65:25:10		NS
Base value in the feed‡	190	25	520	40	
Acid–base excretion in urine (mmol/l)	–86	34	17	20	< 0.001

*Diet fed to pigs contained (g/kg diet): maize, 620; barley, 200; soyabean meal, 120; meatmeal, 30 (150 g/kg crude protein content in diet).

†Diet fed to pigs contained (g/kg diet): silage, 600; wheat, 250; soyabean meal, 120 (140–150 g/kg crude protein content in diet).

‡Determined as the bicarbonate load resulting from consumption of 1 kg of feed.

wheat, barley and Australian sweet lupins that promoted hindgut fermentation (as evidenced by lower pH, increased VFA levels, heavier organ weights) caused the highest incidence of SD.

Subsequent experiments in our laboratory (Pluske *et al.* 1996, 1998) confirmed these findings, and have demonstrated that a diet low in both soluble NSP and RS concentrations generally affords protection against *B. hyodysenteriae* following experimental infection. Diets using rice, sorghum and maize as the sole cereal source appear to be more protective than diets based on wheat, barley and dehulled oats (groats). However, the manner in which the grains have been processed also appears to be important, especially with cereals inherently low in NSP (< 1 g/100 g soluble NSP). Our data suggest that a reduction in RS levels (for example, via extrusion, steam flaking) will only prove effective against SD if the grain in question has an inherently low NSP level to begin with.

Whilst we have shown that the microbial digestion of fermentable carbohydrates in the large intestine facilitates the expression of SD, our research has provided few insights into the underlying mechanisms. It is evident that the varied expression of SD with different diets extends beyond a simple effect of fermentation *per se*. For example, Pluske *et al.* (1996) failed to show any relationships between fermentation indices (for example, pH, VFA levels) and the incidence of SD. Furthermore, Pluske *et al.* (1998) showed no association between ATP levels (an indicator of microbial activity) in the large intestine and expression of the disease. Pigs fed a viscous NSP source (guar gum), for instance, showed the highest incidence of SD, low pH levels and high VFA concentrations, yet the lowest ATP levels.

Numerous hypotheses have been proposed in an attempt to explain these dietary effects on SD. These include factors such as the DM content of colonic contents influencing the survival of spirochaetes (Siba *et al.* 1996), factors affecting the mobility of the spirochaete in the mucosal lining, diet-induced changes in chemotactic-regulated motility (Kennedy *et al.* 1988), factors affecting the ability of *B. hyodysenteriae* to express haemolysins and/or lipopolysaccharides and cause inflammation of the epithelium, and the Zn content of the diet (Zhang *et al.* 1995). More recently, Durmic *et al.* (1998, 2000) reported changes in composition of the hindgut microflora associated with feeding different diets in association with SD. It has long been recognised that other bacterial species, such as *Fusobacterium* spp., *Clostridium* spp. and *Bacteroides* spp. must be present for SD to occur (Meyer *et al.* 1975; Whipp *et al.* 1979).

Durmic *et al.* (1998, 2000) showed differences between diets in the genera and species present in the large intestine, with changes in bacterial populations consistent with those that occur in the natural disease. However, Leser *et al.* (2000), using 16S ribosomal DNA sequence analysis, did not detect the same synergistic bacteria in pigs infected with *B. hyodysenteriae*, although they did report changes in bacterial populations when pigs were fed either a cooked rice diet or a FLF following infection with the causative agent. Furthermore, and in contrast to our data, Kirkwood *et al.* (2000) and Leser *et al.* (2000) did not find a protective effect of feeding a par-boiled cooked rice–animal protein diet following experimental infection of pigs with *B. hyodysenteriae*. Possible reasons for the disparate results between laboratories include differences in *B. hyodysenteriae* strains or virulence factors, variation in diet ingredients and preparation of the rice, and variation in the microflora composition of the hindgut in pigs in the different countries.

An area of research not yet explored in relation to nutritional effects on SD is that of dietary protein content and type. Given the postulated effects of excess protein entering the caecum and colon on bacterial proliferation and production of bacterial metabolites, it is feasible that this component of the diet might also influence the aetiology of SD. This has particular relevance for some countries where many animal protein sources have been banned from diets fed to grow and finish pigs, and only vegetable proteins are available to the feed manufacturer. Studies, for example, to examine possible effects of processing (for example, extruded peas), protein types (for example, soya protein isolate), and appropriate enzyme combinations (for example, proteases plus glycosidases) appear to be warranted in relation to susceptibility to SD.

Enzymes and swine dysentery. Given the purported role of certain carbohydrates in the expression of SD, a logical progression of this work was to examine whether glycosidases added to the diet could reduce the incidence of the disease. In an experimental model of SD, Durmic *et al.* (2000) used an in-feed arabinoxylanase in an attempt to ameliorate the incidence of SD by hydrolysing glycosidic linkages of soluble NSP before their passage into the large intestine. The hypothesis that a reduced load of fermentable substrate entering the large intestine would reduce the incidence of SD was tested in a study comprising a 2 × 2 factorial arrangement of treatments. Wheat was fed to pigs either in extruded form (to reduce the contribution of RS to the expression of SD) or hammer-milled form, and an exogenous arabinoxylanase was added or not added to the diet. Pigs were infected with a virulent strain of *B. hyodysenteriae* at a body weight of around 25 kg, and subsequently monitored for expression of the disease.

Both the extrusion of wheat and addition of arabinoxylanase increased pre-caecal starch digestion, as judged by reduced starch levels in the large intestine (Table 6). Addition of arabinoxylanase to the diet did not reduce the incidence of SD. The failure of extrusion and the enzyme to protect against SD might be related to the apparent increased fermentation in proximal areas of the hindgut, as judged by an increase in bacterial ATP concentrations (Table 6). A significant main effect of enzyme addition on digesta pH was seen, but only in the distal part of the colon, such that pigs fed arabinoxylanase in their diet had a higher pH than pigs not fed enzyme (6.68 v. 6.35, $P = 0.017$). These data suggest that by the time the enzyme had cleaved the arabinoxylan chains, and the digesta had reached the distal colon, there was little or no fermentable carbohydrate remaining and protein fermentation was occurring. Passage of smaller NSP molecules may have then allowed colonisation by *B. hyodysenteriae* in the anterior parts of the hindgut by providing suitable types and levels of substrate, with subsequent expression of SD. In this study, therefore, use of an enzyme targeting wheat NSP exacerbated the incidence of SD.

Table 6. Production data, large intestinal fermentation indices, and incidence of swine dysentery (SD) in pigs fed wheat-based diets subject to different processing and addition of arabinoxylanase (after Durmic *et al.* 1998) (Mean values and standard errors of the difference)

	Diet type				SED	Significance		
	RW	ExtW	RW-Enz	ExtW-Enz		W	Enz	W × Enz
Growth rate (g/d)	427	430	489	423	33.8	NS	NS	*
Starch (mg/g)								
Proximal colon	10.2	0.6	6.2	2.0	2.52	***	NS	*
Distal colon	7.2	0.2	2.1	0.0	2.97	**	NS	NS
pH								
Proximal colon	5.7	6.1	5.7	6.0	0.34	NS	NS	NS
Distal colon	6.1	6.6	6.6	6.8	0.30	**	*	NS
ATP (nmol/g)								
Proximal colon	0.30	0.10	0.42	0.44	0.26	NS	*	NS
Distal colon	0.18	0.14	0.17	0.23	0.18	NS	NS	NS
Incidence of SD (%)	66.7	33.3	100	100	–	–	–	–

RW, raw wheat; ExtW, extruded wheat; RW-Enz, raw wheat + enzyme; ExtW-Enz, extruded wheat plus enzyme; W, wheat.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Porcine intestinal spirochaetosis

The terms porcine intestinal spirochaetosis (PIS) or spirochaetal diarrhoea have been used to describe colitis of growing pigs associated with infection of the large intestine with the weakly β -haemolytic intestinal spirochaete *Brachyspira (Serpulina) pilosicoli* (Trott *et al.* 1996). Infection is characterised by the attachment of *B. pilosicoli* to the colonic epithelium, followed by disruption of the microvilli and, in some cases, local invasion and necrosis of the epithelium. This results in patchy colitis, excess mucus production and the passage of watery, mucoid, and occasionally blood-flecked diarrhoea. Clinically, pigs lose body condition, appear ill-thrifty, have perineal staining and a tucked-up appearance. Pigs initially pass loose, sticky faeces, which develops into diarrhoea with a consistency of wet cement. In weaner or grower pigs the diarrhoea is usually watery to mucoid, green or brown, and can contain tags of mucus and flecks of blood (Hampson & Trott, 1995, 1999). Pigs do not often die of this infection, which may occur as a primary or secondary invader of the large intestine.

Given the close similarity between *B. pilosicoli* and *B. hyodysenteriae*, their very similar habitats in the hindgut, and reports from veterinarians in the field of dietary influences on PIS, an investigation was made into whether the cooked rice diet that protected pigs from SD might also protect from PIS (Hampson *et al.* 2000). Two groups of weaner pigs were fed either a standard commercial wheat-lupin weaner diet ($n = 8$) or a highly digestible diet based on cooked white rice and animal protein sources ($n = 6$) for 3 weeks after weaning. All pigs were then challenged orally over 3 d with 10^{10} active mid-log phase cells of a field strain of *B. pilosicoli* (strain 95/1000). The pigs were killed 3–4 weeks post inoculation. All animals became colonised with *B. pilosicoli* strain 95/1000, but this occurred later (mean of 10 d compared with 3 d post inoculation, $P < 0.001$), and lasted for less time (mean of 5 d compared with 16 d, $P < 0.001$), in the pigs fed the cooked white rice-animal protein diet compared with those fed the wheat-lupin-based diet. One pig fed the wheat-lupin diet developed an acute and severe erosive colitis with severe watery diarrhoea within 3 d post inoculation, and was euthanased. All the other pigs on both diets developed mild transient diarrhoea, lasting only 2–3 d. Small areas of mild patchy colitis were observed at *post mortem*, but no spirochaete attachment to the epithelium was detected. This study demonstrated that, as with *B. hyodysenteriae* in grower

pigs, colonisation by *B. pilosicoli* could be influenced by diet. In this case, the rice-based diet did not prevent colonisation but only retarded it. In contrast with regard to the type of diet fed, but with a similar outcome, Stege *et al.* (2001) reported that the provision of straw in pens for finishing pigs was associated with a reduced prevalence of weakly β -haemolytic spirochaetes. In support, Pluske *et al.* (1998) reported a low incidence of SD when oaten chaff (a largely insoluble source of NSP) was fed to experimentally infected pigs.

DE McDonald, DW Pethick and DJ Hampson (unpublished results) showed that inclusion of CMC in a diet based on cooked white rice for pigs aged 5–6 weeks of age increased digesta viscosity and caused an increase in the duration of shedding of *B. pilosicoli* following experimental infection with the organism. The CMC also increased the duration of diarrhoea. The number of days that faecal swabs were positive for *B. pilosicoli* in pigs fed the rice-based diet without CMC (1.5 out of 9 d tested) was identical to that described in rice-fed pigs by Hampson *et al.* (2000), indicating consistency across experimental trials. In the same study by Hampson *et al.* (2000), the number of positive faecal shedding days for pigs fed a standard commercial diet based on wheat, barley and Australian sweet lupins was 5.3, a value not dissimilar from that recorded in pigs fed rice plus CMC by DE McDonald, DW Pethick and DJ Hampson (unpublished results) (4.2 d). The similarity between the duration of shedding of *B. pilosicoli* in pigs in these two studies suggests that inclusion of CMC exerts physicochemical properties in the gut similar to that seen when a commercially-based diet is fed, further reinforcing the role of viscosity *per se* as a causative factor in the aetiology of PIS.

Non-specific colitis. Before the description of PIS and its association with *B. pilosicoli*, certain cases of what were almost certainly PIS were described as 'grower scour/non-specific colitis' (Smith & Nelson, 1987). Such cases are still reported in the literature (for example, see Thomson *et al.* 1998; Johnston *et al.* 2001). Connor (1992) reported that non-specific colitis was influenced by diet, with pelleting of the diet and wheat-based diets believed to predispose pigs to the condition. The incidence of 'non-specific colitis' can be reduced by using an in-feed arabinoxylanase (Partridge, 1998), suggesting that NSP might play a role in this enteric condition. Processing of cereal grains at high temperature can change the structure of NSP, with Pluske *et al.* (1996) showing chemically that extrusion increased the soluble:insoluble NSP ratio in wheat, but decreased the RS content markedly. Working with barley-based diets but in 78 kg pigs, Fadel *et al.* (1988) showed that extrusion caused a shift from insoluble NSP to soluble NSP, and insoluble β -glucans to soluble β -glucans. The shift from insoluble to soluble NSP after extrusion cooking resulted in increased digestion of soluble NSP at the ileum (proportionally 0.54 more) and increased fermentation of insoluble NSP in the lower tract (proportionally 0.56 more). Moreover, Robertson *et al.* (1997) reported similar changes in barley when it was cooked at various temperatures up to 100°C. Pelleting diets is known to solubilise some of the NSP in cereals (Pluske *et al.* 2001), and may cause starch retrogradation upon drying and cooling.

With extrusion and/or pelleting, it is possible that the major site of digestion in the gastrointestinal tract shifts from being predominately pre-ileal to post-ileal, in the caecum and colon. An increased rate of fermentation of carbohydrates in the hindgut most likely creates a favourable environment for the growth of *B. pilosicoli* and attachment to the colonic epithelium, and contributes to this condition. Greater digestibility of carbohydrates before the caecum is desirable, because entry of substrate into the hindgut might promote the proliferation of this particular pathogen. Alteration of the site of digestion of grains, therefore, by judicious grain selection and processing methodology may ameliorate the incidence of PIS. Even in the

absence of the spirochaete, vigorous fermentation of undigested starch in the large intestine might generate sufficient hydrogen ion concentrations to damage the epithelium and cause a 'non-specific colitis'. In this regard, histological examination of the hindgut tissue might provide valuable evidence of inflammatory reactions that might predispose pigs to this condition.

Work in progress by the Meat and Livestock Commission (UK) (D Armstrong, personal communication) reported that 38 % of farms surveyed had non-specific colitis, with 42 % of farms reporting an infectious colitis. Of these, *B. pilosicoli* was isolated from 58 % of farms, *Lawsonia intracellularis* (the agent of proliferative enteropathy) was isolated from 37 % of farms, and *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* (yersiniosis) were both isolated from 21 % of farms. Earlier, Thomson *et al.* (1998) investigated the possible causes of colitis and typhlocolitis on eighty-five pig units in the UK between 1992 and 1996, and reported that *B. pilosicoli* was the primary agent on 25 % of farms. This bacterium also formed part of mixed infections on another 27 % of farms, with the main co-infections being *Y. pseudotuberculosis*, proliferative enteropathy (see pp. 354 and 355), *Salmonella* species or *B. hyodysenteriae*. Pathogens were not detected on 7 % of farms despite colitis being present. The high incidence of mixed infections on pig farms poses a problem for the nutritional control of enteric pathogens, because it is unlikely that all pathogens will respond in the same way to dietary interventions.

Salmonellosis

Salmonella infections are a concern for the pig industry for two major reasons: (1) clinical disease (salmonellosis); (2) infection of pigs with *Salmonella* spp. serotypes that can be a source of infection on pork products. In contrast to the large number of serotypes isolated from carcasses and pork products, disease in swine is almost always caused by either the H₂S-producing variant of *S. choleraesuis* var. Kunzendorf (manifest as septicaemia) or by *S. enterica* var. Typhimurium (Schwartz, 1999). A variety of serotypes may be isolated from diarrhoeic piglets immediately after weaning, but Schwartz (1999) remarked that most were associated with poor hygiene, concurrent enteric pathogens, inappropriate diets and a poor environment. *Salmonella heidelberg* has been associated with PWC, with lesions more typical of enterotoxigenic diarrhoeal disease than salmonellosis (Reed *et al.* 1985).

Causes of salmonellosis appear to be many and, as with many diseases, infection of pigs is more common than overt disease. A survey conducted recently in the USA (Harris *et al.* 1997) reported that salmonellae were isolated from the feed or feed ingredients from fourteen out of thirty farms and thirty-six out of 1228 samples, with isolation from pelleted feeds being more frequent than that from ground (mash) feed. In addition, isolation of salmonellae from feed was associated with the lack of bird-proofing, on-farm feed preparation, and with housing of pigs in facilities other than total confinement (Harris *et al.* 1997). Currently the major form of control and prevention of salmonellosis is the use of antimicrobial compounds, although Schwartz (1999) commented that nutritional approaches to preventing or alleviating the disease included feeding of propionic or other VFA (to lower gastric pH) and supply of mannose, heavy metals, lactose and probiotics. Evidence to support these claims was not presented.

Of recent interest is a US study by Anderson *et al.* (2001), who reported that oral administration of a sodium chlorate solution 8 h and 16 h after a *Salmonella enterica* var. Typhimurium challenge in weaned pigs reduced caecal concentrations of the organism. *Salmonella* possess respiratory nitrate reductase activity that also catalyses the intracellular reaction of chlorate, an analogue of nitrate, to cytotoxic chlorite. Most other gastrointestinal anaerobes do not possess

respiratory nitrate reductase. Anderson *et al.* (2001) proposed that such a nutritional treatment could be used before transport to the abattoir or in the drinking water during lairage as a way to reduce *Salmonella* carriage onto carcasses. Work by DJ Hampson and DW Pethick (unpublished results) at Murdoch University showed that feeding 7 kg weaner pigs a cooked white rice–animal protein diet in association with oral inoculation with *Salmonella enterica* var. Typhimurium delayed the onset of faecal colonisation (as assessed by plate counts) compared with pigs fed a wheat-based diet higher in NSP.

As discussed earlier (p. 344), FLF has been shown to influence the bacterial ecology of the gastrointestinal tract of pigs (Mikkelsen & Jensen, 2000), particularly in relation to members of the family *Enterobacteriaceae*, and including *Salmonella* spp. For example it has been reported that units adopting liquid feeding of by-products or using FLF have a lower incidence of salmonellosis than herds using dry feed (Stege *et al.* 1997; Van der Wolf *et al.* 1999). Van Winsen *et al.* (2001) recently reported that the numbers of *Enterobacteriaceae* along the gastrointestinal tract were lower in pigs fed FLF compared with dry feed. These authors also reported a significant negative correlation between the concentration of undissociated lactic acid and *Enterobacteriaceae* numbers. Nevertheless, because other issues apart from specific dietary components may be contributing to the effects, information from such studies needs to be confirmed by careful experiments conducted under controlled conditions. For example, in the case of salmonellosis, the relative hygiene of the various diets may be influencing the infectious dose presented to the pigs, rather than the diet itself having protective effects in the gastrointestinal tract (Hampson *et al.* 2001). Consequently, numerous countries have now instigated strict quality assurance schemes in their feed manufacturing industries to limit the contamination of animal feed with *Salmonella*.

Porcine proliferative enteropathies

Porcine proliferative enteropathies (PPE; also known as proliferative ileitis) are a group of acute and chronic conditions of widely differing signs but with a common underlying pathological change visible at necropsy: a thickening of the mucosa of the small intestine and colon. The affected tissues show a proliferation of immature epithelial cells of the crypts, forming a hyperplastic to adenoma-like mucosa. These proliferating cells invariably contain numerous intracytoplasmic *Lawsonia intracellularis*, a Gram-negative, obligate intracellular bacterium (McOrist & Gebhart, 1999). In growing pigs with uncomplicated proliferation of the mucosa, the condition is chronic proliferative enteropathy (or porcine intestinal adenomatosis, or ileitis). A variety of *Campylobacter* species have been recovered from proliferative lesions, but these are thought to be secondary agents in the condition (McOrist & Gebhart, 1999). The main clinical signs of PPE include loose, watery stools with or without blood, puddled faeces with undigested feed, gauntness, and lack of weight-gain uniformity. Clinical signs often appear after stressful events (for example, moving, mixing, transport), with pigs aged 6–20 weeks more commonly affected than pigs younger than 6–8 weeks of age (Knittel, 1999).

PPE is widespread throughout the world, with surveys indicating that the percentage of herds infected varies between 3 and 94 % (for example, Chang *et al.* 1997; Kim *et al.* 1998; Thomson *et al.* 1998; Pearce, 1999a,b; Stege *et al.* 2000). In Denmark, for example, Stege *et al.* (2000) reported that the prevalence of *L. intracellularis* in 1580 faecal samples was 93.7%, with 32 % of all farms surveyed having *L. intracellularis* as the only causative agent of infection. In contrast, a UK survey (Thomson *et al.* 1998) reported that *L. intracellularis* was the primary infective agent and cause of colitis on 3.5% of herds examined, although it was associated

with a mixed infection where *B. pilosicoli* was the primary causative agent in 15 % of farms. The use of antibiotics against *L. intracellularis* is the most common form of control, however McOrist & Gebhart (1999) commented that acute and chronic PPE remains a major problem even in high-health status, minimal-disease herds.

A survey by Stege *et al.* (2001) aimed at identifying risk factors for infection with *L. intracellularis* showed that home-mixed (and/or non-pelleted) feed was associated with a reduced prevalence of the pathogen (as well as of weakly β -haemolytic spirochaetes). Pearce (1999b) provided evidence that the prevalence of *L. intracellularis* in UK pig herds was strongly related to infection with the endo-parasite *Trichuris suis*. In turn, herds that were fed relatively high levels of NSP in their grower diets (i.e. in the top 25 % of levels in all herds studied) were twenty-seven times more likely to be infected with this nematode. Similar observations with regard to endo-parasites in pigs have been made in Denmark (Petkevicius *et al.* 1997). Pearce (1999b) concluded that the control of NSP intake for growers was the most important factor controlling parasite infection in grower-finisher pigs in the UK. Although not pertinent to infection by *L. intracellularis per se*, these data support the work of Stege *et al.* (2001) that the prevalence of *L. intracellularis* might be influenced by diet, although whether this is a direct effect or by modulation of other gastrointestinal organisms remains in question. In this respect, it is interesting to speculate on the possible role of dietary formation of butyrate and cell turnover as a potential modulator of the time available for intracellular *Lawsonia* to proliferate. Furthermore, Møller *et al.* (1998) commented that while the presence of *L. intracellularis* can be predictive of the diarrhoeic status of a pig herd, it does not warrant the conclusion that *L. intracellularis* is necessarily causing the diarrhoea.

Gastric ulcers

Ulceration of the *pars oesophagea* of the stomach of pigs is frequently detected at slaughter, and these lesions are sometimes thought to cause reduced weight gain to slaughter (Ayles *et al.* 1996). Ulceration of the stomach is also a serious welfare issue. Ulcers may lead to perforation of the stomach wall and to peritonitis, as well as haemorrhaging. Such problems are considered a common cause of sudden death in grower pigs and sows (Friendship, 1999). A myriad of factors are implicated in the aetiology of gastric lesions, including low levels of dietary fibre, transportation stress, feed restriction, pelleting of the diet, physical crowding, pig genetics, feed particle size and so on. Recent studies from Brazil have shown a positive relationship between the presence of the spiral bacterium *Helicobacter heilmannii* in the pig's stomach and the occurrence of gastric ulcers. This organism was present in 100 % of all slaughtered pigs showing ulcers, but only in 35 % of macroscopically normal stomachs (Barbosa *et al.* 1995; Queiroz *et al.* 1996).

A considerable body of information exists in the literature describing the effects of cereal type, processing method and particle size on the prevalence of gastric ulceration. A large number of these studies originate from the USA and so have used maize as the base cereal (for example, Healy *et al.* 1994; Wondra *et al.* 1995a,b; Lawrence *et al.* 1998; Eisemann & Argenzio, 1999; Regina *et al.* 1999). It is possible that the influence of particle size and processing method in maize-based diets on the expression of gastric ulceration differs from what might occur if wheat-, barley- and/or sorghum-based diets were fed to pigs, as would happen under Australian and European conditions.

In contrast to studies using maize, there are fewer reports investigating the effects of other cereals such as wheat, barley and sorghum on the prevalence of ulcers in pigs, although inter-

est in this area has increased markedly in the last 5–10 years. Danish work reported by Nielsen & Ingvarsten (2000a) found that pigs receiving rolled barley (50 % of particles > 1 mm) or wheat in non-pelleted form had virtually no gastric lesions as opposed to pigs receiving ground (3 mm screen size) barley or wheat. Interestingly, rolled barley could be pelleted without causing gastric lesions whereas rolled, pelleted wheat resulted in the highest gastric lesion score. Ground wheat in pelleted form resulted in the highest gastric lesion scores. In another study, Nielsen & Ingvarsten (2000b) found that pigs receiving either straw bedding (that they could consume) or rolled barley had a lower incidence of gastric ulceration in comparison with pigs receiving straw bedding where the cereal was finely (3 mm screen) ground. In all instances, groups of pigs having few ulcers had a higher stomach DM content. Ange *et al.* (2000) compared a maize–soyabean-based diet fed as a finely ground and pelleted diet *v.* a coarsely-ground and mash diet, and reported that the average daily water : feed ratio was higher for pigs on the pelleted diet (4.21 litres/d *v.* 3.04 litres/d, $P = 0.02$). Ange *et al.* (2000) suggested that the higher ratio for the pelleted diet might be the cause of a more fluid digesta allowing reflux of irritants (for example, H^+ , bile acids) from the distal stomach to damage the *pars oesophagea*. Lang *et al.* (1998) and Ange *et al.* (2000) associated decreased pH in the proximal stomach with increased prevalence of ulcers. In another US study, Mavromichalis *et al.* (2000) reported that wheat ground to an average particle size of 400 μm supported improved gain:feed and faecal digestibilities of nutrients (ileal digestibilities were not reported) compared with a particle size of 600 μm , but also increased the incidence of stomach keratinisation and lesions in finishing pigs (Table 7).

A possible reason for differences seen between wheat and barley in the Danish work of Nielsen & Ingvarsten (2000a) might be the degree of gelatinisation and retrogradation of starch (*i.e.* RS) that occurred after pelleting and drying and cooling. These interactions could be examined by comparing grains that differ in their amylose:amylopectin ratio, and that behave differently *in vivo* after processing. In addition, we are unaware of any reports investigating the extent of cell-wall rupture that occurs when grain is rolled, and how this compares with grain

Table 7. The influence of enzyme supplementation and particle size on the extent of keratinisation and ulceration in pigs fed wheat-based diets between 63 and 115 kg live weight (after Mavromichalis *et al.* 2000)

Particle size . . .	No enzyme		Enzyme		Statistical contrast*
	600 μm	400 μm	600 μm	400 μm	
Stomach keratinisation					
No. of observations	38	35	33	36	
Normal	26	4	24	2	
Mild	6	12	7	18	
Moderate	6	8	2	7	
Severe	0	11	0	9	
Mean score†	0.47	1.74	0.33	1.64	$P < 0.01$
Stomach ulceration					
No. of observations	38	35	33	36	
Normal	37	21	33	30	
Erosion	1	10	0	2	
Ulcer	0	2	0	4	
Severe ulcer	0	2	0	0	
Mean score‡	0.03	0.57	0	0.28	$P < 0.01$

* Contrast between particle sizes of 600 μm *v.* 400 μm .

† Scoring system: 0, normal; 1, mild; 2, moderate; 3, severe keratosis.

‡ Scoring system: 0, normal; 1, erosion; 2, ulcer; 3, severe ulcer.

that has been hammer-milled. Microscopic examination of grains comminuted by these two methods might provide insights into the extent of starch availability, and how this impacts upon the prevalence of gastric ulcers.

Regina *et al.* (1999) postulated that compounds secreted in the distal stomach and that return to the proximal stomach in finely ground diets may play a role in initiating damage to the stratified squamous epithelium of this region. Eisemann & Argenzio (1999) reported that the generation of hydroperoxidases and stimulation of the antioxidant and prostaglandin defence systems were greater in growing pigs fed a finely ground, maize–soyabean meal diet. Studies in our laboratory have looked for a possible link between diet and bacterial activity in the aetiology of ulceration in the *pars oesophagea*. A weaner model of stomach ulceration was developed in which weaner pigs fed finely ground wheat developed quite severe ulceration after 2–3 weeks, whilst pigs fed the same wheat that had been subjected to high pressure and temperature extrusion did not develop lesions (Accioly *et al.* 1998). Protection was associated with the absence of the urease-producing *H. heilmannii* in the stomach. It is possible that interactions with dietary components that lead to gastric ulcers extend beyond factors such as particle size and processing, although these appear to be definite risk factors. These interactions might include, for example, the interplay between the degree of starch disappearance before the duodenum, the products of fermentation and the commensal stomach flora. We are aware of no studies that have attempted to quantify starch fermentation in the stomach. In this regard, Krakowka *et al.* (1998) postulated that production of organic acids from certain carbohydrates by *Lactobacillus* spp. and *Bacillus* spp. in the stomach was a risk factor for gastric ulceration. This possibility would appear to warrant further research.

Other nutritional perspectives related to enteric bacterial diseases

Our review to date has focused on specific relationships between nutrition and some major enteric bacterial diseases of pigs. The following discussions expand on these general nutritional strategies and concepts and alert the reader to other nutritional approaches to controlling enteric disease.

Minerals and polymers to control enteric diseases

Zinc. Zn is a component of many metalloenzymes, including DNA and RNA synthetases and transferases, many digestive enzymes, and is associated with insulin; as such, it plays a crucial role in lipid, protein and carbohydrate metabolism in the pig. Zn is an essential element for pigs, and the National Research Council (1998) recommended intake for weaner pigs is 100 mg/kg feed DM. The National Research Council (1998) remarked that the bioavailabilities (defined as a percentage of a recognised standard, for example, $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O} = 100\%$, rather than the actual percentage absorbed or retained) of different Zn compounds is generally less than 50%, with the bioavailability of ZnO ranging from 40 to 95% (National Research Council, 1998; Mavromichalis *et al.* 1999). Zn from organic complexes appears to be more available than that from ZnO, and equivalent to Zn from ZnSO_4 (Schell & Kornegay, 1996; Swinkels *et al.* 1996).

Interest in the growth-promoting properties of ZnO came to light after a report by Poulsen (1989; cited by National Research Council, 1998), who showed increased weight gain and

reduced post-weaning scours in pigs when the weaner diet was supplemented with a pharmacological level (3000 parts per million (ppm)) of ZnO for 14 d after weaning. Numerous studies (reviewed by National Research Council, 1998; also Bertol & Debrito, 1998; Carlson *et al.* 1999; Mavromichalis *et al.* 1999) have confirmed this earlier work, and now it is relatively commonplace to include ZnO, at levels ranging anywhere between 2000 and 6000 ppm, in weanling pig diets. However, some other studies (see National Research Council, 1998; Windisch *et al.* 1998) have failed to show an improvement in performance with the use of ZnO.

The growth-promotion attributes of ZnO might in part be due to its effectiveness in controlling PWC (for example, Holm, 1988; Poulsen, 1989; Bertol & Debrito, 1998). With a ban on growth-promoting antibiotics in some countries, the reliance on pharmacological levels of ZnO in weaner diets to control enteric disease, particularly PWC, has increased. Despite its widespread use after weaning, the precise mechanism(s) whereby ZnO exerts its effects is uncertain. Huang *et al.* (1999) offered some insight into this question since pigs fed high doses of ZnO showed reduced bacterial translocation from the small intestine to ileal mesenteric lymph nodes. Other workers (for example, Carlson *et al.* 1999) suggested that high levels of ZnO induced metallothionein in gut enterocytes that, in turn, was involved in up regulation of RNA and DNA cell proliferation. Mavromichalis *et al.* (1999) found no improvement in gut structure due to addition of different sources of ZnO. Other workers have not observed differences in diarrhoea, although changes in the microbial ecology appeared to occur with the use of ZnO.

Jensen-Waern *et al.* (1997) reported that supplementation of a weaner diet with 2500 ppm ZnO improved performance in pigs over a 28 d period following weaning, with no pigs showing any clinical signs of illness. However, this also occurred in the control group that did not receive 2500 ppm ZnO. Use of ZnO had no effects on numbers of excreted *E. coli* or *Enterococci* spp./g of faeces, although these authors suggested that ZnO at growth-promoting levels in the diet might reduce the diversity within the gut microflora that, in turn, promotes a more stable ecology. In turn, Katouli *et al.* (1999) reported that addition of 2500 ppm ZnO to a weaner diet did not reduce total coliform numbers in the faeces, but control pigs showed an increase in both the variety and diversity of coliforms compared with pigs fed ZnO. These authors recommended that ZnO be fed not longer than the first 14 d after weaning.

A major concern with the increasing use of pharmacological (growth-promoting) levels of ZnO is excretion of this mineral into the environment, with possible antibacterial effects on beneficial soil and water bacteria. Some countries, such as Denmark, are restricting ZnO levels in diets to reduce the amount of Zn leaving the pig and entering the environment. Although other forms of Zn such as zinc chelates and organic complexes have higher bioavailabilities than ZnO, it is not known whether they are as effective in preventing enteric disease after weaning as ZnO. Evidence in the literature to support their use is lacking, and they tend to be more expensive. The extent of diarrhoea after weaning obviously depends on a multitude of factors and conditions, and these will influence the effectiveness of any Zn compound relative to ZnO. However, it would appear necessary to investigate alternative forms of Zn (or ZnO) that not only have higher bioavailabilities (leading to reduced faecal excretion), but also have growth-promoting properties and ameliorate or prevent diarrhoea. Mavromichalis *et al.* (1999) compared two sources of ZnO that differed in their relative bioavailability (39 v. 93 %) in weanling pigs, and reported no major differences between the two sources in their growth-promoting properties. No data were reported on the incidence of diarrhoea.

Copper. The pig needs Cu for the synthesis of haemoglobin and for the synthesis and activation of several oxidative enzymes necessary for normal metabolism. The National Research

Council (1998) recommends a level of 6 mg/kg feed DM for weanling pigs. Cu salts with high biological availabilities include the sulfate, carbonate and chloride salts (Cromwell *et al.* 1998). Organic complexes of Cu appear to have equal bioavailability to CuSO₄ (see National Research Council, 1998), although Coffey *et al.* (1994) reported that pigs fed a copper-lysine compound had a faster growth rate than pigs fed CuSO₄, suggesting a difference in bioavailability and/or systemic effects. Coffey *et al.* (1994) also reported that 100 ppm Cu was as efficacious in stimulating growth as 200 ppm Cu.

Braude (1945) was the first to show a growth response to high dietary Cu concentrations fed to growing–finishing pigs. When fed at 100–250 ppm, Cu (as CuSO₄) has a growth-promoting effect in weanling pigs that appears to be independent of, but in addition to, the growth response elicited by antimicrobial compounds (National Research Council, 1998). In addition, the growth response to high levels of CuSO₄ is increased by added fat, an effect attributed to enhanced lipase and phospholipase A activities (for example, Luo & Dove, 1996). As with pharmacological levels of ZnO, the precise mechanism(s) whereby CuSO₄ exerts growth-promoting properties is (are) unknown, although some evidence suggests that it may have a systemic component (Zhou *et al.* 1994), in addition to any direct effect(s) in the gastrointestinal tract.

Interactions between other minerals, such as growth-promoting inclusion levels of ZnO in weanling pig diets, also require consideration. Recently, Hill *et al.* (2000) reported the results from twelve experimental stations in the USA investigating the effects of Zn and Cu supplementation above National Research Council (1998) requirements for weanling pigs (6.55 kg, weaned at 22 d of age). All diets contained chlortetracycline (220 ppm) and were fed for 28 d. Treatments were as follows: (a) control; (b) 3000 ppm ZnO; (c) 250 ppm CuSO₄; (d) 3000 ppm ZnO plus 250 ppm CuSO₄. Pharmacological levels of ZnO and CuSO₄ enhanced growth rate and feed intake, and improved feed efficiency, beyond levels of Zn and Cu intake that met nutrient requirements. The combination of Zn and Cu did not cause an additive growth response. In another study, Hill *et al.* (2001) reported that supplemental ZnO at 1500–2000 ppm plus an antibacterial agent (carbadox) improved post-weaning performance in an additive fashion.

Similar environmental pollution concerns to those expressed with regard to feeding high levels of ZnO have been raised with addition of growth-promoting levels of CuSO₄ in diets, but CuSO₄ may be more of an issue because it is often included in diets through the growing and finishing periods. Investigations into ‘alternatives’ to CuSO₄ and ZnO require a focus on sources of minerals that are cost-effective, provide similar levels of control of enteric disease, and reduce the amount of mineral entering the environment.

Polymers. Work by Hampson *et al.* (2000) demonstrated that the use of a polymer (2-propenal, 2-propenoic acid, which is based on the polymerisation of 2-propenal) was effective in reducing colonisation and the incidence of diarrhoea of weaner piglets following both experimental challenge with *E. coli* and an on-farm trial. This product is thought to inactivate surface proteins on micro-organisms, thereby destroying them in a non-selective way.

Influence of diet processing on enteric diseases

A key influence on the physicochemical properties *in vivo* of ingested feed, especially cereals, is the type and extent of processing that the grain component undergoes before feeding. A multitude of changes occur in the chemistry of cereals (and perhaps oilseeds and legumes) during the various processing procedures, and these are relatively poorly understood. This is especially

the case when trying to unravel factors responsible for maintenance of intestinal health, because it is likely that mechanical events occurring even before the pig eats its diet will have an impact on its capacity to digest and absorb the feed. Furthermore, these effects will also influence the microbial ecology of the gastrointestinal tract. The interactions that might exist between grain type and growing region, grain handling and processing, and the subsequent effects on the intestinal health of the pig require further investigation, particularly in relation to diseases such as salmonellosis and gastric ulceration.

Processing of grains breaks cell walls and reduces particle size. This is important because it determines the surface area that is exposed to the digestive and microbial enzymes, and influences the number of starch granules released from the protein-fibre matrix of the endosperm (Rowe *et al.* 1999). It has generally been considered that a 'fine' particle size is desirable because the surface area available for digestion will be increased. For example, Owsley *et al.* (1981) reported that ileal digestibility of starch in sorghum increased from 72 % for dry rolled sorghum (1.3 mm particle size) to 86 % for hammer-milled sorghum passed through a 3.2 mm screen (500 μm particle size). However, comminuting grain too 'fine' may have adverse consequences for the prevalence of enteric diseases such as gastric ulceration, especially in wheat-based diets (pp. 355–356). Particle size is also important because, again depending on the cereal and even the cultivar within a cereal, it will largely determine the rate, extent and sites of digestion of starch within the gastrointestinal tract of the pig.

While the combination of enzymic activity in the small intestine and a (relatively) high fermentative activity in the hindgut results in a consistently high value for digestible energy content of grains, the pattern of digestion along the gastrointestinal tract may not necessarily be optimum with regard to nutrient absorption and utilisation, and hence the net energy content of a grain. The literature indicates that while there are well-established methods of processing grains to reduce particle size and achieve efficient digestion across the entire gastrointestinal tract (Rowe *et al.* 1999), information pertaining to the effects of processing and grain type on the rate, extent and site of digestion within specific regions of the gastrointestinal tract of the pig is less common. Furthermore, an undesirable pattern of digestion in the gastrointestinal tract might have dire consequences for the proliferation of certain bacteria that cause disease in pigs, such as in PIS and possibly PWC.

Acid-binding capacity of feedstuffs and post-weaning colibacillosis

Aumaitre *et al.* (1995) suggested that the activities of endogenous proteases are reduced in the post-weaning period by the presence of certain protein-containing feedstuffs, but not others. These included fishmeal or fish protein concentrate, by-products of the slaughter industry (for example, meat and bone meal), and the presence of 'high' amounts of soyabean meal in the diet. Animal protein products such as fishmeal and dried milk powders have high acid-binding capacity values (Bolduan *et al.* 1988). These feedstuffs bind more HCl in the stomach than cereals resulting in a higher pH (reduced acidity) and reduced pepsinogen production. As a consequence, proteolysis in the stomach might be diminished and the presence of 'extra' protein in the small intestine might overwhelm the digestive capacity of pancreatic and brush-border proteases in the upper regions of the small intestine, themselves relatively immature in the immediate post-weaning period. More protein would then move distally and be available for fermentation that, in turn, might predispose the young weaned pig to PWC. In addition, acid-binding capacity in the stomach might allow increased survival of pathogenic *E. coli* that is subject to faecal–oral recycling. In this regard there is considerable (re)interest in the use of

organic acids as a means of lowering gastric pH that, in turn, might be associated with reduced proliferation of *E. coli* in the upper small intestine.

Organic acids

For a number of years organic acids have been used with varying success for amelioration of enteric infections. The withdrawal of growth-promoting antimicrobials has forced them back into focus as an alternative or replacement to antimicrobials in pig production. Several recent reviews (Partanen & Mroz, 1999; Partanen, 2001) describe the rationale behind the use of organic acids and present results on digestibility of nutrients and their effects on some bacterial diseases (see Partanen, 2001). In the review by Partanen (2001), a meta-analysis of published data in weaned pigs revealed that improvements in production attributable to organic acids were observed due to an increase in voluntary food intake, although the exact mechanism(s) causing this effect are somewhat difficult to ascribe. This is no surprise given the multitude of factors affecting feed intake after weaning. The situation becomes complicated because different acids cause different effects, and studies have used different diets and conditions of hygiene that might also contribute to the differences observed. Furthermore, data associating the use of organic acids with reductions in diarrhoea are equivocal and data *in vivo* demonstrating that organic acids exert their effects by lowering gastric pH are lacking (Gabert & Sauer, 1994; Partanen & Mroz, 1999). Findings are also equivocal as to whether organic acids exert their effects by influencing dietary buffering capacity (Roth & Kirchgessner, 1989).

In addition, Partanen (2001) commented that: 'It seems that the growth promoting effect of organic acids is primarily associated with effects on gastrointestinal microflora'. There are few data to support this notion, although some studies in both weaner and growing-finishing pigs (Øverland *et al.* 1999; 2000*a,b*; Canibe *et al.* 2001) have reported decreases in measurements such as total coliform counts and total anaerobic bacteria counts with the use of some acids (potassium diformate in this case). Partanen (2001) commented that organic acids not only act on pathogenic bacteria, but also modify beneficial flora, and considered that reduced microbial fermentation allows the pig increased access to the carbohydrates, which in turn improves performance. Considerable work is still needed in this area, particularly in the field of microbiological changes that occur in response to organic acids. It is possible also that other alternatives to in-feed antibiotics, such as enzymes (Partridge & Tucker, 2000), might work in conjunction with organic acids to enhance performance after weaning. Regardless, work from the field in countries such as Sweden and Denmark suggests that certain organic acids have a positive effect on reducing the incidence of diseases such as PWC and salmonellosis, and hence are used widely in commercial practice.

Nutraceuticals, botanicals and fatty acids

There has been considerable interest in the use of these compounds to replace growth-promoting antimicrobials in diets for pigs. Although the scientific rationale and mechanisms behind the antimicrobial properties of these compounds to control enteric infections are not new, and in principle are sound, there are very few scientific data *in vivo* to support their efficacy in controlling enteric bacterial diseases.

A number of papers show marked *in vitro* effects of essential plant oils and extracts on a number of bacterial genera and species (for example, Hammer *et al.* 1999). Duncan *et al.*

(1998) identified several specific metabolites isolated from plants, such as the coumarins esculetin, umbelliferone and scopoletin, which created inhibitory conditions *in vitro* for pathogens such as *E. coli* O157. These authors could not ascertain the exact mechanism(s) whereby the coumarins exerted their effects, though the results appear promising. Nevertheless, the majority of studies investigating nutraceuticals or botanicals have been conducted *in vitro*, and hence validation of these findings *in vivo* is still required.

It is recognised that compounds such as these possess potent anti-inflammatory and immunological actions. Recently, Bassaganya-Riera *et al.* (2001a,b) showed that conjugated linoleic acid enhanced cellular immunity in pigs by modulating phenotype and effector functions of CD8(+) cells involved in both adaptive and innate immunity. This occurred irrespective of whether pigs were in a 'clean' or 'dirty' environment. Development of cell-mediated immunity against pathogens in both the gut and the circulation are key defence mechanisms, and it might be possible to influence the development and duration of some enteric diseases by the addition of compounds such as conjugated linoleic acid.

Exclusion products and probiosis

Many of the successful enteric organisms have developed strategies to resist displacement from the epithelium via the development of anchoring adhesive fimbriae (pili). Approaches to masking these attachment sites from pathogenic bacteria include the feeding of certain lectins or competing carbohydrates (oligosaccharides) that inhibit attachment of certain bacteria to the epithelium (Kelly *et al.* 1994), to the provision of (avian) egg immunoglobulin G immunised against enterotoxigenic strains of *E. coli* (Mroz *et al.* 1999). For example, addition of 2.5% D-mannose to broiler diets reduced the excretion and colonisation of *Salmonella enterica* var. Typhimurium (Oyoyo *et al.* 1988), while the use of a mannan oligosaccharide has been reported to reduce the concentration of caecal coliforms and *Salmonella enterica* var. Typhimurium and *Salmonella* Dublin in chicks (Spring *et al.* 2000). Similar studies in pigs have not been published in peer-reviewed journals.

McCracken & Gaskins (1999) commented that dietary supplementation of human subjects and animals with probiotics has been shown to provide protection against intestinal, diarrhoea-producing pathogens under certain situations, for example in 'dirty' facilities. Simpson *et al.* (2000) reported that the introduction of *Lactobacillus reuteri* strain MM53 to 21 d old piglets caused bacterial changes in the faeces that could be reliably assessed using denaturant gradient gel electrophoresis methodology. In 'clean' conditions though, the use of probiotics based on *Lactobacillus* spp. and *Enterococcus* spp. may in fact cause growth depression and deterioration in gut health because of the production of toxic metabolites (Gaskins, 2001). It would appear that the growth-promoting effects of probiotics in pigs are less consistent and quantifiably less evident than the use of antimicrobial compounds, although the recent technical developments in molecular microbiology have identified other probiotic strains that might have greater potential for enhancing disease resistance (Kelly, 1998).

Conclusions

This purpose of the present review was to describe the major enteric bacterial diseases of pigs in relation to where nutrition, in its broadest sense, has or might be used to prevent or modulate the incidence of disease. The restricted use and, in some parts of the world, the total ban on the

use of antimicrobial agents, such as growth-promoting antibiotics and minerals, has resulted in a search for alternatives or replacements for these compounds in pig diets. This is particularly pertinent to the period following weaning, where diets have traditionally been fortified with antimicrobial agents to control the proliferation of diseases such as PWC. Data from countries such as Denmark (Hansen, 2000) and Sweden (Commission on Antimicrobial Feed Additives, 1997) suggest that banning and/or restricting the use of antimicrobial agents increases the incidence of clinical disease, increases mortality, reduces growth rates and increases the overall cost of production to slaughter weight. In these instances the use of 'nutrition' to compensate, at least in part, for the loss of antimicrobial agents has been adopted. Unfortunately, the underlying basis for many of the reported positive effects of nutrition on controlling enteric infections is poorly understood, so firm dietary recommendations to prevent or reduce clinical enteric disease cannot be made with full confidence. A greater understanding of how nutrition influences the intestinal environment, gut epithelial biology and immunobiology, and their interactions with both commensal and pathogenic bacteria, holds promise as a means of tackling enteric disease without antimicrobial agents. An important first step in defining the bacterial diversity of the gut was achieved recently by Leser *et al.* (2002), who compiled a library of 4270 cloned 16S rDNA sequences representing 375 phylotypes from the ileum, caecum or the colon of pigs aged 12–18 weeks. In addition, it is important to consider the overall system of pig production in the context of controlling enteric bacterial diseases. In this regard, Penny (2000) commented that the pig industry, especially in Europe, needed to consider new management techniques plus a re-evaluation of existing production systems (for example, weaning age, bedding systems, all-in, all-out management) to tackle this issue. In this regard, a postal survey by Pearce (1999a) found a negative association between the use of wet feeding systems and colitis in finishing pigs in a sample of UK pig herds. Although the statistical association between units adopting wet feeding and increased colitis does not necessarily indicate a causal relationship, it does highlight the overall complexity when faced with responding to enteric diseases. It is envisaged that this challenge will become greater if there is a total ban placed on all antimicrobial agents.

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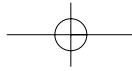
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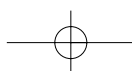
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