



## A Review of Interactions between Dietary Fiber and the Gastrointestinal Microbiota and Their Consequences on Intestinal Phosphorus Metabolism in Growing Pigs

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**ABSTRACT :** Dietary fiber is an inevitable component in pig diets. In non-ruminants, it may influence many physiological processes in the gastrointestinal tract (GIT) such as transit time as well as nutrient digestion and absorption. Moreover, dietary fiber is also the main substrate of intestinal bacteria. The bacterial community structure is largely susceptible to changes in the fiber content of a pig's diet. Indeed, bacterial composition in the lower GIT will adapt to the supply of high levels of dietary fiber by increased growth of bacteria with cellulolytic, pectinolytic and hemicellulolytic activities such as *Ruminococcus* spp., *Bacteroides* spp. and *Clostridium* spp. Furthermore, there is growing evidence for growth promotion of beneficial bacteria, such as lactobacilli and bifidobacteria, by certain types of dietary fiber in the small intestine of pigs. Studies in rats have shown that both phosphorus (P) and calcium (Ca) play an important role in the fermentative activity and growth of the intestinal microbiota. This can be attributed to the significance of P for the bacterial cell metabolism and to the buffering functions of Ca-phosphate in intestinal digesta. Moreover, under P deficient conditions, ruminal NDF degradation as well as VFA and bacterial ATP production are reduced. Similar studies in pigs are scarce but there is some evidence that dietary fiber may influence the ileal and fecal P digestibility as well as P disappearance in the large intestine, probably due to microbial P requirement for fermentation. On the other hand, fermentation of dietary fiber may improve the availability of minerals such as P and Ca which can be subsequently absorbed and/or utilized by the microbiota of the pig's large intestine. (**Key Words :** Dietary Fiber, Bacteria, Fermentation, Phosphorus, Pigs)

### INTRODUCTION

Dietary fiber is an inevitable component in diets of pigs as it is present in a variety of feedstuffs of plant origin including cereal grains and their by-products, grain legumes but also protein supplements produced from various oilseeds. In recent years, there is growing interest to increase the utilization of by-products originating from the production of bio-ethanol, such as distiller's dried grains, wheat-millrun and soy hulls, in the nutrition of ruminants and non-ruminants as well. Both, dry milling and distilling processes, remove most of the starch fraction from cereal grains, accumulating dietary fiber but also protein and minerals in the residuals (e.g. Spiels et al., 2002; Huang et al., 2003; Slominski et al., 2004).

The dietary fiber fraction of these by-products has received growing attention as some fibrous compounds have shown characteristics of prebiotics (Shi et al., 2001;

Konstantinov et al., 2004; Yin et al., 2004; Shim et al., 2007), while others were rather associated with the growth of potential pathogenic bacteria (McDonald et al., 2001). Recently, potential interactions between fibrous feedstuffs and the microbial ecology of the host animal have been described (Konstantinov et al., 2004; Hill et al., 2005; Owusu-Asiedu et al., 2006). It is well accepted that dietary fiber may affect digestive functions in the small intestine with consequences on digestion and absorption of nutrients (e.g. Bach Knudsen, 2001; Grieshop et al., 2001; Wenk, 2001; Montagne et al., 2003), however, there is little information on the consequences of microbial fermentation in the gastrointestinal tract (GIT) of pigs on mineral absorption and metabolism as it has been previously described for rodents (Demigné et al., 1989; Levrat et al., 1991).

In pigs, dietary fiber is the main substrate for bacteria in the gastrointestinal tract (GIT), and inclusion of dietary fiber has shown to promote bacterial growth, resulting in a higher fecal excretion of amino acids, lipids and minerals such as phosphorus (P) and calcium (Ca) of bacterial origin

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Received August 15, 2007; Accepted August 24, 2007

**Table 1.** Characterization of fiber components based on fermentability (adapted from Tunland and Meyer, 2002)

Characteristic	Fiber component	Main source
Partial or low fermentable	Cellulose	Plants (e.g. sugar beet, various brans, vegetables)
	Hemicellulose	Cereal grains
	Lignin	Woody plants
	Resistant starches	Corn, potatoes, grains, bananas, legumes
High fermententable	$\beta$ -Glucans	Grains (oat, barley, rye)
	Pectins	Fruits, vegetables, legumes, sugar beet, potatoes
	Gums	Leguminous seed plants (guar, locust bean), seaweed extracts (carrageenan, alginates), plant extracts (gum acacia, gum karaya, gum tragacanth)
	Inulin	Chicory, Jerusalem artichoke, wheat
	Oligosaccharides	Fructooligosaccharides, galactooligosaccharides, lactulose

(e.g. Mosenthin et al., 1994; Bovee-Oudenhoven et al., 1997b; Wang et al., 2006). During microbial breakdown of complex structures of dietary fiber several nutrients such as amino acids and P may be released from bindings with fiber components (Larsen and Sandström, 1993). These nutrients may be absorbed and/or utilized by the microbiota of the pig's large intestine (LI). Thus, fermentation of dietary fiber may affect the intestinal availability of P and other minerals in pigs. On the other hand, studies in ruminants revealed that bacterial fermentation intensity in the rumen is dependent on the P supply of dietary or salivary origin. In fact, according to *in vitro* studies, fermentation of cellulose and pectin is largely reduced under P deficient conditions (Wider, 2005).

In this review, the main focus will be on potential interactions between dietary fiber and the gastrointestinal microbiota and their effects on the intestinal P metabolism in growing pigs. Where applicable, data from other species are included to complete the discussion.

## DIETARY FIBER

### Definition, classification and microbial fermentability

Dietary fiber is usually defined as the sum of plant polysaccharides and lignin that are not hydrolyzed by endogenous enzymes of the mammalian digestive system (Theander et al., 1994). According to this nutritional concept, the term dietary fiber refers to those polysaccharides that escape enzymatic digestion of the host animal including resistant starch, soluble and insoluble fiber as well as lignin. Dietary fiber represents the main constituent of the plant cell wall which contains a heterogeneous group of polysaccharides, such as cellulose, pectins,  $\beta$ -glucans,  $\beta$ -fructans, pentosans and xylans, differing considerably in terms of type, number and order of monosaccharides, the linkage between monosaccharides and the presence of side chains (Fan and Squires, 2003). These non-starch polysaccharides (NSP) can be hydrolyzed by microorganisms only, with subsequent production of volatile fatty acids (VFA) and various gases, i.e. CO<sub>2</sub>, NH<sub>3</sub>,

CH<sub>4</sub>, and H<sub>2</sub>O (Jørgensen et al., 1996). There is general agreement that the cecum and proximal colon are the main sites of microbial fermentation in the pig. However, there is already substantial microbial activity in the distal part of the small intestine (Leser et al., 2002), so that fermentation of fibrous feed ingredients is assumed to be not restricted to the LI only.

The type and origin of dietary fiber greatly influences the site and degree to which it can be degraded (Table 1), mainly depending on the degree of lignification, solubility and structure of the NSP (Bach Knudsen, 2001). In general, both soluble and insoluble dietary fiber can be degraded by intestinal bacteria, but soluble fiber is more easily, rapidly and completely fermented than insoluble (Bach Knudsen and Hansen, 1991). The higher fermentability of soluble fiber (e.g. pectins, gums,  $\beta$ -glucans) can be attributed to its higher water-holding capacity allowing bacteria to easily penetrate the matrix and start degradation. Thus, with diets containing high soluble fiber levels, the microbial activity is generally increased (Bach Knudsen et al., 1991). By contrast, insoluble fiber (e.g. cellulose) cannot be penetrated easily by bacteria which limits its microbial breakdown in comparison to the soluble fraction (Schneeman, 1987). Hence, degradation of insoluble dietary fiber takes longer, occurring along the full length of the LI. Lignin is neither digestible for enzymes in the small intestine nor fermentable for intestinal bacteria (Graham et al., 1986), but it influences the fermentability of other fibrous components of the diet. As cellulose and lignin are closely associated within plant cell walls, cellulose becomes less accessible for microbial attack which depresses the rate and degree of fermentation in the LI.

### Physiological aspects of dietary fiber

The nutritional significance of dietary fiber and its role in digestive physiology of pigs has been described in detail in previous reviews (e.g. Dierick et al., 1989; Bach Knudsen, 2001; Grieshop et al., 2001; Wenk, 2001; Montagne et al., 2003). Diets high in fiber usually contain a lower energy density than low-fiber diets; thus decreasing

growth rate and feed efficiency in growing pigs. Particularly, the soluble fiber fraction may interfere with the digestion of fibrous and non-fibrous feed components in the small intestine (Graham et al., 1986). Soluble fiber increases the volume and bulk of the small intestinal contents which is related to the water-holding capacity and viscosity of soluble fiber. However, increased viscosity of digesta results in lower transit time in the small intestine due to reduced intestinal contractions (Cherbut et al., 1990). This leads to a reduced mixing of dietary components with endogenous digestive enzymes, resulting eventually in lower nutrient digestibilities. Additional effects of soluble fiber in the GIT include increased total tract transit time, delay of gastric emptying, delay of glucose absorption, increase in salivary, pancreatic and bile secretion (Dierick et al., 1989), whereas insoluble fiber decreases the transit time in the total tract, supports water holding capacity and stimulates fecal bulking in non-ruminant animals (Montagne et al., 2003). Both soluble and insoluble fiber sources increase intestinal epithelial cell proliferation rate. For example, growing pigs fed with 10% wheat straw responded with 33 and 43% increase in jejunal and colonic cell proliferation rate, respectively. Moreover, there was an increase in cell death of jejunal and colonic cells by 65 and 59%, respectively, indicating that dietary fiber may stimulate intestinal cell turnover rate (Jin et al., 1994). As a result, nutrient digestion and absorption may be depressed. Recently, Hedemann et al. (2006) reported that villi and crypts of the small intestine were shorter in weaned pigs fed diets supplemented with pectin, while the villous height/crypt depth ratio was unaltered. Moreover, pectin significantly decreased the area of mucins in the crypts of the small intestine, indicating that pigs fed pectin may be more susceptible to pathogenic bacteria. In contrast, feeding of insoluble fiber diets improved gut morphology by increasing villi length and stimulating mucosal enzyme activity in comparison to piglets fed a diet supplemented with pectin as soluble source of fiber. In addition, it can be derived from the chemical composition of the mucin fraction that piglets fed diets high in insoluble fiber seem to be better protected against pathogenic bacteria than pigs fed diets high in soluble fiber (Hedemann et al., 2006).

Feeding of a high-fiber diet causes earlier satiety than a low-fiber diet due to gastric signals in response to the elongation of the stomach wall. This earlier satiety is of particular interest in pregnant sows. In fattening pigs, a diet low in fiber would be preferred to reach maximum intake of energy and nutrients (Wenk, 2001).

During microbial fermentation of fiber VFA, mainly acetate, propionate and butyrate, are produced to be subsequently absorbed and metabolized by the pig. One of the most important features of VFA is their trophic effect on the intestinal epithelium. Acetic, propionic and butyric acids

are taken up by the colonic mucosa, though butyric acid appears to be the preferred energy source for the colonocytes (Roediger, 1980). After absorption into the portal blood system, VFA play an important role in the intermediary metabolism of the animal. Volatile fatty acids absorbed from the LI may provide up to 30% of the energy requirement for maintenance in growing pigs (Yen et al., 1991). Moreover, they are involved in the regulation of systemic effects, such as changes in glycemia, lipidemia, uremia and overall nitrogen balance (Tungland and Meyer, 2002). However, high production of VFA in the hindgut has been associated with an increased mucin secretion in the LI (Sakata and Setoyama, 1995). Moreover, in a recent study of Pié et al. (2007) a correlation between VFA and proinflammatory cytokines was reported, indicating that the regulation of cytokines may be linked with branched-chain fatty acids which originate from protein fermentation.

#### **General description of interactions between dietary fiber and minerals**

The reported effects of dietary fiber on digestion, absorption and utilization of minerals in pigs are not consistent. It has been generally accepted that the main absorption of minerals occurs in the small intestine. However, according to a study in rats, some highly fermentable dietary fibers, e.g. inulin, pectin and amylo maize starch, may shift the absorption of minerals, such as Ca and P, from the small intestine to the LI (Demigné et al., 1989). Lower pH in digesta of the LI as a result of increased VFA production due to fiber fermentation may improve the solubility of minerals, such as Ca-phosphate, thereby increasing their diffusive absorption via the paracellular route in the LI (Rémésy et al., 1993). In general, the binding of minerals by dietary fiber is related to its origin, and mediated through several mechanisms such as hydration, gelation, physical effects, ion binding capacity and bacterial activity (Van Soest, 1984). Components of dietary fiber and lignin that interact with minerals include the carboxyl group of uronic acids (i.e. hemicelluloses and pectin), carboxyl and hydroxyl groups of phenolic compounds (e.g. lignin), and the surface hydroxyl of cellulose (Kornegay and Moore, 1986). During microbial breakdown of these complex structures, several nutrients such as amino acids and P may be released from bindings with fiber components (Larsen and Sandström, 1993). These nutrients may be absorbed and/or utilized by the microflora of the pig's LI as it has been documented for bacterial nitrogen assimilation (Mosenthin et al., 1992).

### **MICROBIOTA**

#### **Commensal microbiota in the GIT of pigs**

The GIT of pigs harbors a large and diverse population

of aerobic, facultative anaerobic and strictly anaerobic bacterial species. The number and composition of the bacteria in the different segments of the GIT vary considerably (Jensen and Jørgensen, 1994; Leser et al., 2002). Though the cecum and colon represent the main sites of bacterial activity in pigs, the proximal segments are also colonized by a complex indigenous microbiota (Savage, 1986; Jensen, 2001). The epithelium of the stomach is predominantly colonized by lactobacilli, but also by bifidobacteria, streptococci, clostridia and enterobacteria (Henriksson et al., 1995) with a cell population density of approximately  $10^8$  bacteria/gram of digesta (Jensen and Jørgensen, 1994). The composition of the microbiota of the small intestine is similar to that of the stomach, and harbors species like lactobacilli, streptococci, clostridia and enterobacteria (Jensen, 2001). The use of molecular techniques such as Chaperonin-60 gene sequence analysis and quantitative PCR in the study of Hill et al. (2005) confirmed previous characterizations of the ileal microbiota. Accordingly, the most predominant taxa in the ileal community were low G+C gram-positive organisms, particularly the Lactobacillales family, which include *Lactobacillus* spp. and *Pediococcus* spp. among others. Smaller numbers of other low G+C gram-positive bacteria, such as the Clostridiales and Bacillales, and yet smaller numbers of  $\gamma$ -proteobacteria were also identified. In addition, several studies targeting the bacterial composition with molecular tools indicate that bifidobacteria may not be indigenous to the pig (Leser et al., 2002; Loh et al., 2006; Vahjen et al., 2007). The ileal microbiota is distinct in composition from populations associated with the cecum, colon or feces, where microbial populations are more diverse and contain higher numbers of gram-negative bacteria, such as *Bacteroides* (Leser et al., 2002; Konstantinov et al., 2004; Hill et al., 2005). According to Jensen and Jørgensen (1994), the last third of the small intestine in seven months old pigs contains approximately  $10^9$  bacteria/gram of digesta, whereas the corresponding values in the colon amount to around  $10^{10}$  bacteria/gram of digesta.

Major bacterial groups isolated by traditional culture techniques from the cecum/colon or feces of pigs include *Bacteroides*, *Prevotella*, *Eubacterium*, *Lactobacillus*, *Fusobacterium*, *Peptostreptococcus*, *Selenomonas*, *Megasphaera*, *Veillonella*, *Streptococcus* and enterobacteria (Russell, 1979; Moore et al., 1987). However, Leser et al. (2002) could show, using comparative 16S ribosomal RNA sequence analysis that only 17% of the identified phylotypes in the GIT of Danish pigs belong to known species.

The number and composition of bacteria may vary considerably in the different microhabitats of the LI, including lumen, mucus layer and mucosal surface

(Salanitro et al., 1977). For example, Pryde et al. (1999) obtained in five month old pigs total bacterial counts ranging from  $8.8 \times 10^8$ ,  $2.3 \times 10^{10}$  and  $5.3 \times 10^{10}$  cell forming units (CFU)/gram of digesta for the colon wall, colon lumen and cecal lumen, respectively. Despite these differences in the bacterial populations of the microhabitats, the bacteria in the mucus layer and mucosal surface are likely a subset of the luminal bacteria due to normal mucus secretion, epithelial turnover and peristaltic movements in the GIT (Leser et al., 2002). Besides bacteria, yeasts are also known as common inhabitants of pig's GIT with the highest population density in the cecum and colon ( $5.2 \log$  CFU/gram of digesta; Canibe et al., 2005). Finally, it should be mentioned that each individual pig harbors its own specific and unique bacterial composition, even if the animals receive the same diet, are housed in the same environment and are siblings (Hill et al., 2005). Particularly, the establishment of molecular tools, such as sequence libraries and quantitative PCR, has unwrapped opportunities to conduct *in vivo* studies aiming to investigate shifts in the bacterial community as influenced by specific dietary ingredients with the potential to promote animal performance and health (Leser et al., 2002; Konstantinov et al., 2004; Hill et al., 2005).

#### Adaptation of the bacterial community to dietary fiber

The bacterial community structure is largely susceptible to changes in the carbohydrate composition, i.e. fiber content, of pig's diet. Indeed, bacterial composition will adapt to the supply of high levels of dietary fiber by increased growth of bacteria with cellulolytic and hemicellulolytic activities (Varel et al., 1987; Durmic et al., 1998; Leser et al., 2000). Pig's microbiota contains highly active cellulolytic bacterial species, including *Fibrobacter intestinalis* (*succinogenes*), *Ruminococcus flavefaciens*, *Ruminococcus albus* and *Butyrivibrio* spp., which are known to be the predominant cellulolytic bacteria in the rumen (Varel et al., 1984; Varel and Yen, 1997). The fibrolytic bacteria can represent up to 10% of the cultivable bacteria in pigs fed high-fiber diets (Varel and Pond, 1985). Hitherto, the most active fiber degrading bacterium isolated from the GIT of pigs has been identified as *Clostridium herbivorans* (Varel et al., 1995a, b). It is predominant in fecal enrichment cultures of pigs, occurs in relatively high numbers in the GIT of pigs ( $10^7$  cells/g wet weight), and has an equal or better ability to degrade plant cell walls than ruminal cellulolytic bacteria. Bacteria species that degrade hemicellulose such as xylan include *Prevotella* (*Bacteroides*) *ruminicola*, *F. suginogenes*, *R. flavefaciens*, and *Butyrivibrio* spp. (Varel et al., 1987; Varel and Yen, 1997). The study of Varel et al. (1982) showed that total culture counts are initially suppressed when exposed to a high-fiber diet (50% of dehydrated alfalfa). In contrast, the

cellulolytic microbiota increased steadily with time on high-fiber diets, but usually does not represent more than 2% of the total microbiota. In general, the numbers of cellulolytic bacteria from adult pigs are approximately 6.7 times higher than those found in growing pigs (Varel and Yen, 1997). Microbial colonization of fiber is quite rapid; however, the rate and extent to which fiber is degraded is largely determined by a variety of different factors such as microbial accessibility to substrate and physical and chemical composition of the feedstuff (Varga and Kolver, 1997). Cellulolytic bacteria usually degrade cellulose by the synergistic action of endo- and exo-glucanases (Ohmiya et al., 1982; Gardner et al., 1987; Doerner and White, 1990). According to Morales et al. (2002), xylanase and amylopectinase activities in cecal digesta are not only related to the diet composition, but also to the animal's breed.

Durmic et al. (2002) emphasize that the counts of *Bacteroides* spp. and *Peptostreptococcus* spp. were higher when 8.7% of resistant starch was included in the diet, whereas *Eubacterium* spp. increased when 5% of guar gum as a source of soluble dietary fiber was added to the diet. In rats, dietary inclusion of 6.5% of pectin of different degrees of methylation significantly enhanced the counts of *Bacteroides* spp. and total anaerobes after 11 or 21 days on diet (Dongowski et al., 2002). In general, pectinolytic enzymes have been isolated from *Bacteroides* spp. (e.g. Jensen and Canale-Parola, 1985) and the *Clostridium butyricum* - *Clostridium beijerinckii* group (Matsuura, 1991). However, members of the *Bacteroides* genus are probably the most important group in terms of pectin degradation, due to their high numbers and nutritional versatility (McCarthy et al., 1985). The enzymes involved in the breakdown of pectin include pectate lyase, polygalacturonase and pectinesterase. Olano-Martin et al. (2002) showed that different strains of bifidobacteria, lactobacilli, *Bacteroides*, clostridia, enterococci and enterobacteria could grow on pectin and pectic oligosaccharides as well. Moreover, Konstantinov et al. (2006) demonstrated that the recently from the porcine intestine isolated novel *Lactobacillus sobrius* is able to ferment components of sugar beet pulp. In this context, Metzler (2007) reported recently that 25% high-methylated apple-pectin in the diet of growing pigs increased the cell counts of *L. amylovorus*/*L. sobrius* in ileal digesta.

Taking the important physiological role of the small intestine and its associated microbiota in pig's health and performance into account, the microbial populations in the upper digestive tract deserve special attention. Though the main fiber degradation occurs in the LI, dietary fiber already influences the bacterial composition in the ileum. For example, Högberg et al. (2004), in analyzing the ileal microbiota of growing pigs by defining base pair length

with terminal restriction fraction length polymorphism, reported differences in many terminal restriction fragments in pigs as influenced by the fiber content of the diet. Similarly, Owusu-Asiedu et al. (2006) observed increased ileal populations of enterococci, bifidobacteria and enterobacteria in growing pigs fed diets with 7% guar gum or cellulose. Moreover, guar gum increased the numbers of lactobacilli and clostridia in ileal digesta. Also Metzler (2007), using 16S ribosomal DNA, found enhanced cell counts of bifidobacteria in ileal digesta of growing pigs fed a low-P diet supplemented with 25% lignocellulose, whereas the supplementation of 25% apple-pectin increased the population of the *Bacteroides-Prevotella-Porphyrmonas* group. Obviously, the ileal microbiota is susceptible to changes in the level, source and type of dietary fiber. Using 16S ribosomal RNA gene-based approaches, Konstantinov et al. (2004) reported that addition of fermentable carbohydrates (a mixture of inulin, lactulose, wheat starch and sugar beet pulp) to diets for weaned pigs promoted the growth of specific lactobacilli (*L. amylovorus*-like and *L. reuteri*-like) in ileal digesta. Thus, dietary fiber components may contribute to the rapid stabilization of the microbial community in weaned pigs. Accordingly, the addition of sugar beet pulp to diets of pigs has been previously reported to reduce the population of coliforms (Reid and Hillman, 1999), while other authors confirmed an increased proliferation of pathogenic *Escherichia coli* at the distal ileum of piglets which were fed a diet enriched with highly viscous carboxymethylcellulose (McDonald et al., 2001). Moreover, soluble fiber in form of guar gum has also been associated with the development of swine dysentery (Durmic et al., 1998). Consequently, the selection of different types of dietary fiber should aim to promote beneficial bacteria and to inhibit the growth of potential pathogens.

Dietary fiber provides not only a substrate for small intestinal bacteria, but it may also affect the bacterial colonization of the small intestine by changing small intestinal secretions. For example, bile acids are known to inhibit the growth of various intestinal microbes including lactobacilli and bifidobacteria (Kurdi et al., 2006). Hence, different binding kinetics, re-absorption of bile acids and regulation by the host due to dietary inclusion of dietary fiber, particularly soluble fiber (Ide et al., 1990), may affect the bacterial composition in the distal ileum.

## PHOSPHORUS

### Bacteria and their phosphorus requirement

Phosphorus is essential for bacteria due to its function as a constituent of primary cell metabolites such as nucleotides, co-enzymes, teichoic acids in the cell walls of gram-positive bacteria and phospholipids in the cytoplasmic

and outer membranes of gram-negative bacteria (Durand and Komisarczuk, 1988; Lengeler et al., 1999). In typically composed bacteria, P represents 2 to 3% of dry matter (DM) (Ewing and Cole, 1994), and nucleic acids amount to 80% of total P in bacterial cells (Durand and Komisarczuk, 1988). In addition, excessive P can be stored in the form of polyphosphates in bacterial cells to be used as P and energy source as well (Wood and Clark, 1988). Nevertheless, bacterial proliferation is strongly dependent on a sufficient supply of P. For instance, growth yield of *Bacteroides amylophilus*, an amylolytic and pectinolytic rumen bacterium, can be described as a function of bacterial P availability (Caldwell et al., 1973). Hence, growth yield increases *in vitro* with increasing P amounts in the surrounding medium.

The function of P as coenzyme is essential for bacterial degradation of dietary fiber. In this respect, it has been shown that isolated cellulases from a soil bacterium, *Clostridium acetobutylicum*, have specific P requirements (Lee et al., 1985), which was confirmed by Francis et al. (1978) for cellulases isolated from mixed rumen bacteria. Rumen cellulase activity *in vitro* could be stimulated by increasing the concentration of phosphate from 5 to 50 mM, whereas the cellulase activity did not change when cations (i.e. Ca, Mg, Fe, Zn, Mn, Cu and Co) were added. Moreover, it has been demonstrated for one of the main cellulolytic bacteria species in the rumen, *Bacteroides succinogenes*, that P deficiency will reduce its growth rate. ATP production and endoglucanase activity (Komisarczuk et al., 1988). Thus, the activity of bacterial fibrolytic enzymes is strongly dependent on the supply of available P.

There is growing evidence that the bacterial activity in the GIT of pigs depends on a sufficient dietary supply of P and Ca. In fact, Metzler (2007) reported a trend of lower cellulase activity in feces of pigs fed a low-P diet supplemented with microbial phytase. The author suggests a reduction in bacterial P availability in the LI due to the phytase-mediated enhanced P absorption in the small intestine. Similarly, Johnston et al. (2004) reported increased ileal neutral detergent fiber (NDF) digestibility when pigs were fed a phytase supplemented diet with adequate supply of Ca and P. However, when the same diet, but deficient in Ca and P was fed, no increase in ileal NDF digestibility could be observed. In addition, it is known from *in vitro* studies with rumen bacteria that rumen NDF degradation, production of VFA and bacterial ATP as well as microbial protein synthesis is reduced under P deficient conditions (Komisarczuk et al., 1987a, b; Durand and Komisarczuk, 1988). Moreover, in P deficiency, fermentation of cellulose and pectin is more affected than the fermentation of starch, probably due to higher P requirements of the fibrolytic enzymes and for bacterial growth (Komisarczuk et al., 1987a, b; Wider, 2005). In fact,

a minimal P level of 3 and 4.5 g/kg fermentable organic matter is required for bacterial nitrogen assimilation and cellulose fermentation, respectively (Durand and Komisarczuk, 1988).

Overall, considerable variations in P concentrations of mixed rumen bacteria have been reported, ranging from 6.1 to 19.9 g/kg of DM (e.g. Komisarczuk et al., 1987a; Wider, 2005). Different factors have been identified that may influence the chemical composition of rumen bacteria including dietary forage and concentrate levels, growth phases of bacterial populations (growing, stationary phase and cell lysis) and bacterial composition (Van Nevel and Demeyer, 1977; Merry and McAllan, 1983; Legay-Carmier and Bauchart, 1989; Martin-Ort e et al., 1998). In pigs, the supplementation of a corn-soybean meal based control diet with 25% of lignocellulose, cornstarch or apple-pectin resulted in different amounts of P being assimilated in the fecal mixed bacterial mass (Metzler, 2007). The author attributes these differences to different microbial P needs for the fermentation of cellulose, starch and pectin and/or changes in the microbial composition. Particularly, the inclusion of pectin reduced the P amount in the fecal mixed bacterial mass significantly, from 23 g/kg DM in the control treatment to 13 g/kg DM in the pectin treatment. Increasing the intestinal P availability through addition of monocalcium phosphate to a low-P diet up to 150% of pig's P requirement caused a considerable increase in the P content of the fecal mixed bacterial mass from 22 to 37 g/kg DM (Metzler, 2007). In contrast, phytase supplementation to the low-P diet reduced the P content of the fecal mixed bacterial mass from 22 to 13 g/kg DM. This indicates that the microbial P assimilation depends on the P availability in intestinal digesta. Similarly, there is evidence from studies with rats that higher dietary Ca and P levels may stimulate bacterial growth as indicated by increased excretion of N and P of bacterial origin (Bovee-Oudenhoven et al., 1997b). However, no data on the bacterial P requirements for fermentation in the GIT of non-ruminant animals exist so far.

#### **Interactions between intestinal microbiota, dietary fiber and phosphorus absorption in rats**

Comparative studies in conventional and germfree rats were designed to examine the role of the intestinal microbiota on mineral absorption. According to Andrieux and Sacquet (1983), the small intestinal microbiota had a negative impact on P but a positive effect on Ca and Mg absorption. In the cecum, however, the microbiota stimulated P absorption, but reduced the absorption of Ca and Mg. Moreover, there is growing evidence that there exist interactions between dietary fiber, the activity of the intestinal microbiota and the absorption of minerals (Andrieux and Sacquet, 1986). For example, when lactulose

was added to rat diets, P, Ca and Mg absorption in the cecum of conventional rats was reduced. Moreover, cecal absorption of P was lower in conventional rats compared with germfree rats which was attributed to the existence of microbial activity. Furthermore, in conventional rats, the reduction in cecal P absorption was more pronounced when amylo maize starch rather than non-treated cornstarch was fed. In addition, feeding of inulin, pectin, lactulose and amylo maize starch at dietary inclusion levels of 5-20%, 10%, 10% and 25-50%, respectively, drastically increased the cecal pool of P and Ca in conventional rats (Demigné et al., 1989; Levrat et al., 1991), eventually to fulfil the higher mineral requirement of the microbiota. Moreover, the higher cecal pools of Ca and P during fermentation of dietary fiber may be attributed to the buffering functions of Ca and phosphate in order to compensate for the lower intestinal pH due to presence of fermentation products such as VFA and lactate (Bovee-Oudenhoven et al., 1997a). Calcium forms an insoluble complex with phosphate in the upper small intestine at pH values above 6 (Govers and Van der Meer, 1993). This complex increases the buffering capacity throughout the intestinal lumen (Bovee-Oudenhoven et al., 1997a). Thus, the bioavailability of some minerals, such as Ca and P, has been suggested to be an important modulator of microbial fermentation in the LI of rats. Increasing the dietary Ca-phosphate level reduced not only the cytotoxicity and concentrations of bile acids but it also changed the bile acid composition in ileal digesta of rats (Bovee-Oudenhoven et al., 1999). According to these authors, potential shifts to a less cytotoxic bile acid pool might favor the growth of bile acid-sensitive gram-positive bacteria such as lactobacilli.

#### **Interactions between intestinal microbiota, dietary fiber and phosphorus digestibility and absorption in pigs**

It is generally accepted that the small intestine, particularly the jejunum, is the major site of P absorption in pigs (Breves and Schröder, 1991). With respect to the LI, however, its role in the regulation of P absorption has been discussed controversially. Some investigators reported a secretion of P into the LI (e.g. Partridge, 1978a; Partridge et al., 1986; Larsen and Sandström, 1993), whereas others found substantially higher apparent total tract than ileal P digestibilities (e.g. Den Hartog et al., 1988; Bruce and Sundstøl, 1995; Nortey et al., 2007). Previously, Liu et al. (2000) reported that both the cecum and proximal colon may be involved in maintaining P homeostasis in pigs.

Many dietary factors may influence the digestibility and subsequent absorption of P in the GIT of pigs, such as dietary P and Ca level, composition of the diet, phytate-P content, feeding level and the supply with inorganic P sources (Jongbloed, 1987; Li et al., 1999; Fang et al., 2007; Ruan et al., 2007). Thus, relatively large differences in P

digestibility and absorption have been reported among and within feedstuffs and diets, and potential interactions between dietary fiber and bacterial fermentation as influenced by the P supply of the animal may have contributed to this variation (e.g. Jongbloed, 1987; Larsen and Sandström, 1993; Partanen et al., 2001; Metzler et al., 2006).

According to results of studies by Seynaeve et al. (2000a, b), bacterial P incorporation might reduce small intestinal P absorption in pigs. Despite supplementation of exogenous phytase to a corn-soybean meal based diet, the released phytate-P was not absorbed in the small intestine, but only became available in the LI. Evidence for an interaction between fermentation of dietary fiber and bacterial P assimilation can be derived from results of a study by Bovee-Oudenhoven et al. (1997a). These authors observed in rats fed 10% lactulose a higher fecal excretion of N but also of P of bacterial origin. Accordingly, Mosenthin et al. (1994) reported increased assimilation of N and amino acids in bacterial mass isolated from pig's feces fed 7.5% pectin. As both N and P are required for bacterial growth, it can be speculated that fermentation of dietary fiber may stimulate bacterial P assimilation in the digestive tract of pigs.

Reports on effects of dietary fiber on P digestibility in growing pigs are controversial, and the results obtained are strongly influenced by the type and inclusion level of dietary fiber (Table 2). Bacterial fermentation appears to be an important factor in the regulation of P digestibility and absorption in different segments of the GIT. Increasing the dietary content of cellulose from 3 to 9% tended to enhance apparent ileal P digestibility, but the absorption of P from the LI was largely decreased resulting in significant lower total tract P digestibility (Partridge, 1978b). Similarly, chicory roots inulin tended to depress both apparent ileal and total tract P absorption (Vanhoof and De Shrijver, 1996). This is in accordance with the results obtained by Nortey et al. (2007) who reported linearly reduced apparent ileal and total tract digestibilities of P in pigs fed wheat-based diets with 0%, 20% and 40% of wheat millrun. The authors related this reduction in P digestibility to a combined effect of increased phytate content of the wheat millrun diets, antinutritional effects of dietary fiber, and the limited ability of pigs to digest phytate-P. Moreover, Heijnen and Beynen (1998) reported that supplementation of uncooked and retrograded resistant cornstarch depressed the apparent ileal digestibility of P, but greatly enhanced the P absorption in the LI so that the apparent total tract P digestibility did not differ from the control. It can be speculated that P, bound to the resistant starch, may have been released by microbial activity in the LI. In contrast, Den Hartog et al. (1988) found no differences in apparent ileal and total tract P digestibilities when 5% of cellulose and wheat straw meal

**Table 2.** Effect of dietary fiber and carbohydrates on ileal and total tract P digestibilities in growing pigs

Reference	Initial BW (kg)	Type of dietary fiber and carbohydrates	Inclusion level (%)	P content of diet (%)	Ileal P digestibility (%) <sup>1</sup>	Total tract P digestibility (%) <sup>1</sup>
Partridge, 1978a	30	Starch, sucrose, groundnut meal	-	0.48	61.0	41.0
		Barley, weatings, fish meal	-	0.72	45.0	45.0
		Starch, sucrose, casein	-	0.56	65.0	74.0
Partridge, 1978b	30	Cellulose	3	0.52	64.0 <sup>y</sup>	80.8 <sup>o</sup>
			9	0.52	69.0 <sup>w</sup>	73.8 <sup>b</sup>
Den Hartog et al., 1988	40	Control	0	0.75	27.9 <sup>y</sup>	41.7
		Apple pectin	5	0.71	25.0 <sup>w</sup>	46.0
		CMC-cellulose <sup>2</sup>	5	0.71	28.1	42.1
		Wheat straw meal	5	0.72	25.5	44.1
Vanhoof and De Schrijver, 1996	85	Control	0	0.52	37.1 <sup>y</sup>	32.4 <sup>y</sup>
		Chicory roots inulin	6	0.52	34.0 <sup>w</sup>	28.5 <sup>w</sup>
Heijnen and Beynen, 1998	16	Control	0	0.45	69.0 <sup>o</sup>	75.0
		Uncooked cornstarch	28	0.45	56.0 <sup>b</sup>	69.0
		Retrograded cornstarch	61	0.45	63.0 <sup>ab</sup>	75.0
Partanen et al., 2001	39	Medium-fiber <sup>3</sup>	-	0.61	46.6 <sup>y</sup>	46.7 <sup>y</sup>
		Medium-fiber <sup>3</sup> +Carbadox	-	0.64	46.3 <sup>y</sup>	47.7 <sup>w</sup>
		Medium-fiber <sup>3</sup> +formic acid	-	0.63	49.2 <sup>w</sup>	47.4 <sup>w</sup>
		High-fiber <sup>4</sup>	18	0.68	46.2 <sup>y</sup>	46.5 <sup>y</sup>
		High-fiber <sup>4</sup> +Carbadox	18	0.68	48.2 <sup>w</sup>	48.5 <sup>w</sup>
		High-fiber <sup>4</sup> +formic acid	18	0.68	53.4 <sup>z</sup>	49.9 <sup>w</sup>
Metzler et al., 2006	40	Control	-	0.29	26.2 <sup>acv</sup>	28.1 <sup>a</sup>
		Lignocellulose	25	0.22	30.3 <sup>aw</sup>	25.2 <sup>a</sup>
		Cornstarch	25	0.23	21.1 <sup>bc</sup>	29.7 <sup>a</sup>
		Apple-pectin	25	0.24	19.9 <sup>b</sup>	11.3 <sup>a</sup>
Nortey et al., 2007	36	Wheat control	-	0.64	53.8 <sup>a</sup>	59.5 <sup>a</sup>
		Wheat millrun	20	0.64	40.5 <sup>b</sup>	45.3 <sup>b</sup>
		Wheat millrun	40	0.62	34.8 <sup>c</sup>	42.9 <sup>c</sup>

<sup>1</sup> Apparent digestibility. <sup>2</sup> Carboxymethylcellulose. <sup>3</sup> Medium fiber: 18.9% NDF. <sup>4</sup> High fiber: 21.9% NDF, wheat bran-middlings.

<sup>a, b, c, d</sup> Values in a column and study differ significantly ( $p < 0.05$ ), as given in the studies.

<sup>w, x, z</sup> Values in a column and study tend to differ ( $p < 0.1$ ), as given in the studies.

were supplemented. The authors suggested that the inclusion level of cellulose and wheat straw might have been too low to obtain more pronounced effects. The dietary inclusion of 5% pectin, however, tended to decrease the ileal P digestibility compared with the control. In determining the P absorption in the LI, pectin, wheat straw and cellulose caused a higher net P absorption in the LI amounting to 21%, 19% and 14%, respectively, compared with the control diet. Thus, it can be concluded that microbial breakdown of dietary fiber may improve intestinal P availability.

Partanen et al. (2001) reported differences in the apparent ileal and total tract P digestibilities in pigs fed

medium or high in fiber diets, supplemented with formic acid or Carbadox, compared with feeding medium or high-fiber diets without carbadox as antimicrobial substance. Addition of formic acid tended to improve ileal and total tract P digestibilities at both fiber levels compared with the control. The addition of Carbadox, in turn, tended to enhance apparent total tract P digestibility in the medium-fiber diet and both ileal and fecal P digestibility in the high-fiber diet. As both formic acid and Carbadox have the potential to affect bacterial growth, bacterial P incorporation may have been influenced by changes in the bacterial composition and density in the GIT.

Recently, supplementation of a pig diet with 25%



lignocellulose and apple-pectin resulted in higher fecal than ileal P recoveries (Table 2) suggesting that P was secreted into the lumen of the LI in situations of active fermentation (Metzler et al., 2006). Supplementation of 25% cornstarch, in turn, resulted in P absorption of approximately 9% in the LI. Apparently, the direction of the P movements in the LI largely depends on the source of dietary carbohydrates which, in turn, may reflect differences in bacterial P needs for fermentation. A higher bacterial P requirement for fermentation of complex carbohydrates, however, may affect the P availability and thus the P requirement of the host animal.

### IMPLICATIONS

Dietary fiber has specific effects on the digestive physiology in pigs depending on the type and inclusion level of fiber. Feeding pigs with high-fiber diets has shown to change the composition of the microbial ecosystem in the small and large intestine. Thus, evaluation of different inclusion levels and combinations of dietary fiber may provide new insights in terms of selectively stimulating beneficial bacteria in both the small and large intestine. There is evidence that dietary fiber may change the intestinal P absorption in the pig but further studies are warranted to elucidate the role of microbial fermentation of dietary fiber and its effect on P metabolism in the GIT of pigs. Special attention should be paid to the hypothesis that higher microbial P utilization may reduce the P availability for the host animal.

### REFERENCES

- Andrieux, C. and E. Sacquet. 1983. Effect of microflora and lactose on the absorption of calcium, phosphorus and magnesium in the hindgut of the rat. *Repr. Nutr. Dev.* 23:259-71.
- Andrieux, C. and E. Sacquet. 1986. Effects of amylo maize starch on mineral metabolism in the adult rat: role of the microflora. *J. Nutr.* 116:991-998.
- Bach Knudsen, K. E. 2001. The nutritional significance of "DF" analysis. *Anim. Feed Sci. Technol.* 90:3-20.
- Bach Knudsen, K. E. and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. I. Digestibility and bulking properties of polysaccharides and other major constituents. *Br. J. Nutr.* 70:537-556.
- Bach Knudsen, K. E., B. B. Jensen and I. Hansen. 1991. Gastrointestinal implications in pigs of wheat and oat fractions. 2. Microbial activity in the gastrointestinal tract. *Br. J. Nutr.* 65:233-248.
- Barrera, M., M. Cervantes, W. C. Sauer, A. B. Araiza, N. Torrentera and M. Cervantes. 2004. Ileal amino acid digestibility and performance of growing pigs fed wheat-based diets supplemented with xylanase. *J. Anim. Sci.* 82:1997-2003.
- Bovee-Oudenhoven, I. M. J., D. S. M. L. Termont, P. J. Heidt and R. Van der Meer. 1997a. Increasing the intestinal resistance of rats to the invasive pathogen *Salmonella enteritidis*: additive effects of dietary lactulose and calcium. *Gut* 40:497-504.
- Bovee-Oudenhoven, I. M. J., D. S. M. L. Termont, A. H. Weerkamp, M. A. W. Faassen-Peters and R. Van der Meer. 1997b. Dietary calcium inhibits the intestinal colonization and translocation of *Salmonella* in rats. *Gastroenterol.* 113:550-557.
- Bovee-Oudenhoven, I. M., M. L. Wissink, J. T. Wouters and R. Van der Meer. 1999. Dietary calcium phosphate stimulates intestinal lactobacilli and decreases the severity of a *Salmonella* infection in rats. *J. Nutr.* 129:607-612.
- Breves, G. and B. Schröder. 1991. Comparative aspects of gastrointestinal phosphorus metabolism. *Nutr. Res. Rev.* 4:125-140.
- Bruce, J. A. M. and F. Sundstol. 1995. The effect of microbial phytase in diets for pigs on apparent ileal and faecal digestibility, pH and flow of digesta measurements in growing pigs fed a high-fibre diet. *Can. J. Anim. Sci.* 75:121-127.
- Caldwell, D. R., M. Keeney, J. S. Baron and J. F. Kelley. 1973. Sodium and other inorganic growth requirements of *Bacteroides amylophilus*. *J. Bacteriol.* 114:782-789.
- Canibe, N., O. Højberg, S. Højsgaard and B. B. Jensen. 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. *J. Anim. Sci.* 83:1287-1302.
- Cherbut, C., E. Albina, M. Champ, J. L. Doublier and G. Lecannu. 1990. Action of guar gums on the viscosity of digestive contents and on the gastrointestinal motor function in pigs. *Digestion* 46:205-213.
- Demigné, C., M.-A. Levrat and C. Rémésy. 1989. Effects of feeding fermentable carbohydrates on the cecal concentrations of minerals and their fluxes between the cecum and blood plasma in the rat. *J. Nutr.* 119:1625-1630.
- Den Hartog, L. A., J. Huisman, W. J. G. Thielen, G. H. A. Van Schayk, H. Boer and E. J. Weerden. 1988. The effect of including various structural polysaccharides in pig diets on ileal and faecal digestibility of amino acids and minerals. *Livest. Prod. Sci.* 18:157-170.
- Dierick, N. A., I. J. Vervaeke, D. I. Demeyer and J. A. Decuyper. 1989. Approach to the energetic importance of DF digestion in pigs. I. Importance of fermentation in the overall energy supply. *Anim. Feed Sci. Technol.* 23:141-167.
- Doerner, K. C. and B. A. White. 1990. Assessment of the endo-1,4- $\beta$ -glucanase components of *Ruminococcus flavefaciens* FD-1. *Appl. Environ. Microbiol.* 56:1844-1850.
- Dongowski, G., A. Lorenz and J. Proll. 2002. The degree of methylation influences the degradation of pectin in the intestinal tract of rats *in vitro*. *J. Nutr.* 132:1935-1944.
- Durand, M. and S. Komisarczuk. 1988. Influence of major minerals on rumen microbiota. *J. Nutr.* 118:249-260.
- Durmick, Z., D. W. Pethick, J. K. Pluske and D. J. Hampson. 1998. Changes in bacterial populations in the colon of pigs fed different sources of DF, and the development of swine dysentery after experimental infection. *J. Appl. Microbiol.* 85:574-582.
- Durmick, Z., D. W. Pethick, B. P. Mulan, J. M. Accioly, H. Schulze and D. J. Hampson. 2002. Evaluation of large-intestinal parameters associated with dietary treatments designed to reduce the occurrence of swine dysentery. *Br. J. Nutr.* 88:159-

- 169.
- Ewing, W. N. and D. J. A. Cole. 1994. The living gut. Context Publications, Dungannon, UK.
- Fan, M. Z. and E. J. Squires. 2003. Manipulation of hindgut fermentation to reduce the excretion of selected odor-causing compounds in pig manure. Final project report-supported by Canadian Pork Council (CPC), and the Agriculture, Agri-Food Canada (AAFC) Multiple Partners' Hog Environmental Management Strategy (HEMS) Program. Alberta, Canada.
- Fang, R. J., T. J. Li, F. G. Yin, Y. L. Yin, X. F. Kong, K. N. Wang, Z. Yuan, G. Y. Wu, J. H. He, Z. Y. Deng and M. Z. Fan. 2007. The additivity of true or apparent phosphorus digestibility values in some feed ingredients for growing pigs. *Asian-Aust. J. Anim. Sci.* 20:1092-1099.
- Francis, G. L., J. M. Gawthorne and G. B. Storer. 1978. Factors affecting the activity of cellulases isolated from the rumen digesta of sheep. *Appl. Environ. Microbiol.* 36:643-649.
- Gardner, R. M., K. C. Doerner and B. A. White. 1987. Purification and characterization of an  $\text{exo-}\beta\text{-1,4-glucanase}$  from *Ruminococcus flavefaciens* FD-1. *J. Bacteriol.* 169:4581-4588.
- Govers, M. J. A. P. and R. van der Meer. 1993. Effects of dietary calcium and phosphate on the intestinal interactions between calcium, phosphate, fatty acids, and bile acids. *Gut* 34:365-370.
- Graham, H., K. Hesselman and P. Aman. 1986. The influence of wheat bran and sugar-beet pulp on the digestibility of dietary components in a cereal-based pig diet. *J. Nutr.* 116:242-251.
- Grieshop, C. M., D. E. Reese and G. C. Fahey, Jr. 2001. Nonstarch polysaccharides and oligosaccharides in swine nutrition. In: *Swine Nutrition* (Ed. A. J. Lewis and L. L. Southern). CRC Press, Boca Raton, Florida, USA. pp. 107-130.
- Hedemann, M. S., M. Eskildsen, H. N. Lærke, C. Pedersen, J. E. Lindberg, P. Laurinen and K. E. Bach Knudsen. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. *J. Anim. Sci.* 84:1375-1386.
- Heijnen, M.-L. and A. Beynen. 1998. Effect of consumption of uncooked (RS2) and retrograded (RS3) resistant starch on apparent absorption of magnesium, calcium, and phosphorus in pigs. *Z. Ernährungswiss.* 37:13-17.
- Henriksson, A., L. Andre and P. L. Conway. 1995. Distribution of lactobacilli in the porcine gastrointestinal tract. *FEMS Microbiol. Ecol.* 16:55-60.
- Hill, J. E., S. M. Hemmingsen, B. G. Goldade, T. J. Dumonceaux, J. Klassen, R. T. Zijlstra, S. H. Goh and A. G. van Kessel. 2005. Comparison of ileum microflora of pigs fed corn-, wheat-, or barley-based diets by chaperonin-60 sequencing and quantitative PCR. *Appl. Environ. Microbiol.* 71:867-875.
- Högberg, A., J. E. Lindberg, T. Leser and P. Wallgren. 2004. Influence of cereal non-starch polysaccharides on ileo-caecal and rectal microbial populations in growing pigs. *Acta Vet. Scand.* 45:87-98.
- Huang, R. L., Y. L. Yin, K. P. Wang, T. J. Li and J. X. Liu. 2003. Nutritional value of fermented and not fermented material of distiller's grains in pig nutrition. *J. Anim. Feed Sci.* 12:261-269.
- Ide, T., M. Horii, T. Yamamoto and K. Kawashima. 1990. Contrasting effects of water-soluble and water-insoluble dietary fibers on bile acid conjugation and taurine metabolism in the rat. *Lipids* 25:335-340.
- Jensen, B. B. 2001. Possible ways of modifying type and amount of products from microbial fermentation in the gut. In: *Gut environment of pigs* (Ed. A. Piva, K. E. Bach Knudsen and J. E. Lindberg). Nottingham University Press, Nottingham, UK. pp. 181-199.
- Jensen, B. B. and H. Jørgensen. 1994. Effect of DF on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. *Appl. Environ. Microbiol.* 60:1897-1904.
- Jensen, N. S. and E. Canale-Parola. 1985. Nutritionally limited pectinolytic bacteria from the human intestine. *Appl. Environ. Microbiol.* 50:172-173.
- Jin, L., L. P. Reynolds, D. A. Redmer, J. S. Caton and J. D. Crenshaw. 1994. Effects of dietary fibre on intestinal growth, cell proliferation, and morphology in growing pigs. *J. Anim. Sci.* 72:2270-2278.
- Johnston, S. L., S. B. Williams, L. L. Southern, T. D. Bidner, L. D. Bunting, J. O. Matthews and B. M. Olcott. 2004. Effect of phytase addition and dietary calcium and phosphorus levels on plasma metabolites and ileal and total-tract nutrient digestibility in pigs. *J. Anim. Sci.* 82:705-714.
- Jongbloed, A. W. 1987. Phosphorus in the feeding of pigs: Effect of diet on the absorption and retention of phosphorus by growing pigs. PhD, University of Lelystad, Lelystad, The Netherlands.
- Jørgensen, H., X.-Q. Zhao and B. Eggum 1996. The influence of DF and environmental temperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *Br. J. Nutr.* 75:365-378.
- Komisarczuk, S., M. Durand, P. Beaumatin and G. Hannequart. 1987a. Effects of phosphorus deficiency on rumen microbial activity associated with the solid and liquid phases of a fermentor (Rusitec). *Repr. Nutr. Dev.* 27:907-919.
- Komisarczuk, S., R. J. Merry and A. B. McAllan. 1987b. Effect of different levels of phosphorus on rumen microbial fermentation and synthesis determined using a continuous culture technique. *Br. J. Nutr.* 57:279-290.
- Komisarczuk, S., G. Gaudet, G. Hannequart, G. Fonty and M. Durand. 1988. Effects of a sub-deficiency in phosphorus on some aspects of cellulolytic activity of *Bacteroides succinogenes*. *Repr. Nutr. Dev.* 28:79-80.
- Konstantinov, S. R., A. Awati, H. Smidt, B. A. Williams, A. D. L. Akkermans and W. M. de Vos. 2004. Specific response of a novel and abundant *Lactobacillus amylophilus*-like phylotype to dietary prebiotics in the guts of weaning piglets. *Appl. Environ. Microbiol.* 70:3821-3830.
- Konstantinov, S. R., E. Poznanski, S. Fuentes, A. D. L. Akkermans, H. Smidt and W. M. de Vos. 2006. *Lactobacillus sobrius* sp. nov., abundant in the intestine of weaning piglets. *Int. J. Syst. Evol. Microbiol.* 56:29-32.
- Kornegay, E. T. and R. J. Moore. 1986. Dietary fiber sources may affect mineral use in swine. *Feedstuffs* 58:36-49.
- Kurdi, P., H. W. van Veen, H. Tanaka, I. Mierau, W. N. Konings, G. W. Tannock, F. Tomita and A. Yokota. 2000. Cholic acid is accumulated spontaneously, driven by membrane  $\Delta\text{pH}$ , in many lactobacilli. *Appl. Environ. Microbiol.* 182:6525-6528.
- Larsen, T. and B. Sandström. 1993. Effect of dietary calcium level on mineral and trace element utilization from a rapeseed

- (*Brassica napus* L.) diet fed to ileum-fistulated pigs. *Br. J. Nutr.* 69:211-224.
- Lee, S. F., W. Forsberg and L. N. Gibbins. 1985. Cellulolytic activity of *Clostridium acetobutylicum*. *Appl. Environ. Microbiol.* 50:220-228.
- Legay-Carnier, F. and D. Bauchart. 1989. Distribution of bacteria in the rumen contents of dairy cows given a diet supplement with soya-bean oil. *Br. J. Nutr.* 61:725-740.
- Lengeler, J. W., G. Drews and H. G. Schlegel. 1999. Biology of the prokaryotes. Thieme, Stuttgart, Germany.
- Leser, T. D., R. H. Lindecrone, T. K. Jensen, B. B. Jensen and K. Møller. 2000. Changes in bacterial community structure in the colon of pigs fed different experimental diets and after infection with *Brachyspira hyodysenteriae*. *Appl. Environ. Microbiol.* 66:3290-3296.
- Leser, T. D., J. Z. Amenuvor, T. K. Jensen, R. H. Lindecrone, M. Boye and K. Møller. 2002. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl. Environ. Microbiol.* 68:673-690.
- Levrat, M.-A., C. Rémésy and C. Demigné. 1991. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. *J. Nutr.* 121:1730-1737.
- Li, D., X. R. Che, Y. Q. Wang, S. Y. Qiao, W. Johnson and P. Thacker. 1999. The effect of calcium level on microbial phytase activity and nutrient balance in swine. *Asian-Aust. J. Anim. Sci.* 12:197-202.
- Liu, J., D. W. Bollinger, D. R. Ledoux and T. L. Veum. 2000. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. *J. Anim. Sci.* 78:106-109.
- Loh, G., M. Eberhard, R. M. Brunner, U. Hennig, S. Kuhla, B. Kleesen and C. C. Metges. 2006. Inulin alters the intestinal microbiota and short-chain fatty acid concentrations in growing pigs regardless of their basal diet. *J. Nutr.* 136:1198-1202.
- Martin-Ortúe, S. M., J. Balcells, F. Zakraoui and C. Castrillo. 1998. Quantification and chemical composition of mixed bacteria harvested from solid fractions of rumen digesta: effect of detachment procedure. *Anim. Feed Sci. Technol.* 71:269-282.
- Matsuura, Y. 1991. Pectic acid degrading enzymes from human faeces. *Agric. Biol. Chem.* 55:885-886.
- McCarthy, R. E., S. F. Kotarski and A. A. Salyers. 1985. Location and characteristics of enzymes involved in the breakdown of polygalacturonic acid by *Bacteroides thetaiotaomicron*. *J. Bacteriol.* 161:493-499.
- McDonald, D. E., D. W. Pethick, B. P. Mullan and D. J. Hampson. 2001. Increasing viscosity of the intestinal contents alters small intestinal structure and intestinal growth, and stimulates proliferation of enterotoxigenic *Escherichia coli* in newly-weaned pigs. *Br. J. Nutr.* 86:487-498.
- Merry, R. J. and A. B. McAllan. 1983. A comparison of the chemical composition of mixed bacteria harvested from the liquid and solid fraction of rumen bacteria. *Br. J. Nutr.* 50:701-709.
- Metzler, B., T. Baumgärtel, M. Rodehutschord and R. Mosenthin. 2006. Fermentable carbohydrates affect the chemical composition of the faecal mixed bacterial mass, microbial activity and P metabolism in the large intestine of pigs. In: International Conference on Sustainable Animal Health through Eubiosis - Relevance for Man (Ed. C. Wenk and O. Simon), pp. 27 and CD-Rom, Ascona, Switzerland.
- Metzler, B. U. 2007. Effects of fermentable carbohydrates and dietary P supply on bacterial P incorporation, activity and composition. PhD, University of Hohenheim, Stuttgart Germany.
- Montagne, L., J. R. Pluske and D. J. Hampson. 2003. A review of interactions between DF and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol.* 108:95-117.
- Morales, J., J. F. Pérez, S. M. Martín-Ortúe, M. Fondevila and J. Gasa. 2002. Large bowel fermentation of maize or sorghum-acorn diets fed as a different source of carbohydrates to Landrace and Iberian pigs. *Br. J. Nutr.* 88:489-497.
- Moore, W. E. C., L. V. H. Moore, E. P. Cato, T. D. Wilkins and E. T. Kornegay. 1987. Effect of high-fiber and high-oil diets on the fecal flora of swine. *Appl. Environ. Microbiol.* 53:1638-1644.
- Mosenthin, R., W. C. Sauer, H. Henkel, F. Ahrens and C. F. M. de Lange. 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. *J. Anim. Sci.* 70:3467-3472.
- Mosenthin, R., W. C. Sauer and F. Ahrens. 1994. Dietary pectin's effect on ileal and fecal amino acid digestibility and exocrine pancreatic secretions in growing pigs. *J. Nutr.* 124:1222-1229.
- Nortey, T. N., J. F. Patience, P. H. Simmins, N. L. Trottier and R. T. Zijlstra. 2007. Effects of individual or combined xylanase and phytase supplementation on energy, amino acid, and phosphorus digestibility and growth performance of grower pigs fed wheat-wheat based diets containing wheat millrun. *J. Anim. Sci.* 85:1432-1443.
- Ohmiya, K., M. Shimizu, M. Taya and S. Shimizu. 1982. Purification and properties of cellobiosidase from *Ruminococcus albus*. *J. Bacteriol.* 150:407-409.
- Olano-Martin, E., G. R. Gibson and R. A. Rastall. 2002. Comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. *J. Appl. Microbiol.* 93:505-511.
- Owusu-Asiedu, A., J. F. Patience, B. Laarveld, A. G. van Kessel, P. H. Simmins and R. T. Zijlstra. 2006. Effects of guar gum and cellulose on digesta passage rate, ileal microbiota, energy and protein digestibility, and performance of grower pigs. *J. Anim. Sci.* 84:843-852.
- Partanen, K., T. Jalava, J. Valaja, S. Perttilä, H. Siljander-Rasi and H. Lindeberg. 2001. Effect of dietary carbadox or formic acid and fibre level on ileal and faecal nutrient digestibility and microbial metabolite concentrations in ileal digesta of the pig. *Anim. Feed Sci. Technol.* 93:137-155.
- Partridge, I. G. 1978a. Studies on digestion and absorption in the intestines of growing pigs. 3. Net movements of mineral nutrients in the digestive tract. *Br. J. Nutr.* 39:527-537.
- Partridge, I. G. 1978b. Studies on digestion and absorption in the intestines of growing pigs. 4. Effects of dietary cellulose and sodium levels on mineral absorption. *Br. J. Nutr.* 39:539-545.
- Partridge, I. G., O. Simon and H. Bergner. 1986. The effects of treated straw meal on ileal and faecal digestibility of nutrients in pigs. *Arch. Anim. Nutr.* 36:351-359.
- Pié, S., A. Awati, S. Vida, I. Falluel, B. A. Williams and I. P.

- Oswald. 2007. Effects of added fermentable carbohydrates in the diet on intestinal proinflammatory cytokine-specific mRNA content in weaning piglets. *J. Anim. Sci.* 85:673-683.
- Pryde, S. E., A. J. Richardson, C. S. Stewart and H. J. Flint. 1999. Molecular analysis of the microbial diversity present in the colonic wall, colonic lumen, and caecal lumen of a pig. *Appl. Environ. Microbiol.* 65:5372-5377.
- Reid, C. A. and K. Hillman. 1999. The effect of retrogradation and amylose/amylopectin ratio on starches and carbohydrates fermentation and microbial populations in the porcine colon. *Anim. Sci.* 68:503-510.
- Rémésy, C., M.-A. Levrat, L. Gamet and C. Demigné. 1993. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. *Am. J. Physiol.* 264:G855-G862.
- Roediger, W. E. W. 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 21:793-798.
- Ruan, Z., Y.-G. Zhang, Y.-L. Yin, R. L. Huang, S. W. Kim, G. Y. Wu and Z. Y. Deng. 2007. Dietary requirement of dtrue digestible phosphorus and total calcium for growing pigs. *Asian-Aust. J. Anim. Sci.* 20:1236-1242.
- Russell, E. G. 1979. Types and distribution of anaerobic bacteria in the large intestine of pigs. *Appl. Environ. Microbiol.* 37:187-193.
- Sakata, T. and H. Setoyama. 1995. Local stimulatory effect of short-chain fatty acids on the mucus release from the hindgut mucosa of rats (*Rattus norvegicus*). *Comp. Biochem. Physiol.* 111:429-432.
- Salanitro, J. P., I. G. Blake and P. A. Muirhead. 1977. Types and distribution of anaerobic bacteria in the large intestine of pigs. *Appl. Environ. Microbiol.* 37:187-193.
- Savage, D. C. 1986. Gastrointestinal microflora in mammalian nutrition. *Annu. Rev. Nutr.* 6:155-178.
- Schneeman, B. O. 1987. Dietary fiber and gastrointestinal function. *Nutr. Rev.* 45:129-132.
- Seynaeve, M., G. Janssen, M. Hesta, C. van Nevel and R. O. Wilde. 2000a. Effects of dietary Ca/P ratio, P level and microbial phytase supplementation on nutrient digestibilities in growing pigs: prececal, post-ileal and total tract disappearances of OM, P and Ca. *J. Anim. Physiol. Anim. Nutr.* 83:36-48.
- Seynaeve, M., G. Janssen, M. Hesta, C. van Nevel and R. O. Wilde. 2000b. Effects of dietary Ca/P ratio, P level and microbial phytase supplementation on nutrient digestibilities in growing pigs: breakdown of phytic acid, partition of P and phytase activity along the intestinal tract. *J. Anim. Physiol. Anim. Nutr.* 83:193-204.
- Shi, B. M., A. S. Shan and J. M. Tong. 2001. Influence of dietary oligosaccharides on growth performance and intestinal microbial populations of piglets. *Asian-Aust. J. Anim. Sci.* 14:1747-1751.
- Shim, S. B., J. M. A. J. Verdonk, W. F. Pellikaan and M. W. A. Verstegen. 2007. Differences in microbial activities of faeces from weaned and unweaned pigs in relation to *in vitro* fermentation of different sources of inulin-type oligofructose and pig feed ingredients. *Asian-Aust. J. Anim. Sci.* 20:1444-1452.
- Slominski, B. A., D. Boros, L. D. Campbell, W. Guenter and O. Jones. 2004. Wheat by-products in poultry nutrition. Part I. Chemical and nutritive composition of wheat screenings, bakery by-products and wheat mill run. *Can. J. Anim. Sci.* 84:421-428.
- Spiehs, M. J., M. H. Whitney and G. C. Shurson. 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *J. Anim. Sci.* 80:2639-2645.
- Theander, O., P. Aman, E. Westerlund and H. Graham. 1994. Enzymatic/chemical analysis of DF. *J. AOAC Int.* 77:703-709.
- Tungland, B. C. and D. Meyer. 2002. Nondigestible oligo- and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety* 1:73-92.
- Vahjen, W., D. Taras and O. Simon. 2007. Effect of the probiotic *Enterococcus faecium* NCIMB10415 on cell numbers of total *Enterococcus* spp., *E. faecium* and *E. faecalis* in the intestine of piglets. *Curr. Issues Intest. Microbiol.* 8:1-8.
- Vanhooft, K. and R. de Shrijver. 1996. Availability of minerals in rats and pigs fed non-purified diets containing inulin. *Nutr. Res.* 16:1017-1022.
- Van Nevel, C. J. and D. I. Demeyer. 1977. Determination of rumen microbial growth *in vitro* from <sup>32</sup>P-labelled phosphate incorporation. *Br. J. Nutr.* 38:101-114.
- Van Soest, P. J. 1984. Some physical characteristics of DFs and their influence on the microbial ecology of the human colon. *Proc. Nutr. Soc.* 43:25-33.
- Varel, V. H. and W. G. Pond. 1985. Enumeration and activity of cellulolytic bacteria from gestating swine fed various levels of DF. *Appl. Environ. Microbiol.* 49:858-862.
- Varel, V. H. and J. T. Yen. 1997. Microbial perspective on fibre utilization by swine. *J. Anim. Sci.* 75:2715-2722.
- Varel, V. H., W. G. Pond, J. C. Pekas and J. T. Yen. 1982. Influence of high-fiber diet on bacterial populations in gastrointestinal tracts of obese- and lean-genotype pigs. *Appl. Environ. Microbiol.* 44:107-112.
- Varel, V. H., S. J. Fryda and I. M. Robinson. 1984. Cellulolytic bacteria from pig large intestine. *Appl. Environ. Microbiol.* 47:219-221.
- Varel, V. H., I. M. Robinson and H.-J. G. Jung. 1987. Influence of DF on xylanolytic and cellulolytic bacteria of adult sows. *Appl. Environ. Microbiol.* 53:22-26.
- Varel, V. H., R. S. Tanner and C. R. Woese 1995a. *Clostridium herbivorans* sp. nov., a cellulolytic anaerobe from the pig intestine. *Int. J. Syst. Bacteriol.* 45:490-494.
- Varel, V. H., J. T. Yen and K. K. Kreikemeier. 1995b. Addition of cellulolytic clostridia to the bovine rumen and pig intestinal tract. *Appl. Environ. Microbiol.* 61:1116-1119.
- Varga, G. A. and E. S. Kolver. 1997. Microbial and animal limitations to fiber digestion and utilization. *J. Nutr.* 127: 819S-823S.
- Wang, J. F., M. Wang, D. G. Lin, B. B. Jensen and Y. H. Zhu. 2006. The effect of source of dietary fiber and starch on ileal and fecal amino acid digestibility in growing pigs. *Asian-Aust. J. Anim. Sci.* 19:1040-1046.
- Wenk, C. 2001. The role of DF in the digestive physiology of the pig. *Anim. Feed Sci. Technol.* 90:21-33.
- Wider, J. 2005. Untersuchungen *in vitro* zum Phosphor-Bedarf von Mikroorganismen im Pansen. PhD, University of Bonn, Bonn, Germany.

- Wood, H. G. and J. E. Clark. 1988. Biological aspects of inorganic polyphosphates. *Annu. Rev. Biochem.* 57:235-360.
- Yen, J. T., J. A. Nienaber, D. A. Hill and W. G. Pond. 1991. Potential contribution of absorbed volatile fatty acids to whole-animal energy requirement in conscious swine. *J. Anim. Sci.* 69:2001-2012.
- Yin, Y. L., Z. Y. Deng, H. L. Huang, H. Y. Zhong, Z. P. Hou, J. Gong and Q. Liu. 2004. Nutritional and health functions of carbohydrate for pigs. *J. Anim. Feed Sci.* 13:523-538.
- Zijlstra, R. T., C. F. M. de Lange and J. F. Patience. 1999. Nutritional value of wheat for growing pigs: Chemical composition and digestible energy content. *Can. J. Anim. Sci.* 79:187-194.