

Cereal Non-Starch Polysaccharides in Pig Diets

**Influence on Digestion Site, Gut Environment and
Microbial Populations**

Ann Högberg

*Department of Animal Nutrition and Management
Uppsala*

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Abstract

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This thesis is based on three different studies comprising the weaning and the growing period, aiming at monitoring the influence of cereal non-starch polysaccharides (NSP) and dietary enzyme supplementation on gastro-intestinal processes in pigs. The diets were based on cereals and cereal by-products, and composed to contain different amounts of total as well as soluble NSP. Results from these studies have shown that with increased NSP content, the reduction in digestibility of organic matter (OM) was twice as high in the small intestine than in the total tract, both in newly weaned piglets and growing pigs. An increased proportion of insoluble NSP decreased the digestibility of OM and fibre components in the small intestine of the newly weaned piglets and in the total tract of growing pigs. The gut environment, as described by content and proportions of organic acids (OA) and pH, as well as total microbial populations and coliform diversity, was altered by NSP content and solubility, whereas enzyme supplementation influenced the distribution of OA in the small intestine. PVTC-cannulation did not influence the coliform flora, and results obtained from PVTC-cannulated pigs were concluded to reflect true intestinal conditions. In conclusion, these results indicate that the dietary content of total and soluble NSP influence gastro-intestinal processes such as digestion site, gut environment and microbial populations in different ways in newly weaned piglets and in growing pigs. Therefore, NSP constitute an important tool with possibilities to influence gut health in pigs, and may therefore offer prospects to optimise the feed for pigs of different age.

Keywords: weaning, digestibility, organic acids, enzymes, PVTC cannula, gastro-intestinal tract, biochemical fingerprinting, coliform diversity, T-RFLP

Author's address: Ann Högberg, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Box 7024, SE-750 07 Uppsala, Sweden.

To my parents

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List of original papers

Papers I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I** Högberg, A., Lindberg, J. E. & Wallgren P. 2001. Influence of ileo-caecal cannulation and oxytetracycline on ileo-caecal and rectal coliform populations in pigs. *Acta veterinaria scandinavica* 42, 435-440.
- II** Högberg, A. & Lindberg, J. E. 2003. Influence of cereal non-starch polysaccharides on digestion site and gut environment in growing pigs. *Livestock production science. In press.*
- III** Högberg, A., Lindberg, J. E., Leser, T. & Wallgren, P. 2003. Influence of cereal non-starch polysaccharides on gut microbial populations in growing pigs. *(Submitted)*
- IV** Högberg, A. & Lindberg, J. E. 2003. Influence of enzyme supplementation in cereal based diets on digestion site and gut environment in weaned piglets. *(Submitted)*
- V** Högberg, A. & Lindberg, J. E. 2003. Influence of cereal non-starch polysaccharides on digestion site and gut environment in piglets around weaning. *(Submitted)*

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List of abbreviations

PWD	Post-weaning diarrhoea
SD	Swine dysentery
TSE	Transmissible spongiform encephalitis
RS	Resistant starch
β-glucans	Mixed-linked (1→3),(1→4)-β-D-glucans
NSP	Non-starch polysaccharides
DF	Dietary fibre
SCFA	Short chain fatty acids
DM	Dry matter
OM	Organic matter
PVTC cannula	Post valve T-caecum cannula
NFE	Nitrogen free extractives
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
AOAC	Association of Official Analytical Chemists
T-RFLP	Terminal restriction fraction length polymorphism
PCR	Polymerase chain reaction
FISH	Fluorescence in situ hybridisation
BW	Body weight
OA	Organic acids
CP	Crude protein
TRF	Terminal restriction fragment
BPT	Biochemical phenotype

Background

Carbohydrates, fat and protein are the energy yielding nutrients in animal feed. In the average diet for farm animals, carbohydrates are included at levels of 70-80%. Pig diets are mainly based on cereals, and they contain the main part of the energy providing nutrients that are essential for pigs. The nutritive value differs between the cereals due to their chemical composition. There are differences in total content of sugars, starch, β -glucans, non-starch polysaccharides and dietary fibre, as well as in the ratio of soluble and insoluble fibre fractions. Also the physico-chemical properties such as viscosity, and the capacity to bind water and ions, vary between different cereals. Consequently, different cereal properties affect digestion and fermentation as well as microbial populations in the gastro-intestinal tract in various ways.

Gastro-intestinal disturbances comprise a major problem for the modern pig production, and especially newly born and recently weaned pigs are affected. The aetiology of these gastro-intestinal disturbances may vary. Apart from infectious causes, the feed may also be a contributing factor. With suitable dietary composition, cereals may function as a prebiotic tool in promoting a beneficial gut environment and thereby preventing gastro-intestinal disorders from the weaning period and onwards.

Introduction

Pig production

Since 1986, it is forbidden to routinely include feed additives such as antimicrobial growth promoters in the feed in Sweden (Wegener *et al.*, 1999). Also the use of chemo therapeutic substances like ZnO has been restricted since 1998 (Odensvik, Robertsson & Wallgren, 1999). The Swedish animal welfare law prohibits weaning before four weeks, and weaning is usually performed at five weeks of age. Growing pigs in piglet producing herds are mainly reared in one of two different systems. A few herds apply continuous production systems, where the sows farrow continuously and as a result pigs of different ages share rearing facilities. A more common practice is the “all in – all out” system. In this system farrowing is synchronised at certain intervals, leaving time for cleaning of the pens and farrowing units between farrowings. At weaning, the piglets are either moved to a growing unit or remain in the pen of birth. The weaned piglets are sold at approximately 25 kg to other farms specialised in rearing slaughter pigs or are kept at the farm until slaughter. The specialised slaughter pig producers purchase pigs in batches and raise them to mature slaughter weight in an “all in – all out” system.

Traditionally, each farm keeps their own breeding stock, purchased from breeding herds. Lately, the possibility of obtaining gestating sows from a sow pool

has evolved. The sows arrive at the satellite herds three weeks before farrowing, and then return to the central sow pool at weaning, where they remain during the dry period.

Intestinal diseases

Pigs frequently suffer from gastrointestinal disturbances. These disturbances are by nature of multifactorial origin, but are generally associated with microorganisms. Common factors that may contribute to the development of intestinal diseases include stressful situations in combination with feed composition or feed structure that causes gastric ulcers, and create conditions that may lead to bacterial infections.

Post-weaning diarrhoea (PWD) is the most common intestinal disorder in pigs during the post-weaning period. This disease is associated with the proliferation of enterotoxigenic strains of *E. coli* in the small intestine (Melin *et al.*, 2000), that may result in severe secretory diarrhoea (Hampson, Pluske & Pethick, 2001). PWD leads to discomfort, growth depression and even loss of piglets, ending up with animal welfare problems as well as economic losses for the farmer. As PWD is a multifactorial disorder (Melin *et al.*, 2000), the diet also influences this disease. Increased dietary levels of protein have been shown to induce, and fibre to reduce, the incidence of PWD (Bertschinger *et al.*, 1978/1979; Prohászka & Baron, 1980; Bolduan *et al.*, 1988), and the presence of soluble NSP in the diet may influence development of PWD (McDonald *et al.*, 1999; Pluske *et al.*, 2001). Also the structure of the feed is of importance, since it has been shown that pigs fed a coarsely ground diet are better protected against intestinal infections than pigs fed a finely ground diet (Brunsgaard, 1998).

Swine dysentery (SD) is a mucohaemorrhagic disease that affects the caecum and colon of growing pigs. The disease is caused by an anaerobic spirochaete, *Brachyspira hyodysenteriae*, which colonises the mucus layer and crypts of the large intestine. It can lead to major growth depression and variable losses of pigs, resulting in problems for the farmer similar to those with PWD. Also SD is enhanced by the presence of other species of anaerobic bacteria (Meyer, Simon & Byerly, 1975; Whipp *et al.*, 1979). SD can be reduced with diets that decrease microbial fermentation in the large intestine (Siba, Pethick & Hampson, 1996), and diets low in soluble NSP and resistant starch (Pluske *et al.*, 1998).

Weaning

Weaning is a critical period in the life of a piglet. Due to demands for a high productivity domestic pigs are weaned at a significantly younger age than free-living pigs, which are gradually weaned in a process that is terminated at an age of around 16 weeks (Jensen, 1988). The main events in connection with the weaning of domesticated piglets are the sudden removal of the sow in combination with an abrupt dietary change from milk to cereals. This occurs at a time when the piglet's own immune system is not fully developed (Bailey *et al.*, 2001; Stokes, Bailey & Haverson, 2001) and the immunoglobulins provided by the colostrum have decreased to low levels (Kelly & King, 2001). Further, the gastro-intestinal tract is still under development and is far from able to digest solid feed such as cereals

effectively. Together, these facts form the background to why piglets often are affected by PWD. Also the housing system and the immediate environment, such as hygienic conditions and pathogen load, is of importance (Young *et al.*, 1959; Clark *et al.*, 1991; Wallgren *et al.*, 1993).

The weaning procedure itself causes physical changes in the gastro-intestinal tract. A decrease in feed intake is common immediately after weaning, and may contribute to intestinal inflammation (McCracken *et al.*, 1999). Further, increased crypt depth and decreased villus height in the small intestine are often observed (Hampson, 1986a-b; Pluske, Hampson & Williams, 1997). This leads to a reduced digestive and absorptive capacity, thereby increasing the risk for development of diarrhoea (Hampson & Kidder, 1986).

There are also age related changes in the intestinal physiology. The length of the small intestine increases more than the length of the large intestine during the first 35 days after birth. Thereafter, the conditions are reversed, resulting in a more rapid growth of the large intestine (McCance, 1974). This switch in intestinal growth rate coincides with the weaning period, and it is possible that the dietary change from the highly digestible sow milk to the cereal based feed is responsible for these alterations (Bach Knudsen & Jørgensen, 2001).

Pig feeds

The majority of feed ingredients used in diets for pigs are of plant origin, and carbohydrates are the major constituents of plant tissues. Thus, carbohydrates constitute the quantitatively most important energy source for pigs (Church & Pond, 1982). Most pig diets are based on cereals, since their energy and nutrient content profile makes them well suited to create the concentrated diets necessary to sustain a high production level. Sometimes by-products from the human food industry are used. Soybean meal is the most common protein source (Seerley, 1991). Fishmeal can also be used, but the content of unsaturated fatty acids restricts the inclusion in diets for commercial production of slaughter pigs. Recently, also problems concerning transmissible spongiform encephalitis (TSE) have reduced the possibilities to use fishmeal, since it is impossible to distinguish bone remnants from mammals and fish. Synthetic amino acids are available to improve the amino acid content of the feed. Minerals and vitamins are commonly added to the feed via a premix.

Carbohydrates

The general formula for carbohydrates is $(\text{CH}_2\text{O})_n$, and they can be divided into two main groups, sugars and non-sugars. Sugars contain monosaccharides, disaccharides and oligosaccharides, while non-sugars consist of polysaccharides with more than ten monosaccharide units (McDonald *et al.*, 1995a).

Sugars

Monosaccharides are the simplest sugars. They can be further divided into subgroups depending on the number of carbon atoms present in the molecule (trioses, tetroses, pentoses, hexoses and heptoses). There are two isomeric forms

of the monosaccharide molecule, D and L. The D-isomer is most abundant in nature. Further, the monosaccharides can have different shapes, which determine how dense the molecules can be packed in polysaccharides (McDonald *et al.*, 1995a). Arabinose, xylose, glucose, fructose and galactose are examples of monosaccharides (Church & Pond, 1982).

Disaccharides consist of two monosaccharide molecules linked together by the release of one water molecule. Lactose from sow's' milk, sucrose from plant products and maltose are examples of disaccharides (Church & Pond, 1982).

Oligosaccharides consist of two to ten monosaccharides linked together by the release of one water molecule at each linkage (McDonald *et al.*, 1995a). Oligosaccharides may contain similar or different monosaccharides, different linkage structures and be linear or branched. Most of them are soluble in water and physiological fluids (Mul & Perry, 1994). Raffinose, kestose and stachyose are examples of oligosaccharides (McDonald *et al.*, 1995a).

Non-sugars

Polysaccharides are polymers with more than ten monosaccharides. Homopolysaccharides contain the same monosaccharide, as in starch, β -glucans and cellulose, whereas heteropolysaccharides consist of different monosaccharides, as in pectin and hemicellulose (McDonald *et al.*, 1995a).

Homopolysaccharides

Starch is chemically composed of two macromolecules, amylose and amylopectin. Amylose is a long, linear glucose polymer consisting of 1,4-linked- α -D-anhydroglucose units in a helical conformation. Amylose has one reducing and one non-reducing end-chain group (Gallant *et al.*, 1992). Amylopectin is a branched glucose polymer where a chain of α -D-(1-4)-glucopyranose units is linked to glucose molecules by an α -D-(1-6)-glycosidic linkage. This clustered macromolecule has one reducing, but several thousands to millions of non-reducing, end-chain groups (Gallant *et al.*, 1992). There are three different crystalline organisations in starch, resulting in three varying dimensional structures. The A pattern is found in cereal starches, the B pattern is found in potato, tubers and some amylose-rich starch granules, and the C pattern is found in beans, roots and legumes (Gallant *et al.*, 1992; Wiseman, Pickard & Zarkadas, 2001). The crystals forming the A pattern are tightly packed, and contain less water than B crystals (Wiseman, Pickard & Zarkadas, 2001). Conversion of A starch into B starch is only possible after a total recrystallisation of the structure, whereas B starch can be irreversibly altered to A starch at high temperature and low humidity. Some consider the C starch to have a crystalline organisation of its own like the A and B starch, whereas others believe that the C starch is a mixed organisation of granules with A and B starch, or that each granule contains both A and B starch (Gallant *et al.*, 1992).

Resistant starch (RS) refers to starch that is resistant to digestion by pancreatic amylases and reaches the large intestine undegraded (Conway, 1994). It is generally considered as a source of dietary fibre, having three major categories

RS1, RS2 and RS3. RS1 is a physically trapped starch, found in whole or partly ground grains, cereals, seeds and legumes, that prevents or delays access of digestive enzymes to the starch. RS2 refers to RS granules or ungelatinised starch granules that are highly resistant to digestion by α -amylase. RS3 consists of retrograded starch polymers (mainly amylose) which are produced when starch is cooled after gelatinisation or heating (Muir *et al.*, 1993). Through cooking, the starch polymers can be converted from a crystalline structure to a gel structure. The gelatinisation makes the starch more sensitive to enzymatic breakdown, and thereby the amount of RS reaching the large intestine can be reduced (Englyst & Cummings, 1987).

Mixed-linked (1 \rightarrow 3),(1 \rightarrow 4)- β -D-glucans (β -glucans) are constituents of the endosperm cell wall. They consist of glucopyranosyl units. The (1 \rightarrow 3)-linkages cause irregularities that make the molecule more soluble than cellulose (Theander, Westerlund & Åman, 1993).

Cellulose is a linear, unbranched polymer consisting of β -D-(1 \rightarrow 4)-linked glucopyranosyl units (Theander, Westerlund & Åman, 1993), which are rotated 180°, creating a flat ribbon-like structure (Gallant *et al.*, 1992). The cellulose occurs in a crystalline form (Theander *et al.*, 1989), where the molecules attach to each other with hydrogen bonds, and form densely packed microfibrils which in turn adhere to form macrofibrils. This results in molecules that are almost completely resistant to hydration and swelling (Bjergegaard, Sørensen & Sørensen, 1997).

Heteropolysaccharides

Pectins can be defined as those polysaccharides that are solubilised from the cell wall by aqueous solutions of chelating agents such as ethylenediaminetetra-acetate or ammonium oxalate (Selvendran, 1984). They are partially methylesterified rhamnogalacturonans, in which rhamnose units are inserted. Also it occurs attached short side chains of arabinose and galactose (Theander *et al.*, 1989). Pectins are a heterogeneous group of glycans and consist of a large amount of uronic acids and their derivatives. Some of the quantitatively important pectins are arabinans, galactans, arabinogalactans, galactouronans, homogalactouronans and rhamnogalactouronans I and II (Bjergegaard, Sørensen & Sørensen, 1997).

Hemicelluloses also constitute a heterogeneous group of glycans. Their separation from pectins is based on solubility in aqueous systems, but there is no sharp distinction between these groups. Some of the quantitatively important hemicelluloses are xylans, xyloglucans, galactomannans, glucomannans, mannans, galactoglycomannans and arabinogalactan II (Bjergegaard, Sørensen & Sørensen, 1997).

Non-starch polysaccharides

Non-starch polysaccharides (NSP) contain β -glucans, cellulose, pectin and hemicellulose (Souffrant, 2001). NSP consist of both soluble and insoluble fractions (Bach Knudsen, 1997) as defined by the extraction procedure used in the chemical analyses.

Dietary fibre

Dietary fibre (DF) consist of NSP and lignin, which are the principal compounds of cell walls (Bach Knudsen, 2001). The main constituents are cellulose, hemicellulose and lignin. Lignin is not a carbohydrate. It can be described as a group of polymerised aromatic alcohols formed in the cell wall structures from ester-bound phenolic carboxylic acids (Bjergegaard, Sørensen & Sørensen, 1997). Lignin consists of very branched networks tightly linked to the cell wall polysaccharides (Iiyama, Lam & Stone, 1994), and is therefore included in dietary fibre.

Cereal carbohydrate composition

Cereal grains have an endosperm core with a thin wall enclosing the embryo and starch. The endosperm is surrounded by the aleurone layer, consisting of cells with thick cell walls. The outermost protective grain shell is the pericarp, consisting of several cell layers (Bedford, 1995). Arabinoxylans and β -glucans are located in the endosperm, whereas cellulose is included in the pericarp and hull (Bedford, 1993).

The chemical composition of cereals and cereal by-products is variable, as shown in Table 1. A large amount of the cell wall components in barley and oats consist of β -glucans, whereas arabinoxylans are common in wheat, rye and triticale (Bach Knudsen, 1997).

Table 1. Chemical composition (g/kg DM) of cereals and cereal by-products (Bach Knudsen, 1997)

	Barley	Oats	Oat feed meal	Oat hull meal	Rye bran	Wheat	Wheat bran
Starch	587	468	623	213	87	651	222
Total β -glucan	42	28	42	14	45	8	24
Total neutral sugars	138	140	76	272	374	94	287
Arabinose	28	18	10	28	78	29	90
Xylose	56	80	16	212	213	47	148
Glucose	47	33	43	20	66	11	35
Uronic acid	6	10	5	36	10	5	15
Klason lignin	35	66	19	148	68	19	75
Total NSP	186	232	89	505	422	119	374
Total dietary fibre	221	298	108	653	490	138	449

The chemical composition of the cereals is of importance for the physico-chemical properties. The major physico-chemical properties of dietary fibre are the cation exchange capacity, hydration properties, viscosity and compound absorptive properties (Bach Knudsen, 2001).

Feed enzymes

Cell wall components such as β -glucans and arabinoxylans are common in cereals, and they are known to decrease the nutritional value of a diet through an increase of the viscosity in the gastrointestinal tract (Bedford, 1995). Degradation of such cell wall components by supplementation with specific feed enzymes may enhance the nutritional value of the diet for the pig (Partridge, 2001). Enzyme supplementation of chicken diets improves nutrient utilisation as well as growth performance (Pettersson, Graham & Åman, 1990; Guenter, 1993). However, in contrast to the data from chickens, enzyme supplementation of pig diets shows variable results. Probably these differences in the effect of feed enzymes between chickens and pigs depend on the species-related differences in anatomy, digestive capacity and bacterial activity (Dierick & Decuypere, 1994).

Also other factors influence the efficiency of enzyme supplementation. The most beneficial effect of feed enzymes is likely to be found in diets for newly weaned piglets (Thomke & Elwinger, 1998), since the pancreatic enzyme production may be limiting (Lindemann *et al.*, 1986). Thus, the age of the target pigs is of importance. The feed ingredients included determine the chemical composition of the diet (Bach Knudsen, 1997), and thereby which feed enzymes that are likely to be effective. There are several enzymes available for degrading cell wall components, for instance pentosanases, β -glucanases, α -galactosidases (Dierick & Decuypere, 1994), cellulases and xylanases (Bhat & Hazlewood, 2001), designed to target different substrates. There are also enzymes for the degradation of non-cellulosic dietary constituents, for instance α -amylase, oligosaccharidases and phytase (Campbell & Bedford, 1992). Also the quality of the enzymes, such as efficiency, tolerance to digestive processes and feed processing is of importance, as well as the application of specific enzymes or enzyme cocktails (Chesson, 1993).

Digestion

Digestive physiology in the pig

Digestion and absorption are the primary functions of the gastrointestinal tract. The digestive tract of the pig consists of mouth, pharynx, oesophagus, stomach, small intestine, caecum, colon and rectum (Argenzio, 1993a).

Mouth

In the mouth, chewing reduces the size of the feed particles and the feed is mixed with saliva (Argenzio, 1993b). Saliva consists of up to 99% of water and the remaining 1% contains mucin, inorganic salts and the enzymes α -amylase and lysozyme (McDonald *et al.*, 1995b).

Stomach

The pig stomach serves as a place for both storage and digestion. The epithelium in the stomach is divided into four areas with different types of mucosa; the oesophageal area, the cardiac area, the gastric gland region and the pyloric region. The oesophageal area is an enlargement of the oesophagus into the stomach,

which is free from secretory glands (McDonald *et al.*, 1995b). In this area the salivary α -amylase is still active and there is a permanent microbial population of *Lactobacilli* and *Streptococci* (Jensen, 2001). In the cardiac area an alkaline, enzyme free, viscous mucus is secreted that protects the epithelia from acid attack. The gastric gland region secretes mucus and contains the hydrochloric acid and pepsinogen producing cells. The pyloric region contains glands that produce a protective mucous layer (McDonald *et al.*, 1995b). Also some pepsinogens are secreted from this region (Argenzio, 1993c).

Small intestine

The small intestine is divided into duodenum, jejunum and ileum, and the digesta is mixed with secretions from the duodenum, liver and pancreas. Alkaline secretions from Brunners glands are mixed with the digesta in the duodenum, and protect the intestinal wall from hydrochloric acid released from the stomach. This also creates an optimal pH for the released pancreatic enzymes to digest carbohydrates into different saccharides, fat into fatty acids and monoglycerides and protein into oligopeptides and amino acids. The liver secretes bile from the gall bladder into the duodenum. In the pig, there is a relatively continuous secretion of bile. It consists of water, electrolytes and organic compounds such as cholesterol and bile salts, and provides bile acids and neutralising buffer (Argenzio, 1993c).

The absorption of amino acids, saccharides, lipids, vitamins and minerals mainly takes place in the jejunum. The small intestine is supplied with villi, finger-like extensions that increase the absorption capacity. The passage rate in the jejunum is higher than in the ileum, where a greater number of segmental contractions delay the transit. Also the ileo-caecal junction decreases the passage rate. The bile salts are absorbed by active transport in the ileum and about 90% are recirculated (Argenzio, 1993c).

Large intestine

The large intestine has an important role in the absorption of nutrients, electrolytes and water from the digesta. Pigs have a short caecum and long colon compared with other monogastric omnivores. Sacculated areas and antiperistaltic movements decrease the passage rate. This is important in order to give sufficient time for absorption as well as microbial fermentation (Argenzio, 1993b). Water and small particles are retained in the proximal colon, while larger particles move on with a higher passage rate (McDonald *et al.*, 1995b).

Faeces excreted from the large intestine via the rectum consists of water, undigested feed residues, digestive secretions, epithelial cells from the gastrointestinal tract, inorganic salts, bacteria and products of microbial decomposition (McDonald *et al.*, 1995b).

Digestion of carbohydrates

Carbohydrates are digested both in the small and large intestine of pigs. The chemical composition determines whether the degradation is performed by endogenous enzymes or by microbes (Bach Knudsen & Jørgensen, 2001).

Mouth and stomach

Salivary α -amylase initiates the enzymatic starch digestion in the stomach only to a limited extent, since the α -amylase activity in saliva is low and becomes inactivated by the acidic environment in the stomach (Bach Knudsen & Jørgensen, 2001). Also a limited microbial fermentation of sugars and starches takes place in the stomach. This degradation results in the formation of short chain fatty acids (SCFA) and lactic acid, which are partly absorbed by the stomach wall. Both the enzymatic starch digestion and the fermentation of sugars and starches are located in the oesophageal area of the stomach. Substrates of fermentation in the stomach of suckling piglets or young early-weaned pigs are monosaccharides, disaccharides and degradation products of starch (Drochner, 1993).

Small intestine

The main site for starch digestion is the small intestine, where the digestion in the luminal phase is performed through hydrolysis of α -1,4-linkages by pancreatic α -amylase. This results in 1,4-linked di- and trisaccharides such as maltose and maltotriose, and branched oligosaccharides with α -1,6- and α -1,4-linkages, so-called α -dextrins. In the mucosal phase, specific saccharidases located in the brush border of the epithelial cells, degrade the products from digestion in the luminal phase further into monosaccharides (Argenzio, 1993d). According to (Gabert *et al.*, 1995), arabinose, galactose, glucose and xylose are the most abundant monosaccharides in ileal digesta. The breakdown products absorbed are glucose, galactose and fructose. Glucose and galactose can be transported into the epithelial cell by active transport, whereas fructose enters the cell more slowly by help of a carrier mechanism (Argenzio, 1993d). The enzyme activities in the small intestine are specific for α -linked units and are inactive against the β -linked glucose polymers in dietary fibre (Chesson, 1987). However, there are fibre-degrading bacteria in the proximal gastro-intestinal tract, capable of disrupting these cell walls prior to the ileum. This disruption releases nutrients encapsulated within the cell walls (Graham *et al.*, 1986), which thereby become available for digestion within the small intestine.

At the end of the small intestine, the cereal starch digestibility ranges from 83.7 to 100% (Bach Knudsen & Jørgensen, 2001). The digestibility of B and C starch is lower compared with cereal starch (Bach Knudsen & Jørgensen, 2001), as for instance in peas (Gdala *et al.*, 1997) and potato (Just, Jørgensen & Fernández, 1985). Also coarse particles reduce the starch digestibility in the small intestine (Brunsgaard, 1998). The amount of NSP digested in the small intestine varies from -10 to 62%, β -glucans from 17 to 97%, arabinoxylans from -10 to 19% and cellulose from 21 to 50% (Bach Knudsen & Jørgensen, 2001).

Large intestine

Digestion in the large intestine is either performed by the help of enzymes that have followed the digesta from the small intestine, or through microbial fermentation. Small amounts of monosaccharides and disaccharides pass the ileo-caecal valve and are fermented completely by microbes. Also some starches, for instance from raw potatoes, pass only partially digested into the large intestine, where they serve as a substrate for microbial fermentation (Drochner, 1993). Also resistant starch is utilised as a substrate for fermentation by the enteric microflora of the large intestine (Conway, 1994).

The main sites for NSP degradation in the large intestine of both piglets and older pigs are the caecum and proximal colon (Gdala *et al.*, 1997). NSP are degraded to varying degree depending on the botanical origin of the fibre material (Graham, Hesselman & Åman, 1986). The physiological action depends to a large extent on the physical and chemical properties of the individual NSP, and these can vary quite considerably between different polymers or different molecular weights of the same polymer. Some polysaccharides are immediately fermented and removed from the lumen in the colon, but others may be chemically modified by the bacteria, and thereby their physical properties are changed (Read & Eastwood, 1992).

The amount of cereal cellulose digested in the total tract varies from 2 to 84% depending on the source of NSP. Hemicellulose is generally better digested than cellulose, whereas lignified material decreases the digestibility (Bach Knudsen & Jørgensen, 2001). Also pectic substances are digested to a larger extent than cellulose (Bach Knudsen & Jørgensen, 2001), and the digestibility of isolated pectins in the large intestine of pigs is in the range of 80 to 90% (Drochner, 1993). β -glucans are completely digested in the total tract (Bach Knudsen, Jensen & Hansen, 1993a-b).

The amount of cereal NSP digested in the large intestine varies from 48 to 95% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Bach Knudsen, Jensen & Hansen, 1993a-b; Longland, Carruthers & Low, 1994; Jørgensen, Zhao & Eggum, 1996; Gdala *et al.*, 1997). Inclusion of sugar beet pulp gives a digestibility of NSP from 69 to 92% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Longland, Carruthers & Low, 1994; Noblet & Bach Knudsen, 1997; Wang *et al.*, 2002), whereas inclusion of wheat bran decreases the NSP digestibility to 30 to 59% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Mathew *et al.*, 1996; Wang *et al.*, 2002).

The digestibility of cereal arabinose varies from 49 to 93%, whereas xylose digestibility varies from 24 to 94% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Bach Knudsen, Jensen & Hansen, 1993a-b; Longland, Carruthers & Low, 1994; Jørgensen *et al.*, 1996; Gdala *et al.*, 1997). Inclusion of sugar beet pulp gives a digestibility of arabinose from 84 to 97%, and xylose from 17 to 64% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Noblet & Bach Knudsen, 1997; Wang *et al.*, 2002), whereas inclusion of wheat bran gives a digestibility of arabinose from 31 to 53%, and

xylose from 33 to 70% (Graham, Hesselman & Åman, 1986; Chabeauti, Noblet & Carré, 1991; Noblet & Bach Knudsen, 1997; Wang *et al.*, 2002).

The main end products of the microbial fermentation are lactic acid, SCFA and various gases (hydrogen, carbon dioxide, methane) (Bach Knudsen *et al.*, 1991). The SCFA are absorbed in the large intestine and contribute to the energy supply of the pig (Fonty & Gouet, 1989).

Digestion in relation to dietary fibre content

Dietary fibres are defined as feed constituents resistant to degradation catalysed by enzymes, especially hydrolases produced in mammalian cells (van Soest, 1988; Bjerregaard, Sørensen & Sørensen, 1997).

Inclusion of dietary fibre in the diet decreases the digestibility of dry matter (DM), organic matter (OM) and energy in the distal small intestine and in the total tract (Graham, Hesselman & Åman, 1986; Bach Knudsen & Hansen, 1991; Bach Knudsen, Jensen & Hansen, 1993b; Jørgensen, Zhao & Eggum, 1996; Andersson & Lindberg, 1997). The effect of dietary fibre on protein digestibility is more variable, and depends on the type of fibre included. Dierick *et al.* (1983) reported a lower decrease in the ileal digestibility of protein and amino acids with cellulose compared with pectin and sugar beet pulp. Further, the cell walls may enclose intracellular protein and fat, and thereby decrease the digestibility of these nutrients (Bach Knudsen, Jensen & Hansen, 1993a).

The energy provided from organic acids increases with dietary fibre content. This leads to the pig suffering an energy loss, since the relative value of energy derived from the hindgut fermentation is only 0.73 of the energy yield from enzymatic digestion in the small intestine (Jørgensen, Zhao & Eggum, 1996).

Age-related differences in carbohydrate digestion

There are some age-related differences in the digestion between piglets and older pigs. Apart from the size differences of the gastro-intestinal tract, also the carbohydrase activity differs. Lactase activity is present at high levels in the mucosa of the newly born piglet, and reaches its maximum activity level when the piglet is one week old (Aumaitre & Corring, 1978). Thereafter, the lactase activity decreases with age (Manners & Stevens, 1972; Kidder & Manners, 1980). The pancreatic amylase activity is low at birth. When the piglet is three weeks old, the amylase activity is slightly higher than in the newly born piglet, and increases more rapidly from this age until the piglet is six weeks old (Corring, Aumaitre & Durand, 1978). Also the pancreatic juice secretions follow the same pattern and increase in connection with weaning (Pierzynowski *et al.*, 1988). Sucrase activity is not present until one week after birth (Aumaitre & Corring, 1978), and increases until maturity (Manners & Stevens, 1972). Maltase activity is low at birth, but increases until eight weeks of age (Aumaitre & Corring, 1978). Isomaltase activity increases with age, whereas the activity of maltase 2 and 3 and trehalase increase until the pig is 200 days old (Kidder & Manners, 1980).

Microbes in the gastrointestinal tract

The gastrointestinal tract of pigs contains a large and diverse microbial population. The bacteria are mainly obligate anaerobes and their numbers generally exceed 10 billion per gram of wet weight of colon contents. They may comprise as much as one-third of the contents in the large intestine (Allison, 1989).

Both external environmental conditions such as unfavourable temperature and humidity, and internal environmental conditions in the intestine, such as for example nutrient availability, pH, redox potential and microbial interactions, influence the structure and function of the microflora. Alterations in these conditions can manipulate the type of microbes that will establish in the gastrointestinal tract. If the resident populations are disturbed, transient microbes may establish in the digestive tract, and these transient microbes may include pathogens. Weaning serves as a good example of this phenomenon (Conway, 1994).

Piglets

The similarity between the microflora of sows and their offspring on day three after farrowing is high (Katouli *et al.*, 1997b). This suggests that sows are the initial source of the piglets' microflora, presumably via microbial populations from the vagina, milk, skin and faeces of the sow (Melin, 2001). When the piglets are seven days old, the similarity is lost and the piglets develop a new type of flora. The microflora is still similar among piglets within litter, but it is clearly different from that of the sow (Katouli *et al.*, 1995, 1997b). In connection with dietary changes, the fermentation capacity of the microflora decreases. Also relocation of pigs between pen units influences the composition of the intestinal microflora. Generally the metabolic capacity of the intestinal flora in pigs decreases as the animals grow older (Katouli *et al.*, 1997b). Bacteria colonising the gastro-intestinal tract of the newly born piglet include *Lactobacilli*, *E. coli*, *Streptococci* and *Clostridia* (Melin, 2001).

The weaning process represents a number of environmental and physiological alterations, experienced at a time when the immune system is not fully developed. In connection with weaning (Katouli *et al.*, 1995, 1999) and diarrhoea (Kühn *et al.*, 1985), a decreased diversity in the intestinal flora occurs when one or a few bacteria strains dominate the intestine. Therefore, piglets are more sensitive to colonisation by enteropathogenic *E. coli* at this time. However, weaning diarrhoea is a multifactorial disease, and has been shown to be *E. coli* associated rather than caused by *E. coli* (Melin *et al.*, 2000).

Adult pigs

In the healthy adult pig, the microflora is relatively stable under constant conditions. The gastrointestinal tract is densely colonised by a diverse population of aerobic, facultative anaerobic and strictly anaerobic species (Conway, 1994). Bacteria remain in the gut either by attachment to the intestinal epithelial cells, or by simply growing at a faster rate than the peristaltic movements are removing them (Ewing & Cole, 1994). The gastrointestinal microflora contributes to the

resistance to colonisation by pathogens through so-called competitive exclusion (Asplund *et al.*, 1996), and also contribute to the energy supply of the pig (Kass, van Soest & Pond, 1980) through fermentation of ingested nutrients otherwise insensitive to intestinal enzymatic digestion. There is a delicate balance between host factors, diet and microflora, and a disturbance of one factor influences all other factors in the ecosystem (Conway, 1994). As a consequence, a dietary change can cause alterations in the composition or function of the microbes in the digestive tract.

Mouth

The bacteria in the mouth probably originate from the tooth surfaces and the soft tissues as well as from the feed (Ewing & Cole, 1994). Various strains of *Streptococci* are commonly found in the mouth (Gibbons & van Houte, 1971).

Stomach

The acidic conditions in the stomach restrict the number of bacteria at this site, but yeasts are often present (Savage, 1989). The total number of bacteria present in the stomach is 10^7 – 10^9 viable bacteria per gram digesta (Bach Knudsen, Jensen & Hansen, 1993a; Jensen & Jørgensen, 1994). *Lactobacilli* and *Streptococci* constitute permanent microbial populations of the stomach (Jensen, 2001), and associate with the stratified squamous epithelium in the pars oesophagus (Tannock, 1990; Jensen, 1998). Other bacteria commonly found in the stomach are *E. coli*, *Clostridia*, *Eubacterium*, *Bifidobacterium*, *Staphylococcus*, *Actinomyces* and *Klebsiella* (Conway, 1994; Melin, 2001).

Small intestine

The total number of culturable bacteria present in the small intestine is 10^7 viable bacteria per gram digesta in the proximal small intestine, and increases to 10^8 – 10^9 viable bacteria per gram digesta in the distal small intestine (Bach Knudsen, Jensen & Hansen, 1993a; Jensen & Jørgensen, 1994). The intestinal environment is altered by secretions of bicarbonate buffer, bile and digestive enzymes. This, in combination with the high passage rate, especially in the proximal small intestine, affects the conditions for microbial proliferation at this site. The densities of bacteria increase towards the distal small intestine, presumably as an effect of slower passage rate and a greater amount of digesta (Zoric *et al.*, 2002). *Lactobacilli*, *Streptococci*, *Clostridia* and *Enterobacteria* are the most common species in the small intestine, but *E. coli* and *Bacteroides* are also present (Conway, 1994; Jensen, 2001).

Large intestine

The large intestine constitutes the major site of microbial proliferation. Here the passage rate is slowed down by sacculations and anti-peristaltic movements, creating an environment with beneficial pH, humidity and temperature for bacterial growth (Fonty & Gouet, 1989). The total number of bacteria present in the large intestine is 10^{10} – 10^{11} viable bacteria per gram digesta, belonging to more than 500 different species (Jensen, 2001). Bacteria such as *Bacteroides*, *Clostridia*, *Prevotella*, *Streptococci*, *Lactobacillus*, *Sellenomona*, *Mitsoukella*,

Megasphaera, *Acidaminococci*, *Fusobacteria* and *Eubacteria* dominate the microflora at this site (Conway, 1994; Jensen, 2001).

Interactions with dietary carbohydrates

More than 80% of the fermented substrates derives from carbohydrates, and NSP are the main energy source for microbial fermentation in the large intestine (Bach Knudsen & Jensen, 1991; Bach Knudsen *et al.*, 1991). Due to the absence of plant cell-wall degrading enzymes in the pig, and the low density of micro-organisms in the anterior part of the small intestine, the dietary fibre is more or less intact on arrival to the hindgut. Bacteria are considered to be the major plant cell wall degrading agents, and the dietary fibre is degraded to a variable extent in the caecum and colon by a diversified microbial population (Fonty & Gouet, 1989).

Microbial growth is promoted by increased amounts of substrate. In a study where a low fibre and a high fibre diet were compared, digesta from the stomachs of the pigs receiving the high fibre diet contained significantly larger amounts of culturable bacteria than did digesta from the stomachs of the pigs receiving the low fibre diet (Jensen & Jørgensen, 1994). Further, there is a correlation between the microbial activity and the amount of digested carbohydrates in the large intestine (Bach Knudsen *et al.*, 1991). This implies that the diet composition, and especially the amount of dietary fibre in the diet, determines how much substrate reaches the large intestine, thereby influencing the microbial fermentation as well as the microbial activity at this site (Bach Knudsen & Jensen, 1991). Through modifying the diet the composition of the microflora can be altered and although bacteria numbers appear unchanged, the dominant strains or species of bacteria may vary (Conway, 1994).

Techniques for digesta sampling

Different techniques have been developed and used to obtain samples of gut content. One common but drastic method is the slaughter technique, where the animal is sacrificed after being fed the experimental diet for a certain time. This technique allows sampling along the entire gastro-intestinal tract and the possibility to analyse a number of response parameters (Moughan, 2003). However, apart from animal welfare aspects, sampling can only be done once per animal and there may not be a sufficient amount of digesta present in the gastro-intestinal tract at the time of slaughter. Also, this technique can only give momentary information about events occurring at the time of slaughter.

Another method to collect gastro-intestinal samples is to insert a cannula or a catheter into the intestinal section of interest. This technique allows continuous sampling of an animal during a certain time interval and also sampling on several occasions. However, the surgical insertion itself may influence the metabolic processes in the animal and change the conditions in the gastro-intestinal tract (Jacobson *et al.*, 2001). The most common cannulas are the simple T-cannula and the post valve T-caecum (PVTC) cannula. The T-cannula is often inserted in the distal part of the small intestine and the PVTC-cannula is inserted in the caecum, in close proximity to the ileo-caecal valve. When inserting a T-cannula, no part of the intestine is removed (Fuller, 1991). However, some problems concerning the

homogeneity of the digesta samples collected have been reported, especially when feeding high-fibre diets (Yin *et al.*, 2000b). When applying the PVTC-cannula, a part of the caecum is removed (van Leeuwen *et al.*, 1991). This might alter the intestinal conditions, but it has been demonstrated that this technique has no influence on digestibility (Lindberg, 1997; Jansman *et al.*, 2001) as well as animal health and gut function (Jacobson *et al.*, 2001). This cannula has a larger diameter compared with the T-cannula and it is known to function well when sampling all kinds of diets.

Analytical methods for determination of dietary fibre

Over the years, more sophisticated methods for determining dietary fibre have been developed. These methods quantify different parts of dietary fibre.

The crude fibre analysis is the oldest method. Dilute acid and alkali are used for the extraction, followed by drying and gravimetric determination. This method gives information on the cellulose and lignin content. However, this method only measures a small and variable fraction of the fibre components, due to the solubilisation of the structural polysaccharides and lignin (Bach Knudsen, 2001). The crude fibre analysis is a part of the Weende analysis, where the feed is analysed with respect to contents of crude protein, crude fibre, crude fat, ash and nitrogen free extractives (NFE) (Carrier, Cottyn & Aerts, 1976).

The detergent methods measure the fraction of the fibre that is insoluble in two different detergents (van Soest & Wine, 1967; van Soest, Robertson & Lewis, 1991). Neutral detergent fibre (NDF) measures the content of cellulose, lignin and hemicellulose after using neutral detergents, while acid detergent fibre (ADF) measures the content of cellulose and lignin after using acid detergents. Thereby the content of hemicellulose can be determined by calculation. According to Bach Knudsen (2001), there are indications about loss of water-soluble NSP and water-insoluble pectic substances in the NDF procedure, contamination of the NDF residue by starch and protein, and remaining hemicellulose in the ADF fraction, when applying this method for dietary fibre analysis.

The Association of Official Analytical Chemists (AOAC) procedures consist of enzymatic-gravimetric methods that measure dietary fibre after extraction of low-molecular weight sugars and lipids followed by enzymatic degradation of protein and starch. Finally the residue is weighed and corrected for ash and protein (Prosky *et al.*, 1988; Lee, Prosky & De Vries, 1992).

Enzymatic-chemical procedures, such as the Englyst (Englyst, Quigley & Hudson, 1994) and Uppsala (Theander *et al.*, 1994) procedures, measure the dietary fibre constituents after extraction of low-molecular weight sugars, enzymatic removal of starch, acid hydrolysis of DF polysaccharides and determination of their monosaccharide residues by gas-liquid chromatography, high-performance liquid chromatography or colorimetry and estimation of uronic acids by colorimetry. These methods quantify the content of non-starch polysaccharides, cellulose, pectin, β -glucans, pentosans and xylans, as well as insoluble and soluble fractions.

Microbiological methods

Traditionally, culturing of bacteria has been the method applied to assess the microbial populations. The culturing can be both aerobic and anaerobic, depending on the bacteria of interest, and is followed by enumeration and isolation. Further, the culturing media used can be either non-selective or selective, by designing the media according to general or specific requirements for the bacteria of interest. Culturing is the method applied to give a complete phenotypic characterisation of bacteria. However, this method only gives information about the culturable bacteria, leaving the other microbial populations undiscovered (Amann, Ludwig & Shleiffer, 1995).

Biochemical fingerprinting is a computerised method for typing of bacteria. It is based on the quantitative measurements of the biochemical reactions of bacteria as a means of measuring the bacterial activity. Pre-prepared 96 well microtitre plates are used. They contain dehydrated reagents, e.g. specific carbohydrates, selected to be highly discriminative within the group of bacteria studied (Möllby, Kühn & Katouli, 1993). The system is based on interval measurements of the colour changes generated by an indicator due to bacterial utilisation of different sole carbon sources, and production or consumption of acids. This method yields fermentation patterns that differ between bacterial populations, and thereby changes in the bacterial composition can be monitored. Also, the method provides information about the ability of a microbial population to ferment different carbohydrates, the fermentation capacity (Katouli *et al.*, 1997a).

16S rRNA gene sequencing is used to classify and determine the evolutionary relationship between bacteria. The 16S rRNA gene is ubiquitous among bacteria and contains a combination of conserved and variable regions, allowing sequence alignment and detection of evolutionary relationships (Woese, 1987). A continuously expanding number of sequences are becoming accessible through web-based database compilations (Maidak *et al.*, 1996), for instance the Ribosomal Database Project II (<http://rdp.cme.msu.edu/html/>; 14-Aug-2003).

Terminal Restriction Fraction Length Polymorphism (T-RFLP) analysis is suitable to apply for comparing the composition of bacterial communities (Clement *et al.*, 1998). The gene commonly used for this purpose is 16S rRNA. These genes are fluorescently end-labelled and amplified by PCR (polymerase chain reaction), followed by digestion with restriction enzymes, endonucleases. Gel electrophoresis is used to separate the digested gene fragments. The fragments are then detected on an automated sequence analyser, and specific profiles are provided depending on the species composition in the sample (Leser *et al.*, 2000).

16S rRNA hybridisation is used to identify and quantify bacteria. This single cell hybridisation uses fluorescently labelled oligonucleotide probes to target the 16S rRNA in the protein matrix of the small ribosomal sub-unit (Amann, Ludwig & Shleiffer, 1995). The method is generally referred to as Fluorescence In Situ Hybridisation (FISH). Since rRNA is present in all bacteria and the databases on the 16S rRNA genome continue to progress (Maidak *et al.*, 1996), it is possible to increase the number of probes available for this methodology (Harmsen *et al.*, 2002). Inside each cell of an active bacterium, there can be several thousand 16S

rRNA molecules. An equal number of probes can be attached to the 16S rRNA and the intensity of the signal can be correlated to the activity level of the bacterium (Binnerup *et al.*, 2001).

Aims of the thesis

The aims of the present thesis were to study the influence of

- ❖ PVTC-cannulation and oxytetracycline on
 - intestinal coliform populations in growing pigs.
- ❖ total dietary NSP content and NSP solubility on
 - digestibility of nutrient and fibre components,
 - digestion site and
 - gut environmentin piglets around weaning and in growing pigs.
- ❖ total dietary NSP content and NSP solubility on
 - intestinal microbial populations in growing pigs.
- ❖ enzyme supplementation in diets with different NSP content on
 - digestibility of nutrient and fibre components,
 - digestion site and
 - gut environmentin piglets around weaning.

Materials and methods

Animals and housing

All animals came from conventional Swedish herds free from diseases according to the A-list of the International Office of Epizootics (www.oie.int), and from Aujeszky's disease, atrophic rhinitis, *Brachyspira* spp transmissible gastroenteritis, porcine epidemic diarrhoea, porcine reproductive and respiratory syndrome and salmonellosis.

Pure bred Swedish Yorkshire castrates, weaned at the age of five weeks and purchased at the age of 10-11 weeks, were studied in papers **I**, **II** and **III**. Cross-bred Yorkshire x Landrace castrates, purchased before weaning at 27-28 days of age (live weight 8.9 ± 0.6 kg) were studied in **IV**. These animals were housed individually (**I**, **II**, **III**) or pair-wise (**IV**) at the experimental unit at the Department of Animal Nutrition and Management, Uppsala, Sweden.

In **V**, pure-bred Swedish Yorkshire litters from the research herd at Funbo-Lövsta Research Station were studied. A total of 20 entire litters were followed

from the age of three weeks until they were nine weeks old. The piglets were weaned at the age of five weeks by removal of the sow from the pen.

The care and use of the animals included in these studies (**I, II, III, IV, V**) was examined and approved by the Board of Experimental Animal Ethics.

Diets

All diets were based on cereals and cereal by-products milled through a 3 mm mesh screen (**II, III, IV, V**). They were formulated to contain different levels of NSP. TiO₂ was used in all diets as a digesta flow marker at a rate of 2.5 g/kg feed. In both piglet trials (**IV, V**), 3 mm size pellets were available *ad lib*. The growing pigs were fed 5 mm sized pellets at 4% of the group mean live weight until they reached 70 kg. Thereafter they were given 2.8 kg feed per day (**II, III**).

The cereal part of the diets varied between 435-965 g/kg feed, and also the content of NSP differed between the diets (**II, III, IV, V**). The control diets included 139-147 g NSP/kg DM of which 0.687-0.727 was insoluble. Diets with high content of NSP included 160-203 g NSP/kg DM (**II, III, IV, V**). These diets were divided into diets with a normal amount of insoluble NSP (H; 0.705-0.706) and into diets with a large amount of insoluble NSP (Hi; 0.847-0.879) included (**II, III, V**). The diets with low content of NSP included 95-109 g NSP/kg DM (**II, III, IV, V**). These diets were also divided into diets with a normal amount of insoluble NSP (L; 0.674-0.726) and diets with a large amount of insoluble NSP (Li; 0.798-0.804) included (**II, III, V**).

Experimental designs and sample collections

The growing pigs studied in **I, II** and **III** were surgically fitted with a PVTC cannula according to van Leeuwen *et al.* (1991). In **I**, microbial samples were collected with a cotton swab through the cannula and in the rectum during the surgery and on day 3, 7, 14 and 20 post surgery. In **II** and **III**, a 5 x 5 Latin square design was applied, including five pigs, five diets and five periods, each lasting for 17 days. Ileo-caecal digesta and faeces were collected on day 15 and 17 in each period (**II**). Ileo-caecal digesta as well as ileo-caecal and rectal samples collected with cotton swabs were collected at the start of the experiment and on days 9 and 17 in each period (**III**).

A split-litter design was applied in **IV**. There were 32 Y x L castrates originating from eight different litters included in the study. The piglets were 27-28 days old and the mean body weight (BW) was 8.9 ± 0.6 kg when initiated in the study. The four litter mates were distributed onto four different diets. The BW of the piglets was recorded on arrival and on day 3, 7, 10 and 14. Feed consumption was noted every day. On day 14, the piglets were sacrificed and samples were collected from different parts of the gastro-intestinal tract. At termination the piglets were aged 41 or 42 days and the mean BW was 11.6 ± 1.3 kg.

In **V**, a herd study was carried out, comprising 20 pure bred Yorkshire litters containing at least eight piglets. The litters were distributed onto five different diets. The piglets were followed from the age of 3 weeks until they were 9 weeks

old. All piglets were weighed individually at 3, 5, 6, 7 and 9 weeks of age, and feed consumption in each litter was noted three times a week. In each litter, one piglet was sacrificed the day before weaning at 5 weeks of age, one at 6 weeks of age and one at 7 weeks of age, and samples were taken from different parts of the gastro-intestinal tract.

Analyses

Chemical analysis

All samples were freeze-dried and milled through a 1-mm mesh screen before chemical analysis (**II**, **IV**, **V**). These analyses included DM, ash, TiO₂ (Short *et al.*, 1996), crude protein (CP) (Nordic Committee on Food Analysis, 1976), crude fat (Official Journal of the European Communities, 1984), starch and sugars (Larsson & Bengtsson, 1983), energy, total (1→3)(1→4)-β-D-glucans and insoluble β-glucans (McCleary & Glennie-Holmes, 1985), total, soluble and insoluble NSP and their constituent sugars, Klason lignin and total DF (Bach Knudsen, 1997), organic acids (OA) (Andersson & Hedlund, 1983), viscosity and pH.

Terminal restriction fraction length polymorphism

In **III**, T-RFLP was used to define base pair length of microbial DNA in ileo-caecal samples (Leser *et al.*, 2000). DNA fragments were analysed by electrophoresis on an automatic sequence analyser (ABI PRISM 373 DNA Sequencer, PE Biosystems, Foster City, Calif.). The lengths of the terminal restriction fragments (TRFs) were determined by comparison with the internal size standard using GeneScan software (PE Biosystems).

Biochemical fingerprinting

Biochemical fingerprinting was used to define the diversity of the intestinal coliform flora at the ileo-caecal ostium and in rectum (**I**, **III**). This method measures the kinetics of bacterial growth in eleven different liquid media, chosen to differentiate between coliforms (Kühn *et al.*, 1993). The individual metabolic response of 24 isolates from each sample was measured. Simpson's index of diversity (Hunter & Gaston, 1988) was used to measure the phenotypic diversity of the coliforms. When only one biochemical phenotype (BPT) is present, the diversity is considered to be low, with a minimum value of 0. In a population containing different BPTs, the diversity is considered to be high, with a maximum value of 1.

Results

Cereal chemical composition

The chemical composition of the cereals and cereal by-products included in the experimental diets is shown in Table 2. The content of nutrient and fibre

components varied among the ingredients as well as the ratio of soluble and insoluble fibre components.

Table 2. Chemical composition (g/kg DM) of cereals and cereal by-products included in the experimental diets presented as means from two* (papers **II**, **III**, **V**) or three (papers **II**, **III**, **IV**, **V**) batches

	Barley	Oats	Oat meal*	Oat bran*	Rye bran*	Triticale	Wheat	Wheat bran
Crude protein	142	149	134	186	174	138	130	162
Fat	30	47	79	120	33	23	23	46
Starch ¹	572	404	659	467	178	650	702	193
Total β -glucan	55	33	40	108	44	8	6	24
Insoluble β -glucan	14	5	12	59	24	3	2	16
Soluble β -glucan	41	28	28	49	20	5	4	8
Total neutral sugars	160	202	85	198	291	147	100	346
Arabinose	26	23	11	25	74	39	26	95
Xylose	56	90	18	48	138	63	44	162
Glucose	69	79	49	115	61	33	21	74
Uronic acid	6	8	5	8	11	6	5	15
Klason lignin	33	59	10	45	70	27	21	85
Total NSP	185	251	93	199	309	170	102	392
Insoluble NSP	140	240	48	91	311	98	93	325
Soluble NSP	45	11	45	107	-2	72	9	67
Total dietary fibre	218	310	103	243	378	198	123	478

¹Sum of starch and maltodextrins.

Feed intake and daily weight gain

The average feed intake between 3-9 weeks was higher ($P<0.05$) in diet Li compared to the other diets in paper **V**. No difference ($P>0.05$) in feed intake was observed between the diets in paper **IV**.

The daily gain during the post-weaning period and over the total experimental period was higher ($P<0.05$) in diet Li than in diet L, H and the control diet (**V**). In paper **IV**, the high NSP content resulted in a higher ($P<0.05$) daily gain. In paper **II**, the daily gain of the pigs was higher ($P<0.05$) when fed the low NSP diets compared with the high NSP diets and the control diet.

Digestibility of nutrient components

The digestibility of OM and CP increased successively through the gastrointestinal tract in piglets and growing pigs (**II**, **IV**, **V**). The digestibility of starch

was almost complete at the ileum and caecum (0.94-1.00) and was unaffected ($P>0.05$) by NSP content (**II**, **IV**, **V**), except during week 7, when diets Li, L and H showed higher ($P<0.05$) starch digestibility compared with the control diet (**V**).

The low NSP content resulted in higher ($P<0.05$) ileal and total tract digestibility of OM, energy (**II** and **IV**) and CP (**II**). In paper **II**, there was a linear decrease ($P<0.05$) in both ileal and total tract digestibility of OM, CP and energy with increasing dietary NSP content.

NSP solubility had no influence ($P>0.05$) on the ileal digestibility of nutrients, but the total tract digestibility of OM, fat and energy was increased ($P<0.05$) with higher levels of soluble NSP (**II**). Also the caecal digestibility of OM was increased ($P<0.05$) with higher levels of soluble NSP in 6- and 7-weeks old piglets, and in the total tract of 6-weeks old piglets fed diets with high NSP content. So was the caecal digestibility of CP in piglets aged 6 or 7 weeks and fed diets with high NSP content (**V**). The digestible energy was increased ($P<0.05$) with higher levels of soluble NSP in the 6-weeks old piglets (**V**). An overview of these results is presented in Table 3. Enzyme supplementation decreased ($P<0.05$) the digestibility of OM in the stomach, but no influence of enzyme supplementation was observed for the other nutrient components (**IV**).

Table 3. Influence of NSP content and solubility on ileal, caecal and total tract digestibility of nutrient components in 6- and 7-weeks old (**V**) piglets and in growing pigs (**II**)

	NSP content ¹			NSP solubility ²		
	W6	W7	Growing	W6	W7	Growing
<i>Ileum/Caecum</i>						
Organic matter	L/H, Li /Hi	L/H, Li /Hi	L/H, Li /Hi	L/Li, H /Hi	L/Li, H /Hi	ns
Crude protein	L /H	L /H	L/H, Li /Hi	L/Li, H /Hi	H /Hi	ns
Starch	ns	ns	ns	ns	ns	ns
<i>Total tract</i>						
Organic matter	Li /Hi	L/H, Li /Hi	L/H, Li /Hi	H /Hi	ns	L/Li, H /Hi
Crude protein	L /H	ns	L/H, Li /Hi	ns	ns	ns
Energy	L/H, Li /Hi	L/H, Li /Hi	L/H, Li /Hi	L/Li, H /Hi	ns	L/Li, H /Hi

¹ Diet L compared with diet H, diet Li compared with diet Hi.

² Diet L compared with diet Li, diet H compared with diet Hi.

ns = not significant.

Significantly increased digestibility in the diets in bold ($P<0.05$).

Digestibility of fibre

The ileal digestibility of β -glucans was higher ($P<0.05$) in diet Hi than in the other diets (**II**), whereas the caecal digestibility of β -glucans was high (0.89-0.99) and unaffected ($P>0.05$) by NSP content (**IV**, **V**).

The ileal digestibility of total neutral sugars, arabinose, xylose, total NSP and DF was higher ($P<0.05$) for the high NSP diets compared with the control diet, but NSP solubility had no influence ($P>0.05$) on ileal digestibility of fibre components (II). In contrast, the caecal digestibility of total neutral sugars, xylose, glucose and total NSP was higher ($P<0.05$) in diet L than in diet Li and in diet H than in diet Hi, respectively, in the 6-weeks old piglets (V). Also during week 7, the caecal digestibility of total neutral sugars, arabinose, xylose, glucose, total NSP and total dietary fibre was higher ($P<0.05$) in diet L than in diet Li, whereas diet H showed higher ($P<0.05$) digestibility for total neutral sugars, glucose and total NSP compared with diet Hi (V).

Table 4. Influence of NSP content and solubility on ileal, caecal and total tract digestibility of fibre components in 6- and 7-weeks old (V) piglets and in growing pigs (II)

	NSP content ¹			NSP solubility ²		
	W6	W7	Growing	W6	W7	Growing
<i>Ileum/Caecum</i>						
Total neutral sugars	ns	ns	ns	L/Li, H/Hi	L/Li, H/Hi	ns
Arabinose	ns	ns	L/H, Li/Hi	L/Li	L/Li	ns
Xylose	ns	L/H	L/H, Li/Hi	L/Li, H/Hi	L/Li	ns
Glucose	ns	ns	ns	L/Li, H/Hi	L/Li, H/Hi	ns
Uronic acid	ns	ns	ns	L/Li	ns	ns
Total NSP	ns	ns	ns	L/Li, H/Hi	L/Li, H/Hi	ns
Total dietary fibre	ns	ns	ns	ns	L/Li	ns
β -glucans	ns	ns	Li/Hi	ns	ns	H/Hi
<i>Total tract</i>						
Total neutral sugars	ns	ns	ns	ns	ns	L/Li, H/Hi
Arabinose	ns	ns	L/H	ns	ns	L/Li, H/Hi
Xylose	ns	ns	ns	ns	ns	L/Li, H/Hi
Glucose	ns	ns	ns	L/Li, H/Hi	L/Li, H/Hi	L/Li, H/Hi
Uronic acid	ns	ns	Li/Hi	ns	ns	L/Li, H/Hi
Total NSP	ns	ns	ns	ns	ns	L/Li, H/Hi
Total dietary fibre	ns	ns	ns	ns	ns	L/Li, H/Hi

¹ Diet L compared with diet H, diet Li compared with diet Hi.

² Diet L compared with diet Li, diet H compared with diet Hi.

ns = not significant.

Significantly increased digestibility in the diets in bold ($P<0.05$).

Total NSP content had no influence ($P>0.05$) on the caecal digestibility of fibre components in the 6- or 7-weeks old piglets (V). These results are summarised in Table 4. In paper IV, the caecal digestibility of uronic acid was higher ($P<0.05$) when the NSP content was low. Otherwise, NSP content had no influence ($P>0.05$) on the caecal digestibility of fibre components. Enzyme supplementation increased ($P<0.05$) the digestibility of total neutral sugars and total NSP in the caecum (IV).

The total tract digestibility of total neutral sugars, arabinose, xylose, glucose, uronic acid, total NSP and DF was higher ($P<0.05$) in diet L than in diet Li and in diet H than in diet Hi, respectively, but NSP content had no influence ($P>0.05$) on the total tract digestibility of fibre components (**II**). Also the total tract digestibility of glucose during weeks 6 and 7 was higher ($P<0.05$) in diet L than in diet Li and in diet H than in diet Hi, respectively (**V**). Otherwise, the fibre components were unaffected by dietary NSP content and solubility in the total tract of piglets (**IV**, **V**).

Gut environment

NSP content and solubility as well as enzyme supplementation had no influence ($P>0.05$) on ileal viscosity (**II**, **IV**). Feeding pig diets with a high NSP content resulted in a lower ($P<0.05$) pH in the small intestine (**II**), as well as in the large intestine (**IV**), when compared with pigs offered diets with a low NSP content.

The content of total (OA) was highest ($P<0.05$) in ileal samples after feeding diets with a high NSP content (**IV**) and diet Hi (**II**). The total OA content and pH were linearly related in digesta from the ileum ($R^2=0.90$, **II** and $R^2=0.70$, **V**), the caecum ($R^2=0.57$, **V**), and the colon ($R^2=0.83$, **V**). The ileal proportion of lactic acid (of total OA) was higher ($P<0.05$) in pigs given the control diet (**II**) than in pigs offered the other diets. This was also found after feeding the diets with a high NSP content or adding enzymes (**IV**), and after feeding diet Hi in the 7-weeks old piglets (**V**).

Pigs offered low NSP diets showed a higher ($P<0.05$) proportion of acetic acid (of SCFA) in the ileum than the high NSP diets, while the reverse was true for propionic acid (**II**, **IV**). Diet Li induced a higher ($P<0.05$) proportion of acetic acid (of SCFA) in the ileum compared with diet Hi, and the reverse was true for propionic acid (**V**). The proportion of butyric acid (of SCFA) was higher ($P<0.05$) in the ileum, caecum and colon after feeding diets with a high NSP content (**IV**). Also diet Hi showed a higher ($P<0.05$) proportion of butyric acid (of SCFA) in the stomach of the 6-weeks old piglets and in the colon of the 7-weeks old piglets (**V**). Enzyme supplementation decreased ($P<0.05$) the proportion of acetic acid (of SCFA) in the ileum (**IV**).

Microbial populations

The coliform populations at the ileo-caecal ostium and rectum were not influenced by either the administration of oxytetracycline or the surgical insertion of a PVTC cannula at the ileo-caecal ostium (**I**). After feeding diets with different NSP content, the pattern of coliform diversity was influenced both at the ileo-caecal ostium and rectum (**III**). The high NSP content caused a decrease in ileo-caecal coliform diversity between day 0 and day 9, but on day 17 the diversity was restored. The low NSP content showed a minor influence on the coliform diversity at the ileo-caecal ostium during the first nine days, but then the diversity decreased until day 17. In the rectum, the influence of NSP content was less clear. However, an indication of solubility influence was found, since pigs fed diet L showed a higher ($P<0.05$) diversity compared with pigs offered diet Li on day 9.

T-RFLP monitored the influence of different dietary NSP contents on the total gut microbial flora at the ileo-caecal ostium (**III**). A total of 118 different TRFs of between 34 and 672 base pairs in length were discovered. However, only 41 of these TRFs were found in at least three pigs fed the same diet and therefore considered characteristic. When the pigs had been fed a standard feed, there were seven TRFs present that subsequently disappeared after day 0, when introducing the experimental diets. Pigs fed diet H showed the highest number of characteristic TRFs whereas pigs offered the control diet and diet Hi showed the lowest number. The TRF with a length of 490 base pairs was always present, on day 0 as well as after feeding the experimental diets. Two unique TRFs of 298 and 408 base pairs in length were only demonstrated in pigs fed diets with a high content of NSP or the control diet, whereas the TRFs of 519 and 571 base pairs length were only recovered in pigs offered the low NSP diets.

Discussion

Cereal composition

There are differences in chemical composition between feed ingredients. Besides dissimilarities between cereals, the specific nutrient content of a cereal is also affected by genetic factors and environmental factors, such as growing region and season (Seerley, 1991; McCann *et al.*, 2003; Kim *et al.*, 2003). Consequently, if different studies offering similar feed are to be compared, the true quality of all diets must be known, emphasising the importance of chemical analysis. The analysed chemical composition of barley, oats, wheat and wheat bran included in the current experimental diets (Table 2) was similar to results obtained in previous analyses (Table 1) performed by Bach Knudsen (1997). Also oat meal (Table 2) was similar in composition to oat feed meal (Table 1).

In contrast, oat bran and rye bran (Table 2) differed in composition from oat hull meal and rye bran (Table 1), respectively. Probably, the proportion of different parts of the grain, cell wall and hull fractions in these cereal by-products differed, resulting in these variances in chemical composition (Bach Knudsen, 2001). Such variances in cereal composition are of importance for the dietary influence on digestibility, site of digestion, gut environment and microbial populations in the gastro-intestinal tract.

Dietary composition, digestibility and gut health

The present studies (**II**, **IV**, **V**) support earlier findings that an increased level of dietary fibre decreases the digestibility of OM and energy in pigs (Just, 1982; Noblet & Perez, 1993; Li, Sauer & Hardin, 1994; Pettersson, Lindberg & Thomke, 1997; Yin *et al.*, 2000a). Further, the reduction in digestibility of OM with increased NSP content ranged from -0.12 to -0.17 at the ileum and caecum, and from -0.06 to -0.09 at the colon and rectum of piglets. In the growing pigs, the corresponding magnitude of reduced digestibility was -0.13 at the ileum and -0.08 in faeces, representing the total tract. Thus, the NSP-induced reduction in OM

digestibility was twice as high in the small intestine as in the total tract, and the relation obtained between the two sampling sites was in agreement with earlier findings (Drochner, 1991).

An increased proportion of insoluble NSP decreased the digestibility of OM and fibre components in the small intestine in the newly weaned piglets (**V**) and in the total tract of the growing pigs (**II**). This is of importance because the most common intestinal disease in recently weaned pigs is PWD which is induced by a proliferation of pathogenic *E. coli* in the small intestine, and the most significant intestinal disorder in growing pigs is SD, which is induced by an infection with *B. hyodysenteriae* in the large intestine (Hampson, Pluske & Pethick, 2001).

The present results suggest that insoluble NSP decreases the amount of digestible substrate for the pig as well as for the microbes in the small intestine among newly weaned piglets and in the total tract among growing pigs. Since *E. coli* proliferates in the small intestine and *B. hyodysenteriae* in the large intestine this suggests that insoluble NSP may be beneficial for the pig by reducing the incidence and/or severity of PWD and swine dysentery by decreasing the substrate available for microbial growth.

Previously the proliferation of enterotoxigenic *E. coli* in newly weaned piglets (McDonald *et al.*, 1999, 2001), as well as the clinical expression of swine dysentery in growing pigs (Pluske *et al.*, 1996, 1998; Siba, Pethick & Hampson, 1996) have been reduced by highly digestible diets based on cooked white rice and animal protein. These results are in line with the studies presented here, because these authors actually decreased the amount of soluble NSP in their diets. Indeed, the amount of soluble NSP will also be decreased when the amount of insoluble NSP is increased, as was the case in the present studies (**II**, **V**).

In newly weaned piglets the total NSP content had no influence on the digestibility of fibre components, either in the small intestine or in the total tract (**IV**, **V**). Therefore, the total NSP content appears to be of minor importance to protect from PWD, but the proportion of insoluble NSP might be of significance, as also indicated by others (McDonald *et al.*, 1999, 2001).

The results obtained among growing pigs differed somewhat from those obtained among newly weaned piglets. Dietary NSP increased the digestibility of fibre components in the small intestine in growing pigs (**II**). This increase in digestibility after feeding diets with a high NSP content may be due to an increased microbial activity stimulated by suitable substrate for microbial growth. However, no differences in digestibility could be measured in faeces between growing pigs fed diets with different NSP content (**II**).

Still, the severity of SD has been decreased by diets with a low NSP content, based on cooked white rice and animal protein, (Pluske *et al.*, 1996; Siba, Pethick & Hampson, 1996), probably due to a decreased amount of fermentable substrates in the large intestine. However, other studies performed in Canada (Kirkwood *et al.*, 2000) and Denmark (Lindecrona *et al.*, 2003) have failed to repeat these successful results in preventing SD by decreasing the amount of fermentable substrate entering the colon with similar diets.

Another approach has been to prevent clinical signs of *B. hyodysenteriae* with maize silage diets containing high levels of cellulose and hemicellulose (Prohászka & Lukács, 1984). These authors speculated that the increased amount of fermentable substrate in the colon increased the production of SCFA and lowered the pH. Possibly an unfavourable gut environment for *B. hyodysenteriae* was created, and thereby the pigs were protected from SD. Also Kirkwood *et al.* (2000) tried this approach, but failed in repeating the successful results with an increased amount of fermentable substrate in the colon.

Obviously it is not very easy to repeat SD studies with successful results, probably because other factors besides the diet also influence the development of SD. These include synergy with certain anaerobic bacteria, such as species of *Bacteroides* or *Fusobacteria* (Meyer, Simon & Byerly, 1975; Whipp *et al.*, 1979), and the composition and function of the enteric anaerobic microflora. Also a decreased diversity of the microflora is commonly seen in connection with diarrhoea (Kühn *et al.*, 1993; Katouli *et al.*, 1999). Thus the stability of the resident microflora is likely to influence the development of intestinal disease as well. Further, the enteric bacteria present in the gastro-intestinal tract of pigs might differ between pig populations. Also differences in environmental conditions and pig genotype might be of importance.

Gut environment

The intestinal viscosity was low and had a narrow range (1.3-2.3 cP) in the present studies (**II, IV**), corresponding to previous observations (Rainbird & Low, 1986; Johansen *et al.*, 1996; Thacker, Campbell & Scoles, 1999; Guerin *et al.*, 2001). Also the dietary viscosity was low and within a narrow range in the different diets (1.2-3.6 cP). Thus, neither the dietary content of soluble NSP nor enzyme supplementation influenced the intestinal viscosity (**II, IV**).

The content of OA as well as the pH level was altered by the dietary NSP content (**II, IV, V**). An increased production of OA caused a decrease in pH at the ileum, caecum and colon (**II, V**), and pH may therefore be used as an indicator of microbial activity at various sites in the gastro-intestinal tract. Also the mutual proportions of different organic acids were influenced by the diets, indicating an influence of diet on the intestinal microflora. For instance, the proportion of lactic acid and butyric acid increased when feeding diets with a high NSP level (**IV**) as well as in the diet where the NSP content was high and insoluble (**V**). Thus, lactic and butyric acid producing bacteria were probably promoted by these diets. Further, low levels of NSP increased the ileal proportion of acetic acid and decreased the proportion of propionic acid (**II, IV**). Thus, the gut environment was altered by the content and quality of the NSP in the feed.

Pigs fed the diets with a low NSP content showed signs of a decreased microbial activity at the ileum, indicated by a decreased production of OA and consequently a higher pH (**II, IV**). In the growing pigs (**II**), the ileal digestibility of fibre components was lower in the diets with a low NSP content than in the diets with a high NSP content, suggesting that a lower digestibility of fibre components could be responsible for this decrease in microbial activity. This statement is also

supported by a decreased coliform diversity after feeding diets with low NSP content (**III**). This pattern of coliform diversity found at the ileo-caecal ostium in the growing pigs suggests that less substrate for microbial growth was available when a low level of NSP was fed. Thus, digestibility should be interpreted as the sum of degradation by enzymatic and microbial processes. An increased digestibility implies more nutrients digested by the pig, but also more substrate available for microbial growth.

Microbial populations

The surgical insertion of a PVTC cannula did not influence the coliform flora (**I**), which is of importance because the cannulation technique provides possibilities to collect repeated intestinal samples for microbial studies in living animals. Previously, it has been shown that neither digestibility (Lindberg, 1997; Jansman *et al.*, 2001), health status nor gut function (Jacobson *et al.*, 2001) are affected by this method. Taken together, these observations suggest that results obtained from PVTC cannulated pigs truly reflect the intestinal conditions of intact animals.

The total microbial populations as well as the diversity of the coliforms were altered when diets with differing NSP content and solubility were introduced (**III**). Thus, dietary NSP content and solubility influenced the total microflora and the coliform populations at the ileo-caecal ostium as well as the coliform diversity at the rectum of pigs. This suggests that dietary NSP has a potential to be used as a tool to modify pig feed, aiming to prevent gastro-intestinal disorders. However, as already pointed out (Pluske *et al.*, 1998; Kirkwood *et al.*, 2000; Lindecrona *et al.*, 2003), more studies are needed to scrutinise the correlation between NSP and their effects on the intestinal microflora.

Further, the microflora needs a certain time to adjust to newly introduced diets. This was indicated by different coliform diversity patterns between days 0, 9 and 17 after introducing new diets, both at the ileo-caecal ostium and in the rectum (**III**). Similar results have previously been shown by others (Kühn *et al.*, 1993; Katouli *et al.*, 1995, 1997b, 1999). Taken together these findings emphasise the importance of giving pigs sufficient time to adapt to newly introduced feed before evaluating the influence of the diet on the intestinal microflora.

In the present study the diversity of the ileo-caecal coliform flora was restored 17 days after introducing the diets with a high NSP content (**III**), indicating that the microflora had adapted to the new feed at that time. This figure corresponds well to the period of about 14 days required to restore the high diversity of the faecal coliform microflora after the decrease in diversity induced by weaning in healthy piglets (Melin *et al.*, 2000).

Nonetheless, it was difficult to find a dietary related pattern in the rectal coliform flora after 17 days (**III**), possibly indicating that the microbial populations in the rectum had not yet adapted to the diets at that time. The denser microbial population in the rectum than at the ileum (Zoric *et al.*, 2002) may have contributed to the longer time required for adaptation after a dietary change. In this context it is of interest that lower total culture counts were observed in rectal samples 3 weeks after introducing a high-fibre diet with 50% alfalfa meal to pigs

(Varel *et al.*, 1982). After 8 weeks the total culture counts were similar to those before introducing the alfalfa diet, suggesting a time period of 8 weeks required for the microbial adaptation for that diet. However, the dietary composition is certainly of importance for the time needed for microbial adaptation after introduction (Katouli *et al.*, 1997b, 1999; Melin *et al.*, 2000). Therefore, it is important to consider the relevant time required for adaptation when feed compositions are to be evaluated.

Possible influence of NSP on microbial populations

Inclusion of dietary fibre will shift the enzymatic digestion of α -glycosidic linkages in the small intestine to microbial fermentation of β -glycosidic linkages in the large intestine (Chesson, 1987; Bach Knudsen & Hansen, 1991). Further, a large amount of soluble fibre indicates an often completely degraded, easily digestible and available fibre composition, whereas a large amount of insoluble fibre indicates a fibre composition with low digestibility, only partly degraded by time-consuming fermentation (Bach Knudsen & Jørgensen, 2001). Depending on the source of dietary fibre included, it will influence the site of digestion as well as the gut environment (**II**, **IV**, **V**), and thereby also the conditions for proliferation of microbial populations in the gastro-intestinal tract (**III**).

In theory, the dietary content of total and soluble NSP can give four separately specified combinations, a high NSP content with a large amount of soluble NSP, a high NSP content with a large amount of insoluble NSP, a low NSP content with a large amount of soluble NSP and a low amount of NSP with a large amount of insoluble NSP. These four theoretically clear-cut combinations are likely to influence the intestinal microbial populations in different ways, although the microbial density remains unchanged (Bach Knudsen, Jensen & Hansen, 1993a-b). A summary of the possible ways NSP may influence microbial populations is given in Table 5.

Table 5. Overview of possible ways of influence on microbial diversity and density depending on the source of fermentable substrate available

	NSP content		NSP solubility	
	Low	High	Soluble	Insoluble
Microbial diversity	-	+	+	-
Microbial activity	-	+	-	+

A diet with a high NSP content and a large amount of soluble NSP is likely to promote microbial growth, as indicated in the ileo-caecal coliform diversity pattern as well as in the increased number of TRFs in diet H (**III**). There is plenty of substrate present for microbial fermentation in the intestine (Bach Knudsen & Hansen, 1991; Bach Knudsen *et al.*, 1991). Moreover, it is easy to ferment in a relatively short time. This type of diet is likely to function as a general substrate for microbial growth, and thereby encourage many different microbes, resulting in high diversity and activity among the resident microflora. But also transient

microbes may easily find a suitable substrate for growth. Hence the colonisation resistance against transient microbes probably depends on competitive exclusion for substrate as well as suitable proliferation sites, through a dense and stable resident flora with high diversity.

A diet with a high NSP content and a large amount of insoluble NSP provides plenty of substrate (Bach Knudsen *et al.*, 1991), but it is difficult and time-consuming to ferment (Bach Knudsen & Hansen, 1991). Further, the passage rate through the gut is increased by this kind of diet (Kass, van Soest & Pond, 1980; Bach Knudsen, 2001). Therefore it is likely that the microbes capable of fermenting this kind of diet need to be specialised, resulting in a decreased diversity of the resident flora. In the present study, this was indicated by a somewhat lower ileo-caecal coliform diversity and a lower number of TRFs present in diet Hi than in diet H (III). Still, the slow fermentation process encourages the growth of resident microbes, since transient microbes simply do not manage to proliferate on this substrate before they are washed away by the peristaltic movements. Thus the colonisation resistance against transient microbes depends on rather specialised resident bacteria proliferating the best sites in the gastro-intestinal tract.

A diet with a low NSP content and a large amount of soluble NSP provides a limited but highly fermentable substrate for microbial growth, easy to ferment in a relatively short time (Bach Knudsen & Jensen, 1991). Further, the passage rate through the proximal gastro-intestinal tract is decreased by this kind of diet (Bach Knudsen & Hansen, 1991; Glitsø *et al.*, 1998), giving transient microbes more time for possible proliferation. Thus, the growths of both resident and transient microbes are probably promoted on this substrate. However, the diversity as well as the activity of the resident flora may be decreased due to the limited amount of substrate, as indicated by the decrease in ileo-caecal coliform diversity pattern in diet L (III). Thus, the colonisation resistance against transient microbes relies on competitive exclusion for substrate.

A diet with a low NSP content and a large amount of insoluble NSP provides a limited and difficult substrate for microbial growth, that is time-consuming to ferment (Bach Knudsen, Jensen & Hansen, 1993b; Noblet & Bach Knudsen, 1997). Therefore, it is likely that the microbes capable of fermenting this kind of diet needs to be specialised, probably resulting in a decreased diversity of the resident flora, as indicated by the decrease in ileo-caecal coliform diversity pattern in diet Li (III). Thus, this diet provides colonisation resistance against transient microbes, both through competitive exclusion for the limited amount of substrate and by encouraging a specialised resident microflora.

Conclusions

- The surgical insertion of a PVTC cannula did not influence the coliform flora, and samples collected through the cannula were concluded to reflect true intestinal conditions of intact animals.
- The total microbial populations as well as the diversity of the coliforms were altered by different NSP content and NSP solubility. Also, it was shown that the enteric microflora needs a certain time for adaptation to newly introduced diets.
- An increased content of dietary NSP decreased the digestibility of OM and energy. Further, the NSP induced reduction in OM digestibility was twice as high in the small intestine as in the total tract.
- An increased amount of insoluble NSP decreased the digestibility of OM and fibre components in the small intestine of the newly weaned piglets and in the total tract of the growing pigs.
- The gut environment, as described by pH and content and proportions of organic acids, was altered by dietary NSP content and NSP solubility.
- Altogether, dietary NSP content and NSP solubility influenced digestibility, digestion site and gut environment in piglets around weaning and in growing pigs. Also the enteric microbial populations in growing pigs were influenced by NSP content and NSP solubility. Therefore, it is concluded that dietary NSP and NSP solubility can be used as a tool to promote gut health in weaning piglets as well as in growing pigs.

Sammanfattning

Avhandlingen är baserad på tre studier som omfattar både avvänjnings- och tillväxtperioden hos grisar, och avser att beskriva inverkan av icke-stärkelse polysackarider (NSP) från spannmål och tillsats av enzymer i foder på processer i mag-tarmkanalen. Spannmål och spannmålsbiprodukter användes för att ge fodren olika innehåll av totala såväl som lösliga NSP. Resultaten visade att både hos nyligen avvanda smågrisar och hos växande grisar medförde ett ökat innehåll av NSP i fodret en dubbelt så stor minskning av smältbarheten av organisk substans i tunntarmen jämfört med grovtarmen. En ökad andel olösliga NSP i fodret minskade smältbarheten av organisk substans och fiberkomponenter i tunntarmen hos nyligen avvanda smågrisar och i grovtarmen hos växande grisar. Fodrets innehåll av totala och lösliga NSP påverkade tarmmiljön i form av ändrade mängder och andelar av organiska syror samt ändrade pH-värden. Även de totala mikrobiella populationerna och den koliforma diversiteten påverkades av fodrets innehåll av totala och lösliga NSP. Tillsats av enzymer i fodret påverkade andelen organiska syror i tunntarmen. Den koliforma floran var oförändrad när växande grisar försågs med en PVTC fistel i blindtarmen, vilket visar att prover tagna från PVTC fistulerade grisar är jämförbara med prover tagna från intakta grisar. Sammanfattningsvis antyder resultaten att fodrets innehåll av totala och lösliga NSP påverkar processer i mag-tarmkanalen såsom smältbarhet, tarmmiljö och mikrobiella populationer på olika sätt hos nyligen avvanda och växande grisar. Därmed utgör fodrets innehåll av NSP ett viktigt verktyg som kan användas för att förändra digestionsprocesser och tarmmiljö med möjligheter att påverka tarmhälsan hos grisar av olika åldrar.

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