Nutrition and pathology of weaner pigs: Nutritional strategies to support barrier function in the gastrointestinal tract

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Abbreviations: AA: Amino acid, AGP: antibiotic growth promotants, BCFA: branched-chain fatty acids, CMC: carboxymethylcellulose , CP: crude protein, E. coli: *Escherichia coli*, ETEC: enterotoxigenic E. coli, FCR: feed conversion ratio, GIT: gastrointestinal tract, iNO: inducible nitric oxide synthase, N: nitrogen, NDF: neutral detergent fibre, NO: nitric oxide, NSP: non-starch polysacchrides, PE: proliferative enteropathies, PIS: porcine intestinal spirochaetosis, PWC: post-weaning colibacillosis, SD: Swine dysentery, TEER: transepithelial electrical resistance, ZnO: zinc oxide, ZO: zonula occludens.

Abstract

Factors including sub-optimal nutrient and energy intake associated with lowered digestion and absorption, immature immune function, and psychosomatic factors caused by weaning can compromise intestinal barrier function through mucosal damage and alteration of tight junction integrity. As a consequence, pigs at weaning are highly susceptible to pathogenic enteric diseases such as post-weaning colibacillosis (PWC) caused by enterotoxigenic *Escherichia coli.* Dietary components such as protein, non-starch polysaccharides, and minerals are known to influence microbial growth in the gastrointestinal tract as undigested nutrients then become available for bacterial growth. This article reviews the association between dietary components, intestinal bacterial growth, intestinal barrier function, and enteric disease in weaner pigs with special emphasis on PWC. Evidence presented in this review indicates that the pathogen-originated diseases such as PWC are closely associated with dietary components and intestinal barrier functions can be maintained through manipulation of dietary protein, NSP and mineral levels. Especially, the use of a reduced protein diet for at least 7 days immediately after weaning, limitation of viscosity-increasing soluble NSP content while including 20 – 80 g/kg insoluble NSP source in the diet, and limitation of iron to 100 mg/kg are important dietary strategies to maintain intestinal barrier function and to minimise PWC.

Key words: Enteric disease; Intestinal barrier function; Mineral; Non-starch polysaccharides; Post-weaning colibacillosis; Protein.

1. Introduction

Weaning is the most significant event in the life of pigs as they are abruptly forced to adapt to nutritional, immunological and psychological disruptions. Sows' milk that is highly digestible and high in protein, fat and lactose is replaced by a dry and less-digestible starch-based diet (Williams, 2003) causing significantly reduced energy intake for maintenance of epithelial structure (Pluske et al., 1996b), reduced transmucosal resistance (Spreeuwenberg et al., 2001; Boudry et al., 2004) and increased secretory activity in the small intestine (Boudry et al., 2004). Damage to the epithelial layers also decreases nutrient digestibility which provides more substrates for pathogen proliferation (Pluske et al., 2002), increases production of epithelial

irritants such as ammonia (Heo et al., 2009), and increases pathogen attachment and penetration through the transcellular and paracellular pathways (Moeser and Blikslager, 2007). Innate and adaptive immune system of weaner pigs are yet to be fully developed and specialized whilst passive immunity from the sows' secretions are depleted at weaning (King and Pluske, 2003; Gallois et al., 2009). Young pigs also have to cope with psychological stressors at weaning such as separation from the sows, mixing with unfamiliar littermates and establishment of the social hierarchy within the group, which are known to increase cortisol release and corticotrophin-releasing factor receptor expression in the intestine of weaned pigs (Moeser et al., 2007). These stressors can increase paracellular and transcellular permeability and therefore eventually increases translocation of antigen and bacterial lipopolysaccharides across the mucosal barrier (Moeser et al., 2007; Smith et al., 2010). Since the ban of antibiotic growth promotants (AGP) in the European Union, numerous additives, management and dietary strategies have been studied to address the abovementioned consequences at weaning without AGP, and a substantial number of review papers dealing particularly with the range of feed additives available have been published (eg, Gallois et al., 2009; Lalles et al., 2009). Also, associations between amino acids and immune function are reviewed by Li et al. (2007), Ball (2008) and Seve et al. (2008).

Nevertheless, pigs at weaning remain susceptible to a number of bacterial and viral diseases but the most significant diseases that at least partly associated with the dietary components at weaning are the pathogenic bacteria-originated diseases, which can cause diarrhoea after weaning. These diseases include post-weaning colibacillosis (PWC) caused by serotypes of enterotoxigenic *Escherichia coli* (ETEC), the proliferative enteropathies (PE), caused by *Lawsonia intracellularis*, salmonellosis caused by *Salmonella S.*, porcine intestinal spirochaetosis (PIS) caused by *Brachyspira piloscicoli*, and swine dysentery (SD) caused by *Brachyspira hyodysenteriae*. Among these pathogen-originated diseases PWC occurs in the first 2 weeks post-weaning period while others are generally occurs 4-6 weeks after weaning. While the ETEC and *Lawsonia intracellularis* specifically affect the small intestine, *Brachyspira piloscicoli* and *Brachyspira hyodysenteriae* are known to colonize in the large intestine (Hampson and Pluske, 2004; Pluske and Hampson, 2009). Therefore, different dietary components depending on their solubility, digestibility, viscous-forming ability and acid buffering ability can prevent or promote proliferation and

colonization of these pathogens in the different part of the intestine. Although the data predominantly generated from the grower-finisher pig studies, including detailed aetiology of these disease and relationships between dietary treatment and onset, development and severity of the diseases are described elsewhere (Pluske et al., 2002; Hampson and Pluske, 2004; Pluske and Hampson, 2009) and will not be repeated in this review. Moreover, the pathogenic bacterial-originated diseases that are known to be influenced by dietary components but not common at immediate post-weaning period, such as PIS, SD and stomach ulcers caused by *Helicobacter S.* will also be excluded. Also, evidence for relationships between the PE and dietary components are not explored yet at a meaningful level and reviewed recently (Pluske and Hampson, 2009). Therefore, this review will concentrate on the role of nutrition on PWC at immediate post-weaning period (i.e., first 2 weeks after weaning) and will review recent evidence concerning the effects of dietary components that are responsible for intestinal barrier function under ETEC infection.

2. Intestinal barrier function

The mucosal epithelium is the primary 'barrier' between the internal milieu and the so-called "external environment", which consists of nutrients and harmful elements such as pathogens and antigens. This epithelial 'barrier' is protected externally by the unstirred water layer and mucus, and internally by the tight junctions between enterocytes. These external and internal barriers regulate selective passage of molecules, thereby protecting entry of pathogens and antigens into the system. Although, roles of the unstirred water layer as a barrier are generally not well understood, it is known that the unstirred water layer acts as a diffusion barrier and is known to limit the entry of fat-soluble components, except forms that are solubilized by micelles (Farhadi et al., 2003).

Underneath the unstirred water layer, a high molecular weight mucus layer covers the enterocytes and prevents damage by endogenous and bacterial proteases and acidic damage in the stomach and duodenum. In addition to this physical protection, the mucus layer is known to selectively block entry of macromolecules such as enzymes and antigens while being permeable to nutrients, and provides pathogen colonization resistance by adhesion of commensal bacteria in the luminal

surface (Montagne et al., 2004). Therefore, mucus-bound commensal microbes are important for competitive exclusion of intestinal pathogens. For example, feeding probiotic strains such as *Bifidobacterium lactis* and *Lactobacillus rhamnosus* inhibited mucosal adhesion of *E. coli*, *Salmonella enterica* serovar Typhimurium and *Clostridium difficile* in the pig's small and large intestine via pathogen exclusion, displacement and competition in vitro (Collado et al., 2007). Also, the quantity and maturity of mucins covering the epithelial surface are important factors for optimum pathogen resistance. For example, as neutral mucins mature, sulfate and sialic acids are detected and these mature mucins are more acidic and viscous and are highly resistant to the bacterial proteases (Allen et al., 1982; Rhodes, 1989; Montagne et al., 2004). In a rat study, both experimental suppression of mucus production from goblet cells using colchicine and thinning mucous gel layer using mucolytic agent N-acetyl cysteine increased small intestinal permeability of fluorescein isothiocyanate dextran, showing the importance of mucous layer thickness and mucin production for intestinal barrier function (Boshi et al., 1996).

Enterocytes are adjoined by a paracellular diffusion barrier called a tight junction. Tight junctions consist mainly of the transmembrane protein complexes claudins and occludins and the cytosolic proteins zonula occludens (ZO-1, ZO-2 and ZO-3), which join the transmembrane proteins to the cytoskeletal actins. These structural proteins provide connections between the cytoskeletons of adjacent enterocytes (Mitic et al., 2000; Anderson, 2001). Alterations of tight junction protein formation and distribution through dephosphorylation of occludins, redistribution of ZO, and alteration of actomyosin through phosphorylation of myosin light chains causes intestinal paracellular barrier dysfunction. Enteric pathogen and endotoxin translocations are known to increase paracellular permeability through tight junction alterations (Berkers et al., 2003; Groschwitz and Hogan, 2009).

Enterocytes also act as a barrier via the roles of energy-dependent transporters and channels in the apical and basolateral cell membranes. However, the regulatory mechanisms of transcellular pathways are not well understood. ETEC uses extracellular projections of protein poles - pili or fimbria - to attach to the specific receptors in the apical membrane of the enterocytes. Production of exotoxins (heat labile enterotoxin LT, Shiga-like toxin type II) and enterotoxins (heat stable STa and STb) trigger fluid excretion and immune system stimulation (Madec et al., 2000). Subsequent uptake of toxins, antigens and pathogens through transcellular and

paracellular pathways trigger inflammation and post-weaning diarrhoea, and nutrition is known to play important roles for the maintenance of intestinal barrier function (Pluske et al., 2002; Vente-Spreeuwenberg and Beynen, 2003; Pluske and Hampson, 2009).

3. Intestinal responses to *E. coli* infection and PWC

When pigs are infected with ETEC, the primary cellular response is excretion of cell fluid and electrolytes into the lumen as LT induces secretion of chloride, sodium, bicarbonate ions and water through activation of the adenyl cyclase-cyclic AMP system of the enterocytes, and ST (STa and STb) reduces enterocytes' ability to absorb sodium and chloride from the lumen via interaction with the guanyl cyclase-cyclic GMP system (Hopwood et al., 2006). Another pathway that contributes to intestinal chloride secretion is the production of nitric oxide (NO). It has been shown in a colonocyte model that NO stimulates chloride secretion via elevation of intracellular cGMP levels (Rolfe and Milla, 1999). ETEC proliferation can activate inducible nitric oxide synthase (iNOS) expression, which is an enzyme converting L-arginine to L-citrulline to produce NO and hence stimulate chloride secretion. In addition a neuropeptide galanin (Hecht et al., 1999), which is produced by the enteric nervous system and modulates intestinal motility and prostaglandin (Eckmann et al., 1997), is known to increase chloride secretion and induce secretory diarrhoea.

The secondary response of the ETEC infection is increased paracellular permeability either through actomyosin ring alteration or occludin dephosphorylation and ZO-1 redistribution, which significantly decreases transepithelial electrical resistance (TEER, a technique to measure paracellular permeability) of the small intestine (Berkers et al., 2003). The loosening of the tight junction due to ETEC infection, therefore, further increases invasion of antigens, toxins and pathogens into the circulatory system and can trigger inflammatory cascades (or immune system activation) that results in the production of cytokines and chemokins (communication molecules between immune cells and other body cells) and recruitment of inflammatory cells (Berkers et al., 2003).

The inflammatory response and subsequent production of cytokines and acute phase proteins reduces protein deposition and growth in animals. For example, Williams et al. (1997) demonstrated that pigs with high immune system activation

showed decreased daily gain (11%), feed intake (29%), body protein accretion (38%) and increased FCR (20%) compared with pigs with low immune system activation, between 6 and 27 kg body weight. Moreover, Breuille et al. (1994; 1998) used an *E. coli* infection model in rats and reported that infected rats showed enhanced liver protein synthesis (33% vs. 15%) whilst muscle protein synthesis was significantly decreased.

Therefore, nutritional strategies to maintain intestinal barrier function and reduce introduction of pathogens and toxins are pivotal steps for success of weaning production systems. Such nutritional strategies (e.g., manipulation of dietary protein and NSP content) should be able to reduce pathogen proliferation in the gastrointestinal tract (GIT) and prevent pathogen invasion through the intestinal barriers. Therefore, dietary strategies in relation to intestinal pathogen proliferation and intestinal barrier function will be discussed with special emphasis on dietary protein and carbohydrate fractions. Recent findings of some minerals in relation to intestinal barrier functions will also be covered in the later part of the paper.

4. Nutritional strategies to reduce pathogen proliferation

4.1. Dietary protein level

Protein content of the diet for weaned pigs has traditionally been formulated between 210 – 245 g/kg to support maximum lean growth of the modern genotype (Cinq-Mars et al., 1988). However, piglets' ability to digest and absorb high protein diets might be compromised as the pancreatic and brush border proteolytic enzyme systems are not fully developed at weaning (Travid et al., 1993b; 1993a; Pluske et al., 2003). The undigested dietary proteins along with endogenous proteins are subject to bacterial fermentation in the distal small intestine and the large intestine, causing increasing intestinal pH, pathogen proliferation, and production of intestinal irritants such as ammonia (Halas et al., 2007). Consequences of the undigested protein materials and subsequent fermentation on pathogen proliferation and intestinal barrier function are significant during the immediate post-weaning period and have been identified as one of the most important risk factors in the aetiology of PWC (Jeaurond et al., 2008).

4.1.1. Dietary protein and stomach barrier function

Bacterial pathogens are introduced into the GIT mainly from faecal materials, contaminated facilities and food sources through oral ingestion. Ingested bacterial pathogens are generally eliminated by the acidic condition (pH <2) in the stomach, although the gastric pH is increased immediately after feeding and decreases with time. However, the acid producing ability of newly-weaned piglets is not complete (Cranwell, 1995) and pH of the piglet stomach is highly variable between 2.2 to 4.2 (Wellock et al., 2008a), which increase pathogen survival and transit to the small intestine, as acidification of diet (Overland et al., 2008) and water (De Busser et al., 2010) significantly decreased *E. coli* and coliform populations in the small intestinal digesta and faecal shedding of *E. coli,* respectively. This is heightened when piglets overeat after fasting, such as after weaning, and is known as a risk factor for increasing gastric pH (Halas et al., 2007). It is generally believed that dietary protein levels influence gastric pH of the weaned pigs due to the buffering ability of some AA with basic side chains such as Lys, Arg and His (Partanen and Mroz., 1999). However, several weaner pig studies fed high versus low protein diets and consistently reported that dietary protein level did not affect gastric pH but affected pH in the jejunum and ileum (Hoe et al., 2010), ileum (Nyachoti et al., 2006) and proximal colon (Wellock et al., 2008a). These authors fed diets containing a wide range of protein (between 130 g CP/kg to 240 CP/kg). Lack of dietary effects on gastric pH under such conditions suggests that the buffering effect of dietary protein was not a contributing factor for gastric pH, but increased pH in the lower part of the intestine could be due to increased protein fermentation by-products such as ammonia, indoles, phenols, amines, BCFA and sulphuric-containing compounds, that are known to increase pH in the large intestine (Jensen, 2001). Nevertheless, maintaining gastric pH as a first line defence mechanism for introduction of pathogenic bacteria is well documented (Halas et al., 2007), and formulation of diet with lower buffering capacity are broadly accepted in the commercial production system to address the limited stomach barrier function at weaning.

4.1.2. Dietary protein and intestinal response to ETEC infection

It is thought that reducing dietary protein level decreases nitrogen flow in the GIT and decreases protein fermentation (Nyachoti et al., 2006). However, physiological evidence in support for this hypothesis is rarely published. In a recent study, Heo et al. (2010) fed either 239 g CP/kg or 190 g CP/kg weaner diet to 3-week-

old pigs without and with ETEC infection and found that nitrogen (N) digestibility at the terminal ileum was not affected by dietary protein levels but ileal N flow was significantly lower in the pigs fed the lower protein diet due to reduced protein intake, although the feed intake was not different. Consequently, protein fermentation indices such as plasma urea nitrogen and ammonia nitrogen content in all parts of the GIT were significantly lower in pigs fed a lower protein diet (Table 1). Finding that dietary protein level did not affect the ileal N digestibility is consistent with the finding that feeding 173 g CP/kg diet did not affect jejunal brush border peptidase activities compared with pigs fed a 222 g CP/kg diet (Opapeju et al., 2009b). However, when pigs are infected with ETEC, ileal N digestibility was significantly compromised and hence increased ileal N flow and N fermentation by-products in the small and large intestine (Table 1). As a consequence, feeding a high protein diet in ETEC-infected pigs significantly increased the incidence of PWC compared with other treatments including feeding a high protein diet without ETEC infection. These studies clearly demonstrated that feeding a high protein diet under high sanitary conditions and low bacterial challenges may not compromise intestinal barrier function but can extend the severity of the infection in a commercial production system where continual bacterial challenges exist.

[Table 1 about here]

4.1.3. Dietary protein and intestinal microbes

Undigested fermentable protein substrates in the GIT affect microbial populations (Wellock et al., 2006; Bhandari et al., 2010) although the effect could be dependent on the available carbohydrate substrates for microflora in the digesta (Heo et al., 2008). It was suggested that available fermentable protein substrates in the intestinal contents can change the saccharolytic: proteolytic microbes ratio, and can generate potentially harmful epithelial irritants such as BCFA, ammonia, amines, volatile phenols and indoles (Williams et al., 2001) which will compromise intestinal barrier functions. Specifically, ammonia in the GIT can disturb maintenance of gut integrity (Lin and Visek, 1991; Nousiainen, 1991). Effects of dietary protein levels on microbial populations were studied using culture-based technique by Wellock et al. (2006), who demonstrated significantly decreased *Lactobacilli* to Coliform ratio mainly due to increased Coliform populations in the faeces and proximal colon

292 samples of pigs fed increasing dietary crude protein from 103 g/kg to 230 g/kg.
293 However, this culture-based technique provides limited information on microbial
294 population and the information for overall bacterial density and diversity is not readily
295 available. More recently, Opapeju et al. (2009a) used the 16S rDNA fragment-based
296 technique (Terminal-Restriction Fragment Length Polymorphism, T-RFLP) to
297 investigate alterations of bacterial populations in pigs fed either 176 g CP/kg or 225 g
298 CP/kg diets. The pigs were challenged with ETEC K88 on day 8 post-weaning and
299 pigs were euthanized on -1, 3 and 7 day post-infection. Colonic digesta collected 7
300 days post-infection showed that feeding a higher protein diet was associated with
301 higher prevalence of family Clostridiaceae and genus Clostridium, which are
302 predominantly proteolytic microbes, and reduced prevalence of the order Clostridiales,
303 particularly the family Lachnospiraceae and genus Roseburia, which are
304 predominately saccharolytic microbes, especially butyrate producers (Opapeju et al.,
305 2009a). Also, pigs fed a higher protein diet had a more dense and diverse microbial
306 population in the colon at day 7 post-infection. These data support the notion that
307 feeding a lower protein diet can alter the saccharolytic: proteolytic microbe ratio in
308 the GIT in a favourable direction for intestinal barrier functions. In the subsequent
309 measurement of fermentation by-products and ileal histology, it was demonstrated
310 that such microbial manipulation through feeding a low protein diet reduced colonic
311 ammonia production and better maintained intestinal integrity (villous height:crypt
312 depth) (Opapeju et al., 2009a).
313
314 ***4.1.4. Dietary protein and intestinal permeability***
315 We are not aware of any study conducted to date to elucidate whether
316 microbial and histological alterations due to the dietary protein levels affect
317 transcellular and paracellular permeability. However, pigs fed a higher protein diet
318 would have a higher risk of infection than pigs fed a lower protein diet because it is
319 evident that feeding a higher protein diet stimulates proteolytic microbes, such as *E.*
320 *coli* and *C. perfringens*, increases protein fermentation by-products, and reduces
321 intestinal integrity (Lin and Visek, 1991; Nousiainen, 1991). Also, and mounting rat
322 small intestine in an Ussing chamber, Go et al. (1995) found that the presence of
323 bacterial endotoxins (i.e., lipopolysaccharides) in the luminal content increased
324 proportion of rats with labelled *E. coli* permeability from 14% in control rats to 78%
325 in lipopolysaccharide-injected rats. In this regard, an interesting study was conducted

with 21-day-old pigs, where pigs were fed either 63, 103, 151, 208 or 249 g CP/kg diet and euthanized at 43 days of age (Gu and Li, 2004). Unfortunately the diets were not formulated to contain similar essential amino acid levels or ideal patterns of amino acids, which affected epithelial protein content and growth of intestinal organ especially in the diets containing less than 208 g CP/kg. Nevertheless, feeding 249 CP/kg diet significantly increased the intraepithelial lymphocytes in all part of the small intestine and decreased goblet cells in the distal jejunum compared with pigs fed a 208 g CP/kg diet (Gu and Li, 2004). These findings could suggest that increased proteolytic microbes and protein fermentation by-products in the GIT of the pigs fed a high protein diet may have increased intestinal permeability and hence have increased immune response. Pié et al. (2007) also found that an increasing proportion of ileal BCFA content up-regulated pro-inflammatory cytokines such as interleukin 8 (IL-8), IL-12p40 and IL-18 mRNA expression in the homogenised ileal tissue samples, suggesting that small intestinal protein fermentation is associated with increased expression of pro-inflammatory cytokines.

4.1.5. Recommended dietary protein levels

Recommending low dietary protein levels for the pigs in the immediate post-weaning period is sometimes problematic because low protein diets without the appropriate balance of supplemented crystalline amino acids is known to compromise performance of pigs (Nyachoti et al., 2006), and use of a low protein diet with crystalline amino acid supplementation significantly increases diet cost. However, Stein and Kil (2006) concluded that such a low protein diet for prevention of PWC should be formulated to less than 180 g CP/kg, although the performance of pigs would be compromised if crystalline amino acids are not fortified to supply adequate essential amino acid levels for growth. This conclusion is supported with many other studies looking at effects of dietary protein levels on PWC (Nyachoti et al., 2006; Stein and Kil, 2006; Heo et al., 2008; Opapeju et al, 2009b). Research by Heo et al. (2008) for instance examined interactions between the duration of feeding and PWC expression. The data in this study demonstrated that feeding a 180 g/kg protein diet for 5-7 days post-weaning period can minimise expression of PWC. Therefore, reduced growth caused by amino acid restriction for the short period time most likely can be compensated if the pigs are fed a standard protein diet after the short period of protein restriction (Stein and Kil., 2006).

4.2. Dietary non-starch polysaccharides (NSP)

4.2.1. Dietary NSP and enteric disease

Associations between dietary NSP and pathogen proliferation in the GIT of weaner pigs were extensively studied (Hopewood et al., 2006; Kim et al., 2008; Wellock et al., 2008b; Hermes et al., 2009; Molist et al., 2009; 2001) and positive relationship between soluble NSP and the expression of PWC was reported (McDonald et al., 2001; Hopewood et al., 2006). A recent epidemiological study in Scottish farms examined the diet composition between farms without and with non-specific colitis and found that feeding diets containing high NSP, specifically those high in arabinose and xylose, had significantly higher incidence of non-specific colitis (Chase-Topping et al., 2007). Therefore, the involvement of dietary NSP on development of enteric diseases is quite clearly demonstrated but there are number of questions need to be answered to fully understand the background mechanisms for the association. The questions include: (1) does both soluble and insoluble NSP encourage pathogen proliferation?; (2) does solubility or viscous forming ability of NSP attribute to the increased pathogen proliferation in the GIT?; (3) does interaction between dietary protein and NSP, which will eventually determine fermentable carbohydrates:fermentable protein ratio in the lower small intestine, affect substrates for pathogen in the lower intestine?; and (4) how much NSP is optimum for prevention of enteric disease at immediate post-weaning period?

4.2.2. Soluble NSP and expression of PWC

A series of studies conducted in the 1990s at Murdoch University (summarised by Hopwood et al., 2006) examined whether dietary soluble NSP are associated with clinical expression of PWC in weaner pigs experimentally infected with β-haemolytic ETEC (serotype O149:K91;K88, enterotoxins LT, STa, STb). The authors fed a wide range of substrates including cooked rice, raw wheat, extruded wheat, pearl barley, pearl barley with enzyme containing β-glucanase, xylanase and α-amylase, carboxymethylcellulose (CMC) and guar gum, the latter two of which contain viscous-forming soluble NSP, to weaner pigs challenged with β-haemolytic ETEC. The studies showed that increasing amounts of soluble NSP linearly increased intestinal viscosity and small intestinal viable counts of β-heamolytic ETEC (Figure

1). Inferences from these studies suggest: first, dietary soluble NSP but not insoluble NSP is associated with proliferation of ETEC in the small intestine (Figure 1); second, that the structure of soluble NSP is a contributing factor for the proliferation of ETEC in the small intestine. In this regard, alteration of NSP structure by extrusion of wheat increased ETEC proliferation while supplementation of NSP degrading enzymes to a pearl barley-based diet decreased ETEC proliferation in the small intestine. This means manipulation of NSP structure either through available processing techniques or through use of NSP degrading enzymes can alter digesta viscosity and pathogen proliferation in the GIT of young pigs; and third, viscosity but not fermentability of NSP is the contributing factor for the ETEC proliferation in the small intestine as CMC used in the study was non-fermentable but increased viscosity of digesta (McDonald et al., 2001). In fact, unlike protein fermentation in the GIT, carbohydrate fermentation is known to encourage proliferation of beneficial bacteria and produce volatile fatty acids that are used for enterocyte proliferation (Williams et al., 2001). The finding that increasing digesta viscosity contributes to ETEC proliferation suggests that carbohydrate fermentation *per se* in the small intestine is not associated with PWC (Bikker et al., 2006; Jeaurond et al., 2008). Rather the positive relationship between dietary soluble NSP that increase digesta viscosity and small intestinal ETEC proliferation is most likely a secondary effect as soluble NSP decreases digesta transit time, nutrient digestibility and increases endogenous nitrogen flow in the small intestine (Choct, 1997). More time for bacterial proliferation and more nitrogen substrates from dietary and endogenous origins may provide the ideal environment for proliferation of proteolytic microbes including most enteric pathogens in the small intestine of pigs fed increasing dietary soluble NSP. The notion that non-viscous soluble NSP do not encourage pathogen proliferation and hence expression of PWC was supported in a recent study with ETEC-challenged weaner pigs (Wellock et al., 2008b), as increasing NSP content that do not increase intestinal viscosity did not increase PWD and increased colonic *Lactobacillus*:coliform ratio. The same principle may extend to the understanding that fermentable but not viscosity-increasing NSP such as fructooligosaccharides and sugar beet pulp encourage proliferation of microbes such as *Lactobacillus acidophilus* and *Bifidobacteria* and reduce the expression of PWC (Halas et al., 2009; Hermes et al., 2009). Also, a recent study showed that feeding 70 g/kg type 2 resistant starch (resistant granules) as a raw potato starch, which is not digested by host enzyme system but fermented by intestinal

427 microbes, significantly reduced faecal score in the first week after weaning (Bhandari
428 et al., 2009).
429
430 **[Figure 1. about here]**
431
432 ***4.2.3. Insoluble NSP for prevention of PWC***
433 On the other hand, insoluble NSP has long been recognized as a dietary
434 component for decreasing expression of PWC in weaned pigs. Early evidence
435 reported by Smith and Hall (1968) suggested that incorporation of barley hull, which
436 is mostly insoluble fibre, prevented expression of PWC, while barley meal that
437 contains soluble β-glucan increased expression of PWC in ETEC-challenged pigs.
438 The role of insoluble fibre on enteric pathogen proliferation has been highlighted
439 recently. For example, supplementation of 20 g oat hulls/kg in diets for weaner pigs
440 significantly decreased expression of PWC in pigs fed a cooked rice- (Mateos et al.,
441 2006) and extruded rice- (Kim et al., 2008) based diet. Addition of 20 g pure
442 cellulose/kg in a wheat-, barley-, corn- and soybean meal-based diet decreased the
443 incidence of PWC from 50% to 16% (Hanczakowska et al., 2008) and from 23% to
444 8% (Swiatkiewicz and Hanczakowska, 2006), depressed proliferation of *E. coli* and
445 *Clostridium*, and increased villous height:crypt depth ratio by better maintaining
446 villous height and decreasing crypt depth (Swiatkiewicz and Hanczakowska, 2006;
447 Hanczakowska et al., 2008). Molist et al. (2009) demonstrated that supplementation of
448 80 g wheat bran/kg significantly decreased unbound water content and increased
449 butyric acid content in the colonic digesta. Another study by Molist et al. (2010)
450 showed in an *E. coli* K88 infection study that incorporation of both coarse or finely
451 ground wheat bran (40g/kg) decreased the *E. coli* K88 population in the ileal digesta
452 and ileal mucosa, microbial density in the ileal digesta, and decreased production of
453 valaric acids, which is produced from proline fermentation and irritants for epithelium
454 like other BCFA. However, the effects were more profound in the pigs fed coarsely
455 prepared wheat bran (Figure 2).
456
457 **[Figure 2. about here]**
458

Unlike viscous forming soluble NSP, insoluble NSP is known to decrease digesta retention time and decrease small intestinal pathogen proliferation as evident in the abovementioned studies. Although feeding insoluble NSP is known to increase endogenous nitrogen flow as well, proliferation of nitrogen utilisers might be limited due to shorter retention of digesta and presence of fibre which may attract saccharolytic microbes. In fact, the concentration of insoluble NSP progressively increases as the digesta moves posteriorly because other digestible nutrients will be progressively digested and absorbed while insoluble NSP remain intact in the small and large intestine.

4.2.4. NSP and intestinal barrier function

The presence of insoluble fibre in the diet means continuous mechanical interaction between digesta and the epithelial mucus layer. This mechanical contact may cause a 'wash-out' of mucins and mucous-bound microbes, which could be a part of the reason why mucous-bound *E. coli* was significantly decreased in the pigs' ileal epithelium when they were fed a diet containing wheat bran, especially coarsely prepared wheat bran (Molist et al., 2010). Decreased microbial density in the ileal digesta only in pigs fed a coarsely prepared wheat bran but not in pigs fed a finely ground wheat bran in this study was most likely due to the increased digesta transit time, and suggests that particle size of insoluble NSP is an important issue which needs to be considered in GIT health. For example, gastric emptying (measured as % DM in stomach content) in a finely ground barley-based diet was faster compared to a coarsely ground barley-based diet in growing pigs (Simonssonand Bjorklund, 1978). Nevertheless, inclusion of insoluble NSP in the form of cellulose or wheat bran is known to increase mucin production and increase enterocytes and goblet cell turnover in rats (Vahouny et al., 1985). Evidence also showed that insoluble NSP could reduce intestinal permeability and bacterial translocation in the GIT. For example, Mariadason et al. (1999) measured intestinal permeability using conductance and Cr-EDTA flux method and reported a 20 % reduction in intestinal permeability in the distal colon of rats fed a diet containing 100 g wheat bran/kg compared with rats fed a diet without what bran. Another rat study conducted by Spaeth et al. (1990) examined the incidence of bacterial translocation into the mesenteric lymph node in rats orally fed either control TPN (total parental nutrition) solution, control + cellulose powder, control + coarsely ground corn cobs or control + citrus pectin. The incidence of

493 bacterial translocation into mesenteric lymph nodes was significantly decreased in rats
494 fed cellulose (15%) and coarsely ground corncobs (30%) compared to rats fed the
495 control (70%) or citrus pectin (65%) diets. This particular study demonstrated that
496 insoluble fibre in the forms of cellulose or corncobs could reduce bacterial
497 translocation in the GIT, whilst the viscosity increasing soluble fibres did not
498 demonstrate such protected effect. Unfortunately, intestinal permeability in pigs upon
499 feeding varying types and concentrations of NSP has not been investigated to our
500 knowledge.
501
502 ***4.2.5. Recommended NSP levels***
503 Evidence presented to date suggests that the amount of soluble and viscosity-
504 elevating NSP should be restricted and insoluble NSP should be included in the
505 practical formulation of diets for weaner pigs to minimise pathogen-originated enteric
506 disease such as PWC. The questions are, however, how much and what types of
507 insoluble NSP is/are required and at what levels should the soluble and viscosity-
508 increasing NSP be limited for prevention of PWC? High levels of insoluble NSP are
509 known to decrease nutrient digestibility (Lenis et al., 1996) and hence growth of pigs
510 (Degen et al., 2009). Bolduan et al. (1988) recommended 50 g crude fibre/kg weaner
511 diet for optimum hindgut development. Mateos et al. (2006) suggested 60 g NDF/kg
512 and the BSAS (2003) recommended 70 – 130 g NDF/kg weaner diet. However, as
513 NDF contains both soluble and insoluble NSP, these recommendations are not
514 specific enough to be used for the conceptual development of weaner diet for
515 prevention of PWC, although they are less problematic and costly to measure.
516 Nevertheless and based on the data presented above, it can be theorized that the
517 'ideal' weaner diet should contain highly digestible cereal sources with a minimum
518 amount of soluble NSP and 20 – 100 g insoluble NSP sources such as oat hulls, pure
519 cellulose, barley hulls and wheat bran to minimise risk of PWC. However, as
520 discussed by Pluske et al. (2001), practical weaner diets contains cereals, legumes
521 and/or oilseed meals that contain considerable amounts of both soluble and insoluble
522 NSP. It is estimated that a typical wheat-based weaner diet contains between 70 – 110
523 g NDF and 30 – 50 g ADF/kg. Further difficulties in recommending NSP levels also
524 originates from the diversity of fibre structure and the generic lack of information
525 about NSP fermentability and their ability to increase intestinal viscosity. Published
526 studies used diverse types and levels of NSP, not all of which are "practical"

ingredients (eg, semi-synthetic diet or highly digestible cereal sources), which make comparisons difficult.

Nevertheless, several recent studies using mostly insoluble NSP in the form of a large particle size showed promising results to minimise expression of PWC in commercial weaner diets containing considerable soluble NSP. For example, the study conducted by Molist et al. (2010) used a diet containing corn (320 g/kg), wheat (200 g/kg), barley (170 g/kg) and soybean meal (140 g/kg). If the NSP contents of this basal diet are calculated using typical NSP content reported by Bach Knudsen (1997), the total and soluble NSP contents of the diet are 167 g/kg and 45 g/kg. In this "commercial" weaner diet supplementation of 40 g coarsely prepared wheat bran significantly decreased expression of PWC at 48 and 72 hours (faecal score 1.5 vs. 0.5, $P<0.05$) post-infection with *E. coli* K88, through decreasing *E. coli* K88 population in the ileal digesta and ileal mucosa (See Figure 2). This result may indicate that even though this "commercial" weaner diet contained considerable amounts of soluble NSP, their potentially deleterious effects can be mitigated by addition of coarsely prepared insoluble NSP, which may disperse the viscous network formed by the interaction between soluble NSP and water (Choct, 1997) in the digesta and hence increase digesta flow. This notion is supported by the subsequent finding in this trial that supplementation of coarsely prepared wheat bran significantly decreased proline fermentation by product and microbial density in the GIT (see Figure 2). Further research looking at the effects of particle size of a range of insoluble fibre sources to titrate the effects of insoluble and soluble NSP in commercial weaner diets to minimise expression of PWC is warranted.

4.3. Dietary protein and NSP interaction

A recent study showed that clinical expression of PWC could be dependent on the balance of fermentable carbohydrates and proteins available in the GIT rather than absolute amount of protein or NSP in the digesta (Kim et al., 2008). In this study the authors fed diets based on extruded rice or raw wheat without and with 20 g/kg oat hulls containing 190 – 200 g protein/kg diet. The protein sources were all animal proteins to limit other source of NSP in the diet. The basal diets without oat hulls contained 3 g and 11 g soluble NSP and 9 g and 66 g insoluble NSP/kg diet, respectively for extruded rice and raw wheat-based diets. Interestingly, expression of PWC was higher only in pigs fed an extruded rice-based diet without oat hull

supplementation while the pigs fed a wheat-based diet without oat hulls did not develop PWC. This interaction may possibly indicate that the ratio between fermentable protein and carbohydrates in the GIT could affect the development of PWC. Williams and Gidley (2007) mentioned that fermentation in the GIT is an energy-dependent process and energy is the limiting factor for microbial fermentation. Therefore, if energy sources are depleted then intestinal fermentation becomes increasingly proteolytic. Moreover, more protein in the lower GIT is known to increase saccharo-proteolytic microbes, which primarily gain energy from carbohydrate fermentation when the protein:carbohydrate ratio in the ileal chyme is low, but are able to proliferate and ferment protein to gain energy when there is increased availability of fermentable protein (Roy, 1969; Abe et al., 1995; Nollet et al., 1999). However the papers (Bikker et al., 2006; Jeaurond et al., 2008; Hermes et al., 2009) examined the interactive effect of protein and fermentable carbohydrate levels on gut development and intestinal fermentation showed equivocal results. For example, factorial experiments conducted by Bikker et al. (2006) and Jeaurond et al. (2008) found no interaction and increasing fermentable carbohydrate consistently increased carbohydrate fermentation by-products such as straight-chain volatile fatty acids, while increasing dietary proteins increased protein fermentation by-products such as ammonia, biogenic amines and BCFA. In contrast, Hermes et al. (2009) found an interaction between dietary protein and fibre in the incidence of diarrhoea, number of antibiotic treatment and BCFA production (Table2). The results showed that these measures were increased by supplementing partly fermentable fibres (40 g wheat bran + 20 g sugar beet pulp) in a low protein diet (160 g CP/kg), while supplementation of partly fermentable fibres in a high protein diet (200 g CP/kg) decreased diarrhoea, antibiotic treatment and BCFA production. The increased incidence of diarrhoea in the sugar beet pulp-supplemented low protein diet is an unexpected result given the demonstrated positive effects of sugar beet pulp or wheat bran on pathogen population in the GIT (Bikker et al., 2006; Molist et al., 2009, 2010). However, Hermes et al. (2009) was using rice and barley-based diet and had much lower fibre levels compared with the other studies (Bikker et al., 2006; Jeaurond et al., 2008), and found that the viscosity of colonic digesta was higher in the low protein and high fibre diet compared with the high protein and high fibre diet, which may be the cause of the interaction. Nevertheless, clarification is required and interaction between fermentable

protein and carbohydrate for manipulation of intestinal microflora warrants further investigation in relation to the intestinal barrier function and expression of PWC.

[Table 2 about here]

4.4 Minerals and intestinal barrier function

4.4.1. Zinc oxide

Use of pharmacological levels of zinc oxide (ZnO) in diets for weaner pigs is widely accepted in the pig industry worldwide as a first choice for replacement of in-feed antibiotics due to its proven effects on performance and PWC through inhibiting cAMP-stimulated chloride secretion (Hoque et al., 2004). It has been perceived that pharmacological levels of ZnO may decrease ETEC colonisation and bacterial population in the GIT as ZnO exhibits bactericidal effects *in vitro* (Soderberg, et al., 1990). Interestingly, results from early *in vivo* studies using culture-based techniques indicated that 2,500-3,000 ppm of dietary ZnO did not influence *E. coli* population in the faecal (Jensen-Waern et al., 1998) and ileal digesta (Li et al., 2001). Moreover, a more recent study using 16S rRNA gene sequencing technique demonstrated that 2,500 ppm of ZnO significantly suppressed gram positive commensal microbes such as *Lactobacillus amylovorous, Lactobacillus reuteri,* and *Streptococcus alactolyticus* throughout the GIT but did not inhibit growth of potentially pathogenic gram negative microbes (Hojberg et al., 2005). Also, a recent *in vitro* study which examined *E. coli* K88 growth in tryptic soy broth dilute showed that addition of 250, 2,000, 3,000 and 5,000 ppm of ZnO did not suppress growth of *E. coli* K88 while zinc sulphate and copper sulphate did (Hardy et al., 2003). Accordingly, these data suggest that suppression of PWC and growth promotion effects seen with high levels of ZnO supplementation in diets for weaner pigs are not associated with ETEC elimination in the GIT.

Rather, several studies have underpinned the mechanism of the effect of supplemental ZnO on prevention of PWC and suggest that improved intestinal barrier function and immune function could be attributable. For example, Li et al. (2001) reported that supplementation of 3,000 ppm ZnO increased mucus thickness, villous height, villous width, and villous height:crypt depth ratio, and decreased crypt depth in the small intestine of 33-day-old pigs (weaned at 21 days). In an *in vitro* study using the human Caco-2 enterocyte model, Roselli et al. (2003) showed that exposure

to ETEC increased transcellular and paracellular permeability measured as TEER and ^{14}C inulin transfer, and up-regulated expression of inflammatory cytokins such as IL-8 and tumour necrosis factor-α (TNF-α) in the enterocytes. However, addition of 5 mmol/L ZnO maintained the transcellular and paracellular permeability to the pre-ETEC treatment level. Also, addition of 0.2 mmol/L ZnO significantly reduced ETEC adhesion and reversed the RNA expression levels of inflammatory cytokines. An *in vivo* weaner pig study confirmed part of the *in vitro* study conducted by Roselli et al. (2003). This particular *in vivo* study conducted by Zhang and Guo (2009) used piglets weaned at 24 days and fed either control diet or diets containing 2,000 ppm ZnO or tetrabasic zinc chloride. Both zinc products reduced expression of PWC and decreased paracellular permeability measured as urinary recovery of lactulose and mannitol. This study also analysed mRNA expression of tight junction proteins Occludin and ZO-1 in the ileal mucosa, and found that both zinc products significantly increased expression of these tight junction proteins in the ileal epithelium. Furthermore, an *in vivo* study was conducted using piglets weaned at 21 days with a lipopolysacchride injection model to quantify pathogenic bacterial translocation into the mesenteric lymph nodes (Huang et al., 1999). Pigs fed a ZnO supplemented diet (3,000 ppm) significantly reduced the percentage of pigs having translocated pathogens such as *E. coli* and *Enterococcus* spp. into the small intestinal mesenteric lymph nodes (from 89% to 33%), suggesting reduced paracellular permeability in the ZnO fed pigs.

Such evidence suggests that a high level of dietary ZnO reduces expression of PWC through reducing ETEC adhesion and intestinal permeability rather than manipulating pathogen population in the GIT. Although having proven beneficial effects, supplementation of a pharmacological level of ZnO in diets for weaner pigs has been criticised as increased zinc excretion in the effluent system can cause environmental pollution and some European countries have banned the use of high levels of ZnO in diet for pigs. Recently, microencapsulated zinc oxide was released in the market and the lipid-coated ZnO has been claimed to dramatically decrease inclusion of ZnO from 2,500-3,000 ppm to 100 ppm to achieve the same effects on PWC. A recently completed experiment used 21-day-old weaner pigs and fed either control, control + 3,000 ppm ZnO or control + 100 ppm microencapsulated ZnO diet. Half of the pigs in each treatment group were experimentally infected with β-

haemolytic ETEC (serotype O149:K91;K88) and the expression of PWC and plasma and faecal zinc concentrations were measured. The results showed that inclusion of 100 ppm microencapsulated ZnO suppressed the expression of PWC in both ETEC-infected and non-infected pigs, and kept the plasma and faecal zinc levels to the levels of that found in the pigs fed a control diet (Kim et al., unpublished data, Table 3). Although further research is required to elucidate whether the use of low levels of microencapsulated zinc affects intestinal barrier functions *in vivo*, the technology could be pivotal to reduce expression of PWC by supplementing ZnO in the diets for pigs without compromising environmental issues.

[Table 3 about here]

4.4.2. Iron and phosphorus

The published literature suggests that high levels of dietary iron and phosphorus can either directly compromise intestinal integrity and barrier function, or indirectly increase pathogen susceptibility by stimulating bacterial growth. Iron supplementation is known to increase bacterial proliferation in body fluid and increase infection in guinea pig and rat studies (Bullen et al., 1978). An early pig study showed that intramuscular injection of 400 mg iron dextran on day 3 increased serum iron concentration but had significantly lower serum iron binding capacity on day 14 than pigs injected 100 mg iron dextran (Knight et al., 1984). A human study showed that oral supplementation of 120 mg iron as ferrous fumarate but not 200 mg intravenous iron supplementation as iron sucrose significantly increased clinical disease score in patients with inflammatory bowl disease (Erichsen et al., 2005). A recent Holstein calf study showed that dietary supplementation of 750 mg iron as iron sulphate increased hepatic expression of hepcidin, which is a signalling molecule to reduce iron absorption, and decreased duodenal transcellular and paracellular permeability (Hansen et al., 2010). Also, a recent weaner pig study demonstrated that a higher dietary iron level (100 mg vs 500 mg iron as iron sulphate) caused villous atrophy and significantly decreased duodenal transcellular and paracellular permeability (Stahl, 2009). Stahl (2009) stressed that iron content in the diet for weaner pigs can easily exceed by up to 5-fold the NRC recommendation (NRC 1998) by including blood meal, dicalcium phosphate and limestone in diets, for example. Therefore, it appears

694 that iron levels in diets for weaner pigs should be watched closely and be limited
695 within the recommended level of 100 mg/kg to maintain intestinal barrier function.
696 Dietary phosphorus (P) level is another concern for intestinal bacterial growth
697 as P is the fundamental component for formation of bacterial cell membranes.
698 Miettinen et al. (1997) demonstrated that P concentration in water stimulated
699 heterotrophic microbial growth while other mineral sources such as K, Mg, Ca, Na
700 and Cl did not stimulate growth of such microbes. The study suggested filtration of P
701 to reduce water P level for prevention of heterotrophic microbial growth in drinking
702 water. Metzler et al. (2008) conducted an interesting study that fed either a low-P diet
703 (3 g P/kg), a high-P diet (7 gP/kg), and a low-P plus 1,000 FTU phytase/kg diet, to
704 30-kg ileal T-cannulated pigs. The study found that increasing dietary P increased
705 bacterial P assimilation while use of phytase in the low-P diet decreased bacterial P
706 assimilation in the ileal and faecal samples. The authors concluded that reducing
707 intestinal availability of P for bacteria could reduce bacterial activity in the GIT of
708 pigs. A follow up study demonstrated that increased intestinal calcium (Ca)
709 availability decreased the number of gram-positive bacteria, whilst increased small
710 intestinal P availability stimulated growth of strictly anaerobic bacteria such as the
711 *Clostridium coccoides* cluster, *Clostridium leptum* cluster, *Bacteroides-Prevotella-*
712 *Porphyrmonas* group (Metzler-Zebeil et al., 2010). These data suggest that P is an
713 important element for stimulation of certain bacterial growth in the pig's GIT and
714 further study is warranted to clarify whether the intestinal P availability is associated
715 with GIT barrier function in weaner pigs.
716
717 **5. Conclusion**
718 Pigs at weaning are exposed to nutritional, immunological and psychological
719 stressors and consequences of the exposure are damage in intestinal architecture and
720 reduction in intestinal barrier functions. These events at weaning greatly increase
721 susceptibility of the GIT to pathogens and many pathogen related diseases such as
722 PWC are commonly occur in commercial production system. Evidence presented in
723 this review indicates that such pathogen-originated diseases are closely associated
724 with dietary components and intestinal barrier functions can be maintained through
725 manipulation of dietary protein, NSP and mineral levels. Especially, the use of a
726 reduced protein diet for at least 7 days immediately after weaning, limitation of
727 viscosity-increasing soluble NSP content while including 20 – 80 g/kg insoluble NSP

source in the diet, and limitation of iron to 100 mg/kg are important dietary strategies to maintain intestinal barrier function and to minimise PWC. Further research to elucidate roles of available P, protein and NSP interaction, type and level of NSP on intestinal barrier function and development of enteric disease in weaner pigs are warranted.

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Table 1. Effect of dietary protein level (PL), experimental ETEC (serotype O149;K91;K88) infection (I) and duration of feeding after weaning (T) on apparent ileal digestibility (AID) of N, ileal N flow, PUN, ammonia-N contents in the gastrointestinal tract (GIT) and incidence of post-weaning diarrhoea (PWD) (after Heo et al., 2010)

Item	Non-Infected				Infected				SEM[1]	P-value[2,3]		
	HP		LP		HP		LP			PL	I	T
	d 7	d 14	d 7	d 14	d 7	d 14	d 7	d 14				
n=	6	6	6	6	6	6	6	6				
Total dietary N intake[4], g/d	53	118	43	95	51	122	41	97	4.7	***	NS	***
AID, %	56	65	53	62	47	60	46	58	1.5	NS	*	***
Ileal dietary origin-N flow[5], g/d	26	41	23	36	32	45	27	40	1.6	*	*	***
PUN, mmol/L	5.8	4.8	2.4	1.4	4.7	5.6	2.0	1.5	0.15	***	NS	NS
NH_3-N, mg/kg												
Ileum	47	57	32	38	55	65	35	43	1.9	***	*	***
Cecum	113	199	77	122	183	248	125	176	12.4	**	*	**
Proximal colon	273	350	186	233	362	406	246	289	11.5	***	***	***
Distal colon	318	413	271	318	389	449	330	387	13.2	**	*	**
Rectum	376	452	327	385	429	489	390	412	12.3	*	*	*
PWD[6], %	1.2^{a}	0.0^{a}	0.0^{a}	2.4^{a}	7.1^{c}	3.6^{b}	1.2^{a}	1.2^{a}	0.44	*	**	NS

[1]Pooled standard error of mean

[2]Significance level: NS: Not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

[3]2- or 3-way interactions were not significant ($P > 0.05$)

[4]Total dietary N intake was calculated based on ADFI and PL

[5]Ileal N flow of dietary origin was calculated based on daily N intake and the apparent ileal N digestibility

[6]PWD is expressed as the mean percentage of days with diarrhea relative to the total 14 days after weaning. PL x I and PL x T interactions were significant at $P<0.05$.

Abbreviations are; HP = high protein (239 g CP/kg), LP = low protein diet (190 g CP/kg).

Table 2. Interactive effect of dietary protein content and dietary fibre on the incidence of diarrhoea, number of antibiotic treatments and viscosity and volatile fatty acid content in the colon of young pigs (Adapted from Hermes et al., 2009).

	160 g crude protein/kg		200 g crude protein/kg			P-value[3]		
Item	Low fibre[1]	High fibre[1]	Low fibre	High fibre	SEM	Protein	Fibre	Interaction
n=	8	8	8	8				
Diarrhoea incidence	1.5	2.6	1.8	1.1	1.4	NS	NS	†
Number of antibiotic treatment	2.25	6.38	2.75	1.88	2.81	†	NS	*
Colon content								
Viscosity, log of cP, SR11	2.8	3.1	3.1	2.6	0.44	NS	NS	*
Total short chain fatty acid, mmol/L	126	147	146	155	20.2	†	*	NS
Acetic acid, %[2]	51.2	55.5	53.0	63.4	4.47	NS	NS	NS
Propionic acid, %[2]	30.8	27.6	31.7	26.5	4.29	NS	*	NS
Butyric acid, %[2]	12.3	11.7	10.5	14.2	3.49	NS	NS	†
Branched-chain fatty acid, %[2]	0.7	1.1	0.9	0.6	0.31	NS	NS	**

[1]Low fibre: 5.3% NDF, High fibre: 7.15% NDF.

[2]Proportion of total short chain fatty acids.

3Significance level: NS: Not significant, † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$.

Table 3. Effects of feeding diets containing either no ZnO (control), 3,000 ppm ZnO (ZnO) or 100 ppm microencapsulated ZnO (ME-ZnO) to 21-day-old pigs challenge with enterotoxigenic E. coli (ETEC) on the expression of post-weaning colibacillosis and plasma and faecal zinc concentration (Kim et al., unpublished data)[1]

	No ETEC challenge			ETEC challenge				P-value[3]		
	Control	ZnO	ME-ZnO[2]	Control	ZnO	ME-ZnO	SEM	Diet	ETEC	Diet x ETEC
n=	12	12	12	12	12	12				
Diarrhoea index[4]	13.1	0.0	6.0	16.1	5.4	3.4	4.8	***	NS	NS
Days with therapeutic antibiotic treatment[5]	1.8	0.0	0.8	2.3	0.7	0.5	0.41	***	NS	NS
Plasma zinc, mg/L[6]	0.73	2.48	0.65	0.64	2.28	0.83	0.146	***	NS	NS
Faecal zinc, g/kg[6]	1687	13961	2694	1787	14329	2209	412.0	***	NS	NS

[1] Pigs in infection group experimentally were infected with β-haemolytic ETEC (serotype O149:K91;K88) on day 3 post-weaning by orally drenching 10 mL of ETEC (2×10^8 cfu/mL) solution.

[2]Shield Zinc®, Zamira Life Science Ltd., Victoria, Australia.

[3]Significance level: NS: Not significant, *** $P < 0.001$

[4]Expressed as the mean percentage of days with diarrhea relative to the total 14 days post-weaning.

[5]Mean days with therapeutic antibiotic injection to treat diarrhoea during 14 days post-weaning.

[6]Measure at 14 days post-weaning.

Figure 1. Relationship between soluble non-starch polysaccharides and viable small intestinal count of haemolytic ETEC. (Re-drawn from Hopwood et al., 2006)

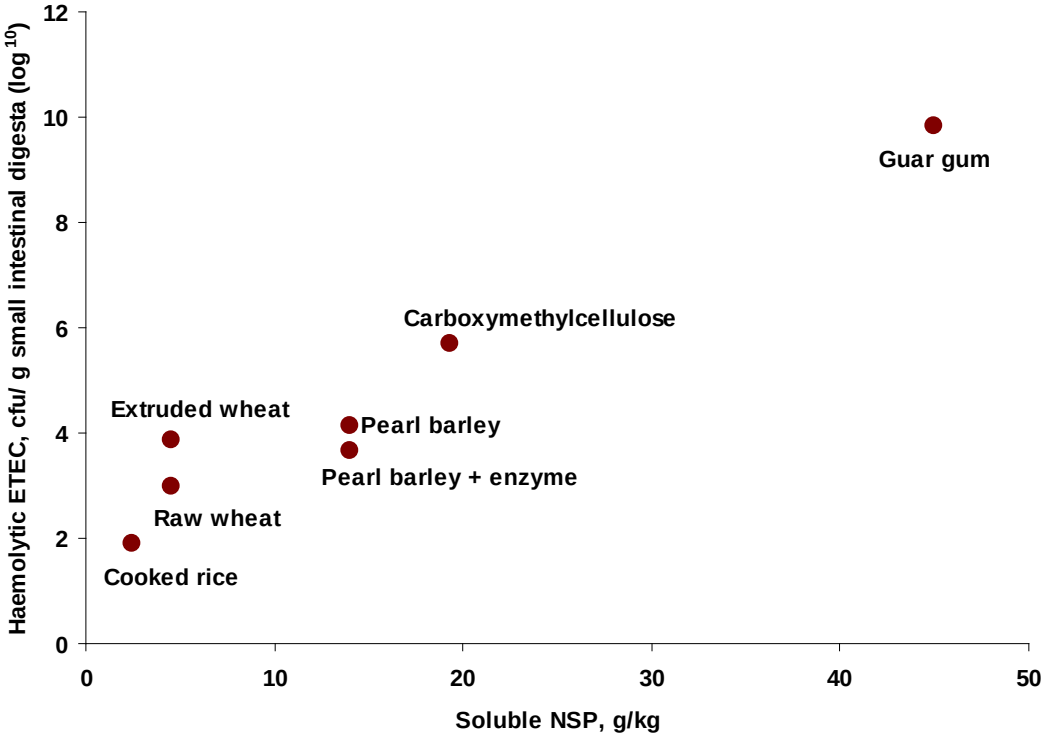
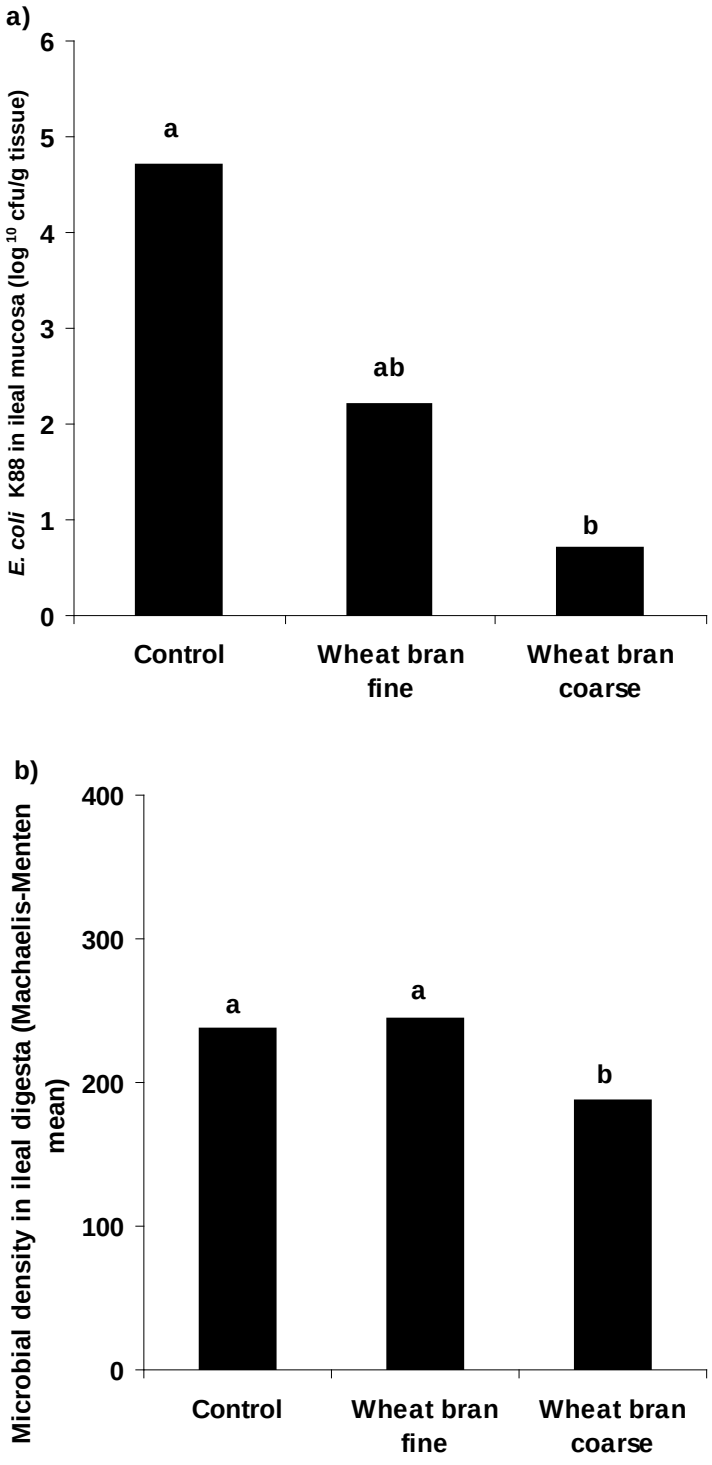
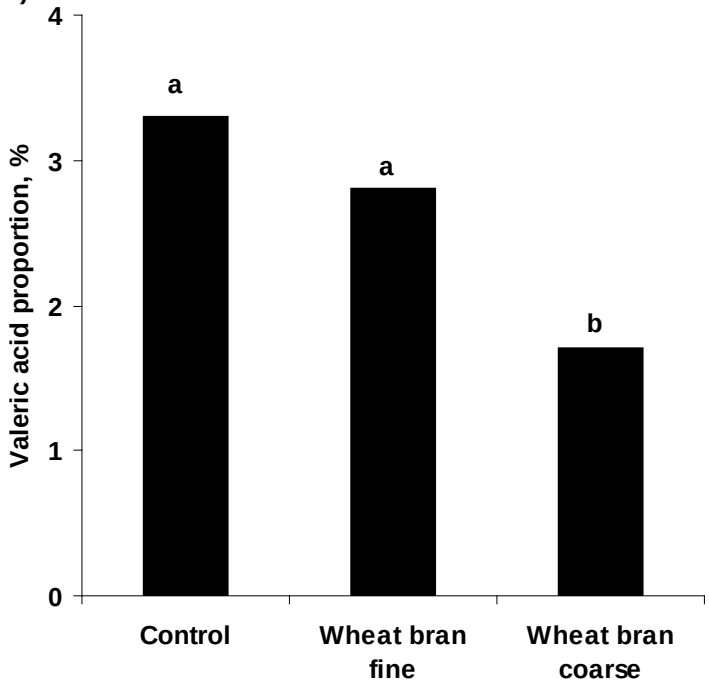


Figure 2. Inclusion of coarsely prepared 40 g wheat bran/kg in a diet for weaner pigs challenged with *E. coli* K88 decreased (a) ileal mucosa-bound *E. coli* K88, (b) microbial density in the ileal digesta, and (c) proportion of valeric acid in the faecal samples (Extracted from Molist et al., 2010). Menten mean (Machaelis-Menten mean) is an estimator of microbial richness



c)


Valeric acid proportion, %
4
3
2
1
0
a
a
b
Control
Wheat bran fine
Wheat bran coarse