Gut health in the pig


*School of Veterinary and Biomedi cal Sciences, Murdoch University, Murdoch WA 6150. **Department of Agriculture and Food, South Perth WA 6151.

Abstract

Gastrointestinal disturbances can cause large economic losses in the pig industry. Diseases and conditions of the gastrointestinal tract (GIT) that can cause economic loss have generally been controlled by the use of dietary (and or in the water) antimicrobial compounds, such as antibiotic feed additives and (or) minerals such as zinc and copper. However the implementation of legislation in some parts of the world, for example the European Union, and a growing sentiment worldwide to reduce the use of dietary antimicrobial compounds, has caused a reassessment of measures to influence GIT 'health' and caused enormous interest in alternative means to control diseases and conditions of the GIT. There are now available a wide array of products and strategies available to the pig industry that influence 'gut health'. The products in the market place are characterised predominately not only by their (claimed) different modes of action, but also by the variation in responses seen when offered to pigs, and not only in the post-weaning period. This variation is presumably a consequence of the many different conditions of management that pigs are under, that in turn influences factors such as composition of the microbiota and mucosal immunity. Other strategies, such as the manipulation of particle size and changing the protein content of a diet, might also be adopted to influence the expression of enteric pathogens and the expression of disease. Ultimately, the cost-benefit of adopting such practices to influence gastrointestinal 'health' requires consideration.

Introduction

'Gut health' is a term that we define as describing a generalised condition of homeostasis in the gastrointestinal tract (GIT) of the pig. The factors and conditions involved in 'gut health' are multifactorial, complex, and currently poorly described and sometimes incorrectly interpreted, although it is evident that perturbations of the GIT that cause disease, even sub-clinical disease, are a disturbance of this homeostasis. In addition to enteric disease, other influences will also impact upon gut health, such as the responses occurring in the GIT in the period immediately after weaning, any changes that might occur after a change in diet, and (or) disruptions to meal patterns and hence the flow of nutrients through the GIT.

Simplistically, 'gut health' can be viewed as an outcome (positive, negative, the status quo) of the complex interactions occurring in the GIT between nutrition (e.g. feed type, feed composition, presence or absence of antibiotic feed additives), the mucosa of the GIT (e.g. type of receptors, mucin, inflammatory activity, cytokine production, barrier function), and the microbiota (e.g. density, location, pathogenicity, extent of colonisation) (Figure 1). The vast array of possible interactions means that analysis that encapsulates the dynamic nature of this organ cannot be achieved with many of the analytical tools currently available. Consequently scientific analysis, and hence interpretation, is usually resistant to a reductionist approach to these issues (Niewold, 2006). Examining the system holistically has not been possible previously, however gene expression profiling using microarray technology (e.g. Niewold, 2006) and (or) the emerging field of metabolomics (e.g. Bertram et al., 2007) hold promise as means of unravelling the regulatory mechanisms and transcriptional networks that underlie these most complex of biological processes. Further description and interpretation of this methodology is beyond the scope of this paper, however.

Nevertheless, when attempting to assess gut function and gut health, it is useful to view any particular assessment in a number of ways that extend from the whole body level to the molecular level. To understand the basic mechanisms during normal and pathological gut development, a useful approach is to assess gut function at as many levels as possible as this will provide maximum knowledge about any treatments applied and each experimental animal used (Figure 2). Analysis of many different parameters can cause conflicting observations that are difficult to interpret. However, relying too much on single parameters is more dangerous as this may lead to erroneous conclusions.
A discussion of 'gut health' cannot proceed without acknowledgement of the various mechanisms mooted for the positive actions of antibiotic feed additives on the host. These have been described previously (see reviews by Visek, 1978; Anderson et al., 2000; Gaskins, 2001; Pluske et al., 2002; Page, 2006; Wegener, 2006), have been covered in the companion paper by Moran (2007), and will not be reiterated in this paper. Nevertheless, the benefits of antibiotic feed additives to pig production can broadly be categorized into production benefits, disease control benefits, prevention of metabolic and/or fermentative disorders, and numerous other related benefits such as protein and energy sparing (Page, 2006). In turn, these can have environmental benefits, for example through reduced nutrient excretion. It is rare therefore, if not impossible, to find a feed additive/nutritional strategy that can elicit the same effects that an antibiotic feed additive has when benefits are realised. In this respect, it is unlikely that any single feed additive/nutritional strategy will fully enhance the 'health' of the GIT to the same degree as an antibiotic feed additive when the GIT is compromised.

The intent in this paper is to provide some context for the use of alternatives to antibiotic feed additives, by briefly discussing modes of action and attempting to define and highlight the basic principles of 'gut health'. The companion paper in this symposium by Dr Colm Moran will cover this in more detail. This paper will then discuss some of the biological issues surrounding the notion of 'gut health', using examples where appropriate. It is hoped that this paper will provide readers with a greater understanding of some of the major issues surrounding 'gut health' and instigate debate and discussion of the topic, particularly in an Australasian context.

Antibiotic feed additives

Antibiotic feed additives have been used extensively and successfully in pig feeds over many years to improve health and performance, but only appear to be effective under certain circumstances. Risks associated predominately with antibiotic resistance of microbes in man have caused the banning or restriction of antibiotics as feed additives for pig production in some parts of the world (the precautionary principle). This has caused an explosion in both the use and development of alternative strategies to antibiotic feed additives, and in particular those feed additives/nutritional strategies that alter (or are claimed to alter) the microbiota of the gastrointestinal tract (GIT) to somehow modulate the 'health' of this organ system. It has also caused a number of large collaborative projects [e.g., "Sustainable Systems for Weaner Pig Production" (a UK-based project), "Defining and validating gut health criteria in young pigs, based on digestive physiology, microbiology and mucosal immunology investigations for testing alternative strategies to in-feed antibiotics" (an EU project), "Feed for Pig Health (Development of Natural Alternatives to Antimicrobials for The Control of Pig Health and Promotion of Performance) and REPLACE (Plants and Their Extracts and Other Natural Alternatives to Antimicrobials in Feeds)" (EU projects)] aimed at understanding further the mechanisms underlying this phenomenon. Although these projects mainly concern the newly-weaned pig, it is important to consider that other pig phases, for example growing-finishing pigs and sows, also suffer from GIT disruptions causing depressed production, morbidity, and sometimes death.
The change in production systems in the 1940s and 1950s from one predominately based on pasture, where plant and animal foods were consumed, to controlled indoor environments, where pigs were fed prepared vegetable-based diets, underscored the inadequacy of diets based on plants for optimum production. Including animal protein products in diets for indoor pigs showed that normal growth could be restored (Page, 2006). Subsequently it was found that the factor responsible for this restoration in growth was vitamin B12. In a case of serendipity, researchers trying to find a reliable and high-producing source of vitamin B12 in *Streptomyces aureofaciens* isolated chlorotetracycline, which was the growth-promoting fraction over and above the independent effect of vitamin B12 (Page, 2006). Since this time a plethora of studies have been conducted attempting to explain the mode(s) of action of antibiotics and, unsurprisingly given the diversity of antibiotics present, no common mechanism(s) has/have ever been found. Coates et al. (1952) commented that "it is unlikely that a single mode of action can explain all the results reported in the literature", with respect to the use of antibiotics in chickens. Little has changed in 55 years.

A feature of using antibiotic feed additives in pig production is the variation in responses that occur. Braude et al. (1953; cited by Page, 2006) summarised a large number of experiments from the time and concluded that the relative improvement in growth rate resulting from adding antibiotics to diets for pigs was inversely related to the growth rate of control animals. This is corroborated by the research of Melliere et al. (1973) (Figure 3). Hays (1991) commented that the "response is greater during critical stages of production such as weaning...", and that "environmental stresses such as inadequate nutrition, crowding, ... poor sanitation ... also contribute to increased responses". A study by Dritz et al. (2002) with more than 24,000 pigs in three multi-site production systems in Kansas showed that only nursery pigs responded to in-feed antimicrobials, noting that the performance of non-supplemented control pigs was very high consistent with decreased microbial challenge and high levels of hygiene.
What does all this mean for ‘gut health’ in pigs? The implication is that responses to any in-feed alternatives to antibiotic feed additives are also likely to be variable, and therefore it is no surprise that such findings are found commercially. The literature is littered with studies that show beneficial responses and enhanced ‘gut health’ in pigs and poultry to alternative dietary products to antibiotic feed additives, but less evident are studies showing a lack of, or even negative, responses. Critically, the circumstances and (or) conditions whereby these (lack of) effects occur are often not cited, making it even more difficult to discern the reasons for the variability that occurs. Examples of exceptions are the meta-analysis described by Miguel et al. (2004) assessing the efficacy of the product BioMos®, and the work described by Rosen (2006) focusing on the construction and application of holistic predictive empirical models to objectively assess alternatives to antibiotic feed additives in the intensive animal industries. Studies such as these at least document the variation that surrounds a response to a feed additive, but they offer little or no comfort to those seeking a unifying product or strategy (elixir) to replace antibiotics in diets, if indeed that is the intention.

Many hypotheses have been proposed to explain the mode(s) of action of the antibiotic feed additives without any consistent consensus. Germ-free animals benefit very little from dietary antibiotics clearly showing that the microbiota is a critical intermediary in any positive effects observed. Page (2006) summarised the hypotheses already proposed and tested to explain these effects, and they include:

- Reductions in total numbers of bacteria in the GIT with decreased competition between the microflora and the host for nutrients;
- Inhibition of ‘harmful’ bacteria that may be pathogenic and (or) capable of producing toxic metabolites, particularly from protein fermentation;
- Inhibition of bacterial urease, to prevent formation of NH3;
- Improved energetic efficiency of the GIT through reduced weight, reduced enterocyte turnover and increased rate of glucose uptake;
- Inhibition of bacterial cholytaurine hydrolase activity, which reduces the level of lithocholic acid;
- Nutrient sparing;
- Improved nutrient absorption effected by morphological changes to the small intestinal epithelium;
- Modification of intestinal enzyme activity, since the characteristics of intestinal enzyme activity are influenced significantly by the microflora;
- Reduced immune stimulation, via a reduction in sub-clinical microbial challenges; and
- Stimulation of intestinal synthesis of vitamins by bacteria.

Saliently, these hypotheses confirm that any alternative/replacement feed additives are most unlikely to fully capture the multitude of effects imparted by the use of antibiotics. Furthermore, it is probable that no one single antibiotic feed additive can achieve all these effects because they have different modes of action.

**Barrier function of the gastrointestinal tract**

Any discussion pertaining to ‘gut health’ cannot occur without reference to the microbiota that inhabit the GIT of the pig and their influence on barrier function. It is not our intention to describe the generalized features of the GIT microbiota because these have been adequately portrayed in many other review articles. However, there is a diverse assemblage of bacteria that varies in population density and diversity in different compartments of the GIT and at different stages in the life of a pig (Hampson et al., 2001; Zoetendal et al., 2004). Additionally, the microbiota (commensal, pathogenic) are intimately involved in ‘cross talk’ between the enteric bacteria and the host, with the chemistry and distribution of bacterial binding sites on gut mucosal surfaces playing key roles in determining host and tissue susceptibility and in triggering host responses, especially in young animals (Kelly and King, 2001). Individual mucin carbohydrates have the capacity either to repel or bind to microbial surface adhesins. In the case of pathogenic bacteria, protection against 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 nature of the diet, the microbiota, and interactions between them influence the composition and functional characteristics of intestinal mucus (Montagne et al., 2004). The taxonomy and distribution of bacterial groups that preferentially reside within the mucous layer must be better defined to ascertain the role of ‘normal’ gut bacteria in mucogenesis and mucolysis (Deplancke and Gaskins, 2001).

A key issue when discussing ‘gut health’ is that of barrier function. A good example where barrier function is compromised occurs in the immediate post-weaning period, where weaning causes a ‘leakier’ small intestine.
Inflammation of the intestine is associated with increased permeability that may lead to translocation of toxins, allergens, viruses or even bacteria. If and when bacteria cross this first line of defence and reach the lamina propria, their metabolites or mediators liberated from epithelial cells may cause an inflammatory response (Gaskins, 1997), and in this case the measurement of pro-inflammatory cytokines provides some information as to the degree of local inflammation (Johnson, 1997). Therefore weaning per se, and essentially the period of anorexia that occurs immediately after weaning, causes an inflammatory response (McCracken et al., 1995; Pie et al., 2004) that initiates perturbations to 'gut health'. In this case, simply encouraging pigs to eat more feed after weaning should ameliorate these responses and stabilize 'gut health'.

Factors influencing the activity and composition of the GI microbiota

Myriad of factors influence the diversity and activity of the GIT microbiota, including the age of the pig and the environment it inhabits, antimicrobial agents (antibiotic feed additives and minerals such as Zn and Cu), dietary composition (e.g., carbohydrate type and content, protein type and content), feed additives (e.g., organic acids), feed processing (grinding, meal vs pellets), feeding methods (e.g., fermented liquid feeding), disease load, weaning, season, stress and genetics. These factors, which can interact, can make the study of the gut microbiota difficult.

Colonization of the GIT by the microbiota plays a critical role not only for the overall well-being of the pig, but also for its nutrition, performance, and quality of the products produced (e.g., production of skatole in the hindgut and effects on meat quality). Apajalahti and Kettunen (2006) reviewed the microbiological activities that could be considered important in assessing the health and (or) performance of the host. These included activities affecting nutrient and energy partitioning between the microbiota and the host, those connected to pathogenesis and disease development, activities supporting or suppressing immunological mechanisms, and activities of various metabolic pathways of bacteria that may manufacture harmful and (or) beneficial end products during their metabolism. Table 1 summarizes these principles and shows that all phenomena are connected to the microbiota, their outer cell wall structure, their growth and their general metabolism. Apajalahti and Kettunen (2006) remarked that because microbiota in the GIT obtain the majority of substrate for growth and metabolism from the host's diet, then it is evident that the listed characteristics of the intestinal microbial community can be influenced by feed composition and (or) feed type. This can have consequences for all phases of pig growth and pig production, from the lactating sow to suckling and newly weaned pigs, through to the finishing pig.

Table 1. Examples of microbial activities relevant for the health and performance of animal hosts (from Apajalahti and Kettunen, 2006)

<table>
<thead>
<tr>
<th>Example of microbial activity</th>
<th>Target Level</th>
<th>Risky Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>General bacterial growth in small intestine</td>
<td>Restricted</td>
<td>Overgrowth</td>
</tr>
<tr>
<td>Effect on intestinal morphology</td>
<td>Stabilising</td>
<td>Disrupting/deforming</td>
</tr>
<tr>
<td>Pathogen growth</td>
<td>Suppressed</td>
<td>Favoured</td>
</tr>
<tr>
<td>Pathogen adherence</td>
<td>Blocked</td>
<td>Facilitated</td>
</tr>
<tr>
<td>Immune activation</td>
<td>Stimulatory</td>
<td>Suppressive</td>
</tr>
<tr>
<td>Lactic acid production</td>
<td>Normal</td>
<td>Accumulation</td>
</tr>
<tr>
<td>Putrefactive activity (e.g., indole, skatole, NH3)</td>
<td>Restricted</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Some influences on the GI microbiota and 'gut health'

It is beyond the scope of this paper to provide a comprehensive discussion of all nutritional influences on the GIT microbiota. A plethora of papers and reviews coinciding with the lead-up to the European Union's ban on antibiotic feed additives have been written (e.g., Jensen, 1998; Gaskins, 1997, 2001; Hillman, 2001; Pluske et al., 2002; Hopwood et al., 2005; Lallès et al., 2007). However, one question that generally arises in discussions relating nutrition to the GIT microbiota, and particularly the area of 'gut health', is: What is 'normal' when referring to the health of the pig GIT?

Hillman (2004) suggested that emphasis should be placed on an 'optimal' microbiota being present in the GIT rather than a 'normal' microbiota, mainly because defining what is 'normal', given the wide array of conditions pigs are grown under and methodological shortfalls, is difficult and problematic. Furthermore, there is widespread use, particularly in the popular press, of the description of bacteria as being either 'beneficial' (good) or 'detrimental' (bad).
For example, it is generally assumed that *Bifidobacteria* and *Lactobacillus* are 'good' bacteria whereas *Enterobacteriacaena* (i.e. the coliforms) are 'bad' bacteria, despite a large number of species and strains in the latter being of a non-pathogenic (avirulent) nature (Chapman et al., 2006). Potential enterotoxigenic strains of *E. coli* exist in all herds, but post-weaning colibacillosis (PWC) does not necessarily occur in these herds. The presence or absence of a pathogenic organism, therefore, may not necessarily predict that disease will occur unless numbers proliferate to such an extent to overwhelm the general microbial population in the GI tract, or more specifically a specific region of the GI tract.

Hillman (2001) showed that intestinal *lactobacilli* having anti-pathogenic ('probiotic') activity against pathogenic *E. coli* that possessed K88 fimbriae were not evenly distributed across 19 Scottish pig farms. The variation in anti-pathogenic efficacy could be responsible for the variation seen across farms in, for instance, the efficacy of probiotics and other additives that purportedly affect specific bacterial populations. This is because adding an antimicrobial to the GIT where there is a population of bacteria already possessing a high indigenous antimicrobial activity is likely to be less effective than adding it where there is a population that shows little or no pre-existing antimicrobial activity (Hillman, 2001). In this regard, Hillman (2001) commented that it is the consistency of activity (e.g., across farms, across diets, across seasons) rather than the degree of activity that is currently poorly understood with respect to the large number of antibiotic replacements/alternatives on the market at present, and this possibly precludes their wider use. A current project within the Pork CRC under the supervision of Dr James Chin (NSW Department of Primary Industries) is investigating this aspect of probiotics further.

With regard to efficacy of probiotics after weaning, is the fact that we are relying on the intake (feed, water) of the bacteria/bacterium at a time when feed intake is both low and variable (Pluske et al., 1997) simply may not be giving the product(s) any chance at all of succeeding? The published data defining the benefits of probiotics for nursery pigs are equivocal, which is no real surprise given the different species and strains that are used and the wide array of weaning and feeding conditions that products work under (Pluske, 2006). Differences in herd-health status undoubtedly contribute to the ambiguity in efficacy seen, as alluded to previously. In addition, recent research (e.g., Liong et al., 2007) shows that oral synbiotics (probiotics + prebiotics) can have powerful metabolic effects in the pig, so should these type of combinations be investigated further? Regardless of this, is there a way of delivering probiotics that can circumvent the usual problems of low feed/water intake after weaning? Indeed, should probiotics be administered at birth when the GIT is sterile?

Work with fermented liquid feed (Demeckova et al., 2002) and *Bacillus cereus* var. toyoi (e.g., Taras et al., 2005) suggests that an altered microbiota in the faeces of the dam caused by changing the microbiota of the sow exerts a beneficial influence on both pre- and post-weaning development of the young pig. There is also some suggestion that feeding spores of *B. licheniformis* and *B. subtilis* (Alexopoulos et al., 2004) alters milk quality in sows, which is pertinent given the current interest in Australia and the Pork CRC regarding the performance of progeny derived from gilt or sow litters. In the study by Taras et al. (2005), one group of sows were fed for 17 weeks, from day 24 after mating to day 28 after farrowing, and the piglets from these sows were fed for 6 weeks, from day 15 of lactation to 8 weeks of age. The control group of sows/piglets did not receive the probiotic strain. The *Bacillus cereus* var. toyoi strain was recovered from the faeces of sows and piglets throughout the trial, including the period 0-14 days of age before introduction of the starter diet occurred, and there was an improvement in FCR of pigs in the post-weaning period derived from sows fed the probiotic during pregnancy and lactation. Of particular interest in the weaned pigs offered the probiotic was a significant reduction in the incidence of liquid faeces (Figure 4) and post-weaning diarrhoea. Diets did not contain any antimicrobial agents, suggesting that this particular probiotic strain reduced the proliferation of enterotoxigenic *E. coli* in the GIT of weaned piglets.

![Figure 4](image-url)

**Figure 4.** Prevalence of liquid faeces (consistency score 4-5) during the total post-weaning period (d 29-56) of piglets in the Control (open boxes) and probiotic (closed circles) group, respectively (after Taras et al., 2005).
Good bacteria versus bad bacteria: is there such a thing?

In an attempt to develop a predictor of ‘gut health’, Hillman (2004) advocated the use of a faecal lactobacilli:coliform bacteria ratio as an indicator of a pig’s ability to resist infection and therefore enhance/maintain ‘gut health’. A higher ratio (at least 100:1) indicates a ‘healthy’ gut while a low ratio (less than 100:1) places the pig at a greater risk of infection. Hillman (2004) commented that a simple test such as this using faeces would help ensure that pigs can be maintained as close as possible to optimum performance because gut ‘health’ is optimised.

Such a predictor obviously requires further validation, however it is seemingly ironic that Anderson et al. (2000) remarked that the class of microbes that appear to depress pig growth the most through toxin production, namely the Gram-positive facultative anaerobes that includes strains of Lactobacillus and Enterococcus, are also often used as probiotics; in the case of Lactobacillus, this would increase the ratio when perhaps it should be decreased. Furthermore, Mikkelsen et al. (2003) reported that the population of Bifidobacteria in GIT samples from piglets was numerically very low, which further complicates any clear explanation of links between ‘good’ bacteria and overall GIT function given the massive microbial diversity and overall population size present.

Further complications arise when the type of antibiotic feed additive is investigated. In an excellent study using molecular PCR-DGGE and quantitative polymerase chain reaction (qPCR) techniques to identify bacteria in the ileum of weaned pigs fed either tylosin or an antibiotic rotation sequence (week 1: chlorotetraacycline sulfathiazole penicillin; week 2: bacitracin and roxarsone; week 3: Encomycin; week 4: carbadox; week 5: virginiamycin), Collier et al. (2003) found that sequence analysis of treatment-specific DNA bands identified three Lactobacillus, one Streptococcus and one Bacillus species that were diminished with the antibiotic rotation treatment, whereas tylosin selected for the presence of L. gasseri. Lactobacillus-specific qPCR was performed and analyzed as a percentage of total bacteria and demonstrated that total bacteria were decreased by tylosin and the antibiotic rotation treatments, whereas the percentage of lactobacilli increased by 14 and through 28 in tylosin-treated pigs. Collier et al. (2003) concluded that the decrease in total bacteria by antibiotics might reduce host-related intestinal or immune responses, which would divert energy that could otherwise be used for growth. Conversely, the ability of tylosin to improve animal growth may relate to its apparent selection for lactobacilli, commensals known to competitively exclude potentially pathogenic species from colonizing the intestine.

An issue that is increasingly discussed with reference to ‘gut health’ and the GIT microbiota is that of diversity, with the general view being that a greater bacterial diversity is beneficial for ‘gut health’. But is this view consistent, and does it provide a meaningful basis for comparing antibiotic feed additives to alternatives? Konstantinov et al. (2003) reported that feeding fermentable carbohydrates in the form of sugar-beet pulp (100 g/kg diet) or fructooligosaccharides (FOS; 2.5 g/kg diet) to piglets after weaning (at 28 days) increased bacterial diversity and promoted a more rapid stabilisation of the bacterial community (by day 5 post-weaning) compared to pigs fed neither of these products. This was related to the predominance of several bacterial genera and species that, the authors postulated, were involved in the utilisation of dietary fibre sources such as sugar-beet pulp and FOS. However, no production benefits or effects on diarrhoea were reported. Conversely, Collier et al. (2003) found that the use of antibiotics caused a homogenization (decrease in diversity) of the microbiota they the authors’ believed might explain, in part, the homogenized growth performance commonly observed in groups of animals fed antimicrobial growth promoters (Schwarz et al., 2001). Collier et al. (2003) commented that microbial homogenization might ultimately prove to be a target mechanism for alternatives to antimicrobial growth promoters in diets for pigs.

Zinc oxide – why does it work?

Zinc oxide (ZnO) is a non-antibiotic product that appears to influence and benefit ‘gut health’. Numerous studies have shown the production and (or) anti-diarrhoeal benefits of including ZnO at supraphysiological levels (i.e. 2000-3000 mg/kg or ppm) in the diet after weaning, but what is less clear is/are the mechanism(s) whereby ZnO exerts its beneficial effects. Reported effects include the increased gene expression of antimicrobial peptides in the small intestine (Wang et al., 2004), positive effects on the stability and diversity of the microflora, particularly with respect to coliforms (Kotoul et al., 1999), increased IGF-I and IGF-IR expression in the small intestinal mucosa (Li et al., 2006), bactericidal functions (Jensen-Waern et al., 1998) and reductions in electrolyte secretion in vitro from enterocytes (Carlson et al., 2006). Hedemann et al. (2006) found changes in some pancreatic enzymes and mucus staining but concluded that there were no definite answers as to how the growth promoting and diarrhoea-reducing effects of excess dietary Zn were exerted. Nevertheless, some studies have reported no benefits of feeding ZnO (e.g., Jensen-Waern et al., 1998; Broom et al., 2006).

A study by Hojberg et al. (2005) using 2500 ppm ZnO showed reduced bacterial activity (ATP accumulation) in digesta from the gastrointestinal tracts of newly-weaned piglets compared to that in animals receiving 100 ppm ZnO. The numbers of lactic acid bacteria and lactobacilli were reduced, whereas coliforms and enterococci were
more numerous in animals receiving the high ZnO dose. These authors surmised that the influence of ZnO on the GIT microbiota resembled the working mechanism suggested for some growth-promoting antibiotics, namely the suppression of Gram-positive commensals rather than potentially pathogenic Gram-negative organisms. Hojberg et al. (2005) suggested that reduced fermentation of digestible nutrients in the proximal part of the GIT might render more energy available for the host animal and contribute to the growth-promoting effect of high dietary ZnO doses. In contrast, dietary CuSO4 inhibited the coliforms and thus potential pathogens as well, but overall the observed effect of CuSO4 was limited compared to that of ZnO.

Despite the ambiguity related to the exact mechanism(s) of action of ZnO to elicit its beneficial effects, it is likely that it will continue to be studied because it is a cost-effective nutritional tool that appears to function well in situations where 'gut health' might be compromised, such as after weaning. Unraveling the mode(s) of action of this chemical compound could provide a means for the rational development of alternatives to antibiotic feed additives.

**Dietary Associations with Enteric Diseases**

The study of a specific bacterial infection offers a means of assessing the usefulness of nutritional strategies on the survival of that particular pathogen in the GIT and its ability to affect production, morbidity and mortality. There are a number of well-known enteric bacterial diseases that occur throughout Australasia and indeed the world, and each is relatively unique in that it generally occurs at different phases of pig growth and/or in different regions of the GIT. There is a relative paucity of studies examining the effects of a specific pathogen on the overall balance and diversity of the GIT microbiota, and how alterations to a community, for example by feeding a particular substrate, can influence pathogenesis. Even where the addition of a dietary component/nutrient/additive is known to stimulate proliferation of specific groups of resident bacteria, little is really known about the way in which these bacteria interact with pathogenic species of bacteria. This lack of information makes it difficult to predict how a given dietary component could be used to indirectly influence a given enteric pathogen (see review by Hampson et al., 2001).

The following section deals with the influences of nutrition/nutritional management on two important diseases, namely swine dysentery (SD; caused by *Brachyspira hyodysenteriae*) and salmonellosis (*Salmonella* spp). We have intentionally highlighted these two diseases because they are pathogenic in different parts of the GIT (SD in the large intestine, salmonellosis mainly in the terminal small intestine/large intestine), highlighting possibly different mode(s) of action, and the approach to their control is quite marked.

**Swine dysentery**

Swine dysentery (SD) is a mucohemorrhagic colitis occurring mainly in grower pigs that involves the caecum, colon and rectum, and is caused by the anaerobic spirochaete *Brachyspira hyodysenteriae* (Hampson et al., 2006). 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, and this can progress 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 gaunt, with a tucked-in abdomen and an arched back. If left untreated, around 10% of affected pigs can die within five days of first showing clinical signs (Hampson and Trott, 1995).

The exact pathogenesis of SD is not clear, however it is apparent that the disease does not always express itself clinically in pig herds despite the presence of the bacterium (Hampson et al., 2006). Numerous factors are implicated in the aetiology of SD (see reviews by Hampson and Trott, 1995; Harris et al., 1999; Pluske et al., 2002; Hampson et al., 2006), including nutrition, and this fact resulted in a considerable body of work being conducted at Murdoch University in the 1990s examining the relationships between cereal type, processing, enzyme addition, the microbiota and the clinical expression of SD following experimental challenge. These data have been reported previously (see Pluske et al., 2002; Hampson et al., 2006).

Briefly, our data showed that feeding a diet low in both soluble non-starch polysaccharides (NSP) and resistant starch (RS) generally afforded protection against *B. hyodysenteriae*. 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 soluable NSP). Our data suggested that a reduction in RS levels (e.g., via extrusion, steam flaking) would only prove effective against SD if the grain in question has an inherently low RS content of the diet. Leser et al. (2000) 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 fermented liquid feed following infection with the causative agent. Kirkwood et al. (2000), Leser et al. (2000) and Lindecrona et al. (2003) failed to duplicate our previous results showing that feeding pigs a diet lower in soluble NSP and RS ameliorated the clinical expression of SD. Subtle diet differences existed between the studies, and these could have accounted for such discrepancies. For example, Kirkwood et al. (2000) and Lindecrona et al. (2003) fed parboiled rice, which has a higher RS content, whereas we fed cooked (autoclaved) medium-grain rice of a lower RS content. In turn, the amount of RS reaching the lower bowel could have been responsible for conditions that favoured/prevented colonisation of the spirochaete (Kim et al., 2006). German researchers (Baumann and Bilkei, 2002) reported that high levels of highly fermentable fiber (9.6% highly fermentable neutral detergent fiber) may increase health and performance of pigs experimentally infected with B. hyodysenteriae compared to pigs fed a diet containing 6.1% low fermentable neutral detergent fiber. Moreover, a recent study by Thomsen et al. (2007) examined the effects of two diets based on triticale and barley and supplemented with either rape seed cake or dried chicory root and sweet lupins. The study showed that diets supplemented with highly fermentable carbohydrates from dried chicory roots and sweet lupins can protect pigs against developing SD. Finally, Piao et al. (2007) concluded that lower concentrations of iso-valerate and iso-butyrate in digesta, associated with feeding non-animal-protein-based diets and therefore in contrast to our general proposition that feeding animal proteins is protective against SD, was “likely associated with development of pathogenic spirochete infection”. However, Piao et al. (2007) made this conclusion based on “3 samples collected from one farm”, and their description of the diets used was vague.

Species differences within a particular pathogen might also influence the success, or otherwise, of a nutritional treatment. For example, Lindecrona et al. (2003) found no protective effect of feeding a (parboiled) cooked rice-based diet on the development of SD in 18-30 kg pigs, but Lindecrona et al. (2004) reported a protective effect of feeding cooked rice on the development of Brachyspira pilosicoli (the agent of porcine intestinal spirochaetosis - PIS - or porcine colonic spirochaetosis) in pigs. In the same studies, Lindecrona et al. (2003) found a protective effect of fermented liquid feed on the development of SD but with Brachyspira pilosicoli, there was no significant effect of fermented liquid feed (Lindecrona et al., 2004).

Less attention has been given to general microbiological changes that occur in association with a specific disease. In the case of SD, other bacterial species such as Fusobacterium, Clostridium and Bacteroides need to be present in the lower GI tract for the disease to occur (see review by Pluske et al., 2002). Durmic et al. (1998) showed diet-associated differences in the genera and species present in the large intestine of pigs experimentally infected with SD, with changes in bacterial populations consistent with those that occur in the natural disease. 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 fermented liquid feed, following infection with the spirochaete and subsequent destabilisation of the colonic bacterial community.

Results such as these make generalisations about the effect of nutrition on SD difficult to make. Unfortunately, such conclusions are not made any clearer when researchers speculate beyond the confines of a particular experiment. For instance, Hogberg et al. (2004) conducted a study using pigs fitted with post-valve T-caecum (PVTC) cannulae on the effects of diets differing in NSP content on the diversity of GIT coliforms, using terminal restriction fraction length polymorphism (T-RFLP). These authors reported that the ratio of soluble and insoluble NSP influenced the coliform diversity in the large intestine, which is plausible, but then drew inferences between their data and the link to SD. They commented that “Previous findings suggest that it is the soluble fraction of NSP that predisposes pigs to swine dysentery. Diets based on cooked rice and animal protein reduced the clinical expression of Brachyspira hyodysenteriae, presumably by limiting the amount of fermentable substrates entering the large intestine (Pluske et al. 2002). However, we found no difference in coliform diversity between the Low NSP diets on day 17, possibly indicating a stabilisation over time. Further, we found no difference in the mean coliform diversity in connection to varying total NSP level measured in the rectal samples. Indeed, other researchers have failed to prevent development of swine dysentery with low fibre diets based on cooked rice and animal protein (Kirkwood et al. 2000, Lindecrona et al. 2003).”

Such data need to be questioned. First, the use of pigs fitted with a PVTC cannula, in which a significant proportion of the caecum is removed, casts doubt on hindgut function, particularly with a disease such as SD that can develop in the caecum. Second, the genus Brachyspira does not belong to the family Enterobacteriaceae, which are commonly called the ‘coliforms’. To then draw conclusions linking coliform ‘diversity’ to SD is clearly wrong, and these authors should be challenged. Nevertheless, Hogberg et al. (2004) did say that the balance of the intestinal flora, as well as pig genotype and microbial environment, are factors of significance for the development of SD, although this has been said previously (e.g., Hampson and Trott, 1995).

What is the way forward with the nutritional effects on SD? It is evident that dietary fibre has an impact on proliferation of the disease, and more precise delineation of this in any diet-related investigations into SD is required. In addition, an area that has received very little attention is the influence of protein entering the large intestine on SD
(although see Jacobsen et al., 2004), and possible interactions with dietary fibre. A comparison of different pathogenic strains species found in field cases coupled to techniques such as T-RFLP and RT-PCR that can identify and enumerate other bacteria might also signal new directions.

Salmonellosis

The major route for transmission of *Salmonella* in pigs is the oral route (Fedorka-Cray et al., 2000) after which the basic virulence strategy common to *Salmonella* is to invade the intestinal mucosa and multiply in the gut-associated lymphoid tissues. From the infected tissue the bacteria are drained to the regional lymph nodes where the host defence mechanism can prevent further spread, in which case the infection remains localised to the gut and often manifests itself as acute enterocolitis. If the macrophages in the draining lymph nodes are unable to limit spread, *Salmonella* can cause systemic disease (Baumler et al., 2000).

Therefore clinical porcine salmonellosis can be separated into two groups with distinctly different symptoms. The first, most severe, form is associated with septicaemia and involves the host-adapted *S. choleraesuis* that is common in the USA but absent in many European countries. The second disease is associated with enterocolitis often connected with *S. typhimurium*, which may be a more important pathogen in a number of countries (Fedorka-Cray et al., 2000; Griffith et al., 2006). A wide variety of other serovars have been isolated from pigs and although they occasionally may cause disease, in general, infected pigs may remain healthy carriers (Fedorka-Cray et al., 2000). In many countries, the problem of *Salmonella* infection in pigs is consequently mainly a question of food safety as sub-clinical *Salmonella enterica* infections in pig herds are recognised as important sources of human salmonellosis and hence a potential threat to human health through food-borne disease outbreaks.

Numerous epidemiological reports have shown that the physical characteristics of feed influence the susceptibility of pigs to *Salmonella*, as the *Salmonella* prevalence was higher in pigs fed heat-treated pelleted feed compared with feeding meal or liquid feed (Dahl, 1997; Wingstrand et al., 1997; Stege et al., 2000). For example, Wong et al. (2004) conducted a survey using 359 finishing-pig herds in Germany, Denmark, Greece, The Netherlands and Sweden, between 1996 and 1998, to find herd factors associated with pigs testing seropositive for *Salmonella*. These data showed that pigs fed non-pelleted feed (dry or wet) had a 2- and 2.5-times lower odds of seropositivity compared to pigs fed pelleted feed, and that pigs given whey (to drink or as the liquid part of the diet) had 2.6-times lower odds to test seropositive than pigs not receiving whey.

To understand this phenomenon further, Mikkelsen et al. (2004) and Hedemann et al. (2005) conducted a 2x2 factorial experiment studying the effects of feed grinding (fine and coarse) and feed processing (pelleted and non-pelleted) on physicochemical properties, microbial populations, morphological characteristics in the small intestine, caecum and colon and *in vitro* survival and adhesion of *Salmonella enterica* serovar *Typhimurium* DT12 in the GIT of pigs. These authors demonstrated that feeding a coarsely ground meal feed to pigs changes the physicochemical and microbial properties of the stomach contents, predominately an increase in undissociated lactic acid concentrations, which caused a higher death rate of *S. enterica* serovar *Typhimurium* DT12 and consequently decreased this bacterium's survival during its passage through the stomach. Knarreborg et al. (2002) previously reported that high levels of undissociated lactic acid inhibited the growth of enterobacteria. In this way the stomach acts as a barrier preventing harmful bacteria from entering and proliferating in the lower part of the gastrointestinal tract. In addition the adhesion was 60% less to the ileal tissue of pigs fed the non-pelleted diets than to those fed pelleted diets. Further, pigs fed pelleted diets secreted mucins capable of binding *Salmonella enterica* serovar *Typhimurium* DT12 and thereby allowing for colonization.

Regarding dry feed, several Danish studies have confirmed that offering meal feed to growing-finishing pigs reduces the *Salmonella* prevalence compared with feeding heat-treated pelleted diets (Hansen, 2004; Jørgensen et al., 1999). However in the same studies it was repeatedly shown that offering a dry meal diet to growing-finishing pigs reduces the performance of the pigs. This poorer performance can be explained by differences in particle size distribution and hence utilisation of the available energy and nutrients, but again it underlines that improving GIT health is often associated with increased cost of production.

Fermented liquid feeding is another means of manipulating the GI microbiota, in both the fermentation tank and the GI tract (e.g., Mikkelsen and 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/bacteriostatic), and typically results in reduced numbers of coliform bacteria in the feed, provided that fermentation conditions are correct. Van Winsen et al. (2001) investigated the effects of fermented (liquid) feed on bacterial populations along the GI tract, and reported significant negative correlation in pigs fed fermented feed between the concentration of disassociated lactic acid and *Enterobacteriaceae* numbers in the stomach. Van Winsen et al. (2001) concluded however that the direct influence of lactobacilli on *Enterobacteriaceae* numbers could not be demonstrated.
Can nutrition of the suckling piglet influence gut health and lifetime performance?

To conclude this part of the symposium, we wish to broach a subject that questions the timing and nature of a dietary intervention to effect changes in 'gut health' that, in turn, might influence whole-of-life performance. Pluske et al. (2005) reviewed the influence of nutrition of the young pig in relation to lifetime performance. In this review, an experiment conducted by Hugh Payne was described where the effects of three pre-weaning nutritional treatments on lactation and lifetime performance were assessed. The three treatments during lactation were: (i) no creep feed, (ii) litters offered a commercial, pelleted creep feed, and (iii) litters offered a mixture of sow feed, fresh straw and soil and organic matter (e.g., faeces, stubble) in a ratio of approximately 5:1:25 (hereafter referred to as the 'outdoor mix'). The third treatment was used to simulate materials that outdoor born and reared piglets might encounter and consume under commercial outdoor conditions. After weaning, piglets were all fed and housed indoors under identical conditions until slaughter at approximately 105 kg. The diets used in treatments (ii) and (iii), and the diet fed after weaning, did not contain any growth promoting antibiotics or pharmacological levels of Zn or Cu.

A summary of the data are presented in Pluske et al. (2005) and Table 2, and shows that feeding the ‘outdoor mix’ during lactation appeared to have a stimulatory effect on both hot carcass weight and dressing (killing out) percentage, after statistical correction for birth weight, sex, P2 backfat depth and final live weight, at slaughter. These data infer that some element(s) associated with young pigs being exposed to an 'outdoor' environment has/have a beneficial effect on carcass conformation 19-20 weeks later.

What might precipitate such a marked effect? A subsequent study using objective PCR-DGGE methodology to assess the diversity of the microbiota in the large intestine of piglets from indoor and outdoor housing systems revealed differences in the gastrointestinal ecology of pigs born outdoors and raised on deep litter (Pluske et al., 2007). These data suggest that changes caused to the GIT microbiota, and hence possibly immune function, by nutrition early in life have whole-of-life effects on growth and production (Payne et al., 2003).

Table 2. Effects of offering no creep food, a commercial creep feed, or an outdoor mix on the performance and carcass characteristics of pigs from birth to slaughter (from Pluske et al., 2005)

<table>
<thead>
<tr>
<th>Treatment in lactation</th>
<th>No creep feed</th>
<th>Commercial creep feed</th>
<th>Outdoor mix</th>
<th>LSDb (5% level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weaning (28 d)</td>
<td>9.1</td>
<td>8.7</td>
<td>8.9</td>
<td>0.9</td>
</tr>
<tr>
<td>7 d post weaning</td>
<td>9.7</td>
<td>9.8</td>
<td>10.0</td>
<td>0.8</td>
</tr>
<tr>
<td>28 d post weaning</td>
<td>18.7</td>
<td>19.3</td>
<td>19.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Final weight</td>
<td>107.1</td>
<td>106.2</td>
<td>107.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Daily gain (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth-weaning</td>
<td>266</td>
<td>256</td>
<td>260</td>
<td>31</td>
</tr>
<tr>
<td>Weaning -7 d after weaning</td>
<td>60</td>
<td>123</td>
<td>131</td>
<td>63</td>
</tr>
<tr>
<td>Weaning - 28 d after weaning</td>
<td>344</td>
<td>366</td>
<td>378</td>
<td>63</td>
</tr>
<tr>
<td>Birth - 28 d after weaning</td>
<td>307</td>
<td>316</td>
<td>325</td>
<td>23</td>
</tr>
<tr>
<td>Birth - finisher</td>
<td>677</td>
<td>677</td>
<td>693</td>
<td>23</td>
</tr>
<tr>
<td>Weight/agec, g/d</td>
<td>686</td>
<td>687</td>
<td>701</td>
<td>24</td>
</tr>
<tr>
<td>HCWd (Trim 13) (kg)</td>
<td>70.4</td>
<td>70.3</td>
<td>71.7</td>
<td>1.27</td>
</tr>
<tr>
<td>P2, mm</td>
<td>12.4</td>
<td>12.7</td>
<td>13.2</td>
<td>1.78</td>
</tr>
<tr>
<td>Dressing, %</td>
<td>65.7</td>
<td>65.9</td>
<td>66.8</td>
<td>1.08</td>
</tr>
</tbody>
</table>

aOutdoor mix consisted of creep feed, straw, and soil + organic matter.
bLSD: least significant difference.
cWeight/age = (Final weight/final age) * 1000.
dHCW: hot carcass weight.

Conclusions

‘Gut health’ is an often contentious and puzzling subject area and one that continues to attract attention worldwide. Important progress has been made in a relatively short period of time in relation to our understanding in this field at the GIT level, with reference to the mechanisms underpinning the physiology, microbiota and localised immune system. Some concepts have emerged, for example, the notion of stimulating/nullifying specific groups of bacteria in the GIT to modify the GIT environment; however it will not be until the system is viewed and analyzed
holistically that major advances will be made. In the post-weaning period, where most attention has been focused, the compromised state of the young pig makes it an ideal candidate for the range of dietary products that might influence 'gut health'. However, pigs in later stages of growth also suffer from diseases and conditions that can dramatically influence production and survival, so 'gut health' needs to be viewed from the whole-of-life perspective. In this sense, and as discussed in this symposium previously, interventions through the sow (gestation, lactation, gestation plus lactation) could be more appropriate in some cases to modify GIT health in the offspring, via colostrum and milk, than via the newly-weaned pig, for example. There is already a large body of research, and this continues to grow, in regard to the various interventions that might influence GIT health. Some of these data are published while other data remains unpublished. Regardless, the issue of variability in responses seen to interventions needs to be considered in any recommendations that might be made.