Animal (2013), **7**:7, pp 1067–1078 © The Animal Consortium 2013
doi:10.1017/S1751731113000062

animal

Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review

V. T. S. Rist, E. Weiss, M. Eklund and R. Mosenthin†

Institute of Animal Nutrition, University of Hohenheim, 70593 Stuttgart, Germany

(Received 4 July 2012; Accepted 10 December 2012; First published online 15 February 2013)

In pigs, the microbial ecosystem of the gastrointestinal tract (GIT) is influenced by various factors; however, variations in diet composition have been identified as one of the most important determinants. Marked changes in fermentation activities and microbial ecology may occur when altering the diet, for example, from milk to solid feed during weaning. In that way, access of pathogens to the disturbed ecosystem is alleviated, leading to infectious diseases and diarrhea. Thus, there is increasing interest in improving intestinal health by use of dietary ingredients suitable to beneficially affect the microbial composition and activity. For example, fermentable carbohydrates have been shown to promote growth of beneficial Lactobacillus *species and bifidobacteria, thereby enhancing colonization resistance against potential pathogens or production of short-chain fatty acids, which can be used as energy source for epithelial cells. On the other hand, fermentation of protein results in the production of various potentially toxic products, such as amines and* NH_3*, and is often associated with growth of potential pathogens. In that way, excessive protein intake has been shown to stimulate the growth of potentially pathogenic species such as* Clostridium perfringens*, and to reduce fecal counts of beneficial bifidobacteria. Therefore, it seems to be a promising approach to support growth and metabolic activity of the beneficial microbiota by developing suitable feeding strategies. For example, a reduction of dietary CP content and, at the same time, dietary supplementation with fermentable carbohydrates have proven to successfully suppress protein fermentation. In addition, the intestinal microbiota seems to be sensible to variations in dietary protein source, such as the use of highly digestible protein sources may reduce growth of protein-fermenting and potentially pathogenic species. The objective of the present review is to assess the impact of dietary protein on microbiota composition and activity in the GIT of piglets. Attention will be given to studies designed to determine the effect of variations in total protein supply, protein source and supplementation of fermentable carbohydrates to the diet on composition and metabolic activity of the intestinal microbiota.*

Keywords: intestinal microbiota, protein fermentation, piglet

Implications

Protein fermentation, as a result of excessive supply of dietary protein to piglets' intestinal microbiota, could be regarded as one factor for the development of enteric diseases. Although some results are inconsistent, it appears that a lower amount of protein reaching the lower gastrointestinal tract (GIT) may reduce the incidence of diarrhea and inhibit proliferation of pathogenic bacteria especially in stress situations, such as a high pressure of infections. Furthermore, dietary inclusion of fermentable carbohydrates shows the potential to reduce detrimental protein fermentation and growth of potential pathogens.

† E-mail: rainer.mosenthin@uni-hohenheim.de

Introduction

The GIT of mature pigs harbors a quite stable microbiota, mainly consisting of beneficial members such as lactobacilli but also potential pathogenic bacteria, for example, *Escherichia coli* (Gaskins, 2001). However, there is a critical period around weaning because of the transition from liquid (sow milk) to solid feed, accompanied by morphological, histological, physiological and microbial changes in the GIT of young mammals (Pluske *et al.*, 1997), often in addition to environmental and social stress on the animal (Etheridge *et al.*, 1984). This period is often associated with a high incidence of post-weaning diarrhea (PWD) caused by potential pathogens such as *E. coli* (Hampson, 1994). Until 2006, in-feed antibiotics have been frequently used in pig production to suppress gastrointestinal

proliferation of pathogenic bacterial strains, thereby reducing the incidence of enteric diseases during the weaning period. Nowadays, it is increasingly being realized that GIT health is largely influenced by the composition of the microbial community and diverse end-products of bacterial metabolism (Klose *et al*., 2010). In this context, diet composition has been identified as a major factor being responsible for differences in composition and function of the microbiota harboring the GIT of pigs (Mosenthin *et al*., 1999). Thus, nutritional strategies to modulate the intestinal microbial ecosystem appear to be a useful tool to improve GIT health. For example, enhancing growth and metabolic activity of beneficial members of the gastrointestinal microbiota and therefore promoting a stable GIT ecosystem by specific dietary ingredients provides one major option (Bauer *et al*., 2006). Accordingly, fermentable carbohydrates such as different types of resistant starch, non-starch polysaccharides (NSP) and nondigestible oligosaccharides have been shown to enhance proliferation of beneficial bacteria in the small intestine (Bikker *et al*., 2007). As a result, there was an increased production of straight-chain short-chain fatty acids (SCFA) that may be used as energy source by the host (Gaskins, 2001), but which are also known to exert selective antimicrobial effects against certain pathogens (Dongowski *et al*., 2002). On the other hand, excessive fermentation of dietary protein should be avoided because of the production of detrimental substances, including ammonia, amines and phenols (Jensen, 2001). These substances have been associated with various detrimental effects in the GIT of the host animal, such as cytopathic effects on epithelial cells (e.g. Gaskins, 2001). In addition, fermentation of protein often coincides with the growth of potential pathogens, such as *Bacteroides* and *Clostridium* species, thereby increasing the risk for infectious diseases (Macfarlane and Macfarlane, 1995). Depending on the source of protein in the diet but also the total amount of dietary protein fed to the animal, the availability of fermentable protein for the gastrointestinal microbiota may vary, thereby furnishing different amounts of substrate for bacterial proliferation and metabolic activity (Etheridge *et al*., 1984). Thus, by selecting feed ingredients containing protein of rather high ileal digestibility, a surplus of protein reaching the large intestine and serving there as substrate for protein-fermenting bacteria, could be avoided (Pluske *et al*., 2002). In that way, both the proliferation of protein-fermenting potential pathogens and the production of detrimental metabolites of protein fermentation can be suppressed. On the other hand, reducing the amount of dietary protein reaching the large intestine by lowering the total supply of dietary protein to the pig has also been described as an alternative option to avoid excessive protein fermentation (Heo *et al*., 2008). It is acknowledged that this approach potentially contributes to improvements in pigs' health conditions and growth performance (Williams *et al*., 2005); however, the implementation of this strategy is limited by the animals' minimum requirement for protein and amino acids (AA; Nyachoti *et al*., 2006).

There is currently considerable interest in scrutinizing the potentials and limitations of different strategies to minimize detrimental protein fermentation and the number of protein-fermenting bacteria in the GIT of pigs (e.g. Heo *et al*., 2008; de Lange *et al*., 2010). The main focus of the present review is directed to the discussion and comparative evaluation of these strategies.

The microbial ecosystem of the GIT: composition and interactions with the host

The GIT of monogastric animals including humans and pigs harbors a complex, numerically dense and metabolically active community of microbes (Macfarlane and Macfarlane, 2007). In pigs, the gastrointestinal microbiota is primarily composed of not only Gram-positive bacteria, such as aerotolerant *Streptococcus*, microaerobe or obligate anaerobe *Lactobacillus*, *Bifidobacterium*, obligate anaerobe *Peptostreptococcus*, *Clostridium* and *Ruminococcus*, facultative anaerobe *Escherichia*, but also obligate anaerobe Gram-negative bacteria such as *Fusobacterium*, *Bacteroides*, *Selenomonas*, *Butyrivibrio* and *Prevotella.* Detailed reports on the composition of the pigs' gastrointestinal microbiota have been published elsewhere (e.g. Gaskins, 2001). Both, the relative proportion of species and the absolute quantity of bacteria, vary considerably along the digestive tract (Savage, 1977), with bacterial densities generally increasing from the proximal to the distal parts of the GIT (Gaskins 2001; Jensen, 2001). In contrast to the human GIT, the pigs' GIT also harbors a substantial number of indigenous bacteria, mainly lactobacilli, in proximal sections, that is, in the stomach (Conway, 1994) and small intestine (Jensen and Jørgensen, 1994). In addition to a proximal to distal gradient in bacterial density within the GIT of pigs, there is also a radial distribution of microbes within each segment of the gut. In total, there are four microhabitats colonized by the commensal microbiota, which include the gastrointestinal lumen, the unstirred mucus layer, the deep mucus in the crypts and the surface of the intestinal epithelial cells (Pluske *et al*., 2002). Consequently, the microbiota present in digesta and feces is not fully representative for the entire GIT microbiota, even though digesta will likely contain microbes from mucosa that have been shed into the digesta as a result of mucosal sloughing or release of attached microbes (Richards *et al*., 2005).

There exist mutual interactions between host organism and gastrointestinal microbiota. This has been shown for different species, for example, men, rats (Collinder *et al*., 2003) and pigs (Hopwood and Hampson, 2003). For example, the composition and activity of the gastrointestinal microbiota is influenced by immune and stress status of the host, pathogen pressure originating from the environment and competition for substrates of endogenous and exogenous origin, such as carbohydrates, AA or minerals (Stewart *et al*., 1993). Moreover, the presence of local defense systems such as specific immune response, as well as gut receptors to which certain bacteria may attach, will also affect the prevailing microbial community (Stewart *et al*., 1993). On the other hand, the gastrointestinal microbiota supports the host animal through immune modulation (Fanning *et al*., 2012), colonization

resistance against pathogens via competition for nutrients and adhesion sites or production of antimicrobial substances (Rolfe, 1997). According to an *in vitro* study by Roselli *et al.* (2007) using porcine intestinal epithelial cells, some lactobacilli strains are able to exert protective effects against damages of the epithelial barrier induced by enterotoxigenic *E. coli*. Furthermore, the gastrointestinal microbiota provides nutrients to the host, for example, by production of straight-chain SCFA, which are rapidly absorbed from the large intestine as energy source (Gaskins, 2001). There are also metabolic costs to the host because of bacterial degradation of dietary or endogenous protein resulting in lower availability of AA and energy for maintenance and protein synthesis (Columbus *et al.*, 2010). Furthermore, bacteria can have a direct impact on the genetic programming of the GIT (Kelly and King, 2001) such as gene expression of epithelial cells, for example, of genes coding for brush border digestive enzymes (Willing and van Kessel, 2009).

Carbohydrate fermentation

Fermentable carbohydrates reaching the GIT are the major source of energy for the gastrointestinal microbiota. They consist of different types of resistant starch, NSP, nondigestible oligosaccharides, such as fructo- or galactooligosaccharides, but also inulin (Houdijk *et al.*, 2002). Fermentation of carbohydrates is considered to be most pronounced in the proximal colon where higher concentrations of SCFA and a more acidic pH have been determined (Williams *et al.*, 2005). However, certain carbohydrates including starch, some soluble β-glucans, oligosaccharides and short-chain inulin may also be fermented in the small intestine. The saccharolytic activity of the gastrointestinal microbiota is considered to be beneficial for the host (Geboes *et al.*, 2006) because of the formation of various straight-chain SCFA including acetic, propionic and butyric acid. In addition, different fermentation gases (H_2, CO_2 and methane) are being produced (Varel and Yen, 1997). The trophic effect of SCFA on the intestinal epithelium has been reviewed elsewhere (e.g. Bauer *et al.*, 2006). Moreover, SCFA create a slightly acidic environment (Unger and Viernstein, 2004), thereby preventing the growth of acid-sensitive detrimental bacteria, including pathogenic *Salmonella* and *E. coli* (Wells *et al.*, 2005). SCFA are rapidly absorbed from the hindgut and may provide up to 30% of the maintenance energy requirements for growing pigs (Morita *et al.*, 2004). The promotion of specific bacterial groups or strains with beneficial properties for the host is mainly directed to the proliferation of *Lactobacillus* and *Bifidobacterium*, also referred to as probiotic bacteria. These genera are known for playing an important role in inhibiting the establishment of pathogenic bacteria via a mechanism known as competitive exclusion, also described as colonization resistance, for example, by better competing for nutrients or adhesion sites (Van der Waaij *et al.*, 1991). However, it has to be considered that the microbiota of the porcine GIT appears to be highly individual (Loh *et al.*, 2006), and that in the intestinal contents of about 60% of the assessed pigs no bifidobacteria were detectable (e.g. Loh *et al.*, 2006). However, according to Loh *et al.* (2006), the role of bifidobacteria in the GIT of pigs is underestimated, maybe because of the analytical detection methods, such as fluorescent *in situ* hybridization, where the detection limit is comparably high, which may partly explain the low number of bifidobacteria.

Furthermore, lactic acid, as produced during carbohydrate fermentation by many intestinal bacteria, such as lactobacilli, bifidobacteria or enterococci, is utilized in the GIT of pigs by *Megasphera*, *Selenomonas* and *Veillonella*, resulting in the formation of acetate, propionate but also butyrate (Duncan *et al.*, 2004), the preferred energy source of colonocytes (Loh *et al.*, 2006). *Eubacterium* spp. and *Clostridium* spp. also seem to be able to utilize lactate, but need extracellular acetate to produce butyrate (Ushida *et al.*, 2002). This cross-feeding between intestinal bacteria plays an important role in preventing intestinal diseases, otherwise lactate may assimilate in the large intestine and cause diarrhea because of dyspepsia (Ushida *et al.*, 2002).

Protein fermentation

In addition to carbohydrates, protein both from exogenous and endogenous sources can be used as a fermentable substrate by the gastrointestinal microbiota (Macfarlane and Macfarlane, 1995). Although for the pig, a considerable part of the carbohydrate fraction is fermented in the proximal parts of the GIT, protein fermentation takes place more distally, in particular, when there are less fermentable carbohydrates available (Williams *et al.*, 2005). However, as protein is present throughout the whole GIT in the form of nondegradable and endogenous sources (Williams *et al.*, 2005), some fermentation may also occur in the small intestine, mainly of AA and peptides. The degradation of protein results – unlike the fermentation of carbohydrates – in a number of additional metabolites such as branched-chain fatty acids (BCFA), but also potentially toxic products including ammonia, amines, phenols and indoles are being formed (Williams *et al.*, 2005; Bikker *et al.*, 2007). The BCFA originate from the deamination of branched-chain AA such as valine, isoleucine and leucine, and include iso-butyrate, 2-methyl-butyrate and iso-valerate (Macfarlane and Macfarlane, 1995). These substances are formed by many bacteria such as *Bacteroides* spp., *Propionibacterium* spp., *Streptococcus* and *Clostridium* species. For example, BCFA are produced in large amounts by clostridia via the Stickland reaction (Macfarlane and Macfarlane, 1995). This chemical reaction involves the coupled oxidation and reduction of AA to organic acids. Thus, intestinal concentrations of BCFA may be used as indicator for the extent of protein fermentation (Macfarlane *et al.*, 1992).

Ammonia. Ammonia present in the GIT of pigs is a toxic catabolite of microbial AA deamination, or it originates from urea hydrolysis due to the action of bacterial urease (Mosenthin *et al.*, 1992). Ammonia is rapidly absorbed into the portal blood, converted to urea in the liver and subsequently excreted in the urine. A second pathway is that ammonia is assimilated by bacteria to synthesize bacterial

protein, provided that fermentable carbohydrates are available to be used as energy source. Such a shift of urinary to fecal nitrogen excretion may help to reduce ammonia emission (Mosenthin *et al*., 1992). Ammonia is one of the major odor components of pig manure contributing to environmental pollution (Otto *et al*., 2003). The concentration of ammonia in the large intestine depends on the balance between AA deamination and bacterial protein synthesis (Mosenthin *et al*., 1992; Hughes *et al*., 2000). Therefore, a reduction of ammonia emission from pig manure seems to be possible by lowering dietary protein (Otto *et al*., 2003).

Amines. A number of gastrointestinal bacteria such as *Bacteroides*, clostridia and bifidobacteria but also enterobacteria, lactobacilli and streptococci are capable of producing amines through decarboxylation of AA. Therefore, high levels of amines in pigs' digesta and feces eventually indicate an increased activity of these bacteria in the GIT (Gaskins, 2001). Amines include monoamines such as tyramine, dimethylamine but also polyamines including cadaverine, agmatine, histamine, spermidine, putrescine and spermine (Pietrzak *et al*., 2002). As was reviewed by Pietrzak *et al*. (2002), they may exert varying physiological effects on the host. For example, some polyamines, such as spermidine and spermine, have been shown to be essential for somatic cell growth. On the other hand, above certain threshold levels, amines present in the GIT may produce detrimental effects such as increasing the incidence of diarrhea (Pietrzak *et al*., 2002). In humans, amines are rapidly absorbed from the colon, and are either detoxified by mono- and diamine oxidases in the gut mucosa or liver, or excreted via urine (Hughes *et al*., 2000). These findings have been confirmed for histamine in pig's colon by Aschenbach *et al*. (2006).

Phenolic compounds. Phenolic and indolic compounds are microbially produced from the aromatic AA phenylalanine, tyrosine and tryptophan. Major genera that have been identified to break down aromatic AA are *Bacteroides*, clostridia and bifidobacteria (Macfarlane and Macfarlane, 1995). In the healthy GIT of humans, phenolic compounds are absorbed in the colon. Thereafter, they are detoxified by means of glucuronide and sulfate conjugation in colonic mucosa or liver and excreted via urine, principally as *p*-cresols (Hughes *et al*., 2000). A positive correlation between dietary protein intake and urinary phenol excretion has been reported in pigs (Otto *et al*., 2003).

Impact of dietary protein on the incidence of PWD

Abrupt weaning of young piglets is characterized by a sudden transit from a milk-based to a solid feed-based diet (Kluess *et al*., 2010). As a result, a marked decrease in voluntary feed intake may occur before the piglets adapt to solid feed (e.g. Hopwood and Hampson, 2003). Changes in feeding behavior and diet composition are also reflected in considerable alterations in the composition of the gastrointestinal microbiota adapting to the new environment (Kluess *et al*., 2010). This period is often associated with a growth check because of a high incidence of gastrointestinal disorders such as PWD (Aherne *et al*., 1992). Commonly, PWD is characterized by an increase in microbial fermentation of undigested protein, resulting in the formation of watery feces in combination with growth impairment, morbidity and even mortality (Wellock *et al*., 2008a). Some studies have been conducted to assess the detrimental effect of protein fermentation on piglets' GIT health. For example, Heo *et al*. (2008) reported elevated plasma urea levels, together with a higher incidence of PWD when feeding a high- (243 g/kg) compared with a low- (173 g/kg) CP diet to weaned piglets. Similarly, Bikker *et al*. (2006) determined higher ammonia concentrations in the small intestine of weaned piglets fed a high- (216 g/kg) compared with a low- (152 g/kg) CP diet. They concluded that protein fermentation was reduced because of the lower supply of dietary protein; however, there was no effect on intestinal morphology, such as villus height and crypt depth. In general, an increase of ammonia concentration is assumed to exert detrimental effects on the health of the GIT, as ammonia can negatively affect growth and differentiation of intestinal epithelial cells (Gaskins, 2001), and may cause disturbances of the intestinal microbial balance during weaning (Bertschinger *et al*., 1979).

It appears that fermentation of protein is often linked to the growth of potential pathogenic bacteria (Ball and Aherne, 1987), such as β-hemolytic enterotoxigenic strains of *E. coli* (ETEC; Heo *et al*., 2008), but rather indirect. They may proliferate in the small intestine and release enterotoxins (Hampson, 1994). However, during weaning, the initially predominant lactobacilli are decreasing; therefore, their beneficial metabolic activities such as the stimulation of GIT immunity and the formation of SCFA are suppressed (Jensen, 1998). As a consequence of these cascading effects, proliferation of *E. coli* is more facilitated. In addition, diets with a high-CP content have a high buffering capacity (Partanen and Mroz, 1999) and will increase small intestinal pH, thereby favoring the proliferation of pathogenic bacteria (Htoo *et al*., 2007). Therefore, *E. coli* has been suggested to be an opportunist rather than a primary pathogen (Li *et al*., 1990).

Strategies to reduce protein fermentation and potential pathogenic proteolytic bacteria in the GIT

Principally, dietary protein becomes available for bacterial fermentation when it escapes digestion by host enzymes (Libao-Mercado *et al*., 2009). Consequently, with decreasing protein digestibility and/or increasing supply of dietary protein to the pig, more protein will become available to the microbiota in the large intestine. This may lead to an increase in protein fermentation, possibly associated with an enhanced proliferation of protein-fermenting bacteria (Libao-Mercado *et al*., 2009). The source of protein, its quality and level in the diet may have an influence on the site of fermentation within the GIT. It is generally accepted that

highly digestible protein sources such as casein are digested by host enzymes in the proximal GIT. As a result, they are not available for microbial fermentation (Pluske *et al*., 2002). On the other hand, plant protein sources are usually not completely digested by host enzymes, which make them available for microbial fermentation more distally, especially at higher dietary protein levels. According to Rist *et al.* (unpublished data), no effects of protein source (i.e. soybean meal (SBM) *v.* casein) on bifidobacteria, lactobacilli and clostridia could be observed in ileal digesta of weaned piglets, whereas in feces these bacterial groups were increased for piglets fed the SBM-based diet. This effect was even more pronounced when piglets were fed diets with elevated protein levels, suggesting increased availability of nutrients to the large intestinal microbiota. It has to be acknowledged that plant protein sources such as SBM also contain relatively high amounts of fermentable carbohydrates. As a result, with any increase in dietary inclusion level of plant proteins, a greater part of these carbohydrates will pass into the distal parts of the GIT. In addition, protein quality may have an influence on the site of fermentation. As determined in humans by Scott *et al*. (2012), heat-damaged pea protein is less digestible by host enzymes in the small intestine and is therefore reaching the more distal parts of the GIT where protein fermentation dominates. In conclusion, the amount and composition of fermentation metabolites in the different sites of the GIT, mainly in the large intestine, depend on the digestibility of the dietary proteins, which in turn is influenced by the quality, source and level of dietary protein (Windey *et al*., 2012). Thus, in the following, dietary strategies will be described, aiming to reduce protein fermentation through reduction of growth and metabolic activities of potential protein-fermenting pathogens.

Source of dietary protein

In piglets fed highly digestible protein, for example, casein, the protein is almost completely digested and absorbed before reaching the large intestine (Morita *et al*., 2004). Therefore, it has been suggested that diets formulated to contain highly digestible protein ingredients would be appropriate to reduce the amount of substrate available for protein fermentation, thereby reducing the frequency of PWD in piglets (Pluske *et al*., 2002). In several studies on pigs, the effect of different dietary protein sources on intestinal protein fermentation including formation of detrimental metabolites, such as ammonia or phenols, has been described (e.g. Blasco *et al*., 2005). In addition, the effect of source of dietary protein on the incidence of protein fermentation-induced diarrhea has been investigated, however, with controversial results. For example, in a challenge study by Owusu-Asiedu *et al*. (2003), piglets were orally infected with ETEC K88 and either fed a diet based on plant protein (pea protein) or animal protein (spray-dried porcine plasma). Piglets fed the pea protein diet had a higher incidence of diarrhea, and mortality was increased compared with piglets fed the plasma diet. In contrast, Wellock *et al*. (2006) observed no different effects of a plant protein source (SBM) in comparison with an animal protein source (dried skim milk powder) on fecal scores of piglets. It can be speculated whether these differences in response to plant and animal protein have been biased by the challenge situation in the aforementioned study by Owusu-Asiedu *et al*. (2003). Another factor that affects fecal consistency, but not mentioned in the study by Owusu-Asiedu *et al*. (2003), may be the temporal hypersensitivity against soy proteins as determined by Li *et al*. (1990). In the study of Li *et al*. (1990), piglets fed a SBM-based diet had shorter villi and a higher immunoglobulin level than piglets fed a dried skim milk-based diet, indicating the antigenic property of SBM. According to Li *et al*. (1990), the formation of complexes between antigens present in the SBM such as glycinin and β-conglycinin and immunoglobulin antibodies may be responsible for the damage to villi. Furthermore, plant protein sources often contain anti-nutritive factors including elevated levels of soluble NSP present in SBM that may increase the incidence of diarrhea in weaned piglets (Choct *et al*., 2010).

As increased intestinal protein fermentation suggests proliferation of potential pathogens (Hughes *et al*., 2000), several studies were designed to examine the effect of dietary protein source on composition (Table 1) of the intestinal microbiota. For example, in a study by Etheridge *et al*. (1984), piglets at 7 days of age were fed either a corn–SBM starter diet or an oat–casein diet or they remained with the sow (control treatment). The control animals were weaned at 21 days of age. At this time, piglets fed the corn–SBM diet showed a lower growth performance associated with a higher incidence of diarrhea than the other treatments. According to the authors, the microbial breakdown of undigested proteins in the lower digestive tract might have been responsible for the observed growth check. In addition, although this was not within the scope of these studies, the occurrence of immune reactions against dietary antigens such as soy protein has to be considered as a possible factor that may negatively affect fecal consistency (Li *et al*., 1990). However, the observed lower growth performance and higher incidence of diarrhea for the corn–SBM group did not correspond to fecal coliform counts, which did not differ from those of the control treatment, but were highest for the oat–casein treatment. In addition, in this study, the counts of total bacteria were higher in piglets fed the corn–SBM or oat–casein diet between days 23 and 27 of age, compared with the control treatment (Etheridge *et al*., 1984). Higher fecal counts of total bacteria might reflect an increase in microbial diversity, which would rather be associated with enhanced stability of the microbial community and therefore an increased resistance to diseases (Konstantinov *et al*., 2004). Kellogg *et al*. (1964) also observed differences in fecal microbial counts in newly weaned piglets as influenced by the protein source in the diet (SBM, dried skim milk, fish meal, meat meal or cottonseed meal). In this study, the plant protein sources resulted in a marked reduction in fecal counts of potential pathogenic coliforms and also of staphylococci. This observation suggests a reduced activity of the proteolytic microbiota upon feeding plant protein sources to newly

Table 1 *Effect of dietary protein source on the composition of the intestinal and fecal microbiota of weaned piglets*[1]

Protein sources	Sampling site	Response	References
SBM[4]	Feces	Total bacteria ↑; yeast and molds ↓[2]	Etheridge *et al.* (1984)
Casein		Total bacteria ↑; yeast and molds ↓; coliforms ↑[2]	
SBM	Feces	Lactobacilli, total aerobes, total anaerobes, streptococci ↑; coliforms, staphylococci ↓[2]	Kellogg *et al.* (1964)
Dried skimmed milk		Lactobacilli, total aerobes, total anaerobes, streptococci ↑; coliforms, staphylococci ↓[2]	
Fish meal		Lactobacilli, total anaerobes, streptococci ↑; coliforms, staphylococci, total aerobes ↓[2]	
Meat meal		Lactobacilli, total aerobes, total anaerobes, coliforms, staphylococci ↓; streptococci ↑[2]	
Cottonseed meal		Lactobacilli, total aerobes, total anaerobes, coliforms, staphylococci ↓; streptococci↑[2]	
Soybean protein isolate	Feces	Staphylococci ↑; coliforms, lactobacilli, total anaerobes, streptococci ↓; total aerobes ↑[2]	Kellogg *et al.* (1964)
Casein		Staphylococci ↓; coliforms, lactobacilli, total anaerobes, streptococci ↓; total aerobes ↑[2]	
Fish meal, fish meal + SBM	Distal jejunum	Enterobacteria, lactobacilli, lactobacilli : *Enterobacteriaceae* ↔[3]	Manzanilla *et al.* (2009)
SBM, dried skimmed milk	Ileum, colon, feces	Coliforms, lactobacilli, lactobacilli to coliforms ratio ↔[3]	Wellock *et al.* (2006)

↑increase, ↓decrease, ↔no effect, SBM = soybean meal.
[1]Microbial plate counts.
[2]Compared with sow milk.
[3]Compared each with the other.
[4]Piglets showed clinical signs of diarrhea.

weaned piglets. In the study by Kellogg *et al.* (1964), SBM and dried skimmed milk increased total anaerobes and total aerobes, whereas the other protein sources resulted in lower fecal counts of these bacteria. Wellock *et al.* (2006) could show that newly weaned piglets fed a diet based on dried skimmed milk powder consumed more feed, resulting in higher BW gain and improved feed conversion ratio than those offered a SBM-based diet. Although all piglets remained clinically healthy, in piglets fed the SBM-based diet, the lactobacilli to coliform ratio in fecal samples was decreased, and there was an increase in gastrointestinal pH, obviously because of the buffering effect of protein (Partanen and Mroz, 1999). An elevated lactobacilli to coliform ratio indicates a more advantageous proportion of beneficial lactobacilli to coliforms, including coliform pathogens (Reid and Hillman, 1999). However, Manzanilla *et al.* (2009) observed no differences in the counts of lactobacilli and enterobacteria in the distal jejunum when feeding newly weaned piglets either a corn–fish meal or a corn–fish meal SBM-based diet. Total counts of both enterobacteria and the lactobacilli to enterobacteria ratio were not influenced by the different protein sources.

Overall, results of currently available studies on the effect of dietary protein source on the composition of the intestinal microbiota are inconsistent and do not allow for recommending ultimate feeding strategies. Nevertheless, under conditions of increased stress such as suboptimal hygienic and housing conditions or exposure to infections (Owusu-Asiedu *et al.*, 2003), plant protein sources of lower CP digestibility might increase the risk for the development of PWD.

Dietary protein intake

In rats, the total protein intake rather than the source of dietary protein has been identified as a major factor eventually affecting the extent of protein fermentation in the GIT, and also intestinal bacterial composition (Le Leu *et al.*, 2006). In addition, experiments in which weaned piglets were fed a low-protein diet resulted in a reduction of protein fermentation activity as indicated by lower ammonia concentrations in the small intestine (Bikker *et al.*, 2006), lower plasma urea levels and reduced contents of SCFA in ileal digesta (Nyachoti *et al.*, 2006) compared with high-protein diets. However, studies in piglets revealed inconsistent results on the effect of protein level in the diet on composition of the gastrointestinal microbiota (Table 2).

Kellogg *et al.* (1964) obtained higher counts of total aerobes and total anaerobes in fecal samples of newly weaned piglets as protein level in a corn–SBM-based diet was raised from 100 to 200 g/kg, and counts of lactobacilli increased when the protein level of the diets was increased from 100 to 150 g/kg. Beyond these levels, total counts of aerobes, anaerobes and lactobacilli decreased. In the same study, changes in fecal coliforms and staphylococci counts were inversely related to those of lactobacilli. Obviously, there was a shift of the microbial community toward potential pathogenic bacteria, such as coliforms, when the CP content of the diet increased beyond a level of 200 g/kg (Kellogg *et al.*, 1964). In more recent studies with newly weaned piglets (Wellock *et al.*, 2006), a reduction of the dietary CP content in a SBM-based diet from 230 to 130 g/kg was associated with lower fecal water content. In addition, fecal samples contained

Table 2 *Effect of dietary protein level on the composition of the intestinal and fecal microbiota of weaned piglets*

CP levels (g/kg, as fed)	Protein source	Sampling site	Response	References
225,[3] 176	Milk protein	Colon	Lactic acid bacteria ↔ Coliforms ↔ 176 g/kg: clostridiales ↑	Opapeju *et al.* (2009)[1,2,4]
130, 180, 230	Milk protein	Colon	Coliforms ↑ until 230 g/kg Lactobacilli ↔ Lactobacilli to coliforms ratio ↓ until 230 g/kg	Wellock *et al.* (2006)[1]
230, 130	Milk protein	Colon	130 g/kg: lactobacilli ↓	Wellock *et al.* (2008b)[1,4]
197, 217	Poultry meal	Colon	Coliforms ↔ Clostridia ↔ Lactic acid bacteria ↔ Lactic acid bacteria : coliforms ↔	Jeaurond *et al.* (2008)[1]
150, 220	SBM	Jejunum, colon	Lactobacilli ↔ Coliforms ↔	Bikker *et al.* (2006 and 2007)[1]
154, 194	SBM	Feces	*Escherichia coli*, enterococci (plate counts) ↔ Enterobacteria, lactobacilli (PCR) ↔	Hermes *et al.* (2009)[1,2]
100, 150, 200, 250, 300	SBM	Feces	Lactobacilli ↑ until 150 g/kg, then ↓ Total aerobes, total anaerobes ↑ until 200 g/kg, then ↓ Coliforms from 150 g/kg ↑, 300 g/kg ↓ Staphylococci from 150 g/kg ↑ Streptococci ↔	Kellogg *et al.* (1964)[1]
170, 190, 210, 230	SBM + fish meal	Ileum	Aerobic and anaerobic sporeformers ↔ *Enterobacteriaceae* ↔ Enterococci ↔ *E. coli* ↔ Coliforms ↔	Nyachoti *et al.* (2006)[1]
147, 200	SBM	Colon	200 g/kg: *Clostridium leptum* ↑ Total bacteria ↔ Lactobacilli ↔ Enterobacteria ↔ *Bacteroides* ↔ *Clostridium coccoides* ↔	Pieper *et al.* (2012)[2]

↑ increase, ↓ decrease, ↔ no effect, SBM = soybean meal.
[1] Microbial plate counts.
[2] PCR.
[3] Piglets showed clinical signs of diarrhea.
[4] Piglets were challenged with *E. coli*.

lower counts of pathogenic bacteria, such as coliforms, and a higher ratio of lactobacilli to coliforms, indicating a decreased risk for the development of PWD.

In several studies with newly weaned piglets, protein-reduced diets were formulated and supplemented with graded levels of crystalline AA to meet piglets' AA requirements (e.g. Heo *et al.*, 2008). Although the supplementation of essential AA to pig diets is becoming increasingly common in commercial practice, some restrictions may apply in their use in protein-reduced starter diets. There is evidence that a decrease in dietary CP content may create serious deficiencies of nonessential AA, resulting in reduced growth performance (Opapeju *et al.*, 2008). For example, Nyachoti *et al.* (2006) observed a decline in weaned piglets' growth performance if the dietary CP content was 190 g/kg or less. At the same time, the authors reported no effect on ileal digesta counts of total coliforms, anaerobic sporeformers, aerobic sporeformers, *Enterobacteriaceae*, enterococci and *E. coli* when weaned piglets were fed diets with graded levels of CP ranging from 170 to 230 g/kg. However, feeding protein-reduced diets was associated with lower contents of ammonia in ileal digesta, indicating reduced fermentation activity of protein in the small intestine but without affecting the composition of the bacterial community. These findings were confirmed by Pieper *et al.* (2012) who measured lower ammonia concentrations in piglets' colon when feeding 147 g/kg CP compared with 200 g/kg diets. However, in this study, the high-CP diet increased counts of the *Clostridium leptum* group in the proximal colon of weaned piglets, but had no effect on total bacteria, lactobacilli, enterobacteria, *Bacteroides* and the *Clostridium coccoides* group in the proximal colon. According to a study of Bikker *et al.* (2007), no effect on counts of lactobacilli and coliform bacteria were found in jejunum and colon of newly weaned piglets fed a potato protein-based diet supplemented with wheat and maize gluten meal with 220 g/kg CP in comparison with piglets fed the basal diet with 150 g/kg CP. However, the authors observed a decrease in ammonia concentration in jejunal and colonic digesta samples of piglets fed the low-protein diet, suggesting reduced protein fermentation.

Obviously, a reduction in dietary protein content may result in a lower intestinal ammonia concentration, but not necessarily in changes of the intestinal bacterial community, maybe because of the ability of the microbiota to adapt to a certain extent to changes in the availability of nutrients (Heo *et al*., 2012). In contrast, in studies with weaned piglets experimentally infected with pathogens, dietary CP supply had more pronounced effects on the composition of the intestinal microbiota. For example, in a study by Opapeju *et al*. (2009), newly weaned piglets were fed corn–SBM–fish meal diets, containing either 225 or 176 g/kg CP. They were supplemented with crystalline AA to meet the animals' AA requirement. Eight days post weaning, piglets were challenged with ETEC K88. Thereafter, they were slaughtered on days 1, 3 and 7 post challenge. Three days after being challenged, ETEC was not present in ileal digesta of piglets fed the 176 g/kg CP diet, but could be found in ileal digesta of 80% of piglets fed the 225 g/kg CP diet. Moreover, piglets fed the 225 g/kg CP diet had a higher ammonia-N concentration in colonic digesta on days 1 and 7 post challenge, indicating a higher protein fermentation activity. On the other hand, in piglets fed the 176 rather than the 225 g/kg CP diet, counts of carbohydrate-fermenting, butyrate-producing *Roseburia* were higher in colonic digesta on day 7. According to the authors, a reduction of dietary protein supply may inhibit the proliferation of those pathogens (e.g. ETEC), which preferentially ferment protein. As a result, there is a shift in intestinal microbial composition toward higher counts of beneficial bacteria, which preferentially ferment carbohydrates (Opapeju *et al*., 2009). In addition, in another challenge study with newly weaned piglets, which were orally infected with ETEC, a reduction of dietary protein content from 230 to 130 g/kg in SBM-based or dried skimmed milk powder-based diets was associated with lower fecal excretion of ETEC (Wellock *et al*., 2008a and 2008b).

In a study by Hermes *et al*. (2009) on 35-day-old piglets, the effects of diets with either 190 or 150 g/kg CP on bacterial composition in feces were assessed. In this study, no differences in fecal samples were found between treatments, on counts of coliforms, enterococci, enterobacteria and lactobacilli. When using finisher boars, O'Shea *et al*. (2010) also observed no differences in total counts of *Lactobacillus* spp. and enterobacteria both at the ileal and fecal level, when the protein content of a wheat–SBM-based diet was decreased from 200 to 150 g/kg CP. Given the stable intestinal microbiota of an adult mammal, one might assume that it would be rather difficult to influence microbial composition by dietary means when compared with a weaner piglet harboring an instable microbiota (Konstantinov *et al*., 2004).

Altogether, results in studies with piglets are rather inconsistent, though it appears that feeding diets with high levels of protein is in favor of increasing the number of protein-hydrolyzing bacteria (e.g. Wellock *et al*., 2006). Furthermore, it appears that, although this must not necessarily be reflected in the intestinal bacterial composition, a reduction in dietary protein content may lower detrimental protein fermentation as indicated, for example, by decreased intestinal ammonia concentrations (Bikker *et al*., 2007; Pieper *et al*., 2012). Furthermore, in piglets challenged with pathogens, changes in dietary protein content are reflected in the composition of the intestinal microbiota, with reduced counts of pathogens for lower protein content. Thus, a reduction of dietary CP in diets for weaned piglets is suggested, in particular in stress situations such as increased pressure of pathogens and increased risk of infections.

Dietary supplementation of fermentable carbohydrates

As was reviewed by de Lange *et al*. (2010), the use of fermentable carbohydrates in pigs' diet seems to be the most promising approach to beneficially affect the composition and activity of the intestinal microbiota. Therefore, in an effort to possibly reduce protein fermentation in the GIT, some authors suggested to add fermentable carbohydrates to the diet (Awati *et al*., 2006; Jeaurond *et al*., 2008), thereby shifting fermentation processes toward carbohydrate rather than protein fermentation (Kim *et al*., 2008). Such a shift in fermentation activity is reflected in lower levels of ammonia and BCFA as indicators of reduced protein fermentation (e.g. Williams *et al*., 2005). To stimulate beneficial bacteria, fermentable carbohydrates must be neither hydrolyzed nor absorbed in the upper GIT of the animal (Branner *et al*., 2004). Fermentable carbohydrates that have been frequently used in pig diets include NSP, such as cellulose, hemicellulose or pectin, resistant starches and nondigestible oligosaccharides (Bauer *et al*., 2006). For example, after inclusion of sugar beet pulp, inulin, lactulose and wheat starch in diets for newly weaned piglets, Awati *et al*. (2006) determined lower fecal concentrations of ammonia and BCFA. Obviously, owing to the preferential fermentation of these carbohydrates, the formation of potentially harmful metabolites originating from protein fermentation was reduced. In addition, in some studies designed to limit protein fermentation in the GIT of pigs, the effect of fermentable carbohydrates on composition of the intestinal microbiota was examined (Table 3).

According to Konstantinov *et al*. (2004), the addition of nondigestible, fermentable carbohydrates (sugar beet pulp and fructooligosaccharides) to the diet of weaned piglets increased the number of lactobacilli in the small intestine, and enhanced stability and diversity of the bacterial community in the porcine colon, thereby eventually suppressing potential pathogenic proteolytic bacteria. The authors suggest that the inclusion of fermentable carbohydrates improved gut health during weaning because of a shift from protein to carbohydrate fermentation. According to Houdijk *et al*. (2002), the supplementation of nondigestible oligosaccharides to the diet of newly weaned piglets affected the microbial population at the ileal, but not at the fecal level. In this study, total aerobes and enterococci were lower upon dietary inclusion of fructo- and transgalactooligosaccharides compared with piglets fed the control diet. Moreover, lactobacilli and *Bacteroides* spp. were not affected, but supplemental oligosaccharides had a reducing effect on potential harmful proteolytic *E. coli* (Houdijk *et al*., 2002). Furthermore, Lynch *et al*. (2009) reported that the inclusion

Table 3 *Selected studies on the effect of fermentable carbohydrates supplemented to the diet of weaned piglets on the intestinal and fecal microbiota*

Carbohydrates	Sampling site	Response	References
Wheat bran, sugar beet pulp	Feces	Coliforms ↓	Hermes *et al.* (2009)[1,2]
Fructo oligosaccharides, transgalactosaccharides	Ileum	Total aerobes ↔ Enterococci ↓ Total anaerobes ↔ *Bacteroides* ↔ Lactobacilli ↔ Bifidobacteria ↔ *Escherichia coli* ↔	Houdijk *et al.* (2002)[1]
	Feces	No effects	
Inulin, lactulose, wheat starch, sugar beet pulp	Colon	Lactobacilli ↑	Konstantinov *et al.* (2003)[2]
Lactose, inulin	Feces	*Lactobacilli* spp. ↑ *Enterobacteria* spp. ↓	Lynch *et al.* (2009)[1]
Wheat bran, sugar beet pulp	Colon	*Bacteroides* ↑ *Clostridium leptum* ↑ *Clostridium coccoides* ↑ Total bacteria ↔ Lactobacilli ↔ Enterobacteria ↔	Pieper *et al.* (2012)[2]

↑ increase, ↓ decrease, ↔ no effect.
[1] Microbial plate counts.
[2] PCR.

of inulin (15 g/kg) and lactose (230 g/kg) to a low- (160 g/kg) and a high- (200 g/kg) CP diet for piglets increased *Lactobacillus* spp. and decreased *Enterobacteria* spp. in feces of weaned piglets. In addition, supplementation of inulin together with high lactose (230 g/kg) decreased BCFA, compared with piglets offered inulin-supplemented diets containing only 50 g/kg lactose. Obviously, a combination of inulin and high lactose was able to beneficially affect microbial composition and reduce harmful protein fermentation. However, different concentrations of inulin in the diets, the degree of polymerization of inulin and other factors affect microbial degradation, which may explain varying effects on the fermentative activity of the intestinal microbiota (Paßlack *et al.*, 2012).

Hermes *et al.* (2009) added two native sources of NSP, wheat bran and sugar beet pulp to a low- (160 g/kg) and a high- (200 g/kg) protein diet fed to piglets. The counts of coliforms and enterobacteria in feces were lower, whereas the lactobacilli to enterobacteria ratio tended to be higher upon supplementing the diets with NSP. There was a lower incidence of diarrhea when NSP was supplemented to the high-protein diet but a higher incidence of diarrhea when NSP was supplemented to the low-protein diet. According to the authors, the higher-dietary CP content was associated with enhanced protein fermentation in the colon, whereas the simultaneous addition of NSP to the high-CP diet increased carbohydrate fermentation (i.e. increased SCFA production) and reduced protein fermentation (i.e. reduced BCFA) in colon. These findings were confirmed in a study conducted by Pieper *et al.* (2012) in which a combination of wheat bran and sugar beet pulp was added to a low- (147 g/kg) and a high- (200 g/kg) protein diet fed to piglets. The authors also determined the lowest incidence of diarrhea for the low-protein diet without supplemental NSP, whereas cell counts of lactobacilli in the proximal colon were lowest for the low-protein diet supplemented with NSP. Moreover, they observed increased numbers of potential proteolytic *Bacteroides* and *Clostridium.* However, despite this increase putrescine and ammonia levels in the proximal colon were lower in piglets fed the NSP supplemented diet, likely due to a reduced protein fermentation activity. Obviously, shifts in microbial composition as observed are not always reflected in changes of metabolic activity as anticipated; however, overall there is growing evidence that supplementation of piglets' diets with fermentable carbohydrates seems to be a promising approach to reduce both intestinal protein fermentation and potential pathogenic bacteria.

Conclusion

Diet appears to be an important factor controlling the composition and metabolic activities of the gastrointestinal microbiota of single-stomached animals. Thus, it is essential to understand the interactions between the host, diet and intestinal microbiota. Although results were not always consistent, it appears that avoiding excessive amounts of protein reaching the lower GIT may reduce the incidence of PWD and inhibit proliferation of pathogenic bacteria, especially in piglets reared under nutritional and environmental stress conditions. In addition, although not always associated with changes in bacterial composition, a moderate reduction of dietary protein level may reduce formation of detrimental fermentation products, for example, ammonia. Moreover, there is evidence that the inclusion of fermentable

carbohydrates seems to be promising in reducing detrimental protein fermentation and proliferation of potential pathogenic proteolytic bacteria. However, studies designed to assess the effect of protein level, protein source or fermentable carbohydrates on occurrence of proteolytic bacteria in the GIT are rather limited. Furthermore, owing to the wide variety of methods used, the results of these studies are sometimes difficult to compare. For example, most of the studies used culture-based bacterial count techniques, whereas only few studies were based on molecular methods for quantitative assessment of bacteria. By using more sensitive and culture-independent methods, at least some of the variation between results of different studies could be reduced. Furthermore, results between studies pertaining to assess the effect of different levels or sources of dietary protein on microbial composition and metabolic activity are often confounded by the use of varying ingredients in the basal diet. For example, supplementation of fermentable carbohydrates to a basal diet, already rich in contents of fermentable carbohydrates (e.g. cereal-based diet), may mask any potential effects of varying protein levels or sources on bacterial composition and activity. Thus, for a better comparability between studies, further research should take into consideration the use of standardized basal diets containing well-defined feed ingredients. Finally, it has to be emphasized that some studies have rather limited practical implications. For example, some experimental designs are based on the use of casein or dried skimmed milk as dietary protein sources. Although these feed proteins are almost completely digested in the small intestine and therefore hardly available for protein fermentation in the large intestine, their use in pigs' diets is rather limited because of their low cost-efficiency.

Acknowledgments

The authors are grateful to AHRHOFF GmbH, Bönen, Germany for the financial support of Vanessa T. S. Rist. They thank Silke Hörner for her technical assistance.

References

Aherne F, Hogberg MG, Kornegay ET, Shurson GC, Brocksmith M, Brocksmith S, Hollis GR and Pettigrew JE 1992. Management and nutrition of the newly weaned pig. Pork industry handbook 111. National Pork Producers Council, Des Moines, IA.

Aschenbach JR, Schwelberger HG, Ahrens F, Fürll B and Gäbel G 2006. Histamine inactivation in the colon of pigs in relationship to abundance of catabolic enzymes. Scandinavian Journal of Gastroenterology 41, 712–719.

Awati A, Williams BA, Bosch MW, Gerrits WJJ and Verstegen MWA 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. Journal of Animal Science 84, 2133–2140.

Ball RO and Aherne FX 1987. Influence of dietary nutrient density, level of feed intake and weaning age on young pigs. II. Apparent nutrient digestibility and incidence and severity of diarrhea. Canadian Journal of Animal Science 67, 1105–1115.

Bauer E, Williams BA, Smidt H, Mosenthin R and Verstegen MWA 2006. Influence of dietary components on development of the microbiota in single-stomached species. Nutrition Research Reviews 19, 63–78.

Bertschinger HU, Eggenberger E, Jucker H and Pfirter HP 1979. Evaluation of low nutrient, high fibre diets for the prevention of porcine *Escherichia coli* enterotoxaemia. Veterinary Microbiology 3, 281–290.

Bikker P, Dirkzwager A, Fleddrus J, Trevis P, le Huërou-Luron I, Lallès JP and Awati A 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. Journal of Animal Science 84, 3337–3345.

Bikker P, Dirkzwager A, Fledderus J, Trevis P, le Huërou-Luron I, Lallès JP and Awati A 2007. Dietary protein and fermentable carbohydrates contents influence growth performance and intestinal characteristics in newly weaned pigs. Livestock Science 108, 194–197.

Blasco M, Fondevila M and Guada JA 2005. Inclusion of wheat gluten as a protein source in diets for weaned pigs. Animal Research 54, 297–306.

Branner GR, Böhmer BM, Erhardt W, Henke J and Roth-Maier DA 2004. Investigation on the precaecal and faecal digestibility of lactulose and inulin and their influence on nutrient digestibility and microbial characteristics. Archives of Animal Nutrition 58, 353–366.

Choct M, Dersjant-Li Y, McLeish J and Peisker M 2010. Soy oligosaccharides and soluble non-starch polysaccharides: a review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Australasian Journal of Animal Science 23, 1386–1398.

Collinder E, Björnhag G, Cardona M, Norin E, Rehbinder C and Midtvedt T 2003. Gastrointestinal host–microbial interactions in mammals and fish: comparative studies in man, mice, rats, pigs, horses, cows, elk, reindeer, salmon and cod. Microbial Ecology in Health and Disease 15, 66–78.

Columbus D, Cant JP and de Lange CFM 2010. Estimating fermentative amino acid losses in the upper gut of pigs. Livestock Science 133, 124–127.

Conway PL 1994. Function and regulation of the gastrointestinal microbiota of the pig. In Proceedings of the VI International Symposium on Digestive Physiology in Pigs (ed. WB Souffrant and HEAAP Hagemeister), pp. 231–240. FBN Dummerstorf, Germany.

De Lange CFM, Pluske J, Gong J and Nyachoti CM 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Science 134, 124–134.

Dongowski G, Huth M, Gebhardt E and Flamme W 2002. Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. Journal of Nutrition 132, 3704–3714.

Duncan SH, Louis P and Flint HJ 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Applied and Environmental Microbiology 70, 5810–5817.

Etheridge RD, Seerley RW and Wyatt RD 1984. The effect of diet on performance, digestibility, blood composition and intestinal microflora of weaned pigs. Journal of Animal Science 58, 1396–1402.

Fanning S, Hall LJ, Cronin M, Zomer A, MacSharry J, Goulding D, O'Connell Motherway M, Shanahan F, Nally K, Dougan G and van Sinderen D 2012. Bifidobacterial surface-exopolysaccharide facilitates commensal–host interaction through immune modulation and pathogen protection. Proceedings of the National Academy of Sciences 109, 2108–2113.

Gaskins HR 2001. Intestinal bacteria and their influence on swine growth. In Swine nutrition (ed. AJ Lewis and LL Southern), pp. 585–608. CRC Press LLC, Boca Raton, USA.

Geboes KP, de Hertogh G, de Preter V, Luypaerts A, Bammens B, Evenepoel P, Ghoos Y, Geboes K, Rutgeerts P and Verbeke K 2006. The influence of inulin on the absorption of nitrogen and the production of metabolites of protein fermentation in the colon. British Journal of Nutrition 96, 1078–1086.

Hampson DJ 1994. Postweaning *Escherichia coli* diarrhea in pigs. In *Escherichia coli* in domestic animals and humans (ed. CL Gyles), pp. 171–191. CAB International, Wallingford, UK.

Heo JM, Kim JC, Hansen CF, Mullan BP, Hampson DJ and Pluske JR 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhea and performance after weaning. Archives of Animal Nutrition 62, 343–358.

Heo JM, Opapeju FO, Pluske JR, Kim JC, Hampson DJ and Nyachoti CM 2012. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition. doi: 10.1111/j.1439-0396.2012.01284.x, Published online by Blackwell Verlag 24 March 2012.

Hermes RG, Molist F, Ywazaki M, Nofrarias M, Gomez de Segura A, Gasa J and Pérez JF 2009. Effect of dietary level of protein and fiber on the productive

performance and health status of piglets. Journal of Animal Science 87, 3569–3577.

Hopwood DE and Hampson DJ 2003. Interactions between the intestinal microflora, diet and diarrhea, and their influences on the piglet health in the immediate post-weaning period. In Weaning the pig (ed. JR Pluske, JL Dividich and MWA Verstegen), pp. 199–219. Wageningen Academic Publishers, Wageningen, The Netherlands.

Houdijk JGM, Hartemink R, Verstegen MWA and Bosch MW 2002. Effects of dietary non-digestible oligosaccharides on microbial characteristics of ileal chyme and faeces in weaner pigs. Archives of Animal Nutrition 56, 297–307.

Htoo JK, Araiza BA, Sauer WC, Rademacher M, Zhang Y, Cervantes M and Zijlstra RT 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. Journal of Animal Science 85, 3303–3312.

Hughes R, Magee EAM and Bingham S 2000. Protein degradation in the large intestine: relevance to colorectal cancer. Current Issues in Intestinal Microbiology 1, 51–58.

Jeaurond EA, Rademacher M, Pluske JR, Zhu CH and de Lange CFM 2008. Impact of feeding fermentable proteins and carbohydrates on growth performance, gut health and gastrointestinal function of newly weaned pigs. Canadian Journal of Animal Science 88, 271–281.

Jensen BB 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. Journal of Animal and Feed Sciences 7, 45–64.

Jensen BB 2001. Possible ways of modifying type and amount of products from microbial fermentation in the gut. In Gut environment of pigs (ed. A Piva, KE Bach Knudsen and JE Lindberg), pp. 181–200. Nottingham University Press, Nottingham, UK.

Jensen BB and Jørgensen H 1994. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Applied and Environmental Microbiology 60, 1897–1904.

Kellogg TF, Hays VW, Catron DV, Quinn LY and Speer VC 1964. Effect of level and source of dietary protein on performance and fecal flora of baby pigs. Journal of Animal Science 23, 1089–1094.

Kelly D and King TP 2001. Luminal bacteria: regulation of gut function and immunity. In Gut environment of pigs (ed. A Piva, KE Bach Knudsen and JE Lindberg), pp. 113–131. Nottingham University Press, Nottingham, UK.

Kim JC, Mullan BP, Hampson DJ and Pluske JR 2008. Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. British Journal of Nutrition 99, 1217–1225.

Klose V, Bayer K, Bruckbeck R, Schatzmayr G and Loibner A-P 2010. In vitro antagonistic activities of animal intestinal strains against swine-associated pathogens. Veterinary Microbiology 144, 515–521.

Kluess J, Schoenhusen U, Souffrant WB, Jones PH and Miller BG 2010. Impact of diet composition on ileal digestibility and small intestinal morphology in early-weaned pigs fitted with a T-cannula. Animal 4, 586–594.

Konstantinov SR, Favier CF, Zhu WY, Williams BA, Klüß J, Souffrant W-B, de Vos WM, Akkermans ADL and Smidt H 2004. Microbial diversity studies of the porcine gastrointestinal ecosystem during weaning transition. Animal Research 53, 317–324.

Konstantinov SR, Zhu WY, Williams BA, Tamminga S, de Vos WM and Akkermans ADL 2003. Effect of fermentable carbohydrates on piglet faecal bacterial communities as revealed by denaturing gel electrophoresis analysis of 16S ribosomal DNA. FEMS Microbiology Ecology 43, 225–235.

Le Leu RK, Brown IL, Hu Y, Morita T, Esterman A and Young GP 2006. Effect of dietary resistant starch and protein on colonic fermentation and intestinal tumourigenesis in rats. Carcinogenesis 28, 240–245.

Li DF, Nelssen JL, Reddy PG, Blecha F, Hancock JD, Allee GL, Goodband RD and Klemm RD 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. Journal of Animal Science 68, 1790–1799.

Libao-Mercado AJ, Zhu CL, Cant JP, Lapierre H, Thibault J-N, Sève B, Fuller MF and de Lange CFM 2009. Dietary and endogenous amino acids are the main contributors to microbial protein in the upper gut of normally nourished pigs. Journal of Nutrition 139, 1088–1094.

Loh G, Eberhard M, Brunner RM, Hennig U, Kuhla S, Kleessen B and Metges CC 2006. Inulin alters the intestinal microbiota and short-chain fatty acid concentration in growing pigs regardless of their basal diet. Journal of Nutrition 136, 1198–1202.

Lynch B, Callan JJ and O'Doherty JV 2009. The interaction between dietary crude protein and fermentable carbohydrate source on piglet post weaning performance, diet digestibility and selected faecal microbial populations and volatile fatty acid concentration. Livestock Science 124, 93–100.

Macfarlane GT and Macfarlane S 2007. Models for intestinal fermentation: association between food components, delivery systems, bioavailability and functional interactions in the gut. Current Opinion in Biotechnology 18, 156–162.

Macfarlane GT, Gibson GR, Beatty E and Cummings JH 1992. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched-chain fatty acid measurements. FEMS Microbiology Ecology 101, 81–88.

Macfarlane S and Macfarlane GT 1995. Proteolysis and amino acid fermentation. In Human colonic bacteria: role in nutrition, physiology and pathology (ed. GR Gibson and GT Macfarlane), pp. 75–100. CRC Press, Boca Raton, Florida, USA.

Manzanilla EG, Pérez JF, Martín M, Blandón JC, Baucells F, Kamel C and Gasa J 2009. Dietary protein modifies effect of plant extracts in the intestinal ecosystem of the pig at weaning. Journal of Animal Science 87, 2029–2037.

Morita T, Kasaoka S and Kiriyama S 2004. Physiological functions of resistant proteins: proteins and peptides regulating large bowel fermentation of indigestible polysaccharide. Journal of AOAC International 87, 792–796.

Mosenthin R, Hambrecht E and Sauer WC 1999. Utilisation of different fibres in piglet feeds. In Recent advances in animal nutrition (ed. PC Garnsworthy and J Wiseman), pp. 227–256. Nottingham University Press, Nottingham, UK.

Mosenthin R, Sauer WC, Henkel H, Ahrens F and de Lange CF 1992. Tracer studies of urea kinetics in growing pigs: II. The effect of starch infusion at the distal ileum on urea recycling and bacterial nitrogen excretion. Journal of Animal Science 70, 3467–3472.

Nyachoti CM, Omogbenigun FO, Rademacher M and Blank G 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. Journal of Animal Science 84, 125–134.

Opapeju FO, Rademacher M, Blank G and Nyachoti CM 2008. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. Animal 2, 1457–1464.

Opapeju FO, Krause DO, Payne RL, Rademacher M and Nyachoti CM 2009. Effect of dietary protein level on growth performance, indicators of enteric health, and gastrointestinal microbial ecology of weaned pigs induced with postweaning colibacillosis. Journal of Animal Science 87, 2635–2643.

O'Shea CJ, Lynch MB, Callan JJ and O'Doherty JV 2010. Dietary supplementation with chitosan at high and low crude protein concentrations promotes Enterobacteriaceae in the caecum and colon and increases manure odour emissions from finisher boars. Livestock Science 134, 198–201.

Otto ER, Yokoyama M, Hengemuehle S, von Bermuth RD, van Kempen T and Trottier NL 2003. Ammonia, volatile fatty acids, phenolics, and odor offensiveness in manure from growing pigs fed diets reduced in protein concentration. Journal of Animal Science 81, 1754–1763.

Owusu-Asiedu A, Nyachoti CM, Baidoo SK, Marquardt RR and Yang X 2003. Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg yolk antibody. Journal of Animal Science 81, 1781–1789.

Partanen KH and Mroz Z 1999. Organic acids for performance enhancement in pig diets. Nutrition Research Reviews 12, 117–145.

Paßlack N, Al-samman M, Vahjen W, Männer K and Zentek J 2012. Chain length of inulin affects its degradation and the microbiota in the gastrointestinal tract of weaned piglets after a short-term dietary application. Livestock Science 149, 128–136.

Pieper R, Kröger S, Richter JF, Wang J, Martin L, Bindelle J, Htoo JK, von Smolinski D, Vahjen W, Zentek J and Van Kessel AG 2012. Fermentable fiber ameliorates fermentable protein-induced changes in microbial ecology, but not the mucosal response, in the colon of piglets. Journal of Nutrition 142, 661–667.

Pietrzak T, Schad A, Zentek J and Mosenthin R 2002. Biogene Amine in der Tierernährung: Entstehung, Stoffwechsel und physiologische Aspekte. Übersichten zur Tierernährung 31, 37–64.

Pluske JR, Hampson D and Williams IH 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Production Science 51, 215–236.

Pluske JR, Pethick DW, Hopwood DE and Hampson DJ 2002. Nutritional influences on some major enteric bacterial diseases of pigs. Nutrition Research Reviews 15, 333–371.

Reid CA and Hillman K 1999. The effect of retrogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. Animal Science 68, 503–510.

Richards JD, Gong J and de Lange CFM 2005. The gastrointestinal microbiota and its role in monogastric nutrition and health with an emphasis on pigs: current understanding, possible modulations, and new technologies for ecological studies. Canadian Journal of Animal Science 85, 421–435.

Rolfe R 1997. Colonization resistance. In Gastrointestinal microbiology, vol. 2 gastrointestinal microbes and host interactions (ed. RI Mackie, BA White and RE Isaacson), pp. 501–536. Chapman and Hall, New York, USA.

Roselli M, Finamore A, Britti MS, Konstantinov SR, Smidt H, de Vos WM and Mengheri E 2007. The novel porcine *Lactobacillus sobrius* strain protects intestinal cells from enterotoxigenic *Escherichia coli* K88 infection and prevents membrane barrier damage. Journal of Nutrition 137, 2709–2716.

Savage DC 1977. Microbial ecology of the gastrointestinal tract. Annual Review of Microbiology 31, 107–133.

Scott KP, Gratz SW, Sheridan PO, Flint HJ and Duncan SH 2012. The influence of diet on the gut microbiota. Pharmacological Research. doi 10.1016/j.phrs.2012.10.020, Published online by Elsevier 09 November 2012.

Stewart CA, Hillman K, Maxwell F, Kelly D and King TP 1993. Recent advances in probiosis in pigs: observations on the microbiology of the pig gut. In Recent advances in animal nutrition (ed. PC Gansworthy and DJA Cole), pp. 197–220. Nottingham University Press, Nottingham, UK.

Unger FM and Viernstein H 2004. Probiotika: Regenerierende, prophylaktische und adjuvant-therapeutische Anwendungen. Journal für Ernährungsmedizin 6, 24–29.

Ushida K, Hoshi S and Ajisaka K 2002. ^{13}C-NMR studies on lactate metabolism in a porcine gut microbial ecosystem. Microbial Ecology in Health and Disease 14, 241–246.

Van der Waaij D 1991. The microflora of the gut: recent findings and implications. Digestive Diseases 9, 36–48.

Varel VH and Yen JT 1997. Microbial perspective on fiber utilization by swine. Journal of Animal Science 75, 2715–2722.

Wellock IJ, Fortomaris PD, Houdijk JGM and Kyriazakis I 2006. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. Animal Science 82, 327–335.

Wellock IJ, Fortomaris PD, Houdijk JGM and Kyriazakis I 2008a. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: performance. Animal 2, 825–833.

Wellock IJ, Fortomaris PD, Houdijk JGM and Kyriazakis I 2008b. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: health. Animal 2, 834–842.

Wells JE, Yen JT and Miller DN 2005. Impact of dried skim milk in production diets on *Lactobacillus* and pathogenic bacterial shedding in growing-finishing swine. Journal of Applied Microbiology 99, 400–407.

Williams BA, Bosch MW, Awati A, Konstantinov SR, Smidt H, Akkermans ADL, Verstegen MWA and Tamminga S 2005. In vitro assessment of gastrointestinal tract (GIT) fermentation in pigs: fermentable substrates and microbial activity. Animal Research 54, 191–201.

Willing BP and Van Kessel AG 2009. Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. Journal of Animal Physiology and Animal Nutrition 93, 586–595.

Windey K, de Preter V and Verbeke K 2012. Relevance of protein fermentation to the gut health. Molecular Nutrition & Food Research 56, 184–196.