EFFECTS OF NON-DIGESTIBLE OLIGOSACCHARIDES IN YOUNG PIG DIETS

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Stellingen

1. Fructooligosachariden en transgalactooligosachariden hebben een verwaarloosbaar effect op de vertering van nutriënten door jonge biggen.
dit proefschrift

2. Fructooligosachariden en transgalactooligosachariden verschillen in fermentatiesnelheid. Zelfs bij een zeer geringe opname in het rantsoen van jonge biggen leidt dit tot verschillen in de ecologie van de darminhoud.
dit proefschrift

3. De opname van fructooligosachariden en transgalactooligosachariden in het rantsoen van jonge biggen leidt tot een verhoogde sacharolytische activiteit van de microflora in de dunne darm. Wanneer deze activiteit niet gehandhaaft kan blijven, kan dit leiden tot een verhoogde proteolytische activiteit van de microflora in de dikke darm.
dit proefschrift

4. Fructooligosachariden kunnen avilamycine niet vervangen als groeibevoorderaar in speenvoeders.
dit proefschrift

5. Zonder een negatieve controle kunnen er geen conclusies getrokken worden uit vergelijkingen tussen alternatieve en traditionele voederbespaarders.

6. In de Westerse wereld is ecologisch verantwoord vlees is niet te duur; het gangbare vlees is te goedkoop.

7. Het feit dat de humane microflora tien keer zo groot is qua aantal cellen als het menselijk lichaam zelf geeft al aan dat we de relatie met deze microflora goed moeten onderhouden. Goede buren zijn immers beter dan verre vrienden.

8. Elke dierwetenschapper dient voor zichzelf een grens te leggen en zich aan deze grens te houden inzake het aandoen van ongerief aan proefdieren.

9. Hoewel de engelse benaming anders doet vermoeden, is de 'small intestine' (dunne darm) langer dan de 'large intestine' (dikke darm).

10. De toegankelijkheid van wetenschappelijke informatie is gebaat bij het inbinden van wetenschappelijke tijdschriften in dunnere volumes binnen een jaargang.

11. Het veranderde groepsgevoel van de vakgroep Veevoeding na de verhuizing van de Haagsteeg naar Zodiac is een typisch geval van genotype-milieu interactie.

12. Als je in de Schotse hooglanden de bergtoppen niet kunt zien, dan regent het. Zie je ze wel, dan gaat het regenen.

Jos Houdijk
Effects of non-digestible oligosaccharides in young pig diets
Wageningen, 22 december 1998

EFFECTS OF NON-DIGESTIBLE OLIGOSACCHARIDES IN YOUNG PIG DIETS

Jos Houdijk

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 22 december 1998
des namiddags te vier uur in de Aula.

Voorwoord

Voor u ligt een proefschrift over niet-verteerbare suikers in biggenvoeders. Waarom iets dat niet verteerbaar is toch boeidend kan zijn, moet u maar in dit boekje lezen. Er blijken veel overeenkomsten te zijn tussen dit onderzoek en het vervoeren van hooi. Het verwerven (hooi laden), verwerken (hooi rijden) en wegschrijven (hooi lossen) van de gegevens heb ik echter niet alleen gedaan.

Er moest eerst gezaaid worden. Het maatschap Seerp Tamminga / Martin Verstegen was al ver voor het seizoen bezig een oogstplan op te stellen. Marlou Bosch was hier intensief bij betrokken, en tijdens de oogst werd Barbara Williams als tweede co-promotor aan het promotieteam toegevoegd. Allemaal hartelijk bedankt voor de uitstekende begeleiding. Zonder jullie was ik waarschijnlijk nog steeds niet met het laatste vrachtje bezig.

Drie andere oogsten werden binnen gehaald door Katrien van Laere (Levensmiddelen Chemie), Ralf Hartemink (Levensmiddelen Microbiologie), en Martine Alles (Humane Voeding). Katrien, Ralf, en Martine, zeer bedankt voor jullie hulp. Het plezier met jullie samen gewerkt te hebben valt niet in woorden uit te drukken. Het einde van de eerste oogst heb ik helaas niet meegemaakt (betonnen balk - Jos Houdijk: 1-0). Gelukkig was Erik Berenpas een uitstekende bijrijder. Meerdere bijrijders hebben geholpen bij de andere oogsten. Hugo van der Linden, Hylke Knoop, Franck Olivier, Jeroen Hoving, en Roel Keursten: bedankt.

Er hebben nog veel meer mensen geholpen met laden. Op het gevaar af dat ik iemand over het hoofd zie (en hiervoor ter verantwoording kan worden geroepen) volgt hier een bloemlezing. Piet Roeleveld en Karel Siebers, bedankt voor de voeders; André Jansen, Ries Verkerk en Sjaak Tijnagel (Zodiac), Dick van Kleef, Casper Deuring (ILOB), en het stalpersoneel van het PV (Rosmalen), bedankt voor het verzorgen van de biggen; Tamme Zandstra en Peter van de Togt, bedankt voor alle technische ondersteuning; Piet van Leeuwen, bedankt voor de operaties en dissekties; Truus Post, Jane-Martine Muijlaart, Marianne van 't End, Meijke Booij, Huug Boer, Pablo Chilibroste, Harmen van Laar, Margaret Bosveld, Yvonne Hogenes, Dick Bongers en Henri Leuvenink, bedankt voor de hulp bij en het uitvoeren van talloze gasmetingen en analyses; Gisabeth Binnendijk, bedankt voor de diarrhee scores; Eric Houdijk, Henk van Wijk, Gerjan Klok, Eric-Jan de Jong en Hung Kee Moon, bedankt voor de hulp bij de dissectie. Tot slot, iedereen bedankt die 's nachts tijdens de gasmetingen gewaakt heeft (jullie hebben in ieder geval achterstallig werk kunnen inhalen).

Tijdens het vervoeren van de vrachten hooi had ik de tijd om over de lading na te denken. Er werden regelmatig hypotheses bedacht die evenzo regelmatig werden verworpen voor dat het lossen kon beginnen. Verschillende visies werden regelmatig 'uitgepraat' voor een volgekalkt 'used-to-be-white-board'. Dit begon in het Basement Intelligence Centre met Jacob Goelema en Menno Thomas. In de nieuwe vleugel van Zodiac werd dit voortgezet en werden Harmen van Laar en Carina Steendam er ook bij betrokken (evenals argeloze voorbijgangers die nieuwschierig om de hoek keken omdat ze dachten dat er ruzie gaande was). Dankzij deze discussies en de koffie is uiteindelijk alle vracht op het juiste adres gelost. Allemaal ontzettend bedankt voor de goede sfeer en het wetenschappelijk verbaal verweer.

Tijdens het lossen van de vracht heb ik steeds dankbaar gebruik gemaakt van de aanwijzingen van het promotie team. Hoewel ik dacht dat het schrijven in het engels steeds beter ging, wist Barbara steeds weer aanwijzingen te geven waardoor de tekst uiteindelijk begrijpelijk werd. Carola van der Peet-Schwering (PV) was betrokken bij de laatste oogst. Carola, je opmerkingen over proefplannen en conceptartikelen waren altijd bijzonder raak, bedankt hiervoor. I would also like to thank Alan Sutton for his help. Alan, your suggestions convinced this American farmer, J.A. Smith, that this load of hay was the best he'd ever get.

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Vroeger wilde ik altijd boer worden. Mijn ouders meenden echter dat ik eerst maar eens door moest leren. 'Boer worden kun je altijd nog wel' was hun motto. Jan en Suze Houdijk, bedankt voor alles. Een speciaal woord van dank voor oma Houdijk. Oma, bedankt dat je de familie er altijd aan herinnerde dat ik niet meer op school zat, maar dat ik, als AIO, een echte baan had.

Lieve Ellen. Als het laden en lossen weer eens tegen zat, dan wist jij me altijd weer op te beuren. Je hebt mij geleerd dat er meer is in het leven dan alleen een baan. Bedankt voor alles, met name voor de hulp bij de laatste loodjes. Met jou wil ik heel erg oud worden.

Ellen, Jan en Suze Houdijk, en Oma Houdijk, aan jullie draag ik graag dit proefschrift op!

Jos

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Some carbohydrates in young pig diets escape enzymatic digestion and form substrates for the gastrointestinal microflora. These include the non-digestible oligosaccharides (**NDO**), which are found in e.g. cereals and legumes. Certain NDO may selectively stimulate the growth and/or activity of one or a limited number of favourable bacterial species, and as such beneficially affect (gut) health of the host. They are referred to as prebiotics. A series of studies was carried out to elucidate the role of dietary NDO as feedstuff components and their potential as prebiotic feed additives in young pig diets.

The two types of NDO studied, fructooligosaccharides (**FOS**) and transgalactooligosaccharides (**TOS**), were included in NDO-free diets up to 40 g/kg. The NDO were also used as substrates for *in vitro* fermentation, using young pigs' digesta and faeces as inoculum. Dietary NDO hardly affected pig performance and apparent ileal and faecal nutrient digestion. More than 90% of the FOS was degraded pre-caecally; this was 30% for TOS (estimated by others). Pre-caecal saccharolytic activity enhanced for both types of dietary NDO, resulting in prebiotic effects at the ileal level (reduced pH, reduced concentration of aerobes). However, the FOS- and TOS diets differed in terms of ileal volatile fatty acid composition and concentration of anaerobes (including lactobacilli). This may have been related to differences in rate of fermentation (FOS>TOS) and types of volatile fatty acids produced, as observed during *in vitro* fermentation.

The observed prebiotic effects at the ileal level were not maintained throughout the total large intestine, due to the fast rate of fermentation of FOS and TOS. As a result, some of the stimulated microflora probably started to use proteins as a source of energy, resulting in the increased proteolytic activity observed at the faecal level (increased pH and concentration of volatile fatty acids of protein origin).

It was discussed that cereal- and legume-based diets may exert a certain level of prebiotic activity. Therefore, the use of FOS and TOS as prebiotic feed additives in such diets may be limited. However, prebiotic carbohydrates which can be fermented throughout the gastrointestinal tract, rather than only in the small intestine and/or the proximal colon, may result in prolonged prebiotic effects and prove useful as feed additive in young pig diets.

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Contents

GENERAL INTRODUCTION

General Introduction

When farmers are feeding their pigs, they usually do not realise that they are also feeding a huge bacterial population. From the moment of birth, piglets are exposed to bacteria. Their first exposure is to bacteria found in the maternal vagina, followed by those in the faeces at the moment of birth itself. From then onwards, the young are continuously exposed to bacteria in their rearing environment. *E. coli* can be found in the faeces of the neonate within two hours after birth. *Clostridium perfringens* appears some hours later, and by 48 hours, the dominant flora consists of lactobacilli. From that moment onwards, large numbers of strict anaerobes, such as *Bacteroides* spp, are also found in the faeces (Ducluzeau, 1985). It has been shown that the total bacterial count reaches a stable level of 10^9-10^{10} colony forming units per g faeces, though, it may take up to at least 120 d before the composition has stabilized (Swords *et al.*, 1993). The gut micro-organisms as a whole are called the gastrointestinal microflora. This microflora forms a dynamic ecosystem with its host (Raibaud, 1992); there are continuous interactions with biotic components (indigenous and transient bacteria, and epithelial cells) and chemical components (endogenous secretory components and undigested nutrients). This last category includes the dietary non-digestible oligosaccharides (**NDO**), which is the subject of this thesis.

Manipulating the microflora

Over thirty years ago, it was shown that germ-free chickens grew faster than their conventional counterparts, and that in-feed antibiotics stimulate growth only in conventional animals but not in germ-free animals (Coates *et al.*, 1963). This suggests that part of the nutrients ingested is used by the gastrointestinal microflora. Indeed, it has been shown that a reduced small intestinal bacterial activity in the pig by in-feed antibiotics enhanced pre-caecal net absorption of amino acids (Dierick *et al.*, 1986). In-feed antibiotics have been used in diets for monogastrics for more than 50 years and generally improve growth performance, though the responses in growth performance vary (reviewed by Rosen, 1995).

It has been suggested that in-feed antibiotics generally lower microbial activity (Rosen, 1995). As such, the potentially beneficial activities of the microflora are also diminished. Probably the most important property of the microflora is its ability to protect the host against infections with pathogens, the so-called colonization resistance (Hentges, 1992). In his review, Hentges cites a classical example of a 10,000-fold decrease in

colonization resistance against *Salmonella enteritis* as a result of oral administration of streptomycin. In addition, the saccharolytic activity is considered to be beneficial, in contrast to proteolytic activity. In the absence of suitable carbohydrates, some bacteria use protein as a source of energy, which lead to the production of potentially toxic compounds, including ammonia, amines, phenol and cresol (Macfarlane and Cummings, 1991).

The increasing insight on the potentially beneficial activities of the gastrointestinal microflora, and the increasing (public) concern about antibiotic resistance and residues in meat products, have resulted in research on alternatives for in-feed antibiotics. It has been suggested that the colonization resistance may be enhanced by the oral addition of beneficial micro-organisms, the so-called probiotics (for a review: Sissons, 1989). Another approach would be to enhance the beneficial activity of the microflora through specific substrates in the diet. This has led to the introduction of the term prebiotics, which are defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health' (Gibson and Roberfroid, 1995). An enhancement of pig's health through the beneficial activities of the it's own microflora may eventually result in less need of in-feed antibiotics. Various studies, especially from Japan, have suggested that certain NDO have prebiotic properties in animal feeds. It has been shown that the NDO-fed pigs had less diarrhoea and an enhanced growth performance (Hidaka *et al.* 1985; Fukuyasu and Oshida, 1986; Deya, 1990; Katta *et al.*, 1993). In terms of pig health, prebiotic feed additives may thus be used to control gastrointestinal disorders such as post-weaning diarrhoea.

Non-digestible oligosaccharides

Table 1 lists some of the NDO that have been reported in the scientific literature. Most of them are now commercially available (Playne and Crittenden, 1996). The NDO have been extensively studied in laboratory animals such as rats and mice. However, some NDO have also been studied in various other animals including horses (Mul and Perry, 1994), in pets such as dogs (Willard *et al.*, 1994), and cats (Terada *et al.*, 1993), and in production animals such as chickens (Baily *et al.*, 1991), turkey (Newman, 1995), pre-ruminant calves (Webb *et al.*, 1992), rabbits (Morrise *et al.*, 1993), and pigs (Hidaka *et al.*, 1985). Studies on NDO in man have been reported since the mid-eighties (Hidaka *et al.*, 1986a). Several reviews on the physiological effects of NDO have been published (e.g. Hidaka *et al.*, 1991; Morgan *et al.*, 1992; Delzenne and Roberfroid, 1994; Gibson *et al.*,

Table 1. *Some of the non-digestible oligosaccharides reported in the scientific literature*

Oligosaccharide	Reference
Fructooligosaccharides	Hidaka *et al.*, 1986
Palatinose	Kashimura *et al.*, 1989
Galactosyllactose	Ohtsuka *et al.*, 1990
α-Galactooligosaccharides[1]	Hayakawa *et al.*, 1990
Xylooligosaccharides	Imaizumi *et al.*, 1991
Galactosylsucrose	Kumemura *et al.*, 1992
β-Cyclodextrin	Flourié *et al.*, 1993
Glucooligosaccharides	Valette *et al.*, 1993
Transgalactooligosaccharides	Ito *et al.*, 1993
Isomaltooligosaccharides	Kaneko *et al.*, 1994
Mannanoligosaccharides	Newman, 1994
Xylosylfructoside	Hoshi *et al.*, 1994
Lactitol oligosaccharides	Yanahira *et al.*, 1995
Xyloglucooligosaccharides	Yamatoya *et al.*, 1996

[1]Also referred to as soybean oligosaccharides, a mixture of raffinose, stachyose, and verbascose

1994; Mul and Perry, 1994). However, the number of studies on the modes of action of NDO in pigs is limited.

In this thesis, two types of NDO were studied in a series of experiments with young pigs. These were the fructooligosaccharides (**FOS**) and transgalactooligosaccharides (**TOS**). Figure 1 shows some chemical structures of FOS. The FOS were available as Raftilose P95® (Orafti, Tienen, Belgium). These types of FOS are derived from chicory inulin (Roberfroid, 1993), and are mixtures of linear GF_n molecules (fructose oligomers with a glucose moiety) and F_m molecules (fructose oligomers without a glucose moiety), with n and m usually ranging from 2 to 8. The TOS were available as Oligostroop® (Borculo Whey Products, Borculo, The Netherlands). These type of TOS are being produced by transgalactosylation of lactose using β-galactosidase (Ekhart and Timmermans, 1996). The TOS-structures found in Oligostroop® consisted of linear and branched β-linked galactose units, sometimes including an α-linked glucose unit. More than 40 different structures can be found in the TOS-mixture used for the experiments in this thesis (K.J.M. van Laere, personal communications). Figure 2 shows some of these TOS-structures.

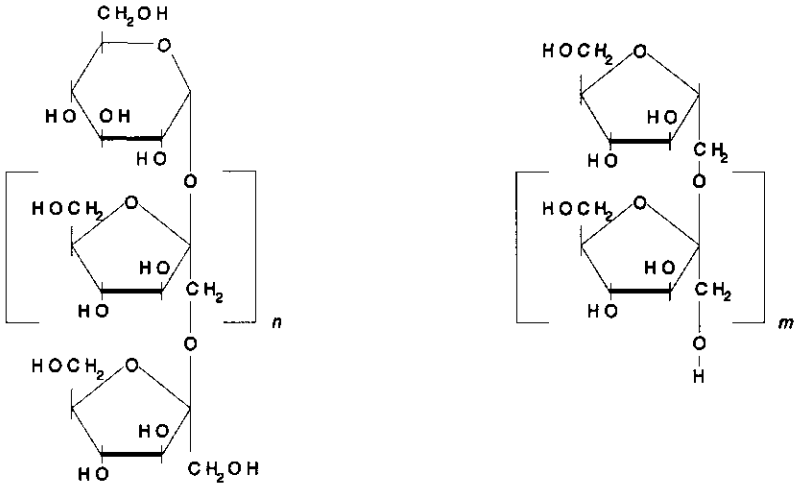

Figure 1. *Examples of fructooligosaccharides, produced by the hydrolysis of inulin* (n and m < *8)*

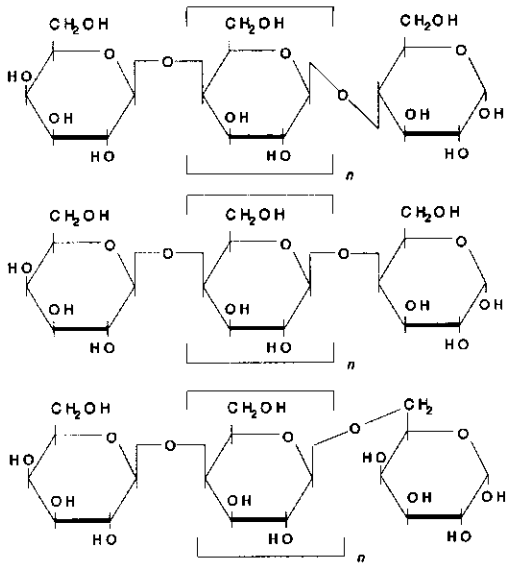

Figure 2. *Examples of transgalactooligosaccharides, produced by the transgalactosidation of lactose using β-galactosidase* (n < *10)*

Objectives of this thesis: NDO in young pig diets

The studies in this thesis focused on the digestive-physiological effects of NDO in young pigs through fermentative degradation by the gastrointestinal microflora. However, it has been suggested that some NDO exert their effects through different modes of actions, such as through pathogen adsorption (receptor analogue theory) and immunomodulation, as suggested for the yeast cell wall derived mannanoligosaccharides (Newman, 1994). It has been shown that the inclusion of NDO in commercial diets did not affect various chemical and microbial digesta characteristics of young pigs, including pH, bacterial counts, volatile fatty acids (**VFA**) and ammonia concentrations (Farnworth *et al.*, 1992; Bolduan *et al.*, 1993; Gabert *et al.*, 1995; Mathew *et al.*, 1997). This suggested that the use of NDO as prebiotic feed additive may be limited in commercial diets. However, the commercial diets used were relatively rich in cereals and legumes (Table 2). These feedstuffs may contain relatively high concentrations of NDO (Reddy *et al.*, 1984; Henry and Saini, 1989). These background levels of NDO may have exerted such a level of prebiotic effects that any effect of the NDO added could not be observed. We studied the effects of FOS and TOS using control diets which were low or devoid of NDO. This allowed us to elucidate the role of dietary NDO as feedstuff components and their potential as prebiotic feed additives in young pig diets, which was the main objective of this thesis.

The NDO are small, water-soluble indigestible carbohydrates, their physical presence in a diet can exert a certain osmotic pressure in the small intestinal digesta (Wiggens, 1984). This may lead to a reduced retention time and affect the pre-caecal enzymatic digestion of nutrients, and thus increase the amount of dry matter arriving in the large intestine. However, since the NDO are not degraded by endogenous enzymes, they are to be fermented by the gastrointestinal microflora. The site of fermentation in the gastrointestinal tract depends various factors, including chemical properties of the NDO such as sugar composition, types of linkages, degree of polymerization, and the physical structure (linear or branched), the presence of NDO-degrading bacteria, the access to the NDO, and the physico-chemical conditions present in the digesta and/or at the gut wall. The same factors that affect the possibility for NDO fermentation may alter as a result of NDO fermentation. These include digestal pH, VFA- and ammonia concentrations, bacterial counts, and bacterial activity. Depending on the site of fermentation, this may affect nutrient digestion. An enhanced saccharolytic activity in the small intestine could reduce the apparent pre-caecal digestion of amino acids, as a result of microbial growth (biomass). However, when this occurs in the large intestine, the apparent faecal digestion of amino

Table 2. *Short outline of studies on NDO in pigs*

NDO	Level (g/kg)	Main diet ingredients (g/kg)				Reference
		Corn	SBM	Wheat	Barley	
Galactosyllactose	5	683	266			Mathew *et al.*, 1997
Fructooligosaccharides	15	440	250		150	Farnworth *et al.*, 1992
Transgalactooligosaccharides Glucooligosccharides	2		210	363	363	Gabert *et al.*, 1995
Fructooligosaccharides Isomaltooligosaccharides Galactooligosaccharides	2		75	380	375	Bolduan *et al.*, 1993

acids could be lowered, though the amount of amino acids available to the host will not be affected. In the latter case, the growth performance may not be affected. A series of experiments were designed to study the effects of dietary NDO in young pigs on the various digestive-physiological aspects mentioned.

Outline of the thesis

This thesis describes three experiments with pigs and two *in vitro* fermentation experiments using digesta obtained from those pigs. The results of these experiments have been written in seven chapters, which are organized by common objectives rather than by experiment (Figure 3). Chapter 1 describes the effects of dietary NDO on growth performance and some faecal characteristics in growing pigs (63-105 d of age). Chapter 2 shows the effects of dietary NDO on the apparent faecal and ileal nutrient digestion in the same pigs, and also in weaner pigs (38-75 d of age). In addition, nitrogen- and mineral balances were studied in the weaner pigs. Chapters 3 and 4 describe the effects of dietary NDO on various physico-chemical and microbial digesta characteristics, obtained from the growing and weaner pigs, respectively. In Chapter 5, a series of *in vitro* fermentation experiments is described, using faeces and digesta from the weaner pigs as inoculum. The *in vitro* fermentation kinetics and end-point measurements of FOS and TOS as substrates are presented, and also the *in vitro* microbial activity of the ileal-, caecal, and faecal microflora as affected by FOS and TOS in the diet.

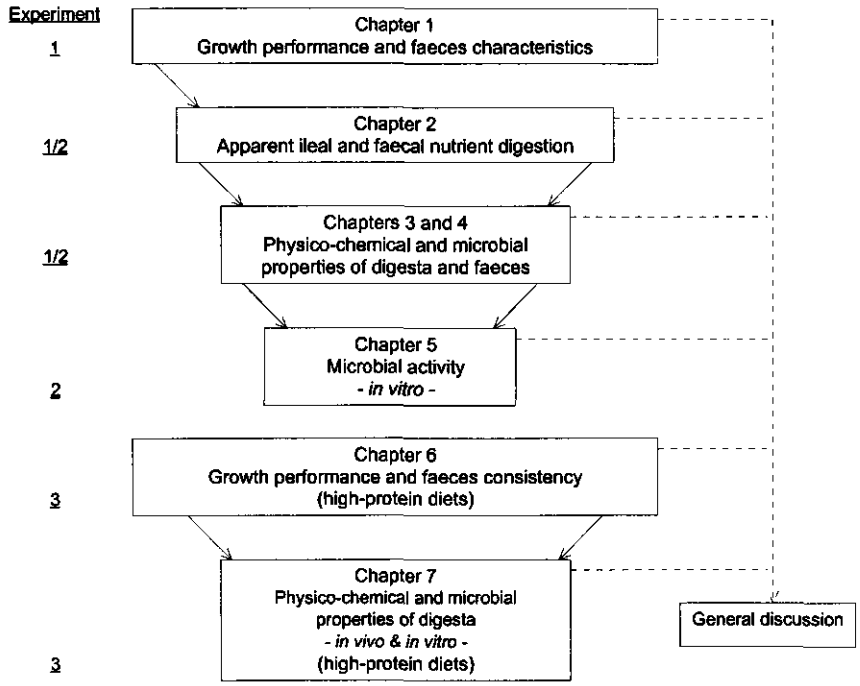

Figure 3. *Schematic representation of this thesis*

The diets used for the Chapters 1 through 5, were balanced in terms of known nutrient requirements for growing pigs. In the last experiment with young piglets around weaning (0-63 d of age), high-protein diets were used to elevate the level of bacterial proteolytic activity. This was done to test the hypothesis that dietary NDO could reduce bacterial proteolytic activity. Chapter 6 describes the effects of dietary FOS and in-feed avilamycin in high-protein diets on growth performance and faeces consistency. A selection of these piglets were sacrificed, and the effects of dietary FOS and in-feed avilamycin were studied on various physico-chemical and microbial digesta characteristics, both *in vivo* and *in vitro* (Chapter 7).

In the last chapter (General Discussion), the main results from the previous chapters are brought together and discussed in the context of the flow of dietary NDO from feed to faeces. Finally, the main conclusions are summarized and some suggestions for further research are given.

It is hoped that the research and ideas presented in this thesis will prove useful in the discussions on alternatives for in-feed antibiotics.

CHAPTER

1

EFFECTS OF DIETARY OLIGOSACCHARIDES ON THE GROWTH PERFORMANCE AND FAECAL CHARACTERISTICS OF YOUNG GROWING PIGS

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Effects of dietary oligosaccharides on the growth performance and faecal characteristics of young growing pigs

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Abstract

The effects of dietary fructo-oligosaccharides (**FOS**) and trans-galacto-oligosaccharides (**TOS**) were studied on growth performance and faecal characteristics of young growing pigs. FOS and TOS are non-digestible oligosaccharides (**NDO**); they are not hydrolyzed by enzymes of endogenous origin but are readily available as substrate for the gastrointestinal microflora.

Dietary levels of NDO-rich products were 7.5 and 15 g/kg diet for FOS, and 10 and 20 g/kg diet for TOS. NDO were included in an experimental corn-based control diet, which was low in NDO (190 mg raffinose/kg) and contained no additional copper, antibiotics or probiotics. NDO-rich products were included at the expense of glucose and purified cellulose. Resulting five experimental diets were fed ad libitum to 9-wk old castrated male pigs (n =10) with an initial body weight (se) of 15.6 ± 0.3 kg. Pigs had received the control diet for 19 d before reaching this body weight. Individual body weights and feed refusals were recorded every 3-4 d during six weeks. Fresh faeces were rectally collected at 0, 14, 35 d of the experiment, and analyzed for NDO, pH, and dry matter content.

Dry matter intake and body weight gain of the NDO-fed pigs were lower than the control pigs (P = 0.039 and P = 0.031 respectively) in week one through three. Dietary NDO did not affect mean growth performance in week one through six. FOS and TOS could not be detected in the faeces. Dietary NDO did not affect faecal pH. Faecal pH increased with time (P < 0.01). NDO-fed pigs had a lower faecal dry matter content than control pigs (P = 0.062). Pigs fed TOS-rich diets had a lower faecal dry matter content than pigs fed FOS-rich diets (P = 0.061).

It is concluded from this experiment that exchanging cellulose for NDO in young growing pigs diet results in a temporary depressed feed intake with little or no effects on faecal dry matter content and pH respectively.

Introduction

Fructo-oligosaccharides (**FOS**) and trans-galacto-oligosaccharides (**TOS**) are water-soluble carbohydrates consisting of 2 to 10 monomeric units. They can be classified as non-digestible oligosaccharides (**NDO**) because the β-linkages between fructose monomers (in FOS) and galactose monomers (in TOS) can not be hydrolyzed by enzymes of endogenous origin (Burvall *et al.*, 1980; Oku *et al.*, 1984). As a consequence, NDO are quantitatively available as substrate for the gastrointestinal microflora. FOS can be found in various feedstuffs such as barley, wheat, and rye (Henry and Saini, 1989). FOS can also be manufactured from inulin via hydrolysis by endoglycosidases, resulting in fructose oligomers (F_m series), which may have a glucose residue (GF_n series). In inulin-derived FOS, m and n, representing the number of fructosyl moieties, vary from 2 to 9 (Roberfroid, 1993). FOS may also be produced via transfructosylation of sucrose, resulting in GF_n series only, with n varying from 2 to 5 (Hidaka *et al.*, 1988). TOS are rarely found in common

feedstuffs. They may be present in low concentrations in yogurts (Toba *et al.*, 1983). They can be manufactured via transgalactosidation of lactose (Burvall *et al.*, 1980).

Certain NDO may improve growth performance of young pigs. Several authors reported increased growth and improved feed conversion ratio together with a reduction of diarrhoea or loose faeces as a consequence of FOS or TOS inclusion in young pigs diets (Hidaka *et al.*, 1985; Fukuyasu and Oshida, 1986; Hidaka *et al.*, 1986b; Katta *et al.*, 1991). Other authors, however, reported no or slightly negative effects of FOS on young pigs growth performance (Farnworth *et al.*, 1992; Kornegay *et al.*, 1992). Control diets used in the latter studies were often based on barley and soybean products. Soybean products contain considerable amounts of NDO such as raffinose and stachyose (Saini, 1988). Therefore, a dilution or masking effect from NDO in the control diet may have contributed to a lack of response. In addition, antibiotics or additional copper may have been part of these diets. These additives can suppress normal gastrointestinal microflora. NDO may be fermented by beneficial members of the normal gastrointestinal microflora, including lactobacilli and bifidobacteria. Therefore, NDO may have less or different effects in diets containing microflora suppressing agents.

Recently, a multidisciplinary project on NDO in food and feed has started, focussing on functional characteristics of dietary NDO. In the present study, effects of FOS and TOS on the growth performance and faecal characteristics of young growing pigs have been investigated.

Materials and methods

Diets

Table 1 gives the composition of the experimental diets. The control diet ingredients were low in NDO. HPLC-analysis of corn revealed a small amount of raffinose (300 mg/kg); FOS or TOS were not found. Inclusion level of corn was 634.6 g/kg. Therefore, the control diet was not completely NDO-free, and contained 0.19 g raffinose/kg. In addition, diets did not contain additional copper, antibiotics or probiotics. Diets and water were available ad libitum. Diets met or exceeded requirements for young growing pigs (National Research Council, 1988).

NDO-rich products were included at a low and high level. Raftilose P95®, a FOS-rich powder (Orafti, Tienen, Belgium), was included at 7.5 and 15 g/kg. Oligostroop®, a TOS-rich syrup (Borculo Whey Products, Borculo, The Netherlands), was included at 10 and 20 g/kg. Table 2 gives some specifications of these products. Raftilose P95® and

Table 1. *Composition of experimental diets fed to young pigs*

	Type	Diets[1]				
		CON	FOS		TOS	
	Level	0	L	H	L	H
Ingredients (g/kg)						
Corn[2]		634.6	-----[3]	-----	-----	-----
Casein		50.0	-----	-----	-----	-----
Fish meal		50.0	-----	-----	-----	-----
Meat meal		50.0	-----	-----	-----	-----
Dextrose® (glucose)		117.8	117.4	117.0	113.2	108.5
Arbocel® (cellulose)		30.0	22.9	15.8	24.6	19.3
Raftilose P95®		0.0	7.5	15.0	0.0	0.0
Oligostroop®		0.0	0.0	0.0	10.0	20.0
Soy oil		10.0	-----	-----	-----	-----
Premix[4]		10.0	-----	-----	-----	-----
Minerals[5]		32.4	-----	-----	-----	-----
Amino acids[6]		5.2	-----	-----	-----	-----
Fumaric acid		10.0	-----	-----	-----	-----

[1]CON = control diet; FOS = fructooligosaccharides; TOS = transgalactooligosaccharides; 0 = no non-digestible oligosaccharides (NDO); L = low level of NDO-rich products (7.5 and 10.0 g/kg for FOS and TOS respectively); H = high level of NDO-rich products (15.0 and 20.0 g/kg for FOS and TOS respectively).
[2]Half of the corn (317.3 g/kg diet) was pressurized toasted (>100°C), and then flaked and pelleted (Cornax®, Schouten/Giessen, The Netherlands).
[3]Same content as in the control diet.
[4]The vitamin/mineral mix provided (per kg feed): 9000 IU vitamin A, 1800 IU vitamin D_3, 40 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 5 mg riboflavin, 12 mg d-panthothenic acid, 1 mg folic acid, 3 mg pyridoxine, 30 mg niacin, 40 µg cobalamin, 1000 mg choline chloride, 50 mg vitamin C, 0.1 mg biotin, 2.5 mg $CoSO_4 \cdot 7H_2O$, 0.2 mg $Na_2SeO_3 \cdot 5H_2O$, 0.5 mg KI, 400 mg $FeSO_4 \cdot 7H_2O$, 60 mg $CuSO_4 \cdot 5H_2O$, 70 mg MnO_2, and 300 mg $ZnSO_4$.
[5]This mineral mix provided (per kg feed) 2.0 g NaCl, 8.0 g $CaCO_3$, 9.4 g CaH_2PO_4, 2.0 g MgO, and 11.0 g $KHCO_3$.
[6]Added synthetic amino acids (per kg feed) were 2.6 g L-lysine HCl, 1.1 g DL-methionine, 0.8 g L-threonine, and 0.7 g L-tryptophane.

Oligostroop® were included at the expense of Dextrose® (glucose) and Arbocel® (purified cellulose) in the control diet, based on the ratio of digestible to (potentially) fermentable carbohydrates found in the NDO-rich products (Table 1). The calculated ratio of digestible to (potentially) fermentable carbohydrates was the same in each diet.

Animals

Fifty-six 38-d-old castrated male pigs, (Great Yorkshire x Landrace)♂ x (Great Yorkshire)♀, weighing (se) 8.9 ± 0.2 kg, were housed individually in an environmentally-controlled animal house (mean temperature 23°C, humidity 60-80%) and received the control diet for 19 d. At the end of this period, the pigs were ranked in body weight. Six pigs with the lowest body weight were excluded. The remaining 50 pigs were divided in 10 weight classes. Within each class, the pigs were randomly allocated to one of the five experimental diets (n = 10). Mean initial body weight was 15.6 ± 0.3 kg.

Table 2. *Specification of non-digestible oligosaccharides (NDO) rich products used to supplement young pigs diets in this experiment*

	NDO-rich products	
	Raftilose P95®	Oligostroop®
Effective NDO	FOS (fructooligosaccharides)	TOS (transgalactooligosaccharides)
Produced from	Chicory	Whey
Compound	Inulin	Lactose
Process	Enzymatic hydrolysis	Transgalactosidation
Physical form	Powder	Syrup
Dry matter (g/kg)	950	741
NDO (g/kg)	903	399
Digestible carbohydrates (g/kg):		
glucose + fructose + sucrose	47	
glucose		176
galactose		13
lactose		153
Producer	Orafti, Tienen, Belgium	Borculo Whey Products, Borculo, The Netherlands

Observations

During the first three weeks, body weights and feed refusals were recorded every 3-4 d. Feed refusals were sampled for dry matter analysis. After three weeks five pigs per treatment were selected to measure faecal digestibility. These pigs were the five closest to the mean growth performance per group. Results of this experiment will be published as part of a series of faecal and ileal digestibility experiments in relation to dietary NDO. Body weights and feed refusals of the remaining pigs were observed for another three weeks (n = 5).

Fresh faecal samples were collected from the rectum at 0, 14, 35 d of the experiment. Samples were frozen in liquid nitrogen and stored at -20°C until analysis. Faecal dry matter content (g/kg) was determined by drying at 103°C until no further weight losses occurred. Faecal pH was measured via inserting a point-shaped electrode from a digital pH meter in the faecal sample (Testo, TYPE 03, Germany). In addition, these samples were analyzed for FOS and TOS. 0.25 g freeze dried faeces were suspended in five ml water and, after vortexing, placed in a waterbath at 100°C for ten minutes. This suspension was centrifuged during 25 minutes at 4500 rpm. The supernatant was analyzed using high-performance anion-exchange chromatography, HPAEC (Dionex B.V., Breda, The Netherlands). The mobile phase of the column used (Carbopack PA-100; 4*250 mm) had a linear gradient from 0 to 300 mM Na-acetate in 25 minutes. Detection was done via PAD-analysis. Samples were spiked with Raftilose P95® and Oligostroop®.

Data analysis and statistical procedures

Individual daily body weight gain (**DWG**), dry matter intake (**DDMI**), and feed conversion (feed to gain ratio, **FC**) were calculated weekly in the first three-week period, per period of three weeks and over the total six-week period. Least square means (LSMeans) and associated standard errors of mean daily body weight gain, daily dry matter intake, and feed conversion were calculated for each diet (D_i) using model 1.

(1) $y_{ij} = \mu + ß^*BW_{ij} + D_i + \varepsilon_{ij}$

where y_{ij} = dependent variable, μ = overall mean, BW_{ij} = individual body weight (i = 1,...,5; j = 1,...,n), D_i = diet effect (i = 1,...,5) and ε_{ij} = residual error. Initial individual body weight (BW_{ij}) at the start of the observation periods was used as covariable. In addition, LSMeans were used to deal with unequal cell sizes (missing values). During the second three weeks, one pig died (stomach ulcer), two piglets developed severe diarrhoea and were treated with antibiotics, and a fourth pig showed extremely slow growth. A fifth pig showed extremely slow growth throughout the entire experiment. Data from these piglets (15 out of 275 observations) were omitted from the statistical analysis.

Effect of sampling day and diet on faecal dry matter and pH were analyzed using model 2.

(2) $y_{ijk} = \mu + D_i + A_k(D_i) + S_j + (D^*S)_{ij} + \varepsilon_{ijk}$

where y_{ijk} = dependent variable, μ = overall mean, D_i = diet effect (i = 1,...,5), $A_k(D_i)$ = animal effect within diet (k = 1,...,n), S_j = sampling day (j = 1,2 or 3), $(D^*S)_{ij}$ = interaction term between diet and sampling day, and ε_{ijk} = residual error. The effect of diet (D_i) was tested against $A_k(D_i)$ as error term; the effect of sampling day (S_j) and the interaction between sampling day and diet $(D^*S)_{ij}$ were tested against the residual error.

The experiment was designed as 2x2 factorial design with added control (Cochran and Cox, 1957). Predetermined orthogonal contrasts were used to study dietary effects. The effect of type (FOS vs TOS), level (low vs high), and the interaction between type and level of dietary NDO as well as the effect of NDO-inclusion per se could be distinguished. Effects were considered significant when contrast probabilities were below 0.05. Sampling days were compared using a Bon-Ferroni adjustment of the P-diff output from the LSMeans procedure (SAS, 1989).

Results

Table 3 shows the growth results from the first three weeks (n = 10). The DWG ranged from 552 - 1162 g in the first three-week period. NDO-fed piglets had a significantly lower mean DWG than the control pigs (P = 0.031). This effect was the most pronounced in the first week (P = 0.016). The DDMI ranged from 903 - 1630 g in the first three-week period. NDO-fed pigs had a significantly lower mean DDMI than the control pigs (P = 0.039). Again, this effect was the most pronounced in the first week (P = 0.104). Individual FC ranged from 1.31 - 1.75 in the first three-week period. NDO-fed pigs had a numerically higher FC than the control pigs. This effect was significant in the first week (P = 0.010). FC was markedly lower in the first week compared with week two and three.

There were no significant differences between FOS-fed and TOS-fed pigs with respect to growth performance during week one through three. In week two, a high level of dietary NDO resulted in a significantly lower FC compared with a low level of dietary NDO (P = 0.016, Table 3). The interaction between type and level of dietary NDO for FC was significant in week one (P = 0.005). In addition, interactions between type and level of dietary NDO for DWG, DDMI, and FC were significant in week three (Table 3).

Table 4 shows the growth results from the total six-week experiment, divided over two three-week periods (n = 5). Mean DWG of the NDO-fed pigs was lower in week one through three compared with the control pigs (P = 0.109). Likewise, NDO-fed pigs had a lower mean DDMI than the control pigs (P = 0.083). FC was not affected. Type, level, and interaction effects were not significant.

Individual DWG ranged from 686 - 1181 g in the second three-week period. NDO-fed pigs had a higher mean DWG than the control pigs (P = 0.089). Individual DDMI ranged from 1228 - 2205 g in the second three-week period. DDMI in NDO-fed pigs was numerically but not significantly higher (P = 0.130) compared with the control pigs. Dietary NDO didn't significantly affect FC, ranging from 1.57 - 1.83, in the second three-week period. Type, level or interaction effects were not significant. Dietary NDO didn't significantly affect the growth results calculated over the total six-week period.

Table 3. *Mean daily weight gain (DWG), daily dry matter intake (DMI), and feed conversion (FC) of young growing castrated male pigs fed non-digestible oligosaccharides-rich diets for three weeks*[1]

	Diets[2]					SEM	Orthogonal contrasts[3]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x
Level	0	L	H	L	H					Level
DWG (g)										
Week 1	953	860	750	765	770	59	0.016	NS	NS	NS
Week 2	709	667	716	603	715	47	NS	NS	0.099	NS
Week 3	854	756	863	971	748	59	NS	NS	NS	NS
Week 1-3	862	766	770	764	740	41	0.031	NS	NS	0.008
DMI (g)										
Week 1	1104	1061	982	1036	957	51	0.104	NS	NS	NS
Week 2	1196	1157	1158	1115	1186	50	NS	NS	NS	NS
Week 3	1396	1240	1383	1446	1261	52	NS	NS	NS	NS
Week 1-3	1268	1163	1161	1175	1126	47	0.039	NS	NS	0.003
FC										
Week 1	1.16	1.23	1.35	1.40	1.24	0.05	0.010	NS	NS	0.005
Week 2	1.71	1.79	1.61	2.03	1.66	0.11	NS	NS	0.016	NS
Week 3	1.70	1.67	1.64	1.49	1.78	0.08	NS	NS	0.095	0.046
Week 1-3	1.48	1.52	1.52	1.56	1.52	0.03	NS	NS	NS	NS

[1]Initial body weight: 15.6 ± 0.3 kg; $n = 10$
[2]See Table 1
[3]Orthogonal contrasts (tendency: $P < 0.10$; significant: $P < 0.05$)
NDO: Control diet vs NDO-rich diets Type: FOS-rich diets vs TOS-rich diets
Level: L-diets vs H-diets Itype x Level: Interaction between Type and Level

Table 5 and 6 show faecal pH and dry matter content (g/kg). Dietary NDO didn't significantly affect faecal pH, which ranged from 5.58 - 7.56 (Table 5). However, faecal pH increased with time, being significantly higher at d 35 compared with d 0 (Table 6, $P <$ 0.01). Faecal dry matter contents ranged from 204.3 - 365.6 g/kg. Dietary NDO did not affect faecal dry matter content by d 14 (Table 5). However, when data from d 35 were included, a tendency towards a lower dry matter content was observed in NDO-fed pigs compared with control pigs ($P =$ 0.062). In TOS-fed pigs, mean faecal dry matter content tended to be lower compared with FOS-fed pigs ($P =$ 0.061). There was no significant effect of sampling day on faecal dry matter content. In addition, there was no significant interaction between diet and sampling day for faecal pH and dry matter content ($P > 0.10$). Both FOS and TOS could not be detected in the faeces (HPAEC).

Table 4. *Mean daily weight gain (DWG), daily dry matter intake (DMI), and feed conversion (FC) of young growing castrated male pigs fed non-digestible oligosaccharides-rich diets for six weeks*[1]

	Diets[2]					SEM	Orthogonal contrasts[3]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x
Level	0	L	H	L	H					Level
DWG (g)										
Week 1-3	885	771	712	772	747	72	0.109	NS	NS	NS
Week 4-6	861	1056	981	964	1032	87	0.089	NS	NS	NS
Week 1-6	885	920	887	881	880	73	NS	NS	NS	NS
DMI (g)										
Week 1-3	1327	1196	1120	1180	1137	84	0.083	NS	NS	NS
Week 4-6	1655	1852	1756	1850	1830	109	NS	NS	NS	NS
Week 1-6	1511	1650	1417	1533	1488	106	NS	NS	NS	NS
FC										
Week 1-3	1.50	1.54	1.58	1.55	1.52	0.05	NS	NS	NS	NS
Week 4-6	1.91	1.73	1.83	1.93	1.78	0.08	NS	NS	NS	NS
Week 1-6	1.71	1.68	1.68	1.72	1.66	0.05	NS	NS	NS	NS

[1]Initial body weight: 15.8 ± 0.6 kg; n =5
[2,3]See Table 3.

Table 5. *Faecal pH and dry matter contents in young growing castrated male pigs fed non-digestible oligosaccharides-rich diets*[1]

	Diets[2]					SEM	Orthogonal contrasts[3]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x
Level	0	L	H	L	H					Level
0-14 d (n = 10)										
pH	5.88	6.04	6.00	5.95	5.96	0.06	NS	NS	NS	NS
DM (g/kg)	259.0	274.3	278.6	261.6	270.5	8.0	NS	NS	NS	NS
0-35 d (n = 5)										
pH	5.98	6.00	6.14	6.08	6.13	0.10	NS	NS	NS	NS
DM (g/kg)	276.1	263.8	264.9	240.5	256.9	8.1	0.062	0.061	NS	NS

[1]Initial body weight: 15.6 ± 0.3 kg for 0-14 d (n = 10); 15.8 ± 0.6 kg for 0-35 d (n = 5)
[2,3]See Table 3

Table 6. *Faecal pH and dry matter contents of young growing castrated male pigs over time*[1]

	Sampling day			
	0	14	35	SEM
pH	5.96[a]	6.08[ab]	6.17[b]	0.04
DM (g/kg)	261.8	264.2	255.3	5.2

[1]Initial body weight: 15.8 ± 0.6 kg (n = 5); means lacking a common superscript differ significant (Bon Ferroni; $P < 0.01$)

Discussion

Ingestion of certain NDO may affect composition and/or activity of the normal intestinal flora. Useful functions of the normal intestinal flora include resistance against potential pathogens, vitamin production, providing energy from non-digestible components, and suppression of intestinal putrefaction, and may be enhanced through NDO-ingestion (Mitsuoka, 1990). Recently, certain NDO have been classified as prebiotics. Prebiotics are "non-digestible food ingredients 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 improve host health" (Gibson and Roberfroid, 1995). FOS may be classified as prebiotics; other non-digestible oligosaccharides, including TOS, need further investigations (Gibson and Roberfroid, 1995). Prebiotics may be used as additives in pigs diet. However, NDO-inclusion does not always result in improvement of growth performances.

Introducing NDO-rich diets to young growing pigs reduced DDMI during the first three weeks of our experiment (Table 3). However, dietary NDO did not affect DDMI over the total six-weeks period (Table 4). This indicates that young growing pigs have a certain adaptation period for NDO. Furthermore, compensation seemed to take place. Mean DDMI was numerically higher in NDO-fed pigs compared to the control pigs in week four through six. The growth figures also showed compensation. NDO-fed pigs had a lower mean DWG than the control pigs in week one through three (Table 3), but mean DWG over the total six-week period did not differ between treatments (Table 4). FC was remarkably lower in week 1 compared with the other weeks. Pigs were fasted overnight at the start of the experiment (for purposes outside the scope of this paper). Therefore, body weight gain in the first week can to a great extent be explained by refilling the gastrointestinal tract in addition to deposition of fat, protein, water, and ash in body tissues. Changes in digesta volume may play an important role in explaining differences in body weight gain (Van Gils, personal communications).

The observed temporary decrease in feed intake after introducing NDO-rich diets to young growing pigs has not been reported in literature until now. However, temporary feed intake depression was observed in rats and broilers given FOS (Mul, personal communications), and in pigs given 1 g of ß-glucan/kg diet (Dritz *et al.*, 1995). In studies where NDO were effective, feed intake was often increased (Hidaka *et al.*, 1985; Fukuyasu and Oshida, 1986; Katta *et al.*, 1991). These studies generally reported feed intake over several weeks and didn't give insight in daily intake during the first days after introducing NDO-rich diets.

The interaction between host and microflora may explain a temporary feed intake depression after introducing NDO-rich diets. During its development, normal microflora presents a major source of antigens to the immune system. As a result, the ecology (presence, composition and/or activity) of the normal microflora primes the immune system (Tannock, 1990). Changes in microflora ecology may be recognized as different from the primed situation, resulting in a non-specific immune response. In this experiment, NDO could not be found in the faeces. Therefore, changes in microbial ecology may have occurred, and thus likely induced an immune responds, during which feed intake may have been reduced (Langhans, 1992).

In the present six-week study, dietary NDO didn't improve the growth performance in young growing pigs. When the results from this experiment are extrapolated in time, it may be speculated that NDO-fed pigs reach slaughter weight in less days than the control group. However, improvement of growth performance will often be marginal during optimal rearing and feeding. Efficacy of related additives such as organic acids (Ravindran and Kornegay, 1993), antibiotics (Rosen, 1995) and probiotics (Stavric and Kornegay, 1995) vary substantially under practical conditions. At high levels of basic growth performance little or no response to these additives can be expected. However, when basic growth performance is reduced due to gastrointestinal factors, the response to these additives may increase (Cole, 1991; Ravindran and Kornegay, 1993; Rosen, 1995). A similar relationship may be found with dietary NDO. Figure 1 shows the negative relation (weighed regression: $Y = 158\text{-}91X$; $R^2 = 0.59$, $P < 0.0001$) between the magnitude of response in gain to feed ratio (Y) and gain to feed ratio in the control pigs (X) derived from several reports (Hidaka *et al.*, 1985; Fukuyasu and Oshida, 1986; Farnworth *et al.*, 1992; Kornegay *et al.*, 1992; Gianotten *et al.*, 1993 [in: Mul and Perry, 1994]; Katta *et al.*, 1993; Orban *et al.*, 1994; Russel *et al.*, 1996). Our results are presented as open triangles (Figure 1). They obviously fit in that region of the graph where the expected impact of NDO is marginal. However, more data are needed to elucidate this relationship, especially data from studies

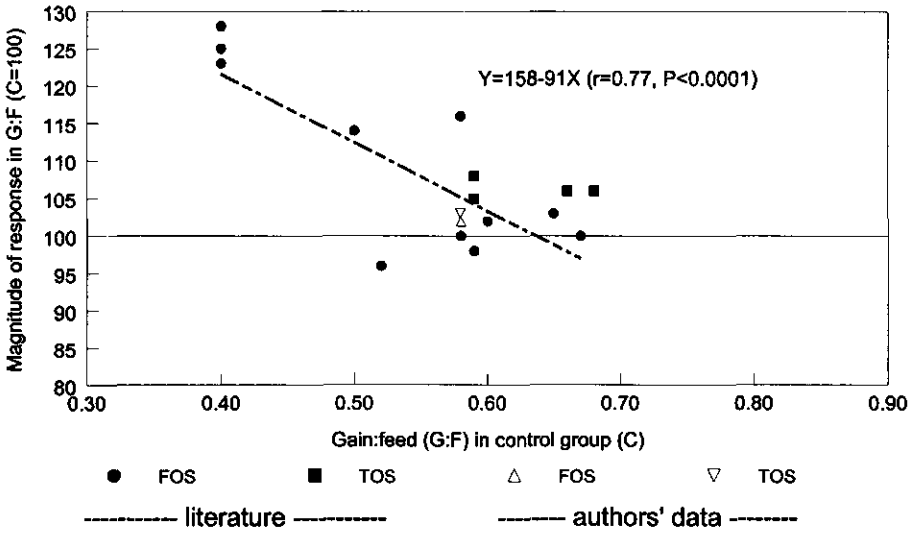

Figure 1. *Magnitude of response in gain to feed ratio to dietary fructooligosaccharides (FOS) and transgalactooligosaccharides (TOS) tested vs. literature findings*

where control pigs have a low gain to feed ratio, as the negative correlation (r = -0.77) completely results from the very few experiments carried out within that region. The high quality diet, including 10 g fumaric acid/kg, the individual housing, and the used commercial pig breed have probably attributed to the relatively high level of feed efficiency observed in our experiment.

In this study, FOS and TOS did not differ with regard to growth performance (Table 3 and 4). However, the comparison between FOS and TOS is not completely clear because dietary NDO contents were not equal (Table 2). Actual dietary NDO contents (g/kg) were 0.68 and 1.35 for FOS, and 0.40 and 0.80 for TOS. Therefore, type effects may partly be level effects. Additional calculations with the low-FOS diet and high-TOS diet, having nearly comparable NDO levels, revealed no significant NDO-type effects. Diets with high NDO levels yielded a significantly lower FC in week two compared to diets with low NDO levels (P = 0.016, Table 3). This may indicate that the adaptation time to high levels of dietary NDO is shorter compared to low levels. Here, the length of adaptation time is judged by the time of elevated FC. Bearing in mind the actual dietary NDO contents and the interaction

between type and level of dietary NDO for FC in week three, the data from our experiment suggest that level of dietary NDO correlates negatively with the adaptation time (Table 3). Similar dose/time dependencies with respect to increased faecal bifidobacteria concentrations in humans were reported for FOS (Mitsuoka, 1986) and xylo-oligosaccharides (Suntory, product information, 1992 [in: Mul and Perry, 1994). However, in our experiment, week-to-week variations in dry matter intake estimations and body weight measurements may have played a role.

Dietary NDO did not affect faecal pH (Table 5). Human studies with FOS (Hidaka *et al.*, 1986a; Alles *et al.*, 1996; Bouhnik *et al.*, 1996), TOS (Bouhnik *et al.*, 1997), and with soybean oligosaccharides (Hayakawa *et al.*, 1990) showed similar results. Conversely, faecal pH decreased in rats fed 100 g FOS/kg diet (Hidaka *et al.*, 1986a). Inclusion levels in current experiment may have been to low to observe an effect on faecal pH. In addition, fermentation of NDO likely results in an elevated luminal concentration of fermentation products in the colon, including volatile fatty acids (VFAs). At faecal level, VFAs may have been completely absorbed, resulting in an unchanged pH. Table 6 shows an increase of faecal pH in older pigs. Increased protein degradation by bacteria, more proximal completion of carbohydrate fermentation or more complete VFA-absorption may have caused this. It is not clear whether our observations on faecal pH are due to changes in carbohydrate fermentation or protein degradation by the intestinal flora.

NDO-fed pigs had a lower faecal dry matter content than the control pigs (Table 5). In elderly people, a decrease in faecal dry matter content may be the mode of action behind the relief of constipation, claimed for FOS (Hidaka and Hirayama, 1991). However, in young men, FOS ingestion did not change faecal dry matter content (Alles *et al.*, 1996). Faecal wet weight increased in rats fed up to 200 g FOS/kg diet (Tokunaga *et al.*, 1986). The presence of non-absorbable sugars in the diet will increase the load of water and electrolytes in the large intestine (Wiggins, 1984). In addition, locally produced VFAs may further increase the osmotic pressure, resulting in accelerated peristaltic movement and decreased transit time (Ruckebusch, 1981). A decreased transit time likely reduces reabsorption of water and electrolytes in the colon, resulting in a lower faecal dry matter content. TOS-fed pigs had a lower faecal dry matter content than FOS-fed pigs. This may indicate that TOS is more distally fermented in the colon than FOS, resulting in less reabsorption of water. However, as discussed previously, NDO-type effects are partly NDO-level effects in this experiment. Therefore, dietary NDO levels should be similar to strengthen this hypothesis, and to study true differences between types of NDO.

From this experiment, it was concluded that exchanging cellulose for FOS or TOS in diets of young growing pigs resulted in a temporary lowered feed intake and apparent daily weight gain. However, dietary NDO didn't affect mean growth performance during six weeks. Dietary NDO did not affect faecal pH but lowered faecal dry matter content. The latter was more pronounced for TOS than for FOS. Faecal pH increases with age. Both FOS and TOS could not be detected in the faeces. Observations on local and systemic immune responses are needed to test the alleged relation between changes in microflora ecology, feed intake depression, and immune system. Effects of NDO on nutrient digestion and microflora ecology in young pigs will be studied in future, elucidating the role of dietary NDO as functional feed ingredients.

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CHAPTER

2

APPARENT ILEAL AND TOTAL TRACT NUTRIENT DIGESTION BY PIGS AS AFFECTED BY DIETARY NON-DIGESTIBLE OLIGOSACCHARIDES

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Table 1. *Composition of the diets fed to the growing pigs*

	Diet[1]				
Type	CON	FOS		TOS	
Level	0	L	H	L	H
Ingredient (g/kg as fed)					
Raftilose P95®	0.0	7.5	15.0	0.0	0.0
Oligostroop®	0.0	0.0	0.0	10.0	20.0
Glucose	117.8	117.4	117.0	113.2	108.5
Cellulose	30.0	22.9	15.8	24.6	19.3
Corn	633.6	633.6	633.6	633.6	633.6
Protein sources[2]	150.0	150.0	150.0	150.0	150.0
Soyoil	10.0	10.0	10.0	10.0	10.0
Fumaric acid	10.0	10.0	10.0	10.0	10.0
Mineral mix[3]	32.4	32.4	32.4	32.4	32.4
Amino acids[4]	5.2	5.2	5.2	5.2	5.2
Premix[5]	10.0	10.0	10.0	10.0	10.0
Chromium oxide[6]	1.0	1.0	1.0	1.0	1.0
DM content[7]					
Period I	893.8	895.7	894.6	899.3	894.1
Period II	889.8	884.2	884.5	888.3	892.2
Analyzed DM composition[8]					
Inorganic matter	60.9	60.3	60.7	60.7	61.1
Crude protein	190.6	190.9	190.1	189.8	191.1
Ether extract	49.0	48.7	49.2	48.7	49.3
Crude fiber	40.4	34.4	29.2	35.5	32.0
N-free extract	659.1	665.6	670.7	665.2	666.5

[1]CON: no non-digestible oligosaccharides; FOS: fructooligosaccharides-rich Raftilose P95® at 7.5 (L) and 15.0 (H) g/kg diet; TOS: transgalactooligosaccharides-rich Oligostroop® at 10.0 (L) and 20.0 (H) g/kg diet.
[2]Casein, fish meal, and animal meal: 50 g/kg diet each.
[3]This mineral mix provided (per kg feed) 2.0 g NaCl, 8.0 g $CaCO_3$, 9.4 g CaH_2PO_4, 2.0 g MgO, and 11.0 g $KHCO_3$.
[4]Added synthetic amino acids (per kg feed) were 2.6 g L-lysine HCl, 1.1 g DL-methionine, 0.8 g L-threonine, and 0.7 g L-tryptophane.
[5]The vitamin/mineral mix provided (per kg feed): 9,000 IU vitamin A, 1,800 IU vitamin D_3, 40 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 5 mg riboflavin, 12 mg d-panthothenic acid, 1 mg folic acid, 3 mg pyridoxine, 30 mg niacin, 40 µg cobalamin, 1,000 mg choline chloride, 50 mg vitamin C, 0.1 mg biotin, 2.5 mg $CoSO_4 \cdot 7H_2O$, 0.2 mg $Na_2SeO_3 \cdot 5H_2O$, 0.5 mg KI, 400 mg $FeSO_4 \cdot 7H_2O$, 60 mg $CuSO_4 \cdot 5H_2O$, 70 mg MnO_2, and 300 mg $ZnSO_4$.
[6]In diets of period II only (at the expense of corn).
[7]Period I (faeces collection) and Period II (digesta collection) after 32 to 36 and 42 to 46 d on the diets, respectively.
[8]For analyses see Material and Methods section.

Growing Pigs. Table 1 gives the ingredients and analyzed chemical composition of the diets fed to the growing pigs. Corn was analyzed via high performance anion exchange chromatography (**HPAEC**; Rocklin and Pohl, 1983), and contained 300 mg GOS/kg (K.J.M. van Laere, unpublished data). Thus, the control (**CON**) diet contained 0.19 g GOS/kg. Raftilose P95® was included at 7.5 and 15 g/kg and Oligostroop® at 10 and 20 g/kg, resulting in (**F-L**) 6.8 and (**F-H**) 13.5 g NDO/kg diet for FOS and (**T-L**) 4 and (**T-H**) 8 g NDO/kg diet for TOS, respectively.

The diets were fed to twenty-five 57-d-old pigs, averaging 15.9 ± 0.6 kg on d 0 of the experiment. Five pigs were used per diet. The pigs had been fed the diets for 3 wk (Houdijk *et al.*, Chapter 1), when they were being moved to the cages. The pigs were allowed a 7-d adaptation period to the cages before faeces were collected. Feeds were offered twice daily as a slurry (1:3 feed:water, prepared 15 min before feeding).

Weanling Pigs. Table 2 gives the ingredients and analyzed chemical composition of the diets fed to the weanling pigs. Oat husk meal was used to study fiber digestion; this fiber source did not contain FOS or TOS, as analyzed by HPAEC (K.J.M. van Laere, unpublished data). Raftilose P95® was included at (**F10**) 11.1 and (**F40**) 44.3 g/kg, and Oligostroop® was included at (**T10**) 22.4 and (**T40**) 89.6 g/kg diet. These inclusion levels resulted in 10 and 40 g NDO/kg diet; this batch of Oligostroop® contained more TOS than that used with the growing pigs. The mass balance was completed by minor changes in the amount of cornstarch.

Thirty 30-d-old pigs were obtained. Before being weaned at 28 d of age, the pigs received an NDO-free creep feed from d 10 of age onwards, without antibiotics or additional copper. After being weaned and transported to the experimental unit, the pigs were allowed an 8-d adaptation period during which they received the same NDO-free diet. Then twenty pigs (selected on BW and health) were ranked on BW and divided into four weight classes, averaging 10.4 ± 0.8 kg. Pigs from each weight class were randomly allocated to the experimental diets (four pigs per diet). Before faeces collection, the pigs were allowed a 13-d adaptation period to the experimental diets, which included a gradual replacement of the creep feed during 3 d. Feeds were offered twice daily; water was available for 1 h during feeding.

Sample Collections

General. After the adaptation period mentioned, faeces were quantitatively collected for 24 h/d on five successive days using big faecal bags, sized 20 x 30 cm, for the growing pigs (Van Kleef *et al.*, 1994), and small bags, sized 14 x 18 cm, for the weanling pigs (Combihesive®, Squibb B.V., Rijswijk, The Netherlands). The bags were replaced twice daily, weighed, and stored at -20°C pending analysis. Weanling pigs' urine was collected via funnels underneath the cages through filters into buckets with 5 mL 6.0 *N* HCl to avoid volatilization of nitrogenous compounds. The urine production was recorded daily and stored at 4°C pending analysis. The small intestinal digesta were collected using different methods for growing and weanling pigs.

Table 2. *Composition of the diets fed to the weanling pigs*

	Diet[1]				
Type	CON	FOS		TOS	
Level	0	10	40	10	40
Ingredient (g/kg as fed)					
Raftilose P95®	0.0	11.1	44.3	0.0	0.0
Oligostroop®	0.0	0.0	0.0	22.4	89.6
Cellulose	42.0	32.0	1.8	32.0	1.8
Glucose	150.0	149.4	147.7	142.9	121.7
Corn starch	470.75	470.25	468.95	465.45	449.65
Casein	185.0	185.0	185.0	185.0	185.0
Oat husk meal	60.0	60.0	60.0	60.0	60.0
Soyoil	25.0	25.0	25.0	25.0	25.0
Amino acids[2]	3.0	3.0	3.0	3.0	3.0
Minerals[3]	54.0	54.0	54.0	54.0	54.0
Premix[4]	10.0	10.0	10.0	10.0	10.0
Chromium oxide	0.25	0.25	0.25	0.25	0.25
DM content[5]					
Period I	888.1	886.9	886.6	880.5	879.2
Period II	887.3	890.6	893.2	889.3	889.7
Analyzed DM composition[6]					
Inorganic matter	50.0	50.3	49.8	50.6	51.4
Crude protein	189.7	191.8	190.1	192.0	198.8
Ether extract	21.1	20.5	19.3	20.3	21.4
Hemicellulose	19.9	20.8	23.1	21.0	23.2
Cellulose	55.3	44.9	19.5	46.0	21.1
Lignine	6.1	6.6	7.4	6.2	6.4
Starch	469.2	467.6	459.1	465.2	452.3
NNSC	188.7	197.5	231.7	198.7	225.4
Ca	9.9	9.9	9.9	10.0	9.9
P	5.9	5.8	5.8	5.9	5.9
Mg, mg/kg	468.9	496.8	484.5	462.6	444.8
Fe, mg/kg	159.2	170.3	162.5	162.8	159.4
Cu, mg/kg	18.4	15.7	18.9	18.3	16.6
Zn, mg/kg	85.8	89.9	93.9	89.8	83.7

[1]CON: no non-digestible oligosaccharides (NDO); FOS: fructooligosaccharides; TOS: transgalactooligosaccharides; both NDO at 10.0 or 40.0 g/kg diet.
[2]Amino acids (g/kg feed): 1.7 L-cysteine, 0.8 L-threonine, and 0.5 L-tryptophane.
[3]Minerals (g/kg feed): 5.0 NaCl, 10.0 $CaCO_3$, 20.0 $CaHPO_4$, 0.5 MgO, 16.5 $KHCO_3$, and 2.0 $NaHCO_3$.
[4]Premix (per kg feed): 9,000 IU vitamin A, 1,800 IU vitamin D_3, 40 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 5 mg riboflavin, 12 mg d-panthothenic acid, 1 mg folic acid, 3 mg pyridoxine, 30 mg niacin, 40 µg cobalamin, 1,000 mg choline chloride, 50 mg vitamin C, 0.1 mg biotin, 2.5 mg $CoSO_4 \cdot 7H_2O$, 0.2 mg $Na_2SeO_3 \cdot 5H_2O$, 0.5 mg KI, 400 mg $FeSO_4 \cdot 7H_2O$, 40 mg $CuSO_4 \cdot 5H_2O$, 70 mg MnO_2, and 200 mg $ZnSO_4$.
[5]Dietary DM in Period I (faeces collection) and Period II (digesta collection) after 13 to 17 and 33 to 37 d on the diets, respectively.
[6]For analyses see Materials and Methods section. NNSC: nonstarch neutral-detergent soluble carbohydrates.

Growing Pigs. Nine to 13 d after faeces collection, 3.0 ± 0.1 h after the morning meal, the pigs were anesthetized with O_2/N_2O and halothane. The abdomen was opened, and the last 7 m of the small intestine (SI2) was isolated with clamps to prevent digesta

movement. The SI2 was removed and the pigs were killed. The SI2 content was determined, and digesta obtained were stored at -20°C pending analysis.

Weanling Pigs. After faeces collection, the pigs were surgically fitted with a post-valve T-caecum cannula (Van Leeuwen *et al.*, 1991). After recovery of at least 10 d, ileal digesta were collected during two periods of two successive days, which were separated by 1 d. Little plastic bags, sized 15 x 20 cm, were attached to the cannula, which had been open for at least 1 h before the morning meal. During 12 h following the morning meal, the bags were hourly replaced and weighed. Digesta were stored at -20°C pending analysis.

Chemical Analysis and Calculations

Feed, faeces, and digesta were analyzed for DM (ISO, 1983), inorganic matter (**IM**; ISO, 1978), CP (6.25 x Kjeldahl N; ISO, 1979), ether extract (**EE**; ISO, 1996), and crude fiber (**CF**; NEN, 1988). The DM - IM equaled **OM**; OM - CP - EE - CF equaled N-free extract (**NFE**). Additional analysis for weanling pigs' samples were ADF, ADL (both according to Van Soest, 1973), NDF, and starch (**St**, both as described by Goelema *et al.*, 1998). The NDF - ADF equaled hemicellulose (**Hemi**) and ADF - ADL equaled cellulose (**Cel**). The ADL was analyzed in feeds only. The OM - CP - EE - NDF - St equaled nonstarch neutral-detergent soluble carbohydrates (**NNSC**), which should include NDO. Indeed, the differences between NNSC of the experimental diets closely reflected the inclusion levels of NDO (Table 2). Most minerals (Ca, Mg, Fe, Cu, Zn, and Cr) were analyzed using atomic absorption spectophotometry (De Ruig, 1986). Phosphorus was analyzed via spectophotometry (AOAC, 1975). Urine was analyzed for N and minerals.

Apparent faecal (**FD**) and ileal digestion (**ID**) were calculated according to Eq. [1] and [2], respectively. The N and mineral balances were described by intake, total excretion (faecal + urinary), and retention (intake - total excretion). Urinary excretion was also expressed as a percentage of total excretion.

$$FD\text{-}a = ((DI_a - DFE_a) / (DI_a)) \times 100 \quad [1]$$

where DI_a = daily intake of nutrient a (g)
DFE_a = daily excretion of nutrient a via the faeces (g)

$$ID\text{-}a = (1 - ((D_a \times F_{Cr}) / (D_{Cr} \times F_a))) \times 100 \quad [2]$$

where D_a = concentration nutrient a in ileal digesta (g/kg)
D_{Cr} = concentration chromium in ileal digesta (mg/kg)
F_a = concentration nutrient a in feed (g/kg)
F_{Cr} = concentration chromium in feed (mg/kg)

Statistical Analysis

The FD was analyzed for 22 pigs (CON: 5, F-L: 4, F-H: 4, T-L: 5, and T-H: 4) and the ID for 18 pigs (CON: 5, F-L: 3, F-H: 3, T-L: 4, and T-H: 3) in the experiment with growing pigs. The FD and balances were analyzed for 17 weanling pigs (CON: 4, F10: 4, F40: 4, T10: 3, and T40: 2) and the ID for 16 weanling pigs (CON: 3, F10: 3, F40: 3, T10: 4, and T40: 3). The excluded pigs had diarrhea; when possible, reserve pigs were included. Hindgut contribution to FD was not calculated from FD and ID as these parameters were determined with different feed intake and different time on the diets.

An analysis of variance was conducted using the model given by Eq. [3]. Results are expressed as least squares means and pooled SEM of D_i. Predetermined orthogonal contrasts were used to locate the effects of dietary NDO. Type (FOS vs TOS), level (L vs H with growing pigs or 10 vs 40 g/kg with weanling pigs), type x level, and the effect of NDO inclusion per se could be distinguished. The contrasts were considered to be significant when $P < 0.05$. The Tukey-Kramer multiple comparisons test was used to clarify observed contrasts. However, contrast statements are much more powerful to locate effects than are multiple comparisons (Lowry, 1992).

$$y_{ij} = \mu + D_i + \varepsilon_{ij} \qquad [3]$$

where y_{ij} = observation
μ = general mean
D_i = effect of diet (i=1, ..., 5)
ε_{ij} = residual error.

Results

Growing Pigs. Table 3 shows apparent FD and ID in the growing pigs at average BW of 34.9 ± 0.8 kg and 45.5 ± 1.3 kg, respectively. Dietary NDO did not significantly affect the FD. An interaction was observed for the ID-CF ($P < 0.001$); an increase of dietary TOS but not of FOS significantly increased ID-CF. Dietary NDO did not affect the ID of the other nutrients.

Table 3. *Feed intake, faeces production, distal small intestinal digesta, and apparent faecal and ileal digestion of nutrients in growing pigs fed diets with or without non-digestible oligosaccharides*

	Diet[1]					
Type	CON	FOS		TOS		
Level	0	L	H	L	H	SEM
Feed intake and faeces production, g/d						
Feed[2]	1228	1174	1194	1148	1102	48
Faeces	561	444	476	501	433	57
Apparent faecal digestion, %						
Dry matter	86.6	86.9	86.8	86.5	86.9	0.6
Inorganic matter	54.6	56.2	53.5	53.4	51.8	1.4
Organic matter	88.6	88.9	89.0	88.6	89.1	0.6
Crude protein	83.3	83.4	82.7	82.2	83.4	1.0
Ether extract	82.8	81.7	82.7	82.1	81.6	2.1
Crude fiber	44.2	43.5	35.8	40.9	44.4	3.7
N-free extract	93.4	93.4	93.5	93.5	93.5	0.3
Feed intake, g/d, and distal small intestinal digesta, g						
Feed[3]	1410	1353	1393	1358	1297	68
Digesta	473	535	485	570	419	63
Apparent ileal digestion, %						
Dry matter	74.4	71.8	73.7	73.6	72.9	2.1
Inorganic matter	37.3	28.5	36.9	32.5	32.0	4.9
Organic matter	76.8	74.6	76.1	76.3	75.6	1.9
Crude protein	64.4	54.4	58.9	60.4	50.9	6.6
Ether extract	82.3	80.0	80.6	82.0	78.0	4.7
Crude fiber[4]	13.0[ab]	8.5[b]	7.4[b]	2.3[b]	20.6[a]	2.4
N-free extract	83.6	83.4	83.6	84.4	85.1	1.2

[1]See Table 1
[2]Diets fed for 28 to 32 d
[3]Diets fed for 42 to 47 d
[4]Contrasts Level (H vs L): $P < 0.001$ and Type x Level: $P < 0.001$; other contrasts throughout the table were not significant
[a,b]LSMeans lacking common superscripts differ significantly ($P < 0.05$ by Tukey-Kramer)

Weanling Pigs. Table 4 shows apparent FD and ID in the weanling pigs at average BW of 15.6 ± 0.3 kg and 20.3 ± 0.3 kg, respectively. The NDO-fed pigs produced less faeces than the control pigs ($P < 0.10$). Further, pigs fed the 40 g NDO/kg diets produced less faeces than pigs fed the 10 g NDO/kg diets ($P < 0.10$) and had higher FD-OM ($P < 0.05$). The FOS-fed pigs had lower FD-CP than the TOS-fed pigs ($P < 0.10$). The FD-Cel was lowered in the NDO-fed pigs compared to the control pigs ($P < 0.10$). Starch was practically completely digested, but the FD-St was higher in NDO-fed pigs ($P < 0.10$) than in the control pigs. The NDO-fed pigs had higher FD-NNSC than the control pigs, and this was higher for the 40 g NDO/kg diets than for the 10 g NDO/kg diets ($P < 0.001$).

Table 4. *Feed intake, faeces and ileal digesta production, and apparent faecal and ileal digestion in weanling pigs fed diets with or without non-digestible oligosaccharides*

	Diets[1]						Orthogonal contrasts[2]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x
Level	0	10	40	10	40	SEM				level
Feed intake and faeces production, g/d										
Feed[3]	608	622	553	587	595	24	NS	NS	NS	NS
Faeces	116	117	82	100	90	10	‡	NS	‡	NS
Apparent faecal digestion, %										
DM[4]	90.9	90.5	92.4	91.3	92.6	0.7	NS	NS	*	NS
IM	78.3	77.6	75.4	76.5	77.6	2.9	NS	NS	NS	NS
OM	91.5	91.2	93.3	92.1	93.4	0.6	NS	NS	*	NS
CP	93.7	93.9	93.6	95.1	94.6	0.6	NS	‡	NS	NS
EE	78.4	79.2	80.4	79.2	83.1	2.3	NS	NS	NS	NS
Hemi	5.3	1.8	21.9	11.2	15.9	8.1	NS	NS	NS	NS
Cel	49.3	29.4	26.2	41.8	24.1	9.6	‡	NS	NS	NS
St	99.98	99.98	99.99	99.99	99.99	0.01	‡	NS	NS	NS
NNSC	94.1^{c}	95.6^{bc}	96.8^{ab}	95.1^{c}	97.2^{a}	0.4	***	NS	***	NS
Feed intake and ileal digesta production, g/d										
Feed[5]	763	793	747	753	727	19	NS	NS	‡	NS
Digesta	575	599	777	699	596	71	NS	NS	NS	‡
Apparent ileal digestion, %										
DM[4]	87.7^{b}	87.4^{b}	88.0^{ab}	86.7^{b}	90.4^{a}	0.5	NS	NS	***	*
IM	74.9	76.0	72.2	73.7	77.6	2.2	NS	NS	NS	‡
OM	88.4^{b}	88.0^{b}	88.9^{b}	87.4^{b}	91.1^{a}	0.5	NS	NS	***	*
CP	89.4	87.7	87.6	86.8	89.1	1.4	NS	NS	NS	NS
EE	86.8	85.2	85.3	84.5	89.6	2.2	NS	NS	NS	NS
Hemi	10.4^{c}	14.5^{bc}	49.7^{ab}	8.8^{c}	60.0^{a}	7.9	*	NS	***	NS
Cel	25.5^{b}	22.4^{b}	64.9^{a}	16.8^{b}	75.9^{a}	4.7	***	NS	***	NS
St	99.7	99.7	99.6	99.7	99.6	0.03	NS	NS	*	NS
NNSC	89.1^{a}	86.3^{ab}	77.6^{c}	86.9^{ab}	83.2^{b}	0.9	***	**	***	*
Ca	78.4	79.2	80.4	80.5	80.9	3.1	NS	NS	NS	NS
P	85.5	84.1	86.5	86.4	86.7	2.2	NS	NS	NS	NS
Mg	34.6	35.7	36.8	33.1	39.5	4.6	NS	NS	NS	NS
Fe	-4.1	-5.9	-39.0	-6.0	-12.3	14.3	NS	NS	NS	NS
Cu	43.5	34.0	44.3	42.9	37.1	2.4	NS	NS	NS	**
Zn	28.8	20.1	30.7	34.2	30.3	3.5	NS	‡	NS	‡

[1]See Table 2
[2]NS: $P > 0.10$, ‡: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$
[3]Diets fed for 13 to 18 d
[4]See Material and Methods
[5]Diets fed for 33 to 37 d
[a,b,c]LSMeans lacking common superscripts differ significantly ($P < 0.05$ by Tukey-Kramer)

Ileal digesta production increased with the increased level of FOS and decreased with the increased level of TOS ($P < 0.10$). The NDO-fed pigs had higher ID of Hemi ($P < 0.05$) and Cel ($P < 0.001$) but lower ID of NNSC ($P < 0.001$) than the control pigs. The ID of OM, Hemi, and Cel was higher for the 40 g than for the 10 g NDO/kg diets ($P < 0.001$); this effect was the opposite for the ID-St ($P < 0.05$) and ID-NNSC ($P < 0.001$). Further, the FOS-fed pigs had lower ID-NNSC than the TOS-fed pigs ($P < 0.01$). However, interactions were observed for ID-OM, ID-NNSC ($P < 0.05$), and ID-IM ($P < 0.10$). On the average, dietary NDO did not affect ID of minerals, though interactions were observed for ID-Cu ($P < 0.01$) and ID-Zn ($P < 0.10$). The FOS-fed pigs had lower ID-Zn than the TOS-fed pigs ($P < 0.10$).

Table 5 shows the balances for N, Ca, and P; table 6 shows the blances for Mg, Fe, Cu, and Zn. The TOS-fed pigs excreted relatively more N via the urine than the FOS-fed pigs ($P < 0.01$) and the control pigs. Dietary NDO did not significantly affect retention of N and minerals on a g/d basis (Table 5 and 6) or when expressed as percentage of intake (data not shown). However, some effects were observed for mineral intake. Magnesium intake was higher in the FOS-fed pigs than in the TOS-fed pigs, and pigs fed the 10 g NDO/kg diets had higher Mg and lower Fe intake than pigs fed the 40 g NDO/kg diets ($P < 0.10$), the latter being more pronounced in FOS- than in TOS-supplemented diets ($P < 0.10$). The NDO-fed pigs had a negative Fe retention, which did not differ from the control pigs. The NDO-fed pigs had lower Cu intake ($P < 0.05$), but Cu balance was not affected.

Discussion

Several NDO may potentially be regarded as prebiotics, recently defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and(or) activity of one or a limited number of bacteria in the colon, and thus improve host health (Gibson and Roberfroid, 1995). In animal nutrition, prebiotics may be used as feed additives with similar beneficial claims. However, information on effects of NDO in pigs is limited, especially with regard to nutrient digestion.

We included NDO up to 40 g/kg diet at the expense of purified cellulose. Therefore, the total amount of potential fermentable components was comparable between diets but its fermentability shifted from slow (cellulose) to rapid (NDO). The NDO fermentation rates are only slightly lower than that of glucose (Houdijk *et al.*, Chapter 5), while fermentation of purified cellulose is low (Sunvold *et al.*, 1995). The NDO inclusion is thus confounded with cellulose removal. A placebo effect has likely been limited; cellulose did not significantly

Table 5. *Nitrogen, calcium, and phosphorus balance in weanling pigs fed diets with or without non-digestible oligosaccharides*

	Diet[1]					
Type	CON	FOS		TOS		
Level	0	10	40	10	40	SEM
Nitrogen, g/d						
Intake[2]	16.36	16.94	14.90	15.86	16.64	0.65
Total excretion[2]	3.78	3.72	3.50	3.56	4.12	0.21
Retention	12.58	13.21	11.39	12.30	12.51	0.59
Excretion via urine[3], %	72.6	72.3	72.9	78.3	78.4	1.8
Faecal digestion[4], %	93.7	93.9	93.6	95.1	94.6	0.6
Calcium, g/d						
Intake	5.34	5.45	4.83	5.14	5.16	0.21
Total excretion	1.61	1.63	1.59	1.66	1.57	0.24
Retention	3.73	3.81	3.24	3.48	3.59	0.37
Excretion via urine, %	13.9	12.0	3.6	9.9	7.8	4.2
Faecal digestion, %	73.3	73.4	67.5	70.2	71.6	5.7
Phosphorus, g/d						
Intake	3.18	3.17	2.83	3.03	3.09	0.12
Total excretion	0.66	0.67	0.65	0.64	0.57	0.10
Retention	2.52	2.51	2.19	2.39	2.52	0.18
Excretion via urine, %	1.2	1.6	3.1	2.0	1.8	0.7
Faecal digestion, %	79.3	79.2	77.5	79.2	81.6	3.8

[1]See Table 2. Diets were fed for 13 to 18 d.
[2]Contrast Type x Level: $P < 0.10$
[3]Contrast FOS vs TOS: $P < 0.01$
[4]Contrast FOS vs TOS: $P < 0.10$

affect ID and FD of crude protein, amino acids, Na, K, Mg, and P in weanling and growing pigs; only FD-Ca, but not ID-Ca, was significantly lowered (Den Hartog *et al.*, 1988; Li *et al.*, 1994).

Dietary NDO did not affect nutrient FD in the growing pigs but in the weanling pigs FD-DM increased with increasing levels of dietary NDO. Younger pigs may be more sensitive to changes in fermentable dietary components, though a more likely explanation is the higher NDO levels used. An increase of the FD-DM may have resulted directly from replacing Arbocel with NDO. Assuming FD-Cel from oat husk meal was 10% (CVB, 1995), the FD-Arbocel for the control diet was 60%. Given that NDO were not recovered from faeces of NDO-fed pigs, the FD-NDO can be assumed 100% (Houdijk *et al.*, Chapter 1). This agrees with the increase of FD-NNSC for NDO-supplemented diets. Since the FD-Cel for the NDO-supplemented diets was reduced ($P < 0.10$), the FD-Arbocel may also have been reduced. Corrected for this, calculated FD-DM was 90.4%, 92.5%, 91.0%, and

Table 6. *Magnesium, iron, copper, and zinc balance in weanling pigs fed diets with or without non-digestible oligosaccharides*

	Diet[1]					
Type	CON	FOS		TOS		
Level		10	40	10	40	SEM
Magnesium, mg/d						
Intake[2,3]	253.0	274.3	237.4	238.9	232.7	10.2
Total excretion	119.6	117.7	96.9	98.8	93.0	10.5
Retention	133.3	156.6	140.4	140.1	139.7	11.9
Excretion via urine, %	6.2	6.4	3.4	8.3	7.5	1.7
Faecal digestion, %	55.6	60.0	60.1	61.8	62.9	3.9
Iron, mg/d						
Intake[3,4]	85.9	94.0	79.6	84.1	83.4	3.5
Total excretion	83.3	96.7	92.1	93.9	89.6	11.8
Retention	2.6	-2.7	-12.5	-9.7	-6.2	14.0
Excretion via urine, %	0.7	0.7	0.6	0.5	0.5	0.1
Faecal digestion, %	3.3	-3.2	-17.1	-8.1	-8.9	16.4
Copper, mg/d						
Intake[4]	9.9	8.7	9.2	9.4	8.7	0.4
Total excretion[5]	7.4	7.0	6.0	6.6	6.4	0.6
Retention	2.5	1.7	3.2	2.8	2.3	0.7
Excretion via urine[6], %	4.8	3.7	3.6	3.7	3.2	0.6
Faecal digestion, %	29.5	22.3	36.0	33.3	28.0	6.9
Zinc, mg/d						
Intake	46.3	49.6	46.0	46.4	43.8	1.9
Total excretion	40.2	37.6	34.7	39.4	32.4	3.1
Retention	6.0	12.0	11.3	7.0	11.4	4.4
Excretion via urine, %	2.5	3.5	3.1	3.6	3.0	0.7
Faecal digestion, %	14.2	26.6	25.8	19.9	27.2	9.3

[1]See Table 2. Diets were fed for 13 to 18 d.
[2]Contrast FOS vs TOS: $P < 0.10$
[3]Contrast 10 vs 40 g NDO/kg diet: $P < 0.10$
[4]Contrast Type x Level: $P < 0.10$
[5]Contrast CON vs NDO-supplemented diets: $P < 0.05$
[6]Contrast CON vs NDO-supplemented diets: $P < 0.10$

92.5%, for the F10, F40, T10, and T40 diet, respectively. These are very close to the FD-DM observed, thus the true effect of NDO on FD-DM may have been limited. Indeed, FOS and TOS did not affect FD-DM in rats (Berggren *et al.*, 1993; Kikuchi *et al.*, 1996) or in pigs with TOS exchanged at the expense of starch (R. Kamelaar, unpublished data).

Dietary NDO per se did not affect the FD-CP. The absence of effects on FD-CP was also observed with lactulose in minipigs (Ahrens and Schön, 1988) and GOS in dogs (Zuo *et al.*, 1996) and in rats (Fleming and Lee, 1983). However, faeces from the FOS-fed pigs contained more CP than faeces from the TOS-fed pigs (65.0 vs 55.1 g/kg, SEM 2.1, $P <$

0.05) and the control pigs (55.6 g/kg). The TOS-fed pigs excreted less of their nitrogen via the faeces and more via the urine compared to the FOS-fed pigs and the control pigs (Table 5). Therefore, the FD-CP was higher for the TOS-fed pigs than for the FOS-fed pigs ($P < 0.05$) and the control pigs, but apparent nitrogen retention was not affected. Apparently, the increased amount of nitrogen absorbed from the intestinal lumen of our TOS-fed pigs was not used and excreted via the urine.

Dietary NDO did not significantly affect nutrient ID in the growing pigs. This was partly due to the relatively large variation observed, especially for CP, which may have been caused by the sampling size used in the slaughter technique. It was expected that digestion of this (calculated) highly digestible diet had completed relatively proximal in the small intestine. Apparently, digestion had not been completed for digesta obtained from the proximal part of the section used for sampling. In addition, transit time may vary between animals, which further attributed to the variation observed for nutrient ID in the growing pigs. In the weanling pigs, the ID-DM for diet T40 was higher than that of the other diets ($P < 0.05$), resulting in the significant contrasts observed. Dietary treatments did not significantly affect the ID-EE and ID-CP. The latter is in agreement with data on TOS (Gabert *et al.*, 1995) and galactosyllactose (Mathew *et al.*, 1997). Since type and level of dietary NDO only tended to interact for the ID-IM, differences in ID-DM between the FOS- and TOS-fed pigs were mainly related to the carbohydrate fraction.

Starch was nearly completely digested pre-caecally; nonstarch polysaccharides (**NSP**) may partly be degraded pre-caecally in pigs. Up to 44 g NSP from various wheat and oat diets were metabolized pre-caecally in 45 to 50 kg pigs, including 4.9 to 6.3 g cellulose from the oat diets (Bach Knudsen and Hansen, 1991). Similarly, pre-caecal loss of cellulose from a rutabaga-based diet was reported in 85- to 90-kg pigs, additionally showing significant concentrations of cellulolytic anaerobes in ileal digesta (Chesson *et al.*, 1985). In our pigs, pre-caecally metabolized cellulose ranged from 3.5 to 11.9 g/d and did not differ between diets. Therefore, the increased ID-Cel resulted from differences in cellulose intake. Similar effects were observed for the TOS diets but not for the FOS diets for the growing pigs. Differences may be due to different CF intake and the possible separation of the digestibility marker and fiber fraction at the time of digesta collection (Graham and Åman, 1986a).

Dietary NDO increased the ID-Hemi, indicating an increased pre-caecal degradation and/or solubility of hemicellulose. Fiber solubility is critical in the determination of hemicellulose as a NDF component. The hemicellulose mainly originated from oat husk meal. The main fibers from oats are (Bach Knudsen and Hansen, 1991) cellulose

(insoluble), arabinoxylan (partly soluble, 25%), and ß-glucan (largely soluble, 85%). *Bacteroides ovatus* may degrade arabinoxylan to oligosaccharides; *Bifidobacterium longum* may ferment arabinoxylan (Van Laere *et al.*, 1997). These species can also ferment FOS and TOS (Hartemink and Rombouts, 1997). Pre-caecal bacterial action may reduce the molecular weight of ß-glucan 7- to 35-fold (Johansen *et al.*, 1993). Both processes may have been enhanced pre-caecally in NDO-fed pigs as a result of an increased bacterial activity.

The FOS- and TOS-fed pigs had different ID-NNSC. This difference may have originated from both diet and endogenous secretions. The first may indicate that the ID-FOS was less than the ID-TOS. However, the ID-FOS averaged 92.3 ± 1.4% in this experiment (HPAEC, data not shown); in another study, the ID-TOS averaged 30% (R. Kamelaar, unpublished results). The FOS may also have stimulated a greater ileal flow of mucin or soluble bacterial products than TOS. Indeed, the FOS-fed pigs had more bacteria in their ileal digesta than the TOS-fed pigs (Houdijk *et al.*, Chapter 4). Further, the ID-NNSC may have decreased due to an increased hemicellulose solubility. More details on ileally recovered carbohydrates are needed to satisfactorily explain the differences in ID-Hemi and ID-NNSC values observed.

Our experiments indicate that the effects of NDO on nutrient digestion of highly digestible diets are small. This apparently contrasts with data on GOS, which have long been known to be detrimental for animal performance (Saini, 1989). While several techniques have been used to remove GOS, only ethanol extraction satisfactorily improved nutritional quality (Coon *et al.*, 1990). Velasse's negative effect on nutrient digestion was not compensated by the addition of α-galactosidase, although the ID-GOS increased from 57 to 93% (Veldman *et al.*, 1993). Replacing high- with low-oligosaccharide soybean meal lowered GOS from 31 to 4 g/kg diet but nutrient digestion was not improved in dogs (Zuo *et al.*, 1996). This may indicate that extraction not only removes GOS but also other more detrimental factors; GOS may act as ANF only when intake exceeds a certain threshold level (Benno *et al.*, 1993).

Dietary NDO did not affect the mineral FD or retention. Rat studies showed improvement of mineral absorption with FOS (Ohta *et al.*, 1995) and TOS (Chonan and Watanuki, 1995), but also with other NDO, including lactulose and raffinose (Brommage *et al.*, 1993). The extent of improvement depended on the type of mineral, mineral deficiency, and type of NDO. The absence of effects of dietary NDO in our study is in agreement with the absence of effects of inulin on faecal mineral availability in pigs (Vanhoof and De Schrijver, 1996). The effects observed in Mg-, Fe-, and Cu intake likely resulted from the

combination of differences in feed intake realized and dietary minerals analyzed. The decreased Cu intake for the NDO-fed pigs seemed to be regulated partly at the renal level (lower excretion of Cu via the urine). Most effects of dietary NDO on mineral ID were not significant. This is in agreement with the lack of significant effects of FOS and inulin on ileal secretion of minerals in ileostomy patients and pigs (Vanhoof and De Schrijver, 1996; Ellegard *et al.*, 1997). The effects observed for ID-Zn were probably due to a different Zn intake, which was 63 and 57 mg/d for the FOS- and TOS-fed pigs, respectively (SEM 2, $P < 0.01$). The effects observed for ID-Zn were likely caused by differences in Zn intake between the TOS- and FOS-fed pigs (63.1 and 57.5 mg/d, SEM 1.8, $P < 0.01$). Similarly with Cu, the interaction between type and level of dietary NDO was significant for Cu intake (data not shown). The Cu intake and ID-Cu were correlated ($r = 0.53$, $P < 0.05$), indicating that the pigs were fed close to their requirements for Cu.

The absence of NDO effects on mineral balances in pigs contrasts with rat studies. The NDO are usually included at 50 to 100 g/kg diet, and are often the only fermentable soluble carbohydrates in rats' diets. Consequently, a higher degree and prolonged fermentation may result, as indicated by a lowered caecal, colonic, and faecal pH (Ohta *et al.*, 1994). In our diets, NDO were included at lower levels and were accompanied by soluble fermentable carbohydrates from oat husk meal; the differences in degree of fermentation created may have been limited. Given that the faecal pH of NDO-fed pigs was not lowered, NDO-fermentation may have been completed proximally (Houdijk *et al.*, Chapter 1). Thus, dietary NDO may have affected luminal conditions for enhanced mineral absorption to a lower extent than in the rat studies cited. Another important factor is the mineral content in the diet. Dietary factors influencing mineral absorption may not be recognized if mineral levels are above requirements. Compared to the mineral requirements of 10 to 20 kg ad libitum fed pigs (NRC, 1988), only iron and copper were slightly in excess. Whether mineral requirements for restricted and ad libitum fed pigs are comparable is not known; the relatively high digestion and the positive correlation between intake and the FD, ranging from $r = 0.45$ ($P < 0.10$) for P to $r = 0.62$ ($P < 0.001$) for Fe, except for Mg, indicated that for most minerals the intake was near the requirements.

The faecal and ileal data should not be compared quantitatively in the experiments described. In addition to previous mentioned time differences between faecal and ileal observations, mean ID-Cel and ID-Hemi were higher than FD-Cel and FD-Hemi. Further, dietary NDO affected ID-Hemi and ID-Cel to a larger extent than FD-Hemi and FD-Cel. This indicates that porcine fiber digestion needs to adapt to dietary NDO, and that this was not completed within 13 d on the experimental diets.

Implications

Dietary fructooligosaccharides and transgalactooligosaccharides, included up to 40 g/kg and exchanged at the expense of purified cellulose, hardly affect apparent nutrient digestion and nitrogen and mineral balances in well-kept pigs fed highly digestible diets. Pigs may need more than 2 wk to fully adapt to non-digestible oligosaccharides. The apparent pre-caecal hemicellulose digestion may be enhanced after feeding diets supplemented with non-digestible oligosaccharides for five weeks. Fructooligosaccharides fermentation is nearly completed pre-caecally. Nitrogen excretion may partly shift from faeces to the urine for diets rich in transgalactooligosaccharides. Knowlegde about the composition of ileally recovered nonstarch carbohydrates is needed to assess the meaning of the lower apparent ileal digestion of nonstarch neutral detergent soluble carbohydrates observed for the diets supplemented with fructooligosaccharides and transgalacto-oligosaccharides.

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CHAPTER

3

EFFECTS OF FRUCTOOLIGOSACCHARIDES AND TRANSGALACTOOLIGOSACCHARIDES ON DIGESTA CHARACTERISTICS AND PORTAL VOLATILE FATTY ACIDS OF GROWING PIGS

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Effects of fructooligosaccharides and transgalactooligosaccharides on digesta characteristics and portal volatile fatty acids of growing pigs

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Abstract

Twenty-five 57-d-old pigs were fed corn-based diets with or without non-digestible oligosaccharides (**NDO**). Fructooligosaccharides (**FOS**, 6.8 and 13.5 g/kg) and transgalactooligosaccharides (**TOS**, 4.0 and 8.0 g/kg) were exchanged at the expense of purified cellulose. Diets were fed for 42 to 47 d, after which pigs were sacrificed, 3 h after the morning meal. Mean BW during dissection was 45.5 ± 1.3 kg. The gastrointestinal tract was divided into 7 sections, from stomach through rectum. Digesta were analyzed for residual NDO, pH, and DM. Volatile fatty acids were determined in caecal- and proximal colon digesta, and in the portal plasma.

No NDO were recovered from stomach and large intestinal digesta, but only from the second part of the small intestine. NDO-fed pigs had a lower stomach pH than the control pigs ($P < 0.10$). Colonic digesta of FOS-fed pigs had a higher pH than that of TOS-fed pigs ($P < 0.01$). The FOS-fed pigs had a significantly lower proximal colon and portal plasma VFA concentration than the TOS-fed pigs. The pH and VFA concentrations of the control pigs was intermediate. Our results indicated that fermentation for the FOS-diets was nearly complete 3 h after feeding, while fermentation was progressing for the TOS-diets.

Introduction

Certain dietary oligosaccharides are not digested, since the endogenous secretions of pigs have no enzymes which can hydrolyze these carbohydrates. Therefore, these non-digestible oligosaccharides (**NDO**) are more likely degraded by the gastrointestinal microflora. It has been claimed that certain NDO may selectively stimulate beneficial microbial activity, and thus improve host health (Gibson and Roberfroid, 1995). As such, these so-called "prebiotics" may form a new class of feed additives. Fructooligosaccharides (**FOS**) are β-linked fructose monomers, and are found in barley and wheat (Henry and Saini, 1989). Transgalactooligosaccharides (**TOS**) are β-linked galactose units. The TOS are not found in feedstuffs, but may be present in yogurts (Toba *et al.*, 1983).

The effect of FOS and TOS on growth performance in well-kept growing pigs was limited. Neither NDO could be recovered from the faeces of these pigs (Houdijk *et al.*, Chapter 1). Thus, FOS and TOS must have been degraded, and therefore may have affected digesta characteristics, including pH and volatile fatty acid (**VFA**) composition. The VFA may be divided in two groups: on the one hand, acetic-, propionic-, and butyric acid, mainly originating from saccharolytic activity (**sVFA**), and on the other hand, *iso*-butyric-, valeric-, and *iso*-valeric acid, which are considered to mainly originate from bacterial proteolytic activity (**pVFA**). However, protein fermentation may also yield sVFA (Mortensen *et al.*, 1988).

Information on the effects of NDO on porcine digesta characteristics is scarce. Diets with 15 g FOS/kg did not affect VFA levels in large intestine digesta of weanling pigs (Farnworth *et al.*, 1992). Similarly, diets with two g FOS, galactooligosaccharides, or isomaltooligosaccharides per kg had no effect on pH, VFA, and lactic acid concentrations in weanling pigs' stomach and colonic digesta (Bolduan *et al.*, 1993). However, the control diets used in these studies were mainly composed of barley, wheat, and soybeanmeal. Considerable levels of NDO in these ingredients may have diluted or masked the effects of NDO added. Experimental diets without NDO-rich cereals and legumes should be used to study dietary NDO, both as feed component as well as feed additive. It has been shown that corn is very low in NDO; the only NDO present was raffinose, at 300 mg raffinose/kg (Houdijk *et al.*, Chapter 1). Here, a corn-based diet was used to study the effects of FOS and TOS on various physico-chemical characteristics of gastrointestinal digesta and portal plasma VFA in growing pigs.

Materials and methods

Diets, animals, and housing

Table 1 shows the ingredients and analyzed composition of the diets. Raftilose P95®, a powder with 90% FOS (Orafti, Tienen, Belgium) and Oligostroop®, a syrup with 40% TOS (Borculo Whey Products, Borculo, The Netherlands) were the NDO-rich products used in this study; other components in these products were digestible sugars, including sucrose and lactose. The diets contained no NDO (**CON**), 7.5 and 15 g/kg Raftilose P95® (**F-L** and **F-H**, respectively), and 10 and 20 g/kg Oligostroop® (**T-L** and **T-H**, respectively). The NDO-rich products were included at the expense of purified cellulose and glucose, based on their contents of NDO and digestible sugars, respectively. No antibiotics or additional copper were added to the diets. The diets met or exceeded the known nutrient and mineral requirements for growing pigs (NRC, 1988).

The diets were fed to twenty-five 57-d-old castrated pigs (Great Yorkshire x Landrace)$^{\male}$ x (Great Yorkshire)$^{\female}$ for 42 to 47 d; the first 21 d pigs were being fed *ad libitum*, followed by 30 d at 2.6 times energy required for maintenance, which was assumed to be 459 kJ ME/kg$^{0.75}$. The pigs were housed in metabolic cages. The feeds were offered in equal parts twice daily as a slurry (1:3 feed:water, prepared 15 minutes before feeding). Ethics approval was given by the Animal Ethics Committee of Wageningen Agricultural University.

$$y_{ij} = \mu + D_i + \varepsilon_{ij} \quad [1]$$

where y_{ij} = observation
μ = general mean
D_i = effect of diet (i = 1, ..., 5)
ε_{ij} = residual error.

Results

Table 2 shows the feed intake, amount of digesta (as percentage of feed intake), and the digesta DM, total protein, and pH. Average BW during dissection was 45.5 ± 1.3 kg. Dietary NDO did not significantly affect gut section nor liver weight, though the NDO-fed pigs had a heavier stomach (% of BW) than did the control pigs ($P < 0.10$, data not shown). Mean feed intake ranged from 648 to 705 g and was not significantly affected by dietary NDO. Pigs being fed the L-NDO diets tended to have less SI2 digesta than those being fed the H-NDO diets and the control diet. On average, the NDO-fed pigs tended to have more CC-digesta than did the control pigs. The T-H fed pigs had more CC digesta than the T-L fed pigs ($P < 0.05$), resulting in an interaction between type and level of dietary NDO as well as an NDO-level effect ($P < 0.05$).

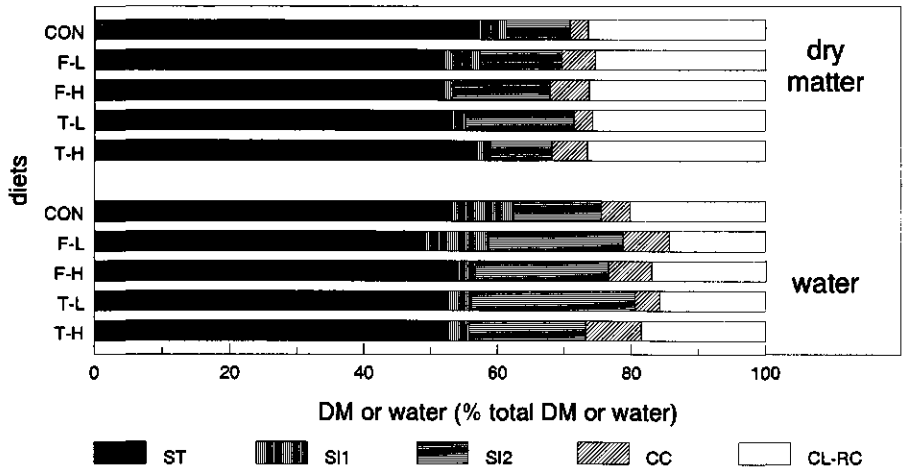

Figure 1. *Distribution of dry matter (DM) and water in the gastrointestinal tract of growing pigs fed diets with or without non-digestible oligosaccharides (for abbreviations, see Material and Methods).*

Table 2. *Feed intake, relative contents, digesta pH, dry matter, and crude protein content of pigs fed diets with or without non-digestible oligosaccharides*

	Diets[1]					SEM	Orthogonal contrasts[2]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x
Level	0	L	H	L	H					Level
Feed (g)										
Intake	705	677	697	679	648	34	NS	NS	NS	NS
Relative segment contents (% feed intake)										
ST[3]	216	226	221	211	219	17	NS	NS	NS	NS
SI1	25	28	8	19	11	10	NS	NS	NS	NS
SI2	67	79	70	83	64	7	NS	NS	‡	NS
CC	16[ab]	25[ab]	23[b]	12[b]	32[a]	3	‡	NS	*	*
CL1	56	46	53	45	58	7	NS	NS	NS	NS
CL2	33	31	26	29	26	8	NS	NS	NS	NS
RC	7	7	5	4	4	3	NS	NS	NS	NS
Digesta dry matter content (g/kg)										
ST	213.2	189.8	196.4	196.0	213.9	12.1	NS	NS	NS	NS
SI1	111.5	123.3	138.7	120.6	125.9	21.9	NS	NS	NS	NS
SI2	145.7	137.9	153.1	143.5	117.4	12.9	NS	NS	NS	NS
CC	130.7	162.7	190.7	171.8	139.1	13.3	*	NS	NS	*
CL1	252.8	293.7	276.3	278.8	252.1	12.0	‡	NS	‡	NS
CL2	297.3	316.0	300.9	316.8	305.6	15.0	NS	NS	NS	NS
RC	308.2	307.9	383.8	305.1	355.5	-	-	-	-	-
Digesta total protein content (g/kg DM)										
SI2	262.5	309.7	295.5	284.5	332.3	25.0	‡	NS	NS	NS
CC	265.2	250.2	242.1	270.7	287.1	21.4	NS	NS	NS	NS
CL1	237.6	225.8	244.9	256.2	266.7	10.5	NS	*	NS	NS
Digesta pH										
ST	4.5	4.1	4.3	4.3	4.4	0.1	‡	NS	NS	NS
SI1	5.9	5.7	5.9	5.7	5.9	0.3	NS	NS	NS	NS
SI2	6.7	6.8	6.6	6.7	6.8	0.1	NS	NS	NS	NS
CC	6.0	5.9	5.8	6.0	5.8	0.1	NS	NS	NS	NS
CL1	6.3[ab]	6.5[a]	6.5[a]	6.3[ab]	6.2[b]	0.1	NS	**	NS	NS
CL2	6.4	6.4	6.7	6.3	6.4	0.1	NS	‡	NS	NS
RC	6.4	5.9	6.5	6.0	6.3	0.2	NS	NS	‡	NS

[1]See Table 1
[2]‡: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$
[3]See Material and methods
[a,b]Data without common superscript differ significantly (Tukey-Kramer, $P < 0.05$)

Figure 1 shows the distribution of DM and water over the GIT. The NDO-fed pigs had relatively more water and DM from SI2 through RC than did the control pigs. The NDO-fed pigs had relatively more DM in SI2 ($P < 0.10$) and CC ($P < 0.05$) and water in SI2

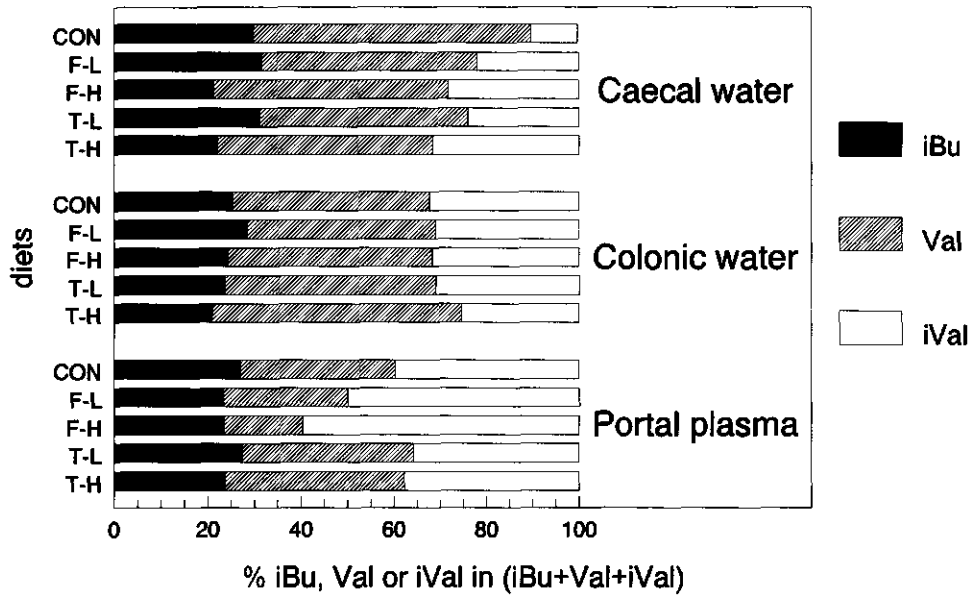

Figure 3. *Composition of protein derived volatile fatty acids in caecal and colonic water, and portal plasma of growing pigs fed diets with or without non-digestible oligosaccharides (for abbreviations, see Material and Methods)*

Discussion

Some Japanese studies have showed that FOS and TOS improved growth performances in young pigs (Hidaka *et al.*, 1985; Katta *et al.*, 1993), while other studies showed no or temporarily negative effects (Farnworth *et al.*, 1992; Kornegay *et al.*, 1992). Most studies lack observations on gastrointestinal digesta. In other studies, the absence of NDO effects may have originated from masking or diluting NDO from the control diet (Farnworth *et al.*, 1992; Bolduan *et al.*, 1993). In our study, we used a control diet with a low level of NDO (190 mg raffinose/kg only). This provided new insight in some effects of NDO on digesta and portal plasma characteristics of growing pigs.

In our experiment, samples were taken and measurements made 3 h after feeding. A significant proportion of the diet may already have reached the terminal ileum by then.

Water-soluble markers have been found in the terminal ileum and hindgut of pigs within 2 h after feeding (Clemens *et al.*, 1975). In humans, breath H_2, an increase of which may indicate active fermentation, may reach a maximum 2-3 h after ingestion of lactulose (Levitt *et al.*, 1987) and FOS (Stone-Dorshow and Levitt, 1987). Further, NDO increase osmotic pressure and consequently oral-caecal transit time may be reduced (Wiggins, 1984). Thus, a significant proportion of the water-soluble part of the NDO-diets may have reached the terminal ileum within 2-3 h after feeding. This is supported by the absence of FOS and TOS in the gastric digesta 3 h after feeding and by the DM and water distribution as shown in Figure 1.

The lower ST-pH may indicate that, to a certain extent, NDO-fermentation takes place in the porcine stomach. Indeed, it has been shown that the porcine stomach may harbour up to 10^6 colony forming units of lactobacilli/cm^2 (Henriksson *et al.*, 1995), which could be able to ferment NDO. Jerusalem artichoke fructan, a polymer of β-linked fructose units, was degraded up to 50 % in the porcine stomach (Graham and Åman, 1986b). The absence of FOS and TOS in gastric digesta may confirm a possible NDO-fermentation. However, disappearance by degradation and passage could not be distinguished in this study. Further, it has been shown that hindgut VFA may inhibit gastric motility (Chuche and Malbert, 1997), allowing longer gastric fermentation of any dietary organic matter. The higher relative stomach weight in NDO-fed pigs may have been an adaptation to the increased stomach retention time.

The pH in the proximal colon digesta increased with increasing dietary FOS, and decreased with increasing dietary TOS. An increased pH could be a result of increased proteolytic activity and/or more VFA absorption. Lack of readily available carbohydrates may cause a shift towards more protein fermentation, resulting in an increase of alkaline fermentation products (ammonia, biogenic amines) and pVFA, and thus a higher pH (Macfarlane and Cummings, 1991). On the other hand, it has been shown that NDO increase proliferation of caecal and colonic epithelial mucosa in neonatal pigs (Howard *et al.*, 1992). Consequently, a larger gut wall surface may allow more VFA to be absorbed.

As already mentioned, NDO may have arrived in the proximal colon within 3 h after feeding. However, no NDO were recovered from the CL1 digesta. Thus either NDO are fermented in the colon digesta at a very fast rate, or they are fermented more proximally, *e.g.* in the terminal ileum. This part of the porcine gastrointestinal tract does indeed harbour an active microflora (Jensen and Jørgensen, 1994). So, some of the dietary NDO may have been fermented before digesta reaches the large intestine. This is supported by the increased concentration of CP in SI2 digesta of NDO-fed pigs, which may indicate that

CHAPTER

4

DIETARY NON-DIGESTIBLE OLIGOSACCHARIDES AFFECT MICROBIAL CHARACTERISTICS OF ILEAL- AND CAECAL DIGESTA, AND FAECES OF WEANER PIGS

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Dietary non-digestible oligosaccharides affect microbial characteristics of ileal- and caecal digesta, and faeces of weaner pigs

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Abstract

The effects of two types of non-digestible oligosaccharides (**NDO**) were studied in relation to the microbial characteristics of porcine intestinal digesta and faeces. An NDO-free diet (control) or this diet containing 10 or 40 g/kg fructooligosaccharides (**FOS**) or transgalactooligosaccharides (**TOS**), were fed to weaner pigs, averaging 10.4 kg on d 0 of the experiment (four pigs per diet). The NDO were included in the diet at the expense of cellulose. Ileal- and caecal digesta, and faeces were collected on d 33 to 37, 19 to 22, and 13 to 17, respectively. The ileal pH was lowered for the NDO-diets ($P < 0.05$). Higher dietary NDO levels yielded relatively more propionic and less acetic acid in the ileal digesta. This was more pronounced for FOS than for TOS ($P < 0.05$). Both NDO-diets yielded less ileal aerobes than the control ($P < 0.05$); the FOS-diets yielded more ileal anaerobes than the TOS-diets. The NDO-diets, especially the FOS-diets, yielded more caecal anaerobes than did the control diet ($P < 0.05$). Caecal pH was increased for the NDO-diets, especially for the TOS-diets ($P < 0.05$). Compared to the control diet, faeces' pH was increased for the NDO-diets, and contained relatively more protein-derived short-chain fatty acids and less butyric acid ($P < 0.05$). The TOS-diets yielded more faecal anaerobes ($P < 0.10$) and less faecal VFA ($P < 0.05$) than did the FOS-diets.

We concluded that dietary NDO affect microbial characteristics of the ileal- and caecal digesta, and faeces of well-kept weaner pigs. Effects of dietary NDO observed in the faeces did not reflect the effects in the ileal digesta. Both FOS and TOS seemed unable to maintain enhanced saccharolytic activity throughout the large intestine, which resulted in an increased proteolytic activity at the faecal level.

Introduction

Fructooligosaccharides (**FOS**) and transgalactooligosaccharides (**TOS**) are small water-soluble carbohydrates, and are not hydrolysable by mammalian intestinal enzymes. However, these non-digestible oligosaccharides (**NDO**) can be fermented by the gastrointestinal microflora. It has been claimed that certain NDO are prebiotics, which 'beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria, and thus improve host health' (Gibson and Roberfroid, 1996). As such, NDO may be applied as prebiotic feed additive for monogastrics. The FOS can be found in most cereals (Henry and Saini, 1989) while TOS are not found in feedstuffs. Both FOS and TOS can be synthesized, from respectively sucrose and lactose, by specific enzymes. The FOS can also be produced via hydrolysis of inulin, a polymer of fructose. Usually, FOS and TOS are mixtures of molecules with different chain lengths, and may include a terminal glucose monomer.

It has been shown that, three h after feeding, the pH of proximal colon digesta of FOS-fed pigs was higher than that of the control pigs, though this pH was lower for TOS-fed pigs. The effects on volatile fatty acid (**VFA**) concentrations in portal plasma and colon

digesta were the opposite, suggesting that fermentation in pigs fed TOS- diets was in progress three h after feeding, while that in pigs fed FOS-diets was nearly complete (Houdijk *et al.*, Chapter 3). This may also have indicated that FOS is fermented more proximally than TOS, eg. in the ileum. This part of the porcine gastrointestinal tract does indeed harbour an active microflora (Jensen and Jørgensen, 1994). Thus, some NDO may be fermented precaecally to a certain extent, and thereby affect the microbial characteristics of the ileal digesta. However, Gabert *et al.* (1995) found no effect of two g TOS or ß-glucooligosaccharides/kg diet on pH, VFA concentrations, and bacterial cell counts in weanling pigs' ileal digesta. Similarly, five g galactosyllactose/kg diet (a TOS component) had no significant effect on these parameters (Mathew *et al.*, 1997). The control diets used in both these studies were composed mainly of cereals and soybean meal. It has been shown that NDO levels (α-galactosides) in soybean meal can reach 50 g/kg (Coon *et al.*, 1990). Consequently, considerable levels of NDO in the control diet may have diluted or masked the effects of the NDO added. Thus, NDO-free diets should be used to study the effects of NDO as feed component and for their potential as a prebiotic feed additive.

In the work reported here, the control diet was based on NDO-free ingredients. The control diet and this diet enriched with FOS and TOS were fed to weaner pigs, and microbial characteristics of ileal- and caecal digesta, and faeces were studied.

Materials and methods

Diets, animals, and housing

Table 1 shows the ingredients and analyzed composition of the experimental diets. The diets contained no NDO (**CON**) or 10 or 40 g/kg FOS or TOS. Raftilose P95®, a powder with 90% FOS (Orafti, Tienen, Belgium) and Oligostroop®, a syrup with 45% TOS (Borculo Whey Products, Borculo, The Netherlands) were the NDO-rich additives used. The NDO were included at the expense of purified cellulose (w/w); the free sugars in the NDO-rich additives were balanced against glucose (w/w). Minor changes in cornstarch content completed the mass balance. There were no antibiotics and additional copper added to the diets. These diets met or exceeded the known nutrient and mineral requirements for young pigs (NRC, 1988).

Table 1. *Ingredients, dry matter, and chemical composition of the experimental diets*

	Diets[1]				
Type	CON	FOS		TOS	
Level	0	10	40	10	40
Ingredients (g/kg)					
Raftilose P95®	0.0	11.1	44.3	0.0	0.0
Oligostroop®	0.0	0.0	0.0	22.4	89.6
Cellulose	42.0	32.0	1.8	32.0	1.8
Glucose	150.0	149.4	147.7	142.9	121.7
Cornstarch	470.8	470.3	468.9	465.5	449.6
Casein	185.0	185.0	185.0	185.0	185.0
Oats husk meal	60.0	60.0	60.0	60.0	60.0
Soyoil	25.0	25.0	25.0	25.0	25.0
Amino acids[2]	3.0	3.0	3.0	3.0	3.0
Minerals[3]	54.0	54.0	54.0	54.0	54.0
Premix[4]	10.0	10.0	10.0	10.0	10.0
Chromiumoxide	0.25	0.25	0.25	0.25	0.25
Dry matter[5] (g/kg)					
Period I	888.1	886.9	886.6	880.5	879.2
Period II	887.3	890.6	893.2	889.3	889.7
Analyzed chemical composition[6] (g/kg DM)					
Inorganic matter	50.0	50.3	49.8	50.6	51.4
Crude protein	189.7	191.8	190.1	192.0	198.8
Ether extract	21.1	20.5	19.3	20.3	21.4
Hemicellulose	19.9	20.8	23.1	21.0	23.2
Cellulose	55.3	44.9	19.5	46.0	21.1
Lignin	6.1	6.6	7.4	6.2	6.4
Starch	469.2	467.6	459.1	465.2	452.3
Sugars	188.7	197.5	231.7	198.7	225.4

[1]CON: no non-digestible oligosaccharides (NDO); FOS: fructooligosaccharides; TOS: transgalactooligosaccharides; both NDO at 10.0 or 40.0 g/kg diet
[2]Amino acids (g/kg feed): 1.7 L-cycsteine, 0.8 L-threonine, and 0.5 L-tryptophane
[3]Minerals (g/kg feed): 5.0 NaCl, 10.0 $CaCO_3$, 20.0 $CaHPO_4$, 0.5 MgO, 16.5 $KHCO_3$, and 2.0 $NaHCO_3$
[4]Premix (per kg feed): 9000 IU vitamin A, 1800 IU vitamin D_3, 40 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 5 mg riboflavin, 12 mg d-panthothenic acid, 1 mg folic acid, 3 mg pyridoxine, 30 mg niacin, 40 µg cobalamin, 1000 mg choline chloride, 50 mg vitamin C, 0.1 mg biotin, 2.5 mg $CoSO_4.7H_2O$, 0.2 mg $Na_2SeO_3.5H_2O$, 0.5 mg KI, 400 mg $FeSO_4.7H_2O$, 40 mg $CuSO_4.5H_2O$, 70 mg MnO_2, and 200 mg $ZnSO_4$
[5]Dietary dry matter (dm) in during faeces and digesta collection after 13-17 and 33-37 d on the diets, respectively
[6]Analyses have been described elsewhere (Houdijk *et al.*, Chapter 2).

Twenty 38-d-old castrated pigs (Great Yorkshire*Landrace)♂ x (Great Yorkshire)♀, averaging 10.4 (SE 0.8) kg on d 0 of the experiment, were individually housed in metabolic cages. Before being weaned, the pigs received an NDO-free creep feed, without antibiotics or additional copper. The pigs were ranked on BW, divided into four weight classes, and randomly allocated to the diets (four pigs per diet) from each weight class. The pigs were given a 13-d adaptation period to the experimental diets. Feeds were offered twice daily;

water was available for 1 h during feeding. Ethics approval was given by the Animal Ethics Committee of Wageningen Agricultural University.

Sample collection

The ileal digesta were collected on d 33, 34, 36 and 37, using a PVTC-cannula (Van Leeuwen et al., 1991). The cannula was opened at least 1 h before the morning meal. Little bags connected to the cannula were replaced hourly for 12 h after the morning meal. During the cannulation procedure on d 19 to 22, the caecum was removed and its digesta collected. The faeces were collected on d 13 to 17 using bags used for quantitative faeces collection during digestibility studies (Combihesive®, Squibb B.V., Rijswijk, The Netherlands). Attachments were checked regularly to minimize airflow into the bags.

Fresh digesta were sampled for bacterial analysis. Fresh samples were used since it has been shown that bacterial counts differed significantly when analyzed in fresh faeces and faeces stored at -80°C in glycerol-enriched broth (Alles, 1997). The latter method has long been is used to store samples for bacterial analysis (Crowther, 1971). Thus, ileal digesta produced 3 to 4 h after the morning meal were sampled, immediately after removal of the bag from the cannula. The caecal digesta were sampled immediately after collection, and faeces were sampled from the faecal bag during the morning meal. These samples were transported in sterile anaerobic buffered peptone water (Oxoid, Haarlem, The Netherlands), and put in an anaerobic chamber within 20 minutes after collection. The remainder ileal- and caecal digesta, and faeces were stored at -20°C pending analysis. After thawing, 50 g (wet weight) pooled faeces or ileal digesta (see below) were placed in a 100 ml centrifuge tube and centrifuged at 30,000 *g* for 2 h at 4°C (Model J2-21M, Beckman, Fullerton, California). The supernatants obtained are referred to as faecal and ileal water, respectively.

Microbial and chemical analysis

Bacterial counts were performed using appropriate dilution and plate culture techniques under aerobic or anaerobic conditions, and expressed as colony forming units (**cfu**) per g fresh sample. The bacterial groups and species determined included total anaerobes (**tAN**, using Faecal Reinforced Clostridial Agar), total aerobes (**tAE**, using Nutrient Agar), lactobacilli (**LAB**, using Sorbic acid-enriched MRS Agar), *Bacteroides* spp (**BAC**, using Bacteroides Bile Esculine Agar), enterococci (**ENT**, using Kanamycin Aeculine Azide Agar), *E. coli* (using Eosine Methylene Blue Agar), and bifidobacteria (**BIF**, using Raffinose Bifidobacterium agar, Hartemink *et al.*, 1996).

The daily faeces production was analyzed for pH, and the results averaged per pig. All faeces produced by each pig were pooled and analyzed for dry matter (**DM**, g/kg) and crude protein (**CP**, g/kg DM). The DM was obtained by oven-drying (at 103°C to constant weight). The CP was analyzed as 6.25 times Kjeldahl-N. The faecal water was analyzed for osmolarity (mOsmol/l), which was based on freezing point depression (HalbMikro-Osmometer, Knauer, Berlin, Germany), and for VFA (Schutte *et al.*, 1992). Acetic-, propionic-, and butyric acid were grouped as **sVFA**, while *iso*-butyric-, valeric-, and *iso*-valeric acid were grouped as **pVFA**. sVFA are thought to have originated mainly from saccharolytic activity, while pVFA are considered to have originated from proteolytic activity (Mortensen *et al.*, 1988).

Fresh caecal digesta were analyzed for pH, while stored digesta were analyzed for DM. In addition, the caecal tissue was analyzed for DM (freeze-drying to constant weight). Ileal digesta were pooled and analyzed for pH, DM, CP, and osmolarity as described for faeces. In addition, viscosity (mPa.s) was analyzed in ileal water using a digital viscometer (Model DV-II+, Brookfield Engineering Laboratories Inc., Stoughton, UK). The VFA and lactic acid (**LA**, mmol/l, via HPLC according to Voragen *et al.* (1986)) were analyzed in ileal digesta of d 37 only. The sum of VFA and LA is refered to as short chain fatty acids (**SCFA**). Digesta obtained hourly were stored on ice, allowing total day production to be sampled without thawing, with minimal fermentation after collection. Ten g digesta was mixed with 0.5 ml 85% phosphoric acid, and then stored at -20°C pending analysis for SCFA.

Data analysis

The ileal digesta were analyzed from 16 pigs (CON: 3, F10: 3, F40: 3, T10: 4, and T40: 3). The caecal digesta were analyzed from 20 pigs (CON: 3, F10: 4, F40: 4, T10: 5, and T40: 4), and faeces from 17 pigs (CON: 4, F10: 4, F40: 4, T10: 3, and T40: 2). The pigs excluded had diarrhoea, blocked cannulae, or an empty caecum; when possible, reserve pigs were included instead. An analysis of variance was conducted using the model shown in Eq. 1. Data are presented as least-square means of D_i with pooled standard errors. Predetermined orthogonal contrasts used to locate D_i-effects were (1) NDO inclusion *per se* (CON *vs.* NDO-diets), (2) Type (FOS *vs.* TOS), (3) Level (10 *vs.* 40 g NDO), and (4) the interaction Type x Level. Contrasts were considered significant at a level of $P < 0.05$. Further, multiple comparisons (Tukey-Kramer, $P < 0.05$) were also applied to clarify contrasts observed. It should be noted that orthogonal contrasts are more powerful to locate treatment effects than are multiple comparisons (Lowry, 1992).

$y_{ij} = \mu + D_i + \varepsilon_{ij}$ [1]

where y_{ij} = observation
μ = general mean
D_i = effect of diet (i = 1, ..., 5)
ε_{ij} = residual error.

Results

Ileal digesta

Table 2 shows the ileal digesta characteristics studied. The digesta of the NDO-fed pigs tended to contain less DM than that of the control pigs; the DM was lower for the 40 than for the 10 g NDO/kg diets ($P < 0.01$). Ileal digesta from the NDO-fed pigs contained more CP than that of the control pigs ($P < 0.05$). A significant interaction between type and level of dietary NDO was observed for ileal osmolarity. Figure 1 shows the relation between ileal osmolarity and SCFA concentration (open circles); a relation such as that in faeces was not observed (see below). Dietary NDO did not affect ileal viscosity. Ileal pH decreased for the NDO-diets ($P < 0.05$), and was lower for the 40 than for the 10 g NDO/kg diets ($P < 0.05$). Digesta from the FOS-fed pigs contained more LA than that of the TOS-fed pigs ($P < 0.05$). Dietary NDO did not significantly affect the digestal VFA concentration. Its composition, however, shifted towards a higher proportion of propionic acid ($P < 0.01$) and a lower proportion of acetic acid ($P < 0.05$) as dietary NDO-levels increased (Figure 2). This was more pronounced for the FOS-fed pigs than for the TOS-fed pigs ($P < 0.05$). *Iso*-butyric acid was not detected in ileal digesta, and valeric acid was found in only two samples, and was not included in the statistical analysis. *Iso*-valeric acid was clearly increased in the FOS-fed pigs (up to 4.5 times higher for the F40 diet than for the control diet), resulting in the significant effects observed. The FOS-fed pigs had more tAN ($P < 0.10$) and LAB ($P < 0.05$) than did the TOS-fed pigs. The concentration of tAN ($P < 0.05$) and LAB ($P < 0.10$) was lower for the 40 than for the 10 g NDO/kg. These level effects did not approach significancy when data were expressed per g DM (P=0.13 and P=0.20, respectively, data not shown). Ileal digesta of the NDO-fed pigs had less tAE and ENT than that of the control pigs ($P < 0.05$), also per g dry digesta. Digesta from the FOS-fed pigs had more *E. coli* than that of the TOS-fed pigs ($P < 0.05$). The *E. coli* counts were higher for the 40 than for the 10 g NDO/kg diets ($P < 0.05$).

Table 2. *Physico-chemical and microbial characteristics of ileal digesta from pigs fed diets with or without non-digestible oligosaccharides*

	Diets[1]					sem	Orthogonal contrasts[2]			
	Type	CON	FOS		TOS			NDO	Type	Level	Type x Level
	Level	0	10	40	10	40					
Dry Matter and Crude Protein content (g/kg)											
DM		145.1^{ab}	152.0^{a}	103.8^{b}	131.2^{ab}	105.4^{b}	10.0	‡	NS	**	NS
CP (in DM)		163.8	186.4	196.9	188.3	225.6	14.5	*	NS	NS	NS
Osmolarity (mOsmol/l),Viscosity (mPa.s), pH, Lactic Acid and VFA (mmol/l)											
Osmolarity		142.7	177.0	145.3	144.0	172.7	10.3	NS	NS	NS	*
Viscosity		1.3	1.4	1.3	1.5	1.4	0.1	NS	NS	NS	NS
pH		7.1^{a}	6.9^{ab}	6.7^{b}	7.0^{ab}	6.8^{ab}	0.1	*	NS	*	NS
LA		6.2	20.5	13.8	6.0	6.7	3.8	NS	*	NS	NS
VFA		17.4	21.7	16.9	16.9	20.8	3.9	NS	NS	NS	NS
pVFA[3]		0.7^{b}	1.7^{ab}	3.4^{a}	0.7^{b}	0.7^{b}	0.2	**	***	***	**
% pVFA		4.2^{b}	8.5^{b}	21.2^{a}	4.4^{b}	3.8^{b}	2.0	*	***	***	**
Bacterial counts (log cfu/g)											
tAN[4]		10.2	10.5	9.9	9.9	9.5	0.2	NS	‡	*	NS
BAC		8.4	7.9	8.8	8.6	8.5	0.2	NS	NS	NS	NS
LAB		9.2	9.5	8.9	8.7	7.9	0.4	NS	*	‡	NS
BIF		6.6	7.2	7.9	6.7	6.9	0.7	NS	NS	NS	NS
tAE		10.2	8.7	9.7	9.3	9.4	0.4	*	NS	NS	NS
E. coli		9.0	8.6	9.0	6.8	8.7	0.4	NS	*	*	NS
ENT		8.9	7.3	8.1	7.5	7.3	0.4	*	NS	NS	NS

[1]See Table 1; Diets were fed for 33-37 d
[2]NS: P>0.10, ‡: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$
[3]Sum of *iso*-butyric acid, valeric acid and *iso*-valeric acid, as such and as % of total VFA
[4]tAN: total anaerobes, BAC: *Bacteroides* spp.; LAB: lactobacilli; BIF: bifidobacteria; tAE: total aerobes; ENT: enterococci
[a,b]LSMeans lacking common superscripts differ significantly ($P < 0.05$ by Tukey-Kramer)

Caecal digesta

Table 3 shows the caecal characteristics studied (pigs were fasted overnight). Dietary NDO did not affect caecum weight or digesta DM, though caecal tissue of the FOS-fed pigs had a higher DM than that of the TOS-fed pigs ($P < 0.05$). The FOS-fed pigs had a larger volume (weight) of caecal digesta than did the TOS-fed pigs ($P < 0.05$). The NDO-fed pigs tended to a higher caecal pH compared to the control pigs, and it was higher for the 40 than for the 10 g NDO/kg diets ($P < 0.001$). The TOS-fed pigs tended to have a higher caecal pH than did the FOS-fed pigs. The NDO-fed pigs had more caecal tAN than did the control pigs ($P < 0.05$); this was more pronounced for FOS than for TOS ($P < 0.05$), and for the 40 than for the 10 g NDO/kg diets ($P < 0.01$). The BIF concentrations were below 10^6 cfu/g. The caecal digesta of the FOS-fed pigs contained more *E. coli* ($P < 0.10$) and less ENT ($P < 0.05$) than that of the TOS-fed pigs.

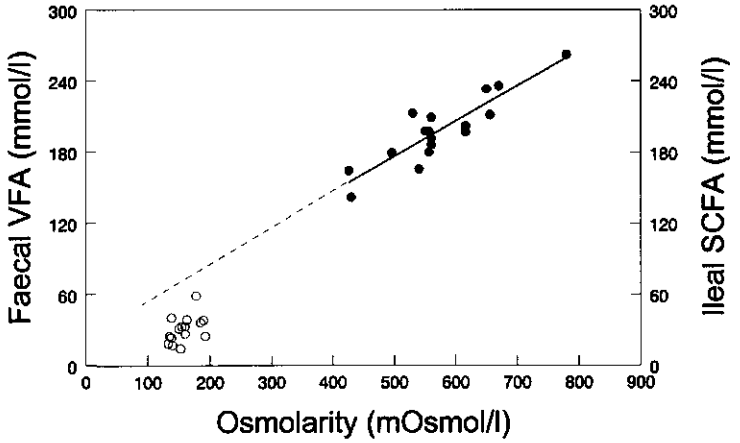

Figure 1. *Relation between total short-chain fatty acid concentrations in and osmolarity of faecal and ileal water of weaner pigs*

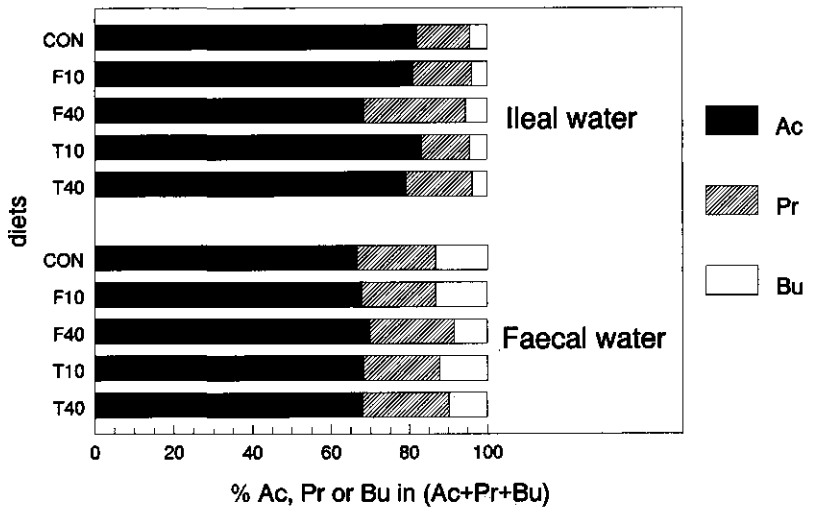

Figure 2. *Composition of carbohydrate derived volatile fatty acids in faecal and ileal water of weaner pigs fed diets with or without non-digestible oligosaccharides*

Table 3. *Physico-chemical and microbial characteristics of the caecum and its digesta from pigs fed diets with or without non-digestible oligosaccharides (after overnight starvation)*

	Diets[1]					sem	Orthogonal contrasts[2]			
	Type	CON	FOS		TOS			NDO	Type	Level	Type x Level
	Level	0	10	40	10	40					
Caecum											
Weight (g)		21	19	24	20	20	2	NS	NS	NS	NS
DM (g/kg)		143.8	159.0	163.8	148.4	126.9	10.4	NS	*	NS	NS
Caecal digesta											
Volume (g)		75	68	99	56	54	11	NS	*	NS	NS
pH		6.3[b]	6.3[b]	6.8[ab]	6.3[b]	7.1[a]	0.1	‡	‡	***	NS
DM (g/kg)		280.3	297.1	309.4	283.3	278.9	16.3	NS	NS	NS	NS
Bacterial counts (log cfu/g)											
tAN[3]		9.3[b]	9.7[b]	10.7[a]	9.4[b]	10.0[ab]	0.1	*	*	**	NS
BAC		8.3	8.1	8.4	8.3	8.4	0.2	NS	NS	NS	NS
LAB		8.3	8.2	8.5	8.3	8.2	0.3	NS	NS	NS	NS
BIF		<6.0	<6.0	<6.0	<6.0	<6.0	-	-	-	-	-
tAE		8.6	8.5	8.9	8.0	8.2	0.4	NS	NS	NS	NS
E. coli		8.3	8.5	9.0	7.9	8.3	0.4	NS	‡	NS	NS
ENT		7.8	7.2	7.2	8.6	7.8	0.3	NS	*	NS	NS

[1]See Table 1; diets were fed for 19-22 d
[2]NS: P>0.10, ‡: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$
[3]See Table 2
[a,b]LSMeans lacking common superscripts differ significantly ($P < 0.05$ by Tukey-Kramer)

Faeces

Table 4 shows the faecal characteristics studied. DM was not affected by dietary NDO, but faeces for the 40 g NDO/kg diets contained more CP than those for the 10 g NDO/kg diets ($P < 0.01$). This effect was more pronounced for the FOS- than for the TOS-diets ($P < 0.10$). The FOS-fed pigs had more faecal VFA and a higher osmolarity than did the TOS-fed pigs ($P < 0.05$) but were comparable with the control pigs. A linear relationship was observed between faecal osmolarity and VFA (closed circles, Fig. 1). Faecal LA was not measured. The NDO-fed pigs had higher faecal pH than did the control pigs ($P < 0.01$), while faecal pH was higher for the 40 than for the 10 g NDO/kg diets ($P < 0.001$). Figure 2 shows the composition of faecal sVFA. The proportion of butyric acid tended to be lower for the 40 than for the 10 g NDO diets ($P < 0.10$); the proportions of acetic- and propionic acid were not affected. Dietary NDO did not significantly affect pVFA concentration, though the NDO-fed pigs had a higher proportion of pVFA than did the control pigs ($P < 0.05$). The proportion of pVFA was higher for the 40 than for the 10 g NDO/kg diets ($P < 0.05$). Within pVFA, the proportion of *iso*-butyric acid was higher for NDO-fed pigs than for control pigs

Table 4. *Physico-chemical and microbial characteristics of faeces from weaner pigs fed diets with or without non-digestible oligosaccharides*

	Diets[1]					sem	Orthogonal contrasts[2]			
Type	CON	FOS		TOS			NDO	Type	Level	Type x Level
Level	0	10	40	10	40					
Dry matter and crude protein content (g/kg)										
DM	423.5	456.0	455.9	452.3	430.5	24.9	NS	NS	NS	NS
CP (in DM)	131.6^{ab}	123.0^{b}	162.4^{a}	109.3^{b}	143.0^{ab}	8.5	NS	‡	**	NS
Osmolarity (mOsmol), pH and VFA (mmol/l)										
Osmolarity	616.6	624.0	581.6	514.0	461.0	42.2	NS	*	NS	NS
pH	5.6	5.7	6.2	5.9	6.2	0.1	**	NS	***	NS
VFA	209.4	215.3	201.1	173.0	171.8	14.9	NS	*	NS	NS
pVFA[4]	24.9	28.4	31.7	21.4	30.1	4.0	NS	NS	NS	NS
% pVFA[4]	11.5	13.1	15.7	12.4	17.6	1.3	*	NS	*	NS
Bacterial counts (log cfu/g)										
tAN[5]	9.6	9.4	9.8	10.1	10.5	0.4	NS	‡	NS	NS
BAC	8.4	7.9	8.8	8.6	8.5	0.2	NS	NS	NS	‡
LAB	8.6	7.7	8.2	8.4	8.3	0.3	NS	NS	NS	NS
BIF	8.1	7.5	8.3	8.3	8.3	0.2	NS	NS	NS	NS
tAE	8.3	7.8	8.2	7.8	8.5	0.4	NS	NS	NS	NS
E. coli	8.0	7.6	8.2	7.7	8.3	0.5	NS	NS	NS	NS
ENT	8.0	7.5	8.1	8.3	7.7	0.5	NS	NS	NS	NS

[1]See Table 1; diets were fed for 13-18 d
[2]NS: P>0.10, ‡: $P < 0.10$, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$
[3,4]See Table 2
[a,b]LSMeans lacking common superscripts differ significantly ($P < 0.05$ by Tukey-Kramer)

and higher for the 40 than for the 10 g NDO/kg diets ($P < 0.05$, data not shown). The TOS-fed pigs tended to have more faecal tAN than did the FOS-fed pigs; the latter was comparable to the control pigs. The interaction between type and level of dietary NDO tended to be significant for faecal BAC. NDO-fed pigs had numerically but not significantly lower LAB than the control pigs. However, when expressed as fraction of tAN, NDO-fed pigs had less LAB than did the control pigs ($P < 0.05$, data not shown). Dietary NDO did not significantly affect the other bacterial species analyzed.

Discussion

The use of cereals and legumes in practical diets for young pigs often results in considerable levels of dietary non-starch polysaccharides (NSP). Apparent faecal digestion of NSP may reach up to 56% in a cereal-based diet (Longland *et al.*, 1994). FOS from cereals (Henry and Saini, 1989) and α-galactosides from legumes (Saini, 1989) also

contribute to the fermentable carbohydrate fraction. If this is not taken into consideration, then the fermentation of these NSP and NDO would mask or dilute the effects of the NDO added. We used an NDO-free, cornstarch-based diet; FOS and TOS were then included up to a level of 40 g/kg diet.

Ileal digesta

The ileal pH decreased for both types of NDO. The SCFA concentration observed did not support this finding, but this may have been due to their rapid absorption across the gut wall. In rats fed FOS, caecal ammonia concentrations decreased, while that of CP increased (Younes *et al.*, 1995). Similar observations were made for pigs fed lactulose (Ahrens and Schön, 1988). The ileal digesta of NDO-fed pigs contained more CP than that of the control pigs, which may indicate more ammonia fixation into microbial biomass. Hence, the ileal pH could also have decreased as a result of a decreased ammonia concentration. This seems to contrast with the rise of pVFA in the FOS-fed pigs, since pVFA are related to the extent of protein fermentation (Macfarlane *et al.*, 1992). In this experiment, mean pVFA for faecal and ileal water were 27.3 and 1.4 mmol/l, respectively. Thus, protein fermentation in the ileal digesta may have been of minor importance. In addition, different amino acids were fermented. The NDO-fed pigs had greater proportions of faecal *iso*-butyric acid, while only *iso*-valeric acid was found in ileal digesta. It has been shown that *iso*-butyric acid originates from the deamination of valine, while *iso*-valeric acid originates form leucine and/or isoleucine (Mortensen *et al.*, 1990).

Ileal disappearance of FOS was 92% in this experiment (Houdijk *et al.*, Chapter 2). That of TOS was estimated at 30% (R. Kamelaar, personal communications). The near complete ileal fermentation of FOS in contrast to that of TOS may have resulted in effects observed for sVFA composition, *iso*-valeric acid concentration, and bacterial counts. Firstly, the ileal digesta of the FOS-fed pigs had relatively more propionic acid and less acetic acid than that of the TOS-fed pigs. This may have originated from lactic acid fermentation, yielding VFA ratios of 2:1 propionic- to acetic acid (Schlegel, 1992). Indeed, ileal lactic acid concentrations were greater in the FOS-fed pigs than in the TOS-fed pigs. Lactic acid fermenting species within porcine anaerobic flora are e.g. *Megasphaera elsdenii*, *Selenomonas ruminantium* (Robinson *et al.*, 1981) and *Veillonella* spp. (Russell and Bruckner, 1991). Secondly, it has been shown that pVFA concentrations increase when the fermentation of easily degradable carbohydrates is complete (Macfarlane *et al.*, 1992). The increased concentration of *iso*-valeric acid thus indicated an absence of carbohydrates as energy source for some bacterial species, and therefore most probably a high degree of

FOS fermentation. Thirdly, ileal digesta of the FOS-fed pigs contained more anaerobes (including lactobacilli) than that of the TOS-fed pigs. The concentration of bifidobacteria was also higher in FOS-fed pigs than in TOS-fed pigs. However, this was not significant, partly due to the large standard error. Digesta from the FOS-fed pigs also contained more *E. coli*. This may have been associated with the greater concentration of *iso*-valeric acid, since *E. coli* can also ferment protein.

The concentration of ileal anaerobes observed in our study are in agreement with results from other workers, though the concentrations of ileal aerobes were generally higher than previously reported (Pollmann *et al.*, 1980; Chesson *et al.*, 1985; Gabert *et al.*, 1995). The presence of a PVTC-cannula may have influenced the ileal microflora composition. Especially aerobes may benefit from an increased exposure to oxygen during digesta collection. Nevertheless, ileal digesta of the NDO-fed pigs contained fewer aerobes than that of the control pigs. It has been shown that FOS-supplemented diets reduced the concentration of aerobes and facultative anaerobes in the small intestinal digesta of German Shepherd dogs with small intestinal bacterial overgrowth (Willard *et al.*, 1994). The effect of dietary NDO on *E. coli* differed from the effect on total aerobes. Ileal digesta of the FOS-fed pigs contained more *E coli* than that of the TOS-fed pigs. Fermentation of FOS by *E coli* has been shown (Hartemink *et al.*, 1997a). Although often associated with disease, *E. coli* are normal intestinal inhabitants, and sometimes even used as probiotics (Stavric and Kornegay, 1995). It has been shown that caecal *E. coli* concentrations increased in rabbits fed FOS, while the rabbits did not show any visible signs of diseases (Morrise *et al.*, 1993). In our experiment, digestive disorders did not differ between dietary groups, indicating that any observed increase in *E. coli* was probably non-pathogenic.

Lower bacterial concentrations are confounded by lower DM contents. Bacterial counts may also be expressed per g DM. As such, total anaerobes and lactobacilli concentrations in the ileal digesta for the 10 and 40 g NDO/kg diets were not significantly different. This may indicate that this effect is mainly, if not completely, due to dilution. The effect of dietary NDO on total aerobes remained ($P < 0.05$), though the concentration of total aerobes for the 40 g NDO/kg diets was greater than that for the 10 g NDO/kg diets (10.5 vs 10.1, sem 0.2, $P < 0.10$), as was the case for *E. coli* (9.8 vs 8.6, sem 0.2, $P < 0.05$). This indicates that a minimal concentration of ileal aerobes may exist between 0 and 40 g NDO/kg. However, dose-response experiments with more inclusion levels in NDO-free control diets are needed to confirm quadratic relations among responses to dietary NDO.

The intake of unabsorbable molecules of low molecular weight can lead to the retention of fluid in the small intestine (Wiggins, 1984). As a consequence, digesta DM will

then decrease. This phenomenon may partly explain the lowered ileal DM observed for the NDO-diets (Table 2). A greater ileal osmolarity was observed as dietary TOS increased from 0 to 40 g/kg. However, this was not the case for the FOS-diets. It is not clear which factors caused this interaction. Various water-soluble components govern the osmotic pressure, including residual NDO, SCFA, electrolytes, and soluble NSP. Faecal osmolarity was highly correlated with faecal VFA (Figure 1). However, if the SCFA were the sole regulators of osmolarity, then the ileal osmolarity would have been lower than that observed.

Thus, the lowered pH and concentration of aerobes suggested a certain extent of FOS and TOS fermentation at the ileal level. The more pronounced increase in the proportion of propionic acid, and the larger concentration of lactic acid, anaerobes and *iso*-valeric acid for the FOS-fed pigs compared to the TOS-fed pigs, indicate that FOS was fermented to a larger extent that TOS at the ileal level.

Caecal digesta

The NDO-fed pigs had a higher caecal pH than did the control pigs. An increase in pH may be explained in (at least) two ways. On the one hand, insufficient readily available carbohydrates as an energy source for the microflora as a whole, may cause a shift towards more protein fermentation, resulting in an increase of alkaline fermentation products such as ammonia and biogenic amines (Macfarlane and Cummings, 1991). On the other hand, luminal pH may also increase as a result of more VFA absorption, *e.g.* as a result of a larger gut wall surface. It has been shown that NDO increase the proliferation of caecal and colonic epithelial mucosa in young pigs (Howard *et al.*, 1993).

The caecal microflora probably had insufficient readily available carbohydrates as a consequence of the overnight starvation. In particular, the number of bifidobacteria was small, probably because they require specific growth factors (Modler *et al.*, 1990). Their counts were more than 2 log less while *E. coli* counts were about 2 log higher than those reported in fed pigs (Orban *et al.*, 1997). An increased *E. coli* count has been observed in fasting rats (Turk *et al.*, 1988). Thus, the caecal microflora of the NDO-fed pigs, which contained more anaerobes than that of the control pigs, may have fermented more proteins as an energy source. Caecal pH was higher in the TOS-fed pigs than in the FOS-fed pigs. Whether this differences was related to proteolytic activity or gut wall proliferation remains unclear.

The NDO-fed pigs, especially those offered the FOS-diets, had more caecal anaerobes than did the control pigs. This difference was not accounted for by any of the

anaerobic species studied. Caecal digesta of the FOS-fed pigs contained more *E. coli* and less enterococci cfu/g than that of the TOS-fed pigs. This suggests that these species may have competed for the same substrates. Whether the substrates involved were FOS, TOS or other carbohydrates was not clear.

Faeces

The faeces of the NDO-fed pigs had an higher pH and more pVFA (as % of total VFA) than that of the control pigs. This was partly a result of the decreased total VFA for the TOS-fed pigs. The lowered proportion of butyric acid may have resulted from a greater utilization in the NDO-fed pigs, since butyric acid was shown to be the primary fuel for colonocytes (Von Engelhardt *et al.*, 1989). This indirectly suggests an increased gut wall proliferation and thus more VFA absorption, probably explaining the lowered faecal VFA concentration of the TOS-fed pigs. Though the proportion of butyrate was also lower in the FOS-fed pigs, total VFA was not. The FOS-fed pigs had more crude protein in the faeces than did the TOS-fed pigs. The difference may have been present as components of fermentation products (ammonia, biogenic amines) rather than as amino acids per se. It has been shown that the proportion of pVFA increases during protein fermentation (Mortensen *et al.*, 1990). Thus, the difference between the FOS- and TOS-fed pigs' faecal VFA was probably a result of more proteolytic activity of the FOS-fed pigs' faecal microflora.

NDO were exchanged at the expense of purified cellulose. Although the fermentability of purified cellulose is low, an increase in faecal pH could result from less dietary cellulose. However, an increased faecal pH and a decreased VFA concentration were also observed for a 30 g TOS/kg diet, when TOS was included at the expense of cornstarch (R. Kamelaar, personal communications).

Bacterial counts from the porcine hindgut have been shown to vary considerably; ranges from 0 to 9 cfu per gram digesta have been reported for certain species (reviewed by Conway, 1994). Animal variation and sample analysis are probably the major factors involved in this variation. The effect of dietary NDO on the faecal microflora was limited to more anaerobes for the TOS-fed pigs than for the FOS-fed pigs. This may indicate a more distal fermentation of TOS than of FOS. However, this increase was not reflected in the lactobacilli and bifidobacteria counts. The proportion of lactobacilli was even smaller for the NDO-fed pigs than for the control pigs (2.6% vs. 7.4%, $P < 0.05$). Lactobacilli, bifidobacteria, and *Bacteroides* spp only accounted for 15% of the faecal anaerobes in our

study. It remains unclear which other species may have led to the differences in total anaerobes between FOS- and TOS-fed pigs.

Thus, faecal pH for both FOS- and TOS-fed pigs increased, which was probably due to an increased VFA absorption and/or an increased bacterial proteolytic activity. The data suggest that the latter was more pronounced for the FOS-fed pigs than for the TOS-fed pigs.

Combining ileal digesta and faeces

The results have been discussed per sampling site. A direct comparison between ileal-, caecal-, and faecal observations is confounded by pig age, body weight, feed intake, and, probably the most important factor, time on the experimental diet. However, if one disregards these factors, our data do suggest that an NDO-induced increase of saccharolytic activity in the ileal digesta (reduced pH and an increased proportion of lactobacilli) is not maintained throughout the hindgut, and may thus result in an increased proteolytic activity in the distal colon digesta (increased pH and a decreased proportion of lactobacilli in the faeces). Differences between FOS and TOS suggest that the more proximally the NDO fermentation is complete, the more pronounced the proteolytic activity reaches in the distal colon. Further, lactic acid fermenting anaerobes may have accounted for part of the increased concentration of anaerobes in the caecum due to an enhanced flow of lactic acid into the caecum, especially for the FOS-fed pigs.

Conclusion

In conclusion, exchanging cellulose for FOS and TOS in weaner pigs diet reduced ileal pH, and changed ileal VFA- (more propionic- and less acetic acid) and bacterial profiles (more anaerobes for FOS, less aerobes for both NDO). Most effects at the ileal level were more pronounced for the FOS-diets than for the TOS-diets. Faecal pH and the proportion of protein-derived short-chain fatty acids increased in the NDO-fed pigs, though faecal bacterial counts were not significantly affected. This increased proteolytic activity of the faecal microflora, which was more pronounced for the FOS-diets than for the TOS-diets, suggest that both FOS and TOS could not maintain enhanched saccharolytic activity at the ileal level throughout the hindgut.

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CHAPTER

5

FRUCTOOLIGOSACCHARIDES AND TRANSGALACTOOLIGOSACCHARIDES HAVE DIFFERENT *IN VITRO* FERMENTATION CHARACTERISTICS WHEN BEING FERMENTED BY THE PORCINE INTESTINAL MICROFLORA

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Fructooligosaccharides and transgalactooligosaccharides have different *in vitro* fermentation characteristics when being fermented by the porcine intestinal microflora

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Abstract

In vitro fermentation of fructooligosaccharides (**FOS**) and transgalactooligosaccharides (**TOS**) was studied, using the cumulative gas production technique, which provides a measure of fermentation kinetics and end-point volatile fatty acid (**VFA**) composition. The inocula were prepared from the ileal- and caecal digesta, and the faeces of weaner pigs fed diets with or without FOS and TOS (40 g/kg). These diets were based on cornstarch, casein, and oats husk meal (**OHM**). Next to FOS and TOS, OHM was also used as a substrate (energy source) for the *in vitro* studies. The effect of FOS and TOS in the diet on *in vitro* microbial activity was studied by comparing fermentation characteristics pooled over substrates.

FOS were fermented at a faster rate than TOS ($P < 0.001$). There was no difference in total VFA production, but FOS yielded significantly more propionic- and butyric acid and less acetic acid than TOS. The caecal inocula of the FOS-fed pigs showed significantly different *in vitro* fermentation characteristics than those of the TOS-fed pigs. Significant interactions were observed between diet and substrate, suggesting that the adaptation of the caecal microflora to dietary TOS was more pronounced than to dietary FOS. The faecal microflora of the pigs fed the FOS- and TOS-diets did not ferment OHM to the same extent as those of the control pigs.

We concluded that the *in vitro* fermentation kinetics and end-point VFA-profiles differed between FOS and TOS as a substrate, and that dietary FOS and TOS can affect the *in vitro* fermentation activity of porcine microflora.

Introduction

Fructooligosaccharides (**FOS**) and transgalactooligosaccharides (**TOS**) are small water-soluble carbohydrates containing up to nine monomeric units, which cannot be hydrolyzed by mammalian enzymes. However, these non-digestible oligosaccharides (**NDO**) can be fermented by the gastrointestinal microflora. Several NDO have been regarded as prebiotics, recently defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health' (Gibson and Roberfroid, 1995). It has been shown that dietary FOS and TOS led to little change in the ileal and faecal nutrient digestion by young pigs (Houdijk *et al.*, Chapter 2). However, the pH, volatile fatty acid (**VFA**) concentrations and profiles, and bacterial counts of the ileal digesta, caecal contents, and faeces were affected (Houdijk *et al.*, Chapter 4). The VFA concentrations *in vivo* are not only a reflection of VFA production but also of absorption through the gut wall and/or utilization by other bacteria.

The cumulative gas production technique is most commonly used to rank ruminant feedstuffs according to their fermentation kinetics (Theodorou *et al.*, 1994). However, this technique can also be used to study differences between inocula (microflora), by using standard substrates. As such, different *in vitro* fermentation characteristics for porcine faeces, colonic- and caecal digesta have been shown (Williams *et al.*, 1998). Further, different *in vitro* fermentation characteristics have been observed for inocula of rats fed different diets (Monsma and Marlett, 1996). The effect of dietary interventions on microbial ecology have often been assessed via traditional bacterial culture techniques. It has been speculated that at present less than a fifth of all microorganisms can be cultured and that only a fraction of them have been described (Ward *et al.*, 1990). It should be realized that microfloral composition does not necessarily provides information on the activity of the microflora as a whole, a feature which may be of more immediate relevance to the host, and which is studied in this paper.

In this study, differences between *in vitro* fermentation characteristics of FOS and TOS as substrates, and differences between *in vitro* microbial activity induced by FOS and TOS as dietary components, were determined using the cumulative gas production technique and ileal- and caecal digesta, and faeces from weaner pigs as inoculum source. In addition, the *in vitro* fermentation of oats husk meal (**OHM**) was studied. This NDO-free fibre source was included in each diet, and dietary NDO may have affected the fermentation of OHM fibres.

Materials and methods

Medium

Air-tight serum bottles of 100 ml, containing 80 ml medium B (Lowe *et al.*, 1985), were prepared one day before inoculation. The study using the caecal inocula was carried out in 50-ml bottles, containing 40 ml medium, given the anticipated small amount of digesta for provision of inocula. The bottles were warmed to 39°C ~ 2 h to inoculation.

Substrates

Four substrates were used: glucose (**GLU**), FOS, TOS, and OHM. The GLU (anhydrous glucose, Merck, Darmstadt, Germany) was used as a carbohydrate source assumed to be fermentable by almost all microorganisms, unlike FOS and TOS. The FOS (Raftilose P95® with 95% FOS, Orafti, Tienen, Belgium) and TOS (spray-dried Oligostroop®

with 85% TOS, Borculo Whey Products, Borculo, The Netherlands) were the contrasting NDO in the diet of the donor pigs (see below). The last substrate was OHM (obtained from a local feed mill), which was included in all diets as fibre source. OHM was milled over a 0.5 mm sieve. The 100 ml bottles contained 0.25 g GLU, FOS, or TOS, or 0.50 g OHM; the 50-ml bottles contained half as much. Each substrate was replicated four times; in addition, two bottles without added substrate (blanks) were used for each inoculum.

Inoculum

The inocula were prepared from ileal- and caecal digesta, and faeces of weaner pigs fed an NDO-free control diet, or the same diet with 40 g FOS or TOS/kg (w/w against purified cellulose). The main ingredients of the diet were casein, corn starch, and OHM. Each diet was fed to three groups of four 35-d-old pigs (for more details about diets, animals, and housing see Houdijk *et al.*, Chapter 2). The ileal digesta were collected via a PVTC-cannula between three and four h after the morning meal. The caecal digesta were obtained during placement of this cannula while the pigs were anaesthetized after overnight starvation (Van Leeuwen *et al.*, 1991). The faeces were collected using small bags, which were firmly attached to a plaquette fixed around the anus (Combihesive®, Squibb B.V., Rijswijk, The Netherlands). The samples collected were immediately placed in CO_2-filled containers for transport to the lab, which took approximately five min. The samples were weighed and mixed with four parts warmed sterile anaerobic saline (w/w) using a blender for 60 s, and the resultant mixture was filtered through two layers of cheese cloth. All these procedures were carried out under a continuous flow of CO_2.

Gas readings and cumulative gas production curves

Four ml of the filtrate (inoculum) were injected into each bottle. The bottles were then incubated at 39°C for a maximum of 144 h. The pressure-volume measurements were carried out manually (Theodorou *et al.*, 1994). The experiments began on different days (balanced over diets) to keep the number of bottles within the limits of the manual system. Due to this and insufficient space in the incubator, gas production in some bottles was monitored for 48, 72, 96, or 120 h. This was balanced over each inocula-substrate combination. The gas production was cumulated according to Theodorou *et al.* (1994).

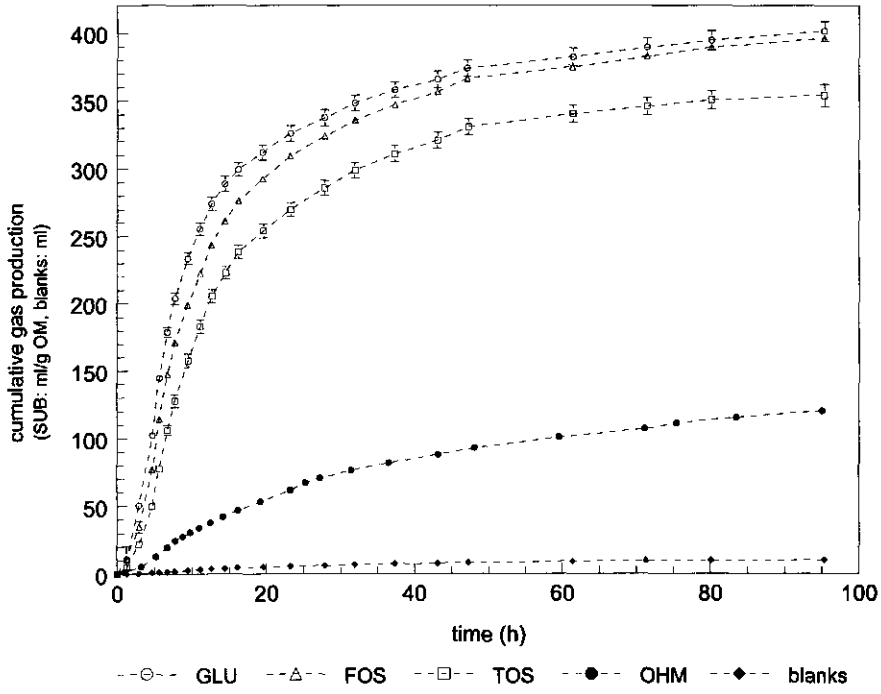

Figure 1. *Observed cumulative gas production for the substrates glucose (GLU), fructooligosaccharides (FOS), transgalactooligosaccharides (TOS), oats husk meal (OHM), and the blanks. The inoculum used was obtained from caecal digesta of a weaner pig fed a diet with 40 g FOS/kg*

Figure 1 shows typical cumulative gas production curves. These curves were fitted to a sigmoidal modified Michaelis-Menten equation (Groot *et al.*, 1996). This equation (Eq. 1) allows multiphasic curve fitting. The A- and B-values obtained were not discussed within the scope of this paper.

$$f(x)=A_i / (1+(C_i / t)^{Bi}) \quad [1]$$

where f(x) = gas produced (ml/g OM weighed in) at time *t* after incubation

A_i = asymptotic gas production for phase *i* (ml/g OM weighed in)

B_i = constant describing the sharpness of the curve's switching characteristic

C_i = half-time of A_i (h)

i = number of phases

Table 1. In vitro *fermentation characteristics of glucose, fructooligosaccharides, and transgalactooligosaccharides using ileal inocula from weaner pigs fed diets with or without these oligosaccharides*

		In vitro fermentation characteristics[1]						
Substrate (S)	Diet (D)[2]	CVOM	R_m	t_{Rm}	VFA	Ac	Pr	Bu
GLU	CON	347.3^{ab}	22.0^{a}	6.5^{c}	7.1^{bcd}	58^{a}	38^{abcd}	4^{d}
	FOS	300.7^{c}	22.7^{a}	6.0^{c}	5.1^{e}	46^{c}	37^{bcd}	17^{a}
	TOS	313.8^{bc}	18.9^{ab}	7.4^{bc}	6.1^{de}	51^{abc}	37^{bcd}	12^{abc}
FOS	CON	374.3^{a}	18.7^{ab}	10.4^{a}	9.3^{a}	47	46^{a}	7^{bcd}
	FOS	348.9^{ab}	22.2^{a}	9.5^{ab}	6.9^{cd}	42^{c}	45^{ab}	13^{ab}
	TOS	324.9^{bc}	18.8^{ab}	9.2^{ab}	8.0^{abc}	45^{c}	43^{abc}	12^{abc}
TOS	CON	324.3^{bc}	14.2^{b}	9.2^{ab}	8.3^{ab}	57^{ab}	37^{abcd}	6^{cd}
	FOS	325.4^{bc}	18.3^{ab}	8.6^{ab}	6.9^{cd}	59^{a}	32^{d}	9^{bcd}
	TOS	302.9^{bc}	15.5^{b}	8.0^{bc}	8.4^{ab}	60^{a}	33^{cd}	6^{cd}
	SEM	10.2	1.2	0.5	0.3	2	2	2
ANOVA[3]								
Main-plot	D_i	NS	NS	NS	‡	NS	NS	NS
	$Pig_k(D_i)$	***	***	***	***	***	***	***
Split-plot	S_j	***	***	***	***	***	***	***
	$(D*S)_{ij}$	**	‡	***	*	***	NS	***
Orthogonal contrasts[3]								
Diet	CON vs NDO	NS	NS	NS	‡	NS	NS	NS
	FOS vs TOS	NS	NS	NS	NS	NS	NS	NS
Substrate	GLU vs NDO	**	***	***	***	NS	*	**
	FOS vs TOS	***	***	***	NS	***	***	***

[1]CVOM = cumulative gas production (ml/g OM weighed in); R_m = maximal rate of gas production (ml/h); t_{Rm} = time of occurrence of R_m (h); VFA = volatile fatty acids, Ac+Pr+Bu (mmol/g OM); Ac = acetic acid, Pr = propionic acid, Bu = butyric acid (all as % of VFA)
[2]CON: no non-digestible oligosaccharides (NDO); FOS: 40.0 g fructooligosaccharides/kg diet; TOS: 40.0 g transgalactooligosaccharides/kg diet
[3]NS: P>0.10; ‡: $P < 0.10$; * $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
[a,b,c,d]Columnwise means without common superscripts differ significantly (Tukey, $P < 0.05$)

The effects of dietary NDO were more pronounced when OHM was used as a substrate (Table 4). The FOS-diet's ileal inocula showed a faster R_m ($P < 0.01$) and later t_{Rm} ($P < 0.05$) compared with the TOS-diets. Both the VFA-production and C-value were smaller ($P < 0.001$). The NDO-diet's caecal inocula showed a earlier t_{Rm} ($P < 0.01$) than those of the control diet; this was significantly shorter for the FOS-diet's than for TOS-diets' inocula. The VFA-production did not differ, but its profile was significantly affected. The NDO-diet's faecal inocula produced less VFA ($P < 0.05$), with a significantly different profile, compared to those of the control diet. Further, the FOS-diet's faecal inocula produced more gas on OHM than those from the TOS-diet ($P < 0.05$). The NDO-diet's faecal- and caecal inocula showed less OM-loss than those of the control diet ($P < 0.10$).

Table 2. In vitro *fermentation characteristics of glucose, fructooligosaccharides, and transgalactooligosaccharides using caecal inocula from weaner pigs fed diets with or without these oligosaccharides.*

		In vitro fermentation characteristics[1]						
Substrate (S)	Diet (D)[2]	CVOM	R_m	t_{Rm}	VFA	Ac	Pr	Bu
GLU	CON	302.7^{ab}	23.3^{c}	8.1^{a}	6.8^{a}	54^{ab}	39^{bcd}	7^{bc}
	FOS	310.7^{a}	31.5^{a}	4.6^{c}	5.7^{b}	49^{cde}	32^{d}	19^{a}
	TOS	296.8^{abcd}	21.6^{cd}	6.1^{b}	7.0^{a}	43^{f}	52^{a}	5^{bc}
FOS	CON	272.4^{def}	21.3^{cd}	8.7^{a}	6.8^{a}	52^{bc}	41^{b}	7^{bc}
	FOS	297.4^{abc}	26.8^{b}	4.7^{c}	5.8^{b}	51^{bcd}	34^{cd}	15^{a}
	TOS	284.4^{bcde}	19.3^{d}	7.9^{a}	7.2^{a}	45^{ef}	51^{a}	4^{c}
TOS	CON	251.0^{f}	19.2^{d}	8.4^{a}	6.8^{a}	54^{ab}	39^{bc}	6^{bc}
	FOS	276.5^{cde}	22.7^{c}	5.8^{bc}	6.5^{ab}	58^{a}	32^{d}	10^{b}
	TOS	268.3^{ef}	18.8^{d}	6.7^{b}	6.9^{a}	46^{def}	50^{a}	3^{c}
	SEM	5.5	0.7	0.3	0.2	1	2	1
ANOVA[3]								
Main-plot	D_i	NS	**	**	‡	*	**	*
	$Pig_k(D_i)$	***	***	***	***	***	***	***
Split-plot	S_j	***	***	***	‡	***	*	***
	$D*S_{ij}$	‡	***	***	**	***	*	***
Orthogonal contrasts[3]								
Diet	CON vs NDO	NS	NS	**	NS	NS	NS	NS
	FOS vs TOS	NS	**	*	*	*	**	**
Substrate	GLU vs NDO	***	***	***	‡	***	NS	***
	FOS vs TOS	***	***	NS	NS	***	***	***

[1,2,3]See Table 1
[a,b,c,d,e,f]Columnwise means without common superscripts differ significantly (Tukey, $P < 0.05$)

Differences between substrates

Most differences between FOS and TOS as substrates were comparable for each inoculum source (Tables 1, 2, and 3). The FOS produced more gas and showed a faster R_m than TOS ($P < 0.001$). While the total production of VFA did not differ between FOS and TOS as a substrate, FOS produced less acetic acid, and more propionic- and butyric acid than TOS ($P < 0.001$). The effect on t_{Rm} depended on the inoculum source; for the ileal inoculum t_{Rm} was later for FOS than for TOS, while this was the opposite for the faecal inoculum ($P < 0.001$).

The difference between GLU and NDO depended on the inoculum source. The ileal and faecal inocula produced less gas on GLU, but the caecal inocula produced more. While t_{Rm} was earlier for GLU than for NDO for each inoculum source ($P < 0.001$), R_m was faster for ileal and caecal inocula only ($P < 0.001$). Glucose always produced less VFA than the NDO. However, the effects on VFA profile differed between inoculum sources (Tables 1, 2, and 3).

Table 3. In vitro *fermentation characteristics of glucose, fructooligosaccharides, and transgalactooligosaccharides using faecal inocula from weaner pigs fed diets with or without these oligosaccharides*

		In vitro fermentation characteristics[1]						
Substrate (S)	Diet (D)[2]	CVOM	R_m	t_{Rm}	VFA	Ac	Pr	Bu
GLU	CON	320.9^{ab}	22.1^{ab}	10.2^{abc}	5.7^{ab}	45^{e}	34^{a}	21^{abc}
	FOS	324.9^{a}	21.8^{ab}	8.2^{bc}	6.3^{ab}	50^{cde}	3^{bc}	12^{bcd}
	TOS	277.0^{b}	20.2^{ab}	8.0^{c}	4.8^{b}	57^{abcd}	16^{c}	26^{a}
FOS	CON	349.4^{a}	24.3^{a}	10.6^{ab}	6.1^{ab}	48^{de}	28^{ab}	23^{abc}
	FOS	333.5^{a}	20.5^{ab}	8.0^{c}	6.6^{ab}	53^{bcde}	36^{a}	11^{cd}
	TOS	333.1^{a}	23.9^{a}	9.0^{bc}	5.0^{b}	57^{abcd}	15^{c}	28^{a}
TOS	CON	312.4^{ab}	18.1^{b}	12.0^{a}	5.4^{ab}	61^{abc}	15^{bc}	24^{ab}
	FOS	322.5^{a}	19.8^{ab}	9.0^{bc}	7.0^{a}	63^{ab}	29^{ab}	8^{d}
	TOS	313.9^{ab}	21.8^{ab}	9.4^{bc}	5.7^{ab}	67^{a}	17^{bc}	16^{abcd}
	SEM	9.9	1.3	0.6	0.4	3	3	3
ANOVA[3]								
Main-plot	D_i	NS	NS	NS	NS	NS	NS	NS
	$Pig_k(D_i)$	***	***	***	***	***	***	***
Split-plot	S_j	***	***	***	‡	***	***	**
	D^*S_{ij}	**	***	**	**	‡	***	***
Orthogonal contrasts[3]								
Diet	CON vs NDO	NS	NS	NS	NS	NS	NS	NS
	FOS vs TOS	NS	NS	NS	NS	NS	‡	NS
Substrate	GLU vs NDO	***	NS	***	*	***	***	NS
	FOS vs TOS	***	***	***	NS	***	***	***

[1,2,3]See Table 1
[a,b,c,d,e]Columnwise means without common superscripts differ significantly (Tukey, $P < 0.05$)

Interaction between diets and substrate

Tables 1 to 3 show that there were significant interactions between diet and substrate for most parameters. The superscripts indicate the location of such effects. For example, the control diet's ileal inocula produced significantly more gas than those of the TOS-diet when FOS but not TOS were used as substrates. The proportions of acetic acid differed for each diet's caecal inocula when GLU was used as a substrate; however, when FOS or TOS were used as substrates, the control diet's and the FOS-diet's caecal inocula did not differ significantly in terms of the proportion of acetic acid, but both were different from those of the TOS-diet ($P < 0.05$). None of the diet's faecal inocula differed in t_{Rm} when GLU was used as a substrate. When TOS were used as substrates, the NDO-diet's faecal inocula showed an earlier t_{Rm} ($P < 0.05$). When FOS were used as substrates, t_{Rm} was significantly earlier for the FOS-diet's faecal inocula and not for the TOS-diet's inocula compared with those of the control diet.

Table 4. In vitro *fermentation characteristics of oats husk meal using faecal-, caecal- and ileal inocula from weaner pigs fed diets with or without non-digestible oligosaccharides*

		In vitro fermentation characteristics[1]								
	Diet[2]	OM-loss	CVOM	C	R_m	t_{Rm}	VFA	Ac	Pr	Bu
Ileal	CON	23.4	63.9	23.3	3.8	2.4	2.1	63	29	9
inoculum	FOS	23.2	81.3	14.3	5.6	3.4	1.8	60	27	12
	TOS	22.8	66.6	26.2	3.3	1.7	2.3	64	27	9
	SEM	0.6	5.7	1.8	0.5	0.6	0.2	1	1	1
Contrasts[3]	CON vs NDO	NS	NS	NS	NS	NS	NS	NS	NS	NS
	FOS vs TOS	NS	‡	***	**	*	***	NS	NS	NS
Caecal	CON	28.6	82.4	19.8	4.0	7.7	1.9	67	25	8
inoculum	FOS	22.4	75.6	21.7	4.1	4.8	1.7	58	33	9
	TOS	25.7	78.8	19.2	3.6	6.5	1.8	58	35	7
	SEM	1.9	2.6	1.3	0.2	0.6	0.1	1	1	1
Contrasts[3]	CON vs NDO	‡	NS	NS	NS	**	NS	***	***	NS
	FOS vs TOS	NS	NS	NS	‡	*	NS	NS	*	**
Faecal	CON	28.7	89.4	27.6	3.3	10.3	1.9	57	26	17
inoculum	FOS	24.7	86.7	22.3	3.9	8.0	1.6	60	28	12
	TOS	24.0	62.3	23.2	3.4	7.6	1.8	62	27	11
	SEM	2.1	6.8	2.4	0.3	1.5	0.1	1	2	2
Contrasts[3]	CON vs NDO	‡	‡	‡	NS	NS	*	***	NS	*
	FOS vs TOS	NS	*	NS	NS	NS	NS	NS	NS	NS

[1,2,3]See Table 1; OM-loss = organic matter loss (%); C = half time of asymptotic gas production (h)

Discussion

This study had several objectives. The differences between FOS and TOS as substrates were studied in terms of *in vitro* fermentation kinetics and end-point VFA-production. A second objective was to determine whether the cumulative gas production technique could identify differences between inocula induced by dietary NDO, by comparing them in terms of *in vitro* fermentation activity on specific substrates. The interaction between dietary NDO and NDO as *in vitro* substrates would then provide information on the adaptation of the microflora to NDO. Finally, OHM was used as a substrate *in vitro* to determine whether fibre-degrading microorganisms could have an altered activity as result of dietary NDO. These *in vitro* observations could provide clues to explain observed effects of dietary NDO *in vivo*.

Rate of fermentation of FOS and TOS

It may be expected that the rate of fermentation depends on the relation between specific microbial activity and the chemical structure of the substrates. Glucose, a monomeric saccharide, is easily available for fermentation. The FOS used in this experiment consisted of linear chains of β-linked fructose units, which may have an α-linked glucose unit (Roberfroid, 1993). Both β-fructosidase and sucrase are needed to release all fructose and glucose and to make them available for fermentation. The structures found in TOS are even more complex: TOS consist of linear and branched β-linked galactose units, sometimes including an α-linked glucose unit. More than 40 different structures can be found in the TOS-mixture used in this experiment (K.J.M. van Laere, personal communications). Thus, a mixture of enzymes is necessary to release all galactose and glucose. Ranking for R_m was similar to that for structure complexity of the substrates used (GLU<FOS<TOS) for the ileal and caecal inoculum. The ranking does not hold for t_{Rm}, which was earlier for TOS than for FOS for the ileal inoculum, but was not different for the caecal inoculum. The combination of t_{Rm} and R_m does suggest that FOS and TOS may be fermented differently in the gastrointestinal tract: fermentation may be completed more proximally as the rate of fermentation increases. Indeed, it has been shown that pre-caecal disappearance of FOS was more than 90% (Houdijk *et al.*, Chapter 2) while that of TOS was estimated at 30% (R. Kamelaar, personal communications). The differences in R_m for FOS and TOS suggest that *in vivo* TOS fermentation is more likely to be completed later in the digestive tract (e.g. proximal colon) than FOS.

The cumulative gas production technique was used to show that the maximal rate of fermentation of FOS as a substrate was significantly faster than that of TOS. This was in agreement with the difference in ileal disappearance of these NDO in weaner pigs.

Volatile fatty acid production from FOS and TOS

There was no difference in total VFA production from the substrates FOS and TOS, though from FOS relatively more propionic- and butyric acid and less acetic acid was produced than from TOS. A high level of propionic acid production has also been observed when FOS were fermented using digesta from neonatal pigs (Bunce *et al.*, 1994). An increased proportion of propionic acid was observed for TOS as well during fermentation *in vitro* by caecal and colonic inocula of pigs (Faisant *et al.*, 1990). It has been shown previously for the weaner pigs used in this experiment, that dietary FOS resulted in relatively more propionic acid and less acetic acid in the ileal digesta than did dietary TOS. However, those relative proportions did not differ between dietary FOS and TOS in the

faeces (Houdijk *et al.*, Chapter 4). This effect in the ileal digesta *in vivo* (FOS vs TOS in the diet) was comparable to that using the ileal inoculum *in vitro* (FOS vs TOS as substrates), suggesting that the *in vivo* VFA profile at the ileal but not at the faecal level directly results of dietary NDO. The substrates FOS and TOS produced significant amounts of butyric acid *in vitro* (up to 1.5 mmol/g). This is very relevant for colonic energy supply as butyric acid is the primary energy source of colonocytes (Von Engelhardt *et al.*, 1989). The butyric acid may have been produced by *Clostridia* spp., which can produce butyrate from glucose and inulin, a fructose polymer (Schlegel, 1992).

Pooled over substrates, the VFA profiles *in vitro* comprised relatively less acetic acid and more propionic- and butyric acid than those *in vivo*. The increased proportion of propionic acid may be a result of a more complete lactic acid (LA) fermentation *in vitro* than *in vivo*. Fermentation of LA can result in a 2:1 propionic- to acetic acid production (Schlegel, 1992). Indeed, significant concentrations of LA were observed in the ileal digesta (Houdijk *et al.*, Chapter 4). Though LA was not measured in our experiment *in vitro*, it has been shown that LA concentrations initially increased and then rapidly declined during a 24 h *in vitro* TOS fermentation (Kikuchi-Hayakawa *et al.*, 1997), which is in agreement with the intermediate nature of this fermentation product. Differences in VFA profile may also be the result of differences in rates of absorption. Dijkstra *et al.* (1993) showed that the rate of absorption of the individual VFA in the rumen of dairy cows increases with the size of the molecule. Thus, the VFA produced *in vivo* may have contained relatively more propionic- and butyric acid than the VFA observed in the ileal digesta at the moment of sampling, which is in agreement with the profiles observed *in vitro*.

Thus, when used as substrates *in vitro*, FOS produced more propionic- and butyric acid and less acetic acid than TOS. This was comparable with differences observed *in vivo* at the ileal but not at the faecal level when these NDO were included in the diet, suggesting that the faecal VFA profile *in vivo* is not the direct result of dietary NDO fermentation.

Effect of dietary FOS and TOS

Significant overall effects of dietary NDO on *in vitro* fermentation of GLU, FOS, and TOS as a substrate occurred only with the caecal inocula. This was in agreement with the effects of dietary NDO on the concentration of anaerobes *in vivo*. The caecal digesta of the NDO-fed pigs, especially that of the FOS-fed pigs, contained significantly more anaerobes than that of the control pigs. Those effects were less pronounced in the ileal digesta and faeces (Houdijk *et al.*, Chapter 4). The fermentation characteristics for inocula from pigs within the same experimental group varied substantially, which is in agreement with other

workers (Robinson *et al.*, 1989; Jensen and Jørgensen, 1994). Highly significant interactions between diet and substrate were observed for each source of inoculum, suggesting that an absence of a significant overall effects of dietary NDO may have been due to different effects for specific substrates. This interaction was used to determine whether pre-exposure to NDO, as part of the diet, had led to adaptation of the microflora.

In the context of this paper, specific adaptation of the microflora to dietary NDO was judged by the difference between control-diet's inocula and the specific NDO-diet's inocula, when fermenting the dietary NDO as a substrate *in vitro*. It was speculated that prior exposure to FOS or TOS in the diet would resulted in a faster R_m and/or earlier t_{Rm} when FOS or TOS were used as substrates *in vitro*. However, adaptation was more likely to be aspecific if such shifts had occurred for GLU and/or for the contrasting NDO as well. At the ileal level, there were no significant differences between t_{Rm} and R_m of the inocula from the control diet and the FOS-diet when FOS were used as substrates. The same was observed for TOS. Thus, there were no suggestions of adaptation to dietary NDO for the ileal microflora (Table 1). The caecal inocula from the FOS-diet showed a faster R_m and an earlier t_{Rm} for each substrate compared with those from the control diet. In contrast, the caecal inocula from the TOS-diet was similar in terms of R_m for each substrate to those from the control diet, but showed an earlier t_{Rm} when TOS and GLU were used as substrates. This suggested that the caecal microflora had adapted to both NDO, but the adaptation to FOS may have been less specific than to TOS (Table 2). The faecal inocula from the NDO-diets were similar in terms of R_m for each substrate to those from the control diet, but showed a significantly earlier t_{Rm} for TOS. In contrast, this was only the case for the FOS-diet when FOS was used as a substrate (Table 3). This suggested that the faecal flora of pigs fed dietary FOS had adapted in such a way that TOS could also be more rapidly fermented, but not vice versa.

The absence of adaptation to dietary NDO at ileal level may have been due to the short residence time of bacteria in the small intestine. Thus, adaptation, if any, is more likely to occur in the caecal or colon flora, the latter being represented by the faeces. The more specific adaptation observed for dietary TOS compared to dietary FOS may have been due to the previously mentioned differences in the chemical structure of these NDO. In addition, the faecal microflora was probably not pre-exposed to FOS at all, given the near complete pre-caecal degradation of FOS (Houdijk *et al.*, Chapter 2).

The effect of dietary NDO on the *in vitro* fermentation characteristics depended on the type of carbohydrates used as substrates. This interaction was used to determine the adaptation of the faecal-, caecal, and ileal microflora to dietary NDO, and suggested that

the adaptation to dietary TOS may have been more specific than the adaptation to dietary FOS.

Fermentation of oats husk meal

The diets of the weaner pigs contained OHM as crude fibre source. Thus, OHM was used as substrate in the *in vitro* fermentation experiment to determine whether dietary NDO had changed the activity of the fibre degrading microorganisms. The t_{Rm} of OHM was earlier, especially for the ileal inocula, and the R_m slower than those observed for the other substrates. This was likely to be a result of the fermentation of a small amount of easily available oat carbohydrates, like ß-glucan (Bach Knudsen and Hansen, 1991), rather than the R_m and t_{Rm} of the more complex cell wall material present in OHM. This should have resulted in a multi-phasic cumulative gas production pattern, as was observed for rye fibre, sorghum, grass, and clover (Groot *et al.*, 1996). More measurements at shorter time intervals could have detected such patterns. The reduced VFA- and gas production was in agreement with the reduced loss of OM from OHM using the faecal and caecal inocula. Though the differences in VFA profile suggest that OHM was used differently, the hindgut microflora of the NDO-fed pigs was not able to ferment OHM to the same extent as that of the control pigs. This may indicate that dietary OHM could not provide the energy needed to maintain NDO-induced changes in microflora activity throughout the large intestine.

Thus, *in vitro* OHM fermentation was less extensive when NDO were included in the diet. This may indicate that the inclusion of NDO in the diet resulted in a lowered *in vivo* activity of the fibre degrading microorganisms.

Conclusions

We concluded that the maximal rate of fermentation of FOS as a substrate was significantly faster than that of TOS, and that FOS produced more propionic- and butyric acid and less acetic acid than TOS. These properties could partly explain differences observed *in vivo* in the ileal digesta when these NDO were included in the diet. The effect of dietary NDO on the *in vitro* fermentation characteristics depended on the type of carbohydrates used as substrates. The adaptation of the caecal and faecal microflora to dietary TOS may have been more specific than the adaptation to dietary FOS. *In vitro* OHM fermentation was less extensive when NDO were included in the diet, suggesting that dietary NDO lowered *in vivo* activity of the fibre degrading microorganisms.

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CHAPTER

6

PREBIOTICS OR ANTIBIOTICS IN HIGH-PROTEIN DIETS FOR WEANER PIGLETS: GROWTH PERFORMANCE AND FAECES CONSISTENCY

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Prebiotics or antibiotics in high-protein diets for weaner piglets: growth performance and faeces consistency

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Abstract

We studied the effects of dietary fructooligosaccharides (**FOS**) on growth performance and faeces consistency of weaner piglets fed high protein diets, to test the hypothesis that FOS discourage protein fermentation in the gut. A FOS-free control diet (**CON**) or the same diet containing FOS at 7.5, 15.0, 22.5, and 30.0 g/kg (w/w cellulose) were formulated. The positive control diet was the same as diet CON but contained avilamycin as in-feed antibiotic at 40 ppm (**AVI**). Sixty litters of 10-11 piglets were used (ten litters per diet). The diets were offered from d 10 of age until 13 d after weaning (at d 28). Then a commercial starter diet was offered for another three weeks. Body weights and feed intakes were recorded regularly, and the faeces consistency (normal, soft, watery) scored until wk 3 after weaning.

Daily weight gain (128 g), feed intake (210 g), and the proportion of normal faeces (66%) of the piglets fed the CON-diet were considerably lower than normally observed from weaning to d 41 of age. There was no effect of FOS on daily feed intake, weight gain, and faeces consistency, though a quadratic effect of FOS on feed conversion was observed in this period ($P < 0.05$). Daily feed intake and weight gain was significantly greater for the AVI-diet, compared to both the CON- and the FOS-diets. In addition, the AVI-diet showed a higher proportion of normal faeces (77%) than did the other diets from weaning to d 41 of age ($P < 0.05$). The proportion of normal faeces increased to more than 90% when the commercial starter diet was offered, and was not affected by the previous dietary treatments. The feed conversion for the AVI-diet was higher than that for the other diets during the feeding of the commercial starter diet. Both the AVI- and FOS-diets did not affect growth performance from weaning to d 63 of age.

The absence of improved growth performance and faeces consistency of weaner piglets fed FOS-supplemented high-protein diets, in combination with the presence of such improvements for in-feed avilamycin, suggested that dietary FOS did not lower protein fermentation in the gut.

Introduction

Some carbohydrates are not enzymatically digested but are fermented by the gastrointestinal microflora. These carbohydrates include the non-digestible oligosaccharides (NDO). Fructooligosaccharides (**FOS**) are found in barley and wheat (Campbell *et al.*,, 1997), and α-galactooligosaccharides (**GOS**) are found in legumes (Reddy *et al.*, 1984). In addition, a range of NDO can be produced chemically or enzymatically, including lactulose, xylooligosaccharides, transgalactooligosaccharides (**TOS**), and lactosucrose (Playne and Crittenden, 1995). Recently, certain NDO have been classified as having prebiotic activity, defined as 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health' (Gibson and Roberfroid, 1995). In terms of pig health, it is thought that prebiotic feed additives could be applied to control post-weaning diarrhoea.

Post-weaning diarrhoea (**PWD**) has multifactorial causes, including nutritional ones. It has been proposed that PWD may originate from a reduced feed intake just after weaning, followed by a period of overeating (Makkink, 1993). This can result in an increased amount of non-digested carbohydrates and proteins flowing into the colon. It has been shown that a threshold level exists for an amount of colon degradable carbohydrates, after which diarrhoea occurs (Saunders and Wiggins, 1981; Holtug *et al.*, 1992). It has also been shown that increasing crude protein levels in the diet led to elevated ammonia concentrations in the colon, and increased diarrhoea (Dong *et al.*, 1996). Furthermore, a negative correlation has been observed between the excretion of protein fermentation products and the growth performance of young pigs (Yokoyama *et al.*, 1982; Fukuyasu and Oshida, 1986).

Several in-feed antibiotics are being used to control PWD and thereby enhance pig performance (Rosen, 1995), including avilamycin (Kyriakis, 1989). Different modes of action have been proposed for this property of in-feed antibiotics, of which the reduction of microbial (proteolytic) activity is one (Rosen, 1995). A reduced proteolytic activity and incidence of diarrhoea have also been reported for FOS in weaner pigs (Hidaka *et al.*, 1985; Fukuyasu and Oshida, 1993), and for lactosucrose in dogs (Terada *et al.*, 1992) and cats (Terada *et al.*, 1993).

In this experiment, we studied the effects of FOS in high-protein diets on the growth performance and faeces consistency of piglets. A diet containing in-feed avilamycin was included as positive control. The diets were high in protein to induce a elevated level of protein fermentation.

Materials and methods

Diets, animals, and housing

Table 1 shows the ingredients and calculated analysis of the control diet used in this experiment (**CON**). The diets were based on corn, as it contains no FOS (Campbell *et al.*, 1997). Several commonly used NDO-free protein feedstuffs were used. Four diets contained FOS-rich Raftilose P95® (Orafti, Tienen, Belgium), which was exchanged at the expense of Arbocel® (purified cellulose) and Dextrose® (glucose). The Dextrose balanced the diets for digestible sugars, since some of these are also found in Raftilose P95®. The four levels of FOS were 7.5, 15.0, 22.5, and 30.0 g/kg. A sixth diet (**AVI**) was essentially the same as diet CON but contained 40 ppm avilamycin (Maxus®, Elanco, Nieuwegein, The Netherlands). The diets met or exceeded the known requirements of nutrients and minerals of young pre-weaned piglets (NRC, 1988).

Table 1. *Ingredients and approximate analysis of the control diet*

	Experimental control diet (CON)
Ingredients (g/kg)	
Corn[1]	452.5
Skimmed milk powder	175.0
Protein sources[2]	175.0
Dextrose® (glucose)	120.0
Arbocel® (cellulose)	32.0
Soyoil	20.0
Amino acids[3]	2.2
Minerals[4]	13.3
Premix[5]	10.0
Calculated analysis (g/kg)	
Dry matter	902.7
Inorganic matter	48.5
Crude protein	222.1
Ether extract	57.7
Crude fibre	39.0
N-free extract	535.4

[1]Half of the corn was pressurized toasted (>100°C), subsequently flaked, and pelleted (Cornax®, Schouten/Giessen, The Netherlands).
[2]Protein sources (g/kg diet): 60.0 potato protein (Protastar®, AVEBE, The Netherlands), 75.0 fishmeal, and 40.0 meat-and-bone meal.
[3]Amino acids (g/kg diet): 1.1 L-cysteine, 0.5 L-threonine, and 0.6 L-tryptophane.
[4]Minerals (g/kg feed): 5.0 NaCl, 6.3 $CaCO_3$, and 2.0 $KHCO_3$.
[5]Premix (per kg feed): 9,000 IU vitamin A, 1,800 IU vitamin D_3, 40 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 5 mg riboflavin, 12 mg d-panthothenic acid, 1 mg folic acid, 3 mg pyridoxine, 30 mg niacin, 40 µg cobalamin, 1,000 mg choline chloride, 50 mg vitamin C, 0.1 mg biotin, 2.5 mg $CoSO_4 \cdot 7H_2O$, 0.2 mg $Na_2SeO_3 \cdot 5H_2O$, 0.5 mg KI, 400 mg $FeSO_4 \cdot 7H_2O$, 40 mg $CuSO_4 \cdot 5H_2O$, 70 mg MnO_2, and 200 mg $ZnSO_4$.

Meat-and-bone-meal was included at a level of 40 g/kg diet. The apparent faecal and ileal digestibility of the protein from meat-and-bone meal are approximately 90 and 55%, respectively (CVB, 1996). Thus, it was anticipated that the inclusion of meat-and-bone meal gave rise to the level of protein fermentation in the colon. The diets did not contain additional copper, probiotics, or organic acids.

The experiment comprised ten replicates of six litters each. The six diets were randomly allocated, one litter per diet. Within each replicate, the litter size was standardized at 10-11 piglets on d 3 after birth. After being weaned, the piglets were moved to ground floor pens without mixing of the litters. The piglets had free access to diets from d 10 onwards. The same diets were offered from weaning (d 28 of age) to d 41 of age. Then the piglets received a commercial starter diet until 5 weeks post-weaning (d 63 of age). Approval for this experiment was given by the Animal Ethics Committee of Wageningen Agricultural University.

Growth performance and faeces consistency

The piglets were weighed on d 0 (at birth), d 10, d 28 (at weaning), d 41, and d 63 of age. Thus, four observation periods were distinguished: Period I from birth to d 10, Period II from d 10 to weaning, Period III from weaning to d 41, and Period IV from d 41 to d 63 of age. The total amount of feed offered was recorded, and the feed refusals were weighed on d 28, d 41, and d 63. The body weights and feed intake were used to calculate daily weight gain, daily feed intake, and feed conversion ratio (feed intake/weight gain). These data were calculated per litter because the individual feed intake was not measured. One person judged the consistency of the faeces per litter, three times per week for two weeks in Period II, two weeks in Period III and one week in Period IV. Three types were distinguished: normal, soft, and watery. The proportion of each faeces type (**PFT**) per week within an observation period was calculated as the number of types observed divided over the total amount of observations per week for each litter. Soft and watery faeces were considered as PWD.

Statistical analysis

The feed conversion during Period III of the control piglets in replicate three was 3.14, which was considered to be an outlier compared to the mean of the other replicates (1.63±0.10). Thus, the data obtained for replicate three were omitted from the statistical analysis. The growth performance data were analysed using the model given by Eq. 1, in which the litter was used as the experimental unit. Mean litter weight at the beginning of each observation period and the proportion of males in the litter were used as covariates.

$$y_{ij} = \mu + R_i + D_j + ß_1{*}S_{ij} + ß_2{*}W_{ij} + \varepsilon_{ij} \quad [1]$$

where y_{ij} = observation
μ = overall mean
R_i = replicate (i = 1, 2, 4, ..., 10)
D_{ij} = diet (j = 1, ..., 6)
S_{ij} = proportion of males in the litter, with coefficient β_1
W_{ij} = mean litter weight, with coefficient β_2
ε_{ij} = residual error

The proportions of faeces types were transformed according to arcsin($\sqrt{}$PFT) to stabilize the variance (Snedecor, 1955). Transformed PFT were analyzed using the model given by Eq. 2.

$y_{ij} = \mu + R_i + D_j + L_k(D_j) + W_l + D^*W_{jl} + \varepsilon_{ijkl}$ [2]

where y_{ij} = observation
μ = overall mean
R_i = replicate (i = 1, 2, 4, ..., 10)
D_{ij} = diet (j = 1, ..., 6)
$L_k(D_j)$ = litter within diet (k = 1, ..., 10)
W_l = week within period II or III (l = 1, 2)
D^*W_{jl} = interaction between D_i and W_l
ε_{ij} = residual error

The effect of D_{ij} was tested against L_kD_j within period I and II (split-plot analysis). The effects of W_l and D^*W_{jl} were tested against the residual error.

A set of five non-orthogonal contrasts was used to locate the effect of dietary treatments. Comparisons were made between the control diet and the FOS-diets (**C-F**), the control diet and the AVI-diet (**C-A**), and the FOS-diets and the AVI-diet (**F-A**). In addition, two orthogonal polynomia were used to study linear ($\mathbf{F_L}$) and quadratic responses to dietary FOS ($\mathbf{F_Q}$). The contrasts were considered significant at $P < 0.05$. The effect of weeks within a period was tested using a t-test ($P < 0.05$).

Results

Growth performance

Table 2 shows the daily weight gain, feed intake, and feed conversion of the piglets during the four observation periods. The piglets averaged 8.1 kg at weaning (28 d). The growth performance in each period was not significantly different for the FOS-diets compared with that for the CON-diet (C-F). A tendency to a higher daily weight gain and a significantly higher feed intake was observed for the AVI-diet compared to the other diets during Period II. Both daily feed intake and weight gain were higher for the AVI diet than for the CON- and FOS diets during Period III. Feed conversion tended to be lower for the AVI diet than for the CON-diet during this period. During period IV, the feed conversion for the AVI-diet was higher than that for the other diets. The body weights of the piglets fed the FOS diets averaged 18.7 kg and those fed diet AVI averaged 20.0 kg (sem 0.6, $P < 0.05$) at the end of Period IV (d 63 of age). Though the final weights were different, both dietary FOS and avilamycin did not significantly affect the growth performance during the total post-weaning period (data not shown).

Table 2: *Growth performance of young piglets fed protein-rich diets with fructooligosaccharides or avilamycin.*

	Diets[1]					SEM	Effects[2]				
	CON	+ FOS				+ AVI		C-F	C-A	F-A	F_L	F_Q
Periods[3]		7.5[4]	15.0	22.5	30.0							
Weight gain, g/day												
I	218	216	218	219	218	214	9	NS	NS	NS	NS	NS
II	252	233	261	251	246	264	9	NS	NS	‡	NS	NS
III	128	141	129	155	137	177	15	NS	*	*	NS	NS
IV	476	502	447	449	446	448	17	NS	NS	NS	‡	NS
Feed intake, g/day												
II	39	44	41	38	38	53	4	NS	*	**	NS	NS
III	210	211	195	222	215	250	14	NS	‡	*	NS	NS
IV	695	738	675	658	681	688	21	NS	NS	NS	NS	NS
Feed conversion												
III	1.68	1.55	1.58	1.43	1.71	1.48	0.09	NS	‡	NS	NS	*
IV	1.46	1.48	1.52	1.48	1.52	1.55	0.02	NS	*	‡	NS	NS

[1]CON = diet without fructooligosaccharides (FOS) or avilamycin (AVI). Presented are the LSMeans for the factor D_i (diet) of the model given in the Material and Methods section
[2]C-F = control vs the FOS-diets, C-A = control vs the AVI-diet, F-A= the FOS-diets vs the AVI-diet, F_L = linear effects of dietary FOS, and F_Q = quadratic effects of dietary FOS. ‡: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$
[3]Observation periods: I = d 0 (birth) to 10, II = d 10 to 28 (weaning), III = d 28 to 41, and IV = d 41 to 63. Each group of piglets was offered a commercial diet during period IV
[4]Level of FOS (g/kg)

Contrast F_Q was significant for feed conversion during Period III ($P < 0.05$). Feed conversion initially decreased as dietary FOS increased from 0 to 22.5 g/kg, but then increased when dietary FOS increased to 30.0 g/kg. Daily weight gain tended to decreased with increasing dietary FOS in the previous diets (F_L, Period IV).

Figure 1 shows the relationship between the feed efficiency (**FE**, gain:feed) for the CON-diets (X-axis) and the maximal magnitude of response in FE to dietary FOS, relative to the level for the CON-diet (Y-axis, CON=100). Each data point represents a replicate. A significant negative relation (r = -0.82, $P < 0.01$) was observed. This correlation was weaker for the AVI-diets (r = -0.43, P>0.10, data not shown). The effect of replicate was also significant for the other observations. The proportion of males in the litter did not significantly contribute to the model (P>0.10). The other covariate, the initial litter weight, was significant for the growth during Period I, and for growth and feed intake during Period IV. Growth and feed intake increased with increasing initial litter weight for these periods.

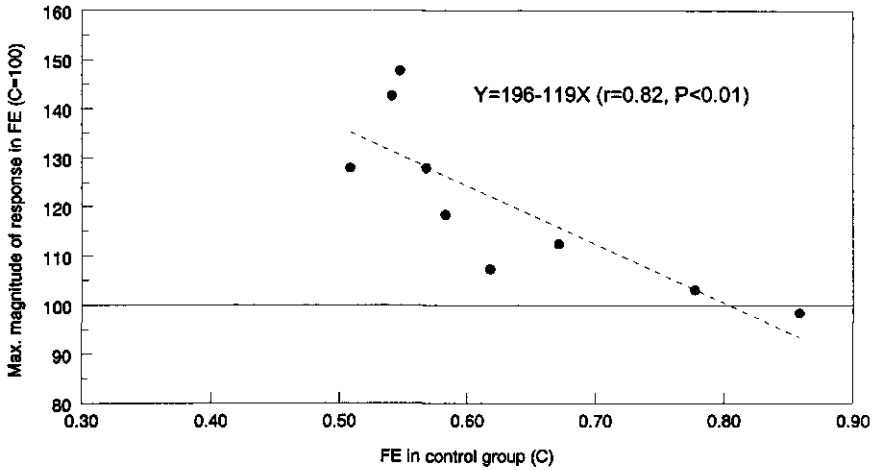

Figure 1. *The relation between the feed efficiency (FE, gain:feed) in the control group and the maximal magnitude of response in FE to dietary FOS. Each dot represents a replicate*

Faeces consistency

Figure 2 shows the proportions of faeces types. The proportion of normal faeces decreased significantly from 95% in week 1 of Period II to 65% in week 2 of Period III. More than 90% of the faeces was normal in Period IV. There were no effects of the FOS-diets per se compared with the control group, and also no linear or quadratic relations between the FOS-diets. The AVI-diet tended to a lower proportion of normal faeces compared to the FOS-diets (Period II), but showed a larger proportion of normal faeces compared to both the control diet and the FOS-diets in Period III ($P < 0.05$).

Discussion

Enhanced protein fermentation for the control diet

It has been shown in an previous experiment that dietary FOS and TOS did not improve growth performance of well-kept growing pigs (Houdijk *et al.*, Chapter 1). These authors suggested that the efficacy of NDO may increase when control performance is low, for instance, due to gastrointestinal disorders. Diarrhoea as a result of an enhanced protein fermentation could be one of such disorders and thus a reason for poor performance. The

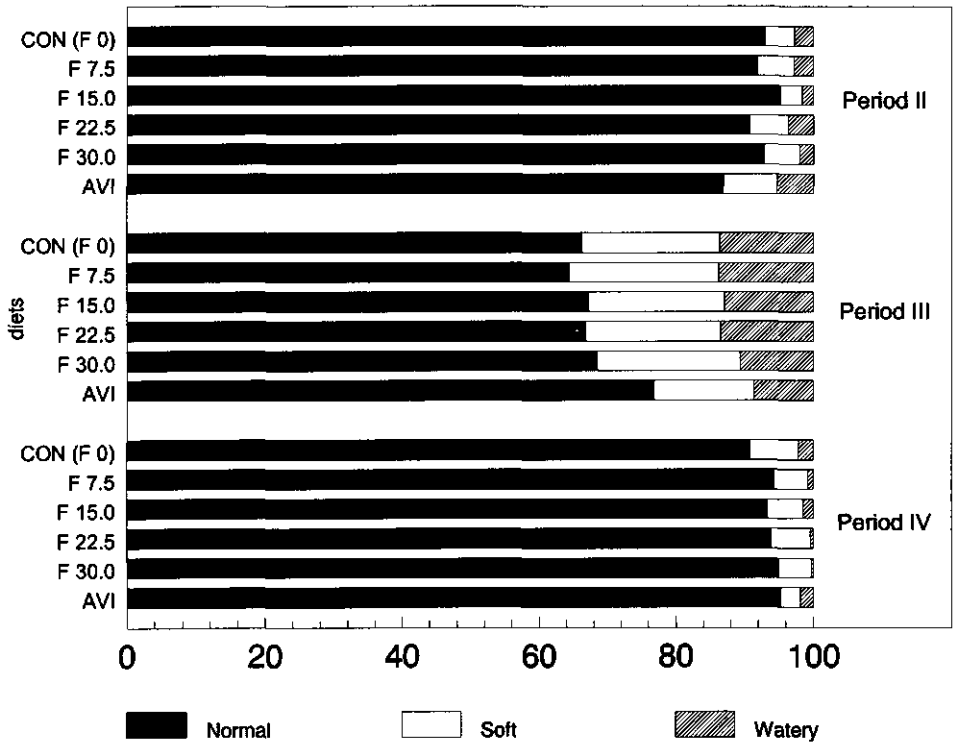

Figure 2. *Faecal consistency for three observation periods (II: two weeks before weaning, III: two weeks after weaning, and IV: wk 3 after weaning. The diets (left) were a control diet (CON), diets with fructooligosaccharides (F) ranging from 7.5 to 30.0 g/kg, and a diet with avilamycin at 40 ppm (AVI), and were fed during periods II and III. In Period IV, the piglets received a commercial starter diet*

control diet used in this experiment was a model for such a situation. Several measures were taken to achieve a relatively high level of protein fermentation. It was calculated that approximately 50 g CP would flow to the large intestine for each kg of the experimental diets ingested. A significant amount of this CP originated from meat-and-bone meal, which has a relatively low apparent ileal CP digestibility (CVB, 1996). The concentration of CP in weaner diets is usually lower than that in creep feed. However, in this experiment, the diets offered post-weaning were the same as those offered pre-weaning (Period III), which should also have increased the CP intake. Finally, no additives such as organic acids, probiotics or additional copper were used. Such additives have been shown to control PWD, though with variable success (Jonsson and Conway, 1992; Ravindran and Kornegay, 1993). Thus, the control diet was expected to result in an elevated level of protein fermentation, and as a result, a low level of performance and a considerable level of PWD.

Lowered performance for the control diet

To judge whether a relatively low performance and proportion of normal faeces were achieved, growth performance and faeces consistency were compared to another study, conducted at the same institute (Van der Peet-Schwering *et al.*, 1996 & personal communications). A diet containing barley, wheat, toasted soybeans and fishmeal as main protein sources, and tylosin as in-feed antibiotic, was fed to weanling piglets for 14 d after weaning (comparable to Period III). The mean growth performance of the control piglets in the present study was poorer during period III: -44% for weight gain, -24% for feed intake, and +39% for feed conversion ratio. In addition, more PWD was observed in Period III (+38%). These comparisons do suggest that the control diet successfully induced poor performance and a considerable level of PWD.

Effects of dietary FOS

Dietary FOS did not reduce PWD and did not improve growth performance in Period III. This is in contrast to earlier reports (Hidaka *et al.*, 1985; Fukuyasu and Oshida, 1986). Therefore, the high-protein diets may have induced a type of PWD that could not be corrected by FOS. It has been shown that FOS are pre-caecally degraded in ten-wk old weaner pigs (Houdijk *et al.*, Chapter 2). If the induced diarrhoea had mainly originated in the large intestine, and the FOS were degraded in the small intestine of the weanling piglets, then the reduction of this type of PWD by FOS would have been limited. Some piglets were sacrificed at the end of Period III, and physical-chemical and microbial properties of the digesta were studied. The pH, volatile fatty acid profile, and bacterial counts were hardly affected by dietary FOS. However, there was an effect of dietary avilamycin, which was in agreement with the lowered PWD. Data on these physico-chemical and microbial digesta characteristics are presented in Houdijk *et al.* (Chapter 7).

Balance between prebiotic and antinutritional properties of dietary FOS

A significant quadratic relation was observed between the level of dietary FOS and the feed conversion ratio in Period III. This suggested that an optimum level of dietary FOS may exist, and that some effects of dietary FOS result from the balance of two different properties, in this case the prebiotic- and antinutritional properties. On the one hand, dietary NDO may reduce the need for protein fermentation by the microflora (Terada *et al.*, 1992 and 1993; Fukuyasu and Oshida, 1993). This would result in less putrefactive products being absorbed from the gut lumen, and thus less energy needed to detoxify these components. Less protein fermentation in the small intestine could also increase the net pre-caecal absorption of amino

acids (Dierick *et al.*, 1986). Moreover, it has been shown that FOS increases villus height in the small intestine (Spencer *et al.*, 1997), which increases the pre-caecal absorption capacity.

On the other hand, some dietary NDO, especially the α-galactooligosaccharides (**GOS**), have long been known as antinutritional factors (**ANF**). High levels of GOS in legumes may induce diarrhoea in monogastric animals (Saini, 1991). Since NDO are small, indigestible, soluble carbohydrates, their ANF properties may be closely related to an increased osmolarity and transit of the small intestinal digesta (Wiggins, 1984). A reduced small intestinal residence time could results in less enzymatic digestion and absorption of nutrients. This effect may be of less importance since a significant overcapacity for enzymatic digestion and absorption is assumed to be present in the small intestine. However, a reduced transit time and increased diarrhoea have been observed in rats fed diets containing as much as 200 g FOS/kg (Tokunaga *et al.*, 1986). Thus, it is suggested that there is a balance between prebiotic- and antinutritional properties of NDO, and therefore an optimum may exist in terms of total levels of dietary NDO.

Level of performance in the control group

It has been suggested that the magnitude of response to dietary NDO is negatively correlated with the FE for the control group (Houdijk *et al.*, Chapter 1). Such a relation was indeed observed in this experiment for FE during Period III (Figure 2). The range on the X-axis reflected a considerable variation between replicates. In addition, the level of performance in the control group affects the expected response to dietary NDO. This could partly explain the variable responses to dietary NDO in practice. As such, dietary NDO do not behave differently from other feed additives, which also aim at controlling gastrointestinal disorders, such as organic acids (Ravindran and Kornegay, 1993), probiotics (Jonsson and Conway, 1992), and in-feed antibiotics (Rosen, 1995), though this was not observed for avilamycin in this study. The dependency of the expected response to such additives, on the level of performance of the control group justify the need of a positive control, especially when potential replacers of in-feed antibiotics are being studied.

Relation between pre-weaning feed intake and post weaning diarrhoea

Several studies have been carried out to test the hypothesis that an increased feed intake during the suckling period results in a decreased PWD (Hampson and Smith, 1986; Kelly *et al.*, 1990). In this experiment, the correlation between pre-weaning feed intake and PWD was not significant (r = -0.21, P = 0.12). However, the correlation for the AVI-diet alone was -0.63 (P < 0.10); those for the CON- and FOS-diets were not significant (P > 0.30).

Although this correlation is calculated with nine observations only, it suggested that creep feed composition should be taken into account when the relation between PWD and pre-weaning feed intake is studied. The increased feed intake observed for the AVI-diet could have been due to a lower level of protein fermentation, since it has been suggested that biogenic amines (protein fermentation products) are involved in lowering the voluntary feed intake (Bergner, 1981).

Conclusion

Dietary FOS did not affect the growth performance and PWD in weaner piglets fed high-protein diets, though a quadratic relation between the level of dietary FOS and feed conversion was observed from weaning (d 28 of age) to d 41. In contrast, in-feed avilamycin reduced PWD and improved growth performance in this period. A negative relation between the level of performance and the maximal magnitude of response to dietary FOS was observed. The absence of improved growth performance and faeces consistency for the FOS-supplemented high-protein diets, in combination with the presence of such improvements for in-feed avilamycin, suggested that dietary FOS did not lower protein fermentation in the gut.

Acknowledgements

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CHAPTER

7

PREBIOTICS OR ANTIBIOTICS IN HIGH-PROTEIN DIETS FOR WEANER PIGLETS: PHYSICO-CHEMICAL AND MICROBIAL PROPERTIES OF THE GASTROINTESTINAL DIGESTA *IN VIVO* AND *IN VITRO*

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Prebiotics or antibiotics in high-protein diets for weanling piglets: physico-chemical and microbial properties of the gastrointestinal digesta *in vivo* and *in vitro*

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Abstract

We studied the effects of dietary fructooligosaccharides (**FOS**) on physico-chemical and microbial propperties of the gastrointestinal digesta of weanling piglets fed high protein diets, to test the hypothesis that FOS lower bacterial proteolytic activity in the gut. A FOS-free control diet (**CON**), and the same with FOS at 30.0 g/kg (FOS), and with avilamycin at 40 mg/kg (**AVI**) were formulated. A total of 27 piglets (three replicates of three piglets per diet) were selected from a growth performance experiment using sixty litters of 10-11 piglets. The diets were offered from d 10 of age until d 13 after weaning (at d 28).

The high concentration of valeric acid found in the gastric- and small intestinal digesta suggested a considerable level of protein fermentation. The AVI-piglets had a lower gastric pH, less valeric acid in the gastric digesta, and less protein derived volatile fatty acids in the portal plasma compared to the CON-piglets. The FOS-piglets had less ammonia in the proximal small intestinal digesta than the CON-piglets. The anaerobe bacterial counts in the distal small intestine, caecum, and proximal colon were not affected by the diet, though the AVI-pigs had a higher count of *E.coli* than did the FOS-piglets. Dietary FOS accelerated the first phase of *in vitro* fermentation compared to the other diets, and both dietary FOS and avilamycin reduced the microbial activity in the second phase. The small intestinal inoculum from the FOS-piglets produced the more volatile fatty acids following *in vitro* fermentation than that from the CON-piglets, while that of the AVI-piglets produced less gas and butyric acid.

We concluded that dietary FOS reduced only some bacterial proteolytic activity in the proximal small intestine, though this was not maintained throughout the total lenght of the gastrointestinal tract. A reduced bacterial protoelytic activity was more pronounced for in-feed avilamycin. As such, dietary FOS cannot be considered to mimic the effects of in-feed avilamycin, though a combination of FOS and more slowly fermentable carbohydrates may prove useful.

Introduction

To test the hypothesis that dietary fructooligosaccharides (**FOS**) discourage intestinal protein fermentation, the effects of FOS on growth performance and the occurrence of post-weaning diarrhoea (**PWD**) were studied in piglets fed high-protein diets. Five diets containing FOS, ranging from 0 to 30 g/kg were studied, and compared to a similar diet without FOS but containing an in-feed antibiotic (avilamycin). The effects on growth performance and faeces consistency have been presented elsewhere (Houdijk *et al.*, Chapter 6). This paper describes several *in vivo* and *in vitro* microbial digesta characteristics of a selected group of piglets.

Biogenic amines are products of microbial proteolytic activity, as are ammonia, indole, and skatol (Macfarlane and Cummings, 1991). The net amine production *in vitro* can be reduced by 80% in the presence of starch (Smith and Macfarlane, 1996). Similarly, ammonia production can be reduced by nearly 40% in the presence of lactulose (Vince and Burridge,

1980). These results suggest that the use of protein as a source of energy, and thus the production of protein fermentation products, is reduced when easily fermentable carbohydrates are present. It has been shown that small intestinal biogenic amine concentrations are elevated in severely diarrhoeic piglets compared to clinically unaffected pigs (Porter and Kenworthy, 1969). Moreover, it has been shown that pre-caecal fermentative degradation of FOS exceeded 90% in weaner pigs (Houdijk *et al.*, Chapter 2). Thus, if dietary FOS were to lower microbial proteolytic activity, then a reduced concentration of putrefactive products would be expected in the small intestine.

The small intestinal bacterial proteolytic activity may also be reduced by the inclusion of in-feed antibiotics (Dierick *et al.*, 1986). In-feed antibiotics probably affect microbial activity throughout the intestinal tract, while that of FOS is probably limited to the site of fermentation itself.

In this paper, we report the effects of dietary FOS or avilamycin on *in vivo* microbial characteristics of the digesta and systemic volatile fatty acids (**VFA**) in weaner piglets fed high-protein diets. In addition, we used the cumulative gas production technique, which measures fermentation kinetics and end-point concentration of fermentation products *in vitro*, on selected substrates as a source of energy. This technique was used to study the effect of diet on the *in vitro* fermentation of FOS, using small intestinal and caecal microflora, and media containing either casein or free amino acids, peptides, and ammonia as the sources of nitrogen.

Materials and methods

Diets, animals, and housing

The work described in this paper was part of a larger experiment, which involved six dietary treatments: a negative control diet, four diets with FOS ranging from 7.5 to 30 g/kg, and a positive control diet (Houdijk *et al.*, Chapter 6). The three dietary treatments used for this part of the experiment were the negative control diet (**CON**), the diet containing 30 g FOS/kg (**FOS**), and the positive control diet containing avilamycin at 40 mg/kg (**AVI**). The FOS (Raftilose P95®, Orafti, Tienen, Belgium) were included at the expense of purified cellulose, and avilamycin was included in the premix (Maxus®, Elanco, Nieuwegein, The Netherlands). The diets were formulated to meet the known requirements of nutrients and minerals of young pre-weaned piglets (NRC, 1988), and did not contain additional copper, probiotics or organic acids. It was anticipated that the inclusion of meat-and-bone meal (40

g/kg) gave rise to the level of bacterial proteolytic activity. More details on the diets, animals, and housing have been presented elsewhere (Houdijk *et al.*, Chapter 6).

The experimental diets were fed ad lib from d 10 of age. The piglets were weaned at d 28 of age and the same creep feeds fed until d 41. Three piglets from each diet were sacrificed on d 41 of age, during three replicates of the ten-replicate experiment (Houdijk *et al.*, Chapter 6). The three piglets selected were those closest to the mean BW of the litter. The three littermates for the same diet were slaughtered within a short time to facilitate fresh sampling of the pooled digesta. The piglets were fed ad lib, as part of the larger experiment they were chosen from. However, this meant that the time between the last feed intake and sampling was not known. To minimize the possible effect of time between the last feed intake and sampling, the slaughter sequences chosen were CON-FOS-AVI (replicate 1), FOS-AVI-CON (replicate 2), and AVI-CON-FOS (replicate 3). Approval for this experiment was given by the Animal Ethics Committee of Wageningen Agricultural University.

Sample collection and measurements

Piglets were anaesthezised with an appropiate mixture of N_2O/O_2 and halothane. The abdomen was opened and blood samples were taken from the portal vein and the aorta. Before removal from the abdomen, the gastrointestinal tract (**GIT**) was divided into nine segments, using clamps and ligatures to prevent movement of the digesta. Firstly, the stomach was isolated. Then the caecum was located and isolated. The small intestine was divided in two, three m anterior to the ileocaecal valve. The *colon ascendens* was divided into four parts: the first and second half of the descending *ansa spiralis* and the first and second half of the ascending *ansa spiralis*. The remainder was considered to be the rectum. Immediately after removal of the GIT, segments were taken out, weighed, emptied, and re-weighed. Digesta for the three littermates were pooled and stored at -20°C pending analysis after sampling for the required fresh analyses.

Digesta from the distal small intestine, caecum, and first quarter of the colon were stored on ice, under a continuous flow of CO_2 during the dissection. When the digesta of the last littermate had been added, the pooled digesta were sampled for bacterial analysis. Approximately 5 g fresh digesta was weighed into airtight bottles containing sterile anaerobic buffered peptone water (Oxoid, Haarlem, The Netherlands). The headspace was gently flushed with N_2 for 30 s, bottles were sealed, and transported to an anaerobic chamber (within 45 min). The bacterial groups and species determined included total anaerobes (using Faecal Reinforced Clostridial Agar), total aerobes (using Nutrient Agar), lactobacilli (using LAMVAB ager, Hartemink *et al.*, 1997b), *Bacteroides* spp (using Bacteroides Bile

Esculine Agar), enterococci (using Kanamycin Aeculine Azide Agar), *E. coli* (using Eosine Methylene Blue Agar), clostridia (using Clostridia Sulfite-reducing Agar), and bifidobacteria (using Raffinose Bifidobacterium agar, Hartemink *et al.*, 1996). Bacterial analyses were performed using appropriate dilution and plate culture techniques.

The stored digesta were thawed at 4°C, and sampled for DM and CP after the pH measurement. The pH meter had been adjusted to the low temperature. The supernatants were obtained by centrifuging at 10,000 rpm. The supernatants were analyzed for VFA (Schutte *et al.*, 1992) and NH_3 as follows. The supernatants were deproteinized with 10% trichloride acetic acid. The NH_3 forms a blue complex with phenol and hypochlorite in an alkaline environment, which is colorimetrically analyzed at 623 nm. The portal and arterial plasma were also analyzed for VFA using the same technique as used for the supernatants, though the plasma was first deproteinized, before being injected into a capillary column (D. Bongers, personal communication). The individual VFA were characterized as belonging to one of two pools. Acetic-, propionic-, and butyric- acids were assumed to have mainly originated from saccharolytic activity and were added together as **sVFA**, while *iso*-butyric, valeric- and *iso*-valeric acids were assumed to have originated from proteolytic activity and were added together as **pVFA** (Rasmussen *et al.*, 1988). The difference between portal and arterial VFA was calculated; this was thought to better reflect the effect of the last feed intake.

In vitro *fermentation*

Fresh digesta from the distal small intestine and the caecum were used as inoculum for *in vitro* fermentation. The gas production technique used was adapted from Theodorou *et al.* (1994) as described by Houdijk *et al.* (Chapter 5). Two types of medium were used, the semi-defined medium B (Lowe *et al.*, 1985) with peptides, free amino acids, and ammonia as sources of nitrogen (**N-PFA**), and an N-free medium with the same amount of N available solely in the form of casein (**N-CAS**). The bottles either contained 0.25 g Raftilose P95® (four replicates) as a source of energy, or no exogenous substrate (two replicates). The cumulative gas production curves were fitted to a two-phasic, modified sigmoidal Michaelis-Menten equation (Groot *et al.*, 1996), through which the maximum rate of gas production ($\mathbf{R_m}$) and the time of occurrence of R_m ($\mathbf{t_{Rm}}$) were calculated for each phase. The curve fitting was carried out using the NON-LIN computer program (Sherrod, 1995). At the end of the fermentation (120 h), the fermentation liquid was sampled and analyzed for VFA and ammonia as described above.

Statistical analysis

The data obtained in this experiment were divided into three groups. The first set of data was obtained from the individual pig (BW, GIT-contents and plasma VFA). For this set, the analysis of variance was carried out according to the model given by Eq. 1. Replicates 1, 2, and 3 in this paper refer to replicates 2, 4, and 5, respectively of the larger experiment (Houdijk *et al.*, Chapter 6).

$$y_{ijk} = \mu + D_i + R_j + (D^*R)_{ij} + \varepsilon_{ijk} \quad [1]$$

where μ = overall mean
D_i = diet (i = 1, 2, 3)
R_j = replicate (j= 1, 2, 3)
$(D^*R)_{ij}$ = the interaction between D_i and R_j
ε_{ijk} = overall error term

The second set of data was obtained from the pooled digesta (DM, pH, CP, VFA, NH_3, and bacterial counts). The analysis of variance for this set of data was carried out according to the model shown in Eq. 2.

$$y_{ijk} = \mu + D_i + \varepsilon_{ijk} \quad [2]$$

where μ = overall mean
D_i = diet (i = 1, 2, 3)
ε_{ij} = overall error term

For both sets of data, least square means were calculated for D_i. The means were compared using non-orthogonal contrasts which were considered to be significant at $P < 0.05$.

The last set of data, *in vitro* fermentation of FOS, was analyzed using the model given by Eq. 3. This split-plot analysis was used to test the effect of diet against the appropriate error term (pooled digesta within diet). The effects of gut segment and medium were tested against the overall error term.

$$y_{ijkl} = \mu + D_i + P_j(D_i) + I_k + N_l + \text{interactions} + \varepsilon_{ijklm} \quad [3]$$

where μ = overall mean
D_i = diet (i = 1, 2, 3)
P_j = pooled digesta within diet (j = 1, ..., 9)
I_k = gut segment (k = 1, 2)
N_l = type of nitrogen source in the medium (l = 1, 2)
ε_{ijklm} = overall error term

The diet effects were located using non-orthogonal contrasts and considered significant at $P < 0.05$. The interactions were broken down into multiple comparison on the twelve combinations of diet, gut segment, and N-source in the medium, using Tukey ($P < 0.05$). All statistical analyses were performed using the SAS GLM procedure (SAS, 1989).

Results

Physico-chemical properties of the gastrointesinal digesta

Table 1 shows the body- and empty gut segments weights, and gut segment content. The AVI-piglets were heavier than the other piglets but had relatively lighter empty stomach and proximal colon weights than the CON-piglets ($P < 0.05$). The FOS-piglets had less total GIT wet contents than the other piglets ($P < 0.05$), especially for the large intestine. The AVI-piglets had more digesta in the small intestine ($P < 0.05$). The digesta DM content was not affected by the diet (data not shown). Figure 1 shows the gastrointestinal CP and ammonia concentrations. The proximal small intestinal digesta of the AVI-piglets contained less CP than that of the other piglets ($P < 0.10$). The FOS-piglets had more CP in the colon digesta than the CON-piglets ($P < 0.05$) and the AVI-piglets ($P < 0.10$). The ammonia concentration was not affected by the dietary treatment for most segments, though the FOS-piglets had less ammonia in their proximal small intestinal digesta than the CON-piglets ($P < 0.05$).

Table 1. *Empty body- and segment weight, and segment content of the gastrointestinal tract of weanling pigs fed diets with or without fructooligosaccharides or avilamycin*

	Diet[1]			SEM	Diet[1]			SEM
	CON	FOS	AVI		CON	FOS	AVI	
Empty body weight, EBW (kg)	9.0[a]	8.9[a]	10.0[b]	0.3				
Segment	Empty segment weights (g/kg EBW)				Segment content (g)			
Stomach	6.7[a]	6.1[ab]	6.0[b]	0.2	153	104	153	18
Proximal small intestine	38.5	34.5	36.0	2.1	119[ab]	79[b]	157[a]	21
Distal small intestine (3m)	16.6	15.8	16.7	1.0	55[b]	48[b]	102[a]	11
Caecum	2.0	2.3	2.0	0.1	27[a]	10[c]	20[b]	2
1st quarter of the colon	6.8[a]	6.0[ab]	5.2[b]	0.4	51	53	50	7
2nd quarter of the colon	3.5	3.6	3.8	0.4	47[a]	25[b]	29[ab]	6
3rd quarter of the colon	1.9	2.0	2.6	0.2	25[a]	11[b]	23[ab]	4
4th quarter of the colon	3.6	2.8	2.9	0.4	30[a]	11[b]	21[ab]	5
Rectum	1.1	1.2	1.2	0.1	9	5	7	2
Total gastrointestinal tract	80.7	74.4	76.5	2.3	517[a]	346[b]	561[a]	42

[1]CON: control diet; FOS: the control diet with 30 g fructooligosaccharides/kg; AVI: the control diet with 40 ppm avilamycin.
[a,b,c]Means per row for each parameter without common superscripts differ significantly (Tukey, $P < 0.05$)

Figure 2 shows the gastrointestinal pH and total VFA concentrations. The CON-piglets tended to have a higher gastric pH and VFA concentration than the AVI-piglets. The FOS-piglets tended to a higher proximal small intestinal pH compared with the CON-piglets. The pH and total VFA concentrations in the other segments were not affected by the diet.

The sVFA in the gastric- and small intestinal digesta was predominantly acetic acid and did not differ between diets. In the large intestine, the sVFA comprised on average 69% acetic-, 22% propionic-, and 9% butyric acid. The CON-piglets tended to have a higher proportion of acetic acid and a lower proportion of propionic acid than the AVI-piglets ($P < 0.10$). The proportion of pVFA was high in the stomach (76%), and decreased gradually until the caecum (4.5%). Then it gradually increased again from the proximal colon (8.8%) until the rectum (11.2%). Valeric acid was the sole component of pVFA in the gastric- and small intestinal digesta. However, averaged over the large intestinal segments, pVFA comprised 12% *iso*-butyric-, 70% valeric-, and 18% *iso*-valeric acid. The AVI-piglets had relatively less pVFA in the gastric digesta than the other piglets ($P < 0.10$), while the FOS piglets had relatively more pVFA in the distal small intestine ($P < 0.05$). The diets did not affect the percentage of pVFA in total VFA in the large intestinal digesta. However, the FOS-piglets had relatively less valeric- and more *iso*-valeric acid than the other piglets ($P < 0.05$).

Systemic volatile fatty acids concentration

Table 2 shows the arterial VFA concentration and the portal-arterial differences. The arterial total VFA concentration was lower in the FOS-piglets than in the CON-piglets ($P < 0.05$). The pVFA comprised about 60% of the VFA, and did not differ between diets. The AVI-piglets had a larger proportion of acetic acid than did the other piglets ($P < 0.05$). The portal-arterial difference of total VFA was not affected by the diet. However, the difference in pVFA was smaller for the AVI-piglets than for the other piglets ($P < 0.05$). The AVI-piglets had relatively less butyric- and more acetic acid than the FOS- and CON-piglets in the portal-arterial difference of sVFA ($P < 0.05$).

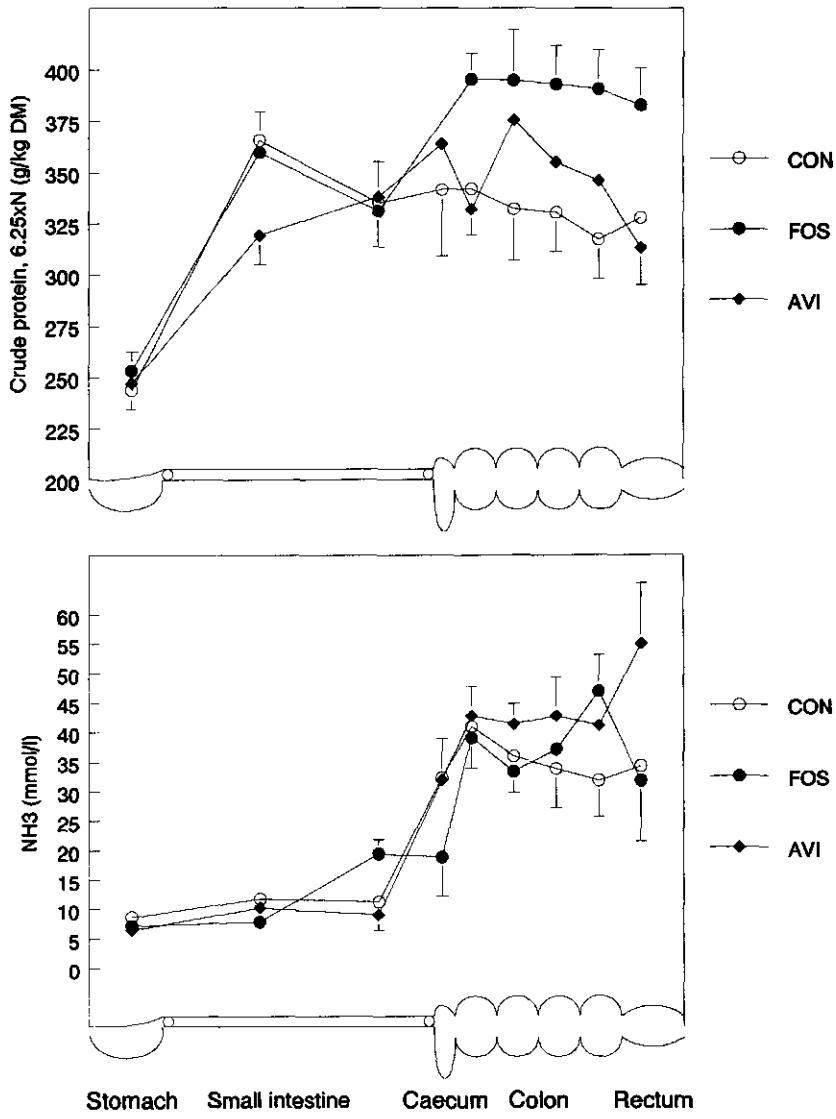

Figure 1. *Mean crude protein and ammonia concentration of the gastrointestinal contents of weanling piglets fed diets with or without fructooligosaccharides or avilamycin; the abscissa represents the various regions of the gastrointestinal tract*

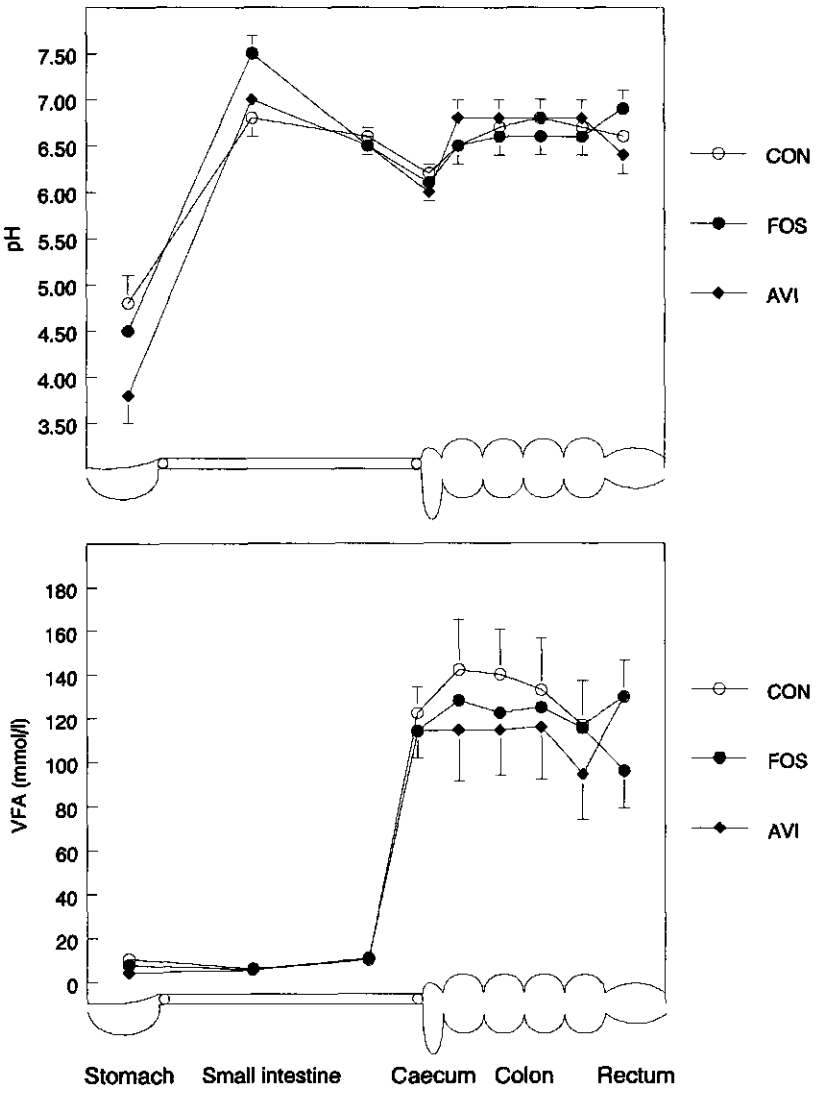

Figure 2. *Mean pH and volatile fatty acid concentration of the gastrointestinal contents of weanling piglets fed diets with or without fructooligosaccharides or avilamycin; the abscissa represents the various regions of the gastrointestinal tract*

Table 2. *Arterial volatile fatty acids and differences between portal and arterial volatile fatty acids in weanling piglets fed diets with or without fructooligosaccharides or avilamycin*

VFA[2]	Diet[1]			SEM	Diet[1]			SEM
	CON	FOS	AVI		CON	FOS	AVI	
	arterial VFA				Portal-arterial difference			
Total VFA, mmol/l	1.37[a]	1.12[b]	1.26[ab]	0.08	2.09	1.53	0.91	0.47
pVFA, mmol/l	0.81	0.67	0.74	0.05	0.44[a]	0.39[a]	-0.19[b]	0.19
Ac, % sVFA	61.0[a]	60.2[a]	65.7[b]	1.4	48.3[a]	52.3[a]	61.6[b]	3.0
Pr, % sVFA	27.0	27.5	26.4	1.4	33.1	29.6	33.6	2.0
Bu, % sVFA	12.0	12.3	7.9	1.7	18.6[a]	18.1[a]	4.8[b]	2.5

[1]See Table 1
[2]VFA: volatile fatty acids; pVFA: sum of *iso*-butyric-, valeric-, and *iso*-valeric acid; sVFA: sum of acetic acid (Ac), propionic acid (Pr), and butyric acid (Bu).
[a,b]Means per row without common superscripts differ significantly (Tukey, $P < 0.05$)

Digestal bacterial counts

Table 3 shows the bacterial counts per gram wet digesta of the distal small intestine, caecum, and the first quarter of the colon. The caecal and colon digesta contained more anaerobes than the small intestinal digesta, while the aerobe counts were comparable between segments. There was no effect of diet on the total and selected anaerobe counts. However, the AVI-piglets had larger counts of aerobes, which were all *E. coli*, compared with the FOS-piglets ($P < 0.05$). Some cultures did not show any colony-forming-units; the dilution factors used may have been too high. Due to these missing values, the statistical analyses could not be carried out satisfactorily for *Bacteroides* spp and *Clostridium* spp in the small intestinal digesta, and *Bacteroides* spp in the caecal digesta.

In vitro *microbial activity: fermentation kinetics*

Figure 3 shows a typical gas production curve from this series of experiments. The curves were fitted to a two-phasic curve, for which the underlying phases are shown as dotted lines in Figure 3. The averaged multiphasic curves are shown in Figure 4. The small intestinal inoculum (a) resulted in clearer two-phasic curves compared to the caecal inoculum (b). Table 4 shows the R_m and t_{Rm} of the first and second phase of these curves, following fermentation of FOS as substrates. There were significant main effects for diet, gut segment, and N-source in the medium. The inoculum from the FOS-piglets had an earlier first phase t_{Rm} than the inoculum from the AVI-piglets and a slower second phase R_m than the inoculum from the CON-piglets ($P < 0.05$). The caecal inoculum showed an earlier t_{Rm} for both phases, a faster first phase R_m, and a slower second phase R_m compared to the small intestinal inoculum ($P < 0.001$).

Table 3. *Bacterial counts in distal small intestinal, caecal, and proximal colon digesta of weanling piglets fed diets with or without fructooligosaccharides or avilamycin*[1]

Gut segment	Bacterial groups	Diets[2]			SEM
		CON	FOS	AVI	
Distal small intestine	Total anaerobes	9.3	9.0	9.4	0.4
	Lactobacilli	8.5	8.1	8.2	0.6
	Bifidobacteria	7.3[3]	7.2	6.3	0.8
	Enterococci	6.9	8.2	7.1	0.5
	Bacteroides spp	6.8[4]	<4.0	6.3[4]	-
	Clostridium spp	5.9[4]	6.3[4]	<4.0	-
	Total aerobes	8.5[ab]	7.4[b]	9.2[a]	0.4
	E. coli	8.4[ab]	7.2[b]	9.2[a]	0.5
Caecum	Total anaerobes	9.8	10.0	9.8	0.3
	Lactobacilli	9.1	8.7	8.7	0.4
	Bifidobacteria	8.2	8.5	7.6	0.3
	Enterococci	7.0[4]	7.4[5]	<4.0	-
	Bacteroides spp	7.2[4]	6.9	7.2[5]	0.8
	Clostridium spp	7.6	9.0	7.9	0.6
	Total aerobes	8.5[ab]	7.5[b]	9.3[a]	0.4
	E. coli	8.5[ab]	7.4[b]	9.3[a]	0.4
First quarter of the colon	Total anaerobes	9.9	10.0	9.9	0.3
	Lactobacilli	8.8	8.8	8.8	0.5
	Bifidobacteria	7.9	8.6	8.1	0.3
	Enterococci	7.2[4]	6.3	6.7	0.4
	Bacteroides spp	7.2[4]	6.7	6.6	0.5
	Clostridium spp	7.8	8.9	8.0	0.5
	Total aerobes	8.3[ab]	7.7[b]	9.4[a]	0.4
	E. coli	8.2[ab]	7.7[b]	9.4[a]	0.5

[1]Bacterial counts in log(colony forming units) per g fresh digesta
[2]See Table 1
[3]n=1; the missing bacterial counts were <5.0 cfu/g
[4]n=2; the missing bacterial counts were <4.0 cfu/g
[5]n=1; the missing bacterial couns were <4.0 cfu/g
[a,b]Means per row without common superscripts differ significantly (Tukey, $P < 0.05$)

The N-PFA medium showed a faster R_m for both phases, an earlier first phase t_{Rm}, and a later second phase t_{Rm} than did the N-CAS medium ($P < 0.001$). In addition to the main effects, significant interactions were also observed, as shown by the superscripts in Table 4. For instance, a significant effect of diet on the first-phase R_m was only observed for the small intestinal inoculum in combination with medium N-PFA, and not for any other combinations.

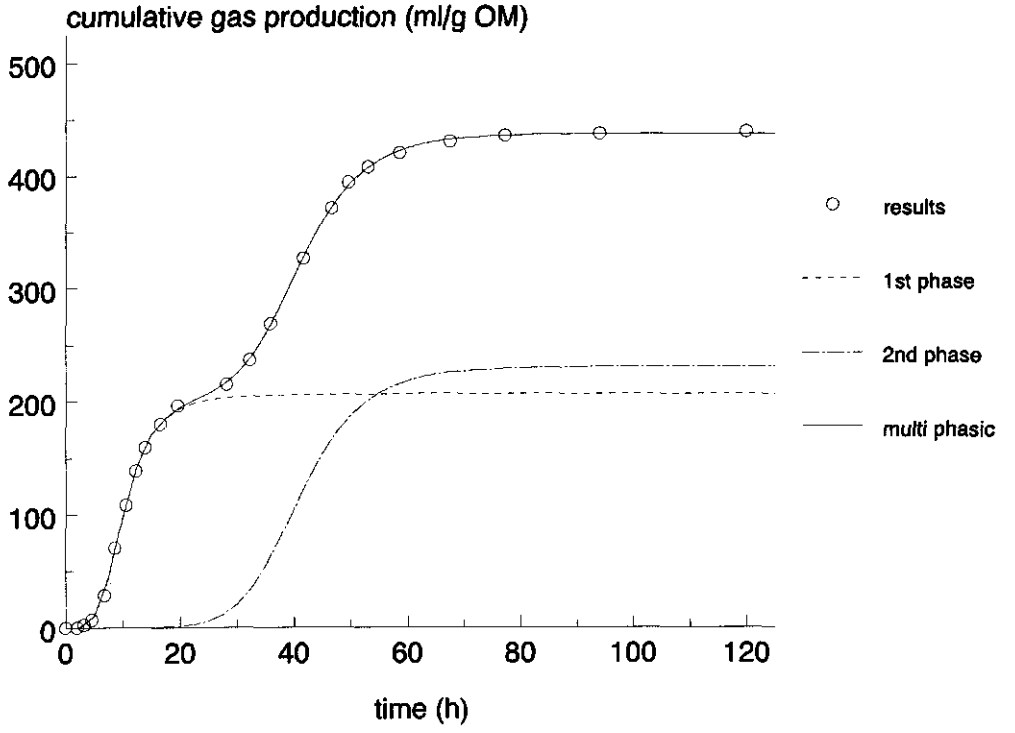

Figure 3. *Measured and multi-phasic fitted cumulative gas production curve, with underlying 1st phase and 2nd phase. Data originated from gas production on fructooligosaccharides in medium B, using distal small intestinal digesta as inoculum source. The piglets which provided the digesta were fed the control diet*

In vitro *microbial activity: end-point measurements*

Table 5 shows the end-point measurements for gas, NH_3, and VFA production, following the fermentation of FOS as substrates. The inoculum from the FOS-piglets produced more pVFA than those of the CON-piglets ($P < 0.05$). The caecal inoculum produced significantly less gas and more NH_3 and sVFA than did the small intestinal inoculum. The sVFA produced by the caecal inoculum comprised relatively more acetic- and propionic acid, and less butyric acid. The N-PFA medium produced significantly more gas, and less NH_3 and pVFA than did the N-CAS medium. The negative NH_3 production indicated that the final NH_3-concentration in the blanks exceeded that in the bottles containing FOS.

can be fermented throughout the gastrointestinal tract, rather than in the small intestine only, may have resulted in a more prolonged reduction of bacterial proteolytic activity.

Bacterial counts

The plasma VFA profile of the FOS- and CON-piglets comprised relatively less acetic acid and more butyric acid than that of the AVI-piglets, suggesting that butyrate producers may have been inhibited in the AVI-piglets. Butyrate is a typical product of carbohydrate fermentation by the Gram-positive *Clostridium* spp (Schlegel, 1992). Indeed, it has been shown that avilamycin is effective mainly against Gram-positive bacteria, while most Gram-negative bacteria, especially *E. coli*, are unaffected (Ohgimoto, 1988 cited by Kyriakis, 1989). This is in agreement with the bacterial counts. The AVI-piglets seemed to have elevated *E. coli* counts, while those in the FOS-piglets were lowered. A reduced activity of several Gram-positive bacteria, such as *Lactobacilli* spp and *Bifidobacteria* spp, may lead to an increase in *E. coli* counts (Ibrahim and Bezkorovainy, 1993). Although often associated with disease, *E. coli* are normal intestinal inhabitants. In this experiment, the increase in *E. coli* counts in the AVI-piglets coincided with a 10% decrease of PWD. The lowered counts of *E. coli* in the FOS-piglets was in agreement with a decrease in aerobe/facultative anaerobe counts when FOS was fed to dogs having small intestinal bacterial overgrowth (Willard *et al.*, 1994). This also suggests that FOS may reduce the incidence of small intestinal disorders. The effect of diet on *E. coli* counts were similar in each GIT segment studied. However, dietary FOS seemed to be effective in the proximal small intestine only. This suggested that a changed microflora population per se is not necessarily beneficial, but that the microbial activity on specific substrates may be of more immediate relevance to the host. The latter was studied using the distal small intestinal and caecal digesta as inoculum for *in vitro* fermentation of FOS as the sole source of energy.

Two phases of gas production

The cumulative gas production curves showed two-phasic patterns. The first phase probably represented the fermentation of FOS, though it can not be excluded that the two types of molecules in the FOS used (Raftilose P95®) have different rates of degradation. These FOS are derived from inulin (Roberfroid, 1993), and are mixtures of GF_n and F_m molecules (fructose oligomers with and without a glucose moiety, respectively). Indeed, it has been shown that some streptococci can degrade this mixture more rapidly than the GF_n series alone, suggesting that the F_m series within the Raftilose P95® are degraded more rapidly (Hartemink *et al.*, 1995). Easily fermentable residual organic matter (**OM**) from the

inoculum may also have contributed to this phase of gas production. Several substrates may have been a source of gas in the second phase. As suggested, the GF_n series within the FOS-mixture may be degraded more slowly. The inoculum inevitably contained also more slowly fermentable OM, though the total amount of gas measured in the blanks was very small (1.6 ± 0.9 ml). In addition, it has been shown that a small amount of lactic acid initially accumulates and then rapidly declines during *in vitro* TOS fermentation (Kikuchi-Hayakawa *et al.*, 1997). When the test-substrate, intermediate fermentation products, and the residual OM are exhausted, some bacteria can alter their internal metabolism to ferment their own storage compounds and RNA (Mason and Egli, 1993). When these have been exploited to their limit, the bacterial cell will lyse and bacterial protein will become available for other bacteria. Thus, for the work reported here, the first phase probably represented mainly fermentation of part of the FOS, while several different substrates may have accounted for the second phase of gas production. To clarify the biological meaning of the two phases, the fermentation liquid could be sampled at different time intervals during fermentation, to elucidate which substrates lead to the development of both phases.

Fermentation kinetics

The small intestinal inoculum from the FOS-piglets showed the earliest first phase t_{Rm}. This probably indicated that the small intestinal microflora had adapted to the FOS in the diet. A slower second phase R_m may suggest that fewer substrates were available for fermentation at the time of R_m. The latter may have been the case for the small intestinal inoculum from the FOS-piglets, and suggested that at least part of the more slowly degradable fraction of FOS may have already been degraded in the first phase. Such a shift may be reflected in more gas being produced in the first phase. This was indeed the case for the FOS-fed piglets (data not shown), in contrast to that shown in Figure 3 (CON-piglets). A slower second phase R_m may also suggest that microbial activity is reduced at the time of R_m. This may have been the case for the small intestinal inoculum from the AVI-piglets, which is in agreement with suggested modes of action for in-feed antibiotics (Rosen, 1995). However, these diet-induced differences in maximum rate of fermentation were apparent only in the medium with easily available peptides, free amino acids, and ammonia as sources of nitrogen. Thus, the differences between inocula, in terms of first-phase *in vitro* fermentation kinetics, suggested that the small intestinal microflora adapted to dietary FOS, while those for the second phase suggested a lowered microbial activity due to in-feed avilamycin.

The less pronounced two-phasic pattern for the caecal- compared to the small intestinal inoculum, was reflected in an earlier second-phase t_{Rm} for the caecal inoculum. This is could be related to a different concentration of detectable bacteria in the digesta (Table 3), resulting in more bacteria per ml caecal inoculum. Moreover, the caecal microflora may be better adapted to ferment proteins, which could also have contributed to the second phase. This is supported by the differences in ammonia concentration *in vivo* (Figure 1) and ammonia production *in vitro* (Table 5).

End-point measurements

The small intestinal inoculum from the FOS-piglets produced more sVFA than that from the CON-piglets, following FOS fermentation *in vitro*, though the total amount of gas was the same. This suggested a shift in the microbial activity to a lower production of metabolic gas as result of FOS in the diet. The inoculum from the AVI-piglets produced less gas, but the same amount of VFA, though with a lower proportion of butyric acid, than that of the CON-piglets, following FOS fermentation *in vitro*. This suggested that the activity of bacteria that produce gas and butyric acid may have been reduced, which is in agreement with the already mentioned inhibition of *Clostridium* spp by avilamycin, and the low *Clostridium* spp counts in the small intestinal digesta for each group of AVI-piglets (< 10^4 cfu/g, Table 3). It should be noted that only one type of substrate was used in this series of experiments. Therefore, the reduced *in vitro* microbial activity of the AVI-piglets does not nessecarily be the same when other substrates were used. Furthermore, the AVI-piglets had no FOS in the diet *in vivo*.

The medium with casein supported the production of more ammonia and pVFA than did the medium with peptides, free amino acids, and ammonia. However, the total amount of gas produced was lower for the casein medium. This suggested that fermentation in general was inhibited (less gas), less microbial biomass was formed (positive ammonia production values), and that more amino acids from casein were being used as a source of energy in the casein medium. The first two may have been due to the absence of suitable sources of nitrogen for microbial growth. *In vitro* casein fermentation by the human faecal microflora also resulted in a net ammonia production (Macfarlane and Allison, 1986). They also found a high proportion of acetic acid, which is in agreement with the sVFA profiles observed in the work reported here. However, this suggested that describing the saccharolytic and proteolytic activity by sVFA and pVFA, respectively, may not always be appropriate. It has indeed been shown that some amino acids can produce acetic-, propionic-, and butyric acids (Mortensen *et al.*, 1990).

The negative ammonia production means that more ammonia was measured for the blanks than for the FOS-bottles, suggesting that more N-sources were taken up by the microbes and used for their growth, rather than as a source of energy. This is in agreement with a lowered ammonia production when TOS were fermented *in vitro* by pigs' hindgut microflora (Faisant *et al.*, 1990). The higher pVFA and net NH_3 production from the small intestinal inoculum from the FOS-piglets compared to those from the CON-piglets suggested that more protein was used as a source of energy after 120 h. This indirectly indicated an earlier complete disappearance of FOS a source of energy for the microflora from the FOS-piglets. The observations discussed are end-point measurements, and do not provide any insight into the kinetics of the production of proteolytic and saccharolytic fermentation products. Such kinetics probably differed between diets, since the gas production profiles were also affected by the diet. In addition, it has been shown that some VFA-profiles can change as the *in vitro* fermentation of amino acids progresses (Mortensen *et al.*, 1990).

Thus, the end-point measurements suggested that dietary FOS enhanced the production of VFA from FOS as a substrate, and that in-feed avilamycin reduced microbial activity towards FOS. In general, the effects observed *in vitro* were more pronounced for the small intestinal- than for the caecal microflora, which supports the suggestion that the FOS are utilized primarily pre-caecally, and never even reach the caecum *in vivo*.

Conclusion

The high-protein diets led to a considerable concentrations of protein fermentation products throughout the gastrointestinal tract of the weaned piglets. In-feed avilamycin and dietary FOS lowered the concentration of some protein fermentation products, though the effects of dietary FOS were less pronounced and limited to the small intestine. The small intestinal *E. coli* counts increased with the addition of in-feed avilamycin and decreased with dietary FOS. The accelerated first-phase of *in vitro* fermentation of FOS suggested that the small intestinal microflora of the FOS-piglets was adapted to the FOS in the diet. However, both dietary FOS and in-feed avilamycin reduced microbial activity in a later stage of fermentation. The effects of diet on microbial activity were more pronounced for the small intestinal microflora than for the caecal microflora, which is not surprising given that FOS were likely to have been completely fermented before reaching the caecum.

Together with the data on performance and faeces consistency (Houdijk *et al.*, Chapter 6), it was concluded that dietary FOS reduced the protein fermentation in the small intestine only, and that those effects were more prolonged for in-feed avilamycin. As such,

dietary FOS may not replace in-feed avilamycin. However, further work using fermentable carbohydrates which can be fermented throughout the gastrointestinal tract, rather than only in the small intestine, may prove useful as alternatives to in-feed antibiotics in order to reduce the bacterial proteolytic activity.

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GENERAL DISCUSSION

General Discussion

Introduction

This thesis has described research on the role of non-digestible oligosaccharides (**NDO**) in young pig diets. These low-molecular, water-soluble carbohydrates are called non-digestible because the enzymes needed to hydrolyse them to absorbable monomers are not found in mammalian endogenous secretions. However, dietary NDO are not recovered from the faeces of the host, suggesting that they must be degraded somewhere in the gastrointestinal tract. It is the gastrointestinal microflora that is responsible for this degradation. It has been estimated that the total number of micro-organisms forming the mammalian microflora is a factor ten more than the number of body cells. From a nutritional point of view, the microflora can be considered as an organism that requires nitrogen, energy, vitamins, and minerals for its maintenance and growth. The main energy sources are dietary carbohydrates which have not (yet) undergone enzymatic digestion, though endogenous mucin also provides substantial amounts of carbohydrates (Monsma *et al.*, 1992). The majority of these dietary carbohydrates are the non-starch polysaccharides (**NSP**). Strictly speaking, this chemical definition does not include NDO. However, a more physiological definition of this carbohydrate fraction is dietary fibre, which should include NDO (Roberfroid, 1993; Thebaunin *et al.*, 1997). Some NDO are being used as prebiotic feed additives. A prebiotic has recently been 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, and thus improve host health' (Gibson and Roberfroid, 1995).

The two types of NDO studied in the series of experiments presented in this thesis were the fructooligosacharides (**FOS**) and transgalactooligosaccharides (**TOS**). These were included in the diets of young pigs up to a level of 40 g/kg. The choice of these from the range of NDO mentioned in the introduction was merely based on their availability, since approximately 25 kg true NDO were needed for a representative animal experiment. The source of FOS was Raftilose P95® (Orafti, Tienen, Belgium) and that of TOS was Oligostroop® (Borculo Whey Products, Borculo, The Netherlands). In addition, the scientific literature has been reporting work using FOS since the early eighties.

The previous chapters have described three pig experiments, which were combined with two *in vitro* fermentation experiments. The study with growing pigs (d 63 to d 105 of age) will be referred to as **PigI** (Chapters 1,2, and 3). **PigII** (Chapters 2, 4, and 5) is the study with weaner pigs (d 38 to d 75), and **PigIII** (Chapters 6 and 7) is the study with young piglets around weaning (d 0 to d 63). The *in vitro* experiments performed in conjunction with PigII will be referred to as **GasI** (Chapter 5), and those associated with PigIII as **GasII** (Chapter 7). Several digestive-physiological aspects of dietary FOS and TOS were studied, including growth performance, nutrient digestion, physico-chemical and microbial digesta characteristics, and *in vitro* microbial activity and NDO degradation. In this chapter, the main results from these experiments will be discussed in the context of the flow of dietary NDO and their effects on the gastrointestinal microflora from feed to faeces (Figure 1). Several feedstuffs used in practical feed formulations for pigs contain NDO. Consequently, background levels of NDO are important when the effects of added NDO are studied. Following feed swallowing, the diet (now called digesta) arrives in the stomach. The digesta then flow through the small intestine, and arrive in the large intestine. In the context of this discussion, the large intestine includes the caecum, colon, and rectum. Each of these three compartments (stomach, small intestine, and large intestine) harbours an active microflora, which will be described briefly in general terms such as size, composition, and functions, before the specific effects of dietary NDO will be discussed. Finally, the effects for the animal will be discussed in terms of growth performance, before the main conclusions and suggestions for further research are given. It should be noted that within and between the three compartments described, digesta backflow might occur, which deviates from the continuous uni-directional flow assumed in the context of this discussion.

NDO in animal feeds

Table 1 shows the content of FOS and α-galactooligosaccharides (**GOS**) in several feedstuffs used in feed formulations for pigs. Legumes are relatively rich in GOS, while cereals usually contain a significant amount of FOS. The NDO are found in the cell contents in the form of storage carbohydrates (Carpita *et al.*, 1991). They probably also contribute to the turgor of plant tissue, due to the osmotic pressure they exert. The NDO content depends on several factors, including variety and stage of maturity of the plant. It has been shown that the content of NDO increases as grain matures (Becker *et al.*, 1997) and decreases during germination (Akinlosotu and Akinyele, 1991).

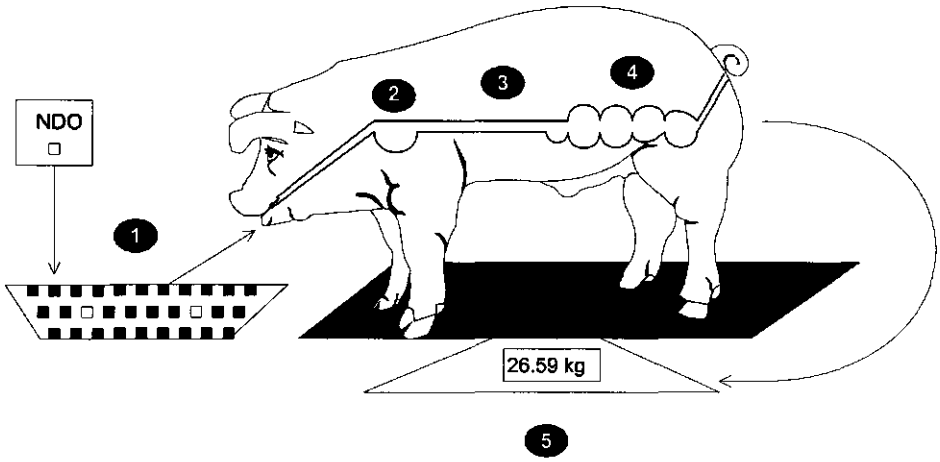

Figure 1. *Schematic representation of the outline of the general discussion. The flow of dietary NDO from feed (1), via the stomach (2), small intestine (3) and large intestine (4) to the faeces. (5) represents the effect on growth performance*

Depending on the factors mentioned and the inclusion levels in the diet, commercial pig rations can contain up to 20-30 g NDO/kg. Consequently, one would expect that commercial diets would either mask or dilute the effects of any NDO added, to such an extent that no effects of the NDO added would be observed. Therefore, diets with a very low level of NDO have to be used if one is to elucidate the role of NDO as feed a component and their potential as prebiotic feed additives. Table 1 shows that corn contains virtually no NDO. For this reason, corn or corn starch were used as the basis for our experimental diets. The requirements for protein were met via protein sources of mainly animal origin (fishmeal, animal meal, casein) with additional amino acids, and soy oil was used as an additional energy source. Although such diets were low or void of NDO, fermentable NSP were present as components of corn (PigI and PigIII) and of oats husk meal (PigII). Oats husk meal (**OHM**) does not contain NDO, though cellulose, arabinoxylan,

Table 1. *Non-digestible oligosaccharides in common feedstuffs*[1]

	Non-digestible oligosaccharides (g/kg DM)	
Feedstuff	*α-Galactooligosaccharides*	*Fructooligosaccharides*
Soybean	29.0 - 58.0	0.0
Soybeanmeal	41.8 - 73.1	0.0
Wheat	4.8	1.4 - 7.9
Barley	2.4	1.9 - 7.2
Peas	30.0-113.0	0.7
Corn	0.3[2]	0.0

[1]Data from Becker *et al.*, 1977; Reddy *et al.*, 1984; Henry and Saini, 1989; Coon *et al.*, 1990; Saini, 1991; Campbell *et al.*, 1997
[2] K.J.M Van Laere, personal communications

and β-glucan can be found (Bach Knudsen and Hansen, 1991). On the one hand, this inevitably resulted in a certain level of background fermentation, but on the other hand, NDO-effects on NSP digestion could obviously not have been studied in the absence of NSP. Negative control diets (no NDO and no NSP) were not included in this series of experiments.

In all experiments described in this thesis, FOS and TOS were included in the diet at the expense of Arbocel® (purified, non-soluble crystalline cellulose). The advantage of this was that the total concentration of calculated digestible energy was kept constant. However, it was inherent to this approach that any effect observed may have been due to the removal of cellulose rather than to the inclusion of NDO. This placebo effect, however, is probably limited in rats, pigs and humans (Slavin and Marlett, 1980; Den Hartog *et al.*, 1988; Dierick *et al.*, 1989; Roberfroid, 1993; Li *et al.*, 1994; Folino *et al.*, 1995), and the effects of fibre in nutritional studies are often more obvious for soluble, viscous fibres (Graham, 1988). In addition, semi-synthetic diets containing 50 g Arbocel®/kg and TOS included at the expense of corn starch, exerted comparable effects of TOS on digestion and microbial digesta characteristics as in PigII (R. Kamelaar, personal communications).

It could be argued that the contribution of dietary NDO to the metabolisable energy (**ME**) content of the diet was limited, since relatively low levels of dietary NDO were used (maximum of 40 g/kg). It has been estimated that FOS provide 4.2-6.3 kJ ME/g (Roberfroid *et al.*, 1993). Using similar calculations based on the volatile fatty acids (**VFA**) production observed in GasI, FOS may provide a maximum of 11 kJ ME/g in the pig. The maximum intake of FOS averaged 30 g/d (ileal digestion experiment in PigII), contributing 0.3 MJ ME/d. The total ME intake averaged 11.0 MJ ME/d. Thus, if the NDO were simply added to the diets, then the ME contents of the diet would have been diluted slightly. Nevertheless,

Arbocel® was used as placebo in the first experiment and this approach was maintained in the other experiments.

It has been shown that the fermentability of purified cellulose is very low (Sunfold *et al.*, 1995), and that of the NDO was expected to be very high. The latter was confirmed in GasI and in a study with human volunteers (Alles *et al.*, 1996). Thus, differences between diets were related to the quality of the fermentable fraction. However, the digestion experiment (PigII) suggested that about 60% of the Arbocel® ingested was intestinally degraded. Short-term studies had confirmed the low fermentability of Arbocel® *in vivo* at the faecal level (P. Van Leeuwen, personal communications), and *in vitro* using pigs' faeces as inoculum (B. Williams and J. Houdijk, unpublished data). However, these faeces were obtained from pigs fed commercial diets that did not contain Arbocel®. The piglets in PigII received experimental diets containing Arbocel® from d10 of age, which suggested that microfloral adaptation to Arbocel® can occur in long-term studies. If Arbocel® is to be used in future experiments as placebo for rapidly fermentable carbohydrates, then more information on its fermentation kinetics is needed.

It was mentioned earlier that NDO in feedstuffs are found in the cell contents. The availability of these NDO largely depends on the extent of plant cell wall damage and/or degradation. Several industrial processes are being applied to increase the access of digestive enzymes to the cell contents, to thus enhance the nutritive value of the feedstuff. These processes may increase the availability of feedstuff NDO at gut level. The NDO added are probably much more directly available to the microflora. However, it is not known whether industrial processes such as pelleting adversely affect NDO availability. It has been suggested that FOS are stable at temperatures up to 140°C (Fujii and Komoto, 1991), although heat treatments may result in Maillard-reactions and subsequently render less NDO available at gut level.

NDO in the stomach

The first micro-organisms, which may interact with the diet, are those found in the oral cavity. The overall effects of oral microbial activity in pigs are probably limited, though it has been shown that porcine oral streptococci can ferment FOS rapidly *in vitro* (Hartemink *et al.*, 1995). An important site of proximal fermentation is the stomach. The porcine gastric non-secreting and secreting epithelium is densely populated by bacteria. The lactobacilli form the major group, up to 2×10^6 colony forming units (**cfu**) per cm^2 (Henriksson *et al.*, 1995), but bifidobacteria, streptococci, clostridia, and enterobacteria also colonize the non-

secreting epithelium (Conway, 1994). The total bacterial count in the gastric digesta can be as high as 10^9 cfu/g. The gastric flora, especially in the pre-weaned piglet, is involved in controlling the number of potential pathogens passing into the small intestine. This is achieved by a low pH, which results from lactic- and acetic acid production from lactose in the sow's milk and by the continuous 'inoculation' of the small intestine with viable lactobacilli (Cranwell *et al.*, 1976; Barrow *et al.*, 1980). It has been suggested that a prolonged retention of digesta in the stomach may also facilitate the fermentation of other simple sugars, starch, and even NSP (Ratcliffe, 1991). The gastric digesta can contain biogenic amines (Dierick *et al.*, 1986). The presence of a certain capacity for protein fermentation in the stomach was further supported by the presence of protein derived volatile fatty acids (**pVFA**) in the gastric digesta in PigIII. Thus, the gastric microflora can and does use various nutrients from the diet, and can be expected that dietary NDO are among those nutrients.

It has been shown that 50% of the fructans from Jerusalem artichoke tubers, which are β1-2 fructose polymers, are degraded in the porcine stomach (Graham and Aman, 1986b). An important factor that determines the extent of gastric NDO fermentation is the retention time of the digesta in the stomach. Since NDO are water-soluble, they will follow the liquid phase of the digesta. It has been shown that the liquid phase reaches the caecum within two h after feeding, though part of this phase can stay in the stomach for more than eight h (Clemens *et al.*, 1975). Large particles were found in the stomach up to 60 h post feeding. Thus, NDO fermentation may take place. This was supported by the observations on the gastric pH, which decreased when NDO were included in the diet (PigI). In addition, the empty stomach of the NDO-fed pigs was heavier than that of the control pigs. This could be due to an increased number of bacteria; one of the effects of the microflora in general is an increased tissue mass (Smith, 1993), probably as a result of responses of the local immune system. The lighter empty stomach and proximal colon weights of the piglets receiving in-feed avilamycin is in agreement with the presence of this mechanism (PigIII). Complete degradation of dietary NDO in the stomach did not occur, as NDO were recovered from the distal small intestinal digesta (PigI). Since the NDO were not recovered in the faeces, one would expect that digestal VFA concentrations as a result of fermentation in the terminal ileum and/or proximal colon increase, at least temporarily. It has been shown that ileal VFA inhibit gastric motility (Chuche and Malbert, 1997), allowing a longer duration of gastric fermentation of any dietary organic matter. Thus, dietary NDO may also indirectly lower the gastric pH, although an actually increased digestal VFA concentration was not observed in the experiments. The latter may be due to a rapid VFA

absorption and/or a dilution of the digesta. Indeed, a lowered ileal digesta DM content was observed in the NDO-pigs (PigII). In both scenarios described, a lowered pH can aid in the resistance of the gastrointestinal tract to passing potential pathogens, and is as such, beneficial for the host.

NDO in the small intestine

The composition of the microflora attached to the small intestinal epithelium is comparable to that of the stomach (Conway, 1994); up to 2 x 10^7 cfu of lactobacilli/cm^2 have been reported throughout the small intestine (Wadström *et al.*, 1987). However, the total bacterial counts in the proximal small intestinal digesta is somewhat lower than that in gastric digesta (Jensen and Jørgensen, 1994), which is probably due to dilution by pancreatic juices. The total bacterial counts can increase in the distal part to approximately 10^{10}/g digesta, and most of the bacteria species found in the ileal microflora are also found in the large intestine, such as *Bacteroides* spp, bifidobacteria, *Clostridium* spp and enterococci. Cellulose- and pectin-degrading bacteria have also been isolated, though at relatively low concentrations of 1.8 x 10^4 cfu/g digesta (Chesson *et al.*, 1985). Obligate anaerobes such as *Bacteroides* spp can be found in reasonable numbers (10^{8-9} cfu/g digesta). It has been suggested that the presence of facultative anaerobes can create bacterial niches which are so low in oxygen that obligate anaerobes can survive, even on the skin (Hentges, 1993). The increased bacterial concentration in the distal compared to the proximal small intestinal digesta, is a direct result of an increased stasis of the digesta, which allows more bacteria to multiply and survive. Obligate anaerobes were also observed in this series of studies, both in small intestinal digesta obtained via a cannula (PigII) and as sampled during dissection (PigIII).

Some remarks need to be made on the use of cannulated pigs. Cannulated pigs have been used in nutritional studies for many years. In this case, the post-valve T-caecum (**PVTC**) cannulation was used (Van Leeuwen *et al.*, 1991), which has been shown to have no effect on a wide range of digestive-physiological parameters (Köhler, 1992). However, the effect of cannulation on microbial activity has not been widely studied. The ileal digesta of PVTC-pigs is exposed to more oxygen than that of intact animals. As a result, the survival and activity of strict anaerobes may be reduced, while that of the (facultative) aerobes may be enhanced. Such changes have been found in ileostomy patients, but ileal stomata may not be directly comparable with ileal cannulae (Ratcliffe, 1991). Indeed, an *in vitro* study with porcine ileal contents showed that the numbers of aerobes and anaerobes

were hardly affected by increasing levels of dissolved oxygen, though the concentration of fermentation products was reduced (Hillman *et al.*, 1994). Clearly, more information is needed about the microbial ecology of ileally cannulated pigs. The effect of PVTC cannulation on microbial activity is currently being studied.

An important benefit the host gains from the small intestinal microflora is protection against potential pathogens. The small intestinal microflora also participates in NSP degradation, which is therefore not solely carried out by the hindgut flora. Up to two-thirds of the NSP ingested from cereal- and legume-based diets are degraded pre-caecally in pigs (Mathers, 1991). The microflora interacts with primary bile acids. It has been suggested that the surplus of bile acids are deconjugated by a range of anaerobic bacteria (Bokkenheuser, 1993). Once deconjugated, the bile acid residues can be absorbed and to some extent re-enter the enterohepatic circulation. However, part of these primary bile acids may be metabolyzed to secondary bile acids (Rowland *et al.*, 1985). In that form, bile acids are no longer of use for the host, but are potentially toxic. The effects of microbial activity on the level of deconjugation has recently been elucidated in broiler chicks (Langhout, 1998). If the small intestinal microflora was to use only NSP, then host nutrition would be unaffected. In terms of enzymatic digestion in the small intestine, the microflora could interact at the level of enzyme activity and/or absorption of hydrolysed nutrients. It has been suggested that the microflora hardly affects enzyme activity (Ratcliffe, 1991). However, host and microflora are in competition for free glucose and amino acids. The impact of the microflora on nutrient availability has been illustrated by the faster growth of germ-free animals (Coates *et al.*, 1963). In addition, in-feed antibiotics show an increased amino acid and glucose absorption (Dierick *et al.*, 1986; Rosen, 1995). Nevertheless, it is generally believed that the gain of having a protective small intestinal microflora outweighs any nutritional loss.

Table 2 shows the apparent ileal nutrient digestion observed in PigI and PigII. Only the data for the highest inclusion levels are presented, compared with the control diet of the specific experiment. The effects of dietary NDO on small intestinal nutrient digestion were limited for the highly digestible diets used (PigI and PigII). With hindsight, it was realized that it would probably be difficult to show effects of NDO on protein- and energy digestion using such diets. Recent pig studies have shown that the removal of GOS with α-galactosidases improved the ileal apparent digestion of several amino acids from lupin-based diets, but not for crude protein as a whole (Gdala *et al.*, 1997). These data are in agreement with PigI and PigII, so it is possible that the digestion of individual amino acids

Table 2. *Apparent ileal and faecal digestion of organic matter, inorganic matter, crude protein, and ether extract in experiment PigI (growing pigs) and PigII (weaner pigs). Data are expressed relative to the control diet (=100)*

	PigI[1]		PigII[2]	
	FOS	TOS	FOS	TOS
Ileal digestion				
Organic matter	99	98	101	102
Inorganic matter	99	86	96	99
Crude Protein	91	79	100	101
Ether extract	98	95	103	106
Faecal digestion				
Organic matter	100	101	101	103[3]
Inorganic matter	98	95	96	104
Crude Protein	99	100	98	100
Ether extract	100	99	98	103

[1]FOS at 14 g and TOS at 8 g/kg diet
[2]FOS and TOS at 40 g/kg diet
[3]Significant different from 100 ($P < 0.05$)

may have been affected. Dietary NDO did increase the apparent ileal digestion of hemicellulose. It was discussed that this could partly have been due to an increased solubility, as suggested for β-glucans (Johansen *et al.*, 1993). This would then result in fewer carbohydrates being analyzed as hemicellulose, which could have been partly responsible for the decreased ileal digestion of the non-starch neutral-detergent soluble carbohydrates (**NNSC**). Thus, the small intestinal microflora did not necessarily completely degrade the increased proportion of soluble hemicellulose, though dietary NDO indirectly affected the composition of the OM available for fermentation in the hindgut. In any case, it did suggest an increased small intestinal bacterial activity.

The use of enzymes to remove GOS from feedstuffs is apparently in contrast to the view of NDO as prebiotics. GOS have long been known as anti-nutritional factors (ANF) since high levels may induce diarrhoea, extensive flatulence and discomfort in monogastric animals (Saini, 1991). However, GOS are in fact bifidogenic (Hayakawa *et al.*, 1990), and are used in health foods (Playne and Crittenden, 1996). It has been suggested that GOS and FOS may act as ANF (induction of diarrhoea) only when the NDO intake exceeds a certain daily threshold level (Benno *et al.*, 1987; Fishbein *et al.*, 1988). This is in agreement with the occurrence of diarrhoea in rats when they were fed diets with 200 g FOS/kg (Tokunaga *et al.*, 1986). Moreover, the presence of a threshold level for NDO strengthens the idea that dietary NDO have both prebiotic and ANF properties, which could result in quadratic responses with an optimum level between 0 and 40 g/kg (PigII and PigIII).

Dietary NDO are to some extent degraded pre-caecally in pigs. More than 90% of FOS was degraded in the small intestine (PigII). This was also shown indirectly by the increased concentration of *iso*-valeric acid (PigII) and valeric acid (PigIII) in the small intestinal digesta. We could not quantify TOS in our experiments, but others showed a 30% pre-caecal degradation of TOS (R. Kamelaar, personal communications). Pre-caecal degradation of GOS in pigs has also been observed, ranging from 57% in velasse-based diets (Veldman *et al.*, 1993) to 65% in pea-based diets (Canibe and Back Knudsen, 1997), and up to nearly 90% in lupin-based diets (Gdala *et al.*, 1997). This high level of pre-caecal GOS degradation was found to be associated with an apparent loss of amino acids. However, such losses may be considered as nutritional investments in an enhanced pre-caecal prebiotic activities, given the bifidogenic nature of GOS.

The differences in the extent of pre-caecal degradation for the different NDO may be due to different rates of fermentation. Indeed, FOS were fermented at a higher rate than TOS *in vitro* (GasI). We suggested that the faster the rate of fermentation, the more proximal degradation will be complete *in vivo*. The observations on the digesta of the NDO-fed pigs (PigI and PigII) confirm this. However, more types of NDO, which differ in their rate of fermentation, should be compared within the same study to test this hypothesis.

The microbial digesta characteristics showed an increased saccharolytic activity due to dietary NDO, with potential beneficial consequences for the host. The ileal pH was decreased for both the FOS- and TOS-diets, and the concentration of aerobes were lowered, both compared to the control pigs (PigII and PigIII). The latter may lower the risk for (subclinical) infections, since the aerobes are often associated with such infections. Most of the other effects in terms of microbial characteristics studied were more pronounced for dietary FOS than for dietary TOS (PigII). Therefore, it was suggested that the decreased pH could also have been the result of a lower concentration of ammonia, since ammonia can be incorporated into bacterial amino acids when fermentable energy is available. Indeed, for both NDO, the concentration of crude protein in the ileal dry matter increased (PigII), and a lowered ammonia concentration was observed in the proximal small intestinal digesta of FOS-fed piglets (PigIII). This crude protein concentration could also have increased due to a decreased concentration of the protein-free OM, as a direct result of the model used (NDO exchanged at the expense of cellulose). However, dietary NDO had little effect on ileal DM digestion, so the flow of DM into the large intestine was unlikely to be affected in terms of total substrates for the hindgut microflora, even though the composition of the digestal carbohydrates changed. Moreover, in terms of the microflora, the digesta flowing into the hindgut of the NDO-pigs, harboured a microflora with

an enhanced level of saccharolytic activity. This probably affected the digesta characteristics throughout the large intestine.

NDO in the large intestine

The total bacterial counts in the large intestine usually exceeds 10^{10} cfu/g wet contents and are predominantly anaerobes (Allison, 1989; Conway, 1994). The microflora of the caecum consists of mainly Gram-negative bacteria (65%) such as *Bacteroides* spp and *Selenomas ruminantum*, but Gram-positive bacteria like lactobacilli and eubacteria are also found. In contrast, it has been reported that the microflora of the colon and rectum of healthy pigs are mainly Gram-positive (90%), and includes Gram-positive cocci, lactobacilli, eubacteria, and clostridia (Russel, 1979; Salanitro *et al.*, 1979; Robinson *et al.*, 1981), though the faecal microflora comprised more eubacteria than the colon microflora. Interestingly, dysenteric pigs had predominantly Gram-negative bacteria in the colon (Robinson *et al.*, 1984). Ideally, these differences would need to be confirmed using the same pigs, since they are now confounded by factors such as diet, age, management, breed, and differences between laboratories. However, they do reflect the large range of the large intestinal microflora. These differences suggest that the faeces is certainly not representative for the caecal digesta, and only tentative represent the colon digesta in healthy pigs, in terms of bacterial profile (Pollmann *et al.*, 1980). However, in terms of bacterial activity, it has been shown that rectal, mid-colon, and caecal microflora were similarly ranked when fermenting a range of substrates *in vitro* (Williams *et al.*, 1998). This suggests that faeces may represent the large intestinal digesta, depending on the purpose of the study.

It has been suggested that gastric- and small intestinal luminal microflora have a similar profile to that of the mucosal associated populations; the luminal flora could be formed by daughter cells of the mucosal associated populations (Conway, 1994). However, this has not been shown the case for the large intestine (Robinson *et al.*, 1984; Allison, 1989). The slow rate of passage probably allows bacteria to multiply in the lumen, and as such attachment is not necessarily essential for survival. The retention time may be as long as 48-54 h in the large intestine compared to 2.5-3 h in the small intestine (Kidder and Manners, 1978).

One of the well-recognized functions of the hindgut microflora is the degradation of NSP to form VFA (Varel, 1987; Rechkemmer *et al.*, 1988; Fonty and Gouet, 1989). Cellulolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and

Clostridium herbivorans and hemicellulolytic bacteria such as *Bacteroides ruminicola* have been isolated (Varel and Yen, 1997). The VFA significantly contribute to the net energy required for maintenance, which may reach up to 15-30% in growing pigs, and up to nearly 100% for adults (Dierick *et al.*, 1989; Varel and Yen, 1997), with butyric acid being the major source of energy for the colonocytes (Von Engelhardt *et al.*, 1989). Several physiological functions have been attributed to VFA as well, including proliferation of the gut wall, enhancement of electrolyte and water absorption, stimulation of the colon blood flow, cholesterol metabolism, and bacterial control (Rombeau *et al.*, 1995). It is generally assumed that amino acids from microbial, endogenous, and dietary origin that enter the large intestine do not quantitatively contribute to the overall amino acid supply. However, its potential contribution should not be ignored (Ratcliffe, 1991). All individual amino acids can be catabolized to VFA, amines, and ammonia. It is generally believed that the latter two, in combination with residual amino acids, provide the microflora as a whole with a plentiful N-supply; microbial growth is usually limited by energy rather than by N-supply in the porcine hindgut (Ratcliffe, 1991). The increasing proportion of protein-derived VFA in the more distal regions of the hindgut indicate that an increasing proportion of proteins is being used as a source of energy, and is thus a reflection of a reduced availability of energy from carbohydrates. This is in agreement with the decreased microbial activity for the microflora as a whole in the distal part of the gut (Jensen and Jørgensen, 1994).

The role of the caecum in nutrient digestion is thought to be limited (Gargallo and Zimmerman, 1981; Pérez-Lanzac *et al.*, 1990), though the faeces of caecectomized pigs contained more water than that of intact pigs. However, Lindberg (1997) observed a decrease in the apparent faecal crude protein and ether extract digestion in PVTC-cannulated pigs. This suggests that the presence of the PVTC-cannula rather than the absence of the caecum modifies apparent faecal digestion. Therefore, faecal nutrient digestion was not studied our cannulated pigs. However, the comparison between the apparent faecal and ileal digestion of some nutrients in PigII suggested that the 13-d adaptation period for the faecal digestion study may have been too short. The apparent ileal digestion of hemicellulose was namely higher than the apparent faecal digestion of hemicellulose. In addition, dietary NDO led to a slight decrease in the apparent faecal digestion of hemicellulose, while significantly increasing its apparent ileal digestion. Indeed, it has been show that microfloral adaptation can take up to five weeks or more (Varel and Yen, 1997). These results suggests that at least for some parameters, a relatively long adaptation period is required to dietary NDO.

A direct comparison between observations at the faecal and ileal level (PigII) can be confounded by several factors, including age, body weight, feed intake, and time of feeding the experimental diets. However, if one disregards these factors, the data from PigII do suggest that an NDO-induced increase of saccharolytic activity in the ileal digesta (reduced pH and an increased proportion of lactobacilli) is not maintained throughout the hindgut, because of the exhaustion of the NDO. As a consequence, some bacteria may start to use protein as a source for energy and/or the competition between the saccharolytic and proteolytic bacteria may settle in favour of the latter. This then results in an increased proteolytic activity in the distal colon digesta (increased pH and pVFA in the faeces) and a reduced proportion of faecal lactobacilli. Differences between the FOS- and TOS-diets suggest that the more proximally the NDO fermentation is completed, the more pronounced the proteolytic activity is at the faecal level (PigII). It was calculated from PigII that the digesta flow (575 g/d) in the control pigs provided the large intestine with 83 g DM/d, which consisted of 16 g crude protein (6.25 x N), 2 g ether extract, 9 g minerals, and 56 g carbohydrates. This is to some extent comparable to the composition of the estimated flow of digesta into the human large intestine, though the composition of the carbohydrate fraction differed: more starch for humans and more NNSC for our pigs (Macfarlane and Cummings, 1991). The ileal digesta the NDO-fed pigs contained less cellulose (as a direct result of the model used), less hemicellulose, but more NNSC than that of the control pigs; that of the FOS-fed pigs contained even less NNSC than that of the TOS-fed pigs (34 vs 24 g/d). Fewer NNSC in the TOS-fed pigs seems to be in disagreement with the longer maintained saccharolytic activity, as suggested by the differences in pH and portal VFA in PigI (Chapter 3). Apparently, the NNSC available in the proximal colon and/or caecum of the FOS-fed pigs is rapidly degraded, while the less enhanced microbial activity of the TOS-fed pigs allows a prolonged activity on the NNSC available. The *in vitro* experiments (GasI), indeed showed that the caecal microflora of the FOS-fed pigs had a higher activity compared to the TOS-fed and control pigs. However, the caecal digesta used for GasI was obtained after overnight fasting, since this was carried out during the placement of the PVTC-cannula. The enhanced *in vitro* microbial activity of the caecal microflora from the FOS-fed pigs needs to be confirmed using caecal digesta of fed-pigs. Although the different treatment groups of piglets in PigIII were offered very different diets compared to PigII, the *in vitro* fermentation kinetics of their caecal microflora was fairly comparable to that observed with the fasted pigs (Figure 2). This suggests that the effects observed in GasI may not have been affected by the overnight fasting.

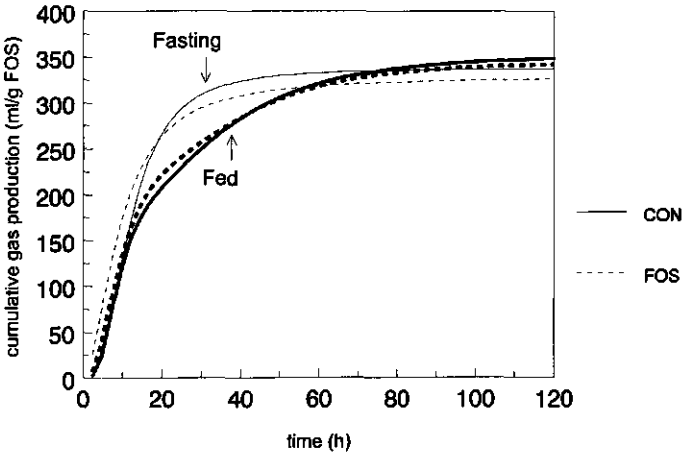

Figure 2. *Cumulative gas production curve using FOS as substrates and a caecal inoculum from pigs fed a diet without FOS (CON) or a diet with 30-40 g FOS/kg. Fasting pigs were from experiment PigII, and the fed pigs were from PigIII*

The enhanced saccharolytic activity in the NDO-fed pigs could not be maintained for the total digestal retention time in the hindgut because suitable sources of energy are exhausted before the digesta are excreted as faeces. This hypothesis was strengthened by the differences in VFA profile between FOS and TOS as dietary NDO on the one hand (*in vivo*), and FOS and TOS as substrates for the *in vitro* fermentation on the other hand (Table 3). The effect in the ileal digesta *in vivo* was comparable to that using the ileal inoculum *in vitro*, suggesting that the *in vivo* VFA profile at the ileal but not at the faecal level directly results of dietary NDO. Pooled over substrates, the VFA profiles *in vitro* comprised relatively less acetic acid and more propionic- and butyric acid than those *in vivo*. It was suggested that this was due to differences in absorption rate for the individual VFA components *in vivo* and the larger extent of lactic acid fermentation *in vitro* (GasI).

Once the preferred energy sources have gone, some bacteria may start to use proteins as a source of energy. It was suggested that the observed increase in faecal pH was a reflection of such increased proteolytic activity, which was more pronounced for FOS

Table 3. *Profile of volatile fatty acids (%) from dietary NDO in vivo and NDO as substrates* in vitro

		NDO in the diet (*in vivo*)			NDO as substrates (*in vitro*)[1]		
Sampling site	VFA	FOS	TOS	P[2]	FOS	TOS	P[2]
Ileal digesta	Acetic acid	68	79	**	45	59	***
	Propionic acid	26	17	**	44	34	***
	Butyric acid	6	4	NS	11	7	***
Faeces	Acetic acid	70	68	NS	53	63	***
	Propionic acid	22	23	NS	27	20	***
	Butyric acid	8	9	NS	20	16	***

[1]Ileal digesta and faeces used as inoculum
[2]P-value for the difference between FOS and TOS. NS: P>0.10; **: $P < 0.01$; ***: $P < 0.001$.

than for TOS (Chapter 4). If the enhanced saccharolytic activity is to be maintained, then more slowly fermentable carbohydrates (**SFC**) would have to be used. A combination of NDO and SFC may result in an enhanced saccharolytic activity at the terminal ileum or proximal colon by the NDO, and further saccharolytic activity throughout the gut maintained by the microbial degradation of the SFC. The *in vitro* fermentation kinetics of OHM, which was part of the diet in PigII, showed that this fibre source could not support such activity (GasI). For example, possible combinations of NDO and SFC could be FOS and inulin, or TOS and galactan (based on the similarity of the chemical structures). It has been shown that faecal bifidobacteria increased and enterococci decreased in humans fed inulin supplements, though no change in pH or VFA profile was observed (Gibson *et al.*, 1995; Kleessen *et al.*, 1997). The effect of inulin on pH, VFA-profile, and lactic acid in the rat caecum were comparable to those observed for FOS in the pig ileal digesta (Levrat *et al.*, 1991). In addition, it has also been shown that galactan decreased ileal pH and *E. coli* counts in weanling pigs (Mathew *et al.*, 1993).

For this concept, the rate of fermentation becomes one of the more important properties of an NDO and SFC. The cumulative gas production technique used for GasI and GasII can be used to screen potential carbohydrates for this property, since it measures fermentation kinetics (Theoderou *et al.*, 1994; Groot *et al.*, 1996). It could be argued that carbohydrates that are fermented rapidly, but have a relatively long lag-phase, might be as useful for this combination of carbohydrates as more slowly fermentable carbohydrates with a shorter lag phase. This approach is currently being tested using a variety of carbohydrates (Voigt *et al.*, 1998).

NDO and growth performance

The experiments did not show a significant improvement of growth performance due to dietary NDO (PigI and PigIII), which is in agreement with the absence of clear effects of nutrient digestion. It was suggested that an absence of an improvement of growth performance was partly due to the already high level of performance in the control group (PigI). The pigs were offered a highly digestible balanced diet, individually housed, kept under a low environmental challenge, and with hindsight, the absence of an improvement of the growth performance was not surprising (PigI). To challenge this, we fed the piglets in PigIII, a diet which was formulated to induce a low growth performance and a high level of diarrhoea. However, there were still no clear effects of NDO on growth performance, though the piglets did respond to the positive control (in-feed avilamycin). Some effects of FOS were observed in the small intestine, though not throughout the entire length of the gastrointestinal tract. This is in agreement with the effects of dietary NDO observed in the other experiments. Compared to the local effects of FOS observed, in-feed antibiotics probably exert their effects more or less throughout the gastrointestinal tract. With hindsight, the absence of clear effects on growth performance and diarrhoea incidence are not surprising. It has been shown that high-protein diets led to elevated ammonia concentrations in the colon (Dong *et al.*, 1996), and the FOS never reach this site of fermentation in the first place. However, young pigs may possibly benefit from dietary NDO when the origin of (subclinical) gastrointestinal disorders lies with the small intestinal microflora.

Growth performance is usually measured in terms of body weight gain, feed intake and feed conversion. However, another approach to measure the benefits of NDO in the diets of young growing monogastrics, would be to regard dietary NDO as a kind of premium for herd health, and record the veterinary costs in a given period. Practical experience with NDO was presented at a seminar during Victam 1998 (Goedhals, personal communications). The reduction of feed intake, as observed in PigI, is also often observed in practice. However, when veterinary costs are included in the final total production costs, it is often observed that the production had been cheaper with added dietary NDO. Such often hidden benefits are very important for the successful application of NDO as a prebiotic feed additive, and those data should be recorded in future experiments or surveys. The reduction in veterinary costs may originate from a lower concentration of aerobes in the small intestine (PigII and PigIII), and thus a reduced incidence of subclinical infections. In addition, dietary NDO may also indirectly interact with the retention time of

drugs in the body. Several drugs are conjugated in the liver and excreted via the bile (Rowland *et al.*, 1985). This conjugation results in a more water-soluble complex which facilitates excretion of active components of the drugs via the faeces. However, β-glucuronidase activity of the microflora can liberate the parent compound from this complex and allow re-absorption (Rowland *et al.*, 1985). One can imagine that such processes could enhance the efficacy of administered drugs, and might result in less drugs being actually used.

Conclusions

This thesis has described research on the effects of NDO in young pig diets. Two types of NDO, FOS and TOS, were included in NDO-free diets up to a level of 40 g/kg. This approach allowed us to study the effect of dietary NDO as feedstuff components and their potential as prebiotic feed additives. Dietary NDO were studied for their effects on growth performance, nutrient digestion, physico-chemical and microbial digesta characteristics, and *in vitro* microbial activity and NDO degradation.

The main conclusions from the presented studies on dietary NDO (FOS and TOS), compared to an NDO-free diet, can be summarized as follows:

- dietary NDO did not improve growth performance and apparent faecal and ileal nutrient digestion of well-kept growing pigs, though the pre-caecal degradation of hemicellulose was enhanced
- dietary FOS did not improve the growth performance and did not reduce the incidence of post-weaning diarrhoea in young piglets fed high-protein diets
- the introduction of diets with additional NDO resulted in a temporarily decreased feed intake, which was compensated for within six weeks
- dietary NDO increased gastric empty weight, lowered gastric- and ileal pH, and increased faecal pH
- dietary NDO yielded fewer aerobes in the small intestinal digesta
- more than 90% of the FOS was degraded pre-caecally
- the FOS-fed pigs had relatively more propionic- and less acetic acid, a higher concentration of lactic acid, more anaerobes, including lactobacilli, and more *iso*-valeric acid in the ileal digesta compared to the TOS-fed pigs
- the maximum rate of *in vitro* fermentation was faster for FOS than for TOS

- the total amount of VFA production *in vitro* did not differ between FOS and TOS, but FOS produced relatively more propionic- and butyric acid and less acetic acid than TOS
- dietary FOS enhanced the caecal *in vitro* microbial activity

The combination of the fast rate of fermentation *in vitro* and the effects on pH, VFA-profile, and bacterial counts at the ileal and faecal level *in vivo* led to the conclusion that FOS, and to a lesser extent TOS, were not able to maintain an enhanced saccharolytic activity throughout the entire length of the gastrointestinal tract.

Figure 3 shows a schematic representation of the fate of dietary NDO in the gastrointestinal tract and the consequences for the microflora as a whole. This figure is compiled from different experiments, and may provide a working model for dietary NDO. It suggests that microbial degradation of NDO starts in the stomach, and is complete, depending on the rate of degradation, relatively proximal in the gastrointestinal tract. The effect of dietary NDO on microbial activity is illustrated with the vertical line along the sites of the gastrointestinal tract where the saccharolytic and/or proteolytic activity is enhanced compared to an NDO-free diet. The proteolytic activity in the more distal part of the gut will be enhanced when no suitable substrates are available to maintain the enhanced saccharolytic activity.

The work reported in this thesis was not designed as a complete study of the possibility for dietary NDO to replace in-feed antibiotics. However, the work does suggest that FOS, and to a lesser extent TOS, cannot replace in-feed antibiotics due to their inability to maintain the enhanced beneficial saccharolytic activity throughout the complete gastrointestinal tract. However, more slowly fermentable carbohydrates, possibly in combination with NDO, may achieve a prolonged beneficial saccharolytic activity, and may prove useful as alternatives for in-feed antibiotics.

Practical implications

Whether the mode of action, proposed in Figure 3, holds for feedstuff NDO, such as the FOS found in cereals and GOS in legume seeds, depends on the access of the microflora to those NDO. The NDO added will be instantly available for the gastrointestinal microflora, and the site of fermentation mainly depends on the rate of fermentation. A high

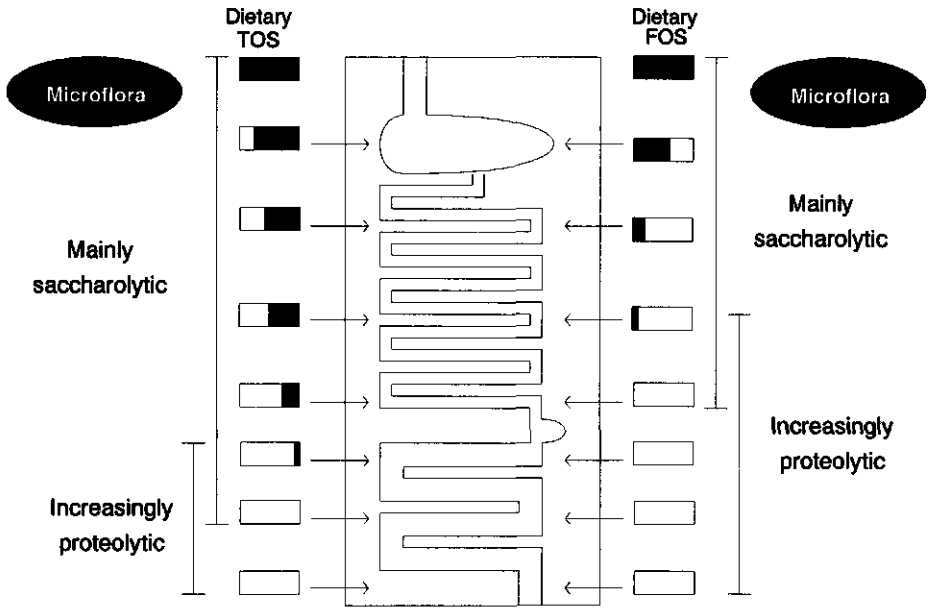

Figure 3. *Schematic representation of the porcine gastrointestinal tract with the fate of dietary NDO and the subsequent changes of the microbial activity. The figure illustrates effects relative to a NDO-free diet*

degree of similarity with added NDO is expected for GOS, since the pre-caecal digestion of in-feed GOS is relatively high. There are no data on pre-caecal degradation of FOS from cereals. However, when the cell contents of the cereals are digested mainly in the small intestine, it might be expected that FOS will be available for the small intestinal microflora.

In contrast to the FOS added, the FOS from cereals could reach the hindgut when digestion of the cereals in the small intestine is limited. The cell contents, including FOS, may then be released due to the (partial) degradation of the cell wall in the large intestine, allowing FOS to exert prebiotic properties in this part of the gut. It has been discussed that the level of in-feed NDO is not constant. However, an effective level of in-feed NDO of approximately 25-30 g NDO/kg can be present in commercial starter diets. The addition of a relatively small amount of NDO to such diets probably do not further increase the already established level of prebiotic properties.

Suggestions for further research

The experimental diets were based on feedstuffs that were low or devoid of NDO. NDO-rich products were included in these diets to achieve a maximum of 40 g NDO/kg. In this case, the NDO were included at the expense of purified cellulose. However, the role of dietary NDO can also be elucidated via the use of specific enzymes. One might prepare a cereal-based diet as a control, and then add β-fructosidases to hydrolyze the FOS present. This approach would be analogous to the inclusion of α-galactosidases to remove GOS from legume- and vellase-based diets (Veldman *et al.*, 1993; Gdala *et al.*, 1997).

The probiotics have not been discussed in this thesis. A probiotic is a 'live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Fuller, 1989). A range of probiotics are being added to animal feeds, though their efficacy is variable (Stavric and Kornegay, 1995). The efficacy of probiotics may be enhanced by combining them with prebiotics. Ideally, the effect of these 'symbiotics' should be studied together with other potential replacers of in-feed antibiotics within the same study. As already mentioned, a combination of NDO and more slowly fermentable carbohydrates may prove useful as an alternative for in-feed antibiotics.

The experimental design of a study to elucidate alternatives to in-feed antibiotics should include a negative control (no additives) and a positive (in-feed antibiotics) control. It was shown that the magnitude of response to dietary NDO, but probably also to other '*microflora modulators*', is negatively correlated with the level of performance in the negative control group (PigI, PigIII). If the animal growth performance does not differ between the in-feed antibiotics and the alternatives, it would be tempting to conclude that the alternatives can be applied without penalizing growth performance. However, if the level of performance of the negative control is not different from both the alternatives and the positive control, then such a conclusion is not justified.

As already mentioned, the level of in-feed NDO in commercial pig diets depends on the type, variety, stage of maturity, and inclusion levels of the feedstuffs used. The question is, however, does an optimum level of dietary NDO exist? The experiments in this thesis suggest that for some parameters the optimum dietary NDO level is about 20-25 g/kg (PigII). However, dose-response experiments with more inclusion levels in NDO-free control diets are needed to confirm the quadratic relations between responses to dietary NDO. Ideally, this should be done under sub-optimal conditions, allowing a certain degree of environmental challenge. If one were to know the optimum level of dietary NDO, eventually in combination with more slowly fermentable carbohydrates, then feed

formulation programs could be used which would optimize feed rations for young pigs in terms of whether additional NDO and/or slowly fermentable carbohydrates are required.

The gastrointestinal microflora has been studied using enumeration techniques on selective media (PigII and PigIII) and *in vitro* fermentation activity (GasI and GasII). In recent years, molecular techniques have been developed to allow for the simple, rapid, and specific detection of microbes. One of these techniques is the temperature gradient gelelectrophoresis, **TGGE** (Zoetendal *et al.*, 1998). As a kind of pilot study, and outside the scope of this thesis, the small- and large intestinal digesta from the CON- and FOS-piglets (PigIII) were analyzed using TGGA (E. G. Zoetendal, unpublished data). Figure 4 shows TGGE profiles of the V6 - V8 regions of 16S rDNA from the small intestinal digesta (SI) and the large intestinal digesta (LI). Each band represents a specific bacterial 16S rDNA amplicon; the darker the band, the higher the concentration of this amplicon. These bands were not identified at this stage. However, fewer bands were detected for the small intestinal- compared with the large intestinal digesta, which suggested a clear difference in bacterial diversity. This was to some extent in agreement with the bacterial counts (PigIII), though differences between the CON- and the FOS-piglets were not clear. Nevertheless, in the future, these types of DNA/RNA techniques are likely to provide meaningful insights into the intestinal microflora and its activities.

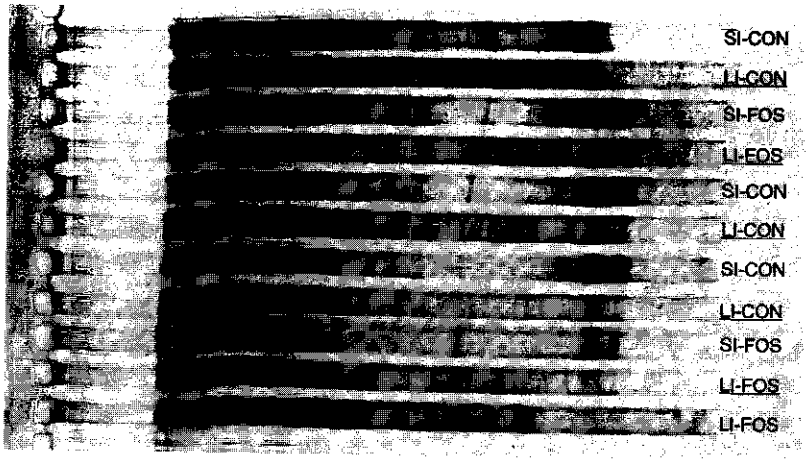

Figure 4. *TGGE profile of the V6 - V8 regions of bacterial 16S rDNA from small intestinal (SI) and large intestinal (LI) digesta of weanling piglets fed high-protein diets with or without FOS, using temperature gradient gelelectrophoresis (with permission from E. G. Zoetendal, Department of Microbiology, Agricultural University Wageningen; unpublished data)*

SUMMARY

Summary

Introduction

This thesis has described research on the effects of dietary non-digestible oligosaccharides (**NDO**) on digestive-physiological and microbial characteristics in young pigs. These low-molecular, water-soluble carbohydrates are called non-digestible because the enzymes needed to hydrolyse these to absorbable monomers are not found in mammalian endogenous secretions. However, dietary NDO are not recovered in the faeces, suggesting that they must be degraded by the gastrointestinal microflora. Some NDO have been classified as prebiotic. A prebiotic is a non-digestible feed ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species. As such, NDO are potential prebiotic feed additives.

Two types of NDO were studied in this thesis, the fructooligosaccharides (**FOS**) and the transgalactooligosacharides (**TOS**). FOS are β-linked fructose oligomers and found in e.g. wheat and barley. The FOS used in this series of experiments were produced by the hydrolysis of chicory inulin. The TOS are β-linked galactose oligomers, not found in common feedstuffs, but trace levels are present in yoghurt. The TOS used here were produced via transgalactosidation of lactose.

It has been shown that FOS and TOS, when included in commercial diets, do not affect digestal pH, ammonia- and volatile fatty acid (**VFA**) concentration and profile, and bacterial counts. This suggested that the use of NDO as prebiotic feed additive in commercial diets may be limited. However, the cereals and soybeanmeal in these diets probably already provided a significant level of NDO, exerting a certain level of prebiotic effects in the control diets. In this thesis, we studied the effects of dietary FOS and TOS using control diets, which were based on corn or corn starch (low or devoid of NDO, respectively). This approach allowed us to elucidate the role of NDO as feedstuff components and their potential as prebiotic feed additives in young pig diets.

Effects of dietary NDO in young growing pigs

Chapters 1, 2, and 3 have described the results of an experiment with young growing pigs (63-105 d of age). Dietary FOS and TOS were studied for their effects on growth performance, apparent faecal and ileal nutrient digestion, physico-chemical and microbial characteristics of the digesta and faeces, and portal plasma VFA concentrations.

The corn-based control diet contained no additional copper, antibiotics or probiotics. The FOS were included in this diet as 7.5 and 15.0 g/kg Raftilose P95®, and the TOS as 10.0 and 20.0 g/kg Oligostroop®. NDO were included in the diets at the expense of purified cellulose (this approach was also used in the other experiments).

Daily feed intake and body weight gain of the NDO-pigs were lower than that of the control pigs in the first three weeks of the growth experiment. However, the mean performance during the total six-week growth experiment, and the apparent faecal and ileal nutrient digestion were not affected. Some pigs were slaughtered, three hours after the last feed intake. Both FOS and TOS were not recovered from the gastric- and large intestinal digesta, and faeces, but only from the digesta of the distal part of the small intestine (7 m). The NDO-pigs had a lower stomach pH than the control pigs. Moreover, the FOS-pigs had a higher colonic pH, and a lower proximal colon and portal plasma VFA concentration than the TOS-fed pigs. The pH and VFA values for the control pigs were intermediate. The faecal pH was not affected, but the faecal DM of the NDO-pigs was lower than that of the control pigs.

It was concluded from this experiment that exchanging cellulose for NDO in young growing pigs diet resulted in a temporary depressed feed intake with no effects on nutrient digestion. However, dietary NDO did affect the microbial ecology of the gastrointestinal tract.

Effects of dietary NDO in weaner pigs

Chapters 2, 4, and 5 have described the results of an experiment with weaner pigs (38-75 d of age). Dietary FOS and TOS were studied for their effects on the apparent faecal and ileal nutrient digestion, N- and mineral balances, and various physico-chemical and microbial characteristics of the ileal- and caecal digesta and faeces. The digesta and faeces were also used as inocula for *in vitro* fermentation, using the cumulative gas production technique, which measures fermentation kinetics and end-point concentrations of fermentation products. The effects of dietary NDO on *in vitro* microbial activity were studied, as were the differences between FOS and TOS as substrates (energy sources). The levels of FOS and TOS in the diet were 10.0 and 40.0 g/kg.

The apparent faecal digestion of the non-starch neutral-detergent soluble carbohydrates (**NNSC**) increased in the NDO-fed pigs. The TOS-pigs tended to have a higher apparent faecal digestion of crude protein than the FOS-pigs and control pigs, though the TOS-pigs excreted more N via the urine. N- and mineral balances were not

affected. The apparent ileal digestion of hemicellulose increased in the NDO-pigs, while that of NNSC decreased. The FOS-pigs had a lower apparent ileal NNSC digestion than the TOS-pigs. FOS were nearly completely degraded pre-caecally (>90%).

The NDO-pigs had a lower ileal pH than the control pigs. The higher dietary NDO levels yielded relatively more propionic- and less acetic acid in the ileal digesta. This was more pronounced for the FOS- than for the TOS-pigs. The FOS-pigs had a higher concentration of lactic acid in the ileal digesta than the TOS-pigs, and a four-fold increase in the concentration of *iso*-valeric acid. The NDO-pigs harboured fewer aerobes than the control pigs in the ileal digesta, and the FOS-pigs had more anaerobes, including lactobacilli, than the TOS-pigs. The NDO-pigs, especially the FOS-pigs, had a higher concentration of caecal anaerobes than did the control pigs. Compared to the control pigs, the faeces of the NDO-pigs had a higher pH and contained relatively more protein-derived short-chain fatty acids (**pVFA**) and less butyric acid. The proportion of faecal acetic- and propionic acid was not affected. The TOS-pigs had more faecal anaerobes and less faecal VFA than did the FOS-pigs, while the lactobacilli as a percentage of the anaerobic flora, were reduced for both types of NDO.

The maximal rate of *in vitro* fermentation of FOS was faster than that of TOS. There was no difference in total VFA production, but FOS yielded more propionic- and butyric acid and less acetic acid than TOS. The overall effect of dietary NDO on *in vitro* fermentation was significant only for the caecal inoculum. That of the FOS-pigs showed a faster maximum rate of fermentation which was reached earlier than that of the TOS-pigs. The diets contained NDO-free oats husk meal as a source of fibre. The faecal microflora of the FOS- and TOS-pigs did not ferment oats husk meal *in vitro* to the same extent as those of the control pigs.

It was concluded from this experiment that dietary NDO in weaner pigs diet hardly affected nutrient digestion, though the apparent pre-caecal hemicellulose digestion did increase. Dietary NDO enhanced saccharolytic activity at the ileal level, and seemed to enhance proteolytic activity at the faecal level. The rate of fermentation and end-point VFA profile differed between FOS and TOS. Adaptation to dietary NDO did not seem to confer any advantage in terms of fibre fermentation.

Effects of dietary FOS in young piglets around weaning fed high-protein diets

Chapters 6 and 7 have described the results of an experiment with young piglets around weaning (0-63 d of age). Dietary FOS were studied for their effects on growth performance, faecal consistency, and physico-chemical and microbial digesta characteristics. In addition, *in vitro* microbial activity was studied using the distal small intestinal and caecal digesta as inoculum and FOS as substrates. The diets were high in protein so as to induce a certain level of protein fermentation. This was done to test the hypothesis that FOS lower bacterial proteolytic activity in the gut. A corn-based control diet (**CON**) or the same diet containing FOS at 7.5, 15.0, 22.5, and 30.0 g/kg were formulated. Diet six (positive control) was diet CON enriched with 40 ppm avilamycin (**AVI**). These diets were fed from d 10 to d 41 of age. Then, a commercial starter diet was offered for three weeks.

There was no effect of FOS on daily feed intake, weight gain, and faecal consistency, though a quadratic effect of FOS on feed conversion was observed from weaning (d 28 of age) to d 41 of age. The daily feed intake and weight gain were greater for the AVI-diet, compared to both the CON- and the FOS-diets. In addition, the AVI-diet showed a higher proportion of normal faeces (77%) than did the other diets (65%), in the period from weaning to d 41 of age. The proportion of normal faeces increased to more than 90% when the commercial starter diet was offered. Both the AVI- and FOS-diets did not affect growth performance from weaning to d 63 of age.

More than 50% of the VFA in the gastric- and small intestinal digesta was valeric acid. The AVI-piglets had a lower gastric pH, less valeric acid in the gastric digesta, and less pVFA in the portal-arterial difference compared to the CON-piglets. The FOS30-piglets had less ammonia in the proximal small intestinal digesta than the CON-piglets. The anaerobes counts in the distal small intestine, caecum, and proximal large intestine were not affected by the diet, though the AVI-pigs had a higher count of *E. coli* than did the FOS30-piglets. The inoculum of the FOS30-piglets showed an earlier first phase of the *in vitro* gas production compared to that of the other piglets. The inocula of the FOS30- and AVI-piglets had a slower maximum rate of gas production in the second phase compared to that of the CON-piglets. These effects were more pronounced for the small intestinal inoculum than for the caecal inoculum. The small intestinal inoculum of the FOS-piglets produced the highest amount of VFA following *in vitro* fermentation of FOS, and that of the AVI-piglets produced less gas and butyric acid than that of the CON-piglets.

It was concluded that dietary FOS reduced the protein fermentation in the small intestine only, and that a reduced protein fermentation was more prolonged for in-feed avilamycin. As a consequence, the growth performance was enhanced for the AVI-diet.

Dietary NDO from feed to faeces

The main effects observed for dietary FOS and TOS have been discussed in the context of the flow from feed to faeces (General discussion). Microbial degradation of dietary NDO probably started in the stomach of the pig. Both FOS and TOS were not recovered from the gastric digesta, while the gastric pH in the NDO-pigs was lowered. The lower pH could also have originated from a longer duration of gastric fermentation of any dietary organic matter. This may have been the result of a reduced gastric motility, which could also be due to dietary NDO.

Dietary NDO hardly affected the apparent ileal digestion of nutrients. With hindsight, it was realized that it would probably be difficult to show effects of NDO on protein- and energy digestion, given the highly digestible ingredients of the experimental diets. However, the apparent pre-caecal digestion of hemicellulose did increase, indicating an increased microbial activity for this substrate. Both dietary FOS and TOS resulted in a reduced ileal pH, suggesting a certain extent of pre-caecal fermentative degradation. Indeed, more than 90% of the FOS were degraded pre-caecally; that of TOS was 30% (estimated by others). The reduced concentration of ammonia and increased concentration of pVFA in the small intestinal digesta are in agreement with the high degree of pre-caecal fermentative FOS degradation. Moreover, the difference between FOS and TOS in the extent of pre-caecal digestion was supported by differences in lactic acid concentration, anaerobe counts (including lactobacilli), and VFA profile in the ileal digesta. In addition, the faster rate of FOS fermentation *in vitro* suggested an earlier complete fermentation *in vivo*, not only in terms of site of fermentation, but also time after feeding. This was in agreement with the differences between dietary FOS and TOS in terms of large intestinal pH and VFA, and portal VFA, measured three h after the last feeding. Despite the differences in the extent of pre-caecal fermentative degradation, both dietary FOS and TOS lowered the concentration of aerobes in the ileal digesta equally. This could be beneficial in terms of gut health.

Dietary NDO had little effect on the apparent faecal nutrient digestion and N- and mineral balances. However, the microbial ecology of the large intestine, as measured in the faeces, was affected. This was probably a direct result of an enhanced pre-caecal saccharolytic activity. The data suggested that dietary FOS and TOS were not able to

maintain the enhanced saccharolytic activity throughout the large intestine (increased pH and lower proportion of lactobacilli in the faeces). This was supported by the lower concentration of ammonia in the proximal small intestinal digesta only, when piglets were fed the FOS-supplemented high-protein diet. In terms of VFA-profile, the differences between FOS and TOS as substrates were comparable to the difference between FOS and TOS as dietary components at the ileal level, but not at the faecal level. This additionally suggested that the effects of dietary NDO in the faeces did not result directly from NDO fermentation. This is not surprising; the fast rate of fermentation *in vitro* suggests that FOS and TOS do not ever reach the rectum in the first place but have been fermented much earlier in the gastrointestinal tract. The increased pH and proportion of pVFA suggested that the effects in the faeces probably resulted from an enhanced protein fermentation. Due to the absence of NDO as a source of energy, some bacteria in the large intestine will start to use protein as a source for energy. Moreover, the differences between dietary FOS and TOS suggested that the more proximal the NDO fermentation was completed, the more pronounced the proteolytic activity reached at the faecal level.

Conclusions

It was concluded that dietary FOS and TOS, included in highly digestible diets and exchanged at the expense of purified cellulose, temporarily reduce feed intake, but have little effect on growth performance and nutrient digestion in pigs. However, the ecology of the gastrointestinal microflora was affected. Dietary FOS and TOS enhanced the pre-caecal saccharolytic activity, resulting in prebiotic effects at the site of fermentation. However, these prebiotic effects are not maintained throughout the total large intestine, due to the fast rate of fermentation of FOS and TOS. It would seem that some of the stimulated microflora will then start to use proteins as a source of energy.

Thus, an enhanced saccharolytic activity at the ileal level that cannot be maintained throughout the large intestine, can result in an increased level of protein fermentation at the faecal level. However, prebiotic carbohydrates which can be fermented throughout the gastrointestinal tract, rather than only in the small intestine and/or the proximal colon, may result in prolonged prebiotic effects in young pigs.

SAMENVATTING

Samenvatting

Inleiding

Dit proefschrift betreft een onderzoek naar verterings-fysiologische en microbiële effecten van niet-verteerbare oligosachariden (*non-digestible oligosaccharides*, **NDO**) in biggenvoeders. Deze laag-moleculaire, water-oplosbare koolhydraten zijn niet verteerbaar, omdat de enzymen, die nodig zijn voor de hydrolyse van deze oligomeren tot absorbeerbare monomeren, niet in de endogene secretie van zoogdieren voorkomen. Echter, deze NDO worden niet in de faeces terug gevonden, hetgeen suggereert dat ze kunnen worden afgebroken door de microflora van het maagdarmkanaal. Sommige NDO zijn geklassificeerd als prebioticum. Een prebioticum is een niet-verteerbaar voederbestanddeel waar de gastheer baat bij heeft, doordat het selectief de groei en/of activiteit stimuleert van één of een beperkt aantal soorten bacteriën. Daarom zouden NDO gezien kunnen worden als potentiële prebiotische voederadditieven.

In de hier beschreven experimenten zijn twee soorten NDO bestudeerd, de fructo-oligosachariden (**FOS**) en de transgalacto-oligosachariden (**TOS**). FOS zijn fructose-oligomeren die verbonden zijn door middel van β-bindingen en komen o.a. voor in tarwe en gerst. De FOS die gebruikt zijn in de hier beschreven experimenten zijn verkregen door hydrolyse van cichorei-inuline. De TOS zijn galactose-oligomeren die verbonden zijn door middel van β-bindingen. De TOS komen niet voor in gangbare voedermiddelen, maar er kunnen lage gehaltes aanwezig zijn in yoghurt. De in dit onderzoek gebruikte TOS zijn verkregen door transgalactosidatie van lactose.

In eerdere studies met praktijkvoeders werden geen effecten van FOS en TOS gevonden op de pH, concentratie van ammoniak en vluchtige vetzuren (**VVZ**), VVZ-samenstelling en bacterietellingen in de darminhoud van jonge biggen. Dit zou kunnen betekenen dat het nut van NDO als prebiotisch voederadditief in praktijkvoeders beperkt is. Echter, de voeders die gebruikt werden in bovengenoemde studies bevatten granen en sojaschroot. Hierdoor kon er al een behoorlijk gehalte aan NDO in de controlevoeders aanwezig zijn, zodat er waarschijnlijk al een zeker prebiotisch effect bewerkstelligd was. Dit proefschrift beschrijft de effecten van FOS en TOS in controlevoeders, gebaseerd op maïs of maïszetmeel (resp. weinig of geen NDO). Hierdoor kan meer inzicht verkregen kunnen worden in de eigenschappen van NDO als voedermiddel ingredient én in de mogelijkheden om NDO te gebruiken als prebiotisch voederadditief in de voeders van biggen.

Effecten van NDO in de voeders van groeiende biggen

De hoofdstukken 1, 2 en 3 beschrijven de resultaten van een experiment met groeiende biggen (63-105 dagen oud). De effecten van FOS en TOS in de voeders werden bestudeerd op de groeiprestaties, schijnbare faecale en ileale nutriënten vertering, fysisch-chemische en microbiële eigenschappen van de darminhoud en faeces, en de concentratie aan VVZ in poortaderlijk bloed. Het controlevoeder was op maïs gebaseerd en bevatte geen extra koper, antibiotica of probiotica. De FOS zijn toegevoegd als 7.5 en 15.0 g Raftilose P95®/kg en de TOS als 10.0 en 20.0 g Oligostroop®/kg. De NDO zijn in de voeders opgenomen door ze uit te wisselen tegen gezuiverde cellulose; deze manier van NDO opname in de voeders is ook toegepast in de andere experimenten.

De dagelijkse voeropname en groei, gedurende de eerste drie weken van de groeiproef, waren lager voor de NDO-biggen dan voor de controlebiggen. Echter, de gemiddelde groeiprestaties gedurende de totale groeiproef (6 weken) en de schijnbare faecale en ileale nutriënten vertering werden niet beïnvloed. Drie uur na de laatste voeropname zijn een aantal biggen geslacht. De NDO werden alleen teruggevonden in het tweede deel van de dunne darm (de laatste 7 meter) en niet in de maag- en dikke darminhoud en de faeces. De pH van de maaginhoud van de NDO-biggen was lager dan die van de controlebiggen. De pH van de dikke darminhoud van de FOS-biggen was hoger dan die van de TOS-biggen. Daarnaast was de VVZ-concentratie in het eerste deel van de dikke darm en in het portale plasma lager dan bij de TOS-biggen. De pH en VVZ-concentraties van de controlebiggen lagen er tussenin. De pH in de faeces werd niet beïnvloed, maar het droge stof gehalte van de faeces van de NDO-biggen was lager dan dat van de controlebiggen.

Uit dit experiment werd geconcludeerd dat de vervanging van cellulose door NDO in de voeders voor groeiende biggen een tijdelijk verlaagde voeropname tot gevolg had, maar dat de nutriënten vertering niet werd beïnvloed. Echter, de NDO in de voeders beïnvloedden de microbiële ecologie van het maagdarmkanaal wel degelijk.

Effecten van NDO in de voeders van gespeende biggen

De hoofdstukken 2, 4 en 5 beschrijven de resultaten van een experiment met gespeende biggen (38-75 dagen oud). De effecten van FOS en TOS in de voeders werden bestudeerd op de schijnbare faecale en ileale nutriëntenvertering, stikstof- en mineraal balansen en diverse fysisch-chemische en microbiële eigenschappen van de dunne- en

blinde darminhoud en de faeces. De darminhoud en faeces werden ook gebruikt als inocula voor *in vitro* fermentatie. De gebruikte cumulatieve gasproductie techniek leverde zowel de fermentatie-kinetiek als de eindconcentraties van fermentatieproducten. Zowel de effecten van NDO in de voeders op *in vitro* microbiële activiteit, als de verschillen tussen FOS en TOS als substraten (energiebron) werden bestudeerd. In dit experiment werden 10.0 en 40.0 g FOS of TOS gebruikt per kg voer.

De schijnbare faecale vertering van de oplosbare, niet-zetmeel koolhydraten was hoger in de NDO-biggen. De schijnbare faecale vertering van ruw eiwit was enigzins verhoogd in de TOS-biggen ten opzichte van de FOS- en controlebiggen. Echter, de TOS-biggen scheidden meer stikstof uit via de urine. De NDO in de voeders beïnvloedden de stikstof- en mineralenbalansen niet. De NDO-biggen hadden een verhoogde schijnbare ileale vertering van hemicellulose, terwijl die van de oplosbare, niet-zetmeel koolhydraten afnam. De FOS-biggen hadden zelfs een lagere schijnbare ileale vertering van de oplosbare, niet-zetmeel koolhydraten dan de TOS-biggen. Meer dan 90% van de FOS werd afgebroken in de dunne darm.

De ileale pH van de NDO-biggen was lager dan die van de controlebiggen. De voeders met het hoge NDO gehalte leverden relatief meer propionzuur en minder azijnzuur in de ileale chymus dan die met het lage gehalte. Dit effect was duidelijker in de FOS-biggen dan in de TOS-biggen. De FOS-biggen hadden een hogere concentratie melkzuur in de dunne darminhoud dan de TOS-biggen en tevens een viervoudige toename van de concentratie aan *iso*-valeriaanzuur ten opzichte van de andere groepen biggen. De NDO-biggen hadden minder aerobe bacteriën in de dunne darminhoud dan de controlebiggen, terwijl de FOS-biggen meer anaerobe bacteriën (en melkzuurbacteriën) hadden dan de TOS-biggen. De NDO-biggen, met name de FOS-biggen, hadden een hogere concentratie anaerobe bacteriën in de blinde darminhoud dan de controlebiggen. De faeces van de NDO-biggen had, ten opzichte van dat van de controlebiggen, een hogere pH en bevatte het relatief minder boterzuur en meer vluchtige vetzuren die afkomstig waren van eiwitfermentatie (**eVVZ**). Er was geen effect op relatieve hoeveelheid azijnzuur en propionzuur. De TOS-biggen hadden meer anaerobe bacteriën en minder VVZ in de faeces dan de FOS-biggen, terwijl het aantal melkzuurbacteriën in de anaerobe fractie relatief lager was voor beide types NDO.

De FOS werden *in vitro* sneller gefermenteerd dan de TOS. De totale VVZ-productie verschilde niet, maar de FOS leverde relatief meer propionzuur en boterzuur en minder azijnzuur op dan de TOS. Het effect van NDO in de voeders op de *in vitro* fermentatie was alleen significant voor het blinde darm inoculum. Er werd een eerdere en hogere maximale

fermentatiesnelheid waargenomen met het blinde darm inoculum van de FOS-biggen ten opzichte van het blinde darm inoculum van de TOS-biggen. De voeders bevatten havermoutafvalmeel, een NDO-vrije ruwe celstof bron. De faecale microflora van de NDO-biggen konden, *in vitro*, dit havermoutafvalmeel niet in dezelfde mate fermenteren als de faecale microflora van de controlebiggen.

Uit dit experiment werd geconcludeerd dat de NDO in de voeders van gespeende biggen nauwelijks een effect hadden op de vertering van nutriënten, hoewel de schijnbare vertering van hemicellulose in de dunne darm toenam. Echter, de NDO in de voeders verhoogden de sacharolytische activiteit in de dunne darminhoud en de proteolytische activiteit in het laatste deel van de dikke darminhoud (gemeten in de faeces). Het VVZ-profiel na fermentatie en de fermentatiesnelheid *in vitro* waren verschillend voor FOS en TOS. De NDO in de voeders hadden geen verbetering van de *in vitro* vezelfermentatie tot gevolg.

Effecten van FOS in eiwitrijke voeders voor biggen rondom het spenen

De hoofdstukken 6 en 7 beschrijven de resultaten van een experiment met jonge biggen rond het spenen (0-63 dagen oud). De effecten van FOS in de voeders werden bestudeerd op groeiprestaties, consistentie van de faeces, en diverse fysisch-chemische en microbiële eigenschappen van de maagdarminhoud. Tevens werd de *in vitro* microbiële activiteit bestudeerd met FOS als substraat. Hierbij werden de inhoud van het distale deel van de dunne darm en de blinde darm gebruikt als inoculum. De voeders bevatten relatief veel eiwit om de eiwitfermentatie in het maagdarmkanaal te bevoorderen. Hierdoor kon de hypothese getoetst worden dat FOS de bacteriële proteolytische activiteit in de darm verlaagd. Het controlevoer (**CON**) was gebaseerd op maïs. Vier voeders met 7.5, 15.0, 22.5 en 30.0 g FOS/kg werden gebruikt. Het zesde voeder (positieve controle) bestaat uit het CON-voer verrijkt met 40 ppm avilamycine (**AVI**). De biggen kregen de experimentele voeders vanaf 10 dagen leeftijd tot dag 41. Daarna kregen ze gedurende drie weken een praktijk speenvoer.

Er was geen effect van FOS op de dagelijkse voeropname, gewichtstoename en faecesconsistentie, hoewel er een kwadratisch verband gevonden werd tussen het gehalte van FOS in de voeders en de voederconversie in de periode van spenen (op 28 dagen leeftijd) tot 41 dagen leeftijd. De dagelijkse voeropname en groei van de AVI-biggen was hoger dan die van de CON- en FOS-biggen. Daarnaast werd bij de AVI-biggen vaker normale faeces waargenomen (77 % van de waarningen) dan bij de andere groepen

(65%), in de periode van spenen tot een leeftijd van 41 dagen. In de periode dat het praktijk speenvoer gegeven werd steeg de proportie normale faeces tot 90%. Er werden geen effecten van de voeders gevonden op de groeiprestaties van spenen tot een leeftijd van 63 dagen.

De VVZ-concentratie van de maag- en dunne darminhoud bestond voor meer dan 50% uit valeriaanzuur. De AVI-biggen hadden een lagere pH in de maag, minder valeriaanzuur in de maaginhoud en minder eVVZ in het verschil tussen het portale- en arteriële plasma. De FOS30-biggen hadden minder ammoniak in het eerste deel van de dunne darm dan de CON-biggen. Er werd geen effect van de voeders gevonden op de anaerobe bacterietellingen van de inhoud van het distale deel van de dunne darm, de blinde darm en de het eerste deel van de dikke darm. Echter, de AVI-biggen hadden hogere *E. coli* tellingen dan de FOS30-biggen. De maximale fermentatiesnelheid in de eerste fase van de *in vitro* gasproductie werd eerder behaald met het inoculum van de FOS30-biggen dan met de inocula van de andere groepen biggen. De maximale fermentatiesnelheid in de tweede fase van de *in vitro* gasproductie was lager voor de inocula van de AVI- en FOS30-biggen dan voor de inocula van de CON-biggen. Deze effecten waren duidelijker voor het dunne darm- dan voor het blinde darm inoculum. Het dunne darm inoculum van de FOS-biggen produceerde *in vitro* de grootste hoeveelheid VVZ uit FOS. Het dunne darm inoculum van de AVI-biggen produceerde minder gas en boterzuur die van de CON-biggen.

Uit dit experiment werd geconludeerd dat FOS in de voeders de eiwitfermentatie in de dunne darm verminderde, maar niet in andere delen van het maagdarmkanaal. Een verminderde eiwitfermentatie was duidelijker aanwezig bij het gebruik van avilamycine in de voeders. Hierdoor waren de groeiprestaties van de AVI-biggen beter.

NDO in de voeders: van voer tot faeces

De belangrijkste effecten van FOS en TOS in de voeders zijn bediscussiëerd in de context 'van voer tot faeces' (algemene discussie). Bij biggen begint de fermentatie van NDO in de voeders waarschijnlijk al in de maag. De NDO werden niet teruggevonden in de maaginhoud, terwijl de pH in de maaginhoud van de NDO-biggen was verlaagd. Deze lagere pH kan echter ook veroorzaakt worden door fermentatie van ander organisch materiaal. Dit kan het gevolg zijn van een lagere motiliteit van de maag, wat eveneens veroorzaakt kan worden door NDO in de voeders.

De NDO in de voeders beïnvloedden nauwelijks de schijnbare ileale vertering van nutriënten. Achteraf gezien zou het moeilijk geweest zijn om een effect op eiwit- en energie verteerbaarheid aan te tonen bij het gebruik van deze zeer goed verteerbare voeders. Echter, de schijnbare vertering van hemicellulose in de dunne darm nam toe, hetgeen wijst op een toegenomen microbiële activiteit. Zowel FOS als TOS in de voeders bewerkstelligden een daling van de ileale pH. Dit suggereerde dat een deel van de NDO voor het eind van dunne darm fermentatief wordt afgebroken. Meer dan 90% van de FOS was inderdaad verdwenen aan het eind van de dunne darm. Voor TOS was dit geschat op 30% (door anderen). De lagere concentratie van ammoniak en de hogere concentratie van eVVZ in de dunne darminhoud wijzen ook op een hoge mate van fermentatieve afbraak van FOS voor het eind van de dunne darm. Het genoemde verschil in afbraak tussen FOS en TOS werd ondersteund door de verschillen in de melkzuurconcentratie, de anaerobe bacterietellingen (incl. melkzuurbacteriën) en het VVZ-profiel van de dunne darminhoud. Daarnaast suggereerde de snellere *in vitro* fermentatie van FOS een eerdere, volledige fermentatie *in vivo*, niet alleen qua plaats van fermentatie maar ook qua tijd na het voeren. Dit werd ondersteund door de verschillen in pH en VVZ-concentraties voor de FOS- en TOS-biggen, gemeten drie uur na de laatste voeropname. Ondanks het verschil in de mate van fermentatieve afbraak voor het eind van de dunne darm, was de concentratie aerobe bacteriën in de dunne darminhoud in dezelfde mate verlaagd in zowel de FOS- als de TOS-biggen. Dit laatste kan gunstig zijn voor de gezondheid van de (dunne) darm.

De NDO in de voeders hadden nauwelijks een effect op de schijnbare faecale nutriënten vertering en stikstof- en mineralenbalanzen. De microbiële ecologie in de dikke darm, gemeten in de mest, werd echter wel beïnvloed. Dit was waarschijnlijk het directe resultaat van de verhoogde sacharolytische activiteit in de dunne darminhoud. De resultaten suggereerden dat FOS en TOS niet in staat waren de verhoogde sacharolytische activiteit in stand te houden in de gehele dikke darm (hogere pH en relatief minder melkzuurbacteriën in de faeces). De daling van de ammoniak-concentratie, die alleen optrad in de dunne darminhoud van de FOS-biggen met het eiwitrijke voeder, bevestigd dit in zekere mate. De verschillen met betrekking tot het VVZ-profiel waren op ileaal nivo vergelijkbaar tussen FOS en TOS als substraten en tussen FOS en TOS in de voeders. Echter, dit was niet het geval op faecaal nivo. Dit wees er eveneens op dat de waargenomen effecten in de faeces van NDO in de voeders niet direct afkomstig waren van NDO fermentatie op faecaal nivo. Dit is niet geheel vreemd; de snelle *in vitro* fermentatie wijst erop dat FOS en TOS het rectum helemaal niet bereiken maar veel eerder in het maagdarmkanaal gefermenteerd worden. De verhoogde pH en proportie van eVVZ

suggereerden dat de effecten in de faeces waarschijnlijk het resultaat zijn van een verhoogde eiwitfermentatie. Vanwege de afwezigheid van NDO als energiebron kunnen sommige bacteriën in de dikke darm eiwitten als energiebron gaan gebruiken. Bovendien wezen de verschillen tussen de FOS- en TOS-biggen erop dat, hoe eerder in het maagdarmkanaal de fermentatie van de NDO is voltooid, des te hoger de proteolytische activiteit op faecaal niveau kan worden.

Conclusies

Uit dit onderzoek kan geconcludeerd worden dat FOS en TOS, indien opgenomen in goed verteerbare voeders en uitgewisseld tegen zuivere cellulose, leidden tot een tijdelijk verminderde voeropname, maar verder nauwelijks effect hadden op de groeiprestaties en nutriënten vertering van biggen. Echter, de microbiële ecologie van het maagdarmkanaal werd wel degelijk beïnvloed. FOS en TOS in de voeders verhoogden de sacharolytische activiteit in de dunne darm, en vertoonden zo prebiotische effecten op de plaats van fermentatie. Deze prebiotische effecten werden echter niet in stand gehouden in de gehele dikke darm. Dit komt door de snelle en volledige fermentatie van FOS en TOS in het eerste deel van het maagdarmkanaal. Een deel van de gestimuleerde microflora zal dan, in de dikke darm, eiwitten als energiebron kunnen gaan gebruiken.

Dus, indien een verhoogde sacharolytische activiteit op ileaal nivo niet in de hele dikke darm in stand gehouden kan worden, kan er een verhoogde proteolytische activiteit op faecaal niveau ontstaan. Dit zou misschien voorkomen kunnen worden door in de voeders koolhydraten op te nemen, die enerzijds langzamer gefermenteerd worden en anderzijds prebiotische eigenschappen vertonen.

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Houdijk J.G.M., K.M.J van Laere, M.W.A. Verstegen, and M.W. Bosch. Effects of fructooligosaccharides and transgalactooligosaccharides on digesta characteristics and portal volatile fatty acids of growing pigs. *J. Sci. Food Agric.* (submitted for publication)

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Houdijk J.G.M., B.A. Williams, S. Tamminga, and M.W.A. Verstegen Fructooligosaccharides and transgalactooligosaccharides have different *in vitro* fermentation characteristics when being fermented by the porcine intestinal microflora. In preparation.

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Curriculum Vitae

Jos (Johannes Gerardus Maria) Houdijk werd geboren op 2 april 1968 te Ter Aar. Hij bracht zijn jeugd door op de Hoeve Maria, waar hij van jongs af aan meehielp met het verzorgen van de dieren op de boerderij. In 1996 behaalde hij het Atheneum-B diploma aan het Ashram College te Alphen a/d Rijn. Datzelfde jaar werd begonnen met de studie Zoötechniek aan de Landbouwuniverstiteit te Wageningen. Hij studeerde af op 29 januari 1993, met als oriëntatie Veehouderij en een afstudeervak Veehouderij (Parasitologie), twee afstudeervakken Veevoeding, en een half jaar stage in Australië. Tijdens zijn studie is hij een jaar voorzitter geweest van de studievereniging 'De Veetelers'. Vanaf 1 september 1993 was hij aangesteld als Assistent In Opleiding aan de vakgroep (later leerstoelgroep) Veevoeding. Tot eind februari 1998 heeft hij daar gewerkt aan het in dit proefschrift beschreven onderzoek. Sinds 26 maart 1998 is hij werkzaam als onderzoeker/projectleider bij de Animal Biology Division, Scottish Agricultural College te Edinburgh, Schotland, waar hij zich voornamelijk bezig houdt met de interacties tussen de voeding en de weerstand tegen maagdarmwormen bij schapen.