

Effects of prebiotics, probiotics and synbiotics in the diet of young pigs

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Effects of prebiotics, probiotics and synbiotics in the diet of young pigs

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ABSTRACT

Effects of prebiotics, probiotics and synbiotics in the diet of young pigs

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Prebiotics are non-digestible carbohydrates that are not metabolized in the small intestine and fermented in the large intestine. Oligofructose are non-digestible oligosaccharides which may stimulate beneficial bacteria in the gut and may affect the gut ecosystem. Prebiotic effects will depend largely on their chemical structure (degree of polymerization). Dietary inclusion of probiotics in young pig diets may beneficially affect gut microbiota. Synbiotics, a combination of prebiotics and probiotics may also stimulate the gut ecosystem. The objective of this thesis was to evaluate the effects of pre-, pro- and synbiotics on the gut ecosystem, and some performance parameters. A series of *in vivo* and *in vitro* experiments were carried out using suckling and weaned piglets. The experimental results are discussed in this thesis. Overall, it was concluded that synbiotics, a combination of multi-strain probiotics and oligofructose, can positively affect performance especially feed intake, and can improve the gut health. However, we did not observe a clear synergistic effect compared to supplementing oligofructose or probiotics alone. A combination of high and low polymer inulin will probably be more beneficial for the intestinal ecosystem and health than using either high- or low polymer inulin alone. The present studies show that the pre-, pro- and synbiotic treatments affect gut microbiota and performance of young pigs.

Keywords: prebiotics, piglets, gut health

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Chapter 1

General Introduction

In many countries, piglets are usually weaned at 3 to 4 weeks of age. At the time of weaning, young piglets are subjected to several stressors such as nutritional, environmental, social and microbial unbalance (Fraser et al., 1998; Nabuurs, 1998). As a result, low feed intake, impaired intestinal morphology and function (Pluske et al., 1995), a high incidence of diarrhea and growth depression are commonly seen immediately after weaning (Van Beers-Schreurs, 1996; Pluske et al., 1997). So weaning is regarded as the most critical period in a pig's life. To help in avoiding problems with adjusting to a different diet after weaning, diets with growth promoting antibiotics are commonly used in both the creep and weaner diet. However, the use of in-feed antibiotics will be banned by the EU from 2006 onward because there is an increasing evidence of microorganism becoming resistant to antibiotics in both animal and human (Cohen, 1992; Newman, 2002). Thus, the development of alternatives to antibiotics growth promoters is urgently needed in commercial pig production. Prebiotics and probiotics, alone or in combination (synbiotics), may be a potential alternative for antibiotic growth promoters.

Prebiotics are defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995). Recently, some researches (Houdijk et al., 1998; Hillman, 2001) have been conducted to manipulate beneficial bacteria in gastrointestinal tract (GIT). Bezkerovainy, (2001) suggested that the use of prebiotics is a promising approach for enhancing the role of endogenous beneficial organisms in the gut. They can be used as potential alternatives to growth promoting antibiotics (Hartemink, 1995). Several reports have shown that supplementing a diet with oligofructose (OF) improved growth in nursery pigs (Chang et al., 2000; Xuan et al., 2001) and in weaned pigs (Bunce et al, 1995b) while other reports (Houdijk et al., 1998; Orban et al., 1997) did not find growth effect in young pigs. The reasons for the different results are not clear yet. It may be due to the different chemical structure (degree of polymerization, DP) and compositions of the OF used. Roberfroid et al (1998) reported that the site in the gut of pigs where the fermentation of OF occurs depend on the molecular structure of the non-digestible carbohydrates. Bifidobacteria may preferentially utilize non-digestible oligosaccharides with a lower DP, whereas bacteroides degrade preferentially oligosaccharides with a higher DP (van Laere et al., 1997). Thus, it was hypothesized that OF with a low DP is (DP= less than 10), may be more beneficial than FOS with a high DP (DP=10~60) for the development of bifidobacteria in the large intestine of young pigs. It appears that the places of fermentation in the small intestine and large intestine will determine the conditions in these parts of the GIT.

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its microbial balance (Fuller, 1989). Probiotics have been

reported to increase feed intake, growth (Lessard and Brisson, 1987), immune responses (Isolauri et al, 1995), the numbers of lactobacilli and decrease the numbers of *E. coli* (Xuan et al., 2001; Deprez et al, 1986). However, experiments have failed to show consistent and beneficial responses (NRC, 1998). Most of probiotic studies that were reported in the literatures used single or two strains probiotics rather than multi-strains bacteria. But Rolfe (2000) suggested that multiple probiotic strains may be more useful than a single strain because they can proliferate more lactic acid bacteria than single strain of probiotic.

A concept of synbiotic, a combination of pre- and probiotics components has been proposed to characterize health enhancing foods and supplements used as functional food ingredients in humans (Gibson and Roberfroid, 1995). A few studies have shown that feeding a diet with synbiotics to young pigs increased lactobacillus and bifidobacterium compared to prebiotics and probiotics alone (Nemcová et al, 1999). It also decreased mortality rate (Nousiainen and Setälä, 1993) in piglets. It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut. However, there is little information on synbiotics and its possible mechanisms in young pigs. The overall hypothesis tested was that dietary OF with a low DP and multi probiotics to either weaner or creep feed will improve gut health and ecosystem, consequently improving growth performance. The synbiotics, a combination of prebiotics and probiotics (multi strains) will have more beneficial effects on gut health and growth than using either pre- or probiotics alone.

The scope of the present thesis was as follows:

The major objective of this thesis was to investigate the mode of action of pre-, probiotics and synbiotics on the gastrointestinal ecosystem, health and performance effects of pigs around weaning. To this aim a number of studies were made to investigate traits in the chyme and morphology of pigs after inclusion of OF, probiotics and synbiotics.

- 1) To study the effects of inclusion of OF in the diet on pH, volatile fatty acids and ammonia concentrations in the digesta. In addition, it was aimed to measure small intestinal morphology in weaned pigs with and without OF.
- 2) To investigate the possible impact of supplementing diets with OF on mucosal enzyme activities, ileal protein digestibility and digesta viscosity.
- 3) To study the effects of antibiotic-free diets with OF, multi-strain probiotics or their synergistic effects (a synbiotics) on hematological traits, nutrient digestibilities and ammonia concentrations of weaned pigs.
- 4) To investigate the effects of supplementing antibiotic-free diets with a synbiotics (combining OF and multi-strain probiotics) in preweaning piglet on changes of microflora, gut structure, VFA, immune responses and weaning weight.
- 5) To study the fermentation characteristics of different sources (DP) of OF with different inocula from weaned and unweaned pigs using the in vitro cumulative gas production technique.

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Chapter 2

Application of inulin–type fructans in animal feed and pet food

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Abstract

The inulin-type fructans are non-digestible oligosaccharides that are fermented in the gastrointestinal tract of farm animals and pets. This review focuses on the various effects of inulin-type fructans in pigs, poultry, calves and companion animals. Effects of the inulin-type fructans on gut microflora, digestion, and availability of nutrients, gut morphology, fermentation characteristics and animal performance are discussed. Inulin-type fructans can support animal performance and health by affecting nutrient digestion, gut microflora and gut morphology. However, results are varying depending on composition of the basal diet, inclusion level, type of fructan, adaptation period and experimental hygienic conditions.

Inulin-type fructans: Feed and pet food: Performance and health

Introduction

For several decades, antibiotics and chemotherapeutics in prophylactic doses have been used in animal feed to improve animal welfare and to obtain economic benefits in terms of improved animal performance and reduced medication costs. However, there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistance in pathogenic bacteria in both humans and livestock linked to the therapeutic and subtherapeutic use of antibiotics in livestock and pets. The European Union has banned all in-feed use of antibiotics from 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. This perspective has stimulated nutritionists and feed manufacturers to search for new and safe alternatives. The primary alternatives studied include; acidification of the feed by organic acids, feeding probiotic organisms and feeding prebiotic compounds.

In the 1980's the possible potential effects of prebiotics in animal feeds was already recognized. Since then the interest in the use of prebiotics in animal feed and pet food has resulted in a high research activity. The use of prebiotics in diets for farm animals and pets has been documented by Mul & Perry (1994 farm and pet animals), Houdijk (1998, swine), Iji & Tivey (1998; 1999, poultry), Flickinger & Fahey (2002, pets, poultry, swine and rabbits) and Patterson & Burkholder (2003, swine). The non-digestible inulin-type fructans are found widely in many vegetable feed and food ingredients and are perhaps the most well studied and documented prebiotics in domesticated animals (Flickinger et al. 2003a).

The aim of this review is to provide an overview of recent developments on the use and application of inulin-type fructans in livestock feed and pet food including: effects on intake, digestion, and availability of nutrients; gut microflora and morphology; immunity and health; and performance in farm animals and pets.

Application of inulin-type fructans in diets for pigs (Table 1).

Weaning is a stressful event for pigs. Under commercial conditions, weaning piglets often face nutritional, social and psychological stress. As a result, abrupt weaning is typically accompanied by low feed intake. Weaning also causes morphological and histological changes of the small intestine of pigs resulting in maldigestion and malabsorption. When feed intake increases, enterotoxemic bacteria may proliferate causing diarrhea. Diarrhea frequently occurs after the weaning transition (Nabuurs, 1991). Supplementing inulin-type fructans to weaning diets may be a practical strategy to reduce weaning related transition of intestinal microflora by supporting beneficial bacteria such as bifidobacteria and lactobacilli and thereby decreasing intestinal pathogens like *Escherichia coli*.

Patterson & Burkholder (2003) and Flickinger et al. (2003) summarized several experiments in which different types of fructans and other prebiotics were supplied to solid feed, formula or drinking water to pigs alone or in combination with a probiotic. The effects of inulin-type fructans were categorized in the effect on performance; availability, digestion and retention of nutrients; gut microflora; host defence and gut integrity. Reported effects on performance of pigs varied from little to no effect (Farnworth et al. 1992; Howard et al. 1993; Olsen & Maribo 1999) mixed effects (Houdijk et al. 1998) to stimulating effects (Russell et al. 1996; Shim & Choi 1997; Estrada et al. 2001; He et al. 2002; Shim et al. (submitted)). Supplementation of inulin-type fructans to the diet or drinking water resulted in fewer cases of diarrhea, reduced mortality, decreased number of pigs shedding the pathogen (Bunce et al. 1995b; Oli et al. 1998) compared to controls.

There is scarce information on the effect of inulin and oligofructose (OF) on nutrient digestion, availability and retention. Studies by Houdijk et al. (1999); Vanhoof & De Schrijver (1996b) and De Schrijver & De Vos (2003) showed that OF and inulin supplementation does not affect protein digestion and nitrogen retention. Mineral absorption and retention was not affected by inulin or OF except for zinc (Vanhoof & De Schrijver 1996a; Houdijk et al. 1999; De Schrijver & De Vos 2003) and iron (De Schrijver & De Vos 2003).

A number of studies have attempted to study the effect of inulin-type fructans on intestinal and faecal microbial populations and in vitro gut tissue association. Some studies in pig evaluated (a limited number of) bacterial populations showed that supplementation had little effect on size and activity of microbial populations (Farnworth et al. 1992; Houdijk et al. 1997). Some studies found enhanced intestinal

bifidobacteria populations (Howard et al. 1993; Klein Gebbink et al. 2001). Others reported modulation of the intestinal flora (Nemcová et al. 1999) and the speeding up of the recovery of the normal intestinal microflora following acute diarrhea (Oli et al. 1998). Konstantinov et al. (2003) studied the changes in time of the predominant faecal bacterial community in weaning pigs that were fed diets containing inulin-type fructans and/or sugar beet pulp using denaturing gradient gel electrophoresis (DGGE) analysis. DGGE was used to describe the microbial diversity in complex ecosystems including the mammalian intestinal tract. Piglets fed diets containing sugar beet pulp (10 g/kg) or inulin-type fructans+sugar beet pulp (2.5 g/kg + 5 g/kg) showed a higher bacterial diversity and a more rapid stabilisation of the bacterial community compared with that of the animals fed the control diet (maize starch).

Recently some experiments have also demonstrated that inulin-type fructans affect *in vitro* fermentation kinetics when used as a substrate (Houdijk 1998; Bauer et al. 2003, van Leeuwen et al. 2003) or affects the inoculum when included in the diet (Houdijk 1998).

Only few studies describe the effect of inulin-type fructans on host defence system and gut integrity. Herich et al. (2002) demonstrated that the combination of OF and probiotic to pigs prior and after birth increased the number of CD4⁺ T-lymphocytes compared to the control diet.

Inulin reduced the *in vitro* association of *E. coli* to jejunal organ tissue and of *Salmonella* spp. (non significant) to ileal tissue (Naughton et al. 2001). Rossi et al. (2001) showed that inulin reduced the *in vitro* adhesion of a pathogenic coliform to intestinal porcine mucosa. Results also suggested a systemic specific immunomodulatory effect of inulin in immunization against bovine thyroglobulin.

Howard et al. (1993) concluded that oligofructose improved the morphological and the cellular kinetics of the epithelial mucosa in the large intestine. Spencer et al. (1997) investigated the effect of supplementation of spray dried animal plasma and inulin-type fructans on the morphology of the small intestine in weaned pigs. Inulin-type fructans did not affect crypt depth (CD) but did increase villous height (VH) and VH:CD ratio.

Shim et al. (submitted) found that OF supplementation (2.5 g/kg and 30 g/kg) for three weeks post weaning (numerically) increased VH but not CD in the small intestine of pigs compared to the control. Brush border enzyme activity was not affected.

Application of inulin-type fructans in diets for poultry (Table 2).

At hatching the gastrointestinal (GI) tract of broilers is sterile. Immediately, bacteria, originating from the mother, the environment, or the diet will colonise in the GI tract. In case of mother contacts, a diverse microbial population will enter the GI tract. As a result after the first colonisation, bacterial species coming later in time will

have more difficulties to colonise (colonisation resistance) than the initial population. Because of the strict separation of generations in broiler chickens, any bacteria from the environment might colonise (e.g. attach to intestinal binding sites or multiply faster than being removed via chyme passage) in the intestinal tract. Those feed components that are resistant to enzymatic degradation, such as inulin-type fructans serve as a substrate for bacterial activity in the intestinal lumen. The interaction between host nutrition and the intestinal microbiota has been clearly illustrated using germ-free animals. Langhout (1998) clearly showed the importance of controlling the activity of the intestinal microbiota to support gut integrity and to avoid (1) bacterial overgrowth, (2) reduced nutrient digestibility and (3) reduced production performance. Feeding inulin-type fructans may be a practical strategy for controlling pathogenic bacteria in chickens. Flickinger & Fahey (2002) and Flickinger et al . (2003a) summarized several experiments in which different types of fructans were fed to broiler alone or in combination with a probiotic to evaluate the effect on colonisation of pathogens (i.e. *Salmonella* spp. and *Campylobacter jejuni*) in caeca (Fukata et al. 1999; Chambers et al. 1997; Oyarzabal and Conner 1996; Bailey et al. 1991) and on prechilled poultry carcasses. Researchers concluded that supplementation of inulin-type fructans in combination with competitive exclusion flora may reduce colonization of the pathogenic bacteria.

In recent experiments with broilers in our lab we (Verdonk and van Leeuwen 2004) evaluated the effect of supplementation of inulin-type fructans on the colonisation and shedding of pathogens. The first broiler study evaluated the effect of the inclusion of 20 g/kg OF and inulin in feed on the colonisation and shedding of *Salmonella typhimurium* and *Campylobacter jejuni* in broilers. The broilers were fed one of four dietary treatments and challenged in the crop on days 10 and 11 of age with a low or high dose of *S. typhimurium* and a single dose of *C. jejuni*. The birds were housed in three-tier battery cages. Feed intake and body weight was measured at the age of 9, 14, 21 and 35 days. During dissection of birds on days 14, 21 and 35, digesta samples of the crop and caeca were taken and the colonisation was determined. On days 18 and 28, *Salmonella* shedding via the excreta was measured. The second study evaluated the effect of inulin at four inclusion levels in a basal diet on the occurrence of lesions due to *Eimeria acervulina* and *Clostridium perfringens*. The broilers were housed in floor pens and given an *Eimeria acervulina* challenge orally at day 10 of age, followed by an oral inoculation of *Cl. perfringens* on days 14, 15 and 16. Intestinal lesions for coccidiosis and necrotic enteritis in the duodenum and jejunum on days 15, 16, 17 and 22 were scored visually.

The supplementation of inulin-type fructans in the diet stimulated the performance of young broiler chickens but did neither clearly affect the colonisation and shedding of *S. typhimurium* and *C. jejuni* nor the occurrence of lesions due to *Eimeria*

acervulina and *Cl. perfringens*.

Yusrizal & Chen (2003a) reported that supplementation of oligofructose and inulin (10 g/kg) to a corn-soy diet did not affect the faecal microbial counts of total aerobe, Lactobacilli, Salmonella and Campylobacter in broilers at 2, 4 and 6 wk of age. Oligofructose (OF) resulted in significant reductions in total faecal aerobes and *E. coli* at wk 4 compared to the control. It also increased the lactobacilli counts in the gizzard and small intestine of female broilers. Inulin or oligofructose supplementation reduced total Campylobacter counts in the large intestine. OF but not inulin resulted in reduced faecal ammonia content and pH during weeks 1 through 4. Same authors (Yusrizal & Chen 2003b) reported that supplementation of OF and inulin improved body weight gain, carcass weight, feed conversion efficiency and gut length in female birds but not in male birds. Also Ao & Choct (2003) reported that birds given drinking water supplemented with oligofructose (0.05%) were heavier and had more efficient feed conversion at day 35 compared to the control birds. Recently, Hartini et al. (2003) concluded that supplementation of inulin-type fructans (2 g/kg) to a wheat-based diet did not affect the feed intake nor egg production in ISA Brown laying hens. Application of inulin-type fructans in diets for (pre ruminant) calves (Table 3).

The common practice of early weaning of pre ruminant calves for veal production, followed by long distance transport and regrouping of animals from different origins may cause a challenge to the natural defence system resulting in dysbacteriosis and digestive disorders. In many fattening systems, starter treatments with antibiotics have become common practice. Mul & Perry (1994) mentioned a large scale use of inulin-type fructans resulting in similar fattening results compared to the in-feed antibiotics. In our lab, we (Verdonk & van Leeuwen 2004) evaluated the effect of inclusion of inulin-type fructans in the milk replacer on health and production performance of pre ruminant calves during the first three weeks after arrival at the fattening farm. Four groups of eight calves, housed individually in wooden boxes with slatted floor, were fed the basal calf milk replacer supplemented with 20 g/kg of either dextrose (DEX), oligofructose (OF), inulin or dextrose supplemented with antibiotics (ANT). Individual body weight of the calves was determined at 7-day interval and feed intake was measured per calf per feeding. The faecal consistency scores were conducted daily. The composition of the micro flora in rectal samples was determined by denaturing gradient gel electrophoresis (DGGE) and nucleotide sequence analysis of rDNA. During the three-week experimental period the oligofructose and the ANT group resulted in higher ($P < 0.05$) body weight gain compared to the DEX group. The faeces consistency over the observed period was best ($P > 0.05$) in the OF group and worse in the DEX group. The DGGE gels showed that the faecal flora in young milk fed calves in fattening farms is very unstable. The dietary treatment did not affect the pattern or the shift in pattern of the bands in the gels of the faecal

samples. Analysis of ileal contents and faeces showed that app. 70% of the dietary inulin-type fructans reached the caecum but that no fructans were recovered in the faeces.

Kaufhold et al. (2000) supplemented 10-week-old calves (average body weight 117 kg) with 0 or 10 g/d oligofructose (with the morning meal). Feed intake was similar between groups but weight gain tended to be higher for the OF supplemented group. They concluded that OF had basically similar effects on metabolic and endocrine traits such as concentration of glucose, lactate, triglyceride and insulin in blood in pre ruminant calves as in animals and humans with diabetes mellitus.

Webb et al. (1992) observed greater weight gains in Holstein bull calves (3-5 d old) by adding a combination of inulin-type fructans (3.75 g/kg), sodium diacetate (10 g/kg) and decoquinatate (50 mg/kg) into milk replacer and starter grain compared with supplementing milk replacer with sodium diacetate and decoquinatate alone. Unfortunately, the effects on rumen and gut microflora were not studied.

Donovan et al. (2002) reported that supplementation of a blend of inulin-type fructans, allicin, and gut-active microbes to the milk replacer had similar effects compared to antibiotics supplemented to milk replacer fed to Holstein male and female calves.

Compared with diets for other species, dietary proteins and carbohydrates for veal calves are usually highly digestible. This was to a large extent related to the soluble/dispersible nature of the proteins used in veal calf diets. Commercial milk replacers were initially made based primarily on skimmed milk powder and animal fat. During the last decade, replacement of milk proteins and lactose by vegetable proteins and carbohydrates has become an important issue both in practice and research (Verdonk et al. 2002). Increasingly, part of the dietary lactose is being replaced by starch and by soy oligosaccharides. Up to 15% starch can be added to veal calves diets with only a minor decrease in starch digestibility. At higher levels (15-25%), the decrease in starch digestibility is more pronounced (van der Honing et al. 1974; van Weerden et al. 1967) and this causes increased fermentation in the large gut. Visual characteristics and pH of the faeces were affected by the quantity of starch fermented in the hindgut. We have demonstrated (Verdonk et al. 1998) that replacement of lactose (65 g/kg) by soluble or insoluble soy carbohydrates resulted in significantly decreased apparent ileal digestibility of dry matter, crude ash and nitrogen free extract. Inclusion of the soy carbohydrates in the diet also tended to increase the endogenous flow of nitrogen at the terminal ileum. It was suggested that this increase might be caused by fermentation in the small intestine increasing the flow of bacterial nitrogen to the large gut. Inulin-type fructans may play a role in creating and maintaining a desired, stable microflora in the rumen (supplementation to solid feed), small and large gut (supplementation to milk replacer) of (pre ruminant)

calves.

Application of inulin-type fructans in pet foods (Table 4).

Several reasons justify the addition of oligofructose and inulin in pet foods Flickinger et al. (2003a):

- * Manipulating the composition of the intestinal flora,
- * Stimulating gut integrity,
- * Affecting nitrogen metabolism and
- * Reducing the offensive faecal odour.

Furthermore, it was indicated that the geriatric pet population is more prone to intestinal irregularities and has diminished digestive microbial balance when compared to younger animals. In their review they summarized the results of studies indicating an effect of supplementation of inulin-type fructans on the intestinal microflora, epithelial cell proliferation, faecal characteristics and nutrient digestibilities. Recently, Hesta et al. (2001) studied the effect of supplementation of OF (30, 60, 90 g/kg) and inulin (30, 60 g/kg) to a commercial diet in cats. Supplementation of 60 and 90 g/kg OF to the diet significantly affected faecal characteristics. Both OF (30 g/kg) and inulin (30, 60 g/kg) resulted in lower apparent faecal protein digestibility. These results are in contrast to Diez et al. (1997), who reported that 40 or 80 g/kg dietary OF did not reduce total-tract digestibility of dry matter (DM), organic matter (OM) or ether extract for dogs fed a beef-, corn-, and vegetable oil-based diet. However, Diez et al. did report that 80 g/kg supplementation of OF reduced the digestibility of crude protein (CP) for supplemented diets compared to the control diet.

At lower inclusion levels of inulin-type fructans (1 to 10 g/kg) results on nutrient digestibility in dogs are conflicting and range from 1) no effect on ileal and total-tract nutrient digestibility (Strickling et al. 2000; Beynen et al. 2002; Swanson et al. 2002c; Grieshop et al. 2002; Propst et al. 2003), 2) a decreased total-tract nutrient digestibility (Flickinger et al. 2003b; Propst et al. 2003) and 3) an increased absorption of magnesium and calcium (Beynen et al. 2002).

Conclusions

Important issues for pet owners and farmers are (1) animal health and veterinary costs, (2) performance and economics and (3) excretion of nutrients into the environment. Inulin-type fructans may play a role in solving these issues. There are many considerations in supplementing inulin-type fructans in animal feed and pet food. The type of diet (i.e. the content of non digestible oligosaccharides), the type and inclusion level to supplement, animal characteristics (species, age, stage of production), hygienic conditions of the farm.

The amount of research evaluating the potential of inulin-type fructans in animal

feed and pet food has increased considerably during last years. Studies indicate a generally positive effect on gut microflora, host health (gut integrity, colonization) and animal performance (digestion, body weight gain, feed efficiency). However, the data on the efficacy of inulin-type fructans are sometimes variable and not fully conclusive yet. Data on the effect of inulin-type fructans on intestinal and systemic immune system as well as the resistance to infections is still scarce.

Costs of animal production will increase when in-feed antibiotics are banned thus there is a need for conclusive data to determine under which conditions inulin-type fructans can reduce the impact of (sub clinical) infections and support animal performance.

There is a need for more standardized studies using both negative and positive controls to study the efficacy of inulin-type fructans. The control groups as well as the experimental setting should be chosen in line with the selected issue of study f.e. animal performance or nutrient excretion. Both technical and economic parameters should be evaluated to be able to conclude on the effectiveness of fructan supplementation.

In order to effectively supplement inulin-type fructans to feed and pet food additional research is also needed to elucidate the mode of action and the relationship between gut microflora, gut and animal health, and performance. Molecular DNA techniques might be helpful in future research to gain further insight in the changes occurring in the composition of the gut microflora and the gene expression in gut tissue and relevant organs.

Table 1. Effect of inulin-type fructans in pigs

Ref1	Design	Type of fructan2 and dosage	Observations2,3
I	weanling pigs fed during 28 days	OF 3 g/kg	OF supplementation: ↑ FI and ↑ ADG
II	weanling pigs fed during 28 days	OF 2.5 g/kg	OF supplementation ↑ FI and ↑ ADG
III	135 weaned (28 day old) pigs fed for 4 weeks	- control (corn, barley, SBM, whey, fat) - Inulin 15 g/kg - OF 15 g/kg	lionsol was replaced by inulin (JAF) and OF. FI. ADG: ↔. VFA levels. bacterial counts in distal colonic digesta: ↔. Pigs fed inulin and OF had non-significant ↑ number total anaerobes and bifidobacteria. The manure samples had different colours depending on the diet. The average panel smell score of the manure ↔ (1.5 (control), 2.4 (inulin) and 2.1(OF)).
IV	20 weaned pigs (36 h postpartum) fed nutritionally complete liquid diets for 15 days	OF 0, 3 g/l	- The number of caecal Bifidobacteria and total anaerobic flora; number of proximal colonic Bifidobacteria and total anaerobic flora, caecal pH and SCFA concentrations ↔ - Cell density and number of labeled cells in cecal epithelial mucosa ↑ - CD. leading edge. labeled cells. proliferation zone, labeling index and cell density in proximal colonic epithelial mucosa ↑. - CD. leading edge. cell density. labeled cells. proliferation zone and labeling index in distal colonic epithelial mucosa ↑ by OF supplementation
V	16 weaned pigs (7 day old) fed a non-medicated milk replacer and orally challenged with E. coli	OF 0, 3 g/d	within 36 hrs 6 of 8 pigs developed symptoms of anorexia, pyrexia, dehydration and diarrhea. 7 of 8 OF pigs showed no visible symptoms. OF supplementation tended to ↑ Bifidobacteria compared to control. In OF pigs E. coli ↓ (numerically), Clostridial populations ↔.
VI	20 weaned pigs (8.8 kg) fed a corn-soy diet during 20 days	OF 0, 0.75 g/d, 1.5 g/d, 1.5 g/d+carbadox+Cu	ADG ↑ linearly (P<0.25) as OF addition increased. Nitrogen digestion and retention ↑. The level of odor metabolites (concentration and excretion in the faeces) ↓.
VII	96 weaned pigs fed solid diets during 4 weeks	2 x 2 factorial design : OF 0, 1 g/d Carbadox (Ab) 0, 50 mg/kg	Growth performance ↑ by OF supplementation
VIII	8 male cannulated pigs (85 kg BWG) individually housed in metabolic cages	Inulin 60 g/kg	Apparent ileal and fecal absorption and retention of Ca and P ↓; apparent faecal Zn absorption ↑ by inulin addition.
IX	16 male cannulated pigs (90 kg BWG) individually housed in metabolic cages	Inulin 60 g/kg	Ileal and fecal nitrogen excretion as well as nitrogen retention ↔ by inulin supplementation.
X	96 weaned (18 day old) pigs fed solid	2 x 2 factorial design: OF 0, 1 g/kg	Pig performance ↔ by inclusion of OF. The diet did not affect CD in the small intestine. VH ↑ by both SDAP and OF. VH:CD ratio ↑ by OF.

	feed during 4 weeks	SDAP 0, 35 g/kg	
XI	weaned (39 day old) pigs fed solid feed during 29 days	OF 50 g/kg	FI ↑ and ADG ↑ by OF supplementation.
XII	20 castrated male ileal cannulated (38 day old, 10.4 kg) weaned pigs were fed solid feed	Control (cornstarch, glucose, casein) OF 10, 40 g/kg TOS 10, 40 g/kg	OF yielded ↑ propionate than TOS in ileal chyme. pH and volatile fatty acid pool of ileal chyme were influenced by type and level of dietary non-digestible oligosaccharide. The OF diets yielded ↑ caecal anaerobes, ↑ faecal pH, ↑ protein-derived SCFA and less butyric acid than the control diet.
XIII	65 castrated male (9 week old, 15.6 kg) pigs fed solid feed during 42 days	Control (corn/dextrose based diet) O F 7 . 5 , 1 5 g / k g T O S 1 0 , 2 0 g / k g	OF was not detected in faeces. OF and TOS supplementation resulted in significantly lower dry matter intake and ADG compared to the control pigs in week one through three. During week one through six mean growth performance was not affected. Faecal pH was not affected but faecal DM content was lower for OF/TOS pigs compared to control pigs.
XIV	41 weaned (21 day old) crossbred standard farm pigs were housed individually in metabolic cages and challenged orally with cholera toxin	oral electrolyte solutions (OES) OES + OF (9.5 g/d)	Supplementation with OF resulted in accelerated recovery of bacteria perceived as beneficial while potentially slowing the recovery of pathogenic forms. OF did not cause an obvious reduction in the duration of diarrhoea and the associated loss of water.
XV	exp 1 25 (57 day old, 16 kg) pigs were fed solid feed as a slurry exp 2 20 (38 day old, 10.4 kg) ileal-cannulated pigs were fed solid feed	control (corn/dextrose based diet) OF 7.5, 15 g/kg TOS 10, 20 g/kg	Apparent faecal and apparent ileal nutrient digestion (DM, Ash, CP, EE) ↔ by supplementation of OF and TOS The OF and TOS fed pigs produced dose dependently less faeces than the control pigs. The apparent faecal digestion of the nonstarch neutral-detergent soluble carbohydrates (including non digestible oligosaccharides) was ↑ by OF and TOS supplementation. Nitrogen and mineral balances were not affected. OF was nearly completely degraded pre-caecally.
XVI	90 weaned (36 days old, 7 kg) pigs during 20 days	control probiotic OF (3 g/d) + probiotic	OF + probiotic resulted in ↑ count of total anaerobes, aerobes, lactobacilli and bifidobacteria as well as decreased clostridia, enterobacteriaceae and E. coli counts in faecal samples. This study indicates a synergistic effect of probiotic (<i>Lactobacillus paracasei</i>) and OF on faecal microflora
XVII	2 exp with 175 group fed pigs during 4 weeks	CON: basal diet AB: Virginiamycin 0.5 g/kg OF: 50 g/kg BP: beef pulp 100 g /kg	Average daily gains were not significantly affected by diet. Overall AB, OF and COM ↑ ADGs by 16%, 9% and 6% respectively compared with CON. In the clean nursery trial. In the dirty nursery trial, OF ↑ feed efficiency (14%) but reduced FI (24%). OF was associated with lower (trend) E coli

		COM: OF 25 g/kg, BP 50 g/kg	concentrations in ileal contents and ↑ bifidobacteria in proximal colon samples. OF tended to reduce the E. coli concentrations and ↑ bifidobacterial and total anaerobic bacteria concentrations in the distal colon of pigs
XVII	Exp 1 in vitro adherence assay Exp 2 20 BT seronegative, weaned pigs (28 day old)	Exp 1: Inulin 0.1%, 1%, 5% to incubation solution Exp 2: Inulin 0, 50 g/kg	Inulin partially inhibited adhesion of F4ac+ coliform to villous brush border (52% inhibition by 5% inulin). Inulin supplementation to the diet did not affect the primary immune response to bovine thymoglobulin immunisation. The secondary immune response after a boost at day 21 in the inulin pigs was numerically ↑
XIX	in vitro inoculation of intestinal tissue (jejunal and ileal sections) from 30 pigs.	Exp 1 incubation solution: Inulin 25 g/kg; GOS 25 g/kg; MOS 25 g/kg Exp 2 dietary inclusion: Inulin 40 g/kg; GOS 40 g/kg	Inulin (25 g/kg) resulted in non significant decrease in association of Salmonella in ileal tissue and in association of E. coli in jejunal tissue. The numbers of E. coli in jejunal tissue and a numerical reduction of Salmonella sp in ileal tissue ↓ in Inulin pigs.
XX	Exp 1 40 weaned pigs (18 days old, 6 kg) were fed solid feed for 21 days Exp 2 80 weaned pigs (18 days old) were fed a solid diet in a 2 x 2 factorial design	control diet control diet + OF (5 g/kg), oral dose of Bifidobacterium longum at day 1 and 3 control diet (C) C+OF (5 g/kg) C+1x10 ⁷ CFU/g B. longum C+OF (5 g/kg)+1x10 ⁷ CFU/g B. longum	The treatment with OF and B. longum improved ADG and FE, reduced the number of faecal total anaerobes and clostridia and ↑ the number of bifidobacteria only from d 0 to 7. ADG and insulin-like growth factor I (IGF-I) ↓ by OF supplementation, while ADG and IGF-I ↑ administration of B. longum
XXI	180 weaned (17 day old) pigs during 4 weeks in 3 phases (wk 1, wk 2, wk 3 - 4)	basal diet (BD, control) BD + Inulin in water (132 g/l) BD + Inulin in feed (5, 2, 1 g/kg) BD + Inulin in water and feed BD + antibiotic in feed	ADG and FE ↑ by supplementation of inulin in water or feed compared to the control. Pig performance ↔ by inulin in both water and feed compared to inulin in water or feed only
XXII	90 piglets (one day old) were fed 10 days after birth and 10 days after weaning (36 days of age) with powdered milk	C = powdered milk L = 2 g/d C+ Lactobacillus paracasei 1x10 ⁹ CFU/g F = 2 g/d C+ Lactobacillus paracasei 1x10 ⁹ CFU/g; + OF (3 g/d)	Supplementation of OF in combination with Lactobacillus paracasei did not stimulate the immune system of newborn piglets compared to C or L. After weaning, OF in combination with Lactobacillus paracasei resulted in highest numbers for almost all measured immune parameters (total count of several types of white blood cells, phagocytic activity of leukocytes and neutrophils)
XXII	36 weaned pigs (25-28 day old) from 9 litters fed	- control (maize starch, fish meal, dextrose) - SBP 10 g/kg - SBP 5 g/kg + OF 2.5 g/kg	piglets fed with SBP or SBP+OF showed a ↑ bacterial diversity (Shannon index of diversity of DGGE bands) and a more rapid stabilization of the bacterial community in faecal samples collected per rectum compared to control pigs.

			Amplicons related to Ruminococcus-like species were found in all DGGE fingerprints derived from SBP and SBP+OF pigs but not in pigs on the control diet.
XXI V	in vitro gas production	Inulin 0, 25 g/kg	The gas production of the Inulin diet was ↑ to 5 ml/q diet/ h compared to <2 ml/ q diet /h for the control diet. Inulin in vitro fermentation comprised a period of less than 10 h while the fermentation of other feed components continued until about 25 h.
XXV	in vitro gas production	Inulin (chicory, Jerusalem artichoke) 0.2 q added to 0.5 q chyme compared with other carbohydrates and antibiotic (Virginianycin)	Except for antibiotic, the fermentation with added carbohydrates resulted in ↑ total gas production and a faster maximum rate of gas production. Addition of all tested carbohydrates except xylan resulted in shorter time at which half of the asymptotic gas had been reached. The amount of ammonia decreased for all added carbohydrates and was nearly the same for the antibiotic compared to chyme only. The branched-chain ratio was significantly lower for all additives compared with chyme whereas the antibiotic led to a significant higher value.
XXV I	12 castrated male ileal-cannulated pigs (20 kg) fed ad libitum	control (barley-sbm diet) GOS Inulin 20 g/kg	Protein and mineral utilization ↔ by inulin and GOS supplementation. The intestinal fermentation (ammonia and VFA concentration) was significantly changed. Inulin degradation reached 45% at the end of the small intestine and 100% in the faeces.

¹ References: I: Nakamura, 1986; II: Fukuyasu & Oshida, 1986; III: Farnworth *et al.*, 1992; IV Howard *et al.*, 1993; V Bunce *et al.*, 1995b; VI Bunce *et al.* 1995c; VII Russell *et al.*, 1996; VIII Vanhoof & De Schrijver, 1996a; IX Vanhoof & De Schrijver, 1996b; X Spencer *et al.*, 1997; XI Shim & Choi, 1997; XII Houdijk *et al.*, 1997; XIII Houdijk *et al.*, 1998; XIV Oli *et al.*, 1998; XV Houdijk *et al.*, 1999; XVI Nemcová *et al.*, 1999; XVII Klein Gebbink *et al.*, 2001; XVIII Rossi *et al.*, 2001; XIX Naughton *et al.*, 2001; XX Estrada *et al.*, 2001; XXI He *et al.*, 2002; XXII Herich *et al.*, 2002; XXIII Konstantinov *et al.*, 2003; XXIV van Leeuwen *et al.*, 2003; XXV Bauer *et al.*, 2003; XXVI De Schrijver & De Vos, 2003.

² Abbreviations: OF: oligofructose; GOS : galacto oligo saccharides; TOS : trans galacto oligo sacchardies; MOS : mannanoligo saccharides; SBP : sugar beet pulp; SBM : soy bean meal; SDAP : spray dried animal plasma; ADG : average daily gain; FE : feed efficiency; FI : feed intake; VH : villous height; CD : crvot depth; VFA : volatile fatty acids; NR : not reported

³ ↑ : increased, ↓ : decreased, ↔ : no change

Table 2. Effect of inulin-type fructans in poultry

Ref1	Design	Type of fructan ² and dosage	Observations ^{2,3}
I	2800 one-day old male broilers fed for 46 days	commercial diet OF in feed (2.5, 5.0 g/kg) BMD	Final body weights numerically ↑ and FE ↑ by OF. (Results are not mentioned in the abstract but are referred to in Flickinger et al. 2003)
II	2400 one-day old male broilers fed for 47 days	Control OF 3.75, 7.5 g/kg antibiotic (virginiamycin) 0.011 g/kg	Supplementation of OF at the inclusion level of 3.75 resulted in heavier birds and improved carcass yield (hot carcass weight, percent breast meat) at 47 days compared to control and antibiotic groups.
III	broilers	Control OF 3.75 g/kg	ADG, FE and carcass quality ↔ by OF supplementation
IV	10 pens with Salmonella negative day-old Cornish broiler chicks	Control Inulin crude Inulin refined lactulose lactosucrose	The Salmonella score ↔ by supplementation of carbohydrates. Salmonella counts at 6 wk in broilers fed crude inulin ↑ compared to all other broiler groups. Infections ↓ in broilers fed refined inulin than in control broilers. The decline of Salmonella infection in broilers fed refined inulin ceased after the dietary additive was discontinued at 5 wk of age. Cecal pH and density ↓ by inulin
V	880 Ross broiler chicks in 16 pens, challenged at day 2 after hatching with Salmonella typhimurium by spraying	control: buffered peptone water OF+ microbial mixture	The treatments were provided in drinking water at 6 weeks of age after feed with drawl. Ceca from treated birds weighed more than from control birds. No difference in Salmonella colonization occurred between the treated and control group
VI	84 one-day old Hubbard x Hubbard male broilers were housed in battery pens and fed a corn-soybean diet with sprayed kestose/sugars for 28 days	control (C) C + 100 g/kg Kestoses (OF), sugars C + 80 g/kg sugars	ADG, FE or concentrations of total aerobic bacteria, coliforms, lactobacilli, total anaerobes or clostridia ↔ by supplementation of kestoses. Cecal bifidobacterial populations ↑ 24 fold in kestose-treated birds.
VII	Exp 1 and 2: two trials with 60 one-day old birds (White Leghorn Hy-Line) challenged orally with Salmonella enteritidis at day 7 (exp	Control CE OF (1 g/kg) CE + OF (1 g/kg)	did not effect cecal Salmonella colonization or translocation in birds challenged at 7 day ↔ by OF supplementation. In birds challenged at day 21 the mean numbers of Salmonella enteritidis in cecal contents on d1 and d7 post infection ↓ by OF supplementation or OF + CE.

	1) or 21 (exp 2) Exp 3 and 4: a total of 20 one-day old birds	control CE OF (1 g/kg) CE + OF (1 g/kg)	The major bacterial population of the cecal microflora ↔ by OF supplementation
VIII	24 ISA Brown laying hens (20 wk old) housed individually	wheat based oat based millrun based rice hull based wheat + OF (2 g/kg) wheat + MOS (2 g/kg)	Gut viscosity and egg production ↔ by dietary treatment
IX	312 one-day old male Cobb broiler chicks in 26 pens during 35 days	Control OF glucose MOS	Supplementation of glucose, OF or MOS tended to result in heavier birds with better FE till day 35
X	98 one-day old male and female Ross broiler chicks in 12 pens	control corn-soy Inulin 10 g/kg OF 10 g/kg	The faecal microbial counts of total aerobe, Lactobacilli, Salmonella and Campylobacter at 2, 4 and 6 wk of age ↔ by the dietary treatment. Total faecal aerobes and E. coli at wk 4 ↓ by OF compared to the control. The lactobacilli counts in the gizzard and small intestine ↑ in female OF birds. Total Campylobacter counts in the large intestine ↓ in Inulin and OF birds. Fecal ammonia content and pH during the wk 1 through 4 ↓ in OF birds but not in Inulin birds.
XI	98 one-day old male and female Ross broiler chicks in 12 pens	control corn-soy Inulin 10 g/kg OF 10 g/kg	ADG, carcass weight, FE and gut length in female birds ↑ but in male birds ↔ by OF and Inulin supplementation.
XII	Exp 1 864 one-day old male Ross 308 birds were fed during 35 days and orally challenged with Salmonella and Campylobacter Exp 2 704 one-day old male Ross 308 birds were housed in 32 floor pens and orally challenged with Eimeria acervulina and Clostridium perfringens	OF 20 g/kg Inulin 20 g/kg High molecular Inulin 20 g/kg Inulin	The performance of young broiler chickens ↑ The colonization and shedding of Salmonella and Campylobacter ↔ The performance of young broiler chickens ↑ The occurrence of lesions due to Eimeria acervulina and Clostridium ↔

- 1 References : I: Ammerman et al., 1988; II: Ammerman et al., 1989; III Waldroup et al., 1993; IV Chambers et al., 1997 ; V Ovarzabal & Conner, 1996; VI Patterson et al., 1997 ; VII Fukata et al., 1999; VIII Hartini et al., 2003; IX Ao & Choct, 2003; X Yusrizal & Chen, 2003a; XI Yusrizal & Chen, 2003b; XII Verdonk & van Leeuwen, 2004
- 2 Abbreviations: OF: oligofructose;; JAF : Jerusalem Artichoke flour; MOS : mannan oligo saccharides; CE : competitive exclusion; ADG : average daily gain; FE : feed efficiency; BMD : bacitracin methylene disalicylate; NR : not reported
- 3 ↑ : increased, ↓ : decreased, ↔ : no change

Table 3. Effect of inulin-type fructans in calves

Ref1	Design	Type of fructan ² and dosage	Observations ^{2,3}
I	40 male Holstein (3-5 day old) fed milk replacer (MR) and calf starter (CS) during a 10 week period	OF 0, 3.75 g/kg in MR and 0, 8.8 g/kg in CS	In winter time, ADG ↑ by adding a combination of OF, sodium diacetate and decoquinat compared with controls. OF source NR
II	Veal calves in practice	OF+ probiotic (5/2.5 g/kg 0-6 w/7-26w)	Authors mention unpublished results in practice (Italy, Netherlands) showing similar fattening results comparing a feeding regime using OF compared to antibiotics.
III	21 calves fed milk replacer and calf starter	0, 3, 7 g OF/d	Plate counts in faecal samples for Bifidobacteria ↑ (P=0.02) by OF supplementation to the milk replacer. Counts for E. coli, Clostridia and the total anaerobes were numerically changed.
IV	14 Simmental x Red Holstein calves (10 week old) fed whole milk plus milk replacer during a 3 week period	GrC = no supplementation GrF = 10 g OF/d	ADG tended to be higher for GrF. The post-prandial increase of glucose concentrations was significantly smaller, of lactate tended to be smaller, whereas maximal insulin concentrations reached were significantly higher in OF calves.
V	45 male and female Holstein calves (new born) fed milk replacer and calf starter	MRA= antibiotics in milk replacer MRE= OF+allicin+probiotics	Total weight gain, faecal scores and serum proteins during 5 week experimental period was not different between groups. OF source NR
VI	32 male black and white (1 week old) calves fed milk replacer for 3 weeks	dextrose (DEX), OF (20 g/kg) Inulin (20 g/kg) dextrose+antibiotics (ANT): 20 g/kg	ADG ↑ for OF and ANT calves compared to DEX. Faecal score numerically better for Inulin compared to DEX. The composition of faecal microflora ↔ and highly variable.

1 References : I: Webb et al., 1992; II Mul and Perry, 1994; III Bunce et al., 1995a; IV Kaufhold et al., 2000; V Donovan et al., 2002;
VI Verdonk and van Leeuwen; 2004;

2 Abbreviations: OF: oligofructose; ADG : average daily gain; NR : not reported

3 ↑ : increased, ↓ : decreased, ↔ : no change

Table 4. Effect of inulin-type fructans in pets

Ref1	Design	Type of fructan ² and dosage	Observations ^{2,3}
I	16 IqA-deficient 15- to 20-months-old German Shepherd dogs with intestinal bacterial overgrowth were fed a chicken based kibble diet for 50 days	OF 10 g/kg	The number of aerobic bacteria in digesta from the duodenum/proximal part of jejunum and in the duodenal mucosa ↓ by supplementation with OF.
II	in vitro fermentation using cat faecal inoculum. Cats were fed a diet with or without supplemental fiber (beet pulp)	OF	OF together with some fermentable fiber sources resulted in highest OM disappearance and acetate, propionate and total SCFA production
III	in vitro fermentation using dog faecal inoculum	OF	OF had greatest OM disappearance (P<0.05; >80%) ,greatest propionate and intermediate total SCFA production
IV	8 castrated 1- to 1.4-year-old young adult male beagles were fed minced meat, flaked maize based diet for 42 days	control (C) C+OF 40g/kg+SBF 10g/kg, C+OF 80g/kg+SBF 20g/kg	Total-tract digestibility of CP ↓ by OF. but digestibility of DM, OM and ether extract ↔. Post-prandial plasma concentrations of insulin and cholesterol ↔.
V	8 beagle dogs (2 intact males and 6 neutered females). 5 year old were fed minced one of four meat, flaked maize based diet in a 4 x 4 latin square design for 20 weeks	control (C) C + Inulin 70 g/kg DM C + SBF 70 g/kg DM C + guar gum 70 g/kg DM	Inulin increased wet faecal output and water consumption compared to C. Supplementation of inulin decreased digestibility of OM, CP and EE. Inulin showed no metabolic effects.
VI	6 male and 6 female healthy specific pathogen free cats were fed a dry diet for 32 weeks	OF 7.5 g/kg	Wide quantitative and qualitative variation in the duodenal flora was observed over time. The duodenal flora ↔ by OF.
VII	6 male and 6 female healthy specific pathogen free cats were fed a dry diet for 32 weeks	OF 7.5 g/kg	Supplementation with OF resulted in alteration of fecal flora of cats. Total bacterial counts, aerobic and anaerobic counts were not affected by diet. Mean counts of lactobacilli and Bacteroides spp ↑ and E coli and Cl pf ↓ by OF.
VIII	10 adult beagle dogs of both sexes were fed a chicken (by) product brewers rice based diet for 6 weeks	cellulose 36 g/kg beet pulp+OF (42+10 g/kg)	Small intestinal dimensions ↑ and in vitro carrier mediated glucose uptake ↑ by fermentable beet pulp and OF.
IX	Exp 1, 8 healthy adult cats, basal diet containing 26.5 g/kg crude fibre	OF 0, 30, 60 and 90 g/kg	supplementation with 60 and 90 g/kg oligofructose significantly affected faecal characteristics
	Exp 2, 8 healthy adult cats, same basal diet, faeces collection	OF 30 g/kg	oligofructose and inulin resulted in lower apparent faecal protein digestibility

		Inulin 0, 30, 60 g/kg	
X	7 adult mixed breed female dogs in a 4 x 7 incomplete latin square design.	Crude inulin, 5 g/kg	Ileal, large intestinal and total tract DM and N digestibility ↔, minor effects on bacterial growth in the large intestine and VFA proportions in the small intestine
XI	in vitro	OF, 4 inulin products	Total production of SCFA ↑ for OF and the inulin products (DP3-5: OF, DP9: Inulin HD, DP9: Inulin IQ, DP>12: Inulin, DP2-8: OF) compared to beet pulp, MOS and soy fiber or cellulose.
XII	5 healthy dogs in a cross over trial	OF 10 g/kg	OF (DP3-20) ingestion changed the faecal microflora. the apparent magnesium and calcium absorption ↑. The faecal pH and the route of nitrogen excretion ↔. The diet used in this study contained rice, corn and beet pulp and probably was rich in non-digestible fermentable carbohydrates.
XIII	Exp 1, 20 healthy female and male dogs Exp 2, 20 healthy female and male dogs	OF, Lactobacillus acidophilus (LAC), OF+LAC 6.7 g/kg (or 2 g OF/d), 1x10 ⁹ CFU, 6.7 g/kg OF +1x10 ⁹ CFU	In exp 1 Clostridium perfringens ↓ (P=0.08) and faecal butyrate (P=0.06) and lactate (P<0.05) concentrations ↑ by OF. In exp 2 FI and faecal DM output tended ↓. OF had lowest faecal concentrations of hydrogen sulfide, methanethiol and dimethyl sulfide. LAC was more effective when fed in combination with OF.
XIV-XV	4 adult female dogs surgically fitted with ileal cannulas	OF, MOS, OF+MOS 5,5,10 g/kg (or 2 g OF/d)	Ileal IgA concentrations ↑ in OF + MOS dogs, Faecal microbial populations ↔ by OF Faecal total indole and phenol concentrations ↓ by OF and OF+MOS. The dose of 2 g OF/d was maybe not high enough to affect the microbial population in the distal colon or faeces
XVI	Exp 1, 16 adult male beagles (12 kg, 3 year old) Exp 2, 4 adult female dogs (20 kg, 3 year old), surgically fitted with ileal cannulas	OF 0,3,6,9 g/kg (or 0, 0.6, 1.2, 1.8 g OF/d) OF 0,2,4,6 g/kg (or 1,2,3 g OF/d)	Total-tract digestibility of DM (P<0.05), OM (P<0.05), lipid (P<0.01) and CP (P<0.07) ↓ Stool quality ↔ Faecal ammonia and urinary ammonia concentration numerically ↓ by 45% Faecal concentrations of propionate (P<0.05), butyrate (P=0.15) and total SCFA (P=0.07) ↑ in OF supplemented dogs. Faecal odor components and total anaerobe concentration ↔ Total aerobe concentration tended ↓ Ileal nutrient digestibility numerically ↑ with increasing OF. Faecal concentrations of SCFA, BCFA, ammonia, phenols and indoles ↔. Faecal total aerobes ↑ and CI pf ↓.
XVII	7 ileal ileal-cannulated adult female dogs, 7 x 7 latin square	OF 0,3,6,9 g/kg Inulin 0,3,6,9 g/kg	- nutrient intake and ileal digestibility ↔, - total-tract digestibility of DM, OM and CP ↓ - faecal ammonia and SCFA (P<0.10) concentrations ↑ - faecal total phenols ↓

			- individual and total amines (only by OF) ↓ by supplementation of OF and inulin:
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1 References : I Willard et al., 1994; II Sunvold et al., 1995a; III Sunvold et al., 1995b; IV Diez et al., 1997; V Diez et al., 1998;
VI Sparkes et al., 1998a; VII Sparkes et al., 1998b; VIII Buddington et al., 1999; IX Hesta et al., 2001; X Strickling et al., 2000;
XI Vickers et al., 2001; XII Beynen et al., 2002; XIII Swanson et al., 2002a; XIV Swanson et al., 2002b; XV Swanson et al., 2002c;
XVI Flickinger et al., 2003b; XVII Propst et al., 2003;

2 Abbreviations: OF: oligofructose; ADG : average daily gain; FI : feed intake; SCFA : short chain fatty acids;

BCFA : branched chain fatty acids; NR : not reported

3 ↑:increased; ↓:decreased, ↔no:nochange.

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Chapter 3

Prebiotics and probiotics or synbiotics in the diets of newly weaned pigs - with regard to gut fermentation

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1. Introduction

Piglets are commonly weaned at around 18-25 days of age in most countries; in some countries weaning is done at an older age. The aim of early weaning is to optimize reproductive efficiency of the sow. When piglets are weaned, they usually face several stressors such as nutritional (change of food from milk to dry feed), environmental (thermal temperature). As a result, piglets often show a variable and low feed intake and suffer from postweaning growth check for the first 1-2 weeks. Sometimes there is a high incidence of diarrhea. Moreover, weaning is associated with villous atrophy in the small intestine. This may impair digestion and absorption of the gut.

Newly weaned piglets need special management and optimal housing environment. Specific feed additives such as growth promoting antibiotics, prebiotics, probiotics and acidifiers are sometimes used in weaner diet to improve feed intake and growth. Among the feed additives, growth promoting antibiotics have been used several decades.

There has been growing concern in the public on the use of growth promoting

antibiotics in the diet of farm animal. This is associated with the fear that antibiotic resistance may develop and that bacteria may transfer that resistance to pathogens. Consequently, the EU has proposed to ban the use of growth promoting antibiotics in farm animal from 2006 onwards. The vast majority of antibiotics which were used as growth promoters until a few years ago are already banned now. The complete ban may lead to a decrease in growth rate, probably also an increase in incidence of diarrhea and mortality (Andreason, 2000). Because of this there has been a keen recent interest in the mechanism of the growth promoting effects of antibiotics. On the basis of that there is also an ongoing search for components which may exert one or more of the actions of antibiotics. Potential feed additives in the diet for young pigs to date are prebiotics, probiotics or synbiotics (a combination of prebiotics and probiotics).

The objectives of this chapter are

1) to identify and quantify the effects of these potential feed additives on postweaning feed intake and growth in pig, 2) to provide information on the effects of prebiotics, probiotics and synbiotics in the diet for young pigs when no antibiotics are given, 3) to verify whether there are some effects which are similar to antibiotics. Including suppression of the growth of pathogenic bacteria like *E. coli*, stimulation of beneficial bacteria (bifidobacteria and lactobacilli), and limit the production of toxins by some microorganisms in the gut of young pigs.

2. Growth of the young pig

2.1 Growth potential of piglets (weaning weight and subsequent growth)

The growth performance of pig is economically very important. Growth rate of the pig is one of the key indicators affecting the profitability of pig meat production. Improvement in growth rate and feed to gain ratio will result in improved profitability due to greater output and reduction in overhead costs (Campbell, 1997). The commercial weights of pigs (7.5 and 13.0 kg at the age of 25 and 45 days, respectively) are well below the potential weights (kg) of pigs at the same age. The potential may be as high as 10.2 and 22.7 kg, respectively, according to Campbell (1997).

It is well known that the age and weight at weaning are closely related to postweaning growth (Quiniou et al. 2002; Mahan et al. 1998). Many studies have demonstrated that weaning weight influences postweaning growth performance and also influence performance during the subsequent grower and finisher phases (Le Dividich, 1999). An increase in pig weight at weaning with 1kg will result in a pig which reaches slaughter weight at least 10 days faster (Cole and Close, 2001). It is also accepted that average daily gain during the first week postweaning has a major

impact on subsequent growth performance (Tokach et al, 1992).

The use of growth promoting antibiotics in pigs results in 4-6.5% increase of their growth and feed efficiency (Muirhead and Alexander, 1997). Prebiotics (nondigestible oligosaccharides such as FOS) may have potential to increase weight gain of young pigs also (Russell et al, 1996). Abe et al (1995) have reported that feeding probiotics (beneficial bacteria) to piglets or calves improved weight gain and feed efficiency. In addition, Nemcova et al (1999) have found that synbiotics, combining FOS and *Lactobacillus paracasei*, increased beneficial bacteria and decreased harmful bacteria in weaned pigs.

2.2 Postweaning growth check

Early weaning imposes stress on the immature piglet. Weaning usually causes a reduction in villous height and in brush border enzyme activity for the first few days after weaning (Pluske et al, 1995). Among others, Spreeuwenberg (2002) clearly showed that the reduction in villous height is associated with a low intake. Thus, low feed intake that is usually seen immediately after weaning causes a serious problem, and this leads to reduction in growth rate which is called "postweaning growth check" (Dunshea et al., 2002; Le Dividich and Seve, 2000). The postweaning check which occurs in pigs is greater and lasts longer in early weaned pigs (Dunshea et al., 2002; Power et al., 1996). This is important because piglets that gained more than 225g/d during the first week postweaning were 7.7kg heavier at market compared to pigs that lost weight during the first week postweaning (Tokach et al., 1992).

Dry matter intake in the first week after weaning significantly affects the 28 day postweaning weight of pig (Geary and Brooks, 1998). Feed intake during 1 week after weaning was only 60-70% of that consumed prior to weaning (Le Dividich and Seve, 2000). The low feed intake after weaning may be avoided partly by feeding a liquid diet instead of dry diet (Odle and Harrell, 1998). However, the above mentioned methods do not have similar effects of growth promoting antibiotics. It is assumed that prebiotics and probiotics could be potential alternative to growth promoting antibiotics. Because prebiotics which are non-digestible carbohydrates may serve to provide nutrient source for bacteria and also influence the gastrointestinal ecosystem (Buddington, 2001). Beneficial lactic acid bacteria may improve gut health and feed intake. The improved gut health by feeding pre- and probiotics may have potential to improve growth of young pigs (Abe et al., 1995)

2.3 Postweaning stressors

After weaning, piglets are commonly exposed to multi-faceted stressors such as nutritional, psychological, microbiological, environmental and immunological stresses. If some of the postweaning stresses can be overcome, then the postweaning

growth check would probably be less severe. This would allow animals to reach their potential weight at weaning. Thus, it is important to understand postweaning stressors for successful adaptation of weanling pigs. We will focus here on nutritional stress.

1) Nutritional stress

The sudden separation of piglets from the mother includes an abrupt change in the pig's diet. This may cause alterations of the gastrointestinal tract of weanling pig. As a result, piglets often do not eat during the first few days after weaning. This is associated with an adverse change in gut histology such as villous atrophy, crept depth increases, reduction in digestive and absorptive functions of nutrient lead to postweaning malabsorption syndrome (Pluske et al., 1997). Thus, a continuous supply of nutrients to the gut after weaning may prevent the detrimental changes of gut morphology and function of newly weaned pig (Bruininx, 2002; Pluske et al, 1996).

The reasons for low feed intake after weaning are not all clear, but several factors may be involved. First, there is a change of food from sow's milk into a solid diet with regard to physical and chemical properties like nutrient composition. In addition, processing temperature of diet, housing (temperature), feeding interval, water content, taste and flavor can be important. Second, gut capacity (biochemical and physical) is limited (Cranwell and Moughan, 1989).

2) Environmental and Psychological stress

Environmental stress and psychological stress are also imposed at weaning. Post-weaning environment has a major influence on the frequency of abnormal behaviors in weaned piglets (Bøe, 1993). When piglets are weaned they are separated from their mother, this will cause psychological stress to pigs (Funderburke, 1985). Piglets are commonly mixed with other litters after weaning. This may also cause psychological stress and depress immunological function (Blecha et al, 1983) and well-being. In addition, behavioral abnormalities such as chewing, massaging pen mates (Fraser, 1978) occur.

3) Effect of weaning on microbiology

Weaning of pigs is associated with the change of diet from sow's milk to a solid weaner diet and other postweaning stressors. The major intestinal flora of pig is lactobacilli, bifidobacteria, streptococci, bacteriodes, clostridium perfringes and E. coli, this microflora changes with age. It has been suggested that it may take 4 to 6 weeks to establish a stable flora (Mul and Ferry, 1994). When piglets are weaned, the intestinal microflora of piglets is altered (Jensen, 1998). It has been well recognized

that *E. coli* populations, especially haemolytic *E. coli*, are markedly increased in the anterior small intestine after weaning, and enteropathogenic *E. coli* is the major infection agent for postweaning diarrhea (Hopwood and Hampson, 2003). Withdrawal of sow's milk that contains natural immunoglobulin will stop preventing the proliferation of haemolytic *E. coli*. (Deprez et al., 1986). The dietary and environmental change after weaning may also be associated with change in intestinal microflora (Jensen, 1998; Conway, 1994).

It is assumed that the inclusion of prebiotics or probiotics in weaner diet may help to smooth the transition of intestinal microflora after weaning by stimulation of beneficial bacteria such as bifidobacteria and lactobacilli. Prebiotics such as FOS and oligofructose are metabolized by bifidobacteria and lactobacilli in the large intestine. This may stimulate their growth (Modler et al, 1990). The most commonly used probiotic strains are lactobacillus, bifidobacterium and streptococcus.

2.5 Small intestine morphology

At weaning there are a number of well documented changes in the histology and morphology of the small intestine. The gastrointestinal tract is the main digestive and absorptive organ in animal. The small intestine has a huge absorptive surface. The gastrointestinal tract permits the uptake of dietary substances into systemic circulation and it also excludes pathogenic compounds simultaneously (Gaskins, 1997). The presence of villi, microvilli and Kerckring's folds in the small intestine results in a much larger surface area than that of a cylindrical tube (Caspary, 1992). The shape of healthy villi in pigs is finger-like (Mouwen, 1972). The total absorptive surface area in the small intestine of piglet (10 day old) is 114m² (Buddle and Bolton, 1992). Moon (1971) found that the villous epithelium in the small intestine in 3 week old pigs is replaced more rapidly (in 2-4 days) than that of one day old piglets (in 7-10 days).

The morphological structure of small intestine from our work (Shim et al., 2004, unpublished) is shown in Figure 1 and 2. There is a reduction in villous height (villous atrophy) and an increase in crypt depth (crypt hyperplasia) at weaning (Kelly et al, 1991; Pluske et al, 1996). Villous atrophy was associated with either an increased rate of cell loss from the villous apex or a reduced rate of cell renewal (Pluske et al, 1997). These changes are clear at 5 days postweaning and continued in the first to second week after weaning (Kelly et al, 1991). The villous height was reduced to 50-75% of pre-weaning values (Hampson, 1996; Kelly et al, 1991).

Our studies (unpublished, 2004) showed that the same feed given at different levels of FOS numerically higher villi in weaned pigs. Several other reports demonstrated that higher feed intake immediately after weaning reduced the histological changes of small intestinal morphology (Spreeuwenberg, 2002; Pluske et

al, 1996; Verdonk et al., 2001). Thus, it is important to increase feed intake immediately after weaning.

From the literature cited above, it can be concluded that early weaned piglets are exposed to rapid changes in nutrition (from a milk based diet to a solid cereal based diet), environment and social environment (separation from the sow and mixing piglets). Low feed intake is usually seen during the first few days after weaning. This is associated with decreased villous height and low brush border enzyme activities. The result is a decreased nutrient absorption, postweaning growth check and high incidence of diarrhea in pigs in commercial early weaning facilities. A nutritional approach can be used to help solving the postweaning stress and growth check. Prebiotics, probiotics or synbiotics may be part of the potential alternatives to antimicrobial growth promoters.

3. Fructo-oligosaccharides as Prebiotics

3.1 Definition and Candidate of Prebiotics

One of the approaches for enhancing the beneficial endogenous microflora in the gut is the use of prebiotics. Generally the term prebiotic can be described as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid, 1995).

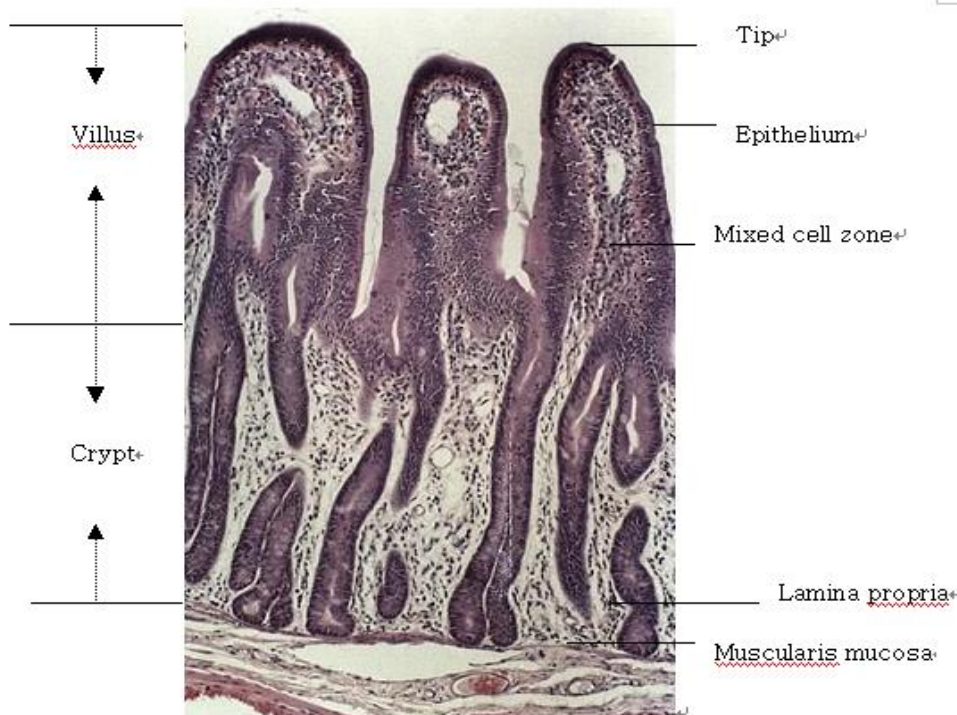


Figure 1. Photomicrograph of histological section of duodenum of a weaned pig (45 days old). The villi, crypts, epithelium and muscularis mucosa are shown (X 573)

* Source: Shim et al, 2004 (Unpublished)

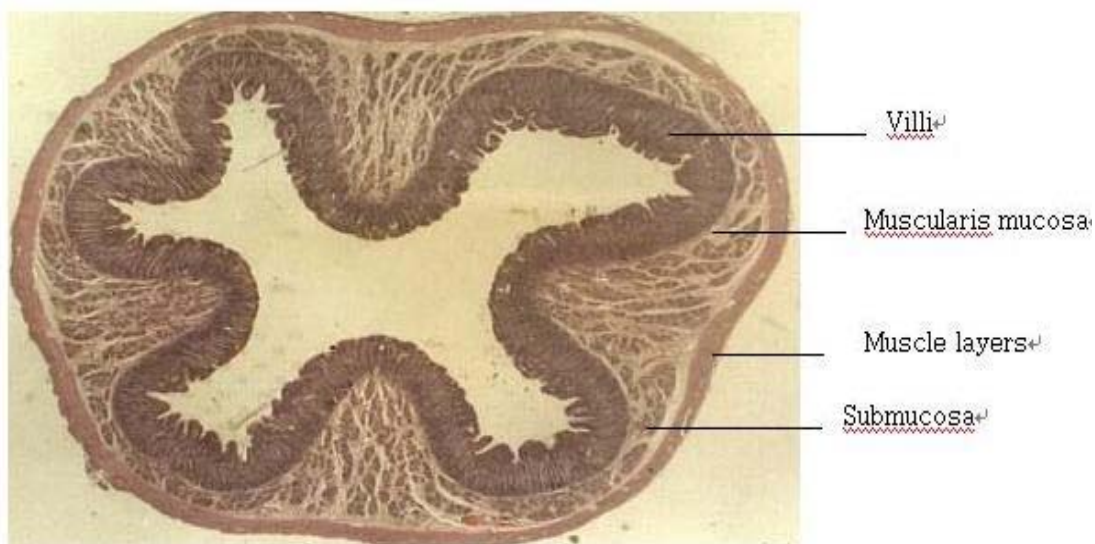


Figure 2. Cross section of duodenum in the small intestine of weaned pig (45 days old), (X 287)

Non-digestible carbohydrates (Figure 3) include non-digestible oligosaccharides and non-starch polysaccharides, resistant starch (Delzenne and Roberfroid, 1994). All of these non-digestible carbohydrates are expressed as non-digestible polysaccharides

because they are not hydrolyzed by endogenous enzyme in the small intestine, but hydrolyzed by colonic bacteria in the large intestine. However, all of these could not be classified as prebiotics but rather colonic food because the process of colonic fermentation in most of these substances is nonspecific (Gibson and Roberfroid, 1995). It should be noted that starch not digested enzymatically but fermented may also be a candidate.

Oligosaccharides are a group of carbohydrates consisting of 2-10 sugar units (Figure 4), and each oligosaccharide has a different chemical structure. FOS are named by the chain length (degree of polymerization=DP). Inulin contains 2-60 DP, and synthetic fructan (FOS) contains 2-4 DP. Oligofructose contains 2-9 DP and can be obtained by partial enzymatic hydrolysis of inulin. It is well known that oligosaccharides are naturally occurring constituent in plants and vegetable, and the most common sources are onions, Jerusalem artichokes, bamboo shoots, chicory roots and bananas. Commercially available prebiotics are mostly fructo-oligosaccharides, isomalto-oligosaccharides, galacto-oligosaccharides, transgalacto-oligosaccharides, inulin and oligofructose etc. (Table 1). Among the candidate of prebiotics, fructo-oligosaccharides are only products that meet the criteria allowing classification as prebiotics (Gibson and Roberfroid, 1995). FOS is one of the most commonly used as prebiotics. Physio-chemical properties of oligosaccharides depend on their chemical structure and composition. Most oligosaccharides are soluble in water or physiological fluids.

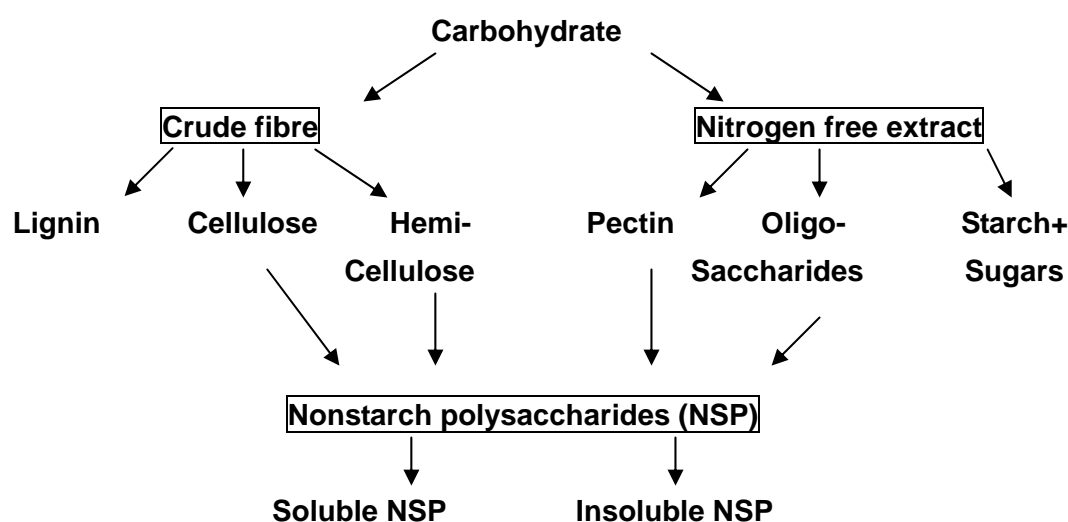


Figure 3. Schematic diagram of total dietary fibre fractions (Adapted from Verstegen and Schaafsma, 1999)

Figure 4. The Classification of Main Dietary Carbohydrates

Monosaccharides
- Glucose, Fructose, Galactose, Mannose
Disaccharides
- Sucrose, Lactose, Maltose
Oligosaccharides (3-10 glucose units)
- α -Galactosides: raffinose, stachyose, verbascose
- Nondigestible oligosaccharides (soluble)
Fructo-oligosaccharides, Oligofructose
Isomalto-oligosaccharides (Gluco-oligosaccharides)
Galacto-oligosaccharides
Lactulose
Polysaccharides (> 10 glucose units)
- Starch: amylose, amylopectin, Modified starches
- Non-starch polysaccharides (NSP) (soluble or insoluble)
cellulose, hemicellulose, gums, pectins
β -glucans, fructans, mucilages

Table 1. Major oligosaccharide candidates for prebiotics

Oligosaccharides	Structure	Linkages	Process	Origin
Xylo-oligosaccharides	(Glu) _n	β -1,4	Hydrolysis	Cereals
Lactulose	Gal-Fru	β -1,4	Isomerisation	lactose
Isomalto-oligosaccharides	(Glu) _n	α -1,6	Hydrolysis	Algae
Gluco-oligosaccharides	(Glu) _n	α -1,2 and α -1,6	Synthesis	Sucrose
Galacto-oligosaccharides	(Gal) _n -Glu	β -1,4 and β -1,6	Synthesis	Lactose
Fructo-oligosaccharides	(Fru) _n -Glu	(β -2,1)- α -1,2	Synthesis	Sucrose
Oligofructose	(Fru) _n -(Fru) _n -Glu	(β -2,1)	Hydrolysis	Inulin

(Adapted from Andrieux, 2001)

3.2 Fructo-oligosaccharides and Bifidobacteria

The chemical structures of FOS consists of short chain polymers of β 1-2 linked fructose units (Figure 5). FOS are produced commercially either by hydrolysis of inulin or by enzymatic synthesis from sucrose or lactose. They are not hydrolyzed by the enzymes of endogenous origin (Oku et al., 1984). Short chain lengths of chicory inulin up to 20 fructose units are called fructo-oligosaccharides. Specific

nondigestible oligosaccharides can selectively proliferate specific bacteria such as bifidobacteria (Hayakawa et al, 1990). A more recent technique is the development of new structurally modified FOS. The degree of polymerization (DP) is relatively low (DP=less than 11) and these components may be used as substrates for the development of specific strains of bifidobacteria in the large intestine of pigs. It is assumed that FOS are not digested in the small intestine. So they will reach to the large intestine of pigs where they stimulate the growth of bifidobacteria (Bunce et al., 1995a). However, FOS may start to digest (ferment) already in the small intestine. It is also thought that FOS is rapidly fermented in the proximal part of the large intestine of weaned pigs (Houdijk et al, 1997).

Bifidobacteria are anaerobic, gram-positive bacteria and they are found in the gastro-intestinal tract of human infants and adults as well as various warm-blooded animals (Rasic, 1983). The organism was first isolated from the faeces of breast-fed infants by Tissier (1900). FOS is selectively fermented by most strains of bifidobacteria (Wang and Gibson, 1993). The predominant species of bifidobacteria in pigs is *bifidobacterium psuedolongum* (Type A) (Mitsuoka, 1984). Bifidobacteria are saccharolytic organisms and all strains of fermented glucose, galactose and fructose. Glucose is fermented via the fructose-6-phosphate shunt to acetic and L lactic acids. Bifidobacteria do not produce CO₂, butyric or propionic acid (Kurmman, 1983). The optimum growth temperature of bifidobacteria is 37 to 43°C and optimum pH for growth is 6.5 to 7.1 (Scardovi, 1986). Bifidobacteria populations in the gastrointestinal tract of piglets range from 10⁴-10⁶/g chyme in the stomach to 10⁸/g chyme in the ileum (Stewart et al, 1993) and 10⁸ to 10⁹ in the large intestine (Borg Jensen, 1993). There is variation Several studies showed that bifidobacteria and lactobacillus may be beneficial and the dominant bacteria in the colon (Jonsson and Conway, 1992; Hidaka et al, 1986). Bifidobacteria have an antibacterial effect because they can suppress potential pathogens like *E. coli*. They do this by producing antimicrobials like bacteriocin or by lowering pH through the rapid production of volatile fatty acids especially acetate and lactate (Wang and Gibson, 1993; Rasic, 1983). The undissociated acid which is presented in a higher proportion where pH decreases in the gut can function as antibacterial agent (Eklund, 1983). It is the use of nitrogenous compounds for the growth of bifidobacteria which will lead to less proteinous substances used for energy. Thus, when there is saccharolytic fermentation, the bacteria does not use as much protein for energy. This may result in less amines and branched chain fatty acids. Non-specific immune activity can be increased by feeding fermented milk products with *bifidobacterium bifidum* (Schiffrin et al., 1995). In an in vitro study, FOS and xylo-oligosaccharides are converted to acids at a high rate by most strains of bifidobacterium, at a lower rate by most lactobacilli, most bacteroides, but not used by eubacteriaceae, most clostridia

(except *Clostridium butyricum*), *E. coli* and staphylococcus (Wada, 1990).

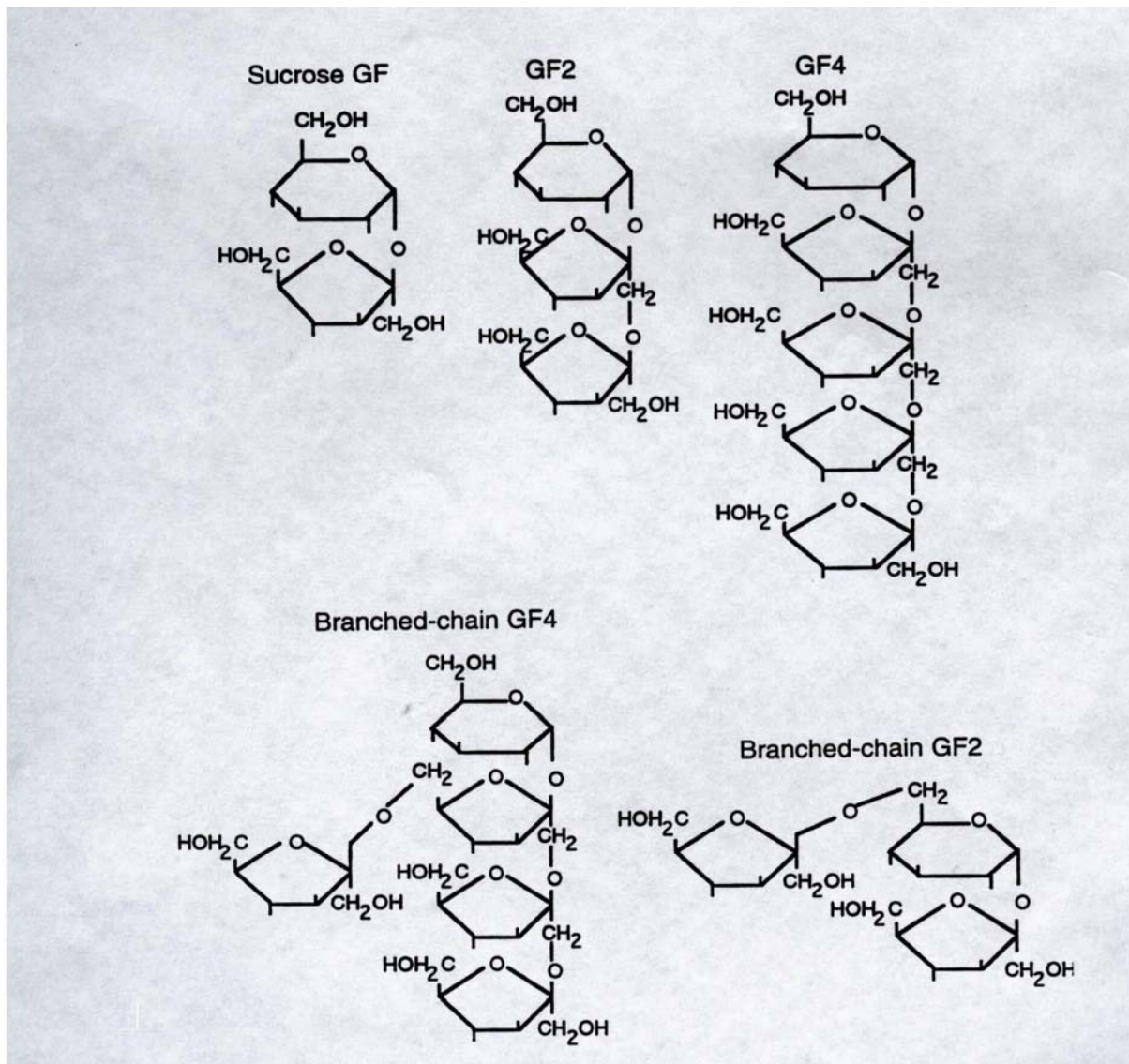


Fig.5. Chemical structures of sucrose and various fructo-oligosaccharides. G= glucose; F= fructose

It is well known that amino acids are absorbed from the small intestine. Nitrogenous products produced by microflora from organic nitrogen in colon are absorbed as well (Niiyama et al., 1979). Nitrogen absorbed in the colon is as ammonia and urea and excreted as urea via urine. It may be more beneficial for pig that the microbes in the colon grow from protein entering the colon and thereby produce biomass. This will only happen if there is a sufficient quantity of carbohydrate which they can use as an energy source. So the best thing for the

animal and human in the colon is to avoid much absorption of nitrogen in the colon and to have the nitrogen excreted with feces as biomass. However, it is in contrast with what is expected nowadays, the absorbed bifidobacterial nitrogen in colon may be beneficial to the pig.

It is believed that dietary prebiotics can increase bioavailability of minerals in the gut (Scholz-Ahrens et al, 2001). Moreover, bifidobacteria produce water soluble vitamin B group (Liescher, 1961).

3.3 Various effects of fructo-oligosaccharides

The main objective of supplementing FOS to the diet of weaned piglets is to help maintaining or proliferating beneficial microorganism such as bifidobacteria and lactobacilli after weaning. Thereby they prevent against intestinal pathogens like *E. coli*. As a result, the gut health of newly weaned piglet might be helped and as a consequence maintain feed intake and growth of pigs. Possible schematic modes of action of FOS in the gastrointestinal tract of weaned pigs are shown in Figure 6. An overview of the experimental results data on various effects of prebiotics is provided in Chapter 2.

4.1 What they are?

Probiotics may be used as one of the potential alternatives to be used as feed additives. The term is used to describe living microbial feed supplements which beneficially affect the host animal by improving its microbial balance (Fuller, 1989). The words probiotics is derived from the Greek (*pro bios*) and means “for life.” Probiotics have been used for several decades since 1970th to improve the gastrointestinal health of animal. It is known to have a stimulating effect on the growth of beneficial bacteria such as lactobacilli and bifidobacteria in the gut. In Europe, European probiotics association was established in 1999 and to help establish proper legislation. Nineteen microorganisms are legislated and marketed in the EU as feed additives.

Various microorganisms to be used as probiotics were isolated from gastrointestinal content, mouth, and feces of animals and humans. The major microorganisms presently used as probiotics strains in animals are *Lactobacillus*, *Bifidobacterium*, *Bacillus* spp, *Streptococcus*, *Yeast* and *Saccharomyces cerevisiae*. They should be non pathogenic, gram positive, acid resistant, strain specific, anti *E. coli*, bile resistant, viable/stable, adhesion to intestinal mucosa, and contain a minimum 30×10 to the power of 9 colony forming unit per gram (Pal, 1999, Salminen et al, 1996).

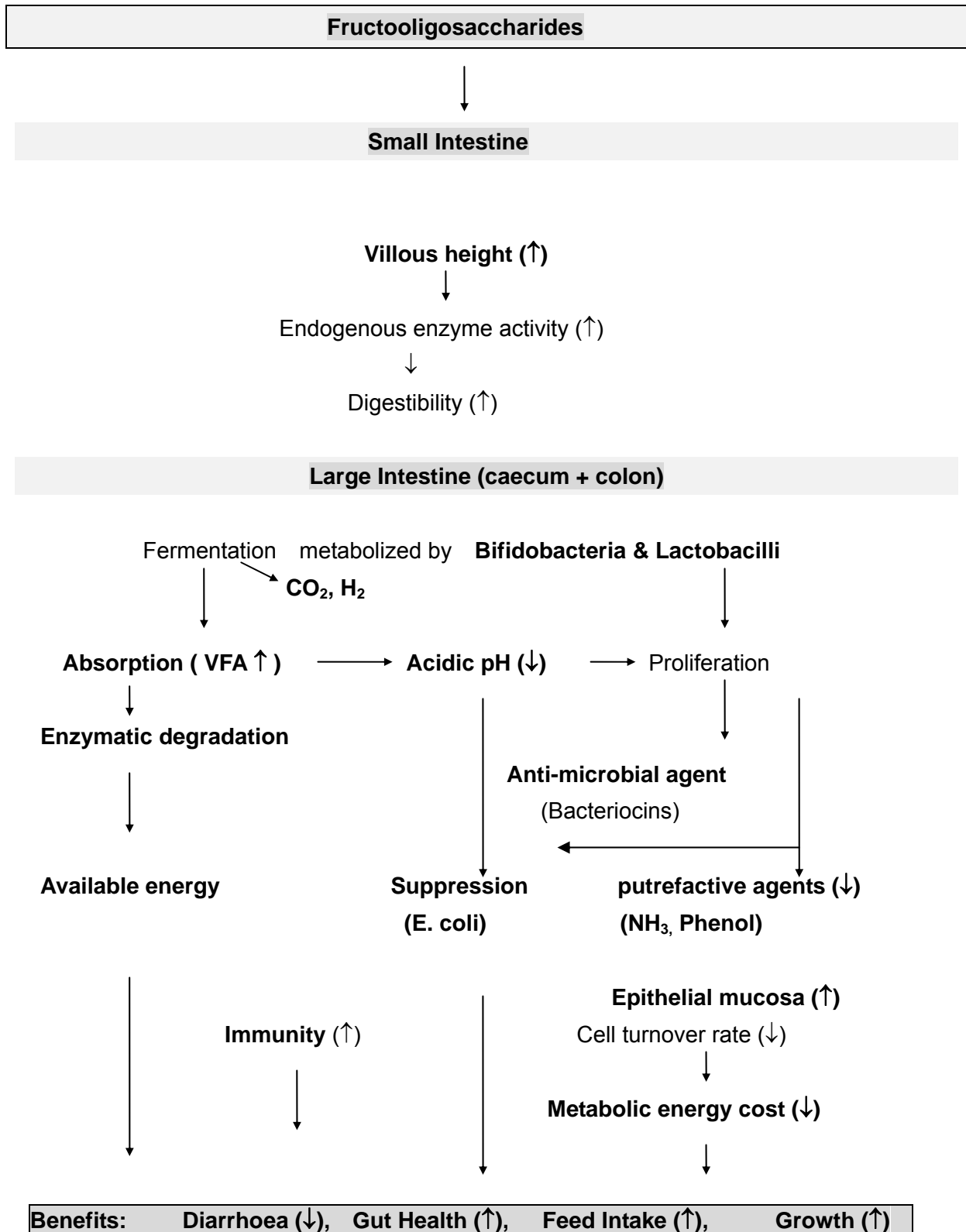


Figure 6. Possible schematic mode of actions of FOS in the gastrointestinal tract of weaned pigs (some pigs produce methane and H₂)

4. Probiotics

Most of works on probiotics in the literature is about using one single or two strains of beneficial bacteria. But multi-strains of bacteria may be more useful to proliferate lactic acid bacteria.

4.3 Mode of action

It has been suggested that probiotics are strain specific, species and dose specific. There are several proposed mechanisms by which probiotics may protect the host from the intestinal disorder (Rolfe, 2000; Lee et al, 1999). Firstly, probiotic microorganisms produce several inhibitory substances such as organic acids, hydrogen peroxide and bacteriocins. These substances may limit the harmful bacteria in the gut. All lactic acid bacteria produce organic acid. Another proposed mechanism is that of competitive inhibition for bacteria (like *E. coli*) adhesion on intestinal epithelial surfaces (Conway et al. 1987) which will allow it to rapidly colonize the intestinal tract. Thirdly, probiotics may prevent the utilization of nutrients by pathogenic bacteria. Fourthly, it is postulated that probiotics can protect against intestinal disease by stimulation of specific and nonspecific immunity (Fukushima et al. 1998).

4.3 Influence on the gastrointestinal microflora

One of the main reasons for using probiotics is to stabilize the digestive microflora and compete with pathogenic bacteria. Several reports demonstrated that administering lactic acid bacteria has an influence on the gastrointestinal microflora. The stimulation of the growth of both bifidobacteria and lactobacilli by supplementing multi-strain probiotics may help to protect young pigs against potential pathogens (Xuan et al., 2001). Summary results of the effects of probiotics on the gastrointestinal microflora are shown in Table 3. From the summary results, it seems that probiotics are beneficial to a certain degree and can influence some beneficial microflora in the gastrointestinal tract of young pigs. Harper et al (1983) have failed to find any beneficial effect of lactobacillous probiotics on growth, feed intake and feed efficiency. In addition Xuan et al (2001) also failed to demonstrate any significant effects of probiotics on microbial population, diarrhea score and growth performance in piglets. Another important consideration is that efficacy of probiotics can negatively be influenced by the presence of antibiotics and anti-mycoplasma drugs in the feed (Pal, 1999). So it is suggested that probiotics should be used after antibiotic therapy and two different routes of administration can be applied with when anti-mycoplasma drugs are used.

Table 3, Summary of the influence probiotic strains on the gastrointestinal microflora in pigs

Animal	Probiotics	Effects	Reference
Suckling piglet	<i>B. thermophilum</i> & <i>B. pseudolongum</i>	Reinforced the normal intestinal flora and alleviated clinical symptoms of scouring	Kimura et al., 1983
Neonate (2-days old)	Yoghurt & milk fermented with <i>L. reuteri</i>	Lactobacilli (↑), <i>E. coli</i> (↓).	Ratcliffe et al., 1986
Piglet	<i>Streptococci</i> , <i>Ent. Faecum cernelle 68</i>	Faecal <i>E. coli</i> and haemolytic <i>E. coli</i> (↓).	Deprez et al., 1986
Weanling	<i>Ent. faecalis</i>	Faecal <i>E. coli</i> (↓).	Danek, 1986
Piglet	<i>L. acidophilus</i>	Lactobacillus and <i>E. coli</i> in stomach (↑), but no influence in other digestive tract	Pollman et al., 1980b
Weanling	<i>Lactobacillus</i>	Scouring (↓).	Hale and Newton 1979
Weanling	<i>Bacillus cereus</i> , <i>Lactobacillus spp.</i> <i>Streptococcus</i>	No influence on mortality, clinical symptoms and fecal hemolytic <i>E. coli</i>	Cupere et al., 1992
Suckling piglet	<i>Lactobacillus</i>	Faecal lactobacillus (↑)	Jonsson, 1986
Weanling	<i>Bifidobacterium globosum A</i>	No consistent effect on scour scores, fecal or gastrointestinal pH and cell-mediated immune response	Apgar et al., 1993
Finisher pig	<i>Bacillus spp.</i>	No influence on intestinal microflora	Spriet et al., 1987
Weanling	<i>Bacillus subtilis</i>	Streptococci and bifidobacteria (↑), Bacteroides (↓).	Ozawa et al., 1983
Suckling piglet	<i>Lactobacillus</i>	Coliforms (↓), lactobacillus (No effect)	Newman, 1990
Suckling Weanling	<i>Bifidus bifidum ID</i>	Less incidence of diseases	Ervolder et al., 1985

* (↓) and (↑) are either significantly increased or decreased.

4.4 Growth performance effects

If the gastrointestinal ecosystem can be altered by feeding probiotics, the health of young pig can be improved and thereby growth performance of host animal can

also be improved.

Table 4, Summary of the influence probiotic strains on growth performance in pigs

Animal	Probiotic	Effect	Reference
Weanling	<i>Lactobacillus sp.</i>	Feed conversion ratio (↑), Nitrogen and fibre digestibility (↑),	Hale and Newton. 1979
Weanling	<i>L. bulgaricus</i> <i>L. casei</i> <i>S. thermophilus</i>	Feed intake (↑), Growth (↑), FCR (No effect)	Lessard and Brisson. 1987
Weanling	<i>L. acidophilus</i> <i>S. faecium</i>	Growth and FCR (↑), Growth (↓).	Pollmann et al, 1980a
Weanling	<i>L. acidophilus</i>	Some depressed growth rate	Harper et al, 1983
Weanling	<i>Lactobacillus sp</i>	Growth (↑)	Ogle and Inborr, 1987
Weanling	<i>L. reuteri</i>	Decreased growth rate and lower FCR	Ratcliffe et al, 1986
Weanling	<i>Bacillus licheniformis</i>	Weight gain (↑),	Collinder et al., 2000
Weanling	<i>Bacillus licheniformis</i> <i>Bacillus toyoi</i>	Growth (↑) Growth (↑)	Kyriakis et al., 1999
Weanling	<i>Bacillus</i>	Growth (↑)	Collinder et al 2000
Growing	<i>Streptococci</i>	Improved growth rate & FCR	Neupert, 1988
Growing Finishing	<i>Lactobacillus sp</i>	No effect	Hale and Newton. 1979
Growing Finishing	<i>L. acidophilus</i> <i>S. faecium</i>	No effect	Pollmann et al, 1980a
Weanling	<i>Bacillus toyoi</i>	Growth and feed efficiency (↑), Diarrhoea, mortality (↓)	Kyriakis et al., 1999
Weanling	<i>L. acidophilus and</i> <i>Streptococcus</i> <i>faecium</i> <i>Or Bacillus toyoi</i>	Growth and feed efficiency (↑), Feed intake (No effect), Nitrogen retention and Biological Value (↑),	Fialho et al., 1998

Growth and health of piglets can be affected by various factors. Some studies found beneficial effect on growth rate (Table 4). One of the main reasons for the conflicting

effects of probiotics is that the strains used as probiotics are very different. Some may not be effective at all and also dose levels vary. So there is a need for a good survey of which ones work under what conditions and which ones do not work.

5. Synbiotics

Effects of prebiotics or probiotics on growth of young pigs are variable. The concept of synbiotic, a combination of pre- and probiotics components has been proposed also to alter beneficial bacteria in the gut, and in turn, improve the health and welfare of young pigs (Gibson and Roberfroid, 1995). The synbiotics are now being used in medical practice in humans (Roberfroid, 1998). This approach may be taken as an alternative to antibiotic growth promoter for young pigs. However, there is scarce information available to date on the effects of synbiotics on pigs. Nemcová et al (1999) have shown that feeding a combination of FOS and probiotics (lactobacillous) to young pig increased lactobacillous, bifidobacterium, total anaerobes, total aerobes count. Other reports also demonstrated that feeding synbiotics to young pigs improved growth rate (Kumprecht and Zobac, 1998) and decreased mortality rate (Nousiainen and Setälä, 1993). Thus, this concept remains open for validation.

6. Conclusions

In this review of the literature, we attempted to provide information regarding postweaning stressors, pre- and post weaning growth and small intestine morphology of young pigs. In addition, information on the various effects of prebiotics and probiotics or synbiotics of young pigs is reviewed. Early weaned piglets are exposed to several stressors such as nutritional, environmental, social, and microbiological. They have resulted in decreased immune response, increases diarrhea, and consequently postweaning growth check. To overcome this suboptimal postweaning performance, subtherapeutic antibiotics are commonly used in commercial pig production. However, there has been growing concern about antibiotics resistance of gut microflora that can be a risk for human health. So the use of growth promoting antibiotics will be completely banned by EU from 2006 onwards. Prebiotics and probiotics are one of the possible approaches to replacing antibiotics growth promoters. Prebiotics are assumed nondigestible in the small intestine and are considered to ferment in the large intestine. Probably there are selectively used by bifidobacteria. For a prolonged saccharolytic activity throughout the large intestine, more slowly fermentable source of carbohydrate may be needed in the diets for better efficacy of FOS. Further research on the effects of different chemical structure (degree of polymerization) of FOS on growth and gut ecosystem is needed to verify and find optimal chemical structure of FOS. Moreover, a combination of relatively

rapid and slowly fermentable FOS on growth and health effects may also be needed. Probiotics is a live microbial feed additive that is supposed to alter beneficial bacterial ecosystem in the gut. For more consistent beneficial effects of probiotic on growth and gut ecosystem in young pigs, probiotic selection criteria must be applied. A new concept of synbiotics is the most promising approach for enhancing beneficial bacteria in the gut. More researches regarding various combinations of multiple probiotic strain and prebiotics are needed to verify possible modes of action and find optimal combination of synbiotics for newly weaned piglets.

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Chapter 4

Effect of dietary fructo-oligosaccharides on viscosity, disaccharidases activity and digestibility in the small intestine of the weaned pig

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Abstract

The objective of this study was to determine the effects of fructo-oligosaccharides (FOS) on digestive enzyme activity, ileal digestibility of protein, viscosity and empty growth weight gain of weaned pigs. Twelve male pigs from three litters were weaned at 24.2 d (average body weight 8.8kg). Animals were individually housed and fed either control, a diet with 0.25% FOS, or a diet with 3% FOS diet for 21 d after weaning. At the end of the experiment, the digesta of small intestine (duodenum, jejunum and ileum) and large intestine (caecum, proximal and distal colon) were collected for determination of disaccharidase activity, viscosity, ileal digestibility. Full and empty weights of gastrointestinal tract were determined. Feed intake and empty body weight gains of young pigs fed with FOS were numerically improved. FOS tends to increase the weight of small and large intestines. Apparent digestibility of protein in ileum (control:68.4, FOS 0.25:75.8 and FOS 3%:72.5) was increased ($P < 0.005$). FOS had no effect on viscosity of the digesta in the small intestine. Disaccharidase activity and mucosal protein content in the small intestine were not affected by FOS addition. FOS increased full weight but not empty weight of large intestine and increased empty weight and length of small intestine. It was concluded that dietary FOS may increase empty growth weight gain and ileal digestibility of protein. FOS did not affect disaccharidase activity, or viscosity of digesta in the small intestine.

Key words: Weaned Pigs, Fructo-oligosaccharides, Enzyme, Viscosity, Digestibility.

Introduction

In different countries piglets are weaned at different ages and in Korea it is at 18 to 23 days of age. Weaning at this age may cause major disruptions to their life because they are exposed to several stressors such as change of food from milk to solid feed, change in environmental temperature, mixing piglets with other litters. Weanling pigs commonly face a decline in brush border enzyme activity (Gay et al, 1976), villous atrophy, changes in gut wall permeability (Hampson, 1986; Spreeuwenberg et al., 2001) and alteration of gut microflora (Vickers et al, 1998). The reduction of both digestive and absorptive capacity can lead to malabsorption (Miller et al, 1984) immediately after weaning. Low feed intake after weaning is often the major problem which can lead to piglet's weight loss for several days after weaning (Barnett et al., 1989), consequently a postweaning growth check. This is a major limitation in pig production.

Fructo-oligosaccharides (FOS) are not hydrolysed by enzymes of mammalian

origin but are hydrolysed by microorganisms (Oku et al, 1984). FOS may stimulate the growth of Bifidobacteria. These bacteria can suppress at least indirectly potential pathogens like *E. coli* (Gibson and Wang, 1994). These changes of intestinal microflora may enhance the health of young pigs. There is conflicting evidence on the effect of FOS on digestion and performance of young pigs. The reasons for this are not completely understood. FOS ferments very rapidly and disappears at the beginning of large intestine (Houdijk, 1997). Some reports have shown that supplementing a diet with FOS improves growth (He et al., 2002; Estrada et al., 2001; Russell et al., 1996) while other reports have found little or no effects (Mikkelsen et al., 2003; Howards et al., 1995) and mixed effects (Houdijk et al., 1998). The reasons why the growth responses are inconsistent are not understood well.

An experiment was conducted to measure the effect of supplementing FOS in a weaner diet on feed intake, activities of disaccharides enzymes and digestibility in the small intestine in weanling pigs.

Materials and Methods

Animals and Managements

A total of twelve male pigs (Large White × Landrace) weaned at 24.2 days old, with an average body weight of 8.2 ± 0.4 kg was used in a 21 day trial. The animals from 3 litters were randomly assigned to each of the treatments on the basis of their litter of origin and live weight. Each litter was represented in each treatment and each treatment had similar mean liveweights of animals. The piglets were housed individually in galvanised wire mesh pens inside a room where the temperature was maintained at $28 \pm 1^\circ\text{C}$ during the first 4 days. The room temperature was maintained between 25 to 27°C . Each pen was equipped with a feeder and water supply from a nipple. Pigs were given free access to feed and water. The piglets did not receive creep feed during lactation. Body weight and feed intake were measured. A completely randomised design with 3 treatments was used. The treatments consisted of a control diet and a control diet supplied with 0.25% or 3% of FOS.

Diets

The experimental diets are shown in Table 1-1. The diets were formulated to meet or exceed protein, lysine and energy requirements for weaned pigs as recommended by NRC (1998). All diets contained 14.6 MJ DE/kg DM, 21.9% crude protein and 1.3% total lysine. The basal diet was a wheat-soybean meal. FOS was substituted for starch according to treatments (either control or 0.25 FOS or 3% FOS). Two different

level of FOS were used in this experiment because a high dose (3%) of FOS in a previous study (Shim and Choi, 1997) showed beneficial effects compared to 0.2 to 0.5% (the levels recommended by the manufacturer). The chemical composition of FOS on a dry-matter basis was 95% dry matter (GF₂:1-kestose = 35%, GF₃:nystose = 50%, and GF₄:1-fructosyl-nystose= 10%), 5% of glucose + fructose + sucrose. GF₂ is FOS with a terminal glucose and two fructose units, GF₃ is FOS with a terminal glucose and three fructose units and GF₄ is FOS with a terminal glucose and four fructose units. Antibiotics were not included in the diets. Total NSP contents (calculated) of the basal diet were 11.6% (control), 11.8% (FOS .25%) and 14.6% (FOS 3%), respectively. Chromic oxide was used at the level of 0.5% in the diets as the marker for the subsequent determination of the ileal protein digestibility.

Table 1. Composition of experimental diets, %(as fed), composition calculated

Ingredients	Diets		
	Control	FOS ¹ 0.25%	FOS 3%
Wheat(12% CP)	69.93	69.93	69.93
Soybean meal	14.84	14.84	14.84
Fish meal	5.00	5.00	5.00
Meat & bone meal	4.79	4.79	4.79
Canola oil	1.00	1.00	1.00
L-lysine-HCL	0.26	0.26	0.26
DL-methionine	0.07	0.07	0.07
L-threonine	0.12	0.12	0.12
Vitamin & mineral premix ²	0.15	0.15	0.15
Chromic oxide	0.50	0.50	0.50
Salt	0.30	0.30	0.30
Corn starch	3.00	2.75	-
FOS	-	0.25	3.00
Calculated analysis			
DE, MJ/kg	14.60	14.60	14.60
Crude protein	21.89	21.89	21.89
Lysine	1.33	1.34	1.34
Ca	0.80	0.80	0.80
P	0.68	0.68	0.68
NSP ³	11.56	11.81	14.56

¹Fructooligosaccharides (Neosugar®, Meiji Seika Kaisha, Ltd., Japan): on a dry basis, composed of 35% GF₂, 50% GF₃, 10% GF₄, and less than 5% of glucose + fructose + sucrose.

²Provided the following nutrients (per kg of air-dry diet): Vitamins: A, 5×10^6 ; D₃, 1.3×10^6 ; E, 10g; K₃, 1g; B₂, 2g; B₆, 0.8mg; B₁₂, 10mg; Ca-pantothenate, 7g; Niacin (nicotinic acid), 10g; Mineral: Cobalt, 0.1g; Se (selenium selenite), 66 μ g; Iodine, 0.3g; Manganese, 30g; Zinc, 100g; Copper, 5g.

³NSP: nonstarch polysaccharides content was calculated by subtracting CP, crude fat, starch, sugar and ash content from the DM content. Total NSP content includes FOS. Chemical composition (%) of NSP in SBM and wheat of basal diets: Cellulose 2.3%, Arabinose 2.42%, Xylose 3.6%, Mannose 0.4%, Galactose 0.9%, Glucose 0.9%, Uronic acids 1.0%, Rhamnose 0.04%), The calculations from the values determined in ingredients of wheat and soybean meal adapted from Bach Knudsen (1997), and Gdala et al. (1997).

Slaughter and Sampling Procedure

At the end of the feeding trial (after 3 weeks), the pigs were fasted for three hours before being euthanised. Each pig was sedated with an intro-muscular injection of ketamine / xylazine (4 mg and 2 mg/kg live-weight respectively) and was anaesthetised with a gas mixture of oxygen and halothane. Ileal digesta was flushed from the terminal 15 cm of the ileum and samples of the small intestine were taken at 25, 50, and 75% of length from the duodenum to ileum for enzymology. After collecting the ileal digesta sample, the piglets were then slaughtered by injection of barbiturate (Lethabarb; 0.5ml/kg live body weight, 325 mg/mL Sodium Pentobarbitone, Virbac Australia Pty. Ltd., Peak Hurst, NSW, 2210) into the heart. The entire gastrointestinal tract of each pig was removed. The small intestine was dissected free of mesenteric attachment and then each section of tract was ligated (silk suture) and cut into the following segments: stomach, duodenum, jejunum, ileum, caecum and colon. Each segment was weighed while full. The length of the small intestine was measured and approximately 10g of the intestinal contents was collected from the duodenum, the jejunum, the ileum, the caecum, the proximal colon, distal colon and the rectum. The digesta samples allowed to flow freely into a collection container (no stripping). The digesta samples were frozen and stored at -20°C until assayed. The empty weights of the small intestine, caecum, and colon were taken. All the intestinal segments were washed with water and the outer intestinal surface was carefully cleaned and dried using absorbent tissue paper. Empty weights were recorded.

Determination of activities of disaccharidases

Three small intestine sites were sampled at equidistant distances of 25, 50 and 75% from the pylorus to the ileo-caecal valve. The samples (10cm) from the sites were excised and frozen in liquid nitrogen for enzyme determination. For the

determination of enzyme activities, the entire mucosa from partially-thawed 10cm lengths of small intestine were scraped with a glass slide and spatula and the weight was noted. The mucosa was then placed in a plastic vial containing 50ml of deionised water and homogenised for 30 seconds in a Polyron®. The content was transferred to a centrifuge tube and spun at 3,000rpm for 15 minutes. The supernatant was then decanted and a dilution of 5ml supernatant plus 5 ml of deionised water was prepared and kept in a plastic tube at 4°C for determination of lactase, sucrase and maltase. The methods for the determination of disaccharidase activities were adapted from Miller (1981) and Kelly et al. (1991).

Determination of apparent protein digestibility

After three weeks of feeding the diet, the terminal ileum and the rectum were removed 3.5h after the morning feed. Terminal ileal digesta 15cm from the ileo-caecal valve were collected and the ileal digesta samples were freeze-dried and then ground through a 1-mm mesh screen. The feed samples were ground and analysis for protein and dry matter according to AOAC (1990). Chromium contents in the diets and ileal digesta was determined according to Williams et al (1962). All analyses were performed in duplicated and calculations were carried out on a DM basis.

Determination of digesta viscosity

Ileal digesta were collected and stored at -20°C. For measurement of viscosity the samples were thawed and approximately 1.5g was centrifuged at 10,000g for 15 min at 20°C. Viscosity was determined on an aliquot (0.5 ml) of fresh supernatant using a Brookfield DVIII Viscometer (Brookfield Engineering Laboratories INC, USA) equipped with a CP40 cone and plate head. All apparent viscosity values were expressed as milliPascal second (mPa. s).

Statistical Analysis

The data from each of the parameters were analysed by ANOVA as a complete randomised design. ANOVA was conducted using the procedure of GENSTAT 5 (Second edition, Release 3.2). When ANOVA revealed a significant effect of mean differences among treatments, means were tested using paired t-tests. Statistical significance was accepted at $P < 0.05$.

The experiment was reviewed and approved by the Animal Experimentation Ethics Committee of The University of Western Australia. The animals used in these experiments were maintained in accordance with the recommendation of The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Results

Empty body weight gain and digestible energy intake

There were no significant differences among treatment (Table 2).

Full and empty weight of the gastrointestinal tract and length of small intestine

Table 2. Empty body weight gain (EBWG), digestible energy feed intake (DEFI) and FCR for weaner pigs fed diets with or without FOS¹

Item	Control	FOS 0.25%	FOS 3%	SEM	<i>P</i>
Weight, kg					
Initial weight	8.2	8.2	8.1	0.27	0.96
Final EBWG	11.0	14.1	13.3	1.17	0.24
EBWG, g/d	132	282	247	52.1	0.18
DEFI, MJ/pig	5.6	8.4	8.1	0.94	0.15
FCR	1.56	1.40	1.52	0.19	0.53

¹A total of 12 weanling pigs were used, one pig per pen, four pens per treatment

Table 3. Full and empty weight (wt) of small intestine and large intestine, and length of small intestine of pigs (g, wet weight)¹ fed diets with or without FOS

Item	Control	FOS 0.25%	FOS 3%	SEM	<i>P</i>
Small intestine					
Full wt	751	936	874	65.6	0.21
Empty wt	662 ^b	918 ^a	802 ^{ab}	42.4	*
Length, m	10.5 ^b	12.2 ^a	12.5 ^a	0.45	*
Caecum					
Full wt	136.3 ^b	233.5 ^a	233.0 ^a	17.5	**
Empty wt	78.0	95.0	122.5	15.6	0.27
Colon					
Full wt	502.0 ^b	694.3 ^a	652.3	44.0	*
Empty wt	260.3	373.8	373.8	35.3	0.11
Large intestine					
Full wt	638.3 ^b	927.8 ^a	885.3 ^a	47.7	**
Empty wt	338.3 ^b	468.8 ^a	490.3 ^a	37.7	0.06

¹A total of 12 weaner pigs were used, one pig per pen, four pens per treatment.

^{a,b}Mean values within row with different superscripts differ significantly ($P < 0.05$), * $P < 0.05$;

** $P < 0.01$

Table 5. Disaccharidase activity of the small intestine and apparent ileal digestibility of protein¹

Item	Control	FOS 0.25%	FOS 3%	SEM	<i>P</i>
Ileal digestibility of CP, %	68.4 ^c	75.8 ^a	72.5 ^b	4.16	0.005
Enzyme activity ²					
Duodenum					
Mucosal protein content, mg/g	71.7	79.7	72.1	9.61	0.44
Maltase	21.2	22.8	29.2	8.97	0.44
Lactase	3.5	7.1	7.1	4.25	0.43
Sucrase	4.6	6.0	7.0	3.24	0.52
Jejunum					
Mucosal protein content, mg/g	73.6	73.4	71.1	8.67	0.90
Maltase	31.1	32.3	25.3	10.21	0.40
Lactase	3.8	6.6	3.7	3.26	0.40
Sucrase	11.0	9.8	7.5	4.65	0.58
Ileum					
Mucosal protein content, mg/g	72.7	77.3	73.5	6.79	0.61
Maltase	24.2	27.8	16.9	9.47	0.30
Lactase	1.3	0.4	1.5	1.73	0.65
Sucrase	7.5	8.2	3.7	3.43	0.19
Mean value of disaccharide ³					
Maltase	26.4	27.3	23.8	2.87	0.65
Lactase	3.0	4.7	4.1	1.07	0.45
Sucrase	7.7	8.0	6.2	1.18	0.48

¹A total of 12 weaner pigs were used, one pig per pen, four pens per treatment.

^{a,b}Mean values within row with different superscripts differ significantly ($P < 0.05$).

²Enzyme activities (mM/m./g mucoa)

³Mean values of enzyme activities over the small intestine (mM/m./g mucoa).

The full and empty weights of the small intestine, caecum, colon, and the length of small intestine are shown in Table 3. Supplementing the diet with FOS at the level of 0.25 or 3% significantly increased the weight of the empty small intestine ($P < 0.05$), full ($P < 0.01$) and empty large intestine ($P < 0.05$), and the length of small intestine ($P < 0.05$). Empty weight of the large intestine ($P < 0.058$) was larger after 3 weeks of feeding with FOS when compared to control.

Determination of viscosity of digesta

There were no significant differences among treatments (Table 4). Viscosity was lower in the duodenum and increased along the small intestine to the ileum.

Disaccharidase activities in the small intestine

There were no significant effects of FOS on the mean values of maltase, lactase and sucrase over three sites (duodenum, jejunum and ileum) of small intestine in Table 5. Large variations of enzyme activities were observed within treatments.

Total disaccharidase activity was slightly lower in the duodenum, especially sucrase, which was low in the duodenum. The average ratio of lactase:maltase, maltase:sucrased and lactase:sucrased were 0.15, 3.5 and 0.53 over the small intestine. The protein content of the mucosa over the small intestine was similar between treatments (Table 5).

The apparent ileal digestibility of protein was significantly higher ($P < 0.001$) for diet with FOS at the level of 0.25 or 3% than that of control (Table 5). The ileal digestibility was 68.4, 75.8 and 72.5% for the control, 0.25 FOS and 3% FOS diet respectively.

Discussion

Empty body weight gain and feed intake

In this experiment we tested the hypothesis that FOS changes digestion and as a consequence also empty body weight gain. Average daily intake of digestible energy (calculated values) was slightly improved in pigs fed FOS compared with the control (NS). As a result, there was a tendency of improved empty body weight gain in pigs fed FOS. This agrees with other reports that feed intake and body weight of weaner pigs may be improved with the addition of FOS (Orban *et al.*, 1994; Bunce *et al.*, 1995b; Russell, 1996). Our previous report shows similar growth promoting effects of FOS (Shim and Choi, 1997). Several other studies (Farnworth *et al.*, 1992; Orban *et al.*, 1994; Houdijk *et al.*, 1998) have failed to demonstrate a growth response with additions of FOS to weaned pigs. The reason for differences in literature might be that there are different forms of FOS chemical structure (linear or branched, type of linkages between monomeric sugars), and the origin of FOS (produced from inulin by enzymatic degradation, bio-synthetic product etc.) can also vary. Piglets used in this experiment were not fed creep.

The differences in NSP contents of the diets as used in different studies in literature may partly affect the responses (Total NSP contents of basal diets: Orban *et al.*, 1997 = from 10.40 to 11.95; Houdijk *et al.*, 1998 = from 7.20 to 9.14%). FOS in a diet with very low levels (because commonly used main ingredients of diet for young pig are specially processed) of NSP alone may not work because the fermentation goes so quick that the FOS is already disappeared in the beginning of large intestine (Houdijk, 1997). So diets which containing a mixture of quick and slowly fermentable carbohydrates may ensure continuous supply of carbohydrates as an energy source

to the microbes.

After weaning, pigs commonly face several problems which are associated with several weaning stressors. Post-weaning growth check is the most serious problem that occurs after weaning. This results from low voluntary food intake. This is a major limitation to reach the potential growth of pigs. If voluntary food intake can increase by the addition of FOS, this may reduce or minimise post-weaning problems. In addition, digestive capacity of weanling pigs can be improved (Kelly et al., 1991; Pluske et al., 1996).

The mechanisms of increased feed intake are unknown, but several factors might be involved. One can be taste. One possibility is that FOS might have beneficial effects on the health of the gastrointestinal tract by stabilization of intestinal microfloral population in the early weaned pigs. If that occurs then disturbances are less likely to occur. If post-weaning stressors in the gut are less by NDO's and if feed intake is increased, then early weaned pigs will have better development and adaptation of the digestive capacity.

Viscosity

It is thought that a low viscosity in the digesta of the small intestine will allow easier penetration of pancreatic enzymes in the chyme. This may increase the digestibility of nutrients such as starch and protein. In the present experiment, the digesta viscosity in duodenum, jejunum and ileum was not affected by FOS treatments. Chyme increases in viscosity as it passes through the GI tract (Dusel et al., 1997). It may be assumed that the non-viscous components are absorbed. Moreover, this would lead to lower viscosity. DM content changes (reduced) and microflora can change composition into more viscous components. Bedford and Classen (1992) have shown that there is a clear beneficial effect of low digesta viscosity in the small intestine with regard to growth in broilers. Choct *et al* (1996) also reported that the digesta viscosity in the small intestine is strongly affected by specific bacterial growth. FOS are not digested in the small intestine in humans and reaches the large intestine intact where they can be fermented by bifidobacteria. Freezing and thawing of digesta samples will reduce digesta viscosity when compared with a measurement of viscosity with fresh digesta samples but relative value of viscosity will be the same (H. Graham, personal communication).

In pigs dietary FOS may partly ferment in the small intestine (Houdijk et al, 1997). This will clearly affect the microflora ecology in the small intestine of weaned pigs. Houdijk *et al.* (1997) suggested that non-digestible oligosaccharides such as FOS are fermented in the ileum of weaner pigs. As a result, the ileal and faecal VFA profiles can be changed compared to animals with no dietary FOS. This result is shown both *in vivo* and *in vitro* (Houdijk et al, 1997). The presence of a viscous

polysaccharide solution in the gut lumen may result in a slowing of nutrient absorption. This can be due to a decreased rate of diffusion of substrates and digestive enzymes (Edwards *et al.*, 1988; Ikegami *et al.*, 1990).

Disaccharidase activities in the small intestine.

It was anticipated that FOS might increase the feed intake as a consequence of the activities of disaccharidases. We also expected increased villus height in the small intestine with the FOS diet. Large variations were found between individual values of enzyme activities in individual animals and no significant differences between treatments were found. The enzyme activity of maltase, lactase and sucrase in the duodenum tended to be higher in animals with FOS compared to the controls. The enzyme activities are stimulated by substrate. Feed intake was higher in pigs fed diet with FOS and therefore more disaccharide was eaten. As a result, this stimulates the enzyme activity. There was however not a significant correlation found between feed intake and pooled enzyme activity in the small intestine among treatments. The intestinal disaccharidase activities responsible for the enzymatic breakdown of dietary carbohydrates can play an important role in the nutritional processes of the young animal. Abrupt weaning around the third to fourth week of life causes a fall in the production of amylases and proteases (Lindemann *et al.*, 1986; Owsley *et al.*, 1986). In this experiment, maltase activity was high but lactate activity was low over the different segments of the small intestine. The lowered lactase activity may be due to the low level of lactose in diet.

Lactase activity reaches its maximum at 1 week after birth, but decreases gradually with age (Aumaitre and Corring, 1978). In this experiment, lactase activity compared to disaccharidase activities in the small intestine was small. The reason for the progressive decline in lactase activity in the pigs is undoubtedly related to the absence or reduction of lactose in the diet (Hampson and Kidder, 1986). The effects of the hormones or the nervous impulses may be stimulatory or inhibitory to secretion of digestive enzymes (Longland, 1991). The relative development of the ratio (lactase:maltase = 0.15, maltase:sucrase = 3.5, and lactase:sucrase = 0.53) of different intestinal disaccharidases activities (Mean values over three sites in the small intestine) as found in this experiment was in agreement with the results reported by Moog *et al.* (1973). Jones *et al.* (1972) have shown that the ratio of maltase:sucrase increased with the age of the piglet. This is a logical development because the primary substrates of the maltase are the end-products of α -amylolytic starch digestion.

Specific enzyme activity can be affected by the different weight of mucosa recovered, so the measurements of specific enzyme activity alone are likely to be a poor indicator of digestive capacity (Widdowson, 1984). The protein of the mucosa

was markedly reduced during the first week after weaning (Hampson, 1983; McCracken, 1984) which probably related to intake. The activity of intestinal enzymes is also important in relation to occurrence of scouring. The incidence and severity of post weaning diarrhoea was reduced by supplementing diet with carbohydrate degrading enzymes (Inborr and Ogle, 1988).

Ileal digestibility is commonly regarded as a reference for reflecting the recovery rate of protein (amino acids) for absorption through the villi of the small intestine. The apparent ileal digestibility of protein was significantly higher ($P < 0.001$) for diet with FOS at the level of 0.25 or 3% than that of control. Previous reports showed that non-digestible oligosaccharides did not affect ileal digestion of nutrient per se (Mathew et al. 1997; Houdijk et al. 1999). The reasons why different results were obtained in the different experiments are not clear. Ileal digestibility measurements are subjects to some limitations like the use of markers, and the representativeness of digesta collection (Just, 1980).

The empty weights of the small intestine and full and empty weight of the large intestine were increased significantly by the addition of FOS, and the length of small intestine was also significantly increased by the FOS inclusion. The empty weight of each organ expressed as % of body weight or as % of empty body was not significantly different among treatments. Pigs fed higher dose levels (3%) of FOS tended to have heavier organs compared with the control or 0.25% FOS.

The results show that apparent ileal digestibility of protein was increased for pigs given diet with FOS. From this experiment, it can be concluded that dietary fructo-oligosaccharides might be used as a potential effective growth promoter for early-weaned pigs especially with a wheat-soybean diet.

Implications

Commercially available fructo-oligosaccharides may have potential as a growth promoter in early weaned pigs, especially under stressful environments. Recommended dose levels of fructo-oligosaccharides for weaned pigs may be around 0.25% but it will depend largely on the chemical structure of oligosaccharides and the age and weight of pigs at weaning, the nature of creep or weaner diet.

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Chapter 5

Feeding diets supplemented with fructo- oligosaccharides for 3 weeks postweaning increased villous height and VFA concentrations in the large intestine of pigs

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Abstract

The effects including fructo-oligosaccharides to the diet of weaning piglet on morphometric change of small intestine, VFA, ammonia concentration, and on pH, and scour scores of gastrointestinal tract contents and feces were studied. Twelve male pigs weaned at 24 days of age (average initial weight 8.2 kg) were housed individually and fed either a control diet, a control diet with 0.25% FOS, or a 3% FOS diet. Duration was 21 d after weaning. At the end of the experiment, digesta of the small intestine (duodenum, jejunum and ileum) and large intestine (caecum, proximal and distal colon) were collected for determination of pH, VFA, ammonia concentration, and for morphometric measurements. Villous height was higher in all sites of the small intestine in pigs fed FOS diets compared with the control animal. The pH in chyme of the proximal colon (control: 5.94, FOS 0.25:5.91 and FOS 3%:5.53) was lower ($P < 0.001$) when pigs had been fed a diet with 3% FOS compared to other treatments. These results showed that dietary FOS may lower pH and ammonia concentration in the gastrointestinal tract of weaned pigs.

Introduction

Early weaned piglets are commonly exposed to post weaning stressors. These are nutritional, microbiological, and environmental origin. These stressors are associated with adverse changes to the gut which include villous atrophy, crypt hyperplasia. This may result in less digestive and absorptive function (Hampson, 1986, Miller et al., 1984). Postweaning piglets often experienced proliferation of *E. coli* in the gastro-intestinal tract (Tzipori et al., 1980; Miller et al. 1984; Nabuurs, 1998), and a lowered immune response (Barnett et al., 1989). As a result, early weaned piglets frequently show growth check and diarrhoea problems (Kats et al., 1994).

Fructo-oligosaccharides (FOS) are known to have the potential to improve gut health in humans. Health and welfare of young pigs may also be influenced. According to Orban et al. (1994), most of dietary FOS are not digested in the small intestine and they may reach the large intestine where they stimulate the growth of bifidobacteria (Bunce et al., 1995a). In piglets, however, they may already be fermented in the small intestine (Houdijk, 1997). This process can suppress potential pathogens like *E. coli* either by producing bacteriocin or by lowering pH through the rapid production of volatile fatty acids (Gibson and Wang, 1994). Villous atrophy impairs digestive and absorptive function of the gut that results in a postweaning growth check (Pluske et al., 1997). Villous atrophy can be reduced if feed intake after

weaning is improved (Van Beers et al., 1998). In the literature the effect of dietary FOS on growth of young pigs are various and conflicting. Some reports have shown that supplementing a diet with FOS improves growth (He et al., 2002; Estrada et al., 2001; Russell et al., 1996) while other reports have found little or no effects (Mikkelsen et al., 2003; Howards et al., 1995) and mixed effects (Houdijk et al., 1998). The reasons why the growth responses to FOS, as found in different studies, are contradictory are not clear. It is possible that other carbohydrate components may play a role (Houdijk et al., 1998).

The objective of this study was to investigate the effect of dietary FOS on villous height and crypt depth in the small intestine. In addition we investigated FOS fermentation in the large intestine and in relation to VFA, pH and ammonia.

Materials and Methods

The experiments were approved by the Animal Experimentation Ethics Committee of The University of Western Australia. The animals used in these experiments were maintained in accordance with the recommendation of The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Animals and Management

Twelve male pigs (Large White × Landrace) weaned at 24 days of age with a mean initial weight of 8.2 ± 0.4 kg were used. The animals were randomly assigned on the basis of their litter of origin and live weight so that each litter was equally represented in each treatment and each treatment had a similar liveweight of the animals. The piglets were housed individually in wire mesh pens inside a room where the temperature was maintained at $28 \pm 1^\circ\text{C}$ during the first 4 days. The ambient temperature was maintained between 25 to 27°C . Each pen was equipped with a feeder and a nipple type waterer. Pigs were given free access to feed and water.

Diets

The experimental diets are shown in Table 1. The diets were formulated to meet or exceed protein, lysine and energy requirements for weaned pigs as recommended by NRC (1998). All diets contained 14.6 MJ DE/kg DM, 21.9% crude protein and 1.3% total lysine. The basal diet was a wheat-soybean meal. FOS substituted starch according to levels (either control or 0.25 FOS or 3% FOS). The FOS product (Neosugar®, Meiji Seika Kaisha, Ltd., Japan) is a mixture of glucose, sucrose and FOS with a terminal glucose unit. The chemical composition of FOS product on a dry-matter basis was 95% dry matter ($\text{GF}_2 = 35\%$, $\text{GF}_3 = 50\%$, and $\text{GF}_4 = 10\%$), 5% of

glucose + fructose + sucrose. GF₂ is FOS with a terminal glucose and two fructose units, GF₃ is FOS with a terminal glucose and three fructose units and GF₄ is FOS with a terminal glucose and four fructose units. Antibiotics were not included in the diets. Chromic oxide (at a level of 5g/kg feed) was used in the diets as marker for the determination of the ileal protein digestibility.

Experimental design

A randomised design with 3 treatments was used. The treatments consisted of a control, 0.25% FOS or 3% FOS and the treatment levels were chosen based on previous results (Shim and Choi, 1997). We found that a 3% level of FOS tended to improve feed intake and body weight gain. FOS at the level of 0.25% was arbitrarily chosen.

Slaughter and Sampling Procedures

At the end of the experimental period of 21 days, Feed was removed from each pig 3h before the expected slaughter time and final live weight was recorded. Each pig was sedated with an intro-muscular injection of ketamine / xylazine (4 mg and 2 mg/kg live-weight respectively) and was anaesthetised with a gas mixture of oxygen and halothane. The piglets were sacrificed by injection of an overdose of barbiturate (Lethabarb; 0.5ml/kg live body weight, 325 mg/mL Sodium Pentobarbitone, Virbac Australia Pty. Ltd., Peak Hurst, NSW, 2210) into the heart. The gastro-intestinal tract of each pig was removed, different sections of the gastro-intestinal tract were ligated (silk suture) and cut into the following segments: stomach, duodenum, jejunum, ileum, caecum and colon. Approximately 10g of the intestinal contents was collected from the duodenum, the jejunum, the ileum, the caecum, the proximal colon, distal colon and the rectum. The digesta samples were frozen at -20 °C until subsequent analysis.

pH Measurements

The digesta samples from the stomach, duodenum, medial part of the jejunum, ileum, cecum, proximal and distal colon contents and faeces were emptied into 50g containers. pH was measured immediately using an electrode with a portable digital pH meter (Suntex Instruments Co., Ltd.). Calibration of the pH electrode was checked every 4 measurements using standard solutions.

Morphometric measurements

Approximately 2 cm² samples from each site of the small intestine (at distances of 25, 50 and 75% from the pylorus to ileo-caecal valve) were excised and placed in 10 % buffered formalin at room temperature for 24h. The fixed material was processed through graded ethanol, chloroform and embedded in paraffin wax. Paraffin

Table 1. Composition of experimental diets, % (as fed), composition calculated

Ingredients	Diets		
	Control	FOS ¹ 0.25%	FOS 3%
Wheat(12% CP)	69.93	69.93	69.93
Soybean meal	14.84	14.84	14.84
Fish meal	5.00	5.00	5.00
Meat & bone meal	4.79	4.79	4.79
Canola oil	1.00	1.00	1.00
L-lysine-HCL	0.26	0.26	0.26
DL-methionine	0.07	0.07	0.07
L-threonine	0.12	0.12	0.12
Vitamin & mineral premix ²	0.15	0.15	0.15
Chromic oxide	0.50	0.50	0.50
Salt	0.30	0.30	0.30
Corn starch	3.00	2.75	-
FOS	-	0.25	3.00
Calculated analysis			
DE, MJ/kg	14.60	14.60	14.60
Crude protein	21.89	21.89	21.89
Lysine	1.33	1.34	1.34
Ca	0.80	0.80	0.80
P	0.68	0.68	0.68
NSP ³	11.56	11.81	14.56

¹Fructooligosaccharides (Neosugar®, Meiji Seika Kaisha, Ltd., Japan): on a dry basis, composed of 35% GF₂, 50% GF₃, 10% GF₄, and less than 5% of glucose + fructose + sucrose.

²Provided the following nutrients (per kg of air-dry diet): Vitamins: A, 5x10⁶; D₃, 1.3x10⁶; E, 10g; K₃, 1g; B₂, 2g; B₆, 0.8mg; B₁₂, 10mg; Ca-pantothenate, 7g; Niacin (nicotinic acid), 10g; Mineral: Cobalt, 0.1g; Se (selenium selenite), 66µg; Iodine, 0.3g; Manganese, 30g; Zinc, 100g; Copper, 5g.

³NSP: nonstarch polysaccharides content was calculated by subtracting CP, crude fat, starch, sugar and ash content from the DM content. Total NSP content includes FOS. Chemical composition (%) of NSP in SBM and wheat of basal diets: Cellulose 2.3%, Arabinose 2.42%, Xylose 3.6%, Mannose 0.4%, Galactose 0.9%, Glucose 0.9%, Uronic acids 1.0%, Rhamnose 0.04%), The calculations from the values determined in ingredients of wheat and soybean meal adapted from Bach Knudsen (1997), and Gdala et al. (1997).

embedded tissue was sectioned at 6 µm and the subsequent slides stained with Gill's

haematoxylin and eosin (a modified method of Nabuurs et al. (1993). Measurements of villous height (from the tip to the crypt-villous junction), crypt depth and epithelium thickness (from the medial part of each villus) were made on eleven well-orientated villi. Morphometric measurements were made using Optimus (tm) software via an Olympus-BX50 Compound Microscope (Japan) with JVC video camera and a computer programme.

Determination of VFA in digesta

A modification of the method of Pethick et al. (1981) was used to determine the VFA concentrations in digesta samples. These were quantified using Gas Chromatography. Approximately 0.5 g of sample was added to 0.5 g of 10 N phosphoric acid in a 1:1 (w/v) ratio and mixed. The mixture was then centrifuged at 4°C for 15 min at 12,000 rpm in a micro centrifuge. The supernatant was collected into eppendorf and was kept at -20°C until analysis. A Shimadzu Gas Chromatograph (model: GC-17A, Japan) and an FFAP 15m × 0.53mm × 1.2µ film capillary column (Cat. No: 19684, Alltech Associates Pty. Ltd., Sydney, Australia) was used. Glass wool (5 cm) was packed in a guard column to prevent blockages in the column. For VFA analysis, the frozen sample was thawed and a 0.5 µL sample was withdrawn from the supernatant and injected into the column for the Gas Chromatography.

Ammonia analysis

Ammonia in the digesta samples from the caecum and faeces were measured for putrefactive bacteria activity using the procedure as described in Methods of Enzymatic Analysis (Bergmeyer and Beutler, 1985). Concentrations were determined using an automated clinical chemistry analyser (Cobas Mira, Roche Diagnostics) and an Ammonia enzyme kit (Cat. No.1112732, Boehringer Mannheim). Frozen samples were thawed at room temperature and approximately 1.25g of sample was centrifuged at 13000 rpm for 10 minutes. Fifty µL supernatant was diluted in 1 to 10 with water to bring into the working of the assay on the Cobas Mira for the analysis. The Cobas analyser temperature was set to 39°C.

Bacteriology (E. coli)

Each swab from an intestinal site (medial part of small intestine, caecum, and distal colon) was streaked onto a 5% sheep blood agar plate (Columbia base) and then this was incubated in air at 37°C for 24 hours. After incubation the plates were examined, with presence of haemolytic *E. coli* and visual estimations (as a semi-quantitative method) of the percentage of haemolytic *E. coli* on the plate.

Dry matter content

Two g of each sample was placed on a preweighed foil tray and put in a drying oven at 105°C for 48h. Each sample was reweighed and the dry matter content calculated.

Statistical Analysis

The data from each of the parameters were analysed by ANOVA as a complete randomised design. ANOVA was conducted using the procedure of GENSTAT 5 (Second edition, Release 3.2). When ANOVA revealed a significant effect of mean differences among treatments, means were tested using paired t-tests. Statistical significance was accepted at $P < 0.05$.

Results

pH values, and concentrations of VFA and ammonia in the large intestine

The pH in the caecum and proximal colon were significantly lowered ($P < 0.001$) in pig fed 3% FOS. The pH values in the distal colon and faeces tended to decrease in pigs fed the diet with FOS 3% compared with other treatments (Table 2). The diets with FOS did not significantly increase VFA concentrations in chyme of the different Dry matter content of caecum, colon and faeces

Pigs fed diet with 0.25% FOS significantly increased ($p < 0.01$) the dry matter content of faeces than those pigs fed control diet.

Villous height, crypt depth and epithelium thickness

There were no significant difference in villous height, crypt depth and epithelium thickness between treatments (table 3).

Diarrhoea and haemolytic E.coli.

Only very mild faeces was observed in a few animals during the first several days of the experiment. The presence of haemolytic E.coli in the digesta of small intestine, caecum and distal colon was determined by the percentage of haemolytic E.coli on the plate. There were no differences between treatments in regard to haemolytic E.coli in any part of the small intestine, caecum and distal colon.

Table 2. pH values, mean concentrations of VFA (digesta), ammonia concentration in the large intestinal contents, and dry matter contents of the caecum, colon and faeces for pigs fed diets with or without FOS¹

Item	Control	FOS 0.25	FOS 3%	SEM	<i>P</i>
pH					
Caecum	5.83 ^b	5.81 ^b	5.42 ^a	0.03	***
Proximal colon	5.94 ^b	5.91 ^b	5.53 ^a	0.05	**
Distal colon	6.39	6.35	6.25	0.21	0.89
Faeces	6.71	6.60	6.45	0.12	0.36
Total VFA (mmol/kg)					
Caecum	82.6	104.7	127.2	13.2	0.14
Proximal colon	99.4	119.5	124.9	21.0	0.68
Distal colon	91.2	144.2	131.6	15.4	0.11
Molar proportions of VFA					
Large intestine ² , %					
Acetate	61.12 ^a	51.21 ^c	56.14 ^b	1.11	***
Propionate	25.40	27.92	25.53	1.17	0.25
Butyrate	10.30 ^b	15.04 ^a	12.95 ^{ab}	1.17	*
Isobutyrate	0.47 ^b	1.17 ^a	0.45 ^b	0.17	**
Valerate	2.09 ^b	2.58 ^b	3.66 ^a	0.27	***
Isovalerate	0.65 ^c	2.08 ^a	1.29 ^b	0.27	**
Ammonia (mg/g/wet)					
Caecum	0.64	0.52	0.61	0.04	0.19
Faeces	0.82	0.75	0.74	0.04	0.44
Dry matter content (%)					
Caecum	15.75	19.99	17.72	2.7	0.57
Proximal colon	19.96	25.02	19.63	2.0	0.19
Distal colon	27.98	33.14	27.16	1.7	0.09
Faeces	27.89 ^b	32.67 ^a	26.54 ^b	0.9	**

¹A total of 12 weaner pigs were used, one pig per pen, four pens per treatment.

²Mean molar proportions (%) of total VFA values over three sites (caecum, proximal colon and distal colon) along the large intestine.

^{a,b}Mean values within row with different superscripts differ significantly ($*P < 0.05$), $***P < 0.01$; $***P < 0.001$.

Table 3. Villous height, crypt depth, the ratio of villous height and crypt depth, and epithelium thickness in the small intestine of pigs fed diets with or without FOS¹

Item	Control	FOS 0.25%	FOS 3%	SEM	P
Villous height(μm)					
Proximal (25%)	333	412	352	26.0	0.16
Medial (50%)	361	368	438	29.1	0.20
Distal (75%)	342	383	413	41.4	0.51
Crypt depth(μm)					
Proximal(25%)	269	269	258	19.7	0.91
Medial (50%)	270	326	269	28.2	0.33
Distal (75%)	249	252	280	16.8	0.41
Villous height/crypt depth ratio					
Proximal(25%)	1.25	1.55	1.38	0.07	0.26
Medial (50%)	1.35	1.20	1.62	0.07	0.20
Distal (75%)	1.37	1.56	1.47	0.09	0.64
Epithelium thickness(μm)					
Proximal(25%)	27	29	29	2.0	0.77
Medial (50%)	32	33	33	1.2	0.85
Distal (75%)	37	33	37	2.2	0.30

¹A total of 12 weaned pigs were used, one pig per pen, four pens per treatment.

Discussion

It was hypothesized that FOS can prevent to a certain extent the decrease in height of villi in the small intestine after weaning. This phenomenon may contribute an increase in growth of piglets. Although there were no significant differences between treatments, the villous height tended to increase by additions of FOS. Pigs fed 0.25% FOS had longer villi (24%) in the proximal small intestine than pigs fed the control. This result is in agreement with the recent report by Spencer et al. (1997) that FOS maintains villous height better than control diet. Villous height at the proximal site of the small intestine is significantly correlated with the total empty body weight gained in piglets in the study of Pluske et al. (1996). In the experiment there was no correlation between villous height in the duodenum and empty body weight gain of weaned piglets. Feeding diet with FOS to the weaned pigs tended to increase feed intake. Overall, the ratio of villous height and crypt depth tended to increase in pigs fed the FOS diets rather than those fed the control. The ratio was greater in the proximal and distal sites of small intestine in pigs fed 0.25% FOS than the control.

It is well known that the villous height in the small intestine is indicative for improved nutrient absorption (Pluske et al., 1996). Several reports have demonstrated that weaning is associated with villous atrophy and crypt hyperplasia in the small intestine (Hampson, 1983; Miller et al., 1986, and Pluske et al., 1996). Low feed intake immediately after weaning may be responsible for the villous atrophy and crypt hyperplasia because the absence of luminal stimulation and malnutrition of the enterocytes after weaning caused the less villous height and longer crypt depth (Kelly et al., 1991; Pluske et al., 1997).

There were no differences in the thickness of the epithelium in the villi between treatments at 25, 50 and 75% of the small intestine. The thickness in the ileum was slightly lower in pigs fed 0.25% FOS compared to control. A thin epithelium may mean less protein synthesis for their wall and less cell turnover rate. This may permit more protein absorbed and this may partly contribute to the improved growth of weaned pigs. A similar result is observed when antibiotics are given to animals (Armstrong, 1986). If the rate of enterocyte turnover is higher, it may result in less mature enterocyte at the tip of villous. Consequently, efficiency of nutrient absorption in the gut is decreased.

Several possible mechanisms have been proposed to explain the change in cell proliferation in gut with different diets (Sakata, 1987; Howard et al., 1995; Fleming et al., 1992). It was found that the level of enteroglucagon in the blood is affected by fermentable carbohydrates, and this may be responsible for the stimulation of mucosal cell proliferation of the small intestine (Gee et al., 1996; Goodlad et al., 1987). Enteroglucagon is a small family of peptides derived from proglucagon by post-translational processing in the L-cells of the distal small intestine and colon (Gee et al., 1996). The fermentative properties of FOS are similar to dietary fibre and this may have similar physiological effects as a dietary fibre (Roberfroid, 1993). Thus, it can be hypothesised that the slightly increased villous height in pigs fed FOS inclusion diets may be associated with the increased plasma enteroglucagon.

In the design of our study we hypothesised that FOS stimulates fermentation and lowers the pH by increasing the content of short-chain fatty acids, acetate, propionate and butyrate. Dietary FOS effects in piglets will also depend on the NSP components in diet. In the present results, the total VFA concentrations in the caecum, proximal and distal colon tended to be increased at FOS levels of 0.25%. The concentrations of acetate, butyrate, except propionate, as % of total VFA values over three sites (caecum, proximal colon and distal colon) along the large intestine were significantly affected by dietary FOS treatments. Acetic acid as % of total VFA in the large intestine was significantly higher in control compared with the dietary FOS either 0.25 or 3%. Butyric acid and isobutyric acid as % of total VFA in the large intestine were significantly increased in FOS 0.25% when compared to the control

diet. These results were similar to the other reports on VFA profile in FOS addition diets (Levrat et al. 1991; Farworth et al. 1992). The increased butyrate may act as a trophic factor for intestinal epithelium. Butyrate produced in the colon by microbial fermentation may be used as energy source for the colonocytes (Roediger, 1980; Csordas, 1996). Several other studies also failed to show increases in VFA concentrations in the large intestine with FOS (Farnworth et al., 1992; Howard et al., 1995). The reason for this has been shown by Houdijk et al.(1997). They found that VFA was already produced at the end of the small intestine. In the large intestine the VFA may have already disappeared. Thus, VFA concentrations at a point in time *in vivo* do not reflect production rates (Edwards, 1991). *In vitro* however, FOS will stimulate the production of VFA (Hidaka et al., 1986; Oku and Tokunaga, 1986). Rats fed diets with FOS at 10% had markedly increased total VFA contents and lowered pH in their faeces (Hidaka et al., 1986). Another reason why it is difficult to detect differences in the concentrations of VFA between treatments is that VFA are very rapidly absorbed and rapid absorption would reduce any variation between treatments (Comming et al., 1987; Von Englehardt et al., 1989; Howard, 1995).

The pH in caecum and proximal colon tended to be lower in pigs fed 3% FOS diet compared with the 0.25% FOS and control. The lower pH in the caecal and proximal colon of 3% FOS is most likely due to an increased VFA production especially, acetate and lactate (Gibson et al., 1995). The lowered pH in the large intestine may provide a better gut environment for bifidobacteria and other gram-positive bacteria, and suppress the growth of potential pathogenic bacteria such as *E. coli*.

Ammonia in the gastrointestinal tract is considered as a putrefactive product. Degradation of endogenous urea to ammonia has been postulated to be the negative effect of the microflora in the gut (Visek, 1978). This may be harmful to enterocytes (Smith, 1985). Caecal and faecal ammonia concentrations appeared to be reduced slightly by the addition of FOS. Several reports demonstrated that dietary FOS can reduce ammonia concentration in the large intestine of piglets (Fukuyasu and Oshida, 1986). The possible reason for reduced ammonia in the large intestine is that microbial growth requires protein and less protein is degraded. Low pH suppresses putrefactive bacteria. Hidaka and Eida (1988) also reported ammonia, phenol and amines are reduced when intestinal pH is low.

Clinical signs of diarrhoea and visual examinations of haemolytic *E. coli*.

No clinical signs of diarrhoea developed in the present experiment.

Dry matter content of caecum, colon and faeces

High levels of dietary FOS fed to weaned pigs will stimulate fermentation in the large intestine. This may increase the passage rate of digesta, which, in turn, might

lead to soft faeces (Mul and Perry, 1994). Thus, the dry matter content in the faeces is likely to decrease in pigs fed high level of FOS in the diet. The present results show that when pigs were fed a high level of FOS (3%), the dry matter content in the caecum, colon and faeces was not affected, but at the lower level of 0.25% the dry matter was increased, especially in the faeces. This result is not consistent with the results of Mul and Perry (1994).

A high VFA concentration in the large intestine may have increased the absorption of Na, K and water absorption in the large intestine (Ruppin et al, 1980). A mixture of acetate and propionate or acetate and butyrate has been shown to stimulate cecocolonic calcium and magnesium absorption in rats (Lutz and Scharrer, 1991). We postulate that NSP in the diet consists of different rates of fermentation. The best way is to have continuous fermentation in the gastro-intestinal tract of piglets (Houdijk, 1997). Thus, supplementing effects of FOS in piglets will also depend on the other NSP components in the diet.

We concluded that the dietary addition of fructo-oligosaccharides to a diet which also had NSP of soybean meal (Average total NSP content= 21.7%; Bach Knudsen, 1997) and wheat (Total NSP= 11.9%) favour villous height in the small intestine. It increased VFA concentration, and reduced ammonia content and pH in the large intestine of weaned pigs.

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Chapter 6

Influence of supplementation of probiotics and oligofructose to antibiotic-free diets on growth, nutrient digestibility, and fecal microflora in weaned pigs

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Abstract

The objective of this study was to determine the impact of different supplementation of oligofructose (OF) and probiotics alone or combined on feed intake, nutrient digestibility and growth performance in weaned pigs. Hematological characteristics, fecal microbial population and ammonia content were measured. Sixty pigs weaned at 25 days of age (average body weight, 6.4kg) were used in a 21 d trial. The treatments consisted of a control (antibiotic-free corn-soy diet), an OF diet (0.25% OF), a M-probiotics diets (0.2% multi-strain probiotics), a synbiotics diet (0.25% OF and 0.2% multi-strain probiotics), and a T-probiotics diet (0.2% two-strain probiotics). All supplementations significantly increased the body weight gain compared to the control. The apparent digestibility of dry matter, crude protein and ash as well as the apparent absorption of calcium and phosphorus in all treatment groups were higher than those of the control ($P < 0.05$). Platelet count in blood was higher in synbiotics fed pigs than with OF ($P < 0.05$). The number of fecal coliforms was decreased in all treatments animals compared to control animals ($P < 0.05$). The number of microbial populations was significantly altered by treatments. Synbiotics diet significantly increased the number of fecal anaerobic lactobacilli compared to the control diet. Fecal ammonia concentrations were reduced significantly in all treatments compared to the control ($P < 0.05$). In general the supplementation of the synbiotics (combining OF and probiotics) had the greatest effect on nutrient digestibility, fecal microflora and growth. But the effect of synbiotics was not additive than the effects of OF and probiotics added. These results showed that supplementation of pre- and probiotics or synbiotics (combination of OF and multi-strain probiotics) to an antibiotic-free diet (negative control) stimulate beneficial bacteria in the gastrointestinal tract and may also have beneficial effects on nutrient digestibility and growth performance in weaned pigs.

Introduction

The first 2 weeks of the postweaning represent a period of adaptation to stressors including nutritional, psychological and environmental stress. These stressors may result in low feed intake and this is associated with poor weight gain, villous atrophy and malabsorption (Pluske et al, 1997; Hampson and Kidder, 1986). Growth promoting antibiotics are often used in prophylactic doses to improve animal performance and health and to obtain economic benefits (Cromwell and Dawson, 1992). However, the use of growth promoting antibiotics in pig diets will be banned in the EU from 2006 onwards. Thus, the new development of alternatives to growth promoting antibiotics has a high priority in research. Prebiotics and probiotics are

potential alternatives to growth promoting antibiotics and it has been shown that they can affect the gastrointestinal ecosystem in pigs (Jensen, 1998).

OF are non-digestible oligosaccharides (NDO) and can be regarded as prebiotics because there are available as substrates for the gastrointestinal microflora (Oku et al., 1984). FOS may beneficially affect the host by selectively stimulating the growth of one or a limited number of bacteria in the colon and may improve the health of host animals (Gibson and Roberfroid, 1995). The effect on growth of OF ranged from little to no effect (Farnworth et al, 1992; Olsen and Maribo, 1999), mixed effects (Houdijk et al. 1998), to stimulation effects (Estrada et al. 2001; He et al. 2002) in weaned pigs. It is not fully clear why the effects of FOS on growth performance in weaned pigs are inconsistent. In this respect differences in degree of polymerization of OF between studies may have influenced the variable response between studies. OF with a different degree of polymerization (DP, 2-4) may differently stimulate bifidobacteria in the large intestine. It is thought that bifidobacteria initially utilize NDO with a low degree of DP. On the other hand, bacteroides degrade preferentially oligosaccharides with a high DP (van Laere et al., 1997).

Probiotics may be candidates as a substitute for antibiotics because they may affect growth in nursery pigs (Xuan et al., 2001; Chang et al., 2000). In several studies a multi-strain probiotics had more effect on growth of the host animal when compared to one-strain probiotics (Fuller, 1999). Rolfe (2000) suggested using a combination of pro- and prebiotics (the so-called synbiotics) because the prebiotics may stimulate the probiotics. In that respect, Nemcová et al. (1999) found an additional effect of supplementing OF and lactobacillus to weaner diet increased beneficial bacteria and decreased harmful bacteria when compared to supplementing lactobacillus only. A review by Vente-Spreeuwenberg and Beynen (2003) concluded that gut microflora can be influenced by the feeding of probiotics or prebiotics. It is not known whether the beneficial effect to weanling pigs is a direct effect on gut integrity or on digestion or absorption. Moreover, there is only scarce information on the effect of OF on nutrient digestion and mineral absorption in pigs. Thus, it is interesting to investigate the effect of supplementation of OF and probiotics or the combination of OF and a multi-strain probiotics on fecal microflora, nutrient digestion and performance in weaned pigs.

This experiment was conducted to investigate the effects of a). antibiotic-free diet with OF as prebiotics, b). multi-strain probiotics, c). two strain probiotics or d). synbiotics (combining OF and multi-strain probiotics) on growth, nutrient digestibility, gastrointestinal and hematological traits in weaned pigs.

Materials and Methods

Animals and Managements

A total of sixty pigs (Duroc×Yorkshire) × Landrace weaned at 25 days, with an average initial body weight of 6.37 ± 0.2 kg were used in a 21 day trial. The pigs from 10 litters were randomly assigned on the basis of their litter of origin, sex and body weight to one of 20 pens with 3 pigs each. Each pen was assigned to one of 5 treatments with 4 replicates (two pens with gilts and two pens with barrows for each treatment). The piglets were housed with 3 pigs per pen in a galvanized wire mesh pen inside a room. The room temperature was maintained between 26 and 28 C. Pigs were allowed to consume feed and water *ad libitum* from a two-hole self-feeder and from a nipple waterer, respectively. Five days after the onset of the experiment, all pigs were injected with 1ml of hog cholera live virus vaccine (HC-VAC, Choong-Ang Animal Disease Lab., Korea).

Experimental Design and Diets

The treatments consisted of a control (antibiotic-free corn-soy diet), a FOS diet (0.25% FOS), a M-probiotics diet (0.2% multi-strain probiotics strains), a synbiotics diet (a combination of 0.25% FOS and 0.2% multi-strain probiotics strains), and T-probiotics (0.2% two strain probiotics). FOS and probiotics were added to the diet at the expense of corn starch. The experimental diets (Table 1) were formulated to contain 14MJ/kg of ME, 20.2% of CP, 1.50% of lysine, 0.42% of methionine, 0.9% of calcium and 0.8% of phosphorus. The non starch polysaccharides (NSP) content of the basal diet was 9.8%. The NSP in the control diet originated from corn and soybean meal. The diets were fed in meal form and formulated to meet or exceed the NRC (1998) recommendations for all nutrients. Chromic oxide was added (0.2% in the diet) as an indigestible marker to allow digestibility determinations.

Chemical composition of FOS on a dry-matter basis was 95% dry matter (GF₂: 1-kestose 35%, GF₃: nystose 50%, and GF₄: 1-fructosyl-nystose 10%), 5% of glucose + fructose + sucrose. GF₂ is FOS with a terminal glucose and two fructose units, GF₃ is FOS with a terminal glucose and three fructose units and GF₄ is FOS with a terminal glucose and four fructose units. Multi-strain probiotics and two strain probiotics used in this study were microbial mixtures manufactured by Chungmi Bio Co. Ltd., Korea (Table 2). The multi-strain probiotics was composed on lactobacillus from the faeces of weaned pigs; bifidobacteria from the infant faeces; bacillus from the soybean malt; yeast and aspergillus from the leaven. The selected probiotics strains had been cultured by BL (bifidobacterium), MRS (lactobacillus), Nutrient (Bacillus), Potato dextrose (Yeast and Aspergillus) mediums (Williams, 1994). The process of probiotics manufacture is as follows. Kozi cultivation (rice bran, wheat bran, soybean meal, molasses) of the 15% Starter was applied at 30 °C for 5-7days,

then drying at 40 °C until a moisture content is 11%. The probiotics strains were subsequently counted.

Table 1. Diet composition (as-fed basis)

Ingredients (g/kg)	Control	OF ¹	M-Pro ¹	Synbio ¹	T-Pro ¹
Corn	405.0	405.0	405.0	405.0	405.0
Soybean meal (CP 48%)	152.1	152.1	152.1	152.1	152.1
Dried whey	250.0	250.0	250.0	250.0	250.0
Soy flour	95.5	95.5	95.5	95.5	95.5
Spray-dried blood meal	20.0	20.0	20.0	20.0	20.0
Fish meal	25.0	25.0	25.0	25.0	25.0
Animal fat	25.0	25.0	25.0	25.0	25.0
Tricalcium phosphate	13.0	13.0	13.0	13.0	13.0
Limestone	1.5	1.5	1.5	1.5	1.5
Vitamin/mineral premix ^a	2.2	2.2	2.2	2.2	2.2
Salt	2.0	2.0	2.0	2.0	2.0
L-Lysine-HCl	1.5	1.5	1.5	1.5	1.5
DL-Methionine	0.7	0.7	0.7	0.7	0.7
Corn starch	4.5	2.0	2.5	-	2.5
OF	-	2.5	-	2.5	-
M-Probiotics	-	-	2.0	2.0	-
Probiotics-2	-	-	-	-	2.0
Chromic oxide ^b	2.0	2.0	2.0	2.0	2.0
Chemical composition ^c					
ME, kcal/kg	3,340	3,340	3,340	3,340	3,340
Crude protein, %	22.0	22.0	22.0	22.0	22.0
Lysine, %	1.50	1.50	1.50	1.50	1.50
Methionine, %	0.42	0.42	0.42	0.42	0.42
Calcium, %	0.90	0.90	0.90	0.90	0.90
Phosphorus, %	0.80	0.80	0.80	0.80	0.80

¹Abbreviated: OF: 0.25% oligofructose; M-PRO: 0.2% multi-strain probiotics; Synbio: 0.25% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two-strain probiotics.

^aProvided per kg diet: 10,000 IU of vitamin A, 2,000 IU of vitamin D₃, 42 IU of vitamin E, 5 mg of vitamin K, 9.6 mg of vitamin B₂, 2.45 mg of vitamin B₆, 40 µg of vitamin B₁₂, 27 mg of pantothenic acid, 49 mg of niacin, 0.05 mg of biotin, 140 mg of Cu, 145mg of Fe, 179 mg of Zn, 12.5 mg of Mn, 0.5 mg of I, 0.25 mg of Co and 0.4 mg of Se.

^bUsed as an indigestible marker.

^cCalculated values.

Table 2. Specification of multi-strain probiotics and two strain probiotics used in this experimental diets

Item	Bacteria	Content, cfu/kg
Multi-strain probiotics		
Bifidobacterium sp.	<i>Bifidobacterium longum</i>	4.6×10^9
Lactobacillus sp.	<i>Lactobacillus acidophilus</i>	8.0×10^{10}
Bacillus sp.	<i>Bacillus subtilis</i>	9.0×10^{10}
Yeast	<i>Saccharomyces cerevisiae</i>	5.0×10^9
Aspergillus sp.	<i>Aspergillus oryzae</i>	6.8×10^8
Two strain Probiotics		
Bifidobacterium sp.	<i>Bifidobacterium longum</i>	5.0×10^8
Lactobacillus sp.	<i>Lactobacillus acidophilus</i>	5.0×10^8

Measurements and Sampling

Body weights and feed consumption were measured on day 7, 14 and 21 to determine daily gain, average daily feed intake and feed conversion ratio. On day 18 of the experiment, fecal samples were collected from the three pigs per pen by rectal massage; the sample was pooled within pen, dried and ground. Laboratory analyses of feed and feces included dry matter (DM), nitrogen, crude ash, calcium and phosphorus (AOAC, 1994). The chromium concentration in the feed and in fecal samples was determined using spectrophotometry (Shimadzu, UV-1201, Japan). The red blood cell (RBC) count, white blood cell (WBC) count, hematocrit value, hemoglobin content, platelet count, lymphocyte, neutrophil and monocyte concentrations in whole blood were measured. Blood samples (5mL) were collected via jugular vein into heparanized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) from five pigs in each treatment at the termination (chosen as random) of the feeding trial. All blood samples were analyzed with an automated haematology analyzer (ADVIA 120, Bayer, Tarrytown, USA) using commercial reagent kits (lymphocytes, monocytes, neutrophils: ADVIA[®] 120 PEROX 1, 2 and 3 reagents, Bayer, USA; WBC: ADVIA[®] 120 BASO reagent; RBC and platelet: ADVIA[®] 120 BASO reagent; Hemoglobin: ADVIA[®] 120 HGB reagent). On day 18 of the experiment, rectal fecal samples were collected in 50ml centrifuge tubes for ammonia nitrogen analysis. The feces were weighed and diluted about 1:4.5 (w/w) with distilled water. An aliquot of 0.134ml 6N H₂SO₄ was added for inactivation of urease in feces. The samples were centrifuged at 2,000×g for 15 minutes and the supernatants were collected into plastic vials and immediately stored

at -20°C for further analysis. The ammonia nitrogen concentration in supernatants was determined by using a calorimetric method (Cheny and Marbach, 1962).

The populations of total coliforms, anaerobic lactobacilli and total anaerobic microbiota were done as described by Orban et al. (1997). Ten grams of fresh feces were serially diluted 1:10 (1g in 9ml) in sterile phosphate buffered saline (pH 7.2). Coliforms (MacConkey Agar, Difco Laboratories, USA) were enumerated by inoculating 100mm plates of the respective media with 0.1ml of the appropriate dilutions. Total anaerobes (Nutrient Agar, Difco), anaerobic lactobacilli (Rogosa, Difco) were enumerated by inoculating triplicate 60-mm plates of the respective anaerobically prepared media with 0.025ml of the appropriate dilutions inside a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) under a 97% CO₂ : 3% H₂ atmosphere.

The parameters were analyzed as a randomized complete block design with analysis of variances performed using the general linear model procedure of SAS (1996), with pen as the experimental unit. Duncan's multiple range test (Steel and Torrie, 1980) was used to determine significant differences among treatments.

Results

The results of feed intake, body weight gain and feed efficiency are presented in Table 3. For days 0 to 7, all four treatments (supplemented with FOS or probiotics or synbiotics) significantly improved weight gain compared to the control ($P<0.05$). FOS or multi-strain probiotics did improve ($P<0.05$) feed efficiency (gain/feed). For d 7 to 14, body weight gain of pigs fed synbiotics significantly improved ($P<0.05$) compared with either two strain probiotics or control. Average daily feed intake of pigs fed synbiotics was higher than control ($P<0.05$) but not compared to other treatments. For d 14 to 21, daily gain of pigs fed either multi-strain probiotics or synbiotics was higher ($P<0.05$) than that of control animal. There were no significant differences in feed intake and gain/feed ratio between treatments. During the total experimental period, the supplemented animals had similar gain but synbiotics had the highest weight gain.

Results on the apparent fecal digestibility of dry matter (DM), crude protein (CP) and crude ash as well as apparent fecal absorption of calcium and phosphorus are presented in Table 4. Supplementation of the diet with pre- and/or probiotics improved ($P<0.05$) the apparent digestibility of DM, CP, crude ash and absorption of Ca and P compared to the control. Fecal ammonia concentrations are presented in Table 4. All supplementations significantly reduced the fecal ammonia concentrations compared to the control fed animals ($P<0.05$).

The fecal microbial populations are shown in Table 5. The number of fecal

Table 3. Average daily weight gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) for weaned pigs¹ fed diets with OF, multi-strain probiotics, two strain probiotics or synbiotics

Item	Control	OF ²	M-PRO ²	SYNBIO ²	T-PRO ²	SE ³
0-7 days						
ADG, g	129 ^b	196 ^a	177 ^a	184 ^a	169 ^a	17
ADFI, g	300	335	289	331	328	27
FE (Gain/feed)	0.43 ^b	0.59 ^a	0.61 ^a	0.56 ^{ab}	0.52 ^{ab}	0.03
7-14 days						
ADG, g	244 ^b	291 ^{ab}	293 ^{ab}	332 ^a	264 ^b	15
ADFI, g	418 ^b	487 ^{ab}	468 ^{ab}	518 ^a	474 ^{ab}	17
FE (Gain/feed)	0.58	0.60	0.63	0.64	0.56	0.02
14-21 days						
ADG, g	430 ^b	465 ^{ab}	483 ^a	489 ^a	461 ^{ab}	10
ADFI, g	630	656	628	727	685	40
FE (Gain/feed)	0.68	0.71	0.77	0.67	0.67	0.04
0-21 days						
ADG, g	267 ^b	317 ^{ab}	318 ^{ab}	335 ^a	298 ^{ab}	12
ADFI, g	449	493	462	525	496	20
FE (Gain/feed)	0.59	0.64	0.69	0.64	0.60	0.03

¹Sixty pigs with an average initial body weight of 6.4kg.

²OF: 0.1% oligofructose; M-PRO: 0.2% multi-strain probiotics; SYNBIO: 0.1% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two strain probiotics

³Pooled standard error.

^{ab}Means in the same row with different superscripts differ (P<0.05).

Table 4. Nutrient digestibility and ammonia content for weaned pigs¹ fed diets with OF, multi-strain probiotics, two strain probiotics or synbiotics

Item, %	Control	OF ²	M-PRO ²	SYNBIO ²	PRO-2 ²	SE ³
Dry matter	83.9 ^c	87.1 ^{ab}	87.3 ^{ab}	88.5 ^a	86.1 ^b	0.47
Crude protein	76.5 ^c	81.6 ^b	82.8 ^{ab}	84.4 ^a	81.1 ^b	0.75
Crude ash	35.9 ^c	55.0 ^a	55.2 ^a	55.7 ^a	48.2 ^b	1.87
Calcium	82.3 ^b	85.6 ^a	85.2 ^a	85.9 ^a	84.9 ^a	0.51
Phosphorus	82.4 ^c	86.9 ^{ab}	87.0 ^{ab}	88.2 ^a	86.2 ^b	0.54
Ammonia (mg/kg)	333 ^a	195 ^b	235 ^b	228 ^b	241 ^b	16.97

¹Fecal samples were collected from sixty pigs with an average initial body weight of 6.37kg.

²OF: 0.1% oligofructose; M-PRO: 0.2% multi-strain probiotics; SYNBIO: 0.1% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two strain probiotics.

³Pooled standard error.

^{abc}Means in the same row with different superscripts differ (P<0.05).

Table 5. Number of coliform, anaerobic lactobacilli and total anaerobic bacterial cells for weaned pigs¹ fed diets with OF, multi-strain probiotics, two strain probiotics or synbiotics

Item	Control	OF ²	M-PRO ²	SYNBIO ²	T-PRO ²	SE ³
Aerobic population ⁴						
Coliform	7.9 ^a	6.8 ^b	6.6 ^b	6.1 ^b	6.2 ^b	0.23
Anaerobic population ⁴						
Total	9.8	10.0	10.1	10.3	9.8	0.16
Lactobacilli	9.6 ^b	9.9 ^{ab}	10.0 ^{ab}	10.7 ^a	9.9 ^{ab}	0.12

¹Fecal samples were taken at day 18 after weaning from five weaned pigs per treatment.

²OF: 0.1% oligofructose; M-PRO: 0.2% multi-strain probiotics; SYNBIO: 0.1% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two strain probiotics.

³Pooled standard error.

⁴Bacterial numbers are expressed as log₁₀ cfu/g of fresh feces

^{ab}Means in the same row with different superscripts differ (P<0.05).

Table 6. Hematological traits and immune response for weaned pigs¹ fed diets with OF, multi-strain probiotics, two strain probiotics or synbiotics

Item	Control	OF ²	M-PRO ²	SYNBIO ²	T-PRO ²	SE ³
Lymphocyte, %	64	59	52	56	61	7.0
Neutrophil, %	28	36	44	36	29	6.0
Hematocrit, %	36	35	39	36	36	2.0
Platelet, ×10 ³ /mm ³	284 ^{ab}	178 ^b	271 ^{ab}	422 ^a	285 ^{ab}	66.0
Hemoglobin, g/dl	10.7	10.7	11.6	11.3	10.7	0.4
WBC, ×10 ³ /mm ³	28.1	25.6	27.0	23.0	21.3	3.2
RBC, ×10 ⁶ /mm ³	5.9	5.5	5.9	5.1	5.8	0.2
Monocyte, %	6	3	3	4	4	2.0

¹Blood samples were taken from five weaned pigs per treatment.

²OF: 0.1% oligofructose; M-PRO: 0.2% multi-strain probiotics; SYNBIO: 0.1% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two strain probiotics.

³Pooled standard error.

^{ab}Means in the same row with different superscripts differ (P<0.05).

coliforms were significantly decreased in all supplemented groups compared to the control group. The total number of anaerobes was not affected by diets. The number

of lactobacilli was significantly increased in pigs fed the synbiotics diet compared with the control pigs ($P < 0.05$) but not when compared to pigs fed the diet supplemented with pre- or probiotics only.

Table 6 shows the effect of FOS and multi-strain probiotics supplementation on hematological traits. During the experimental period, neutrophil concentration was numerically higher in all treatment groups compared to the control. Platelet count in blood for pigs fed synbiotics was higher ($P < 0.05$) than that of FOS pig. There were no significant differences in RBC count, WBC count, hematocrit, hemoglobin, lymphocyte, neutrophil and monocyte concentrations of whole blood.

Discussion

We hypothesized that FOS, probiotics and synbiotics would help the beneficial microflora by stimulating the good microflora or by adding beneficial microbes in the gut. This might improve gut health and in that aspect indirectly cause an increase feed intake. As a result animals may have increased growth because they eat more. We also investigated whether a specific synbiotics, a combination of OF and multi-strain probiotics, have a higher effect on weight gain and feed intake compared to other treatments in antibiotics-free diets for weaned pigs. Gibson and Roberfroid (1995) have proposed the synbiotics concept to characterize health-enhancing foods and supplements used as functional food ingredients in humans. Some researchers (Kumprecht and Zobac, 1998; Nemcová et al., 1999) have suggested that synbiotics may be better for growth (and health) than either pre- or probiotics alone. In the present experiment, synbiotics are similar to pro- or prebiotics alone. The supplementation of synbiotics (combining pre- and probiotics) significantly increased BWG compared to the control ($P < 0.05$). The increased BWG for the synbiotics group can be explained by a numerically increased daily feed intake.

The supplementation of a prebiotics (OF) did not significantly affect growth. This result was similar to other studies (Olsen and Maribo, 1999; Houdijk et al., 1998). But other studies in literature reported that FOS has clear stimulating effect (He et al., 2002; Estrada et al, 2001) on weight gain. The difference may be associated with the different chemical structure (degree of polymerization) of FOS used in the different studies, or length of oligosaccharides and presence of other fermentable sources especially non-starch polysaccharides in the diets used in the literature. In the present experiment, the NSP contents in the basal diet was 9,8% and the NSP content was similar compared to the NSP content (9,1%) in the basal diet of the study of Houdijk et al. (1998). It may also be the environmental conditions were too optimal to show the effect of prebiotics similar to Houdijk et al (1998).

We also investigated if feeding a multi-strain probiotics to weanling pigs would result in a large effect compared to a two strain probiotics. Supplementation of a multi-strain probiotics tended to result in higher BWG and improved feed efficiency compared to the two strain probiotics. It has been hypothesized that multi-strain probiotics may also be active at different sites. In this experiment the multi-strain probiotics contained species of microorganisms but it also resulted in a higher number of CFU/g of diet compared to the two strain probiotics. In non-optimal conditions probiotics may improve growth and health (Fuller, 1999). This experiment was conducted under optimal conditions, but animals have been transported. So they experienced some stress. The weaned pigs had been transported (about 20 min. by truck with cover) from a commercial farm to the experimental room at the day of weaning and were not allowed adaptation period before the experiment. Some studies have shown indeed that feeding probiotics improved performances (growth and feed conversion) in young pigs (Collinder, et al., 2000; Kyriakis et al., 1999; Fialho et al., 1998). Other studies, however, did not find effects of probiotics in weanling piglets (Jost and Bracher-Jakob, 1998; Harper et al., 1983).

Overall, the apparent fecal digestibility of DM and CP was higher in the synbiotics pigs when compared to other treatments ($P < 0.05$). It is not known whether the protein digestibility in the ileum is different. Fecal digestibility of CP would be less with prebiotics because there is more fecal biomass. This is considered beneficial. It may be that longer villi and thinner epithelium in the villi of the small intestine (Shim et al, 2004, unpublished) may lead to improved digestion and absorption of nutrients in the gut. Houdijk et al. (1999) did not find any effect by both oligofructose and transgalactooligosaccharides (TOS) on ileal digestibility of protein and fat. They have found an increased ileal digestibility of dry matter and fiber (cellulose and hemicellulose). Houdijk et al. (1999) have shown that feeding either FOS or TOS as prebiotics did not affect ileal digestion of dry matter or protein (amino acids) in weaned pigs. Mathew et al. (1997) also found no effect of galactosyl lactose on protein digestibility.

Scientific evidence has revealed that NDO can increase the availability of calcium, magnesium, zinc and iron in human (Scholz-Ahrens et al., 2001). In this experiment, the supplementation of pre- and probiotics alone or in combination of both improved the apparent absorption of calcium compared to the control diet ($P < 0.05$). Synbiotics significantly increased ($P < 0.05$) digestibility of phosphorus compared to other treatment groups. These results coincide with the result (Morohashi et al, 1998) that feeding FOS to weaned pigs enhances calcium and phosphorus absorption in rats. Other reports (Scholz-Ahrens et al., 2001; Tunland and Meyer, 2002) also suggested that prebiotics, such as inulin and FOS may increase bioavailability of minerals by an increased solubilization of minerals by the production of VFA and

lower pH resulting from the increased fermentation (Kashimura et al., 1996; Rémésy et al., 1993). So the addition of synbiotics may stimulate the fermentation more in the gut and this may partly contribute the increased digestibility of mineral. However, Houdijk et al (1999) found similar ileal digestibility of calcium and phosphorus by oligofructose or TOS.

We expected that dietary FOS and probiotics or synbiotics would suppress the population of coliform in the gut of weaned pigs. In this experiment indeed, the fecal coliform population was decreased ($P < 0.05$) in all the treatments compared to the control. Synbiotics increased ($P < 0.05$) the population of anaerobic lactobacilli compared to the control but not compared to other treatments. These results are in agreement with other studies (Herich et al., 2002; Nemcová et al., 1999). Herich et al. (2002) found that supplementation of synbiotics, the combination of lactobacillus paracasei and FOS, significantly increased the number of total anaerobes, total aerobes and lactobacilli in the feces of weaned pigs, when compared with the supplementation of lactobacillus paracasei alone or control. Other studies reported that feeding FOS in pig diets increased in number of lactobacilli and bifidobacteria (Howard et al. 1995) and reduced the *E. coli* (Klein-Gebbink et al., 2001). But some studies did not find stimulating effects of FOS on lactobacilli and bifidobacteria in the gut of young pigs (Orban et al., 1997; Bolduan et al., 1993). This may be due to differences in other fermentable carbohydrate levels between studies. The source and content of NSP in pig diets may influence the microbial population. A diet containing rapid and slow fermentable carbohydrates may ensure continuous supply of energy source to the gastrointestinal microflora and thereby prevent protein fermentation.

Ammonia is one of the malodorous products which are produced by putrefactive bacteria such as *E. coli* or clostridia in the large intestine. The fecal ammonia concentration was less for all supplemented groups. This result was consistent with the studies on supplementation of inulin (Vanhoof and De Schrijver, 1996; De Schrijver and De Vos, 2003). However, Bolduan et al. (1993) have found that feeding oligofructose, isomaltooligosaccharides or galactooligosaccharides did not decrease the ammonia concentration in the stomach and colon of weanling piglets. Feeding FOS increased bifidobacteria and decreased both number of *E. coli* and clostridia (Gibson and Roberfroid, 1995, Russell et al, 1995). Because NSP in diets can also stimulate microbial growth, they will use nitrogenous compounds for growth. As a result, putrefactive products like ammonia, phenol and amines may be reduced (Hidaka and Eida, 1988).

In this experiment the haematological traits were unaffected by FOS, M-Probiotics and synbiotics supplementation. There were no significant differences in lymphocyte, neutrophil and monocyte, WBC count, RBC count, hematocrit, and hemoglobin

concentrations in the whole blood. Platelet count was only significantly higher with synbiotics supplementation than that of FOS ($P < 0.05$). However, Herich et al., (2002) found that immune parameters such as lymphocytes, leukocyte, neutrophils, CD4⁺ T cells tended to increase in supplementation of synbiotics (combining lactobacillus and FOS) compared to the single administration of lactobacillus and the control in weaned pigs. Pierre et al. (1997) demonstrated that oligofructose enhanced the T-lymphocyte function in mice. It has been suggested that prebiotic such as FOS or inulin may be beneficial for the immune system and health of weaned pig.

In conclusion, each of the supplementations of synbiotics (combining low DP of OF and multi-strain probiotics) prebiotics and probiotics to an antibiotics-free diet resulted in increased growth, increased phosphorus and calcium absorption and a slight increase in nutrient digestibility. Supplementation of synbiotics also increased the number of lactobacilli and decreased the number of *E. coli* compared to the control or other supplementations with pre- or probiotics alone.

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Chapter 7

Feeding antibiotic-free creep feed supplemented with oligofructose or synbiotics to suckling pigs increases the preweaning body weight gain and changes the intestinal microbiota

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Abstract

The objective of this study was to determine whether feeding an antibiotic-free creep feed supplemented with either oligofructose, probiotics or synbiotics to suckling piglets influences growth performance, changes the gut microflora, gut morphology and hematological traits at weaning. Twenty sows with 10 piglets each were randomly assigned to one of 4 treatments. The treatments consisted of a control (antibiotic-free) diet or the control diet supplemented with 0.2% of oligofructose (OF), 0.3% probiotics or 0.5% synbiotics (mixture of 0.2% OF + 0.3% probiotics). Piglets were offered the diet (dry, mash) ad libitum from 7 days after birth until 1 day after weaning (21 days of age). At 1 day after weaning, blood samples were collected from the jugular vein to determine the immune response. Digesta samples of the ileum and colon were collected to determine the microbial composition. Tissue segments from the duodenum and ileum were collected for morphometric measurements of the small intestine. The average daily weight gain was significantly higher ($P < 0.05$) for piglets fed the OF or synbiotics diet compared with the pigs fed the control diet. The hematological traits (the concentration of lymphocytes and neutrophils in whole blood) were not affected by the diet. Piglets fed the OF, probiotics or synbiotics diet had significantly ($P < 0.05$) decreased number of *E coli* in the colon. Feeding OF, probiotics or synbiotics significantly ($P < 0.05$) increased the population of bifidobacteria in the ileum compared to the control. In the colon, the probiotics and synbiotics diet significantly ($P < 0.05$) increased the number of bifidobacteria compared with the control diet. The results of this experiment showed that supplementation of oligofructose or synbiotics to an antibiotic-free creep feed during the preweaning period affected gut microbial population and performance of piglets.

Keywords: Pigs, Oligofructose, Probiotics, Synbiotics, Bifidobacteria

Introduction

In practice, creep feed is often provided to suckling piglets. It is aimed to have beneficial effects such as an increased preweaning body weight gain and increased early postweaning feed intake. Antibiotic growth promoters are commonly used in commercial creep feed for the purpose of growth or prophylactic action (Cromwell, 2001). The use of in-feed antibiotics will be banned in the EU from 2006 onward due to the public concern about the food safety and antibiotic resistance (Corpet, 1988). Constraints on the use of antibiotics in the EU and probably other countries would require an alternative to antibiotics for improved growth and gut health in young pigs. Prebiotics, probiotics and synbiotics may provide potential alternatives to antibiotic

growth promoters for young pigs.

In several studies (He et al., 2002; Collinder et al., 2000), stimulating effects of pre- or probiotics on postweaning growth were reported. In other studies however little or no effect on growth was found (Olsen and Maribo, 1999; Houdijk et al., 1998). It has been suggested that the use of the synbiotics concept (a combination of pre- and probiotics) may give the synergistic effects of both pre- and probiotics on growth of young pigs (Nemcová et al., 1999). Our recent study (Shim et al., 2004, unpublished) showed that supplementation of synbiotics, combining oligofructose (low degree of polymerization) and multi-strain probiotics to an antibiotic-free diet had beneficial effect on growth and the number of lactobacillus in weaned pigs. In the light of this result, it is interesting to investigate whether supplementing antibiotics-free creep feed with synbiotics (combining oligofructose and multi-strain probiotics) during the preweaning period increases weaning weight and the population of beneficial bacteria such as bifidobacteria and lactobacillus, and decreases the number of *E. coli* in the intestine. In the literature, there is no information available on the impact of synbiotics on preweaning growth of piglets. So supplementing antibiotics-free creep feed with synbiotics to suckling piglets on growth and change of microflora needs to be evaluated.

The objective of this experiment was to study the effect of feeding an antibiotic-free creep feed supplemented with prebiotics, probiotics or synbiotics to suckling piglets on preweaning body weight gain and on the composition of the intestinal microflora, gut structure and immune responses of piglets at weaning.

Materials and Methods

Animals and Managements

This study was carried out in a large pig unit. Two hundred piglets from twenty sows within 3 to 5 parity (Yorkshire × Landrace) were used. Each litter with 10 piglets was weighed at two days of age. The sow with 10 piglets (Yorkshire × Landrace) × Duroc) each were then randomly assigned to one of 4 treatments. Each treatment had similar mean liveweights of animals. The treatments consisted of a control diet (CON), a control diet supplemented with 0.2% oligofructose (OF), 0.3% probiotics (PRO) or 0.5% synbiotics (SYN, a combination of 0.2% oligofructose and 0.3% probiotics). The farrowing rooms were mechanically ventilated to maintain a thermoneutral environment for the sows. Room temperature was maintained between 18 to 24°C. The sows were kept loose (0.61 × 2.13m) all the time and fed twice daily at 07:00 and 14:00 h. The piglets had a safe zone (0.98m²) in a corner of each farrowing pen where a lamp provided heat. Each pen was equipped with a feeder

and water supply from a nipple. Creep feed (dry meal) was provided 3 times a day (08:00, 13:00 and 18:00) in plastic creep feeders (round shape, diameter 21cm) from day 7 after birth to weaning (21 days of age). Immediately after weaning, 5 piglets from each treatment (1 piglet per sow) were selected for further measurements and transported to the Animal house by a truck (with cover) for 20 minutes and were kept until one day after weaning for slaughter. The piglets were given free access to feed (creep feed given three times a day) and water. The feeders were cleaned daily. The piglets were weaned and weighed at 21 days of age.

Diets

The experimental diets are shown in Table 1. The creep feed was formulated to meet or exceed nutrient requirements for weaned pigs as recommended by NRC (1998) and contained no antibiotic substances. The creep feed contained 14.94 MJ DE/kg, 22.0% crude protein and 1.61% total lysine. The creep feed was based on extruded rice and soybean meal used commercially in Korea. The sow diet contained 14.64 MJ DE/kg, 14.0% crude protein and 0.9% total lysine. The sow diet was a corn-soybean meal and was not included in-feed antibiotics. Corn starch was substituted by oligofructose and probiotics according to treatments. The level of OF used in this experiment was based on a previous study with weaned piglets (Shim et al, submitted to JAP&N, 2004). The chemical composition of OF on a dry-matter basis was 95% dry matter (GF₂:1-kestose 35%, GF₃:nystose 50%, and GF₄:1-fructosyl-nystose 10%), 5% of glucose + fructose + sucrose. GF₂ is OF with a terminal glucose and two fructose units, GF₃ is OF with a terminal glucose and three fructose units and GF₄ is OF with a terminal glucose and four fructose units (Neosugar®, Meiji Seika Kaisha, Ltd., Japan). Probiotics used in this study were microbial mixtures (Economix®, Chodae F&P, Ltd, Korea) as shown in Table 1. The selection of the probiotic strain was done as follows: lactobacilli from the faeces of weaned pigs; bacilli from the soybean malt; saccharomyces cerevisiae from soil. The selected probiotics strains had been cultured on the media MRS (lactobacillus), Nutrient (Bacillus), and Sabourade (saccharomyces cerevisiae) (Williams, 1994). The process of probiotics manufacture is as follows. Kozi cultivation (rice bran, wheat bran, soybean meal, molasses) of the 15% Starter at 30 °C for 5-7days, drying at 40 °C until moisture content is at 11%, and then counting the probiotics strains.

Measurements

One day after weaning, 20 piglets (5 piglets per treatment) were slaughtered for the measurements (morphology, hematology and microflora). The concentrations of lymphocyte and neutrophil concentrations in whole blood were measured to investigate the effect of OF, multi-strain probiotics or synbiotics supplementation on

health of the piglets. Blood samples (5mL) were collected via the jugular vein into heparinized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) from five pigs (one pig per pen) in each treatment at one day after weaning when weanling pigs faced some weaning stress. All blood samples (lymphocytes and neutrophils) were analyzed with an automated hematology analyzer, ADVIA 120 (Bayer, Tarrytown, USA) using commercial reagent kits (ADVIA[®] 120 PEROX 1, 2 & 3 reagent, Bayer, USA).

Tissue samples of the small intestine were taken at weaning for morphometric measurements. At the end of the trial, the pigs were fasted for three hours before being euthanised. The piglets were euthanised by an injection of barbiturate (Lethabarb; 0.5ml/kg live body weight, 325 mg/mL Sodium Pentobarbitone, Virbac Australia Pty. Ltd., Peak Hurst, NSW, 2210) into the heart. The entire gastrointestinal tract of each pig was removed. Approximately 3cm segments were collected from the two cross-sections of proximal (duodenum) and distal parts (ileum) of small intestine. Each small intestine was excised and placed in 10% buffered formalin at room temperature for 24h. Each sample was processed in low melt paraffin. Paraffin embedded tissue was sectioned at 6 μ m. The section were cut and stained with haematoxylin and eosin. And then each sample was mounted on glass slides. Measurements of villous height (from the tip to the crypt-villous junction) and crypt depth were made on 15 well-orientated villi in the duodenum and ileum. Measurements of villous height and crypt depth were made using a binocular microscope (Olympus, CH-30, Japan) with mounted CCD camera (Toshiba, Japan) and a computer software programme.

A modification of the method as described by Orban et al (1997) was used to determine the populations of total coliforms, lactobacilli and bifidobacteria. Ten grams of mixed ileal or colonal contents were serially diluted 1:10 (1g in 9ml) in sterile phosphate buffered saline (pH 7.2). Appropriate serial dilutions were used to enumerate total coliforms, lactobacilli and bifidobacteria. Aerobic total coliforms (MacConkey Agar, Difco Laboratories, USA) and aerobic lactobacilli (Bacto Lactobacilli MRS Agar, Difco) were enumerated by inoculating triplicate 100mm plates of the respective media with 0.1ml of the appropriate dilutions. Anaerobic bifidobacteria (Tryptic Soy Agar, Difco) were enumerated by inoculating triplicate 60-mm plates of the respective anaerobically prepared media with 0.025ml of the dilutions inside a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) under a 97% CO₂ and 3% H₂ atmosphere.

Data was analyzed as a randomized complete block design using the general linear model procedure of SAS (1987), with pen as the experimental unit. Duncan's multiple range test (Steel and Torrie, 1980) was used to determine significant differences among treatments. Microbial data were converted to the log₁₀ base and

then analyzed using the GLM procedures.

Results

Feeding diets supplemented with OF and synbiotics significantly increased ($P < 0.05$) the preweaning body weight gain of piglets compared to the control diet. Supplementation of probiotics to the diet did not affect body weight gain. Blood parameters (concentrations of lymphocytes and neutrophils in blood) were not significantly different between treatments (Table 2).

Table 1. Composition (as-fed basis) of the experimental diets

Ingredients (g/kg)	Control	OF ¹	Probiotics ²	Synbiotics
Extruded rice	300.0	300.0	300.0	300.0
Lactose	173.1	173.1	173.1	173.1
Processed soybean meal	90.0	90.0	90.0	90.0
Precooked corn	60.0	60.0	60.0	60.0
Whey powder	100.0	100.0	100.0	100.0
Fish meal (CP 60%)	40.0	40.0	40.0	40.0
Protamyl (potato protein)	34.5	34.5	34.5	34.5
Soybean oil	30.0	30.0	30.0	30.0
Blood plasma powder	50.0	50.0	50.0	50.0
Egg powder	25.0	25.0	25.0	25.0
Cookie (biscuit)	24.8	24.8	24.8	24.8
Infant food (byproduct)	20.0	20.0	20.0	20.0
L-lysine	0.3	0.3	0.3	0.3
DL-methionine	0.8	0.8	0.8	0.8
Choline	2.8	2.8	2.8	2.8
Salt	2.0	2.0	2.0	2.0
Sweetener	1.9	1.9	1.9	1.9
Dicalcium phosphate	12.4	12.4	12.4	12.4
Lecithin	3.5	3.5	3.5	3.5
Vitamin and Mineral premix ³	3.0	3.0	3.0	3.0
Antioxidant (Ethoxiquin)	0.2	0.2	0.2	0.2
Zinc oxide	3.2	3.2	3.2	3.2
Dairy concentrate (Serolat-20A)	17.5	17.5	17.5	17.5
Corn starch	5.0	3.0	2.0	-
Oligofructose	-	2.0	-	2.0

Probiotics	-	-	3.0	3.0
Chemical composition ⁴				
ME, MJ/kg	14.94	14.94	14.94	14.94
Crude protein, %	22.00	22.00	22.00	22.00
Lysine, %	1.61	1.61	1.50	1.50
Methionine, %	0.48	0.48	0.48	0.48
Calcium, %	0.85	0.85	0.85	0.85
Phosphorus, %	0.75	0.75	0.75	0.75

¹OF:Oligofructose.

²Probiotics (Lactobacillus acidophilus 9x10⁸; Lactobacillus bulgaricus 2x10⁶, Bacillus subtilis 9x10⁸, Saccharomyces cerevisiae 8x10⁶).

³Provided per kg of complete diet: 20,000 IU of vitamin A; 4,000 IU of vitamin D3; 80 IU of vitamin E; 16 mg of vitamin K3; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B12; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin; 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.

⁴Calculated values.

Table 2. Growth and hematological characteristics of piglets¹ fed diets with control, OF, probiotic or synbiotics

Item	Control	OF	Probiotics	Synbiotics	SE ²
Growth performance, No. of pigs	50	50	50	50	
Initial body weight (Day 2), kg	1.47	1.54	1.49	1.52	0.10
Weaning weight (Day 21), kg	5.25	5.79	5.55	5.82	0.28
Average daily weight gain, g	199 ^b	224 ^a	214 ^{ab}	226 ^a	5.22
Hematological traits, No. of Pigs	5	5	5	5	
Lymphocytes, %	33.50	33.75	30.25	27.50	5.37
Neutrophils, %	59.50	61.75	63.50	61.25	5.17
Neutrophil / Lymphocyte ratio	1.94	1.84	2.52	2.43	0.52

¹Each treatment had fifty piglets (5 sows/treatment). The hematological measurements were done from 5 piglets per treatment.

²SE (standard error) is pooled within treatment.

^{ab}Means in the same row with different superscripts differ (P<0.05).

Results of the morphological measurements in the proximal and distal parts of small intestine are shown in Table 3. The dietary treatment did not affect the villous height in the duodenum and ileum at weaning. Pigs fed the OF diet had significantly increased (P<0.05) crypt depth in the duodenum compared to the control.

The log₁₀ of the number of bacterial cells per gram of fresh digesta samples from the ileum and colon are shown in Table 4. Piglets fed antibiotics-free diet with OF, probiotics or synbiotics significantly ($P<0.05$) decreased the numbers of *E. coli*. in the colon when compared to the control. The number of *E. coli*. in the ileum was not affected by the dietary treatments. The OF, probiotics or synbiotics diet significantly increased ($P<0.05$) the number of bifidobacteria in the ileum compared to the control. The number of bifidobacteria was also significantly increased ($P<0.05$) in the colon of piglets fed antibiotics-free diet with either probiotics or synbiotics when compared with the control.

Table 3. Villous height and crypt depth in the small intestine of piglets¹ fed diets with control, OF, probiotic or synbiotics

Item	Control	OF	Probiotics	Synbiotics	SE ²
No. of pigs	5	5	5	5	
Doudenum					
Villous Height, μm	319	380	302	331	28.81
Crypt Depth, μm	290 ^b	378 ^a	300 ^{ab}	333 ^{ab}	24.94
Ileum					
Villus Height, μm	250	349	278	400	71.17
Crypt Depth, μm	250	350	283	403	72.42

¹The morphological measurements were done from 5 piglets per treatment.

²SE (standard error) is pooled within treatment.

^{ab}Means in the same row with different superscripts differ ($P<0.05$).

Table 4. Numbers of *E. coli*, *L. acidophilus* and Bifidobacteria in the terminal ileum and colon of piglets¹ fed diets with control, OF, probiotic or synbiotics

Item	Control	OF	Probiotics	Synbiotics	SE ²
Ileum (Bacteria) ³					
<i>E. coli</i> . ⁴	-	-	-	-	-
<i>L. acidophilus</i>	7.40	7.77	7.53	7.74	0.45
Bifidobacteria	6.40 ^b	8.87 ^a	8.23 ^a	8.10 ^a	0.32
Colon (Bacteria) ³					
<i>E. coli</i> .	7.18 ^a	6.30 ^b	6.50 ^b	6.05 ^b	0.13
<i>L. acidophilus</i>	8.71	7.79	8.38	8.62	0.36
Bifidobacteria	6.71 ^b	6.83 ^b	7.53 ^a	7.62 ^a	0.28

¹The microbial populations were determined from 5 piglets per treatment.

²SE (standard error) is pooled within treatment.

³Bacterial numbers are expressed as mean colony forming units $\times \log_{10}$ /g of fresh (wet) digestive contents.

⁴Bacterial numbers were below $\log_{10} 4$ CFU/g.

^{ab}Means in the same row with different superscripts differ ($P < 0.05$).

Discussion

We tested the hypothesis that supplementing antibiotics-free creep feed with OF, probiotics and synbiotics to piglets would help microflora balance and alter weight gain during the suckling period compared to the control. In this experiment, the body weight gain of piglets during the preweaning period was significantly higher ($P < 0.05$) in both OF and synbiotics supplementation compared to the control. Multi-strain probiotics also showed a numerically higher weight gain than the control. The heavier weight at weaning by the supplementation of either OF or synbiotics in antibiotics-free creep feed may be the results of improved gut health (possibly by stimulating beneficial bacteria) of pigs by the presence of both biologically active microorganism and prebiotics as a substrate for this. So from our study some beneficial effects from each supplementation were found. But we did not find an extra effect of synbiotics compared to OF or the multi-strain probiotics. If pig weight at weaning is improved then, this may positively affect the postweaning growth (Lawlor et al., 2003; Miller et al., 1999). Some reports in literature (Nemcová et al., 1999; Kumprecht and Zobac, 1998) suggested that synbiotics may have beneficial effect on growth of weanling pigs. But there is little or no information whether dietary synbiotics affect the growth and health of preweaned piglets.

The effect of creep feeding on growth is still equivocal because the effect is generally small and very variable from little or no effect (Pluske et al., 1995). The inconsistent effect of creep feeding is related to variation in creep feed intake between individual piglets (Barnett et al., 1989) and the nature of creep feed (Bruininx et al., 2002).

Effects of OF, multi-strain probiotics and synbiotics supplementation to antibiotics-free diets on lymphocyte and neutrophil concentration in the blood of weaned pigs were determined. An increase in lymphocyte and neutrophil concentrations was anticipated as an indicator of immune response in the piglets. There were no significant differences in lymphocyte and neutrophil concentrations in the blood. This result was in agreement with other reports that either dietary inulin (Rossi et al., 1998) or probiotics (Apgar et al., 1993) did not affect immune response in weaned pigs. However, Kaila et al. (1992) reported that the immune system of weaned pigs was stimulated by probiotics supplementation. The ratio of neutrophils

to lymphocytes was numerically higher in pigs fed the diet supplemented with probiotics and synbiotics compared to the pigs fed the diet supplemented with OF or the control diet. It is known that neutrophil can mediate the action of the innate immune system (O' Carra, 1998). There is scarce information available to date regarding the interaction between OF and immune system in pigs.

The results found on gut morphology and microflora in this study agrees with the finding of Spencer et al. (1997) that piglets with oligofructose in the diets had higher villous height in the small intestine of weaned pigs. It is known that oligofructose are not digested by mammalian enzymes and may reach the large intestine where they act as specific nutrients for bifidobacteria or lactobacilli (Modler et al., 1990). However, oligofructose starts to ferment to some extent in the small intestine and is rapidly fermented in the distal part of small intestine and/or in the proximal part of large intestine of piglets (Houdijk et al., 2002). Villous height in the small intestine is important because it is regarded as an indicator of nutrient absorption in pigs. If a good villous height can be maintained during the weaning transition, nutrient digestibility might be optimal. Villous height is positively correlated with empty body weight gain (Pluske et al., 1996), nutrient intake level (Verdonk et al., 1999) and the health status of postweaning pigs (Nabuurs, 1991). However, in this study there was no significant correlation observed between the growth and villous height of medial or distal sites of small intestine. Several studies (Howard et al., 1995; Fleming et al., 1992) have postulated the possible mechanisms on the changes of cell proliferation in the gut. Enteroglucagon in the blood as stimulated by dietary fermentable carbohydrates may be responsible for the stimulation of mucosal cell proliferation of the small intestine (Gee et al., 1996). The properties of FOS are similar to physiological effects of a dietary fiber (Roberfroid, 1993). It can be speculated that the fermentative property of dietary FOS may have positively influenced villous height. This may be associated with the increased plasma enteroglucagon of the pigs.

We tested the hypothesis that dietary supplementation of OF, probiotics or synbiotics during preweaning period may increase the population of bifidobacteria and lactobacilli, and decrease the population of *E. coli*. at weaning. The population of bifidobacteria in the ileum was significantly higher in OF, probiotics or synbiotics supplementation compared to the control. The supplementation of both probiotics and synbiotics significantly increased ($P < 0.05$) the number of bifidobacteria in the colon compared with the control. This result agrees with other studies (Smiricky-Tjardes et al., 2003; Nemcová et al., 1999). The OF supplemented diet did increase the bifidobacteria population in the ileum in this study. This result may partly be explained by the result of Houdijk et al. (1997) that oligofructose affects bifidobacteria populations more proximally than does transgalactooligosaccharides which fermented more distally in the colon. However, Mitsuoka et al. (1987) reported that

dietary oligofructose stimulates lactobacilli, but not stimulates bifidobacteria in pigs. Mikkelsen et al (2003) also reported that fecal bacterial populations were not affected by dietary FOS. The bifidobacterial numbers in the range of 10^7 to 10^8 bacteria per gram of feces of newly weaned pigs shown in this experiment was similar to other studies (Nemcová et al., 1999). The increased bifidobacteria by supplementing pre- and probiotics or synbiotics may help to suppress potentially pathogenic bacteria like *E. coli*. The population of *E. coli* in the colon was significantly ($P < 0.05$) lower in all treatment groups compared to the control, but the *E. coli* population in the ileum was not affected by all treatment groups. The reasons for the contradictory results of the effect of prebiotics in literature are not clear yet. These discrepancies may be related by several factors such as the nature of prebiotics or probiotics (strains and populations), creep feed (antibiotics and NSP content) and selective agar media for the enumeration and isolation of intestinal microbiota.

In conclusion, feeding an antibiotic-free creep feed supplemented with OF and probiotics or synbiotics (a combination of OF and multi-probiotic strains) to suckling piglets resulted in increased body weight gain and changed gut morphology and microflora at weaning.

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Chapter 8

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

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Abstract

An *in vitro* experiment was conducted to evaluate the differences in microbial activity of five faecal inocula from weaned and one faecal inoculum from unweaned pigs in combination with 6 substrates. The substrates tested were negative control diet, corn, soybean meal, oligofructose (OF), grinded chicory roots and a mixture (60% chicory pulp and 40% OF). The inocula used were derived from pigs fed either a corn-soy based diet without antibiotics (NCON), the NCON diet supplemented with oligofructose (OF), a mixture of chicory pulp (40%) and OF (60%) (MIX), grinded chicory roots (CHR) or the NCON diet supplemented with antibiotics (PCON). The cumulative gas production measured fermentation kinetics, and end products such as total gas production, ammonia and volatile fatty acids were also determined. Both the substrate and the inoculum significantly affected the fermentation characteristics. The cumulative gas production curve showed that different substrates caused more differences in traits of fermentation kinetics than the different inocula. Inoculum of weaned pigs had a higher rate of fermentation and more gas than unweaned inoculum. OF showed the highest fermentation kinetics and the lowest NH₃, pH and OM loss compared to other substrates. It was concluded that the microbial activity significantly affected by substrate and inoculum. Inoculum from weaned pigs had more potential for microbial fermentation to the carbohydrate ingredients and oligofructose than that of unweaned pigs. A combination of high and low polymer inulin may be more beneficial to the gut ecosystem than using high- or low polymer inulin alone.

Introduction

Oligofructoses (OF) are non-digestible oligosaccharides and regarded as prebiotics which are not metabolized by endogenous enzymes in the gastrointestinal tract (GIT) of human and monogastric animals. It can be fermented by beneficial bacteria such as bifidobacteria and lactobacilli in the large intestine (Gibson and Roberfroid, 1995; Tomomatsu, 1994). However, Houdijk et al (1999) reported that oligofructose may be partly or completely fermented in the distal part of the small intestine in pigs. The site in the gut of pigs where the fermentation of OF occurs may depend on the molecular structure (chain length) of the non-digestible carbohydrates which can have a major impact on fermentation processes (Roberfroid et al., 1998).

The principal energy sources for microbial fermentation are dietary carbohydrates which are not digested in the small intestine (Pluske et al., 1999). The major end products of carbohydrates fermentation in the large intestine are volatile fatty acids (VFA), CH₄, H₂, CO₂ and NH₃. OF influences the microbial populations and activities

and by the production of VFA and may beneficially affect the gut ecology and health (He et al., 2002; Estrada et al., 2001; Houdijk et al., 1997). Porcine faecal, colonic- and caecal digesta have shown to differ for *in vitro* fermentation characteristics (Houdijk et al., 1998; Williams et al., 1998). The knowledge on the site of fermentation in the GIT of different sources of OF is important because it may differently affect the intestinal ecology and health of pigs.

The major end products of fermentation in the gut, VFA and NH₃ may indicate the beneficial effect on gut health. The cumulative gas production technique can be used to study the effect of inocula (as source of microflora) and substrates on *in vitro* fermentation characteristics between inocula and substrate. Bauer et al (2004) reported that faecal inocula can be used for *in vitro* assessment of large intestinal fermentation in pigs. The better understanding on the site of fermentation by different OF sources will give opportunities to manipulate the gut ecology and health in young pigs.

The aim of this study was to determine the differences in *in vitro* fermentation characteristics between feces from weaned and unweaned pigs as inoculum and different sources (degree of polymerization) of inulin derived fructans (OF, chicory roots and chicory pulp) and pig feed ingredients as substrates. Fermentation kinetics were assessed using the cumulative gas production technique. End products of fermentation (VFA and NH₃) were measured to identify their potentially beneficial effects on GIT health.

Materials and Methods

Collection and preparation of inocula

Faecal samples were collected from five treatment groups of 6 – 8 (8 week-old) pigs. The pigs were weaned at 4 weeks of age and had a body weight ranging between 14 -15kg. Pigs were randomly selected for faecal sample collection, in order to obtain sufficient amounts of inocula for each treatment group. In case of unweaned piglets, pigs from two litters of suckling piglets (14 -16 days old and 4.0 – 4.5kg) were sampled. The weaned pigs were fed commercial creep feed before weaning. The selected litters of unweaned piglets did not receive any creep feed. Weaned pigs were fed the experimental diets for 28 days after weaning (and had creep feed before weaning) according to the treatments. The faeces were collected directly from the rectum of each pig with a gloved finger and immediately placed in CO₂ filled containers to maintain an anaerobic condition, and transported in an insulated container on ice to the lab within 1.5 h of sample collections. Equal amounts of faeces from each animal per treatment were pooled and diluted with an anaerobic

sterile saline, pre-warmed to 39 °C. The faecal samples were diluted 1:2. Unweaned piglets yielded less faeces so the dilution for unweaned pigs is 1:25. Samples were then homogenized for one minute using a hand-blender. The mixture was filtered through two layers of sterile cheese cloth, and the resultant filtrate used as inoculum. All these procedures were carried out under a continuous flow of CO₂ to maintain strictly anaerobic conditions (Awati et al., 2005).

Diets

Weaned pigs were fed either a basal corn-soy diet supplemented with either corn starch (NCON), oligofructose (OF), grinded chicory roots (CHR), a mixture of chicory pulp and oligofructose (MIX) or corn starch and antibiotics (PCON). The NCON diet did not include antibiotics and inulin type oligofructose. The main ingredients (g/kg) of the basal diet were corn (600.0), soy flakes (160.0), tapioca (73.2), potato protein (46.9), whey powder (40.0) and molasses (35.0). In addition, vitamins, minerals and amino acids were supplemented to meet or exceed the requirements of weaner pigs (NRC, 1998).

Substrates

Six substrates were tested: five substrates were chosen from experimental treatments, the basal diet (Negative control), a blend of high- and low-polymer inulin (Raftifeed IPE, average DP=10, Orafti, Tienen, Belgium), grinded chicory roots, and a blend of chicory pulp and oligofructose (60:40), All the inulin-type fructans were obtained from Orafti (Tienen, Belgium). The air dried substrates were ground to pass a 1 mm sieve. The basal diet (negative control) and the mixture (60% chicory pulp + 40% oligofructose) were also used as substrates in combination with the inoculum of unweaned pigs.

Cumulative gas production

Cumulative gas production by the pressure-volume measurements was carried out manually according to a modified *in vitro* fermentation method of Theodorou et al. (1994). 0.5g of the test substrate was weighed into fermentation bottles (100 ml serum bottles), and pre-warmed (39 °C) semi-defined anaerobic medium (82 ml; Lowe et al., 1985) was added. Five ml of the filtrate (Inoculum) was injected into each bottle within 1.5 h of feces collection from the pigs. The bottles were then incubated at 39 °C and incubation was terminated at 48 h. Four replicates per inoculum-substrate combination were used.

Analyses

Following *in vitro* fermentation, samples of fermentation fluid were collected for

analyses of the content of volatile fatty acids (VFA) and ammonia (NH₃). VFA were analyzed by GC (Fisons HRGC Mega 2, CE Instruments, Milan, Italy) using a glass column fitted (6 ft x 2 mm) fitted with Chromosorb 101 (80 -100mesh), as carrier gas N₂ saturated with formic acid, at 190 °C with *iso*-caproic acid as the internal standard.

Ammonia was determined according to a method described by Houdijk et al. (1998). Supernatants were deproteinized using 10% trichloroacetic acid. NH₃ forms a blue complex with phenol and hypochlorite in an alkaline environment, which was measured colorimetrically at 623 nm using a UV spectrophotometer (Beckman-Coulter DU 64, Fullerton, USA). Diet and feces were analyzed for dry matter (DM; ISO 6496) and inorganic matter (IM; ISO 5984). The pH was measured at the end of fermentation process. The pH of faecal samples was measured by direct insertion of the pH electrode (Hanna instruments) into the bottle of samples.

Statistics

The data for cumulative gas production (as ml of gas accumulated g⁻¹ OM over time) were fitted to the monophasic model described by Groot et al. (1996). (Eq. 1) using the nonlinear least squares regression procedure PROC NLIN (SAS Inst. Inc., Cary, NC).

$$G = A / (1+(C/t)^B) \quad \text{----- (1)}$$

Where G= total gas, A= asymptotic gas production, B= switching characteristic of the curve, C= time at which half of the asymptotic has been reached (T_{1/2}), t= time.

The maximum rate of gas production (R_{MAX}) and the time at which it occurred (T_{MAX}) were calculated according to the following equations (Eq. 2 and 3; Bauer et al., 2001):

$$R_{MAX} = \frac{\{A \times (C^B) \times B \times (T_{MAX}^{-(B-1)})\}}{\{1+(C^B) \times (T_{MAX}^{(-B)})\}^2} \quad \text{-----(2)}$$

$$T_{MAX} = C \times \{ (B-1) / (B+1) \}^{1/B} \quad \text{----- (3)}$$

A normal mixed model analysis was used to analyses the data of the present study (SAS, 1989). All parameters were tested for significance by analysis of variance using the Tukey multiple range test.

$$Y = \alpha + I_i + S_j + (I \times S)_{ij} + \epsilon_{ijk} \quad \text{----- (4)}$$

Where Y is the dependent variable, α represents the mean, I_i is the effect of inocula

($i=5$), S_k is the effect of substrate j ($j = 6$), $(I \times S)_{ij}$ is the interaction between substrate and inoculum, ε_{ijk} denotes the error term. All statistical analyses were performed using the GLM procedure of SAS version 8.1.

Results

The values for DM, ash and pH content measured in the faecal samples used as inocula from the five treatments are shown in Table 1. The values for DM and ash content of each substrate are shown in Table 2.

Fermentation kinetics

Table 3 shows the mean values for parameters of fermentation kinetics. A significant interaction between substrate and inoculum was found for most parameters except pH and NH_3 . The fermentation characteristics of different substrates were significantly different ($p < 0.001$). Both fermentation kinetics and end products are affected more by substrates than by different inocula.

Table 1. DM, ash and pH content measured in fresh faecal inocula of weaned and unweaned pigs[†]

Inoculum	DM (g/kg)	Ash (g/kg)	pH
Rectal faeces of pigs fed:			
Negative control	48.5	17.3	6.49
Oligofructose (OF, Raftifeed IPE)	60.4	20.4	6.45
Grinded chicory roots	45.6	16.2	6.60
Mixture (60% chicory pulp+40% Raftifeed IPE)	58.7	19.8	6.50
Positive control	58.9	20.5	6.46
Rectal faeces of suckling pigs			
Sow milk	- ‡	9.7	5.78

[†]The values of DM, ash and pH after being diluted.

[‡]The values was not determined.

OF (a blend of high- and low polymer inulin) and MIX had the highest value for total gas production (OMCV), and soybean meal had the lowest gas production ($p < 0.001$). All the oligofructose and inulin type substrates had a higher gas production compared to the NCON diet or soybean meal ($p < 0.001$).

Table 2. DM and ash contents of substrate (air-dried)

Substrate	DM (g/kg)	Ash (g/kg)
Negative control	973.0	71.3
Oligofructose (Raftifeed IPE)	985.2	64.3
Chicory roots (grinded)	978.6	42.1
Blend of chicory pulp+ OF (60:40)	977.4	92.3
Corn	968.9	13.4
Soybean meal	976.0	70.2

Corn as a substrate had a large gas production comparable to the CHR substrate, but later C and T_{MAX} . OF and CHR almost completely disappeared with OM losses of 97.7 and 95.3%, respectively. The highest OM loss by fermentation of OF resulted in the lowest pH and NH_3 also significantly lowered ($p < 0.001$). Soybean meal had the highest NH_3 and pH, and also had the lowest OM loss (76.0%). CHR had the highest R_{MAX} and the lowest C and T_{MAX} .

The inoculum affected most fermentation characteristics expect for the pH. Total gas production (OMCV), C, R_{MAX} and T_{MAX} were highest with faeces from PCON, CHR, OF and CHR, as inoculum respectively. OM loss was significantly lower for both PCON and CHR compared to all other inocula ($p < 0.007$). pH values were similar between the inocula, but ammonia concentrations were lower for CHR and NCON than that of other inocula ($p < 0.001$).

The mean values of fermentation characteristics for the faecal inocula of weaned and unweaned pigs and two substrates are shown in Table 4. Total gas production and fermentation kinetics expect for NH_3 were significantly affected by substrate. The substrate MIX had higher total gas production (OMCV) and R_{MAX} , and lower C and T_{MAX} compared to the NCON substrate. The pH was significantly lower in the MIX than that of NCON. Ammonia was numerically in the MIX compared to the NCON. There were significant differences in fermentation characteristics of C, T_{MAX} , ammonia and OM loss between inocula of weaned and unweaned pigs. The inocula of unweaned pigs had higher C, T_{MAX} and lower OM loss and ammonia compared with the inocula of weaned pigs.

Volatile fatty acids and branched-chain ratio

The main effects of substrates and fecal inocula on VFA production and BCR are shown in Table 5. The production and proportion of VFA were significantly different according to substrate ($p < 0.001$). The oligofructose and inulin substrates produced the highest total VFA, and had the lowest BCR compared to the NCON diet, corn and soybean meal. This is logic because substrates with high protein have mostly more

BCR. Corn as a substrate produced the highest butyric acid, and soybean meal produced the highest BCR ($p < 0.001$) between substrates. The oligofructose and inulin substrate had significantly lower BCR compared to the grain group substrates (Corn, soybean meal and NCON).

Fermentation characteristics by inocula from CHR had significantly lower acetic acid, and OF inocula produced the highest butyric and valeric acids. The NCON produced the highest propionic acid. Total VFA were not significantly different among the inocula. PCON had the highest BCR between inocula ($p < 0.001$). There were significant interaction between substrate and inocula for propionic and valeric acids and BCR ($p < 0.001$).

Table 3. Mean values of the fermentation characteristic parameters according to the effects of substrate and faecal inocula of the weaned pigs

Factor	OMCVT ^a (ml/g OM)	C ^β (h)	R _{MAX} ^γ (ml/h)	T _{MAX} ^δ (h)	OM loss (%)	pH	NH ₃ (mg/ g OM)
Substrate							
NCON ^ε	253 ^c	19.5 ^b	10.5 ^{de}	21.8 ^b	80.5 ^e	6.49 ^b	75.7 ^b
Corn	287 ^b	22.4 ^a	12.3 ^d	18.2 ^a	84.4 ^d	6.41 ^c	63.1 ^{cd}
Soybean meal	172 ^d	17.6 ^c	9.5 ^e	3.1 ^d	76.0 ^f	6.65 ^a	136.5 ^a
OF ^θ	305 ^a	8.7 ^e	24.0 ^b	2.9 ^{de}	97.7 ^a	6.31 ^e	64.1 ^d
CHR ^π	287 ^b	5.8 ^f	32.9 ^a	2.0 ^e	95.3 ^b	6.35 ^d	67.7 ^c
MIX ^ω	298 ^{ab}	11.5 ^d	18.1 ^c	4.2 ^c	88.2 ^c	6.39 ^c	75.9 ^b
SEM	4.2	0.34	0.79	0.55	0.30	0.013	1.19
Probability	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Inoculum							
NCON	262 ^b	14.8 ^b	16.2 ^c	7.8 ^a	87.5 ^a	6.41	76.7 ^b
OF	267 ^b	13.9 ^c	19.9 ^a	6.5 ^b	87.5 ^a	6.46	81.4 ^a
CHR	253 ^b	16.2 ^a	16.6 ^{bc}	8.0 ^a	85.9 ^b	6.42	76.3 ^b
MIX	268 ^b	14.0 ^c	17.9 ^{abc}	7.0 ^b	88.2 ^a	6.44	82.1 ^a
PCON ^Ω	285 ^a	12.4 ^d	18.9 ^{ab}	6.7 ^b	85.8 ^b	6.44	85.9 ^a
SEM	3.9	0.31	0.72	0.32	0.28	0.012	1.10
Probability	0.001	0.001	0.002	0.003	0.007	0.109	0.001
Interaction							
Sub x Inoc ^φ	0.001	0.001	0.004	0.001	0.001	0.29	0.08

^aOMCV= total gas produced and corrected to ml gas / g OM.

^βC= time at which half of asymptotic has been reached.

^γR_{MAX}= maximum rate of gas production.

^δT_{MAX}= time at which maximum rate of gas production occurs.

^εNCON, Negative control; ^θOF, Oligofructose (Raftifeed IPE, Orafti, Belgium); ^πCHR, grinded chicory roots; ^ωMIX, 60% chicory pulp + 40% Raftifeed IPE; ^ΩPCON, Positive control; ^ΦSub x Inoc, substrate X inoculum.

^{abcdef}Means in the same column for substrate or inocula without a common character in the superscript differ significantly.

Table 4. Mean values of the fermentation characteristic parameters according to the effects of substrate and faecal inocula of weaned and unweaned pigs

Factor	OMCV ^α (ml/g OM)	C ^β (h)	R _{MAX} ^γ (ml/h)	T _{MAX} ^δ (h)	OM loss (%)	pH	NH ₃ (mg/ g OM)
Substrates							
NCON ^ε	257	23.8	9.7	16.2	77.9	6.45	71.0
MIX ^ω	317	14.2	16.4	7.4	88.5	6.39	68.6
SEM	7.9	0.91	0.84	0.72	0.33	0.02	1.19
Probability	0.001	0.001	0.001	0.001	0.001	0.08	0.17
Inoculum							
Weaned pigs	281	16.2	13.8	8.8	85.5	6.42	72.2
NCON							
Suckling piglets	294	21.7	12.2	14.8	80.9	6.42	67.4
SEM	7.6	0.91	0.84	0.72	0.33	0.022	1.19
Probability	0.26	0.001	0.19	0.001	0.001	0.95	0.02
Interactions							
Sub x Inoc ^Φ	0.47	0.14	0.68	0.77	0.07	0.19	0.01

^αOMCV= total gas produced and corrected to ml gas / g OM.

^βC= time at which half of asymptotic has been reached.

^γR_{MAX}= maximum rate of gas production.

^δT_{MAX}= time at which maximum rate of gas production occurs.

^εNCON, Negative control; ^ωMIX, 60% chicory pulp + 40% Raftifeed IPE; ^ΦSub x Inoc, substrate x inoculum.

Mean values of VFA and BCR for the substrates and faecal inocula of the weaned pigs and unweaned pigs are shown in Table 6. The substrate MIX had significantly higher acetic acid, and also had significantly lower butyric and BCR compared to the NCON substrate. Fermentation by faecal inocula of weaned pigs produced significantly more VFA, total VFA and BCR compared to the inocula from unweaned pigs. There were significant differences in propionic, butyric acids and BCR between the inocula of weaned and unweaned pigs.

Table 5. Mean values of VFA and BCR according to the effects of substrate and faecal inocula of the weaned pigs

Factor	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Total VFA	BCR ^a
	mmol / g OM					
Substrate						
NCON ^Φ	4.34 ^c	1.97 ^d	2.09 ^b	0.42 ^c	9.23 ^c	0.045 ^b
Corn	3.90 ^d	1.96 ^d	2.28 ^a	0.36 ^d	8.87 ^c	0.041 ^c
Soybean meal	4.49 ^c	1.88 ^d	1.39 ^{cd}	0.49 ^b	8.84 ^c	0.066 ^a
OF ^ω	5.30 ^b	3.53 ^a	1.47 ^c	0.55 ^a	11.06 ^a	0.020 ^e
CHR [Ⓜ]	5.49 ^{ab}	3.06 ^b	1.29 ^d	0.49 ^b	10.54 ^a	0.020 ^e
MIX ^Ω	5.74 ^a	2.36 ^c	1.17 ^e	0.40 ^c	9.91 ^b	0.025 ^d
SEM [⊥]	0.102	0.057	0.035	0.009	0.200	0.001
Probability	0.001	0.001	0.001	0.001	0.001	0.001
Inocula-faeces						
NCON	4.85 ^a	1.32 ^a	1.54 ^{ab}	0.47 ^b	9.96	0.035 ^b
OF	4.99 ^a	1.10 ^{bc}	1.68 ^a	0.54 ^a	9.85	0.035 ^b
CHR	4.52 ^b	1.06 ^c	1.53 ^b	0.41 ^c	9.18	0.034 ^b
MIX	5.07 ^a	1.16 ^b	1.68 ^a	0.45 ^b	9.75	0.036 ^b
PCON ^Σ	4.96 ^a	1.21 ^b	1.65 ^a	0.39 ^d	9.93	0.040 ^a
SEM	0.095	0.001	0.032	0.008	0.185	0.001
Probability	0.003	0.03	0.004	0.001	0.06	0.001
Interaction						
Sub X Inoc [§]	0.47	0.001	0.09	0.001	0.32	0.001

^aBCR= branched-chain ratio calculated as total straight chain fatty acids to branched-chain fatty acids.

^ΦN-control, Negative control; ^ωOF (R-IPE), Oligofructose (Raftifeed IPE, Orafiti, Belgium);

[Ⓜ]CHR, grinded chicory roots; ^ΩMIX, 60% chicory pulp + 40% Raftifeed IPE; [⊥]SEM, standard error of mean; ^ΣPCON, Positive control; [§]Sub x Inoc, substrate x inoculum.

^{abcde}Means in the same column for substrate or inocula without a common character in the superscript differ significantly.

Table 6. Mean values of VFA and BCR according to the effects of substrate and faecal inocula of the weaned pigs and unweaned pigs

Factor	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Total VFA	BCR ^a
	mmol / g OM					
Substrate						
NCON ^ϕ	4.04	1.94	1.70	0.34	8.33	0.036
MIX ^ω	5.58	2.00	1.19	0.28	9.23	0.020
SEM [⊥]	0.224	0.103	0.094	0.021	0.422	0.001
Probability	0.001	0.71	0.003	0.08	0.16	0.001
Inoculum						
Weaned pigs NCON	5.22	2.55	1.67	0.45	10.2	0.034
Suckling pigs	4.40	1.39	1.21	0.17	7.3	0.021
SEM	0.224	0.103	0.094	0.021	0.42	0.001
Probability	0.02	0.001	0.006	0.001	0.001	0.001
Interaction						
Sub x Inoc [§]	0.57	0.003	0.007	0.69	0.70	0.003

^aBCR= branched-chain ratio

^ϕN-control, Negative control; ^ωMixture, 60% chicory pulp + 40% Raftifeed IPE; [⊥]SEM, standard error of mean; [§]Sub x Inoc, substrate x inoculum.

Discussion

Differences between substrate

As was expected oligofructose and inulin type substrates produced a higher rate of fermentation kinetics (total gas production) and end product (VFA) than that of the NCON or feed grain substrates. There were significant differences by the main effects of substrate and inoculum. The different rate of fermentation certainly related to differences in microbial activity and in the chemical structure (degree of polymerization) of substrates. The production of VFA which produce as end products of fermentation by microbiota may be beneficial for GIT health (Cummings and Macfarlane, 1991). The individual SCFA produced in the large intestine may have a specific role. For instance, acetic acid acts as an energy sources for muscle tissue, and propionic acid is converted in the liver. In addition, butyric acid is preferred substrate for the colonic epithelial cells (Roediger, 1980). The present results suggest that type of fermentable carbohydrate is the important in determining the amount and

profile of VFA in the large intestine.

The fermentation patterns between substrates indicated that both OF (a blend of high and low polymer inulin) and MIX (a mixture of 60% chicory pulp and 40% OF) were the most fermentable in both kinetics and end products between substrates. This result suggests that a mixture of slowly fermentable (high polymer inulin) and rapidly fermentable (low polymer inulin) may produce more total gas production and also may help maintain the fermentation for a longer time (Van Loo, 2004). If then, it may help maintain the fermentation throughout the large intestine of pigs and may also reduce proteolytic microbial activity in the large intestine.

Soybean meal was the least fermentable (OMCV) between substrates, but had almost double the ammonia than that of all other substrates. There was also high BCR for this substrate. The greater proportion of branched chain fatty acids (BCFA) in soybean meal substrate suggested an increased fermentation of protein during fermentation of soybean meal compared to other substrates. Because BCFA are mostly produced as end products by the metabolisms of branched-chain amino acids (valine, leucine and iso-leucine). It is thought that this can be the result from a shortage of energy from carbohydrates (Macfarlane et al., 1992). The greater ammonia concentration in soybean meal most probably related to the relatively high crude protein content of the substrate. Corn as a substrate had greater kinetics and fermented very slowly (greater C and T_{MAX}). Corn also had the highest butyric acid but the least acetic acid between substrates. Bauer et al (2001) have shown that feed grain such as maize, barley and wheat had similar VFA concentrations at the end of fermentation for both unweaned and adult pigs. The composition and activity of microflora in the large intestine can be manipulated by use of dietary prebiotics, and fermentable carbohydrates (Gibson and Roberfroid, 1995).

When comparing the kinetics and end products of substrates from weaned and suckling pigs, substrate from MIX had greater fermentation, C, OM loss and BCR than that of substrate from NCON. As a result, MIX significantly lowered pH and ammonia was numerically lowered compared to the NCON. Most fermentation kinetics and end products parameters of substrates between weaned and unweaned pigs were significant.

Differences between faecal inocula

There were significant differences in fermentation characteristics (fermentation kinetics and end products) between inoculums, but not for pH. The differences between inocula are a consequence of differences in microbial activity. Unexpectedly, the PCON (with antibiotics) had the highest gas production, NH_3 and the maximum rate of gas production. The BCR was the highest for the PCON and this is a result from fermentation of amino acids. This result is in agreement with the result of study

by Bauer et al (2003). They showed that pig chyme with antibiotics (Virginiamycin) led to a significantly greater BCR. Houdijk (1998) reported that addition of antibiotics (avilamycin) to weaner diets reduced the level of protein fermentation, but the concentration of ammonia was slightly reduced.

Dilution of the faecal samples in weaned pigs was 1:2 and in unweaned pigs was 1:25 because of the differences in the amount of faecal samples. This will probably affect lag time at the beginning of fermentation.

Inocula of CHR had the lowest acetic acid and least total VFA compared to other inocula. The total VFA and pH between inocula were not affected. The OF and MIX had the highest butyric acid between inocula. This agrees with the report of Houdijk (1998) that substrate from FOS and transgalacto-oligosaccharides produced significant amounts of butyric acid *in vitro*. The butyrate may have been produced from glucose, inulin and a fructose polymer by *Clostridia* spp. (Schlegel, 1992). Generally, substrate effects are tended to greater than inoculum effects on fermentation kinetics and end products. The OM loss of inoculum from PCON and CHR were significantly lower than that of other inocula, and this result suggests that microbial activity of the inocula during fermentation may be to some extent inhibited by in-feed antibiotics in the inoculum.

The comparison of cumulative gas production curve for different substrates with inocula from weaned and unweaned pigs, weaned pigs fermented both NCON diet and MIX (chicory pulp and OF) were much better than the inocula from unweaned pigs. The inocula from weaned pigs produce more total gas in a faster rate. The unweaned pigs were not offer creep feed and only consumed sow's milk. Initial colonization of bacteria in the GIT of young piglet is mainly lactic acid bacteria, and they remain as long as sow's milk is consumed (Mathew et al., 1998). So ability to ferment the both inocula by weaned and suckling pigs differs probably due to different microbial activity, microbial population in the inocula from weaned and suckling pigs. The less gas production and slow fermentation that occurred in unweaned pigs suggested a difference. The lower gas production and a lower rate of fermentation with inocula from unweaned pigs suggest that the 15 day-old suckling pigs had not yet reached the microflora of weaned or adult pigs. Most fermentation kinetics and end products parameters of inoculum between weaned and unweaned pigs differed significantly but some parameters such as OMCV, R_{MAX} and pH were not affected. Generally, inoculum of suckling pigs produced more total gas and used longer time at which maximum rate of gas production occurs than that of weaned pigs.

It was concluded that the different fermentation kinetics (T_{MAX} , R_{MAX}) from different substrates in the present study suggest that microbial flora is different. Inocula from inulin and oligofructose type substrates fed pigs produce more gas at a faster rate

than that of control or feed grain substrates. OF may reduce bacterial proteolytic activity at the actual site of fermentation. Both combined oligofructose substrates, OF, and MIX showed the highest fermentation kinetics and total VFA between substrates, and this result may help to maintain fermentation happen at the distal part of the large intestine or it may last longer. So a combination of slowly fermentable (high polymer fructan) and rapidly fermentable (low polymer fructan) may be more beneficial to the gut ecosystem and health than single type of OF. However, this needs to be evaluated in *in vivo* experiments.

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Chapter 9

General Discussion

Introduction

Weaning piglets at around 3 to 4 weeks of age often results in low feed intake and is associated with villous atrophy (impaired small intestinal integrity) and a high incidence of diarrhea. As a result, a growth check is commonly seen immediately after weaning. So weaning is regarded as the most critical period in a pig's life. To overcome these problems, growth promoting antibiotics are commonly used in both creep and weaner diet. However, the use of in-feed antibiotics will be banned by the EU from 2006 onward. The development of alternatives to antibiotics growth promoters is urgently needed in commercial pig production. Prebiotics and probiotics, alone or in combination (synbiotics), may be potential alternative for antibiotic growth promoters. The major objective of this thesis was to investigate the mechanisms of pre-, probiotics and synbiotics on the gastrointestinal ecosystem, health and performance effects of pigs around weaning. In this chapter, the main findings of this thesis are discussed in relation to the nature of prebiotics in swine feeds, gastrointestinal effects, performance effects and effects of creep feed on weaning weight and postweaning growth.

Prebiotics in animal (swine) feeds

There is an increasing interest in the use of prebiotics in feed for animals especially young pigs, poultry and pet dogs for the purpose of either improving gut health or reducing malodors. Inulin and oligofructose are major prebiotics in commercial uses. The major feed ingredients for pig diets differ by country. For instance, the major feedstuffs for pig diets in Korea and the USA are corn and soybean meal, but some EU countries and Australia are wheat and barley. Inulin and oligofructose are present in significant amounts in various foods and food ingredients (Van Loo et al., 1995). The oligofructose and inulin content of selected feeds and food ingredients are shown in Table 1. Wheat by-products (middlings, germ and bran) contained the highest contents of oligofructose. In addition, alfalfa meal, barley and wheat also contained relatively higher contents of oligofructose than remaining feed and food ingredients. However, oligofructose was not found in several foodstuffs such as corn, soybean meal and rice (Hussein et al., 1998). Garlic and onion also have higher contents of inulin and oligofructose compared to other foodstuffs (Van Loo et al., 1995).

Houdijk (1998) reported that commercial pig diets can contain up to 20-30g of non-digestible oligosaccharides (NDO) per kg feed. He also postulated that commercial diets would either mask or dilute the effects of any NDO added. He suggested using diets with very low level of NDO to have any beneficial role of oligosaccharides as feed components and as potential prebiotics. Most of the basal

diets used in the experiments of this thesis contain little (9.5g / kg basal diet in Chapter 4 and 5) or no oligofructose (Chapter 6 and 7). Because major ingredients of the basal diets used in most experiments described in this thesis were either wheat-soybean meal, corn-soybean meal or extruded rice-soybean meal-corn. But they have a considerable level of non-digestible oligosaccharides. The nature of oligosaccharides contained in commonly used feed and food ingredients will be medium chain length. Therefore, to find any beneficial prebiotics effects in young pigs, a very low degree of polymerization (DP 2-5) of oligofructose were used in our experiments described in this thesis. In addition, the combination of short chain and long chain fructans (average DP 25) has more beneficial (or synergistic) effects than that short chain or long chain fructans alone. Oligofructose with different average DP will be fermented at different sites in the gastro-intestinal tract. Roberfroid et al. (1998) reported that chicory inulin which has long chain fractions (DP 10 to 60; average DP of 25) is fermented twice as slowly as the short chain oligofructose fraction (DP 2 to 8; average DP of 4). Thus, when comparing the effects of oligofructose in the literature, the chemical structure (DP) of oligofructose products must be considered and also all scientific studies relating prebiotics should give information on the chemical structure of the oligofructose product used.

The amount and type of substrate available to the microbiota strongly affect the microbial fermentation in the gastrointestinal tract (GIT) of pigs. Non-digestible carbohydrates, especially non-starch polysaccharides (NSP), in the diet may affect the efficacy of prebiotics in animals because specific NSP components in diets are not digested in the small intestine and reached in the large intestine will contribute more saccharolytic fermentation. The continued saccharolytic fermentation that occurs in the large intestine may be beneficial to the host animals because the activity of proteolytic microorganisms can be reduced due to competition with carbohydrate fermenting bacteria for common resources (Jensen, 1999). This may prolong the effects of prebiotics in the gut. Table 2 shows the NSP contents in commonly used feed ingredients for pig diets. Soybean meal contains the highest NSP compared to other feedstuffs. Soluble carbohydrates are more rapidly fermented than insoluble (Jensen, 2001). NSP in carbohydrate fractions will be fermented in the more distal parts of the large intestine compared to short chain oligofructose which was used in the experiments of this thesis.

The combination of easily fermentable short chain oligofructose (average DP of 4) and slowly fermentable NSP components in the diet may allow continuing fermentation by saccharolytic bacteria throughout the large intestine. However, if there is a lack of preferred substrates as energy sources for saccharolytic bacteria then, some bacteria may start to use proteins as a source for fermentation (increased proteolytic activity).

Table 1. Oligofructose and inulin content of selected feeds and food ingredients

Item	Fructan content ¹ , mg/g DM	Fructan content ² , mg/g (as-is basis)
Alfalfa meal	2.24	
Barley	1.92	0.05-0.10
Beet pulp	0.05	
Canola meal	0.04	
Corn gluten feed	0.09	
Corn gluten meal	0.34	
Garlic		0.98-1.60
Oats	0.36	
Oat groats	0.12	
Onion, dried		0.11-0.75
Peanut hulls	2.40	
Rice barn	0.14	
Rye flours		0.05-0.10
Soybean hulls	0.12	
Wheat	1.36	0.10-0.40
Wheat bran	4.00	
Wheat germ	4.68	
Wheat middlings	5.07	

¹Determined by Hussein et al. (1998) as sum of 1-kestotriose + 1,1-kestotetraose + 1,1,1-kestopentaose

²Determined by Van Loo et al. (1995) as sum of oligofructose and inulin with DP of 2 to 60.

The basal diets that were used in our experiments for Chapter 4 and 5 were a wheat-soybean meal based diets which has relatively high NSP contents. This may partly contribute to fermentation in the more distal part of the large intestine as was supported by the numerically higher content of the volatile fatty acids (VFA) in the caecum and colon. However, the impact of fermentable carbohydrates especially specific NSP components in pig diet on prebiotics efficiency remains to be investigated.

Table 2. NSP contents in major feed ingredients¹ (g/kg DM)

	Wheat	Barley	Maize	Soybean meal	Tapioca
Total NSP	119	186	97	217	84
Raffinose	4	5	2	10	1
Stachyose	2	1	1	47	1
Verbascose	-	-	-	3	-

¹Data adapted from Back-Knudsen, 1997

Gastrointestinal effects of prebiotics and probiotics or synbiotics

1. Microbial Population in the Small intestine and Large intestine

At birth, the GIT of piglets is germfree but is rapidly colonized by anaerobic bacteria during the first weeks of life (Moughan et al., 1992). In neonatal piglets, the colonizing lactobacilli originate mainly from the feces of the sow (Tannock et al., 1990). More than 400 different species with numbers as high as 10^{10} - 10^{11} culturable bacteria per gram digesta were found in the colon of pigs (King and Kelly, 2001). The microbial population in the large intestine is more numerous per g of chyme than in the stomach and small intestine mainly due to the slower transit time of digesta in the large intestine, which enables microorganisms to multiply there. The predominant bacterial species present in the stomach and small intestine of a healthy pig are enterobacteria, streptococci and lactobacilli in the stomach and small intestine. The predominant bacterial species in the large intestine are bacteroides, prevotella, eubacteria, lactobacilli, fusobacteria, peptostreptococci. Bifidobacteria makes up less than 1% of the total population of bacteria in the pig gut (Jensen, 1999). The microbial balance between beneficial and harmful organisms may be disturbed when pigs are under stress conditions such as weaning, alterations in environmental conditions and change of diet. These conditions allow potentially pathogenic bacteria to colonize the gut, resulting in diarrhea, gastroenteritis and reduced growth performance (Cromwell, 2001).

Prebiotics are non-digestible food ingredients that positively affect the host by selectively stimulating the activity of a limited number of beneficial bacteria, resulting in improved host health (Gibson and Roberfroid, 1995). The purpose of supplementing oligofructose in diet of weaned pigs is to maintain a healthy microbial balance by stimulating beneficial bacteria such as bifidobacteria and lactobacilli. Oligofructose and inulin may selectively stimulate the growth of beneficial bacteria like bifidobacteria in the colon and may maintain healthy gut ecology by producing VFA and decreasing pH. Consequently, the growth of harmful bacteria such as *E. coli* may be suppressed (Kruse et al., 1999). This may help to improve the gut health thereby reduces the incidence of diarrhea of weaned pigs. Several studies (Howard et al., 1995; Bunce et al., 1995; Nemcová et al., 1999) reported that supplementing diets with oligofructose increased the population of bifidobacteria. The number of *E. coli* in the large intestine was decreased in weaned pigs (Bunce et al., 1995; Rossi et al., 2001). The results of our experiments in Chapter 6 and 7 (Table 3) showed that the number of lactobacilli or bifidobacteria increased. But dietary oligofructose did not affect the colonic population of bifidobacteria while the ileal bifidobacteria population

of newly weaned pigs increased. The results suggested that the oligofructose (low DP) that was used in our experiments is more rapidly fermentable than long chain oligofructose and may have been already partly fermented before it reached the colon. To maintain more continued fermentation of prebiotics throughout the large intestine, a combination of rapidly (low DP) and slowly (high DP) fermentable oligofructose may be needed. The reported inconsistent results of microbial responses by including prebiotics may be due to the different composition of the basal diets, different type of prebiotics and different sanitation condition of pigs.

In Chapter 6 and 7, the synbiotics approach (a combination of prebiotics (oligofructose) and probiotics (multi-strains)) showed more consistent stimulatory effects on beneficial bacteria than that of either oligofructose or probiotics alone. Consequently, the population of *E. coli* that is often regarded as potentially harmful bacteria was reduced in the gut of weaned pigs (Table 3). These results suggested that synbiotics approach may have some more synergistic effects on gut ecosystem compared with supplementing pre- or probiotics alone.

Table 3. Summary of the effects of oligofructose (OF), multi-strain probiotics (M-Pro), two-strain probiotics (T-Pro) and synbiotics (SYN) on microbial populations of weaned pigs^{1,2}

Age	Diet	Site	Source	Level,%	Lacto	Bifido	E. coli	Anaero
Weanling	Corn- Soybean meal	Feces	OF	0.25	↔	NM	↓	↔
			M-Pro	0.2	↔	NM	↓	↔
			T-Pro	0.2	↔	NM	↓	↑
			SYN	0.45 ³	↑	NM	↓	↔
Suckling to weaning	Creep Feed: Extruded	Ileum	OF	0.2	↔	↑	↔	NM
			M-Pro	0.3	↔	↑	↔	NM
			SYN	0.5 ⁴	↔	↑	↔	NM
	rice- Lactose	Colon	OF	0.2	↔	↔	↓	NM
			M-Pro	0.3	↔	↑	↓	NM
			SYN	0.5 ⁴	↔	↑	↓	NM

¹Abbreviations used: Lacto, lactobacilli; Bifido, bifidobacteria; Anaero, Total anaerobic bacteria; NM, No measurement.

²↑ = increase; ↓ = decrease; ↔ = no change.

³A: Synbiotics (0.25% OF + 0.2% Multi-strain probiotics).

⁴B: Synbiotics (0.2% OF + 0.3% Multi-strain probiotics).

2. Fermentation characteristics and Gut Morphology

Fermentable carbohydrates such as NSP, resistant starch and oligosaccharides

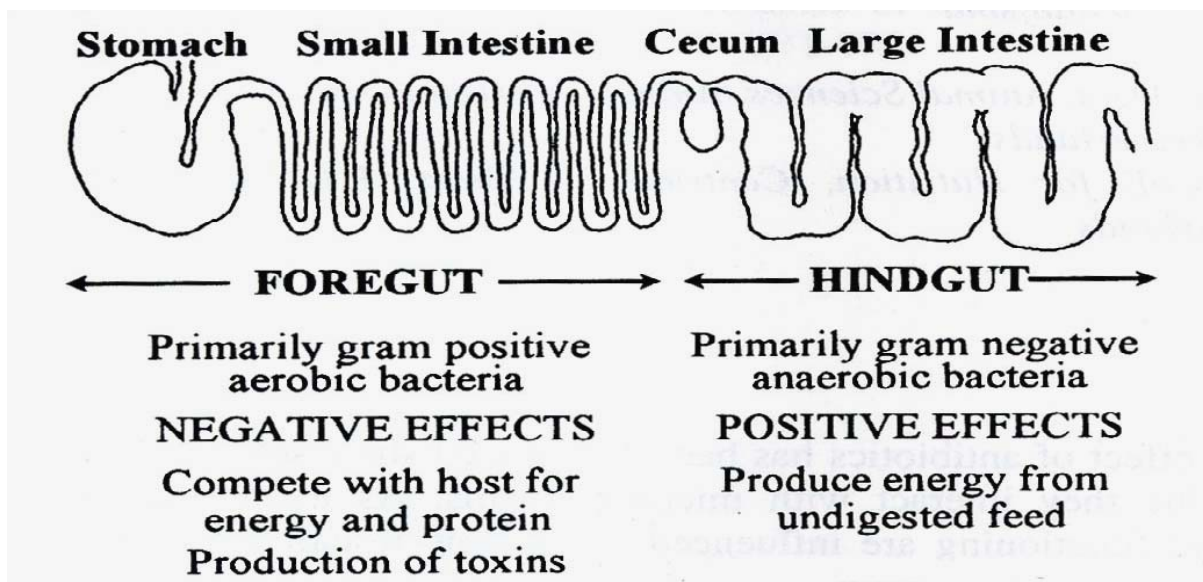
which are not digested in the small intestine and pass through to the hind gut are the major source of energy for microbial fermentation in the large intestine (Mathers and Anison, 1993). As a result of fermentation in the large intestine, VFA (mainly acetate, propionate and butyrate), CH₄, H₂, CO₂ and ammonia are produced (Jensen and Jørgensen, 1994). The production of VFA is generally considered to be beneficial for the GIT because it can supply energy to the host animal (Bach Knudsen and Hansen, 1991). The VFA also contribute to the net energy for the maintenance requirement of around 20% for growing pigs (Varel and Yen, 1997). The increased amounts of acetic and lactic acids produced by the fermentation of prebiotics by lactic acid bacteria will decrease the pH in the large intestine. The decreased pH may suppress the growth of pathogenic bacteria like *E. coli*. and thereby reduce the incidence of diarrhea may be reduced (Gabert et al., 1994). The ratio of short chain fatty acids (SCFA) and branched SCFA could be used as an indicator of saccharolytic activity relative to proteolytic activity (Swanson et al., 2002). Butyrate is a preferred energy source for cell proliferation (Lupton and Kurtz, 1993). Therefore, the increased butyrate concentration by dietary oligofructose might positively affect epithelial cell proliferation and increased the gut's absorptive area. The results of the fermentation characteristics in our experiments in Chapter 4 and Chapter 5 showed that dietary oligofructose increased the VFA concentration in the large intestine. Villous height in the small intestine was also numerically higher by the inclusion of oligofructose in the diet compared to the control diet. This finding is in agreement with the results of Howard et al. (1995) that oligofructose enhances indices of epithelial cell proliferation along the entire length of the hind gut in very young pigs. If villous atrophy which is common immediately after weaning can be ameliorated by dietary oligofructose then, the nutrient absorption capacity could be maintained and the postweaning growth check could be reduced. Bolduan et al (1993) suggested that feeding prebiotics likely to reduce the concentration of ammonia and an increase in VFA concentration in the colon of weaned pigs. The results from our experiment in Chapter 5 showed that the concentration of ammonia was not significantly reduced by feeding oligofructose, but in Chapter 6, the ammonia concentration was significantly reduced by addition of pre-, probiotics and synbiotics when compared to the control.

In vitro, Mitsuoka et al. (1987) have found that a range of bifidobacteria can utilize oligofructose and some enteric bacteria especially *Bacteroides* species were also able to grow on a range of prebiotics. However, utilization of oligofructose by lactobacilli, *E. coli* and *Clostridium perfringens* was poor. The results of our *in vitro* fermentation experiment described in Chapter 8 clearly show (Figure 2) that the fermentation characteristics of oligofructose and inulin were different depending on their molecular structure (DP) of prebiotics.

3. Nutrient digestibility

Little information is available regarding the effect of oligofructose on nutrient digestibility. A study by Houdijk et al (1999) revealed that feeding oligofructose to weanling pigs did not affect both ileal and fecal digestibility in well-kept weanling and growing pigs. But digestion of dietary NSP may be affected and also ileal digestibility of dry matter and fiber was affected. Vanhoof and De Schriver (1996) reported that dietary inulin to the growing pigs increased apparent faecal Zn absorption but decreased apparent ileal and faecal absorption and retention of Ca and P. Our results in Chapter 4 have shown that the dietary oligofructose may affect ileal digestibility of protein. Our results of oligofructose on the apparent faecal digestibility of dry matter, crude protein and ash as well as on the apparent faecal absorption of calcium and phosphorus in all treatment groups were significantly higher than those of the control (Chapter 6). The reason why the nutrient digestibility increased is not clear. A decreased pH enhances mineral solubility which increases the absorbability. Moreover, higher villous height that shown in both experiments may partly have been affected the nutrient absorption of weanling pigs. In a human study, dietary oligofructose have been shown to improve the absorption of calcium, magnesium and iron absorption (Cumming and Macfarlane, 2002).

Figure 1. Gut microbial characterization in the foregut and hind gut of pig



Adapted from Verstegen and Schaafsma (1999).

Figure 2. Cumulative gas production curves of inoculum 1 (Negative control) with different substrates

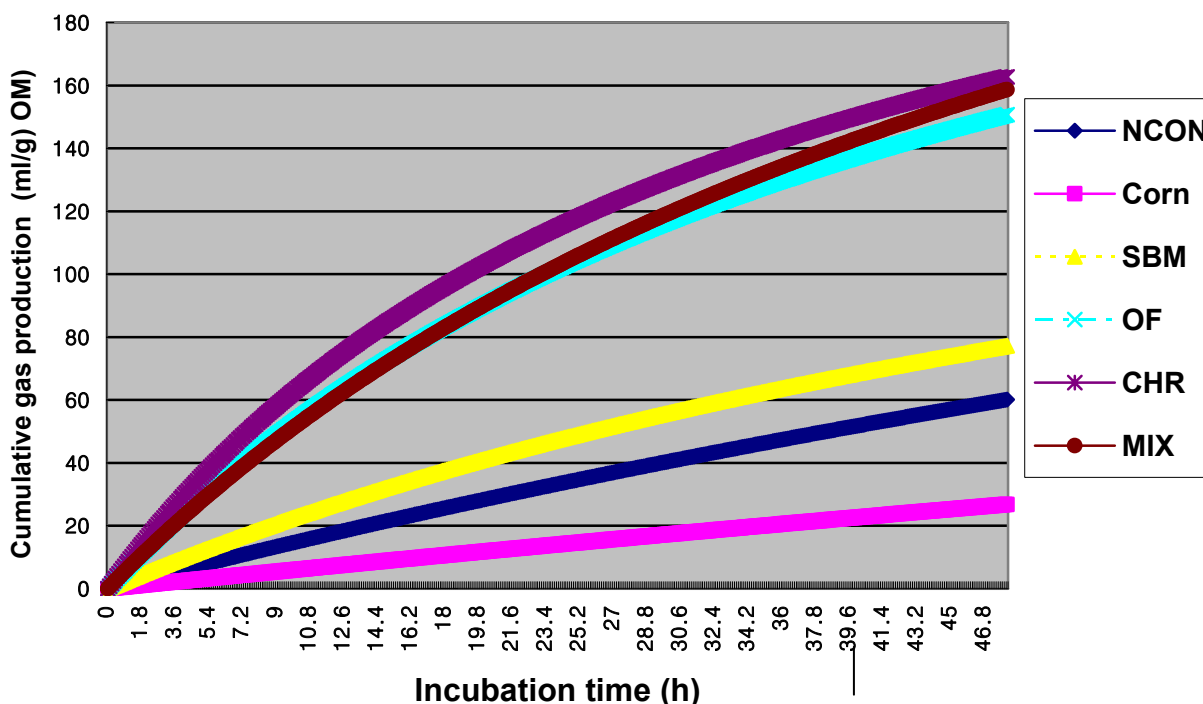


Figure 3. Schematic representation of the fate of dietary oligofructose (OF) with different polymers and the subsequent changes of the microbial activity in the porcine gastrointestinal tract

	Stomach	Small intestine			Large intestine		
		duodenum	Jejunum	Ileum	caecum	colon	rectum
Dietary OF							
Low polymer	████████	████████	████████	████████	████████	████████	████████
Microbiota	Mainly saccharolytic					Increasing proteolytic	
High polymer	████████	████████	████████	████████	████████	████████	████████
Microbiota	Mainly saccharolytic					Increasing proteolytic	

Performance effects of prebiotics and probiotics or synbiotics

Effects of oligofructose on growth performance reported in literature are not inconsistent. Some studies (Russell et al., 1996; Estrada et al., 2001) reported that

weaning pigs fed oligofructose numerical increased in body weight gain while other studies (Howards et al., 1995; Houdijk et al., 1998) found little or no effects on growth of weanling pigs. However, pigs fed synbiotics diet, combining prebiotics (oligofructose) and probiotics, increased lactobacilli and bifidobacteria (Nemcová et al., 1999) and improved growth performance (Kumprecht and Zobac, 1998). This result is in agreement with our results shown in Chapter 6. The main reasons why growth effects of prebiotics are still not clearly understood. Several factors such as molecular structure (DP) of oligofructose, NSP content in the diet, the dosage level of oligofructose, sanitation and health condition of the pigs may be involved. Our results in Chapter 6 on growth performance effects of dietary prebiotics (oligofructose), two or multi strain probiotics, and synbiotics in weanling pigs are shown in Table 5. Among the treatments, the synbiotics diet showed the best growth performance compared to either prebiotics or probiotics alone.

Table 5. Average daily weight gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) for weaned pigs¹ fed diets with OF, multi-strain probiotics, two strain probiotics or synbiotics. (Data are expressed relative to the control diet (=100), Duration: 21days.

Item	Control	OF ²	M-PRO ²	SYN ²	T-PRO ²
ADG, g	100	119	119	125	112
ADFI, g	100	110	103	117	110
FE (Gain/feed)	100	108	117	108	102

¹Sixty pigs with an average initial body weight of 6.4kg.

²OF: 0.1% oligofructose; M-PRO: 0.2% multi-strain probiotics; SYN: 0.1% OF + 0.2% multi-strain probiotics; T-PRO: 0.2% two strain probiotics

Conclusions

Based on the results of our experiments described in this thesis, it was concluded that

- Feeding oligofructose to weanling pigs did not affect disaccharidase activity and viscosity of digesta in the small intestine, but villous height was numerically increased.
- Dietary oligofructose decreases pH and ammonia concentration in the gastrointestinal tract of weaned pigs.
- Supplementation of pre-, probiotics or synbiotics (combining OF and multi-strain probiotics) to an antibiotic-free diet (negative control) stimulate beneficial bacteria especially bifidobacteria and decreases the population of *E. coli* in the large

intestine.

- Dietary oligofructose improves ileal digestibility of protein as well as the apparent absorption of calcium and phosphorus and may improve growth performance of newly weaned pigs.
- Supplementation of oligofructose or synbiotics to an antibiotic-free creep feed during the suckling period affects gut microbial population and performance of piglets.
- Oligofructose did not affect hematological traits except platelet count.
- *In vitro*, the microbial activity differs significantly between substrates and between inocula, and the differences in fermentation characteristics were more profound between substrates than between inocula.
- *In vitro*, inoculum of weaned pigs had more capability of microbial fermentation to the carbohydrates ingredients and oligofructose than that of unweaned pigs.
- A combination of high- and low polymer inulin can be maintain fermentation more distal part of large intestine than that of single polymer oligofructose alone, and it may more beneficially affect to the gut ecosystem.
- Overall, it was concluded that synbiotics, a combination of multi-strain probiotics and oligofructose showed the highest growth performance and was also more beneficial to alter beneficial microflora and gut ecosystem compare to supplementing oligofructose or probiotics alone. A synbiotics approach could be used as potential alternative to antibiotic growth promoter for young pigs.

Future Research Direction

- 1 Differences in molecular structure and chain length may influence where in the intestinal tract they are fermented. So combination of different molecular structure (DP) of prebiotics, for example, combining low DP (rapidly fermented) and high DP (slowly fermented such as inulin) may have different fermentation characteristics, and also have different rates and sites of fermentation in the gut. This may allow prolonged fermentation of prebiotics throughout the large intestine. This may result in different physiological and microbiological effects to host animals. Thus, a combination of rapidly or slowly fermented prebiotics may be more beneficial than supplementing one source (same chain length) prebiotics.
- 2 Effects and role of NSP contents especially stachyose and raffinose in swine diet on oligofructose efficacy needs to be elucidated for practical use (optimal dosage level) of oligofructose in the diet for young pigs.
- 3 For precise understanding of the effect of oligofructose on pig gut microbiota, a molecular approach rather than classical plate counting is needed for

accurate monitoring of changes in the composition of the gut microflora by supplementing oligofructose.

- 4 Optimal inclusion levels of various types of oligofructose in pig diets have to be established in commercial pig farm environments according to sanitation level and health status of pigs, the nature of pig diets (nutrient contents including NDO and NSP, ingredients and antibiotics) and housing (room temperature).
- 5 A mixture of different molecular structure of oligofructose (high and low degree of polymerization) together with multi-strain probiotics may be most beneficial for a new synbiotics concept in newly weaned pigs. This needs to be evaluated in future studies.

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SUMMARY

Piglets are commonly weaned at around 18-25 days of age in most countries. When piglets are weaned, they are usually exposed to several stressors such as nutritional, environmental, social and microbiological stressors. This results in villous atrophy in the small intestine, decreased immune response, increased incidence of diarrhea, and consequently a growth check. To prevent these suboptimal postweaning events, subtherapeutic antibiotics are still commonly used in commercial pig production in some countries. However, there has been a growing concern about antibiotics resistance of pathogenic gut microflora. That may have consequences for which antibiotics can be used therapeutically for humans and for animals. So the use of growth promoting antibiotics will be completely banned by EU from 2006 onwards. Prebiotics and probiotics alone or in combination (synbiotics) may be potential alternative for antibiotic growth promoters. A concept of synbiotics (a combination of prebiotics and probiotics) is a promising approach for enhancing beneficial bacteria in the gut.

The aim of the five experiments described in this thesis was to evaluate the effects of prebiotics, multi-strain probiotics and synbiotics on gastrointestinal ecosystem, health and performance effects of pigs around weaning. This research has been conducted to find effective alternatives to growth promoting antimicrobials in young pigs. It was hypothesized in the project that oligofructose (OF, DP=2-4, 95% of OF) and multiple-strain probiotics may beneficially alter gut ecosystem and health. This may be beneficial for growth of young pigs.

The following experiments were done.

Experiment 1. The effects of OF on growth, small intestinal morphology and pH, volatile fatty acids, ammonia concentrations in the digesta of large intestine of weaned pigs were investigated (Chapter 3).

Experiment 2. The effects of dietary OF on digesta viscosity, digestibility and mucosal enzyme activities are described in Chapter 4.

Experiment 3. In this study we investigated the effects of antibiotic-free diets supplemented with OF, multi-strain probiotics or synbiotics on hematological traits, nutrient digestibilities, ammonia concentrations for weaned pigs growth were investigated (Chapter 5).

Experiment 4. The effects of feeding an antibiotic-free creep feed supplemented with either oligofructose, probiotics or synbiotics on changes of microflora, gut structure, VFA, immune responses 1 day after weaning were investigated (Chapter 6).

Experiment 5. An *in vitro* study on the differences in *in vitro* fermentation characteristics of faeces from weaned and unweaned pigs as inoculum and different sources (degree of polymerization) of oligofructose, chicory and pig feed

ingredients as substrates were determined. Fermentation kinetics were assessed using the cumulative gas production technique. End products (VFA and NH₃) were also measured to identify their potentially beneficial effects on gastrointestinal tract health (Chapter 7).

The literature review in Chapter 2 discusses the application of inulin-type fructans in animal feed and pet food. In addition, the effect of inulin-type fructans on the performance and health of various animals (pigs, poultry, calves and pets) was discussed. In Chapter 3 the weaning and its effect on growth performance, gut morphology and gut microflora in pigs was discussed. This review shows that the morphology of small intestine and the growth of young pigs are influenced by nutrition. Information on the various effects of prebiotics and probiotics or synbiotics of young pigs is reviewed. In Chapter 3, the first part of the review describes the growth of the young pigs. The growth of piglets (weaning weight and subsequent growth), postweaning growth check, postweaning stressors such as nutritional, environmental, and microbiological are described. In addition, the adaptation of integrity of the small intestine of young pig is described. The second part of the review focused on the effects of OF as prebiotics. Generally the term prebiotic can be described as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon, and thus improves host health. OF are known to stimulate beneficial bacteria like bifidobacteria. The definition, candidate and schematic mode of actions OF were described. The third part of the review describes the potential of probiotics in pig feeds. The term probiotics is used to describe living microbial feed supplements which beneficially affect the host animal by improving its microbial balance. The modes of action in probiotics are described. Moreover, effects of probiotics on the gastrointestinal microflora and growth were also discussed. The fourth part of the review describes the effects of synbiotics. It was concluded that inulin-type fructans can support animal performance and health by affecting nutrient digestion, gut microflora and gut morphology although results are varying depending on composition of the basal diet, inclusion level, type of fructan, adaptation period and experimental hygienic conditions. For a prolonged saccharolytic activity throughout the large intestine, more slowly fermentable source of carbohydrate or a combination of relatively rapid and slowly fermentable OF on growth and health effects may be needed. For more consistent beneficial effects of probiotics on growth and gut ecosystem in young pigs, the selection criteria for probiotics must be applied. The concept of synbiotics is the most promising approach for enhancing beneficial bacteria in the gut. More research regarding various combinations of multiple probiotics strain and prebiotics are needed to verify possible modes of action and find

optimal combination of synbiotics for newly weaned piglets.

To elucidate the possible mechanisms of OF, effects of inclusion of OF in the diet on digestive enzyme activity, ileal digestibility of protein and viscosity of weaned pigs were determined (Chapter 4). Animals were individually housed and fed either a control diet, a diet with 0.25% OF, or a diet with 3% OF diet for 21 d after weaning. At the end of the experiment, the digesta of the small intestine (duodenum, jejunum and ileum) and large intestine (caecum, proximal and distal colon) were collected for determination of disaccharidase activity, viscosity and digestibility coefficients. Full and empty weights of the gastrointestinal tract were determined. Feed intake and empty body weight gain of young pigs fed with OF were numerically improved. OF tended to increase the weight of the small and large intestines. Apparent ileal digestibility of protein (control:68.4, OF 0.25:75.8 and OF 3%:72.5) was increased ($P < 0.005$). OF had no effect on viscosity of the digesta in the small intestine. The disaccharidase activity and the mucosal protein content in the small intestine were not affected by OF addition. OF increased the full weight but not the empty weight of the large intestine and increased the empty weight and length of small intestine. It was concluded that dietary OF may increase empty growth weight gain and ileal digestibility of protein. OF did not affect disaccharidase activity, nor viscosity of digesta in the small intestine.

The effects including fructo-oligosaccharides to the diet of weaning piglet on morphometric change of small intestine, VFA, ammonia concentration, and on pH, and scour scores of gastrointestinal tract contents and feces were investigated (Chapter 5). Pigs were housed individually and fed either a control diet, a control diet with 0.25% OF, or a 3% OF diet during 21 d after weaning. At the end of the experiment, digesta of the small intestine (duodenum, jejunum and ileum) and large intestine (caecum, proximal and distal colon) were collected for determination of pH, VFA, ammonia concentration, and/or for morphometric measurements. The villous height was higher at all measured sites of the small intestine in pigs fed OF diets compared with control animal. The pH in the digesta of the proximal colon (control: 5.94, OF 0.25:5.91 and OF 3%:5.53) was lower ($P < 0.001$) when pigs had been fed a diet with 3% OF compared to other treatments. These results showed that dietary OF may lower pH and ammonia concentration in the gastrointestinal tract of weaned pigs.

From the results of studies in Chapter 4 and 5, an experiment was designed to study the effects of supplementation of oligofructose (OF) and probiotics alone or in combination (OF and probiotics) on feed intake and nutrient digestibility in weaned

pigs (Chapter 6). Hematological characteristics, the fecal microbial population and ammonia content were measured. Sixty pigs weaned at 25 days of age (average body weight, 6.4kg) were used in a 21 d trial. The treatments consisted of a control (antibiotic-free corn-soy diet), an OF diet (0.25% OF), a M-probiotics diets (0.2% multi-strain probiotics), a synbiotics diet (0.25% OF and 0.2% multi-strain probiotics), and a T-probiotics diet (0.2% two-strain probiotics). All supplementations significantly increased body weight gain and numerically increased feed intake compared to the control. The apparent digestibility of dry matter, crude protein and ash as well as the apparent absorption of calcium and phosphorus in all treatment groups were higher than those of the control animals ($P<0.05$). Platelet count in blood was higher in synbiotics fed pigs than with OF ($P<0.05$). The number of fecal coliforms was decreased in all treatment animals compared to control animals ($P<0.05$). The number of microbial populations was significantly altered by treatments. The synbiotics diet significantly increased the number of fecal anaerobic lactobacilli compared to the control diet. Fecal ammonia concentrations were reduced significantly in all treatments compared to the control ($P<0.05$). In general the supplementation of the synbiotics (a combination of OF and probiotics) had the greatest effect on nutrient digestibility, fecal microflora and body weight gain. However, the effect of synbiotics was smaller than the sum of the effects of OF and probiotics alone. These results showed that supplementation of pre- and probiotics or synbiotics (combining OF and multi-strain probiotics) to an antibiotic-free diet (negative control) stimulate beneficial bacteria in the gastrointestinal tract and may also have beneficial effects on nutrient digestibility and growth performance in weaned pigs.

The objective of the study reported in Chapter 7 was to determine whether feeding an antibiotic-free creep feed supplemented with either oligofructose, probiotics or synbiotics influences growth performance, composition of the gut microflora, gut morphology and immune responses in suckling piglets. Two hundred piglets from twenty sows were used. Ten piglets from each sow were each randomly assigned to one of 4 treatments. The treatments consisted of a control (antibiotic-free diet), 0.2% oligofructose, 0.3% probiotics or synbiotics (mixture of 0.2% OF + 0.3% probiotics). Piglets were offered the diet (dry, mash) ad libitum from 7 days after birth until 1 day after weaning (21days old). At 1 day after weaning, blood samples were collected from the jugular vein to determine the immune response. Digesta samples of the ileum and colon were collected from 5 animals per treatment to determine the microbial composition. Tissue segments from the duodenum and ileum were collected for morphometric measurements of the small intestine of the piglets. The average daily weight gain was significantly higher ($P<0.05$) for piglets fed the OF or synbiotics

diet compared with the pigs fed the control diet. The hematological traits, the concentrations of lymphocyte and neutrophils in whole blood were not affected by the diet. Piglets fed the OF, probiotics or synbiotics diet significantly ($P < 0.05$) decreased the population of *E. coli* in the colon. OF, probiotics or synbiotics significantly ($P < 0.05$) increased the population of bifidobacteria in the ileum compared to the control. In the colon, the probiotics and synbiotics significantly ($P < 0.05$) increased the number of bifidobacteria compared with the control diet. The results of this experiment showed that supplementation of oligofructose or synbiotics to an antibiotic-free creep feed during the preweaning period affected gut microbial population and performance of piglets. It was concluded that dietary OF may increase empty growth weight gain and ileal digestibility of protein. OF did not affect disaccharidase activity and viscosity of digesta in the small intestine, but villous height was numerically higher. Dietary OF may lower pH and ammonia concentration in the gastrointestinal tract of weaned pigs. Supplementation of pre- and probiotics or synbiotics (combining OF and multi-strain probiotics) to an antibiotic-free diet (negative control) may stimulate beneficial bacteria in the gastrointestinal tract and may also have beneficial effects on nutrient digestibility and growth performance in weaned pigs. Supplementation of oligofructose or synbiotics to an antibiotic-free creep feed during the preweaning period affected gut microbial population and performance of piglets.

In chapter 8, an *in vitro* experiment was conducted to evaluate the differences in microbial activity of six different substrates with five different fecal inocula from weaned and unweaned pigs. The substrates tested were a negative control diet, corn, soybean meal, oligofructose, grinded chicory roots and a mixture (60% chicory pulp and 40% OF). The inocula were feces collected from weaned pigs fed a negative control diet, the control diet supplemented with either OF, a mixture (60% chicory pulp and 40% OF), grinded chicory roots or an antibiotic (positive control). The cumulative gas production measured fermentation kinetics, and end products such as total gas production, ammonia and volatile fatty acids were also determined. There were significant differences in the fermentation characteristics both between substrates and between inocula. A significant interaction between substrate and inocula was also found for most characteristics. Cumulative gas production curves showed that substrates generally more affected the fermentation kinetics than inocula. Inoculum from weaned pigs had lower gas production compared to inoculum from unweaned piglets. Raftifeed IPE (a blend of high and low polymer inulin) showed the highest fermentation kinetics and the lowest NH_3 , pH and OM loss between substrate. It was concluded that the different fermentation kinetics (T_{MAX} , R_{MAX}) from different substrates in the present study suggest that microbial flora is affected differently by different substrates. Inocula from pigs fed diets containing inulin and oligofructose

produced more gas at a faster rate than that of pigs fed a control diet or feed grain substrates. OF may reduce bacterial proteolytic activity at the actual site of fermentation. Both combined oligofructose substrates, OF, and MIX showed the highest fermentation kinetics and total VFA compared with other substrates. This result may help to select a combination of substrates which will be able to support prolonged fermentation throughout the gut. A combination of slowly fermentable and rapidly fermentable OF may be more beneficial by supporting prolonged fermentation in more parts of the small and large intestine of pigs compared to either slow or rapid fermentable components alone. However, this needs to be evaluated in *in vivo* experiments.

Overall, it was concluded that synbiotics, a combination of multi-strain probiotics and oligofructose can have some benefit with regard to performance. We also concluded that the synbiotics may have some synergistic effects on stimulating beneficial microflora and improving gut ecosystem compare to supplementing oligofructose or probiotics alone. A combination of high- and low polymer inulin will have a more beneficial effect on gastrointestinal ecosystem and health than using either high- or low polymer inulin alone. A synbiotics approach could be used as potential alternative to antibiotic growth promoter for young pigs.

SAMENVATTING

In de meeste landen worden varkens gespeend op een leeftijd van 18-25 dagen. Tijdens het spenen worden de varkens blootgesteld aan verschillende stressoren, zoals voedings- en omgevingsstressoren, maar ook sociale en microbiologische stressoren. Dit resulteert in villusatrofie in de dunne darm, een verminderde immuunrespons, een hogere frequentie van diarree, en dientengevolge een groeidepressie. Om dergelijke suboptimale omstandigheden na spenen te voorkomen worden in sommige landen nog steeds subtherapeutische antibiotica gebruikt in de varkenshouderij.

Er is echter een groeiende bezorgdheid over resistentie van pathogene darmmicroflora tegen antibiotica. Dit kan gevolgen hebben voor antibiotica die therapeutisch worden gebruikt voor zowel mens als dier. Deze potentieel verstrekkende consequenties hebben geleid tot een totaalverbod op het gebruik van de antimicrobiële groeibevorderaars in de EU vanaf 2006. Prebiotica en probiotica of een combinatie van beide (synbiotica) kunnen een mogelijk alternatief vormen voor antimicrobiële groeibevorderaars. Het concept van synbiotica is een benadering om de groei van goede bacteriën in de darm te stimuleren door dagelijks zowel microben als voedingsstoffen voor deze microben te verstrekken.

Het doel van de vijf in dit proefschrift beschreven experimenten was het evalueren van de effecten van prebiotica, een multi-strain probiotica (dus probiotica die uit meerdere soorten microben bestaat) en synbiotica op het ecosysteem van het maagdarmkanaal, de gezondheid en groei van varkens rondom spenen.

Dit onderzoek werd uitgevoerd om effectieve alternatieven te vinden voor antimicrobiële groeibevorderaars in jonge biggen. Het werd gehypothetiseerd dat oligofruuctose (OF, DP=2-4, 95% OF) en multi-strain probiotica goed is voor het ecosysteem in de darm en de diergezondheid positief kan beïnvloeden. Dit kan bevorderend zijn voor de groei van jonge biggen.

De volgende experimenten werden uitgevoerd:

Experiment 1. De effecten van OF op groei, morfologie van de dunne darm, pH en concentraties van vluchtige vetzuren en ammoniak in de digesta van de dikke darm werden onderzocht (Hoofdstuk 3).

Experiment 2. De effecten van OF in de voeding op de viscositeit van digesta, de verteerbaarheid en enzymactiviteiten in de mucosa werden beschreven (Hoofdstuk 4).

Experiment 3. In deze studie onderzochten we de effecten van antibioticavrije voeders gesupplementeerd met OF, multi-strain probiotica of synbiotica op hematologische kenmerken, verteerbaarheden en ammoniakconcentraties in gespeende biggen (Hoofdstuk 5).

Experiment 4. Een antibioticavrij voer werd gesupplementeerd met oligofruuctose,

probiotica of synbiotica. Effecten op microflora, darmstructuur, concentraties van vluchtige vetzuren en de immuunrespons één dag na spenen werden onderzocht (Hoofdstuk 6).

Experiment 5. Een *in vitro* studie met feces van gespeende en zogende biggen als inoculum en verschillende soorten (mate van polymerisatie) oligofruuctose, chichorei en varkensvoeringrediënten als substraat werd uitgevoerd. Fermentatiekarakteristieken werden bepaald met de cumulatieve gasproductietechniek. Tevens werden eindproducten (vluchtige vetzuren en ammoniak) gemeten om de mogelijke effecten op darmgezondheid te kunnen beoordelen.

In het literatuuroverzicht (Hoofdstuk 2) werd de toepassing van inulineachtige fructanen in voeding voor landbouwhuisdieren en huisdieren bediscussieerd. Ook werd het effect van inulineachtige fructanen op de technische resultaten en gezondheid van verschillende diersoorten (varkens, pluimvee, kalveren en huisdieren) beschreven. In Hoofdstuk 3 werd het effect van spenen op de groei, darmmorfologie en darmflora in varkens bediscussieerd. Uit de resultaten blijkt dat de morfologie van de dunne darm en mogelijk ook de groei van biggen wordt beïnvloed door voeding van de bestudeerde componenten. Informatie over de verschillende soorten effecten van prebiotica, probiotica of synbiotica in de darm wordt behandeld in het eerste deel van het literatuuroverzicht. In Hoofdstuk 3, wordt de groei van jonge biggen beschreven. Deze groei (speengewicht en de daaropvolgende groei) en stressoren rondom spenen, zoals een veranderende voeding, omgeving en microbiologie, zoals in de literatuur aangegeven zijn in dit hoofdstuk samengevat. Ook wordt ingegaan op de aanpassing van de integriteit van de dunne darm. Het tweede deel van het literatuuroverzicht behandelt de effecten van OF wanneer dit als prebiotica fungeert. In het algemeen kunnen prebiotica worden omschreven als onverteerbare ingrediënten die een positief effect hebben op de gastheer door selectieve stimulatie van de groei en/of activiteit van een beperkt aantal bacteriën in de dikke darm en die daardoor de darmgezondheid verbeteren. OF stimuleert positieve bacteriën zoals bifidobacteriën. Het derde deel van het overzicht beschrijft de mogelijkheden van probiotica in varkensvoerders. De term probiotica wordt gebruikt voor levende microbiële voedersupplementen met een positief effect op de microbiële balans in het maagdarmkanaal van de gastheer. De werkingsmechanismen van probiotica worden beschreven. Tevens worden effecten van probiotica op de microflora in het maagdarmkanaal en groei bediscussieerd. Het vierde deel van het overzicht beschrijft de effecten van synbiotica. Het werd geconcludeerd dat inulineachtige fructanen de gezondheid van varkens en technische resultaten mogelijk kunnen bevorderen door de verteerbaarheid,

darmmicroflora en –morfologie te beïnvloeden. De resultaten verschillen echter afhankelijk van de samenstelling van het voer, de dosering van inulineachtige fructanen, het soort fructanen, de aanpassingsperiode en de experimentele hygiënische omstandigheden. Een combinatie van relatief snel en langzaam fermenteerbare OF zou de saccharolytische activiteit beter over de darm kunnen verspreiden en resulteren in een betere darmgezondheid en misschien een hogere groei. Voor effecten van probiotica op groei en het ecosystem in de darm van jonge biggen is de literatuur niet eenduidig. Het concept van synbiotica is wellicht meest veelbelovende benadering om de groei van goede bacteriën in de darm te promoten. Nader onderzoek naar de verschillende combinaties van multi-strain probiotica en prebiotica zijn nodig om de werkingsmechanismen te identificeren en een optimale combinatie synbiotica voor pasgespeende biggen te formuleren.

Effecten van opname van OF in het voer op activiteit van verteringsenzymen, ileale eiwitverteerbaarheid en viscositeit werden onderzocht om mogelijke mechanismen te kunnen ophelderen (Hoofdstuk 4). Individueel gehuisveste varkens kregen een controlevoer, een voer met 0.25% OF of een voer met 3% OF verstrekt gedurende 21 dagen na spenen. Aan het einde van de proef werden de digesta uit de dunne darm (duodenum, jejunum en ileum) en dike darm (caecum, proximal and distal colon) verzameld voor de bepaling van disaccharidase-activiteit, viscositeit en verteerbaarheidscoëfficiënten. Het maagdarmkanaal werd vol en leeg gewogen. Voeropname en groei (gecorrigeerd voor maagdarminhoud) van biggen met OF in het voer waren numeriek hoger dan van controledieren. Er was een tendens voor een hoger gewicht van de dunne en dikke darm in varkens met OF in de voeding. De schijnbare ileale eiwitverteerbaarheid was hoger ($P < 0.005$) wanneer OF in het voer was verwerkt (controle:68.4%, OF 0.25%:75.8% and OF 3%:72.5%). OF had geen effect op de viscositeit van de digesta in de dunne darm. De disaccharidase-activiteit en het eiwitgehalte van de darmwand in de dunne darm werden niet beïnvloed door toevoeging van OF aan het voer. Toevoeging van OF resulteerde in een toename van het volle gewicht van de dikke darm en het leeggewicht van de dunne darm. Er werd geconcludeerd dat toevoeging van OF aan varkensvoer de voeropname en dus groei (gecorrigeerd voor maagdarminhoud) en ileale eiwitverteerbaarheid kan verhogen. OF had geen effect op disaccharidase-activiteit en viscositeit van digesta.

De effecten van toevoeging van fructo-oligosacchariden aan de voeding van gespeende varkens op morfologische veranderingen van de dunne darm, concentraties van vluchtige vetzuren en ammoniak, pH van de maagdarminhoud en diarrhee score van feces werden onderzocht (Hoofdstuk 5). Individueel gehuisveste varkens kregen een controlevoer, een voer met 0.25% OF of een voer met 3% OF verstrekt gedurende 21 dagen na spenen. Aan het einde van de proef werden de

digesta uit de dunne darm (duodenum, jejunum en ileum) en dikke darm (caecum, proximale en distale colon) verzameld voor de bepaling van pH, concentraties van vluchtige vetzuren en ammoniak en morfologische metingen.

Varkens met OF in het rantsoen hadden op verschillende meetpunten in de dunne darm hogere villi dan controledieren. De pH van de digesta in het proximale deel van de dikke darm was lager ($P < 0.001$) voor varkens met 3% OF dan voor de andere behandelingen (controle: 5.94, OF 0.25%:5.91 en OF 3%:5.53). Deze resultaten tonen aan dat toevoeging van OF kan resulteren in een lagere pH en ammoniakconcentratie in het maagdarmkanaal van gespeende varkens.

Naar aanleiding van de uitkomsten van de studies uit Hoofdstuk 4 en 5 werd een experiment ontworpen om de effecten van supplementatie van OF en probiotica apart of een combinatie van beide (synbiotica) op voeropname en verteerbaarheid in gespeende varkens te bestuderen (Hoofdstuk 6). Hematologische kenmerken, de fecale microbiële populatie en ammoniakconcentratie werden gemeten. Zestig biggen werden gespeend op een leeftijd van 25 dagen (gemiddeld lichaamsgewicht, 6.4kg) en gebruikt in een proef gedurende 21 dagen. De behandelingen bestonden uit een controlevoer (antibioticavrij mais-sojavoer), een OF-voer (0.25% OF), een M-probioticavoer (0.2% multi-strain probiotica), een synbioticavoer (0.25% OF and 0.2% multi-strain probiotics) en een T-probioticavoer (0.2% twee-strain probiotica). Alle supplementen resulteerden in een significante toename van groei en een numeriek hogere voeropname vergeleken met de controlebehandeling. De schijnbare verteerbaarheid van drogestof, ruw eiwit, as en ook de schijnbare absorptie van calcium en fosfor waren hoger voor alle supplementatiegroepen dan in de controlegroep ($P < 0.05$).

Het aantal bloedplaatjes was hoger in bloed van varkens met synbioticasupplementatie dan in dat van varkens met OF in het voer ($P < 0.05$). Het aantal fecale coliforme bacteriën was lager voor alle gesupplementeerde dieren dan voor de controledieren ($P < 0.05$). Het aantal microbiële populaties was significant beïnvloed door de behandelingen. Het aantal fecale anaerobe lactobacilli was hoger bij varkens met het synbioticavoer dan die met het controlevoer. Fecale ammoniakconcentraties waren lager ($P < 0.05$) in alle behandelingen dan in de controledieren. Over het algemeen had supplementatie met synbiotica (een combinatie van OF en probiotica) het grootste effect op verteerbaarheid, fecale microflora en groei. Het effect van synbiotica was echter kleiner dan de som van de effecten van OF en probiotica afzonderlijk. Deze resultaten tonen aan dat supplementatie van pre- en probiotica of synbiotica (combinatie van OF en multi-strain probiotica) bij een antibioticavrij voer (negatieve controle) de groei van positieve bacteriën in het maagdarmkanaal stimuleren. Ook kunnen dergelijke

toevoegingen de verteerbaarheid en groei in gespeende biggen verhogen.

De doelstelling van de studie in Hoofdstuk 7 was het vaststellen of supplementatie van een antibioticavrij voer met oligofruuctose, probiotica of synbiotica de groei, samenstelling van darmmicroflora, darmmorfologie en immuunrespons in zogende biggen beïnvloedt. Tweehonderd biggen van twintig zeugen werden gebruikt. Tien biggen van elke zeug werden willekeurig toegewezen aan één van de vier behandelingen. De behandelingen bestonden uit een controlevoer (antibioticavrij), een voer met 0.2% oligofruuctose, een voer met 0.3% probiotica en een voer met synbiotica (0.2% OF + 0.3% probiotica). Het voer (droog, meelvorm) werd ad libitum verstrekt vanaf 7 dagen na geboorte tot 1 dag na spenen (totaal 21 dagen). Op dag 1 na spenen werden bloedmonsters genomen via de halsader voor de bepaling van de immuunrespons. Digesta uit het ileum en de dikke darm werden verzameld van vijf dieren per behandeling voor bepaling van de microbiële samenstelling. Weefselmonsters van het duodenum en het ileum werden genomen voor morphologische metingen van de dunne darm van de biggen. De gemiddelde dagelijkse groei was hoger ($P < 0.05$) voor biggen met OF of synbiotica in het voer dan voor biggen op het controlevoer. Hematologische kenmerken en concentraties van lymfocyten en neutrofielen in bloed waren niet verschillend tussen de behandelingen. Biggen met OF, probiotica of synbiotica in de voeding hadden een kleinere ($P < 0.05$) *E coli.*-populatie en hogere ($P < 0.05$) populatie bifidobacteriën in het ileum dan de controledieren. In de dikke darm was het aantal bifidobacteriën hoger ($P < 0.05$) voor biggen met probiotica en synbiotica in het voer dan voor de biggen op de controlebehandeling. Uit dit experiment blijkt dat supplementatie van oligofruuctose of synbiotica bij een antibioticavrij voer tijdens de zoogperiode de microbiële populatie in de darm en de prestaties van de biggen kan beïnvloeden. Het werd geconcludeerd dat toevoeging van OF aan het voer de groei (gecorrigeerd voor maagdarminhoud) en ileale eiwitverterbaarheid kan verhogen. OF had geen effect op disaccharidase-activiteit en viscositeit van digesta in de dunne darm, maar de villushoogte was numeriek hoger bij supplementatie van OF. Verstrekking van OF via de voeding kan de pH en ammoniakconcentratie in het maagdarmkanaal van gespeende biggen verlagen. Deze resultaten tonen aan dat supplementatie van pre- en probiotica of synbiotica (combinatie van OF en multi-strain probiotica) bij een antibioticavrij voer (negatieve controle) de groei van positieve bacteriën in het maagdarmkanaal stimuleren. Ook kunnen dergelijke toevoegingen de verteerbaarheid en groei in gespeende biggen verhogen. Supplementatie van oligofruuctose of synbiotica bij een antibioticavrij voer tijdens de zoogperiode beïnvloedde de microbiële populatie in de darm en de groei van biggen.

In Hoofdstuk 8 werd een *in vitro* experiment uitgevoerd om de verschillen in microbiële activiteit van zes verschillende substraten met vijf verschillend inocula van gespeende en zogende biggen te bestuderen. De geteste substraten waren een negatief controlevoer, mais, sojameel, oligofruuctose, gemalen chichoreiwortelen en een mengsel van 60% chichoreipulp en 40% OF. De geteste inocula waren feces van gespeende varkens op een negatief controlevoer, het controlevoer met OF, het controlevoer met gemalen chichoreiwortelen, het controlevoer met een mengsel van 60% chichoreipulp en 40% OF of het controlevoer met een antibiotica (positieve controle). De kinetiek van het fermentatieproces en de fermentatie-eindproducten, zoals totale gasproductie, ammoniak en vluchtige vetzuren, werden bepaald met de cumulatieve gasproductietechniek. Er waren significante verschillen in fermentatiekarakteristieken tussen zowel substraten als inocula. Een significante interactie tussen substraat en inoculum werd gevonden voor de meeste karakteristieken. De cumulatieve gasproductiecurves toonden aan dat substraten in het algemeen een groter effect hadden op de fermentatiekinetiek dan de inocula. Het inoculum van gespeende varkens resulteerde in een lagere gasproductie dan het inoculum van zogende biggen. Raftifeed IPE (een mengsel van een hoog en laag polymer inuline) gaf het hoogste niveau van fermentatie en de laagste ammoniakconcentraties, pH en verliezen van organische stof tussen substraten. Het werd geconcludeerd dat de verschillende fermentatiekinetiek (T_{MAX} , R_{MAX}) tussen verschillende substraten in deze studie aangeeft dat de microbiële flora verschillend wordt beïnvloed door verschillende substraten. Inocula van biggen met inuline en oligofruuctose in de voeding produceerden meer gas, en met een hogere snelheid, dan die van biggen op het controlerantsoen of graansubstraten in het voer. OF kan de bacteriële proteolytische activiteit op de plek van fermentatie verminderen. Beide voeders met OF, alleen of als mengsel, resulteerden in de hoogste gasproductie en de hoogste productie van vluchtige vetzuren vergeleken met andere substraten. Deze bevindingen kunnen relevant zijn voor het selecteren van een geschikte combinatie van substraten om fermentatie over het gehele darmkanaal te stimuleren. Een combinatie van langzaam en snel fermenteerde OF kan nuttiger zijn dan gebruik van slechts een langzame of snelle fermenteerbare component, omdat fermentatie in meerdere delen van het darmkanaal kan worden gestimuleerd. Echter, dit moet worden geëvalueerd in *in vivo* experimenten.

Samenvattend, er werd geconcludeerd dat synbiotica, een combinatie van multi strain probiotica en oligofruuctose de technische resultaten in positieve zin kunnen beïnvloeden. Wij concludeerden ook dat synbiotica effecten kunnen hebben op stimulatie van positieve microflora en verbetering van het ecosysteem in de darm vergeleken met oligofruuctose of probiotica afzonderlijk. Een combinatie van een hoog en laag polymeer inuline zal een sterker positief effect hebben op het ecosysteem en

Samenvatting

de gezondheid dan één van beide polymeren afzonderlijk. De benadering van het gebruik van synbiotica kan worden gebruikt als mogelijk alternatief voor antimicrobiële groeibevorderaars voor jong.

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CURRICULUM VITAE

I was born on February 7, 1959 in Iksan, Jeonbuk, Korea. In February 1984, I obtained a BSc degree in Animal Science, Jeonbuk National University. During the undergraduate student period, I served in the army service for 27 months which is compulsory for young men in Korea. Shortly after graduation, I was awarded a scholarship from the Danish Agricultural Cooperation Federation which allowed me to take a dairy management course for four months in Odense Agricultural University, Denmark. After returning to Korea, I decided to study a Master degree in swine nutrition which is one of the most important fields of livestock industry in Korea. After obtaining a MSc degree in Animal Science (Animal Nutrition) at the Jeonbuk National University in 1988, I started to work for the largest food and feed company, CJ Corporation in Korea as a manager. After returning to Korea from Australia, I worked for Daesang Feed Co. and Samyang Corporation as swine product manager for 5 years. In 1995, I was a recipient of the Overseas Postgraduate Research Scholarship by the University of Western Australia which funded by Australian Government for his PhD study. After three years of my research, I decided to transfer and continue my research project on probiotics at Wageningen University (Animal Nutrition Group) under supervision of Professor Martin Verstegen. I fulfilled the requirement of PhD degree in Animal Nutrition and defended his thesis on April 29, 2005 at Wageningen University, The Netherlands.

Notes

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