Utilisation of Fibre-rich Feedstuffs for Pigs in Vietnam

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Abstract

This thesis examines the chemical composition and water holding capacity (WHC) of fibrous feeds and evaluates the effects of fibre level, fibre source, particle size and enzyme supplementation on the digestibility, performance, total tract mean retention time (MRT), as well as gut development, morphology, environment and microflora of pigs.

The content (g/kg dry matter, DM) of crude protein, ether extract, starch, total sugars and non-starch polysaccharides was found to vary between feedstuffs. The content of individual neutral sugars varied between feed ingredients, with the highest content of arabinose, galactose and glucose in tofu residue, the highest xylose content in brewer's grain and the highest mannose content in coconut cake. The content of soluble non-cellulosic polysaccharides was positively correlated to the WHC.

Pigs (Landrace x Yorkshire, LY) fed a diet containing cassava residue had higher the total tract apparent digestibility (TTAD) of nutrients and average daily gain (ADG) and lower feed conversion ratio (FCR) than those fed a diet containing sweet potato vines. A reduction in particle size and multi-enzyme addition improved the TTAD of dietary components and growth performance in the post-weaning period, but not the in growing period. An increased fibre content in the diet decreased ADG, the nutrient digestibility and MRT, and increased FCR and gut weight. Moreover, there was a possible impact of fibre properties on nutrient digestibility and MRT, but not on gut size. Mong Cai (MC) pigs had a greater relative gut weight and content than LY pigs and a longer MRT, resulting in higher nutrient digestibility. Across diets within breeds the MRT was negatively related to DMI, while the TTAD of nutrients was positively related to MRT. Fibre level and fibre source affected small intestinal morphology, in particular in the ileum. This effect occurred in parallel with fibre-related effects on lactic acid bacteria (LAB) and *E. coli* counts in the gastrointestinal tract (GIT), and on the gut environment. There were differences between MC and LY pigs in small intestinal morphology, counts of LAB and *E. coli* along the GIT and gut environment.

In short, this thesis shows that fibrous feedstuffs from green plants and agro-industrial by-products can be used in pig diets as common feed ingredients, particularly in indigenous pig diets.

Keywords: Digestibility, enzyme supplementation, fibre level, fibre source, gut size, gut environment, Landrace x Yorkshire, mean retention time, Mong Cai, particle size.

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Dedication

To my parents
My husband Le Minh Linh
My daughter Le Thi Phuong Quynh
My son Le Minh Tuan

Ngọc kia chẳng giũa chẳng mài
Cũng thành vô dụng cũng hoài ngọc đi
[If the ruby stone is not polished, it is worthless]
Vietnamese folk verse

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ngoc, T.T.B., Len, N.T. and Lindberg, J.E. 2012. Chemical characterization and water holding capacity of fibre-rich feedstuffs used for pigs in Vietnam. *Asian-Australian Journal of Animal Science* 25 (6), 861-868.

II Ngoc, T.T.B., Len, N.T., Ogle, B. and Lindberg, J.E. 2011. Influence of particle size and multi-enzyme supplementation of fibrous diets on total tract digestibility and performance of weaning (8-20 kg) and growing (20-40 kg) pigs. *Animal Feed Science and Technology* 169, 86-95.

III Ngoc, T.T.B., Len, N.T. and Lindberg, J.E. 2012. Impact of fibre intake and fibre source on digestibility, gastro-intestinal tract development, mean retention time and growth performance of indigenous (Mong Cai) and exotic (Landrace x Yorkshire) pigs. *Animal* (in revision).

IV Ngoc, T.T.B., Hong, T.T.T., Len, N.T. and Lindberg, J.E. 2012. Effect of fibre level and fibre source on gut morphology and micro-environment in local (Mong Cai) and exotic (Landrace x Yorkshire) pigs. *Asian-Australian Journal of Animal Science(accepted*).

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Abbreviations

BG	Brewer's grain
BW	Body weight
CC	Coconut cake
CF	Crude fibre
CR	Cassava residue
DF	Dietary fibre
DM	Dry matter
EBW	Empty body weight
EE	Ether extract
FCR	Feed conversion ratio
GE	Gross energy
GIT	Gastrointestinal tract
IAD	Ileum apparent digestibility
LAB	Lactic acid bacteria
LY	Landrace x Yorkshire
MC	Mong Cai
MRT	Total tract mean retention time
NCP	Non-cellulosic polysaccharides
NDF	Neutral detergent fibre
NSP	Non-starch polysaccharides
OA	Organic acids
RB	Rice bran
SBM	Soybean meal
SCFA	Short chain fatty acids
SPV	Sweet potato vines
TFR	Tofu residue
TTAD	Total tract apparent digestibility
WHC	Water holding capacity
WSP	Water spinach

1 Introduction

Pig production plays a very important role in livestock production in Vietnam, because pork contributes around 80% of total meat consumption (MARD, 2008). The pig population in Vietnam increased from 21.8 million in 2001 to 27.4 million in 2010, representing an average annual growth rate of 3.2% (GSO, 2011). It has been estimated that around 80% of the total pig population are raised in traditional smallholder systems (Lapar *et al.*, 2003). Raising pigs in the traditional system results in low production performance and revenues, but requires low investment, resulting in a low level of risk. This may be an advantage in a situation with unstable pork prices.

Commercial feeds are rarely used on smallholder farms because they are expensive, and therefore agricultural by-products have become an important feed resource, especially for pigs. Various plants and agricultural by-products, such as sweet potato vines (SPV), water spinach (WSP), cassava leaves, rice bran (RB), cassava residue (CR), tofu residue (TFR), brewer's grain (BG), and other locally available by-products, which are characterised by high fibre content and low nutritional value, are used in almost all households for feeding pigs. A number of studies have evaluated the effect of using diets based on local fibrous feeds such as SPV, CR and RB (Len *et al.*, 2009a; Len *et al.*, 2009b; Giang, 2003) for post-weaning and growing pigs, indicating both reduced digestibility and performance. In order to overcome these shortcomings, better utilisation of locally available feed resources for pigs is thus very important. Various techniques, such as pelleting, reduction of particle size and supplementation with exogenous enzymes can be applied to enhance the nutritional value and utilisation of available feed resources (Kim *et al.*, 2007; Brufau *et al.*, 2006; Mavromichalis *et al.*, 2000).

Today, the use of exotic breeds such as the Landrace, Yorkshire and Duroc has expanded rapidly, especially in the urban areas of Vietnam, in order to meet the higher demand from consumers for both quantity and quality of meat.

However, the Mong Cai (MC) breed has better characteristics with regard to reproduction, is adapted to the hot climate and is more tolerant of high-fibre diets than exotic breeds. Therefore, pure-bred MC sows are still commonly mated with boars of an exotic breed, usually Yorkshire or Landrace, using artificial insemination, and the offspring are fattened in small-scale semi-intensive or intensive systems. Earlier studies have shown that the MC breed digests high fibre-diets better than exotic breeds (Len *et al.*, 2009a; Len *et al.*, 2009b; Borin *et al.*, 2005). Consequently, different breeds probably exhibit different responses to the same diets in terms of nutrient digestibility and growth performance. This difference in response between breeds could be related to their digestive capacity (Len *et al.*, 2009a) and to the composition of the diet, especially dietary fibre (DF) level and fibre sources (Len *et al.*, 2009a; Len *et al.*, 2009b; Borin *et al.*, 2005; Freire *et al.*, 2003).

Objectives of the thesis:

- To evaluate variations in the nutritional properties for pigs of fibrous feedstuffs originating from green plants and agro-industrial by-products.
- To evaluate the digestibility of dietary components and performance of post-weaning and growing pigs fed fibrous diets containing different fibre sources with different particle sizes and multi-enzyme supplementation.
- To investigate the influence of fibre source and fibre level on:
    - ✓ Digestion site, gut development and total tract mean retention time (MRT) of MC and Landrace x Yorkshire (LY) pigs.
    - ✓ Bacteria counts and mucosa morphology in different segments of the digestive system of MC and LY pigs.

Hypotheses examined in the thesis:

- The chemical characteristics and water holding capacity (WHC) differ between feedstuffs made from green plants and agro-industrial by-products, and there are differences within feedstuffs related to collection time.
- Reducing the particle size of a fibrous diet can improve the total tract apparent digestibility (TTAD) of nutrients and the performance of post-weaning and growing pigs.
- Multi-enzyme supplementation of a fibrous diet can improve the TTAD of nutrients and the performance of post-weaning and growing pigs.
- Gut ecology and morphology and the bacteria counts in different segments of the gastrointestinal tract (GIT) are affected by differences in fibre source, fibre level and pig breed.
- Mong Cai pigs have a larger GIT and longer MRT, and can therefore utilise high-fibre diets better than exotic LY pigs.

2 Background

2.1 Fibre-rich feed resources used for feeding pigs in Vietnam

In Vietnam, commercial complete feeds meet 30-35% of the total feed requirements for pig production throughout the country and the remaining 65-70% is provided by home-made feed using locally available feed resources (Kopinski *et al.*, 2011). However, the available feed resources such as WSP, SPV, RB, CR, TFR, BG and other by-products are often of poor quality and have low nutritional value. Thus, it is of great importance for the pig industry in Vietnam to find ways to improve the utilisation of fibrous diets in terms of higher digestibility of dietary components and thereby make it possible to improve pig performance.

Rice is the most important crop in Vietnam, with around 7.5 million ha planted and 40 million tons of rice produced in 2010 (GSO, 2011). Rice bran is the most important rice by-product. It is available in rural areas of Vietnam where smallholder pig production is dominant. Rice bran is a good source of B-vitamins and is palatable to farm animals. For pigs, it is recommended that RB should not exceed 20-30% of the total ration to avoid soft pork and that in the final weeks of fattening, lower levels should be used (Trujillo, 2009). Rice bran is often adulterated with rice hulls, resulting in a variable crude fibre (CF) content ranging from 7 to 25% (NIAH, 2001).

In Vietnam, cassava is the second most important crop after rice, with about 496,200 ha planted and cassava root production of about 8.5 million tons in 2010 (GSO, 2011). Cassava residue is the by-product from the processing of cassava root and it comprises about 45% of cassava root production (Van *et al.*, 2008). Cassava root is processed for starch at both large scale (factories) and small scale (household level). Utilisation of CR as an animal feed contributes to the development of sustainable agriculture due to the decrease in pollution from decomposition of the residue, which contains a high level of moisture

(80%). Cassava residue has a high content of fibre (160-200 g CF/kg dry matter (DM)) and a metabolisable energy content of 11.7 MJ/kg DM (NIAH, 2001). Owing to its relatively high energy value, CR is also used in the feed industry to reduce feed costs.

Soybean contains 160-210 g/kg oil and is regularly solvent-extracted, and the residual meal has an oil content of about 10 g/kg DM (McDonald *et al.*, 2002). Soybean meal (SBM) is an important source of dietary protein and energy for livestock. The protein contains all the essential amino acids but the concentrations of cystine and methionine are suboptimal for growing pigs. However, SBM contains a number of toxic, stimulatory and inhibitory substances, including allergenic, goitrogenic and anticoagulant factors. Of particular importance in nutrition are the protease inhibitors (anti-trypsin and anti-chymotrypsin). Thus, to increase the protein nutritive value, these anti-nutritional factors must be destroyed. Trypsin inhibitors disrupt protein digestion, which results in decreased release of free amino acids, and their presence is characterised by compensatory hypertrophy of the pancreas due to stimulation of pancreatic secretions. Fortunately, the heat treatment (roasting) applied during processing is usually enough to destroy the trypsin inhibitors and other anti-nutritional factors such as lectins (haemagglutinins). Lectins exert their inhibitory effect by binding to the epithelial cells lining the small intestine, disrupting the brush border and reducing the efficiency of nutrient absorption (McDonald *et al.*, 2002). Soybean meal with added DL-methionine is equivalent to fish meal in protein quality, and may comprise up to 250 kg/ton of pig diets (McDonald *et al.*, 2002).

Tofu residue, a by-product from soybean processing, is obtained from the extraction of soybean milk. Tofu residue is a low-cost and nutritious feed (more than 200 CP g/kg DM (NIAH, 2001)) for livestock and is widely available in a number of Asian countries. Although SBM is commonly used for livestock, it is fairly expensive, and therefore a much cheaper alternative is to utilise TFR from local food industries.

Two main feedstuffs can be produced from coconut. One is the by-product from the extraction of the oil from the seed, known as coconut oil meal, coconut cake (CC) or copra meal which represents approximately 34-42% of the weight of the nut. The other is the broken kernel, usually known as raw copra. The main difference between the two products is the amount of protein and fat. Coconut cake is ground to meal for use in feed for poultry, cattle, sheep and swine. Coconut cake is generally low in lysine and sulphur amino acids (Sundu *et al.*, 2009), and therefore utilisation of CC as a source of protein can lead to amino acid deficiency. With amino acid supplementation, CC can be used as a substitute for fish meal in pig diets, but may cause constipation

(Pickard, 2005). As the amount of cake in the diet increases, its palatability decreases.

The brewing industry generates relatively large amounts of by-products, with malt culms, BG, spent hops and yeast being the most common. Brewer's grain is the most abundant brewing by-product, corresponding to around 85% of total by-products generated (Mussatto *et al.*, 2006). Brewer's grain has a relatively high crude protein (CP) content in DM (300-320 g CP/kg DM), but it is also high in fibre (133-147 g CF/kg DM) (NIAH, 2001). According to Gohl (1981), BG is not commonly used for pigs, and it is generally recommended that it should not exceed 50% of dietary protein, as otherwise growth rate and feed efficiency may be reduced.

Sweet potato vines and WSP are green plant feeds that are commonly used in the rural areas at household and smallholder farm level, but not by the animal feed industry. They are characterised by high fibre content and sometimes a relatively high CP content. Traditionally, farmers plant sweet potato in their garden and harvest the vines for their pigs on a daily basis. In addition, SPV can be collected as a by-product at harvesting of the root, and then processed and preserved by ensiling or sun-drying for later use as animal feed. Water spinach is also a valuable protein feed for growing pigs and sows in Vietnam.

2.2 Determination of dietary fibre

The fibre fraction in feedstuffs used in pig feed is still commonly characterised by use of CF and more frequently by use of the Van Soest detergent system (Van Soest *et al.*, 1991), in which the fibre fraction is separated into neutral detergent fibre (NDF), acid detergent fibre and lignin. However, despite considerable refinement in describing the fibre fraction compared with CF, the detergent system has the limitation of not accounting for soluble carbohydrates. In order to more accurately describe the whole fibre fraction in feed and food, including both soluble and insoluble NSP, the Uppsala method (Theander *et al.*, 1995) is today accepted as being the most relevant method to apply (Bach Knudsen, 1997). Sugars and starch are removed from samples in acetate buffer using thermostable α-amylase and amyloglucosidase. Soluble polymers are precipitated with ethanol. Precipitated and insoluble polysaccharides are hydrolysed with H_2SO_4. Subsequently, determinations of monomeric constituents are performed either by GLC or HPLC for neutral sugars, by colorimetry for uronic acids, and by gravimetry for Klason lignin, which is the insoluble residue. These analytical steps allow total NSP, cellulose, insoluble and soluble NCP, and Klason lignin to be distinguished. Moreover, the monomeric residue of soluble NCP and insoluble NCP provides information about individual sugars including the separation into neutral and acidic sugar monomers.

2.3 Exogenous enzymes, interaction with particle size

In order to achieve high production efficiency in the pig industry at low cost, a continuous improvement in the utilisation of diets and of a wide range of dietary ingredients is crucial. Smallholder pig farms use commercial feeds in combination with certain by-products, and profitability is highly dependent on the relative cost and nutritive value of the selected feedstuffs. For these reasons, a continuous effort has been made to understand the complex nature of commonly used feed ingredients. Feed enzymes can increase the digestibility of nutrients, leading to improved pig performance (Len *et al.*, 2009b; Partridge, 2001) and can thus contribute to better utilisation of available feed resources. However, the effect of feed enzymes on digestibility and pig performance varies, depending on age and pig breed, feed particle size and type of enzymes used to target substrates (Len *et al.*, 2009b; Kim *et al.*, 2005; Mavromichalis *et al.*, 2000; Thomke & Elwinger, 1998). According to Kim *et al.* (2005) and Oryschak *et al.* (2002), enzyme supplementation increases nutrient digestibility to a greater extent in larger particle size diets than in smaller particle size diets. The longer retention of larger particle size diets in the gut may provide a longer time of exposure to the enzyme supplement, resulting greater efficacy of enzyme addition for these diets.

Enzymes, as biological catalysts, are involved in all anabolic and catabolic pathways of digestion and metabolism. They enable the pathways to operate efficiently under metabolic conditions and act as regulators of individual processes. Because of these characteristics, interest is growing in the use of enzymes to improve animal performance.

There are a number of enzymes available for degrading specific components in the feed, such as for instance pentosanases, ß-glucanases, α-galactosidases, cellulases and xylanases designed to target different fibre fractions, amylase for starch, and proteinases for protein. The quality of the enzymes, such as efficiency, tolerance to digestive processes and feed processing, is important, as are the specific enzymes or enzyme mixture used (Chesson, 1993).

2.4 Dietary fibre

Non-starch polysaccharides (NSP) together with lignin have been defined as the DF fraction in feedstuffs and food, and can be used as a collective measure of the fibre content (Bach Knudsen, 1997; Theander *et al.*, 1995). The main constituents of NSP are cellulose, pectins, β-glucans, pentosans and xylans (Souffrant, 2001), which differ considerably in terms of type, number and order of monosaccharides, the linkage between monosaccharides and the presence of side chains. The NSP consist of both soluble and insoluble fractions (Bach Knudsen, 1997), as defined by the extraction procedure used in chemical analyses. Lignin is not a carbohydrate but consists of branched networks of poly-phenols tightly linked to the cell wall polysaccharides (Iiyama *et al.*, 1994), and is therefore included in DF.

The physico-chemical properties of the feed are very important for effective digestion in the animal and can be characterised by the viscosity, swelling capacity, solubility, WHC and water binding capacity. The terms WHC and water binding capacity are used differently in the literature, but both reflect the ability of a fibre source to incorporate water within its matrix. According to Kunzek *et al.* (1999), the water binding capacity describes the ability of a sample to bind water when exposed to an external stress, while WHC describes the ability to hold water within the feed matrix under atmospheric pressure. There are several methods available for measuring WHC, such as centrifugation, dialysis bags and filtration (Elhardallou & Walker, 1993) and each gives different results. It is therefore difficult to make a direct comparison of the numerical values of WHC obtained in different studies.

2.5 Dietary fibre in relation to digestion

It is well known that inclusion of DF in the diet decreases the digestibility of energy and dietary components in the ileum and in the total tract (Len *et al.*, 2009a; Len *et al.*, 2009b; Högberg & Lindberg, 2004a; Högberg & Lindberg, 2004b). The negative effects of DF are partly the result of increased digesta flow rate and inhibition of the exposure of digesta to enzymes, and also increased endogenous nitrogen, which is an additional factor reducing apparent nitrogen digestibility (Jørgensen *et al.*, 1996; Rainbird & Low, 1986). The effect of DF on the digestibility of dietary components is variable, and depends on the type of fibre included. Different feed sources have different characteristics, such as solubility and degree of lignification (Bach Knudsen & Jørgensen, 2001). In general, soluble fibre is more easily and rapidly fermented than insoluble fibre (Montagne *et al.*, 2003; Bach Knudsen & Hansen, 1991). Besides, soluble DF may increase mean retention time (Le Goff *et al.*, 2002;

Glitsø *et al.*, 1998), resulting in greater nutrient absorption from the intestine. Lignin is highly undegradable and probably hinders digestion of other cell wall components, either by physical encapsulation or by chemical binding (Cornu *et al.*, 1994). Thus, the digestibility of cellulose and arabinoxylans is much higher in cell wall materials from non-lignified materials than from lignified materials (Glitsø *et al.*, 1998; Bach Knudsen & Hansen, 1991).

2.6 Dietary fibre in relation to fermentation, intestinal morphology and gastrointestinal tract development

The main products of fermentation of DF are short chain fatty acids (SCFA), which are mainly acetate, propionate and butyrate, and the gases H_2, CO_2 and CH_4. As shown by some researchers (Jensen, 2001; Simon, 2001; Bach Knudsen *et al.*, 1993; Bach Knudsen *et al.*, 1991), diets with varying fibre content and fibre properties may lead to changes in the SCFA due to interactions between the diet and the gut microflora. The energy available to the host animal after microbial fermentation is the energy found in the SCFA and it provides up to 24% of the maintenance requirement for growing pigs (Yen *et al.*, 1991) and potentially even more for adult pigs.

The SCFA, and especially butyrate, have roles in connection with animal health (Jensen, 2001). Butyrate stimulates the development and growth of the large and small intestine by stimulating epithelial cell proliferation (Montagne *et al.*, 2003). Almost all SCFA are completely absorbed from the lumen of the GIT, leading to stimulation of resorption of water and sodium from the large intestine (Montagne *et al.*, 2003), thus reducing the risk of diarrhoea. Furthermore, SCFA are capable of promoting the proliferation of beneficial bacteria species, which can inhibit the development of some pathogenic species (Bauer *et al.*, 2006).

The effect of DF on epithelial morphology and cell turnover is variable, and depends on the level and physico-chemical properties of the DF in the diet, the duration of ingestion, the animal species and age, and the site in the intestinal tract (Montagne *et al.*, 2003). In growing pigs, inclusion of 10% wheat straw in a low-fibre diet has been found to result in longer villi and deeper crypts in the jejunum and ileum, and increased cell division and crypt depth in the large intestine (Jin *et al.*, 1994). In contrast, the fibre concentration has no influence on morphological characteristics, and the mitotic counts in the small intestine are lower in pigs fed a high-fibre diet (Hedemann *et al.*, 2006). Feeding a diet containing high viscosity carboxy-methyl cellulose reduces the villus height and increases the crypt depth, whereas feeding low viscosity carboxy-methyl cellulose results in villus elongation (McDonald *et al.*, 2001). There are also conflicting results on the effect of soluble fibre on the intestinal morphology of growing pigs (Glitsø *et al.*, 1998).

It is well known that when pigs are fed a high-fibre diet, the development of the GIT, especially the large intestine, increases relative to that of pigs on a low-fibre diet (Len *et al.*, 2009a; Len *et al.*, 2009b; Freire *et al.*, 2003; Freire *et al.*, 2000). The increase in GIT size is probably due to the prolonged presence of fibre in the gut stimulating an increase in mucosa weight and hypertrophy of the gut, which facilitates the development of bacterial mass (Eastwood, 1992). Alternatively, the increase in GIT size could be due to the production of SCFA, which stimulate epithelial cell proliferation, resulting in growth of the intestine. Source of fibre also influences on the length and weight of the intestine (Len *et al.*, 2009a; Freire *et al.*, 2000), and may be related to fibre properties such as the ratio between soluble and insoluble fibre (Bach Knudsen & Jørgensen, 2001; Freire *et al.*, 2000).

2.7 Dietary fibre in relation to gut microbiota

Dietary fibre that escapes digestion in the upper part of the GIT is potentially available for bacterial fermentation in the large intestine (Jensen, 2001). As ingested feed remains in the stomach only a short period of time, the pig as a non-ruminant has a smaller population of micro-organisms in the stomach than in the lower parts of the digestive tract, leading to limited microbial activity. In the lower part of the small intestine and particularly in the large intestine, an increased number of micro-organisms can be found. The total number of bacteria in the pig GIT ranges from 10^7 to 10^9 viable bacteria per gram digesta in the stomach and small intestine, and from 10^{10} to 10^{11} viable bacteria per gram digesta in the large intestine (Jensen & Jørgensen, 1994; Bach Knudsen *et al.*, 1993).

Microbial growth depends on the availability of substrates that can be metabolised (Wenk, 2001). The NSP are the main energy source for microbial fermentation in the large intestine (Bach Knudsen & Jensen, 1991; Bach Knudsen *et al.*, 1991), and the amounts and chemical and structural composition of the DF are important factors for the microbial activity in the digestive tract. In a study by Jensen & Jørgensen (1994), greater microbial activity was found in the stomach, caecum and colon in pigs fed a high-fibre diet than in pigs fed a low-fibre diet. Moreover, the amount of digested carbohydrates in the large intestine is correlated to microbial activity (Bach Knudsen *et al.*, 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).

2.8 Dietary fibre and pig breed

Effects of genotype on the digestibility of nutrients in high-fibre diets have been reported (Len *et al.*, 2009a; Len *et al.*, 2009b; Borin *et al.*, 2005; Ndindana *et al.*, 2002; Fevrier *et al.*, 1992). The results indicate that indigenous pigs can digest fibrous diets better than improved pig breeds. This has been attributed to the fact that indigenous pigs have higher digestive capacity and higher microbial activity in the hindgut than improved pigs (Freire *et al.*, 2000; Jørgensen *et al.*, 1996). Freire *et al.* (2003) found that the native Alentejano breed in Spain had a higher digestibility of fibrous diets than an improved breed (Duroc x Landrace) and attributed this to a better adaptation of the enzymatic activity, namely xylanase and cellulase, of the caecal microflora to degrade the cell wall constituents of the diets. In contrast, Ly *et al.* (1998) found that Creole indigenous pigs in Cuba did not have better digestibility of high fibre-diets than improved pigs.

Fermentation of fractions of NSP by pig breeds may differ due to differences in the composition of the microbial populations in the large intestine, resulting in different volatile fatty acid patterns (Morales *et al.*, 2002). Moreover, the effect of breed on digestibility also depends on DF level, because in some breeds this effect is only seen when they are fed a high-fibre diet (Fevrier *et al.*, 1992; Kemp *et al.*, 1991).

Differences in digestive ability between pig breeds may also be due to the size of the GIT and digesta transit time in the gut. It has been shown that local pig breeds have greater GIT size when expressed relative to body weight (BW) compared with improved breeds, resulting in higher digestibility of dietary components, in particular when pigs are fed a high-fibre diet (Len *et al.*, 2009a; Len *et al.*, 2009b; Freire *et al.*, 2003; Freire *et al.*, 2000). Pigs with heavier, longer and larger GIT (relative to their BW) usually have longer retention time of digesta in the GIT (Guixin *et al.*, 1995). This should contribute to more efficient digestion due to longer contact between digesta, digestive enzymes and absorptive surfaces.

3 Summary of materials and methods

3.1 Location

The studies described I-IV were carried out at the Station of Research and Testing of Animal Feed, National Institute of Animal Sciences (NIAS), Hanoi, Vietnam, and were approved by the Animal Science Committee of NIAS.

3.2 Experimental feeds

Paper I: A total of 64 samples, comprising RB, CR, BG, TFR, SBM, CC, SPV and WSP were collected during two years for chemical analysis and assessment of WHC.

Paper II: In the diets used, CR and SPV were the main fibrous ingredients, and were milled with different screen sizes (1.0 mm and 3.0 mm diameter). The diets were calculated to contain around 220 g NDF/kg DM in the first period (post-weaning) and 300 g NDF/kg DM in the second period (growing). The experiment had two diets containing either CR or SPV, with small and large fibrous feed particle size and with or without a supplementation of an enzyme mixture. The enzyme mixture included α-amylase (from *Bacillus amyloliquefaciens,* activity 788 U/g), β-glucanase (from *Aspergillus aculeatus,* activity 5602 U/g), cellulase (from *Trichoderma reesei,* activity 9007 U/g) and protease (from *Bacillus subtilis*, activity 922 U/g)

Papers III and IV: Three experimental diets were used: one low-fibre diet (LF), containing around 200 g NDF/kg DM, and two high-fibre diets (HF-CR and HF-BG), containing around 270 g NDF/kg DM. The high-fibre diets contained CR and BG as fibre sources.

All the experimental diets were based on maize meal, fish meal, SBM, full fat soybean, soybean oil, RB and CR, and SPV or BG as fibre sources. The feeds were in mash form. Celite was included as an indigestible marker and was top-dressed on the diets (30 g/kg as fed)

3.3 Animals and experimental design

Before the experiment started, all animals were vaccinated against hog cholera, pasteurellosis, pneumonia and paratyphoid. The pigs were kept individually in concrete floored pens (1.8 m x 0.8 m), fully covered with wooden slats, in an open-sided house. The pens and the troughs were cleaned every day. The animals had free access to feed and water.

A summary of the experimental designs used in Paper II, III and IV is shown in Table 1.

Table 1. *Summary of experimental design in Papers II, III and IV*

	Paper II	Paper III and IV
Experimental design	Completely randomised 2 x 2 x 2 factorial design, two fibre sources (CR or SPV), with two particle sizes (small and large) and without or with multi-enzymes, and 5 replicates.	Completely randomised 2 x 3 factorial design, with two breeds (MC and LY) and three diets (LF, HF-CR and HF-BG), and 6 replicates.
Initial body weight of pigs	At 30 days of age, the pigs weighed 8.0 ± 0.5 kg	At 60 days of age, the MC pigs weighed 9.5 ± 0.4 kg and of the LY pigs 16.5 ± 0.4 kg.
Experimental time	60 days	27 days

3.4 Measurements and data collection

Papers II and III: Feed intake and refusals were recorded daily at around 08.30h. The animals were weighed in the morning before feeding at the beginning and at the end of each experimental period. Growth rate and feed conversion ratio (FCR) were calculated.

During the last five days of the experiment, the faeces was collected from individual pigs in the morning and stored at -4^{o}C. At the end of the study, all five faecal samples collected from each pig were pooled, mixed and sampled for analysis.

Paper III: On day 20 of the experiment, digesta retention time of liquid and solids were estimated on four selected pigs in each treatment. The pigs were simultaneously given a single dose in the morning meal of chromium (Cr) mordanted NDF (500 mg) from rice bran as a solid marker and Co-EDTA (500 mg) as liquid marker, respectively. The Cr-mordanted rice bran and Co-EDTA were prepared according to Udén *et al.* (1980). The Cr content of the mordanted rice bran was 40 g/kg. Faecal samples were collected three times per day during three days.

In Papers III and IV, at the end of experiment, after a 4-hour fasting period in the morning, the pigs were injected with Thiopental (20 mg/kg body weight, BW), exsanguinated and immediately eviscerated. The GIT was removed and segmented into stomach, small intestine, caecum and colon plus rectum. The removal of GIT started from the anus, using scissors to remove each segment in turn (rectum, colon, caecum, small intestine and stomach).

Segments of full and empty digestive tract were weighed to calculate digesta weight, and the length of each segment of intestine was recorded. Digesta was collected from the stomach, ileum (about 100 cm of small intestine before the ileo-caecal ostium), caecum and colon (about 50 cm beyond the caecal-colon junction), and immediately transferred to plastic jars, frozen at -20^{o}C and kept frozen until preparation for chemical analysis (Paper III), and bacteria counts and gut environment (Paper IV).

The enumerative experiment on bacteria in digesta (Paper IV) was carried out by culture methods using selective media. Digesta samples were diluted with sterile 0.9% NaCl solution. For enumeration of the total number of *E. coli*, the suspensions were spread on EMB (Eosin Methylene Blue) agar (Difco) and incubated under aerobic conditions at 37^{o}C for 24 h. For total number of lactic acid bacterial (LAB), the suspensions were spread on MRS (de Man, Rogosa and Sharp) agar (Merck), and incubated at 37^{o}C for 48 h under anaerobic conditions.

Sections of the duodenum (approximately 10 cm from stomach sphincter), the jejunum (5.5 m from stomach sphincter) and the ileum (10 cm prior to the ileo-caecal orifice) were excised and opened along their length at the mesenteric border and fixed in 10% formaldehyde solution and embedded in paraffin wax. These tissue samples were cut in transverse section in 5µm thick slices and were stained with haematoxylin and eosin. Villous height and width, and density of crypts were measured at 50x magnification using a Zeiss Axioplan 2 microscope and image-analyzing software KS 400 2.0 (Koltrol Eletronic, Munique, Germany). A total of 3 slides were cut for each tissue sample and villus height and width were evaluated on 10 well-oriented villus on each slide from ileum samples.

3.5 Data calculations

In Paper III, the data on faecal marker concentration (C) were first fitted using TableCurve^{TM} 2D (Jandel Scientific, 1996) to a two-pool model of the function:

$C= A + B(k_1(e^{-k_1 t} - e^{-k_2 t}))/(k_2 - k_1)$,

where A, B and k are constant and t is time (h).

The MRT was then calculated according to Thielemans *et al.* (1978):

$MRT = \sum^{n}_{i=1} t_i C_i \Delta t_i / \sum^{n}_{i=1} C_i \Delta t_i$,

where t_i is the time elapsed (h) since dosage of marker to the mid-point in the i^{th} collection interval, C_i is the concentration of marker (>0) excreted in the i^{th} sample, at time t_i over a time interval Δt_i set at 0.1h.

The size of the GIT (Paper III) was expressed as weight (g/kg) or length ($cm/kg^{0.33}$) relative to empty BW (EBW). The EBW was calculated as live BW minus the digesta weight of stomach, small intestine, caecum and colon + rectum.

The digestibility of the diets (Papers II, III) at each sampling site was calculated using the indicator technique (Sauer *et al.*, 2000) according to the equation:

$CAD_D = (1 - (DC_F * AIA_D / DC_D * AIA_F)) * 100$

where CAD_D is the coefficient of apparent digestibility of dietary components in the assay diet; DC_F the dietary component concentration in ileal digesta or faeces (g/kg DM); AIA_D the acid insoluble ash concentration in the assay diet (g/kg DM); DC_D the dietary component concentration in the assay diet (g/kg DM); and AIA_F the acid insoluble ash concentration in ileal digesta or faeces (g/kg DM).

3.6 Chemical analysis

All samples were dried at 60°C and milled through a 1-mm sieve before analysis. Dry matter (967.03), CP (984.13), ether extract (EE) (920.39), CF (962.09), acid insoluble ash (942.05) and ash (942.05) were analysed according to standard methods (AOAC, 1990), and CP content was calculated as nitrogen x 6.25. Neutral detergent fibre was determined by the method of Van Soest *et al.* (1991). Amino acids were determined by HPLC using an ion exchange column (AQ, 1990). Gross energy (GE) was determined with a bomb calorimeter (Model: C2000 Basic – IKA Co., Staufen, Germany). Total starch content was determined using the Megazyme total starch assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland) (Paper II) and was analysed by an enzymatic method (Larsson & Bengtsson, 1983) (Papers I, III, IV). Concentration of acetic, propionic, butyric and lactic acids in digesta samples from different segments was determined by HPLC (Shimadzu, Japan) (FCAC, 2002) (Paper IV).

Total, soluble and insoluble NSP and their constituent sugars were determined as alditol acetates by GLC for neutral sugars, and by a calorimetric method for uronic acids using a modification of the Uppsala method (Theander *et al.*, 1995), as described by (Bach Knudsen, 1997). Klason lignin was determined as the 12 M H_2SO_4 insoluble residue. Total DF is the sum of Klason lignin and total NSP (Papers I-IV).

3.7 Statistical analysis

The data were analysed using Minitab Software, version 13.31 (Minitab, 2000).

Data on chemical composition of individual feedstuffs was described by mean values and their standard deviation using descriptive statistics. The variation between years within the individual feedstuffs was analysed by one-way analysis of variance. Mean values were considered differently at a P-value <0.05. The correlation between WHC and soluble non-cellulosic polysaccharides (NCP) in the whole data set was analysed using linear regression analysis (Paper I).

The General Linear Model procedure was used for analysis of variance (ANOVA) of independent and interaction factors. Treatment means which showed significant differences at the probability level $P<0.05$ were compared using Tukey's pair-wise comparison procedure (Papers II, III, IV).

4 Summary of results

4.1 Chemical composition and water holding capacity of green plants and agro-industry co-products

The content (g/kg DM) of CP, EE and total NSP varied between feedstuffs and ranged from 21 to 506 for CP, from 14 to 118 for EE and from 197 to 572 for NSP. Cassava residue had a high starch content (563 g/kg DM), while SPV, WSP, CC and SBM had a high content of sugars (63-71 g/kg DM). The content of individual neutral sugars varied between feed ingredients, with the highest content of arabinose, galactose and glucose in TFR, the highest content of xylose in BG and the highest content of mannose in CC. The content of uronic acid was high for CR, TFR, SPV and WSP (57-88 g/kg DM).

The content of soluble NCP was positively correlated (R^2 = 0.82) to WHC. The content (g/kg DM) of CP, NDF, neutral sugars, total NSP, total NCP, soluble NCP and total DF in TFR, WSP and CC varied ($P<0.05$) between years.

4.2 Growth performance and nutrient digestibility of pigs: effect of fibre level, fibre source and breed

Fibre source did not affect DM intake (DMI) ($P>0.05$) in any of the periods (Paper II). However, fibre source and fibre level had effects on DMI (Paper III), with pigs fed diet LF having the lowest DMI ($P<0.001$), followed by the pigs fed diet HF-CR and diet HF-BG (Table 2).

The diet containing CR supported faster growth and better feed efficiency in post-weaning and growing periods than the diet containing SPV ($P<0.001$) (Paper II). However, in Paper III, the ADG was similar for the fibrous diets containing CR and BG ($P>0.05$), while pigs fed diet HF-CR had lower FCR than pigs fed diet HF-BG. In general, the ADG was higher ($P<0.01$) and the FCR lower ($P<0.001$) in pigs fed the low-fibre diet than in pigs fed the high-fibre diets (HF-CR and HF-BG) (Paper III).

Table 2. *Effect of fibre level and fibre source on growth performance of pigs*

	Diet	Post-weaning (30-60 days of age)			Growing (60-90 days of age)		
		DMI[a]	ADG[b]	FCR[c]	DMI	ADG	FCR
Paper II	CR	559	422	1.33	1289	597	2.16
	SPV	550	385	1.44	1271	540	2.36
Paper III[d]	LF	-	-	-	814	449	1.83
	HF-CR	-	-	-	835	386	2.21
	HF-BG	-	-	-	899	375	2.45

[a] DMI, g/day; [b] ADG, g; [c] FCR, kg feed/kg gain; [d] Values in Paper III were the mean values from Mong Cai and Landrace x Yorkshire pigs.

Table 3. *Effect of fibre level and fibre source on ileal and total tract apparent digestibility of pigs*

Item	Paper I		Paper II[a]		
	CR	SPV	LF	HF-CR	HF-BG
At ileum (Growing, 60-90 days of age)					
OM	-	-	0.73	0.70	0.67
CP	-	-	0.76	0.72	0.69
Total NSP	-	-	0.33	0.47	0.29
Cellulose	-	-	0.26	0.35	0.35
Total NCP	-	-	0.41	0.56	0.34
DF	-	-	0.31	0.45	0.28
GE	-	-	0.71	0.68	0.66
At total tract (Post-weaning, 30-60 days of age)					
OM	0.82	0.78	-	-	-
CP	0.81	0.76	-	-	-
CF	0.52	0.49	-	-	-
NDF	0.57	0.54	-	-	-
GE	0.81	0.77	-	-	-
At total tract (Growing, 60-90 days of age)					
OM	0.85	0.82	0.85	0.82	0.77
CP	0.84	0.80	0.84	0.82	0.77
CF	0.56	0.53	-	-	-
NDF	0.63	0.60	-	-	-
Total NSP	-	-	0.51	0.76	0.62
Cellulose	-	-	0.39	0.69	0.64
Total NCP	-	-	0.62	0.81	0.66
DF	-	-	0.45	0.67	0.53
GE	0.82	0.80	0.83	0.80	0.76

[d] Values in Paper III were the mean values for Mong Cai and Landrace x Yorkshire pigs.

In both post-weaning and growing periods, the TTAD of dietary components and fibre fractions was higher for the CR diet than the SPV (Paper II) and the BG (Paper III) diet ($P<0.05$), with the exception of cellulose ($P>0.05$) (Table 3). In Paper III, the ileum apparent digestibility (IAD) of OM, CP, GE and cellulose was not significantly different between diets HF-CR and HF-BG ($P>0.05$), while pigs fed diet HF-CR had higher IAD of total NSP, total NCP, soluble NCP and DF than pigs fed diet HF-BG ($P<0.005$).

Increased fibre level (diets HF-CR and HF-BG) resulted in decreased IAD and TTAD ($P<0.05$) of OM, CP and GE (Paper III). Overall and within breed, there was a linear decrease ($P<0.01$) in the TTAD of OM, CP and GE with increasing NDF, DF and total NSP level in the diet (Paper III).

In Paper III, DMI (expressed as g/kg $BW^{0.75}$) was higher ($P<0.001$) for MC pigs than for LY pigs, while ADG was lower ($P<0.001$) and FCR was higher ($P<0.01$) for MC pigs than for LY pigs. The IAD and TTAD of OM, CP, GE, total NSP, cellulose, total NCP and DF differed between breeds ($P<0.05$), with the exception of the TTAD of soluble NCP ($P>0.05$). Generally, MC pigs showed greater digestibility than LY pigs.

4.3 Growth performance and nutrient digestibility of pigs: effect of fibrous feed particle size and enzyme supplementation

As shown in Paper II, in the post-weaning period, enzyme supplementation had a positive effect on ADG and FCR ($P<0.05$), while reducing particle size of fibrous ingredients improved only ADG ($P<0.05$). However, there were no effects of enzyme addition and fibrous feed particle size on performance in the growing period ($P>0.05$).

In the post-weaning period, particle size and enzyme addition affected the TTAD of all dietary components ($P<0.05$), with the exception of EE ($P>0.05$). A reduction in fibrous ingredient particle size and supplementation with multi-enzymes improved the TTAD of CP, CF, NDF, OM and GE ($P<0.05$). The TTAD of CP and NDF showed a particle size by enzyme supplementation interaction ($P<0.05$), such that enzyme supplementation had no effect on the TTAD of CP and NDF for the smaller particle size, while there were considerable improvements for the larger particle size.

In contrast to the TTAD in the post-weaning period, there were no differences in the TTAD of dietary components between large and small particle size fibrous ingredients ($P>0.05$). Enzyme supplementation had no effect on the TTAD of EE, CF, OM and GE ($P>0.05$), whereas the TTAD of CP and NDF was improved considerably by enzyme addition ($P<0.05$).

4.4 Mean retention time and its relation to dry matter intake and total tract digestibility

The MRT (Paper III) of solid ($P<0.001$) and liquid ($P<0.05$) markers were higher in MC than LY pigs. Pigs fed diet LF had the highest MRT for solids and liquid ($P<0.001$). For solids this was followed in descending order by the MRT for pigs fed diet HF-CR and diet HF-BG ($P<0.01$), while the MRT for liquid was comparable between diets HF-CR and HF-BG.

In both MC and LY pigs, the MRT of solids was linearly related to DMI (g/kg $BW^{0.75}$) ($R^2 \geq 0.70$, $P<0.05$) and to the TTAD of OM, CP and GE ($R^2 \geq 0.59$, $P<0.05$).

4.5 Gastrointestinal tract development, gut morphology, gut environment and bacterial counts: effect of fibre level, fibre source and breed

Gastrointestinal tract development (Paper III): Breed had an impact ($P<0.05$) on the weight and length of the whole gut (sum of all segments), with greater weight and length in LY than in MC pigs. However, the scaled weight (stomach, small intestine, caecum and colon + rectum) of individual segments were greater ($P<0.05$) in MC than in LY pigs, while the scaled length of individual segments (small intestine, caecum and colon + rectum) was greater ($P<0.01$) in LY than in MC pigs.

Diet had an impact ($P<0.05$) on the scaled weight of the whole gut (sum of all segments), with higher values for diets HF-CR and HF-BG than for diet LF. The scaled weight (stomach, caecum and colon+rectum) of individual segments was greater ($P<0.05$) for pigs fed diets HF-CR and HF-BG than for pigs fed diet LF, with exception of the scaled weight of small intestine ($P>0.05$). The scaled length of colon + rectum was higher for diet HF-CR than for diet LF, while the scaled length of small intestine, caecum and total intestine was unaffected ($P>0.05$) by diet.

Gut content was higher for MC pigs than for LY pigs ($P<0.01$). The pigs fed diets HF-CR and HF-BG had greater gut content than the pigs fed diet LF ($P<0.01$).

Gut morphology, gut environment and bacterial counts (Paper IV): Crypt density and villus width in the duodenum and jejunum were not affected by diet ($P>0.05$), whereas increased DF level in the diet resulted in a decrease in the crypt density and an increase in the height and width of villi in the ileum ($P<0.05$).

Lactic acid bacteria counts in the colon and *E. coli* numbers in the stomach were not affected by the diet ($P>0.05$). However, the LAB counts in the stomach and ileum were lower for pigs fed diet LF than for the pigs fed diets HF-BG and HF-CR ($P<0.001$), whereas the reverse was true for the *E. coli* counts in the ileum ($P<0.01$). The *E. coli* counts in the colon were similar for diets LF and HF-BG ($P>0.05$), but lower for diet HF-CR ($P<0.001$). There was an interaction between diet and breed on the counts of LAB in the ileum ($P<0.05$), with increased fibre level in the diet having an effect on LAB count for MC pigs but not for LY pigs.

The DF level and fibre components also had effects on the concentration of total and individual organic acids (OA) in all intestinal segments ($P<0.05$), except for the concentration of lactic acid in the caecum ($P>0.05$). The proportions of lactate and total SCFA and individual SCFA in different gut segments were not affected by the diet ($P>0.05$), with the exception of the proportions of lactate and SCFA in the ileum ($P<0.01$).

In the ileum, there was a diet by breed interaction for the concentration of propionic acid ($P<0.05$), such that the difference in propionic acid concentration between diets HF-CR and HF-BG was higher in MC pigs than in LY pigs.

The diet had an effect on pH at all sites in the gut ($P<0.05$). The lowest pH value at all intestinal sites was found for diet HF-CR.

Pig breed had an effect on crypt density and the height and width of villi in the ileum ($P<0.05$), while there were no significant differences in crypt density, villus height in the duodenum and jejunum and villus width in the duodenum between MC and LY pigs ($P>0.05$). The LAB counts in stomach and ileum were greatest, and *E. coli* counts in ileum were lowest, in MC pigs ($P<0.05$). The concentration of total OA in ileum, caecum and colon was greatest ($P<0.05$) and the pH was lowest ($P<0.05$) in MC pigs.

5 General discussion

5.1 Chemical characterisation and water holding capacity of fibrous feeds

In general, the contents of CP, EE and ash in green plants and agro-industry co-products were similar to those previously reported from Vietnam (NIAH, 2001) and other tropical countries (Lekule *et al.*, 1990; Gohl, 1981). However, for some of the feed ingredients (*e.g.* TFR, CC and WSP) there was variation between years.

The agro-industry co-products represent a very heterogeneous group of plant residues. They come from different plant families of different botanical origin (grain, tubers, roots, fruits, culms, shells and hulls) and during processing they are exposed to a wide variety of physical and chemical treatments for extraction of the economically important components (Serena & Bach Knudsen, 2007). Thus, the chemical composition of the co-products depends largely on the botanical origin of the plants, time of harvesting and type of processing applied. Brewer's grain in Paper I was higher in CP and lower in total NSP than reported by Serena & Bach Knudsen (2007). Despite the high arabinan content in BG, xylan and glucan were the main polysaccharides found in this material, in agreement with earlier studies (Serena & Bach Knudsen, 2007; Mussatto & Roberto, 2006). Protein is highly concentrated in BG because most of the starch is removed during mashing (Mussatto & Roberto, 2006).

More than 60% of the nutrient components of CC were analysed as NDF. This indicates that the major component of coconut carbohydrate is in the form of DF. The total NSP content in CC in Paper I was lower than reported by Bach Knudsen (1997). Saittagaroon *et al.* (1983) and Bach Knudsen (1997) reported that mannans are the major polysaccharides in CC representing 61 to 73% of total NSP. Supporting this finding, the present study showed that individual neutral sugars in the NSP fraction of CC had a predominance of mannans (70% of total NSP) and with about 15% glucose and trace amounts of other neutral sugars (rhamnose, fucose, arabinose and xylose).

Starch is not botanically present in the outer pericarp layers, but because of endosperm breakage during milling it appears in RB. The quantity of starch varies according to amount of breakage and degree of milling. The RB analysed in Paper I had a starch content of 219 g/kg DM, which was in range (100-550 g starch/kg DM) reported in previous studies (Bor *et al.*, 1991; Saunders, 1986). Free sugars in RB are concentrated in the aleurone layer and the values reported in Paper I are low compared with values (3-5%) reported earlier (Saunders, 1986). However, the total NSP and cellulose content in RB were in line with data by Saunders (1986).

Due to different processing method, TFR is lower in CP and sugars, and higher in EE and NSP fractions than SBM. Cellulose and xylans, which are the major NSP in cereal grains, are only found in the hulls or husks of most grain legumes (Choct, 1997). The major NSP in the cotyledon of legumes are pectic polysaccharides. The starch content in TFR and SBM was low and was also lower than reported by others (Bach Knudsen, 1997; Lekule *et al.*, 1990).

Cassava residue was low in protein and high in fibre and starch. The high content of starch (560 g/kg DM) could be due to entrapment in the cellulose-hemicellulose matrix (Divya Nair *et al.*, 2011). The fibre fraction was high in uronic acids and more than 50% of the total NCP fraction was soluble. A significant amount of the total NSP (306 g/kg DM) was also detected and the predominant sugars in the NSP fraction were glucose, galactose and xylose. The present results (Paper I) are similar to those reported by Kosugi *et al.* (2009).

The water spinach and SPV analysed in Paper I were high in CP and fibre (NDF and total NSP). However, the CP values were lower and NDF values were higher than those reported by Dung *et al.* (2002). This was probably due to differences between studies in time of harvest and in the sampling protocol (*i.e.* the cutting height of WSP and the length of SPV). The content of total sugars (63-71 g/kg DM) was high in both WSP and SPV, while the starch content was low (13-27 g/kg DM). The total NSP fraction was composed of 38% cellulose and 62% total NCP in WSP, and 46% cellulose and 54% total NCP in SPV. Woolfe (1992) reported that the NSP fraction in SPV was composed of 34% cellulose and 66% total NCP and that the contents (DM basis) of starch and sugars in SPV were 3.6-3.7% and 8.8-13.0%, respectively, which was higher than in Paper I.

The WHC of a feedstuff is the amount of water retained in a feedstuff when water is in excess (McConnell *et al.*, 1974), and thus is a measure of its ability to immobilise water within its matrix (Singh & Narang, 1991). The WHC is usually obtained by centrifugation at high speed (Robertson & Eastwood, 1981; McConnell et al., 1974) and sometimes by filtration (Robertson & Eastwood, 1981). The WHC values reported in Paper I were obtained by filtration. The filtration method is robust and easy to perform, and has been suggested to match more closely the conditions likely to be found in the gastro-intestinal tract and should resemble normal physiological conditions (Robertson & Eastwood, 1981). The WHC of plant material and agro-industry co-products analysed in Paper I ranged from 2.0 to 9.5 kg/kg DM. There was also a strong correlation between content of soluble NCP and WHC in the samples analysed. This could be due to the occurrence of more gaps within the cell matrix that can retain excess water in feed ingredients which are high in soluble NCP. This suggests that it is the polysaccharide content of the plant that determines the ability to hold water. Similarly, Serena & Bach Knudsen (2007) have shown that the soluble NCP fraction in co-products from vegetable food and agro-industries is linearly related to selected hydration properties, such as swelling and water binding capacity.

5.2 Effect of dietary fibre source and level on performance, digestibility, mean retention time, size of GIT, gut morphology, gut environment and bacterial count

The use of CR or SPV in the diet (Paper II) resulted in similar DMI. However, fibre source and fibre level had effects on DMI (Paper III), with pigs fed diet LF having the lowest DMI, followed by pigs fed diet HF-CR and diet HF-BG. Len *et al.* (2009a) found no difference in DMI of pigs fed low-fibre and high-fibre diets based on RB, SPV and CR. The lower DMI (Paper II) on diet HF-CR compared with diet HF-BG could be related to the higher WHC of CR compared with BG (Paper I). The WHC of a feedstuff is related to its bulking properties and affects the feed intake capacity (Kyriazakis & Emmans, 1995).

The effect of DF on pig performance depends on fibre level, fibre source and the age of the animals. A diet containing CR supported higher ADG in both post-weaning and growing periods compared with a diet containing SPV (Paper II), whereas ADG was similar between the diets containing CR and BG in growing pigs (Paper III). This could be because the same level of CR and SPV (20% and 35% of diet in post-weaning and growing period, respectively) was included in the diet in Paper II, whereas in Paper III the level of CR and BG in the diets was used different (35% CR and 30% BG of diets). Besides, the chemical properties were different between SPV and BG used in Paper II and III, especially Klason lignin content (16% of DM for SPV and 11% for BG) (Paper I). Similar to Paper II, Shriver *et al.* (2003) showed that performance of finishing pigs was similar between diets containing soybean hulls (insoluble diet) and dried beet pulp (soluble diet). In general, the fibrous diets had a negative impact on ADG and FCR (Paper III), and the higher DMI on the high-fibre diets could not fully compensate for the lower energy utilisation and reduced nutrient availability. Increased fibre level in the diet has been shown to reduce total tract energy digestibility and to result in a higher proportion of digestion taking place in the large intestine (Högberg & Lindberg, 2004b). According to Jørgensen *et al.* (1996), increased DF level results in less absorbed energy being derived as monosaccharides from the small intestine, and relatively more of the absorbed energy being derived from fermentation by bacteria as volatile fatty acids and lactic acid in the hind-gut. This reduces the net energy content of the diet more than expected based on reduced digestible energy content.

Fibre source had an effect on the TTAD of pigs in Papers II and III. There was a higher TTAD of OM, CP and GE for the CR diet than for the diets SPV and BG. Differences in lignin content and soluble fibre content between the diets could be the reason for the difference in digestibility. For example, Len *et al.* (2009a) and Dung *et al.* (2002) found that a diet containing CR resulted in higher digestibility of nutrients than diets containing SPV and RB. However, the different solubility and lignification properties of diets HF-CR and HF-BG did not have any impacts on the IAD of OM, CP, GE and cellulose. It is possible that the difference in soluble DF between diets HF-CR and HF-BG (29 compared with 47 g/kg DM) was too small to have an effect on the IAD. In contrast, Serena *et al.* (2008) indicated that there was a difference in the IAD of CP between fibrous diets with contrasting fibre properties (such as soluble fibre).

The results obtained in Paper III are in good agreement with previous findings that feeding a high-fibre diet reduces ileal or/and TTAD of OM, CP and GE (Len *et al.*, 2009b; Len *et al.*, 2007; Högberg & Lindberg, 2004a; Högberg & Lindberg, 2004b). This could be due to specific properties of fibre, such as high viscosity or high WHC (Whittemore *et al.*, 2003; Kyriazakis & Emmans, 1995), and the related negative effects of high fibre content on digesta retention time in the GIT (Wilfart *et al.*, 2007; Le Goff *et al.*, 2002; Jørgensen *et al.*, 1996) and on endogenous nitrogen losses (Schulze *et al.*, 1994).

The decrease in TTAD of OM, CP and GE was linearly related to the dietary content of DF, total NSP and NDF. This was in agreement with results from other studies where fibrous feed ingredients had been included in the diet (Len *et al.*, 2007; Högberg & Lindberg, 2004b). However, the study by Högberg & Lindberg (2004b) showed a negative impact of fibre level on the digestibility of dietary components at both ileum and total tract, while it was only seen at total tract in Paper III. This could be due to different chemical properties of the fibre sources used or to difference in fibre content among the diets used in each study.

Dietary fibre NSP increased the digestibility of fibre components in the total tract. The increase in digestibility after feeding a diet with a high NSP content may be due to an increased microbial activity stimulated by suitable substrate for microbial growth (Högberg & Lindberg, 2004b).

Retention time of digesta is assumed to be reduced when a diet contains a high level of fibre. This was confirmed by the results in Paper III. The reduction in retention time is mainly caused by the higher viscosity and WHC of the digesta, and higher quantities of digestive juice being secreted when feeding a high-fibre diet (Le Goff *et al.*, 2002; Freire *et al.*, 2000; Glitsø *et al.*, 1998; Stanogias & Pearce, 1985). The longer MRT was associated with higher digestibility of OM, CP and GE across diets, in agreement with previous studies (Kim *et al.*, 2007; Ravindran *et al.*, 1984). This could be due to the increased time available for enzymatic digestion and intestinal absorption. Moreover, Paper III showed that a slower passage rate reduced feed intake within pig breed, probably due to distension of GIT wall (Lepionka *et al.*, 1997), confirming earlier studies (Stanogias & Pearce, 1985; Castle & Castle, 1957).

The effect of fibrous diets on the size of the digestive tract was investigated in Paper III. Feeding a high-fibre diet increased the weight of the GIT of growing pigs, in good agreement with previous studies (Len *et al.*, 2009a; Freire *et al.*, 2003; Whittemore *et al.*, 2003; Freire *et al.*, 2000), while the scaled length of GIT was unaffected. However, fibre source had no effects on the weight and length of the GIT between diets HF-CR and HF-BG, which contradicted our starting hypothesis. This could be due to the small differences in soluble fibre content in our study.

The effect of DF on gut morphology is variable, and depends on the level and type of DF and the digestion site in the GIT (Montagne *et al.*, 2003). The villus height and width in the ileum were higher for the fibrous diets than for diet LF (Paper IV), which could be related to the higher LAB count with the high-fibre diets and the resulting higher concentration of SCFA in the ileum than on diet LF. The SCFA stimulate cell proliferation and cell growth in the gut. Thus, changes in epithelial morphology in the small intestinal induced by DF can be due to the trophic effect of SCFA (Montagne *et al.*, 2003). Greater villus width in pigs fed high-fibre diets has also been reported by others (Liu *et al.*, 2012; Martins *et al.*, 2010; Jin *et al.*, 1994).

Increasing fibre level resulted in increased LAB counts and decreased *E. coli* counts (Paper IV). In addition, OA and SCFA tended to be higher for the high-fibre diets compared with the low-fibre diet, while the reverse pattern was found for pH. This indicates that increased fibre level in the diet can be a mean of reducing the harmful effects of pathogens, by stimulating growth of LAB and production of OA. In an acidic environment, SCFA are capable of inhibiting the growth of some intestinal bacterial pathogens, and thus reducing the risk of pathogenic diarrhoea (Bauer *et al.*, 2006; Lidbeck & Nord, 1993). The highest LAB counts in stomach and ileum were found in the diet with a high content of soluble NSP (HF-CR), which suggests that this diet provided good substrate for LAB development. In contrast, Wang *et al.* (2003) found lower counts of LAB in a diet containing sugar beet pulp (soluble diet) than in a diet containing wheat bran (insoluble diet). However, it appears likely that the differences in the physico-chemical properties of DF sources (such as degree of branching between monosaccharides, monosaccharide composition of DF sources and WHC) and/or the entry rate of fermentable substrates into the different parts of the GIT influence the gut environment and microbiota (Pluske *et al.*, 2003). In Paper IV, the content of total OA, SCFA and individual SCFA and the pH in all intestinal sites were affected by DF. According to Awati *et al.* (2005), the structure and availability of substrates appear to be more important for the fermentation end-products rather than the microbial community presented. Furthermore, the pigs fed different diets in our study responded with different MRT of digesta (Paper III) and thus the rate of entry of digesta into the different intestinal segments could influence the fermentative capacity of pigs fed different experimental diets.

5.3 Effect of breed on performance, digestibility, mean retention time, size of GIT, gut morphology, gut environment and bacterial count

In general, MC pigs can digest nutrient, particularly fibre components, better than LY pigs (Len et al., 2009a; Len et al., 2009b; Len et al., 2007; Borin et al., 2005). The results in Paper III support this finding. It is generally believed that local pigs can utilise high-fibre diets better than exotic breeds, because they have been kept under poorer environmental conditions, feeding and management for a long time. Therefore, MC pigs should be better able to adapt to diets of low quality and high fibre content than exotic pigs (Rodríguez & Preston, 1996). Interestingly, MC pigs had longer MRT of digesta than LY pigs (Paper III). This may help improve the activity of microbiota in the GIT (Freire *et al.*, 2000), due to an increased time of contact between digesta and digestive enzymes and between digestion products and absorptive surfaces (Guixin et al., 1995). In Paper III, the IAD of dietary components was different between breeds, which was in contrast to Len *et al.* (2009b). This could be due to the age of the pigs used in the respective experiments. The pigs used in Paper III were growing pigs, while those used by Len *et al.* (2009b) were post-weaning piglets. It can be assumed that older pigs can utilise high-fibre diets better than post-weaned piglets due to their better developed GIT, and will therefore show a greater response to DF level.

As shown in Table 4, the difference between breeds in term of the TTAD of fibre components was higher than in the IAD of these components. Similar results were obtained by Len *et al.* (2009b) (Table 5). This is probably due to a larger microbial population in the hindgut and higher microbial activity, which is of particular importance for degradation of fibre fractions (Bach Knudsen & Hansen, 1991).

Table 4. *Summary of the effects of breed on digestibility and performance of pigs from Paper III*

	Growing pigs				Difference between MC and LY	
	IAD		TTAD		IAD	TTAD
	MC	LY	MC	LY		
OM	0.71	0.69	0.82	0.81	0.3	0.1
CP	0.74	0.7	0.83	0.79	0.4	0.4
GE	0.7	0.67	0.81	0.78	0.3	0.3
Total NSP	0.41	0.31	0.64	0.61	1	0.3
Cellulose	0.36	0.28	0.59	0.56	0.8	0.3
DF	0.4	0.28	0.58	0.53	1.2	0.5
ADG, g	-	-	337	469	-	-132
FCR, kg	-	-	2.27	2.05	-	0.22

Table 5. *Summary of the effects of breed on digestibility and performance of pigs from Len et al. (2009b)*

	Post-weaning pigs				Difference between MC and LY	
	IAD		TTAD		IAD	TTAD
	MC	LY	MC	LY		
OM	0.75	0.73	0.84	0.81	0.2	0.3
CP	0.74	0.73	0.81	0.78	0.1	0.3
GE	-	-	0.8	0.77	-	0.3
CF	0.17	0.17	0.51	0.45	0	0.6
NDF	0.21	0.2	0.53	0.48	0.1	0.5
ADG, g	-	-	197	293	-	-96
FCR, kg	-	-	1.91	1.56	-	0.4

In spite of higher nutrient digestibility (Paper III), MC pigs exhibited a lower growth rate and poorer feed-to-gain ratio than LY. This was in line with Len *et al.* (2009a) and Len *et al.* (2007), and can be explained by higher growth potential of LY pigs than MC pigs. Evaluation of the digestive tract of MC and LY pigs in response to fibrous diets in Paper III showed the weight and length of the GIT (expressed as g/kg EBW) differed between the two breeds, with higher GIT weight for MC pigs compared with LY pigs, confirming previous studies (Len *et al.*, 2009a; Freire *et al.*, 1998). This can be related to a higher intake and gut fill of MC pigs. However, the GIT (expressed cm/kg $EBW^{0.33}$) was longer for LY pigs than for MC pigs. A more developed gut in relation to EBW compared with the LY pigs supported the longer MRT in MC pigs (Paper III). Morales *et al.* (2002) reported differences in caecal and colon transit time between Iberian and Landrace pigs, with higher values for Landrace pigs.

The epithelial morphology (Paper IV) in the ileum was affected by pig breed, while there were no breed-related differences in the duodenum and jejunum. Barea *et al.* (2011) did not observe significant differences in villus length or crypt depth in the upper parts of the small intestine (*i.e.* duodenum and mid-jejunum) between Iberian and Landrace x Large White pigs. However, MC pigs had shorter villi, smaller villus width and greater crypt density in the ileum than LY pigs. The differences in gut morphology between MC and LY pigs could be due to differences in digesta transit time, LAB and gut fermentation. The majority of the digested nutrients are absorbed in the proximal small intestine. This, together with a rapid digesta passage and low microbial activity, results in less exposure to digesta components compared with the situation in the more distal small intestine.

There were interactions between breed and diet on LAB count and concentration of propionic acid in the ileum, with increased fibre level in the diet in combination with a high soluble fibre content having a greater impact on the LAB count and the concentration of propionic acid in the ileum of MC pigs than of LY pigs. This suggests differences in the gut microbiota activity and/or composition between MC and LY pigs. The concentrations of OA, lactic acid, SCFA and individual acids (acetate, propionate and butyrate) differed significantly between local (MC) and exotic (LY) pigs, in line with previous studies (Freire *et al.*, 2003; Morales *et al.*, 2002; Ly *et al.*, 1998). Furthermore, the LAB counts were higher and *E. coli* counts were lower in MC than in LY pigs.

5.4 Effect of feed particle size and enzyme supplementation on performance and digestibility

Larger particles provide less surface area per unit of mass for digestive enzymes to interact with their substrates than smaller feed particles (Mavromichalis *et al.*, 2000). Larger particle size thus require more time for complete digestion. However, the time available during digesta transit in the intestine is limited. Positive effects have been observed with reducing particle size of cereal grains for nursery pigs (Healy *et al.*, 1994), grower pigs (Oryschak *et al.*, 2002; Mavromichalis *et al.*, 2000; Owsley *et al.*, 1981), and lactating sows (Wondra *et al.*, 1995a; Wondra *et al.*, 1995b). Fine grinding of grain used in pig diets optimises performance and improves feed efficiency regardless of age. Here, grinding to achieve a smaller mean particle size improved the TTAD of most nutrients and performance in the post-weaning period (Paper II), but not in the growing period. The lack of effects of particle size on digestibility and performance in the growing period may be due to fibrous ingredients only representing 35% of the diet in Paper II.

Pigs do not produce endogenous enzymes to digest fibre. However, digestion of fibre can be achieved by enzymes provided in feeds or by enzymes produced by intestinal bacteria, which are abundant in the large intestine. In general, diets with high fibre levels are associated with reduced digestibility of nutrients. Therefore, supplementation of exogenous enzymes, especially fibre-degrading enzymes, to high-fibre diets in order to increase the efficiency of digestion can be considered a useful strategy to help animals utilise nutrients better. In the present study (Paper II), the mixture of enzymes used included mainly fibre-degrading enzymes (cellulase and β-glucanase), and improved the TTAD of nutrients and performance in the post-weaning period, but not in the growing period, except for the TTAD of CP and NDF. In general, the negative effect of high fibre levels in the diet on digestibility and performance is more pronounced in piglets than in growing pigs. Piglets have an immature digestive system, which may result in inadequate production of endogenous enzymes (Lindemann *et al.*, 1986), and less development of bacterial flora (Graham *et al.*, 1988). Indeed, Varel & Yen (1997) indicated that piglets have smaller populations of cellulolytic bacteria than older pigs when fed a high-fibre diet.

In Paper II, a particle size by enzyme interaction for the TTAD of CP and NDF was found in post-weaning pigs, where the TTAD of CP and NDF was improved by enzyme supplementation in the larger particle size fibrous feed. Similar results were obtained in earlier studies (Kim *et al.*, 2005; Oryschak *et al.*, 2002). In addition, enzyme supplementation (Table 6) had a larger effect than particle size on the TTAD of nutrients and on performance in the weaning period. This could indicate a limited capacity to digest dietary components that is age-related and is due to the stage of maturity of digestive functions.

Table 6. *The difference in digestibility and performance between small and large particle size or with and without enzyme supplementation in post-weaning pigs*

	CP	CF	NDF	OM	GE	ADG, g
Particle size						
Small	0.79	0.51	0.57	0.81	0.79	416
Large	0.78	0.49	0.55	0.80	0.78	391
Enzyme						
-	0.77	0.48	0.53	0.79	0.78	387
+	0.80	0.53	0.58	0.81	0.80	420
Difference						
Particle size	0.1	0.2	0.2	0.1	0.1	25
Enzyme	0.3	0.5	0.5	0.2	0.2	33

5.5 Feed hygiene and fibre-rich feedstuffs

It is well known that a wide range of agricultural products and their by-products, such as rice, cassava, soybean, maize, peanut and other legumes, are commonly found to be contaminated by mycotoxins during harvesting, drying, and storage. The impact of mycotoxins on animal health can be considerable, depending on the level consumed (Lawlor & Lynch, 2001). They may have negative effects on feed intake, performance and reproduction functions, and can cause decreased liver and kidney function and suppression of the immune system (Thieu, 2008; Akande *et al.*, 2006). In tropical countries with warm and humid climate conditions, contamination by aflatoxins and fumonisins is regularly detected in agricultural products (Devegowda *et al.*, 1998). Thieu et al. (2008) found that feedstuffs and pig complete feeds in Vietnam had very a high incidence of aflatoxins and zearalenone, but the mean concentrations and range were low, except in maize samples. To reduce the high incidence of mycotoxins, drying at harvesting time and suitable techniques and equipment for drying and storage should be considered.

6 General conclusions and implications

6.1 Conclusions

- There are marked differences in chemical composition and WHC between potential feed ingredients derived from green plants and agro-industry co-products. In particular, there are major differences in the composition and properties of the carbohydrate fraction of potential feed ingredients. It appears reasonable to assume that diet formulation to pigs can be improved if the variation in chemical composition, in particular the fibre fraction, is taken into account.
- Diet containing CR supports a higher TTAD of dietary components and better performance in post-weaning and growing pigs than diet containing SPV. A reduction in particle size can improve the TTAD of nutrients and growth performance in the post-weaning period, but not in the growing period. Addition of a multi-enzyme mixture to diets based on CR or SPV improves the TTAD of nutrients and growth performance in the post-weaning period. The improvement in TTAD is most pronounced when larger particle sizes of the fibrous ingredients are included.
- Dietary fibre level and soluble DF have an impact on the digestibility of nutrients, MRT and the morphology of the small intestine. This effect occurs in parallel with fibre-related effects on LAB and *E. coli* counts in the GIT, and on the gut environment. Increasing the fibre content in the diet increases gut size, but soluble DF does not affect gut size.
- Mong Cai pigs have a greater relative gut weight and content than LY pigs and a longer MRT, resulting in higher digestibility of dietary components. The MRT appears to be the major factor influencing the digestibility of dietary components across diets within pig breed.
- There are differences between pig breeds in small intestine morphology, counts of LAB and *E. coli* along the GIT and gut environment.

6.2 Implications and future research

The data presented in this thesis on the chemical composition (especially the fibre fractions) and WHC of green plants and agro-industry co-products, and the impact of fibre level and fibre source on digestibility and growth performance, can be of great value in practical diet formulation for pigs. Moreover, the data on the impact of enzyme supplementation and feed particle size on digestibility, growth performance and feed conversion can be applied to improve the utilisation of fibre-rich diets for pigs, particularly post-weaning pigs. It is recommended that soluble fibrous feed ingredients be used in diet formulation rather than insoluble fibrous feed ingredients.

The data obtained support the contention that it can be economically feasible for resource-poor pig farmers to use available and cheap fibre-rich feed ingredients. This can be particularly successful if combined with the use of indigenous breeds, such as the Mong Cai pigs, which appear to be better adapted to low quality feeds than exotic breeds in terms of development of the gastro-intestinal tract, mean retention time of digesta and gut environment.

The DF level and fibre properties can be used as a tool to manipulate the gut ecosystem in order to prevent enteric disease in pigs, in particular in diets for post-weaning piglets.

The impact of feeding high fibrous diets, based on available fibre-rich feedstuffs, on performance, health and production economy should be further studied in different pig breeds to evaluate the potential and to identify limitations.

More knowledge is needed about the impact of fibre level and fibre properties on nitrogen and phosphorus excretion and emissions of hydrogen sulphide, ammonia and greenhouse gases from pig manure.

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