FIBRE FERMENTATION IN THE PIG INTESTINE:
EFFECT ON METABOLITE PRODUCTION AND NITROGEN EXCRETION

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EXECUTIVE SUMMARY

Fine tuning a nutritional strategy by incorporating dietary fibre (DF) in pig diets can help to improve gut health. Fermentation of DF, especially the soluble fraction, in pig intestines yields short-chain fatty acids (SCFA) and lactic acid, which have been found to improve gut health by favouring the growth of health-promoting bacteria such as *Lactobacilli* and *Bifidobacteria*, at the expense of pathogenic ones like *Clostridium* or *Salmonella*, which may enhance the health of host species. The presence of fermentable fibre in the pig diet can also contribute to reducing nitrogen (N) excretion, which can have a positive impact on the environmental footprint, one of the main concerns of a modern commercial pork production.

The overall objective of this thesis project was to evaluate the fermentation characteristics of a selection of feedstuffs in the pig intestines and their potential impact on the gut environment and nitrogen excretion. The evaluation was performed by executing two projects using both *in vitro* and *in vivo* studies.

The first project focused on the fermentation characteristics of hulless barley in comparison to hulled barley and oats and their effects on the gut environment, especially the production of fermentation metabolites. The rate of fibre fermentation in the intestines was first studied by means of an *in vitro* gas production technique. The results demonstrated that hulless barleys have higher fermentability and produce higher amounts of SCFA than hulled barley and oats. An experiment carried out on pigs confirmed that the fermentation of the soluble fibre fraction of hulless barley in the gut leads to increased production of SCFA and lactic acid, which in turn contribute to the growth of potentially beneficial microbiota and decrease potentially harmful bacteria, an indicator of improved gut health. This finding shows that gut health parameters may be modulated. Thus gut health could potentially be improved through feed formulation by a judicious selection of feed ingredients with specific fibre fractions, not only by the addition of isolated fibres, which is commonly recommended at present.

The second project was executed to study the effect of some feedstuffs differing in their DF and protein content on fermentation characteristics and N excretion in pigs. The feedstuffs included wheat bran, wood cellulose, peas, pea hulls, pea inner fibre, sugar beet pulp, flax seed meal and corn distiller's dried grains with solubles. The results showed that peas and pea fibre-based diets produced higher amounts of SCFA and reduced N excreted, compared to others. In a

parallel *in vitro* study, fermentation characteristics and bacterial protein synthesis was also studied using the same feed ingredients. The findings of the *in vitro* study corraborated the results of the *in vivo* experiment. These studies showed that peas and pea fibres have the potential to be used in pig diets in order to gain gut health-benefits and reduce N excretion.

From this thesis, it can be concluded that sources and type of dietary fibre have a significant effect on the production of fermentation metabolites in the pig intestine and on N excretion. Among the feed ingredients studied, hulless barley and pea fibres seem to have the greatest potential to be included in pig diets as a source of fermentable fibre to modulate the gut environment, which in turn, extend possibly health-promoting properties and reduce N excretion from pigs. However, further research is needed to understand the specific health benefits of these fibre sources and to quantify the specific fibre components required to achieve these benefits.

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This Thesis is

Dedicated

to

My Parents

Father Raghbendra Jha

and

Mother Nageshwori Devi Jha

Who provided me the eternal power to come up to this stage

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LIST OF ABBREVIATIONS

AA	acetic acid
AD	apparent digestibility
ADF	acid detergent fibre
AGP	antibiotic growth promoters
AIA	acid-insoluble ash
AOAC	Association of Official Analytical Chemists
ATP	adenosine triphosphate
AX	arabinoxylans
BA	butyric acid
BBG	isolated barley β-glucan
BCFA	branched-chain fatty acids
βG	β-glucan
BNI	bacterial nitrogen incorporation
CDC	Crop Development Centre
CHO	carbohydrate
CIAD	coefficient of ileal apparent digestibility
CP	crude protein
CTTAD	coefficient of total tract apparent digestibility
DDGS	distillers dried grains with solubles
DE	digestible energy
DF	dietary fibre
DGGE	denaturing gradient gel electrophoresis
DM	dry matter
EE	ether extract
FC	fermentable carbohydrate
FOS	fructo-oligosaccharides
FSM	flax seed meal
GC	gas chromatography
GE	gross energy

GIT	gastrointestinal tract
hB	hulless barley
HB	hulled barley
iNSP	insoluble non-starch polysaccharides
iCP	indigestible crude protein
IVDMD	*in vitro* dry matter degradability
LA	lactic acid
ME	metabolisable energy
N	nitrogen
NA	not available
NCP	non-cellulosic polysaccharides
NDF	neutral detergent fibre
NSP	non-starch polysaccharides
OG	oat groat
OM	organic matter
PA	propionic acid
PH	pea hulls
PIF	pea inner fibre
PWD	post-weaning diarrhoea
RS	resistant starch
SBP	sugar beet pulp
SCFA	short-chain fatty acids
SF	Solka-floc®
sNSP	soluble non-starch polysaccharides
TDF	total dietary fibre
TOS	transgalacto-oligosaccharides
WB	wheat bran

CHAPTER 1

INTRODUCTION

Intensive pork production, coupled with selective breeding programs and a better knowledge of swine nutrition has brought tremendous growth in pork production in the last half century. Feeding strategies based on antibiotics used as growth promoters (AGP) aimed at improving the pig growth rate by improving its gut health status. However, there is a growing concern about the resistance of numerous bacteria to antibiotics used in human medicine, which is claimed to be due to the consumption of meat from animals grown with in-feed antibiotics, as meat from antibiotic-fed animals were found to have antibiotic-resistant bacteria (Gebreyes et al., 2006). This has led to increased demand for pork produced using antibiotic free diets. Thus, looking for alternatives to AGP to promote animal health, improving growth rate and feed efficiency through beneficial modification of microbial fermentation in the gastrointestinal tract of the pig is a subject of great interest in order to prevent the lack of competitiveness of the pig industry. In addition, odour emission from commercial pig barns as a nuisance factor for the neighbourhood is also an issue. Thus, measures to mitigate odour emissions from pig facilities require attention while maintaining the competitiveness of the pork industry.

To deal with these socio-technical challenges, several alternative approaches have been proposed. These are based on better nutritionally balanced diets, use of technology (processing, enzymes) and the choice of feedstuffs with favourable properties. Among the alternatives, inclusion of isolated fermentable fibre in pig diets is much discussed with some proven benefits. However, use of isolated fibres in pig diets to get such benefit is neither a practical nor a sustainable approach and using feed ingredients with fermentable fibres having such properties might be more applicable at the farm level.

Plant carbohydrates represent a major fraction of the pig diet, accounting for more than two-thirds of the dry matter (Bach Knudsen, 1997). However, the dietary fibre (DF) portion of the carbohydrates is not digested by endogenous enzymes in the small intestine and become available as a substrate for bacterial fermentation, mainly in the large intestine. DF in pig diets has been found to reduce the digestibility of dietary components and energy (Just, 1982; Bach Knudsen, 2001), the effect is based on the properties of the fibre fraction available in the gut (Graham et al., 1986; Bach Knudsen and Hansen, 1991; Chabeauti et al., 1991).

Non-starch polysaccharides (NSP) serve as the main energy source for microbial fermentation in the large intestine and account for over 80% of the fermented substrates at this site (Bach Knudsen et al., 1991; Bach Knudsen and Hansen, 1991). Despite the negative impact

on energy and nutrient digestibility, there is growing interest for including DF in pig diets due to its potential health benefits and possible contribution to reducing nitrogen (N) excretion. Moreover, fermentable DF is a potential alternative to AGP (Verstegen and Williams, 2002) due to their "prebiotic properties". Inclusion of fermentable fibre in diets results in the promotion of fibre fermenting-bacteria (Konstantinov et al., 2003), increases associated metabolic end-products like short-chain fatty acids (SCFA) (Houdijk et al., 2002; Awati et al., 2006) and decreases protein fermentation and the release of ammonia (Le et al., 2005). This, in turn, may stimulate the growth of health promoting-bacteria such as *Lactobacilli* and *Bifidobacteria,* at the expense of pathogenic ones like *Clostridium* or *Salmonella,* thus enhancing the health of host species (Charalampopoulos et al., 2002; Konstantinov et al., 2004).

The inclusion of fermentable DF and reduction of protein content in pig diets are found to reduce nitrogenous gaseous emissions (Aarnink and Verstegen, 2007). The fermentable fibre shifts N from urine to faeces as it increases the bacterial mass in the hindgut, consecutive to an increased rate of fibre fermentation (Bindelle et al., 2009). Urea, continuously released from blood to the intestines, is used by bacteria for protein synthesis. This increases the amount of N present in the faeces and decreases the N excreted in urine, in the form of urea (Zervas and Zijlstra, 2002). Consequently, less ammonia is emitted in the air, since the latter comes from urea hydrolysis (Nahm, 2003). Thus, incorporating fermentable DF in pig diets can be an effective strategy to improve gut health and reduce the environmental footprint from pork production (Verstegen and Williams, 2002).

All the functional benefits mentioned above have been obtained with diets supplemented with isolated fibre, while little information is available on the fermentation characteristics of fibres from different feedstuffs used in swine nutrition.

There are several feed ingredients with diverse carbohydrate compositions. These feedstuffs include cereals and legume grains, distillery by-products, milling by-products, and other fibrous feeds. These feedstuffs contain high amounts of fermentable fibres. The rate of fermentation of DF in the pig intestine depends on its composition and physical properties; the soluble fraction and the non-cellulosic polysaccharides are more fermentable than the insoluble fraction and cellulose. Among the available fibrous feed materials, most studies have been done with wheat bran and sugar beet pulp while little information is available on other grains like barley, oats and peas which have potential as feed ingredients due to their composition and

production level. Several researchers mentioned that the level of DF in the commonly available feedstuffs varied in relation to type and quality. In general, barley (*Hordeum vulgare* L.) grains are relatively rich in DF such as β-glucan and arabinoxylans. In particular, hulless barleys contain high levels of soluble fibre, such as β-glucan (Izydorczyk et al., 2000), that are associated with health benefits for the gut (Brennan and Cleary, 2005; Snart et al., 2006). Thus, it is imperative to know the effect of inclusion of different types of fibre before the latter can be used in swine nutrition.

Determining the rate of fibre fermentation in the digestive tract and their impact on the bacterial population and the faecal N excretion is difficult and expensive. Thus, it would not be possible to screen a series of feedstuffs that differ in their fibre content by *in vivo* techniques. *In vitro* gas production techniques, in stead, can be used as standard procedures to measure the fermentability of feedstuffs (Theodorou et al., 1994; Bindelle et al., 2007a) or the microbial activity of source inocula (Awati et al., 2005; Bindelle et al., 2007a). *In vitro* methods are becoming increasingly popular for determining fibre fermentation characteristics because they are ethically superior, faster and less expensive than *in vivo* techniques (Coles et al., 2005).

This thesis aimed to fill the gap of the limited information on the fermentation characteristics of the fibre fraction from feedstuffs, when that fibre lies in the grain matrix (**Chapters 3 and 4**) as well as the interaction of carbohydrate and protein fermentation (**Chapters 5 and 6**) in the pig gastrointestinal tract, using both *in vitro* models and animal experiments. This thesis also aimed at screening some feedstuffs to explore the possibility of incorporating them in pig diets and evaluating their functional properties, especially related to the fermentability of fibre in the pig intestine (**Chapters 3 through 6**).

The overall objective of this thesis was to examine different alternative feed ingredients for their fermentation characteristics in the pig intestine and their effects on bacterial protein synthesis and N excretion. Therefore, two projects were executed. The first project (**Chapters 3 and 4**) aimed at studying the effects of fibre fermentation from different types of cereals on the gut environment, especially in the production of fermentation metabolites. The second project (**Chapters 5 and 6**) focused on evaluating the effect of feedstuffs differing in their DF and protein content on fermentation characteristics and bacterial protein synthesis in the pig intestine and N excretion. In the first project, it was hypothesied that hulless barley will have higher fermentability, resulting to higher concentrations of short-chain fatty acids (SCFA) and lower

ammonia in the pig intestine, as compared to hulled barley and oat. In the second project, it was hypothesised that the fermentation of fibre from different sources will increase bacterial protein synthesis in the pig gut and decrease N excretion. The thesis also hypothesised that both *in vitro* and *in vivo* techniques will have similar results in terms of fermentation characteristics. The results and the conclusions from the different studies are presented in **Chapters 3 through 6** of this thesis.

CHAPTER 2

DIETARY FIBRE FERMENTATION AND ITS EFFECT ON THE GUT ENVIRONMENT AND NITROGEN EXCRETION: A REVIEW

Abstract

Although dietary fibre (DF) negatively affects energy and nutrient digestibility, there is growing interest for the inclusion of its fermentable fraction in pig diets due to its potential health benefits and its contribution to the reduction of the emission of nitrogenous gases from piggery. This paper reviews some of the relevant information available on the role of DF on digestion and fermentation process and its affect on gut microbiota and health. The fermentation kinetics of DF and the associated production of metabolic compounds are discussed in relation to protein fermentation, their impact on bacterial protein synthesis, nitrogen excretion and the role in reducing emission of nitrogenous gases.

Keywords: Bacterial protein synthesis, Dietary fibre, Fermentation, Nitrogen excretion, Non-starch polysaccharides, Pig

2.1 Introduction

During the last half century, there has been tremendous development in the field of pork production, resulting in more than 100 million tons of pork produced per year (FAO, 2008). This progress has been achieved by an intensification of the pork production system, coupled with selective breeding programs and a better knowledge of pig nutrition. Feeding strategies based on antibiotics used as growth promoters (AGP) aimed at improving pig growth rate by improving its gut health status. However, there is a growing concern about the resistance of numerous bacteria to antibiotics used in human medicine, and is claimed to be due to consumption of meat from animals grown with in-feed antibiotics, as meat from antibiotic-fed animals were found to have antibiotic-resistant bacteria (Gebreyes et al., 2006). This can also select mobile genetic elements carrying resistant detriments which can be transferred to human when AGP fed grown pork is consumed (White and McDermott, 2001). Thus, there is increased demand for pork produced without in-feed antibiotics. It has increased pressure on pig nutritionists and pork producers to look for alternatives to AGP so that the competitiveness of the pig industry will be maintained. Modern pig production systems are also criticised for their negative impact on the environment due to ammonia and odour emission from barn. However, the environmental impact is also partly due to the fact that pigs are raised at commercial scale, even at places with not enough land to disseminate the slurry. Moreover, due to urbanization, people come to live closer to the barns and later complain about odours. Thus, measures to mitigate odour emissions from pig facilities need attention. To deal with these socio-technical challenges, several alternative approaches have been forwarded, including nutritional manipulation. Diets are now formulated to meet the requirements of pigs at each production level. Feed technology (processing, enzymes) is improving and feed producers also select feedstuffs with favourable properties like digestibility and fermentability.

In commercial pig production, plant carbohydrates (CHO) represent the main fraction of a pig diet, accounting for more than two-thirds of the dry matter (Bach Knudsen, 1997). However, part of the CHO is not digested by the digestive enzymes of the small intestine and becomes available as a substrate for bacterial fermentation, mainly in the large intestine. This fraction, i.e. dietary fibre (DF), reduces nutrient and energy digestibility (Just, 1982; Bach Knudsen, 2001; Noblet, 2007). Its physico-chemical properties (like viscosity, water-holding

capacity) also has a marked effect on nutrient digestibility along the gastro-intestinal tract (Graham et al., 1986; Bach Knudsen and Hansen, 1991; Chabeauti et al., 1991).

Thus, despite its negative impact on nutrient and energy digestibility (Bach Knudsen, 2001; Noblet, 2007), there is growing interest to include fermentable fibre in pig diets, due to its possible effects on gut health, welfare and the environment. Fibre has been found to be an effective alternative to AGP (Verstegen and Williams, 2002), to improve gut health (Williams et al., 2001) by modulating gut microbiota and reducing ammonia emission (Aarnink and Verstegen, 2007). Moreover, during the last decade, there has been dramatic shift in the landscape of the feed industry, in terms of price and availability of feed ingredients for animal feeds. There is increased availability of different alternative ingredients and by-products from distillers and milling industries, which are rich sources of fibre as well. However, it is important to know the implications of the use of these relatively new and potential fibre sources in pig nutrition. Therefore, nutritionists are attempting to gain a more thorough understanding of inclusion of DF in pig diets.

To address the concerns and have a better understanding of DF and its role, this paper has reviewed different aspects of DF in swine nutrition. More specifically, DF and its composition are first briefly reviewed. The digestibility and fermentability of the CHO and DF components in the gut of monogastric animals; especially that of the pig and their effect on gut physiology, microbial environment and health are highlighted. At last, the impact of DF and crude protein (CP) fermentation on bacterial protein synthesis, nitrogen (N) excretion and environment are also discussed.

2.2 Dietary fibre

2.2.1 Definition and composition of dietary fibre

The term “dietary fibre” was first used by Hipsley in 1953 (DeVries et al., 1999) for “the non-digestible constituents that make up the plant cell wall”. In 1972, Trowell defined DF as “the skeletal remains of plant cells that are resistant to digestion by the enzymes of man”. Thereafter, it has been re-defined by several workers based on their interests in chemical, physical and physiological characteristics. However, there has been consensus among scientists

since the late 70's that "dietary fiber consists of the remnants of edible plant cells, polysaccharides, lignin and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans" (DeVries et al., 1999), which is basically applicable to monogastric animals as well.

Broadly, DF includes compounds like cellulose, hemicelluloses, mixed linked β-glucan (βG), pectins, gums and mucilages (Davidson and McDonald, 1998). Lignin, a complex phenolic compound, is also included in DF because it is a constituent of the plant cell walls that can greatly affect the digestibility of plant-derived foods (Theander et al., 1989). From a physiological point of view, non-starch polysaccharides (NSP) and resistant starch (RS) have to be included in the DF fraction because they are not hydrolysed by endogenous enzymes. They become available as substrates for microbial fermentation in the intestines (Cummings and Stephen, 2007). NSP and RS are discussed in detail below.

Dietary fibre can be further classified, based on its solubility and nature of reaction with water (Davidson and McDonald, 1998). The Joint Food and Agriculture Organization / World Health Organization Expert Consultation on Carbohydrates in Human Nutrition held in Rome in 1997 proposed to classify the chemical composition of the carbohydrates, based on their molecular size, as determined by the degree of polymerization, type of linkage (α or non- α) and character of individual monomers (Cummings and Stephen, 2007). For this review, the physiological definition of DF is taken as the centre of discussion but other definitions are also considered.

NSP are "non-α-glucan polysaccharides, consisting of a large number of monosaccharides (glycose) residues joined to each other by glycosidic linkages" (IUB-IUPAC and Joint Commission on Biochemical Nomenclature, 1982, cited by Cummings and Stephen, 2007) and are principally found in the plant cell wall (Theander et al., 1989). Upon hydrolysis, NSP produce monomers, pentoses (arabinose, xylose), hexoses (glucose, galactose and mannose), 6-deoxyhexoses (rhamnose and fucose) and uronic acids (glucuronic and galacturonic acids or its 4-O-methyl ether). The ratio of these monomers determines the properties of NSP (Cummings and Stephen, 2007). However, the physiological properties and fermentability of the NSP is not only affected by the proportion of its monomer constituents but also by their physico-chemical properties like physical structure, solubility, viscosity and water-holding capacity (Asp, 1996).

Another major component of plant polysaccharides is starch, which is hydrolysed by an α-amylase enzyme in the upper gut, resulting in end products: maltose, maltotriose and α-limit dextrins, which are further degraded to glucose in the intestine. However, some portion of starch escapes hydrolysis in the small intestine and is available for fermentation in the large intestine. It is termed as "resistant starch" or RS (Englyst et al., 1992). Starch may become RS, due to its physical inaccessibility to enzymes (RS_1), crystalline structure (RS_2), after retrogradation of amylose after cooking and cooling (RS_3) or after chemical modification (RS_4) (Englyst et al., 1992; Sajilata et al., 2006).

The components of plant polysaccharides, which comprise RS and NSP, are illustrated in Figure 2.1.

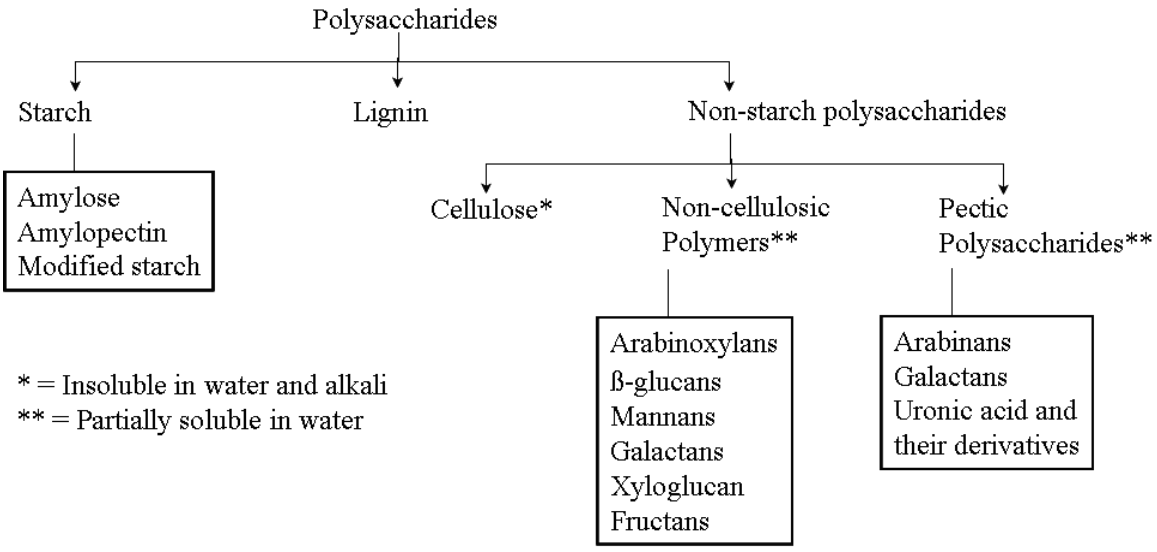

Figure 2.1 Components of plant polysaccharides, modified from Choct (1997)

2.2.2 Sources of dietary fibre and interest for their use in pig diet

It is well established that DF negatively affects nutrient and energy digestibility (Bach Knudsen, 2001; Noblet, 2007). However, the origin and composition of DF could be responsible for large variations in their utilization (Chabeauti et al., 1991). The acceptability of these alternative feed ingredients in pig diets depends on several factors, like the cell wall content of the plant, the degree of microbial fermentation in the large intestine, and the extent of absorption and utilization of the SCFA produced (Pond, 1987). These fibres are fermented in the intestine

and produce SCFA, which in turn positively affects gut health (Williams et al., 2001). Wellock et al. (2007) noted that gut health may benefit most from diets containing appropriate sources of predominantly soluble NSP (sNSP) rather than insoluble NSP (iNSP). Pectins, gum and hemicelluloses are soluble DF, while cellulose and lignin are the insoluble fraction (Davidson and McDonald, 1998). Thus, it is imperative to know the source and type of fibre being supplied in pig diets.

There is also increasing interest and incentive for the identification and characterization of alternative feed resources containing significant amounts of fermentable fibre in pig diets. These alternative sources of feed include cereals and legume grains, distillery by-products, milling by-products and other fibrous feeds.

Cereal grains and their by-products account for the major part of pig rations as main sources of energy. They are rich in fibre as well (Bach Knudsen, 1997). The NSP of cereal grains are mainly composed of arabinoxylans (AX), βG and cellulose, with small amounts of pectic substances found in the stems and leaves of cereals (Choct, 1997). Several workers mentioned that the level of DF in the commonly available feedstuffs vary in relation to type and quality. As a reference to show the variation found in fibre components, composition of some of the most common cereals and by-products is presented in Table 2.1. Wheat, barley, rye and triticale contain significant amounts of both soluble and insoluble NSP while corn and sorghum contain very low levels of NSP. More specifically, AX is the main sNSP in wheat while that of barley and oats is βG.

Barley (*Hordeum vulgare* L.) grains are relatively rich in DF such as βG and AX. In particular, hulless barleys contain high levels of soluble βG (Izydorczyk et al., 2000), that are associated with health benefits for the gut (Brennan and Cleary, 2005; Snart et al., 2006). Even within hulless barleys, the variability in total fibre, soluble and insoluble NSP is very wide (Holtekjolen et al., 2006). There is also wide variation in βG content within and between cereal types, ranging from approximately 19-110 g/kg in barley, 17- 70 g/kg in oats and 2-7 g/kg in wheat (Lee et al., 1997; Brennan and Cleary, 2005; Havrlentova and Kraic, 2006). The rate of solubility also varies. Lambo et al. (2005) found that most of the βG in barley fibre were insoluble (about 85%) while in oat, the opposite is observed, where about 68% of βG were found in soluble fibre fractions.

Oat (*Avena sativa* L.) contains both soluble and insoluble dietary fibres, with high levels of βG, AX and cellulose (Johansen et al., 1997; Drzikova et al., 2005). Among the DF components of oat, βG play an important role because of their functional properties in the gastrointestinal tract (Dongowski et al., 2005).

Grain legumes are used in pig diets primarily as sources of protein and contain significant amounts of NSP as well. Cellulose and xylans are found in the hulls, while pectic polysaccharides are found in the cotyledons (Choct, 1997).

Table 2.1 Types and levels of fibre components in some common cereal grains and by-products (g/kg DM) (Englyst, 1989; Bach Knudsen, 1997; Choct, 1997; Serena and Knudsen, 2007; Murray et al., 2008)

	Wheat	Barley		Oats		Corn	Triticale	Rye	Wheat bran	Rice bran (defatted)	Sugar beet pulp
		Hulled	Hulless	Hulled	Hulless						
Starch	651	587	645	468	557	690	550	613	222	na	81
Cellulose	20	39	10	82	14	20	25	15	72	112	203
NCP[1]											
Soluble	25	56	50	40	54	9	na[3]	42	29	na	290
Insoluble	74	88	64	110	49	66	na	94	273	na	207
NSP[2]											
Arabinoxylans	81	12	48	98	36	52	108	89	238	85	165
β-glucan	8	43	42	28	41	1	17	20	24	na	8
Mannose	1	2	4	3	3	2	6	3	5	4	8
Galactose	3	2	3	7	4	6	5	3	9	12	38
Uronic acids	2	2	2	10	5	1	2	2	15	4	199
Total NSP	114	167	124	232	116	81	163	132	374	218	700
Lignin	19	35	9	66	32	11	8	21	75	na	37
Dietary fibre	133	202	133	298	148	92	171	153	449	na	737

[1]Non-cellulosic polysaccharides

[2]Non-starch polysaccharides

[3]Not available

2.3 Dietary fibre and digestion

2.3.1 Effect of dietary fibre on digestion and physiological functions

2.3.1.1 *Effect of dietary fibre on digestibility*

Digestibility of DF is more variable (40 to 60%) and lower than that of other nutrients like starch, sugars, fat and CP (above 80% in general). It is negatively affected by the amount and source of DF content in the diet (Noblet, 2007). Consequently, the digestible energy content of diets is negatively and linearly affected by DF (Le Goff and Noblet, 2001). DF is better digested in adult sows than in growing pigs. The difference reaches 0.6 MJ/kg DM, on average (Le Goff and Noblet, 2001). This is ascribed to differences in the physiological stage of pigs as there is a higher rate of degradation of DF in the hindgut of sows, compared to growing pigs, due to longer retention time consecutive to their higher gastrointestinal tract volume, combined with a lower feed intake per live weight (Le Goff et al., 2002). Just et al. (1983) found that every 1% of additional crude fibre in the diet decreases the gross energy digestibility by 1.3% and metabolizable energy by 0.9%. NSP, both in purified form or embedded within the matrix, also reduce CP digestion in pigs (Bedford et al., 1992). There is a linear decrease in apparent ileal digestibility of DM and CP with increased levels of purified neutral detergen fibre (NDF) in the diet (Schulze et al., 1994) and lower organic matter (OM) and CP digestibility in diets containing hulled barley, as compared to hulless barley-based diets in pigs (Baidoo and Liu, 1998).

2.3.1.2 *Effect of dietary fibre on physiological functions*

The presence of DF in the diet does not only affect digestibility but also other physiological functions in the gut. High fibre diets increase endogenous N losses (Schulze et al., 1994). The latter are affected by the level and type of fibre (Schulze et al., 1995) and their physico-chemical properties, like water-holding capacity (Leterme et al., 1996). The increased endogenous N loss in the gut is also due to increased digesta viscosity as a result of the presence of sNSP in the diet (Larsen et al., 1994; Mariscal-Landin et al., 1995). The high viscosity of the gut chyme stimulates epithelial cell proliferation and may contribute to some loss of epithelial cells (Gee et al., 1996). In the presence of fibre, there is also an increase in pancreatic secretions and the number of goblet cells (Schneeman et al., 1982). Moreover, there is an increase in mucus secretion in the small intestine (Lien et al., 1994; Mariscal-Landin et al., 1995), which might be due to the mechanical effect of fibre on the gut wall that

affects the integrity of the mucus layer, resulting in superficial cell lesions (Schmidt-Willig et al., 1996). However, Leterme et al. (1998) did not observe any influence of iNSP with high water-holding capacity on protein digestion and absorption, as opposed to sNSP with high water-holding capacity. In these studies, the contrasted result of the effect of NSP on endogenous N losses was due to different sources of fibres used, which supports the findings that the source of fibres with different physico-chemical properties behave differently and have different effects on nutrient digestibility and physiological properties in the gut.

It can be concluded that DF content negatively affects nutrient and energy digestibility, which varies according to the amount and source of DF and their physico-chemical properties. Moreover, different types of fibre also exert their effect on different physiological functions in the gut.

2.3.2 Degradability of dietary fibre in the intestine

2.3.2.1 *Degradability of dietary fibre in the upper gut*

Dietary fibre escapes enzymatic digestion in the small intestine and becomes available for fermentation by bacteria in the caecum and colon. However, substantial degradation of fibre may also occur in the small intestine. Fibre-degrading bacteria are present in the proximal small intestine. They can partially disrupt the cell wall components of fibre, which leads to partial digestion (Varel and Yen, 1997). Bach Knudsen et al. (2008) summarised the ileal digestibility of NSP and their components from different studies (Table 2.2). The results clearly indicate a wide variation in the digestion of NSP components, within and between different cereal sources. The wide variation in the fibre degradability can be ascribed to the physico-chemical properties of DF, the complexity of digestion/fermentation process, differences in experimental design, sample collection and analytical techniques. Gdala et al. (1997) reported lower digestibility of xylose, arabinose and uronic acids in the small intestine of piglets compared to glucose when fed diets based on cereals and soybean meal. This might be due to the high digestibility of mixed linked β-glucan, which is highly degradable in the upper gut, due to its soluble nature (Graham et al., 1989; Bach Knudsen and Hansen, 1991).

Table 2.2 Apparent ileal digestibility of non-starch polysaccharides from different cereals in pigs (Bach Knudsen et al., 2008)

	Digestibility, %	
	Average	Range
β-glucan	65	
Barley	79	40 to 97
Oat	43	17 to 73
Rye	48	-
Arabinoxylans	13	
Barley	40	17 to 51
Oat	1	-8 to 11
Wheat	2	-10 to 12
Rye	8	-7 to 16
Cellulose	16	-47 to 56
Total NSP[1]	21	-10 to 62

[1]Non-starch polysaccharides

2.3.2.2 *Degradability of dietary fibre in the lower gut*

There is wide variation in the degradation of fibre in the large intestine, ranging from 48 to 95% (Bach Knudsen et al., 1993a; Jorgensen et al., 1996; Gdala et al., 1997). Similarly, the total tract digestibility of cellulose varies widely (2 - 84%). Soluble pectin and hemicelluloses are digested to a greater extent than cellulose, while soluble βG from barley are almost completely digested by the end of the gut (Bach Knudsen et al., 1993a). On the other hand, insoluble branched-chain arabinoxylans from wheat, rye and oat are less digestible in the pig gut (Bach Knudsen and Hansen, 1991; Glitsø et al., 1998). There are also noted effects of the DF source on variation in NSP digestibility. Chabeauti et al. (1991) found that the NSP digestibility in growing pigs varies from 16% for wheat straw, 44% for wheat bran (WB), 70% for sugar beet pulp (SBP) to 79% for soybean hulls. The poor digestibility of wheat straw is ascribed to their high lignin content, while wheat bran NSP contain less fermentable hemicelluloses and cellulose, compared to highly-digestible pectic substances of SBP and soybean hulls (Karr-Lilienthal et al., 2005).

Table 2.3 Apparent digestibility (%) of non-starch polysaccharide residues from ileum to faeces of piglets fed a cereal-soybean meal diet (Gdala et al., 1997)

	Terminal ileum	Caecum	Colon1	Colon2	Colon3	Faeces
Arabinose	6.8	49.9	56.2	67.2	70.3	71.8
Xylose	4.1	23.5	45.5	52.5	58.2	60.5
Mannose	23.9	65.8	78.7	81.9	86.3	89.9
Galactose	14.2	81.6	87.9	89.3	90.5	92.1
Glucose	13.0	12.1	36.5	43.1	50.2	54.9
UA[1]	4.1	56.6	71.6	75.6	81.0	83.5
Total NSP[2]	8.2	40.9	56.5	62.4	66.8	68.0

[1]Uronic acids

[2]Non-starch polysaccharides

Gdala et al. (1997) analysed the digestibility of different NSP residues (Table 2.3) in piglets and found that the rate and overall degradation of the polymers in the large intestine was largely influenced by the chemical nature of the fibre, especially its solubility and degree of lignification. Similar results were obtained by Johansen et al. (1997) while studying the degradation of βG and AX from oat bran in the pig gut. However, the total loss of NSP from the anterior to terminal ileum was lower than reported by other workers in old pigs (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993a), possibly due to lower microbial activity in young piglets. Among the NSP components, soluble βG, AX and pectins are rapidly degraded in the caecum and proximal colon while insoluble components of NSP like cellulose and insoluble AX are degraded slowly and at the distal part of the colon (Bach Knudsen et al., 1993a; Canibe and Bach Knudsen, 1997; Glitsø et al., 1998). Moreover, soluble and non-cellulosic mannose and galactose are highly digestible and fermentable compared to the insoluble cellulosic components of NSP (Serena and Knudsen, 2007).

2.4 Fibre fermentation in the gastrointestinal tract

2.4.1 Fibre fermentation

Dietary fibres are not digested by endogenous enzymes but are available for microbial fermentation in the large intestine. In monogastric animals, the large intestine is the most important site of fermentation (Cummings, 1984; Williams et al., 2001). However, substantial fermentation of soluble DF has been observed in the pig's small intestine (Drochner, 1991; Jensen and Jorgensen, 1994; Konstantinov et al., 2004). Fibre fermentation is an extremely complex process, affected by many factors in the gastrointestinal tract, including the host, its microflora, and their interaction, which takes place between them (Williams et al., 2005a).

2.4.1.1 *Fibre fermentation and production of metabolites*

Fibre fermentation results in the production of SCFA like acetic, propionic and butyric acids, along with some gases such as hydrogen, carbon dioxide and methane (Macfarlane and Macfarlane, 1993; Williams et al., 2001). However, the extent of fermentation and the profile in SCFA depend on the substrate (Salvador et al., 1993) while the rate of fermentation of DF in the pig's intestine depends on its composition and physico-chemical properties (solubility in water, water-holding capacity and viscosity), the degree of lignification and particle size (Le Goff et al., 2003) and transit time in the digestive tract (Stanogias and Pearce, 1985a). The soluble fraction and the non-cellulosic polysaccharides are more fermentable than the insoluble fraction and cellulose (Glitsø et al., 1999), as complex cell walls limit the accessibility of bacteria and hydrolytic enzymes to these substrates. Thus, these characteristics are directly dependent on the botanical origin and (or) processing of the fibre source (Johansen et al., 1997). The fate of anaerobic fermentation of CHO can be summarised by the following general equation (Ewing and Cole, 1994):

$$57.5\ C_6H_{12}O_6 + 45\ H_2O \rightarrow$$
$$65\ \text{acetate} + 20\ \text{propionate} + 15\ \textit{n}\text{-butyrate} + 140\ H_2 + 95\ CO_2 + 288\ \text{ATP} \qquad (2.1)$$

In general, the molar ratio of these acids is 1 acetate: 0·31 propionate: 0·23 *n*-butyrate. However, these ratios vary widely, depending on the type of substrate available, the composition of the anaerobic flora, and the prevailing pH (Williams et al., 2001). According to equation (2.1), acetate is the major SCFA produced during fibre fermentation in general. However, the fermentation of pectin yields 80:12:8 (acetate: propionate: butyrate) (Drochner

et al., 2004) while other NSP yield 63:22:8 and starch yield 62:15:23 (Cummings et al., 2001). RS consistently produces relatively more butyrate whereas oligofructose and inulin are the lowest butyrate producers (Sajilata et al., 2006). Arabinogalactan and polydextrose yield relatively more propionate and oligofructose yields predominantly acetate (Cummings and Macfarlane, 1997).

In addition to SCFA, other metabolites such as lactate, ethanol and succinate are also produced from bacterial fermentation of DF (Drochner et al., 2004). The majority of these metabolites (possibly except ethanol) are further converted into SCFA by cross-feeding mechanisms (Macfarlane and Gibson, 1995), as illustrated in Figure 2.2.

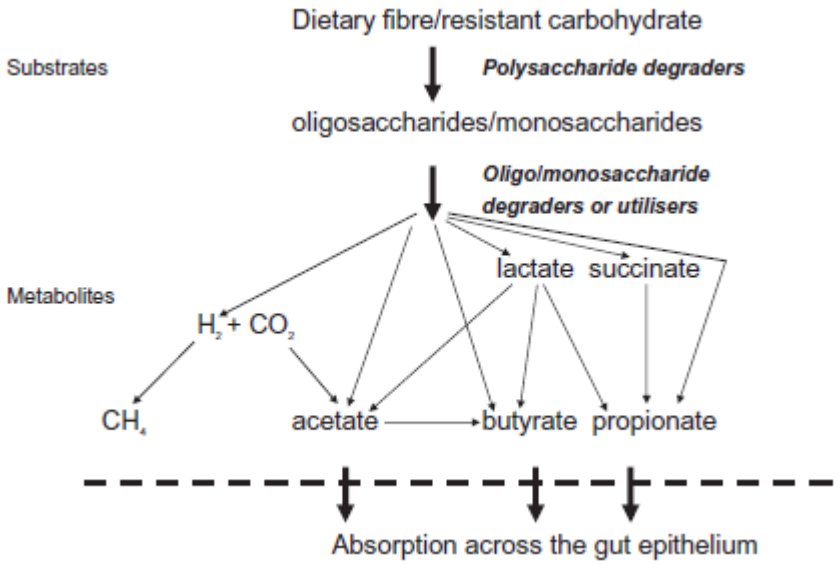

Figure 2.2 The sequential degradation of dietary substrates, and the intermediate and final metabolites formed, by the bacteria inhabiting the large intestine (Scott et al., 2008)

2.4.1.2 *Fate of fermentation metabolites in the pig large intestine*

The main site of SCFA absorption in pigs is the large intestine (Imoto and Namioka, 1978) where the majority (about 90%) of the SCFA are absorbed and subsequently undergo a variety of metabolic fates within the body in humans (Macfarlane and Macfarlane, 1993) while SCFA are much efficiently absorbed and metabolised in the hind gut of the pig (Jorgensen et al., 1997).

SCFA are absorbed in the gut through passive diffusion. The exact mechanism for absorption is still unclear. However, several mechanisms have been proposed, stating their dependence on luminal pH, CO_2, as well as the fluxes of water, protons and inorganic ions (Bugaut, 1987). The fermentation metabolites are taken up by the cells in the intestine and

also used for bacterial growth (Bach Knudsen, 2001). Although SCFA are primarily taken up and metabolised by colonocytes, they are also used as a source of energy by other tissues (Wong et al., 2006). The absorbed SCFA are basically metabolised in three major sites: in the caeco-colonic epithelial cells that use butyrate for their energy production pathway, liver cells that metabolise residual butyrate and propionate for gluconeogenesis, as well as 50 to 70% of acetate, and muscle cells, mainly from skeletal and cardiac muscles that oxidise the residual acetate (Roberfroid, 2007). The energy produced from SCFA may contribute up to 15% of the maintenance energy requirements of growing pigs (Dierick et al., 1989) and up to 30% in gestating sows (Varel and Yen, 1997).

2.4.2 Methods to measure fermentation activity

Several methods exist to measure fermentation. They can be divided into two groups: *in vivo* and *in vitro*. *In vitro* methods are becoming increasingly popular as they are faster, less expensive and have less ethical concerns than *in vivo* methods (Coles et al., 2005).

It would be difficult and expensive to screen a series of feedstuffs that differ in NSP or RS content by *in vivo* techniques. Similar work can be performed by means of *in vitro* techniques in comparatively less time. Moreover, enzymatic methods based on substrate disappearance during incubation (Boisen and Fernandez, 1997) succeed in the prediction of *in vivo* OM and energy digestibility, but limit the understanding of fermentation in the large intestine. On the other hand, it has been established that processing (grinding, etc.) has significant effect on the amount of NSP liberated from the fibre matrix and available for fermentation, which is possible only with *in vitro* techniques. Moreover, *in vitro* anaerobic fermentation methods can suggest the rate and extent of SCFA formation (Barry et al., 1995; Karppinen et al., 2000).

In vitro fermentation techniques (Theodorou et al., 1994; Mauricio et al., 1999; Davies et al., 2000; Bindelle et al., 2007a) are often used as a standard procedure to measure the fermentability of feedstuffs or the microbial activity of source inoculum. These techniques are based on the *in vitro* fermentation of a substrate (*i.e.* the fibre source) by microorganisms collected from the colon of monogastric animals (Awati et al., 2005) or the rumen of ruminants (Menke and Steingass, 1988), instead of an enzymatic complex such as viscozyme. These techniques allow recording the substrate disappearance during fermentation and also bacterial accumulation, SCFA production and gas release, which are of first importance when the functionalities of DF are considered. They are also used to evaluate

the capacity of the NSP to generate metabolites (Barry et al., 1995; Karppinen et al., 2000; Awati et al., 2005), influence the intestinal microbial population (Crittenden et al., 2002; Awati et al., 2005) and affect the rate of bacterial protein synthesis (Bindelle et al., 2009) in pigs. However, these methods need to be validated before they can be used routinely because several factors might affect the fermentation process *in vitro* and *in vivo* (Williams et al., 2005b).

A novel *in vitro* gas production technique developed by Bindelle et al. (2007a) allows for the measurement of the rate of fermentation of soluble fibres in any part of the digestive tract by intestinal bacterial, the monitoring of the evolution of the bacterial population and the estimation of the rate of bacterial nitrogen incorporation in the pig intestine. However, the sample must be predigested *in vitro* with pepsin and pancreatin in order to simulate digestion in the stomach and the small intestine. Moreover, the mathematical models used do not always fit with the patterns observed.

2.4.3 Kinetics of fibre fermentation

Dietary fibre is fermented by microbiota in the intestine; thus microbial ecology becomes an important factor to explain the rate and kinetics of fibre fermentation. In addition, diet also influences the composition and diversity of the gut microflora (Varel and Yen, 1997). So, both the available substrates and microbial population determine the fermentation characteristics in the gut.

Polysaccharides also break down at different rates in the intestine and this will strongly influence the rate at which CHO becomes available to the bacteria.

The differences in fermentation kinetics and end-products observed between ingredients are ascribable to the nature of their respective fibre fractions. In an *in vitro* study, Awati et al. (2005) tested different isolated fibres (inulin, lactulose, SBP and wheat starch) and observed different kinetics of fermentation between substrates. Lactulose had the fastest maximum rate of gas production (*R*max) while SBP had the slowest *R*max. Similar results were found in an *in vitro* study by Bindelle et al. (2007a): WB started fermentation sooner than SBP. However, SBP degraded faster and produced higher total gas volume than WB. Differences in fermentation characteristics and kinetics are explained by differences in solubility of the fibre fractions and their degree of polymerization. Although most of the fibre fermentation occurs in the large intestine, fermentation may be complete within the small intestine for rapidly fermentable ingredients. Houdijk et al. (2002) found that non-digestible

fructo-oligosaccharides (FOS) disappear before the terminal ileum of growing pigs. Considering the risk that the solubility of a particular CHO is directly linked to its fermentability, Williams et al. (2005a) suggested that potential feed ingredients should be screened for their fermentability before being added to animal diets in order to gain the benefit of improving gut health.

The fermentation of fibres from legumes differs from that of cereals, due to their chemical composition. NSP fractions from legumes are only fermented to a limited extent in the stomach and the small intestine and the undegraded portion of NSP is available for fermentation in the large intestine. The degradation of the CHO fraction of peas occurs throughout the large intestine. Oligosaccharides, residual sugar and resistant starch, as well as the soluble and pectic polysaccharides of the cotyledons are readily available to the microbiota present in the distal small intestine and in the proximal segments of the large intestine. Acidic xylans and cellulose from pea hulls require longer transit times, and are thus fermented in the distal part of the large intestine, to a less extent (Canibe et al., 1997; Canibe and Bach Knudsen, 2002).

2.4.4 Effect of source of fibre on metabolite production

Dietary fibre fermentation and the composition of SCFA depend on the composition of the substrate, its physico-chemical properties (water solubility, viscosity), chemical structure, the physical form of the cell walls, processing, the degree of polymerization of the carbohydrates and the amount of fibre entering the gut (Canibe et al., 1997).

Among the fibre sources, FOS are the most extensively studied. They contain two to seventy fructose residues and some of the *Bifidobacteria* can digest them as they produce the enzyme fructofuranosidase (Gibson et al., 2004). FOS are fermented by bacteria, yielding energy for bacterial growth. Houdijk et al. (2002) evaluated the effect of FOS and transgalactooligosaccharides (TOS) in comparison to non-fibre control diets in weaned pigs and found that both FOS and TOS increased SCFA production in the gut. However, there were differences in the concentration between FOS and TOS in different sections of the gut, which supports the view that FOS and TOS have different fermentation characteristics in the pig's gatrointestinal tract.

Among the fibre fractions, β-glucan is gaining more attention as it is a source of easily fermentable energy for intestinal microbiota. It yields higher levels of SCFA (Brennan and Cleary, 2005) due to a relatively high concentration, soluble state and high molecular

weight and results in several beneficial physiological effects to the host (Dongowski et al., 2002). Oat bran, a rich source of soluble DF in the form of βG, produces almost twice as much SCFA per gram DF as WB in the pig intestines (Bach Knudsen and Hansen, 1991; Bach Knudsen et al., 1993a). However, AX and not βG in the cell walls of oat bran are responsible for the enhanced butyric acid production of oat bran (Bach Knudsen et al., 1993b). Bach Knudsen and Canibe (2000) found higher concentrations and flows of lactic acid in the ileum of cannulated pigs after feeding a diet supplemented with soluble fibre from oat bran, which supports the view that βG stimulates the production of lactic acid in the small intestine, which is found to promote the development of *Lactobacilli*, a family of health-promoting bacteria.

There are noted effects of sources of DF on SCFA production and profile. Carneiro et al. (2008) compared the effect of two fibre sources, WB and maize cobs in weaned pigs and found no difference in the amounts of SCFA in the small intestine. However, there was higher acetic acid and lower butyric acid production in the caecum when WB was replaced with maize cobs. Similarly, Stanogias and Pearce (1985b) found a linear relationship with the levels of NDF intake and the concentration of total SCFA in the proximal colon of pigs in a study with different fibre sources (lupin hulls, maize cobs, wheat bran and alfalfa stems in a basal fibre-free diet). However, the molar ratios were significantly affected by the level of NDF intake only in the cases of acetic and butyric acids, whereas the source of dietary NDF had a marked influence on the molar ratios of all the acids. Findings of these studies clearly indicate that not only the amount and type of substrate, but also the source of fibre fraction is important to determine the amount and type of SCFA production.

In an *in vitro* study, Awati et al. (2005) evaluated different fermentable fibres (inulin, lactulose, sugar beet pulp and wheat starch) and found no difference in SCFA concentrations between fibre sources. However, lactulose produced the highest ammonia concentrations of all substrates. In a companion *in vivo* study, they found similar results in terms of metabolite concentration in the intestines of piglets fed the same substrates (Awati et al., 2006). There was higher SCFA concentration and lower ammonia and branched-chain fatty acids (BCFA) in all fibre sources, compared to diets based on non-fermentable fibre, which suggests that fibre fermentation increases SCFA production and decreases protein fermentation.It also confirms that *in vitro* methods can give similar results than *in vivo* studies in terms of metabolites production.

Hogberg and Lindberg (2004) compared different levels and types of fibre (low insoluble, low normal, high insoluble and high normal in comparison to barley- and wheat-

based control diets) using different cereals and cereal by-products in ileal-cannulated pigs and found higher concentrations of total organic acids (SCFA and lactic acid) in the digesta of pigs fed with sources of highly insoluble fibre, which suggests that diets high in DF stimulate microbial activity and fermentation in the distal small intestine.

Similar to NSP, RS is fermented by numerous saccharolytic bacteria residing in the lower gut and produces more SCFA, but the proportion of butyrate in the SCFA is higher (Cummings et al., 1996; Sajilata et al., 2006). Supporting this view, Brown et al. (1997) reported that faecal concentrations and excretion of total SCFA are higher in pigs fed with high amylose cornstarch, with higher propionate and butyrate ratio. Similar result was reported by Bird et al. (2007) when using different maize starch sources with varying levels of amylose content in pig diets. Moreover, there is a positive linear relationship between the level of RS, the total SCFA concentration and the proportion of butyrate, and a negative relationship with the proportion of acetate (Hedemann and Bach Knudsen, 2007). It confirms that RS is primarily a butyrogenic substrate, which is of special interest for improving intestinal health (Sajilata et al., 2006).

Enzyme supplementation influences the utilization of fibre and their fermentation characteristics. Carneiro et al. (2008) evaluated the fermentation characteristics of WB and maize cob, supplemented or not with exogenous enzymes (endo-1,4 β-cellulase, endo-1,3(4)-β-d-glucanase and endo-1,4-β-xylanase) in pig diets. The results showed that enzyme supplementation affected the molar proportion of SCFA in the small intestine, tended to increase acetate, propionate and total SCFA concentrations in the colon of piglets fed WB, along with higher xylanolytic and cellulolytic activities in the caecum and colon of piglets fed WB than for piglets fed maize cob. There is noted interaction effect of cereal type and enzyme supplementation on total SCFA production with effect on the individual SCFA. O'Connell et al. (2005) found higher proportions of iso-valerate and iso-butyrate in the caecum and colon of pigs fed with barley-based diets without enzyme supplementation. These BCFA are products of amino acid metabolism. The use of enzyme makes these NSP monomers more degradable, thus more CHO substrate is available for fermentation. As a result, less proteolytic fermentation takes place in the gut of pigs fed diets supplemented with enzymes.

2.4.5 Effect of fibre fermentation on gut microbiota and health

The influence of diet on microbial communities in the pig intestines has been of interest for long time. More than 90 years ago, for example, a technical bulletin from the Kansas State Agricultural College (1917) reported that certain saprophytic types of microbes (Spirilla, *Fluorescens liquefaciens*, molds, etc.) tended to disappear when pigs were fed with corn only. In due course of time, there has been great achievement in this field. We now have a better knowledge and a lot is known about the interaction of diet and microbiota in the intestines of the pig but several aspects are still not well understood.

Potential beneficial effects of DF on animal health are manifested through changes in microbiota of the gastrointestinal tract (GIT). Several studies report that DF and protein fermentation in the intestines modulates the gut environment, especially microbial ecology. The GIT microbiota of the pig is composed primarily of bacteria. The microbial population increases from 10^3-10^5/g of digesta in the stomach to 10^9-10^{10} in the distal small intestine, and further to 10^{10}-10^{11} in the large intestine of pigs, belonging to more than 50 genera and over 500 species of bacteria (Jensen and Jorgensen, 1994; Gaskins, 2001). The majority (about 90%) of the cultivable bacteria are Gram-positive, strict anaerobes belonging to the *Streptococcus*, *Lactobacillus*, *Eubacterium*, *Clostridium* and *Peptostreptococcus* genus while the remaining 10% of total flora belongs to Gram-negative of *Bacteroides* and *Prevotella* groups (Jorgensen and Just, 1988; Gaskins, 2001; Leser et al., 2002). Each bacterial species occupies a particular niche with numerous interrelationships between them (Flint et al., 2008).

2.4.5.1 *Effect of fibre fermentation on microbial composition*

The population and activity of bacteria in the gut is influenced by several factors, the main one being diet (Varel and Pond, 1985). More specifically, the structure and composition, solubility (Hogberg and Lindberg, 2004) and amount and type of substrate available (Macfarlane and Macfarlane, 1993) affects the gut microbial ecology. Among the different constituents of diets, fermentable carbohydrates (FC) are found to affect the gut environment (Awati et al., 2005). The source of fibre affects the digestion site and gut environment, thereby affecting the conditions for the proliferation of microbiota in the gut (Hogberg and Lindberg, 2004). Moreover, fibres serve as an energy source for microbes and support their proliferation. Jorgensen and Just (1988) reported 5.5 times (as measured by ATP concentration) increased microbial activity in the GIT of pigs when fed with a high-fibre

diet containing WB (102 g NSP/kg feed) or oat bran (93 g NSP/kg feed). In addition, there was increased (5-9 times) carbon dioxide and methane production, suggesting increased microbial fermentation that takes place in the GIT of pigs fed a high-fibre diet. Similar increased microbial activity was observed in the intestines of pigs fed pea fibres and pectin, as indicated by higher bacterial counts, ATP concentration, adenylate energy charge and low pH (Jensen and Jorgensen, 1994). However, Varel et al. (1982) noted that there was initially a decrease in the bacterial population of the pig intestines when the animals were fed with high-fibre diet (50% alfalfa meal) in lean genotype pigs. However, the microbial population increased after continuous fibre-feeding for 17 weeks, compared to the starting period of feeding DF. It suggests that there is some kind of adaptation of the microflora in the pig intestines when fed with high fibre diets.

Dietary fibre affects fermentation in the GIT by stimulating the growth or metabolism of special bacterial species (Williams et al., 2001). These increased numbers of cellulolytic bacteria enhance hindgut fermentation and the production of SCFA, which decreases the pH of the gut content. A decrease in pH favours the growth of beneficial bacteria (e.g. *Bifidobacteria, Lactobacilli*), at the expense of pathogenic ones like *Clostridium* or *Salmonella,* which contribute to enhance the health of host species (Brouns et al., 2002; Charalampopoulos et al., 2002; Bouhnik et al., 2004). This phenomenon is termed the "prebiotic effect" (Gibson and Roberfroid, 1995).

The potential "prebiotic effect" of DF has been studied in several monogastric species, including swine. The results are quite variable from one study to another in terms of their effect on microbial population, diversity and gut health, which can be ascribed to the type of substrate available for fermentation and the gut environment of the host. At the increased level of FC in the large intestine, there is an increase in activity of the entire microbial community. However, some types of DF may have selective effects and stimulate particular niches of microorganisms (Louis et al., 2007). Oligofructose, galacto-oligosaccharides and lactulose increase *Bifidobacteria* and *Lactobacilli* in the large intestine of humans (Macfarlane et al., 2006). In an *in vitro* study using human intestinal bacteria, Al-Tamimi et al. (2006) found that arabino-oligosaccharides increase *Bifidobacteria* and *Lactobacilli* and decrease *Clostridia*, which are influenced by the molecular weight of the substrate.

Similar results are reported from several studies with pigs. For example, Estrada et al. (2001) found increased numbers of *Bifidobacteria* and decreased numbers of total anaerobes and *Clostridia* in faeces of pigs fed with diets containing 0.5% FOS in conjunction with

Bifidobacterium longum. Drew et al. (2002) compared the effect of CHO sources (corn, wheat and barley) in weaned pigs and found that the bacterial population was significantly related with ADF and NDF contents of the diets. There were increased *Lactobacilli* and decreased *Enterobacteria* populations in barley-fed pigs, as compared to corn-fed pigs. Moreover, barley-based diets increased *Lactobacilli* spp. and *Bifidobacterium* spp. in the caecum, compared to corn-based diets. One possible explanation might be the higher amount of β-glucan in barley-based diets, compared to corn-based diets, which is found to enhance the growth of beneficial bacteria at the cost of harmful bacteria. Interestingly, wheat-based diets had higher numbers of *Bifidobacterium* spp. and lower numbers of total aerobes and *Clostridium* spp., compared to barley-based diets. These can be ascribed to the complexity of the various bacterial species and fermentable substrates present, especially the monomers of the fibre which are selective for certain microbes. Kostantinov et al. (2003) found *Ruminococcus*-like species in the faeces of pigs fed diets containing fibres (SBP and FOS), but not in pigs fed a control diet, suggesting that these bacteria may play a role in the utilization of DF. Moreover, there was a specific response of a novel and abundant *Lactobacillus amylovorus*-like phylotype to dietary oligosaccharides in the gut of weaning pigs (Konstantinov et al., 2004). Similarly, Owusu-Asiedu et al. (2006) reported increased *Bifidobacteria* and *Enterobacteria* populations in the ileal digesta of growing pigs fed diets supplemented with guar gum or cellulose to a standard diet.

Pierce et al. (2007) found interaction effects of lactose (215 and 125 g/kg) and CP concentrations (160, 185 and 210 g/kg) on post-weaning piglets. There was a linear increase in faecal *Escherichia coli* population and a linear decrease in faecal *Lactobacilli* population at the low lactose level with increasing CP. However, there was no effect of CP concentration on either *E. coli* or *Lactobacilli* populations at high lactose levels. Pigs offered 215 g lactose/kg diet had a significantly higher *Bifidobacteria* population compared to pigs offered 125 g lactose/kg. There was a linear decrease in *Bifidobacteria* population as CP increased. In a study by Lynch et al. (2009), similar interaction effect of DF and CP level was found on *Lactobacilli* spp. and *Enterobacteria* spp. populations.

There is limited information available on the effects of fibre when they reside in their matrix. To evaluate the effect of NSP components, especially βG embedded in a matrix, as compared to the isolated form, Pieper et al. (2008) conducted an experiment with weaned pigs and found that hulless barley varieties with high soluble NSP content favoured xylan- and β-glucan-degrading bacteria whereas β-glucan-supplemented hulled barleys favoured *Lactobacilli*. Moreover, there was a decrease in the number of *Lactobacilli* in the ileum of

pigs fed hulless/high β-glucan barley-based diets. This suggests that both type and form of βG affect the bacterial population in the pig intestines.

Processed fibres are also found to exert a positive response on health-promoting characteristics. As an example, oat fibre and βG isolates, fermented ropy oat-based products containing both native and microbial βG, stimulated *Bifidobacteria* in the GIT apart from other health benefits like reduced blood cholesterol level to host (Martensson et al., 2005). Like the NSP, RS is also found to affect the bacterial population in the pig intestines. Brown et al. (1997) found higher *Bifidobacteria* counts in faeces of pigs fed with a high amylose cornstarch diet, than in faeces of pigs fed with a low amylose cornstarch. Part of the high amylose corn-starch becomes RS, which is fermented in the large intestine and exerts prebiotic effects.

2.4.5.2 *Effect of fibre fermentation on health*

Dietary fibre plays an important role in the function of the pig gastrointestinal tract. It is evidenced by several studies reporting the positive role of DF in controlling bacterial infections, particularly reducing post-weaning diarrhoea (Williams et al., 2001; Lalles et al., 2007), which is a major problem for the pig industry in many parts of the world. Thomsen et al. (2007) observed that pigs fed with highly fermentable carbohydrates, like fructan-rich chicory roots and sweet lupins were completely protected against the development of swine dysentery. Similarly, the inclusion of FC in piglet feeds enhanced intestinal populations of *Lactobacilli* and reduced the incidence and severity of diarrhoea (Edwards, 1996). These studies support the view that diets supplemented with fibre can protect pigs against swine dysentery. However, there is continuous debate on whether fibre exerts beneficial or detrimental effects on the development of post-weaning enteric dysentery. Pluske et al. (1998; 2003) noted increased incidence of clinical swine dysentery in growing pigs and diarrhoea in weanling pigs fed with diets high in fermentable NSP and RS. Similar negative effect on gut health was also noticed with supplementation of isolated soluble fibre. Nursery pig diets supplemented with 0.025% βG increased growth performance but also increased the susceptibility to *Streptococcus suis* infection (Dritz et al., 1995). These authors suggest that a complex interaction exists between growth performance and disease susceptibility in pigs fed β-glucan.

There is some information available on the fibre activity of other grains like peas, chick peas, faba beans and lupins and their effect on microbial population and gut health. Queiroz-Monici et al. (2005), while working with rats, found that peas and chickpeas have a

bifidogenic effect due to the DF and RS supply in diet from these legumes. Fermentation of RS produces more butyrate, which is utilised by the colonic cells as fuel, thus strengthening the first line of defence in the gut (Topping and Clifton, 2001). Thus, higher butyrate yielding RS sources have a potential to improve gut health.

2.4.5.3 *Mechanism of health improvement*

As discussed above, the role of fibre in enhancing the growth of "beneficial bacteria" is well reported. However, how these "beneficial bacteria" help the host is still poorly understood. To explain the possible role of commensal bacteria in gut health, some theories have been forwarded with some evidences. The theory of "colonization resistance" is one of those, which suggests that commensal bacteria in the gut protect the host from pathogens by suppressing colonization, and entering new bacteria and pathogenic microbiota (Van der Waaij et al., 1971). However, the effect of colonization resistance differs between microbial species and may even differ between strains of the same species. Another theory is the "competitive exclusion", which refers to the fact that commensal bacteria in the gut compete with pathogenic bacteria for adhesion sites, nutrients, stimulate the immune system and intestinal motility and secret anti-microbial compounds like organic acids. There is a delicate balance between beneficial and pathogenic bacteria in the GIT, and many symbiotic and competitive interactions occur between them (Ewing and Cole, 1994). Moreover, commensal bacteria salvage energy from dietary fibre, which is otherwise indigestible and protects the host from pathogens by forming a front line of mucosal defence (Zoetendal et al., 2004). SCFA produced from the fermentation of DF create an acidic environment, which in turn, inhibits the growth of some intestinal pathogens like *E. coli*, *Salmonella* spp. and *Clostridium* spp. (May et al., 1994; Montagne et al., 2003). Studies with pigs indicate that SCFA, especially butyrate, play a selective antimicrobial role. *Lactobacillus* spp. and *Streptococcus bovis* are less sensitive to butyrate, compared to *E. coli*, *Salmonella* spp., *Clostridium acetobutylicum*, *Streptococcus cremoris*, *Lactococcus lactis* and *Lactococcus cremoris* (Williams et al., 2001).

There are some other mechanisms proposed by which fibre promotes pig health, apart from its effect on the microbial community. Inclusion of fibre will have positive effects due to its physico-chemical properties, thus exerting a local effect. It might help in the protection from ulcers as it retains digesta in the stomach for prolonged periods of time, avoids the separation of the solid and liquid phases of digesta, which results in reduced risks for reflux of irritants like bile acids from the distal part of the stomach, to damage the relatively

protected region of the pas-oesophagus (Hansen, 2004). SCFA were also found to stimulate epithelial cell proliferation and differentiation (Sakata, 1987). Butyrate, the fermentation product, serves as the principal oxidative fuel for the colonocytes. It could also have beneficial tropic effects on inflamed caeco-colonic mucosa (Topping and Clifton, 2001) and could improve the immune status of animal (Brouns et al., 2002).

It can be summarised that the presence of fibre in the gut significantly affects the gut microbial environment, creates more favourable lumen conditions for gut health by stimulating the growth of "beneficial bacteria" at the cost of "harmful bacteria", with the possibility of some negative impact on gut health, which depends on the type of fibre substrate available for fermentation. However, there is no straightforward answer of the benefits of fibre on gut health and direct evidence for enhanced resistance to unfavourable conditions is still lacking.

2.5 Relationship between carbohydrate and protein fermentation

2.5.1 Protein fermentation in the gut

Available energy is considered as the most limiting factor for microbial fermentation in the lower gut of the pig, where FC serves as the main substrate (Houdijk et al., 2002). There is a gradual decrease of FC in the intestinal content as it passes through the caecum and colon. When there is depletion of CHO source available for bacterial fermentation, or when the ratio between CHO and protein substrates available to microbes as energy sources is unbalanced in the medium, there is a shift of fermentation from CHO to protein in the gut (Piva et al., 1996; Macfarlane and Macfarlane, 2003). However, fermentation shifts back from proteolytic to saccharolytic when FC is made available in the gut (Houdijk et al., 1998).

Proteins available to bacteria for fermentation are dietary proteins that escape digestion in the small intestine, along with some proteins of endogenous origin (Macfarlane et al., 1992). The end-products of protein are also different from those of CHO. The proteolytic fermentation results in the production of SCFA, especially BCFA (mainly iso-butyrate, valerate and iso-valerate) which are formed by the metabolism of branched-chain amino acids such as valine, leucine and isoleucine. Among the fermented proteins, about 30% are converted to SCFA, of which BCFA constitute between 16 and 23%, depending on the substrate type (Macfarlane et al., 1992; Cone et al., 2005). In addition, some potentially toxic

metabolites like ammonia and amines (Cone et al., 2005) as well as malodorous compounds such as skatole and indole (Jensen et al., 1995) are produced.

The gas production caused by protein fermentation is not the same as that of carbohydrates. Cone and van Gelder (1999) compared protein and CHO fermentation on the gas production profile and found that the fermentation of casein produced only one third of the amount of gas produced by carbohydrates. Moreover, protein fermentation occurred mainly in the initial hours of incubation, apparently because the major part of protein was soluble.

2.5.1.1 *Nutritional strategy to reduce protein fermentation*

Some nutritional strategies like lowering the amount of CP in the diet (Nyachoti et al., 2006; Htoo et al., 2007) or the inclusion of FC, such as lactitol (Piva et al., 1996), FOS (Houdijk et al., 1998), RS and WB (Govers et al., 1999) are found to be effective to reduce protein fermentation in the gut. Moreover, the pattern and site of fermentation can also be influenced by using a combination of different sources of DF (Henningsson et al., 2002). Awati et al. (2006) investigated such a relationship and found that the inclusion of FC in diets for weanling pigs reduced protein fermentation along the GIT as well as the faecal ammonia concentration. Moreover, less fermentable substrates like WB used in combination with highly fermentable NSP or RS, maintained the microbial activity throughout the entire large intestine and decreased proteolysis occurring in the distal colon (Govers et al., 1999). On the other hand, the protein content of pig starter diets can be reduced safely by about 20% while balancing with limiting amino acids according to ideal protein ratios, which will also reduce the production of potentially harmful microbial metabolites in the caecum (Htoo et al., 2007). In similar line, Bikker et al. (2006) reported that an increase in fermentable fibre content, and to a lesser extent a decrease in CP content, reduce ammonia concentrations and change the fermentation patterns of the gastrointestinal tract of weaned pigs. Moreover, the presence of FC stimulates microbial growth, leading to an increased demand for amino acids by micro-organisms (Hawe et al., 1991). Therefore, the presence of FC will likely reduce the production of potentially harmful compounds, such as tryptophan catabolism to skatole.

Thus, the inclusion of FC and a reduction in protein seems to be an effective nutritional strategy that may counteract the negative effects of protein fermentation in weaned pigs fed high-CP diets, without negatively affecting the performance of pigs.

2.5.2 Effect on gut health

Both undigested dietary and endogenous proteins pass to the distal gastrointestinal tract and enhance the growth of N-utilizing bacteria (Reid and Hillman, 1999) that ferment the available protein. This leads to increased ammonia and amine concentration in the colon (Macfarlane et al., 1992), whereas, normally, these compounds are found only in small amounts in a healthy colon (Rasmussen et al., 1988). The increased ammonia concentration in the gut can negatively affect the development of the intestinal mucosa (Visek, 1984) and villus height (Nousiainen, 1991), which in turn, affect the digestion and absorption processes in the intestinal lumen adversely. The impaired absorption due to reduced villus absorptive area leads to diarrhoea in piglets. Moreover, ammonia generated in the colon readily passes across the gut wall, thereby gaining access to other tissues of the body (Rowland, 1992) which can be detrimental for the host's health (Nollet et al., 1999; Cone et al., 2005). It has also been found to be a predisposing factor for post-weaning diarrhoea (PWD) in pig (Porter and Kenworthy, 1969; Gaskins, 2001).

Among the several factors responsible for PWD in pigs, enterotoxigenic strains of *E. coli* are major ones (Williams et al., 2001). *E. coli* colonise the small intestine in multifactorial conditions and is influenced by the diet composition (Hampson, 1994). A high level of dietary protein predisposes to the condition (Prohaszka and Baron, 1980) while various types of DF can reduce the incidence and severity of PWD (Pluske et al., 2003). Thus, the manipulation of diets to control the growth of *E. coli* might be an effective strategy to control PWD in piglets, which should be focused toward balancing CHO and protein fermentation.

In a review, Williams et al. (2001) mentioned that FC inclusion in the diet influences the composition and activity of microbiota in the gastrointestinal tract. This, in turn, may provide some protection against PWD. Pluske et al. (1996) reported that pigs fed diets based on steam-flaked corn and steam-flaked sorghum had a lower incidence of dysentery (11-33%) than pigs fed diets based on other grains (barley, groats, corn, sorghum and wheat) (75-100%). In a companion study, they also found that pigs fed a diet based on cooked white rice were fully protected against swine dysentery (Pluske et al., 1996). Similar finding was reported by Siba et al. (1996) with the conclusion that there is reduced fermentation in the large intestine of pigs fed cooked rice, the latter providing protection against dysentery. Moreover, feeding diets low in soluble NSP, oligosaccharides or RS reduces clinical dysentery in pigs experimentally infected with *Serpulina hydysentriae* (Pluske et al., 1996;

Siba et al., 1996) which was confirmed later by the same research group (Pluske et al., 1998). This series of studies suggests that carbohydrate fermentation from different sources play a significant role in reducing PWD. McDonald et al. (1999) evaluated the effect of a highly-digestible diet based on cooked white rice supplemented with animal protein, and a diet with added soluble fibre provided as guar gum in pigs challenged with enterotoxigenic *E. coli*, and found that more of these organisms were recovered from pigs fed rice-based diets supplemented with guar gum. The findings of the study suggest that guar gum has a detrimental effect on the proliferation of enterotoxigenic *E. coli* in the small intestine of weaned pigs. Similarly, adding oat hulls to weaner pig diets based on extruded rice decreases protein fermentation and tends to decrease total biogenic amine concentrations, and decreases the incidence of PWD (Kim et al., 2008). It suggests that insoluble fibre sources like oat hulls can also reduce PWD, when there is unbalance in the CHO:protein ratio entering the pig's large intestine.

High FC content increases the number of *Lactobacilli* and tend to decrease the number of some coliforms and reduced ammonia concentration (Bikker et al., 2006). Similar trend was found in the colon for ammonia concentration. It shows that an increase in dietary FC content and, to a lesser extent, a decrease in CP content, reduces ammonia concentration in the gut and alters the microbiota in the gastrointestinal tract of weaned pigs. To have a more specific dose response of protein fermentation, Heo et al. (2008) studied the effect of different protein levels on protein fermentation and its impact on PWD and found that low protein diets (173 g CP/kg) supplemented with amino acids reduce the incidence of PWD at day 8 after weaning, without compromising growth. In another experiment, Heo et al. (2009) found that pigs challenged with *E. coli* had lower growth rate and decreased the gain-to-feed ratio compared with non-challenged pigs in a 4-week period after weaning. However, there was a marked reduction in the incidence of PWD after infection with β-hemolytic *E. coli*. and feeding a low protein diet for 7 or 14 d after weaning. It suggests that protein fermentation selectively affects the microbiota in the gut and can influence the pig's health condition.

From the above discussions, it can be noted that reducing protein and including FC in diets for weanling pigs may reduce the negative impact of proteolytic fermentation on gut health and function. However, FC and fermentable proteins show independent effects on newly weaned pigs in metabolite production and plasma nitrogen level but also show partly-related effects on gut microbiota (Jeaurond et al., 2008). It can thus be concluded that a planned reduction in dietary protein content combined with the inclusion of fermentable fibre will reduce the production of potentially harmful microbial metabolites in the caecum and the

incidence and severity of PWD in piglets. Therefore, the inclusion of DF and reduction in protein content can be used as a nutritional strategy to optimise intestinal health of early-weaned pigs.

2.5.3 Effect on bacterial protein synthesis and nitrogen excretion pathway

Microbiota in the large intestine synthesise their nitrogenous cell components and secretions from simpler molecules such as ammonia, amino acids and peptides (Payne, 1975; Nolan *et al.* 1976; cited by Mason, 1984), using FC as the principle energy source. With increased supply of FC as an energy source, an excess of indigestible protein is more likely to be incorporated into bacterial proteins rather than being fermented and used as a source of energy (Houdijk et al., 1998). It is reported that 60 to 90% of faecal N is of bacterial origin (Mason, 1984; Mosenthin et al., 1992; Rubio, 2003). Similarly, Cummings (1984) mentioned that most of the N excreted through the faeces in man and animals fed DF is considered to come from bacterial fermentation in the large intestine. The undigested protein available in the large intestine, which is of both dietary and endogenous origin, is utilised by resident bacteria as a source of N, resulting in increased bacterial proteins. Moreover, blood urea is the key supply of N for bacterial proliferation in the colon. In presence of highly ureolytic bacteria in the caecum, the urea concentration gradient favours a net transfer of urea into the caecal lumen (Younes et al., 1995), thus bacteria enhance the urea transfer from blood to the large intestine (Mosenthin et al., 1992). The ammonia generated by bacterial urease is used by bacteria for protein synthesis, which increases the amount of N present in the faeces and decreases N excretion in urine, in the form of urea (Kirchgessner et al., 1994; Younes et al., 1995). Thus, the inclusion of fermentable fibre in diets shifts N excretion pathway from urine to faeces.

With an increase in fibre intake, there is increase in faecal N excretion and decrease in urinary N excretion, expressed as a proportion of N intake (Morgan and Whittemore, 1988). Kreuzer and Machmüller (1993) found that the addition of 10 to 22% NSP in pig diets reduced urinary N excretion by 20 to 28%. This function is affected by the source of fibre as well. In a study with pigs, Kirchgessner et al. (1994) reported the effect of different fibre sources on bacterial protein synthesis and N excretion. A higher increase in faecal N per gram of N intake at the cost of urinary N was found in alfalfa meal, compared to WB. In the same study, bacterial protein excretion per 100 g of fermented matter was 13, 20 and 28 g for the

basal diet, wheat bran and alfalfa meal, respectively; clearly indicating the varying effect of different fibre sources on bacterial protein synthesis and shift in N excretion pathway.

Pigs fed with highly fermentable by-products such as SBP excrete less N via urine and more N via faeces than pigs fed grains and tapioca (Canh et al., 1997). Zervas and Zijlstra (2002) reported that the inclusion of fermentable fibre from SBP and soybean hulls in low protein wheat- and barley-based diets increased faecal N output from 5.1 to 7.7 g/d and lowered urinary:faecal N excretion ratio, decreasing from 2 for the control diet to 1.3 with soybean hulls and 1 for SBP-based diets. However, there was no affect of diets on total N excretion. Rubio (2003) used a rat model to evaluate the effect of different legume seeds (raw or germinated faba bean and chickpea seed meals) on the N excretion pattern, and found higher faecal N excretion in rats fed legume seeds, compared to rats fed lactalbumin.

Not only fibre, but also a low protein diet (173 g CP/kg) fed for 5 days after weaning, decrease plasma urea N and faecal ammonia-nitrogen contents (Heo et al., 2008). The combination of both the addition of fibre and reduction of CP and amino acid content result in greater proportions of N excreted through faeces, as compared to the control diet (Shriver et al., 2003). Recently, Bindelle et al. (2009) examined the response of fibre on bacterial protein synthesis and reported a linear increase of bacterial N incorporation with graded levels of SBP: 2.01, 2.06, and 2.35 mg/g diet with 10, 20, and 30% SBP, respectively, as compared to 1.51 mg for the control diet. In another experiment, they also found that the inclusion of SBP linearly decreases urinary N excretion from 0.285 to 0.215 g of N excreted in urine per gram N ingested and decreases the urinary:faecal N excretion ratio from 2.17 to 1.18 (Bindelle et al., 2009). Further, they concluded that the *in vivo* observation of N excretion shifts with graded concentrations of fermentable DF in pig diets were correlated with enhanced bacterial N uptake in the large intestine, as measured by the *in vitro* gas production technique. They suggest that the *in vitro* technique can be used to predict the N excretion from the pig intestines using bacterial nitrogen incorporation.

There is very limited information on the role of RS on the shift of N excretion pathway. Heijnen and Beynen (1997) worked with post-valve T-caecum cannulated pigs to study the influence of different types of RS (uncooked high amylose corn starch, RS_2; retrograded high amylose corn starch, RS_3; and glucose as reference) on the route of N excretion. They concluded that RS_3 reduces urinary N excretion; mainly in the form of urea while RS_2 does not affect the N excretion pattern, compared to glucose. In another experiment, De Schrijver et al. (1999) found similar results while working with ileum-cannulated pigs fed the same conventional diets with 6% dietary raw (RS_2) or retrograded

high-amylose corn starch (RS_3). From these studies, it can be concluded that RS_3 as compared to RS_2 exerts more pronounce effects in terms of shifting N excretion from urine to faeces. This shift of N excretion after consumption of RS is explained by the same phenomenon as with DF, i.e. there is increased bacterial protein synthesis and a subsequent decrease in colonic absorption of N in the form of ammonia.

It can be summarised that there is high bacterial nitrogen uptake when pigs are fed with fermentable fibre sources, suggesting that, in presence of higher levels of CHO substrate for fermentation, the resident microbiota in the large intestine retain more N for their own growth. There is thus increased bacterial protein mass and also a shift in N excretion pathway from urine to faeces.

2.6 Impact on nitrogenous gases emission

Intensive pig farming systems have largely contributed to increased pork production. However, they are criticised for their negative impact on the environment. The major concern of commercial pig production on odour emission arises from surplus nutrients in the excreta and gaseous losses in the environment. The main nutrients of environmental concern are nitrogen, phosphorus and heavy metals while the main gaseous losses are ammonia and methane (Aarnink and Verstegen, 2007). However, this review has limited its scope to nitrogenous gases, especially ammonia where fibre and protein fermentation have a major role to play.

Nitrogenous gases emitted from piggery have been criticised by a large group of the human society, apart from their negative impact on the health of barn workers as well as pigs. Even low levels of ammonia (25 ppm) in pig barns induce nasal irritation and depression of growth in pigs (Urbain et al., 1994), while air quality in the confinement of a pig facility can cause acute respiratory responses in humans (Zhang et al., 1998).

Most of the ammonia in pig manure originates from the breakdown of urea while small parts come from the breakdown of protein in faeces (Aarnink et al., 1993). The activity of urease determines the rate at which urea is converted into ammonia. Urease is present only in faeces, not in urine. Thus, the conversion of urea to ammonia only starts when urine mixes with faeces or comes in contact with soiled floors (Aarnink et al., 1997). At the manure storage pit, protein breakdown from manure is a slow process which might take weeks or even longer. But, the degradation of urea to ammonia and carbon dioxide can occur only in few hours (Spoelstra, 1979) but the process is affected by several factors like ammonia

concentration, pH, temperature, air velocity and emitting surface area (Aarnink and Verstegen, 2007).

2.6.1 Strategy to reduce nitrogenous gases emission

To deal with the negative impact of intensive pig production on the environment, three nutritional approaches have been proposed with some noted success: reducing N excretion by lowering CP intake, shifting N excretion from urea in urine to protein in faeces and lowering the pH of manure by lowering the pH of urine and faeces (Aarnink and Verstegen, 2007). Inclusion of DF (Canh et al., 1997) and reduction of CP, supplemented with essential synthetic amino acids (Gatel and Grosjean, 1992) are also effective.

2.6.1.1 *Reducing protein in diets*

The concentration of urea in urine and the pH of faeces and urine are important characteristics of excreta to determine ammonia emission from a pig facility. The urea concentration of urine highly depends on the protein level of the diet (Jongbloed, 2008). It can thus be changed by manipulating the protein content. In practice, protein levels generally exceed the pig requirements and are thus not totally utilised by the pigs. Several studies have shown that the protein content of diet can be reduced by 3-4% without any negative effect on pig performance (Canh et al., 1998b; Ball and Möhn, 2003; Htoo et al., 2007). Some portions of proteins, for example ~25% of protein in a typical corn-soybean meal diet, cannot be utilised, due to unbalanced amino acids (Rademacher, 2000). These overfed or unbalanced proteins/amino acids are broken down to nitrogen and excreted as urea in urine. Thus, supplying balanced protein/amino acid contents and matching the closest possible amount to the requirement of the pig can contribute to reduce N excretion.

Using a mathematical model, Aarnink et al. (1993) estimated that the reduction of CP by 1% in a diet can reduce ammonium nitrogen in manure by 9%, which is almost double that of the first documented report on the role of reduced CP level on reducing N excretion (Lenis, 1989). Confirming the model estimate, Mohn and Susenbeth (1995) found that a reduction of 20% in dietary protein can reduce N excretion by up to 35%, if the low protein diets offer adequate amino acid concentration. Thereafter, several attempts have been made to explore the in-depth relationship of CP content in diet and N excretion. Canh et al. (1998b), while comparing three levels of protein content (16.5, 14.5 and 12.5 %), found that every 1% reduction in CP in diet reduced ammonia content of the manure by 10% and ammonia

emission by 10-12%. The effect of CP and ammonia reduction ratio was a little higher in this study than in studies reported by other workers, which might be explained by the fact that the pH of the manure had been lowered. Similarly, Sutton et al. (1999) reported a 28% reduction of ammonium and total N concentration in manure when CP was reduced by 3% (from 13 to 10%) in corn-soybean meal-based diets supplemented with lysine, tryptophan, threonine, and methionine in growing pigs. Such reduction in N can be expected from sows as well (Ball and Möhn, 2003). Reviewing several studies, Kerr (1995) noted that there is wide variation in the effect of reducing the CP content with amino acid supplementation on N excretion, ranging from 3.2 to 62% (average around 8.4%), which depends on the size of the pig, level of dietary CP reduction, and initial CP level in the control diet.

2.6.1.2 *Including dietary fibre in diets*

Dietary fibre inclusion in pig diets has been used extensively to reduce ammonia emission. The addition of NSP from soybean hulls and/or SBP in pig diets reduces the urinary-to-faecal N ratio and, thereby, ammonia emission (Mroz et al., 2000). Quantitatively, the addition of 10 to 22% NSP in pig diets reduce urinary N excretion by 20-28% (Kreuzer and Machmüller, 1993). Similar results were found in a study by Canh et al. (1997), where the increase in NSP content from 14 to 31% in the diet decreased the urinary-to- faecal N ratio from 3.8 to 1.2 and the apparent N digestibility from 85 to 75%. In a follow-up study, Canh et al. (1998c) found a linear relationship between NSP intake and ammonia emission, with decrease of 5.4% ammonia for each 100 g increase in NSP intake.

Feeds with high contents in pectin and hemicellulose, like citrus pulp and SBP, are more effective DF sources to reduce N loss in manure, as compared to cellulose from rye bran and RS from cassava (Kreuzer et al., 1998). This can be ascribed to their fermentative capacity, which affects the N utilization pattern in the intestines. Sutton et al. (1999) reported that the manure of pigs fed a grain-based diet lost 2.4% of the initial N in the form of ammonia, as compared to 1.4% with SBP-based diets during a 7 d-storage period. Supplementing enzymes in cereal-based diets also affects ammonia emission. Garry et al. (2007) studied the effect of diets based on barley or wheat, supplemented or not with exogenous enzymes (endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase) on ammonia emission in growing-finishing pigs and found that the addition of an enzyme to the wheat-based diet decreased the rate of ammonia emission while the addition to the barley-based diet increased both odour and ammonia emissions. Similar results were found in a study of Leek et al. (2007). Ammonia production from manure during 10 d was higher with maize and

wheat-based diets (11.3 and 12.1% of the N intake, respectively) than with barley-based diets (6.6%). But when exogenous enzymes (endo-1,3(4)-β-glucanase and endo-1,4-β-xylanase) were added to the diets, the emission of ammonia from maize- and wheat-based diets decreased while the emission from barley-based diets increased. This confirms the role of the NSP in the reduction of ammonia emission.

2.6.1.3 *Lowering the pH of faeces and manure*

Lowering the pH of faeces and manure is also an efficient means for reducing ammonia emission since it is soluble under its protonated form (NH_4^+) (Aarnink and Verstegen, 2007). Microbial fibre fermentation in the large intestine results in the acidification of the digesta due to SCFA production, resulting in the reduction of the pH of faeces and manure while the opposite is found when protein is fermented, due to ammonia production. In a series of experiments, Canh et al. (1997; 1998a; 1998c) reported reduced pH of faeces and manure when pigs were fed diets containing FC, apart from the shift in N excretion pathway. For each increase of 100 g NSP in pig diets, the pH of the slurry decreased by 0.12 units and ammonia emission was reduced by 5.4%. The decrease in pH was partly attributed to the decrease in the NH_4-N content of the slurry as well as increased SCFA concentration of the slurry following carbohydrate fermentation (Canh et al., 1998a). Similarly, lowering the pH of urine can lower the pH of manure, thus reducing ammonia release form the storage. The urine pH can be altered by changing the electrolyte balance of the diet (Patience et al., 1987).

There is scarce information available on the role of fibre fermentation on urinary pH reduction. Mroz et al. (2000) used different fibre sources in conjunction with buffering agents (alkalogenic limestone and acidogenic Ca sulfate) in pig diets and reported indications of acidification of urine in pigs fed Ca-sulfate in presence of NSP-rich carbohydrates from soybean hulls and sugar beet pulp. However, there was no effect of dietary factors on the acidity of manure or on ammonia emission.

It can be concluded that dietary manipulation is an effective tool to reduce nitrogenous gases from piggery. N excretion and ammonia emission from pig can be reduced substantially by strategically decreasing dietary protein and increasing fermentable fibre in pig diets.

2.7 Conclusions

There is incentive for including sources of fermentable fibre in pig diets due to their functional properties, i.e. value beyond supplying energy and nutrients to the animals. It is also of special interest for the pork industry to add fibre to the diet in the changing context of consumers looking for pork produced without antibiotics used as growth-promoters and are concerned about odours emitted from pig barns. To some extent, diets containing fermentable fibre can contribute to reduce the necessity of antibiotics in diets and to reduce the emission of nitrogenous gases from pig barns.

However, DF negatively affects nutrient and energy digestibility. Moreover, there is wide variation found in the digestibility and fermentation characteristics of the different fibre fractions, due to their physico-chemical properties and degree of polymerization. In general, soluble DF fractions are fermented faster, produce higher amounts of SCFA and lower ammonia concentrations than insoluble fractions. Resistant starch is primarily butyrogenic, which improves gut health and the immune system, apart from exerting other health benefits to the host animal. Some of the fibre fractions are found to exert "prebiotic effect", enhancing "beneficial bacteria" at the cost of "harmful bacteria" in the pig gut. Inclusion of fibre in pig diets also shifts nitrogen excretion pathway from urine to faeces and contributes to reduce ammonia emission. Moreover, reducing protein level and including fermentable carbohydrates to weanling pigs may reduce the negative impact of proteolytic fermentation on gut health and function. Therefore, the inclusion of dietary fibre and reduction in protein content in diets can be used as a nutritional strategy to optimise the intestinal health of pigs.

CHAPTER 3

IN VITRO EVALUATION OF THE FERMENTATION CHARACTERISTICS OF THE CARBOHYDRATE FRACTIONS OF HULLESS BARLEY AND OTHER CEREALS IN THE GASTROINTESTINAL TRACT OF PIGS

Submitted to *Animal Feed Science and Technology*

Abstract

An *in vitro* model was used to study the fermentation characteristics of carbohydrate fractions of hulless barley (hB), in comparison to hulled barley (HB), hulled oat and oat groats (OG) in the pig intestines. For this purpose, 6 hulless barley cultivars (hB), varying in β-glucan content (36-99 g/kg DM), were compared to 3 HB cultivars, 2 oat groat samples (OG), 3 oat varieties and a reference sample of wheat. The residue of a pepsin-pancreatin hydrolysis was incubated in a buffered mineral solution inoculated with pig faeces. Gas production, proportional to the amount of fermented carbohydrates, was measured for 48 h and modelled. The fermented solution was analysed for short-chain fatty acids (SCFA) and ammonia concentrations. *In vitro* dry matter degradability varied according to ingredient ($P<0.001$). Higher values were observed for OG, ranging from 0.88 to 0.99 as compared to oat, hB and HB, for which degradability ranged from 0.63 to 0.73, 0.68 to 0.80 and 0.69 to 0.71, respectively. A "cereal type" effect ($P<0.05$) was observed on fermentation kinetics parameters. Total gas production was higher ($P<0.05$) with hB (224 ml/g DM incubated) than with HB and oat (188 and 55 ml/g DM incubated, respectively). No difference was observed between hB cultivars ($P>0.05$) for total gas production but differences ($P<0.001$) were found for lag time and the fractional rate of degradation. CDC Fibar (waxy starch) and CDC McGwire (normal starch) started to ferment sooner (lag time 0.7 and 0.9 h respectively) but the lag time for SH99250 (high amylose starch) was longer (1.7 h). The fractional rate of degradation was similar in both hB and OG (0.15/h, on average) which was higher than that of HB (0.12/h). The production of SCFA was also higher ($P<0.05$) with hB (6.1 mM/g DM incubated, on average) than with HB and oat (4.9 and 2.9 mM/g DM incubated respectively). Similar trends were found for SCFA production expressed per g fermented carbohydrates, with higher butyrate and lower acetate ratio. In contrast, oat fermentation generated higher ($P<0.05$) ammonia concentration (1.4 mM/g DM incubated, on average) than hB (1.0 mM/g DM incubated). In summary, hulless barleys, irrespective of cultivar type had higher *in vitro* fermentability and produced more SCFA and less ammonia than hulled barley and oat. Thus, hulless barleys have a better potential to be used in pig nutrition with gut health- promoting properties.

Key words: β-glucan, Hulless barley, *In vitro* fermentation, Non-starch polysaccharides, Pig intestine

3.1 Introduction

Although dietary fibre (DF) negatively affects nutrient digestibility (Bach Knudsen, 2001), there is growing interest in incorporating fermentable DF in pig diets, including resistant starch (RS) and non-starch polysaccharides (NSP), due to its potential prebiotic properties (Verstegen and Williams, 2002). The intestinal fermentation of NSP results in the formation of short-chain fatty acids (SCFA) (Awati et al., 2006), a decrease in protein fermentation and the release of ammonia (Le et al., 2005). It also stimulates the growth of enteric bacteria, which have positive effects on gut health (Dongowski et al., 2002).

Isolated DF were reported to have prebiotic effects in pigs (Awati et al., 2006) and to enhance the development of health-promoting bacteria, such as *Lactobacillus* and *Bifidobacteria* species (Charalampopoulos et al., 2002). However, little information is available on the fermentation characteristics of the carbohydrates (CHO) embedded within the fibrous matrix of the cereals. Wellock et al. (2007) reported that gut health may benefit most from diets containing appropriate sources of predominantly soluble NSP (sNSP) rather than insoluble NSP (iNSP). Thus, it is imperative to know the source and type of fibre incorporated in pig diets to obtain optimum benefit for an improved gut health.

Various cultivars of hulless barleys (hB) with different levels of β-glucan (βG) and/or amylose/amylopectin ratio are now available. These are interesting sources of fermentable DF due to the high proportion of NSP (Izydorczyk et al., 2000). Within the hB cultivars, differences in fermentation characteristics can be expected based on the composition of their CHO fractions. As pig diets are formulated with whole grains, the inclusion of hB cultivars containing highly-fermentable NSP, especially βG, could possibly contribute to an increase in SCFA production, a decrease in ammonia production and an increase in the growth of beneficial microbiota in the pig intestines, compared to common barley varieties. This, in turn, may contribute to improve gut health.

An *in vitro* experiment (based on enzymatic digestion and use of gas technique) was conducted to compare the fermentation characteristics of different hB cultivars varying in their carbohydrate fractions, in comparison to hulled barleys (HB), hulled oats, oat groats and wheat (used as a reference) as a first step to screen the potential beneficial properties of hB on pig intestines. It was hypothesised that the carbohydrate fraction of hB, especially amylose and soluble NSP (βG) are better fermented and yield higher levels of SCFA and less ammonia than that of hulled barley and oat.

3.2 Methodology

3.2.1 Cereal samples

A total of 15 cereal samples (6 cultivars of hB, 3 of HB, 2 of dehulled oats (i.e. oat groats; OG), 3 of oats and wheat (reference) were tested. Hulless barleys were obtained from the Crop Development Centre (CDC) at the University of Saskatchewan (Saskatoon, SK, Canada) while hulled barleys, oats and wheat were collected from different farms of Saskatchewan in 2005 and 2006. The samples were chosen according to their different fermentable carbohydrate composition (Table 3.1).

3.2.2 Enzymatic hydrolysis

The cereal samples, ground with a Retsch ZM1 mill (Newton, PA, USA) to pass a 1 mm mesh screen, underwent an *in vitro* pepsin and pancreatin hydrolysis, following the first steps of the protocol of Boisen and Fernandez (1997). Briefly, 2 g samples were weighed in conical flasks. A phosphate buffer solution (100 ml, 0.1M, pH 6.0) and an HCl solution (40 ml, 0.2M) were poured into the flasks. The pH was adjusted to 2.0 with 1M HCl or 1M NaOH. Two ml of a chloramphenicol (Sigma C-0378) solution (0.5 g/100 ml ethanol) was added to prevent bacterial growth during hydrolysis. Fresh pepsin solution (4 ml, 20 g/l porcine pepsin, Sigma P-0609) was added and the flasks were placed in a water-bath at 39^{o}C for 2 h under gentle agitation (50 rpm).

Afterwards, 40 ml phosphate buffer (0.2M, pH 6.8) and 20 ml of a 0.6 M NaOH were added into the solution. The pH was adjusted to 6.8 with 1M HCl or 1M NaOH. Fresh pancreatin solution (2 ml, 100 g/l pancreatin, Sigma P-1750) was added and hydrolysis was continued for 4 h under the same conditions.

After hydrolysis, the residues were collected by filtration on a nylon cloth (42 µm), washed with ethanol (2 x 25 ml 95% ethanol) and acetone (2 x 25 ml 99.5% acetone), dried for 24 h at 60^{o}C and weighed. The enzymatic hydrolysis was repeated from 5 to 11 times, depending on the degradability of each cereal in order to get enough samples for the *in vitro* fermentation and their analysis. Hydrolysed residues from the different replicates and batches of same ingredients were pooled for subsequent analyses (dry matter, DM; crude protein, CP; βG and starch) and *in vitro* fermentation.

Table 3.1 Chemical composition of the cereal samples and resulting hydrolysed substrates (g/kg DM)

Cereal type	Cultivar	Starch type[1]	Cereal samples									Hydrolysed substrates			
			DM (g/kg)	Ash	EE	CP	NDF	ADF	βG	NSP	S	CP	βG	NSP	S
hB	SB94893	H	932	19	24	176	150	27	81	118	525	79	145	345	137
hB	CDC Fibar	Z	933	22	30	154	110	17	99	122	515	91	140	425	95
hB	SH99250	H	935	22	27	134	155	21	91	137	513	68	161	365	129
hB	CDC McGwire	N	930	19	22	153	113	23	52	120	587	81	89	309	137
hB	SH99073	H	933	22	30	144	156	30	96	114	476	72	161	425	142
hB	SB90300	N	933	21	19	131	103	21	36	82	593	81	69	312	79
HB	CDC Bold	N	930	27	19	108	172	64	38	120	594	75	68	354	140
HB	McLeod	N	932	25	17	139	175	58	49	113	561	63	85	391	136
HB	CDC Helgason	N	935	24	22	129	145	49	47	96	542	76	76	365	139
OG	Morgan		938	20	57	138	89	23	51	153	558	147	116	345	102
OG	CDC Sol-FI		934	20	64	174	117	29	63	261	549	145	154	335	104
Oat	Morgan		946	37	41	99	390	198	31	148	276	54	23	601	7
Oat	CDC Sol-FI		942	38	46	127	324	161	46	265	324	57	40	579	31
Oat	CDC Baler		899	32	40	165	310	145	29	220	458	74	38	576	49
Wheat	Unknown		915	18	17	160	134	36	7	80	513	72	18	358	129
	Feed[2]		917	47	30	201	119	52	12	NA	426	NA	NA	NA	NA

Abbreviations: DM, dry matter; EE, ether extract; βG, β-glucan; NSP, non-starch polysaccharides; S, total starch; hB, hulless barley; HB, hulled barley; OG, oat groat; NA, not available

[1] H, high amylose (~40% of total starch); N, normal amylose (~25%) and Z, no amylose (~0%); Source: Rossnagel et al., (2005) and reports of Crop Development Centre, University of Saskatchewan, Saskatoon, Canada

[2] Feed sample of pig used as donor of faeces used as inoculum for *in vitro* fermentation

3.2.3 *In vitro* fermentation

The rate of fermentation of the hydrolysed substrates was assessed *in vitro,* using a cumulative gas-production technique adapted to the pig by Bindelle et al. (2007a): 200 mg samples were incubated at 39°C (in a shaking water-bath with 50 rpm) in a 125 ml-glass bottle with 30 ml buffer solution containing macro- and micro-minerals (Menke and Steingass, 1988) and a faecal inoculum. Three weaned piglets (4-5 weeks age) from the herd of the Prairie Swine Centre (Saskatoon, SK, Canada), fed a standard commercial diet devoid of antibiotics (Table 3.1), were used as donors for the faecal inoculum. Faecal samples were collected directly from the rectum and immediately placed in an air tight plastic syringe. The inoculum prepared from faeces was diluted 20 times in the buffer solution and filtered through a 250 µm-screen and transferred into bottles with the fermentation substrates. Bottles were sealed with a rubber stopper and placed for incubation. An anaerobic environment was maintained thoroughout, from the inoculum preparation till the incubation step by flushing with CO_2 gas. The gas generated by the fermentation process and the CO_2 released by the buffering of the SCFA produced during the fermentation were measured at 0, 2, 5, 8, 12, 18, 24, 36 and 48 h by means of a pressure transducer (GP:50 SIN-54978, Grand Island, NY) (Mauricio et al., 1999), fitted with digital data tracker (Tracker 211, Intertechnology Inc., Ontario, Canada). The bottles were vented after every measurement. Fermentation was stopped at 48 h of incubation by quenching the bottles in iced water.

The experimental scheme was as follows: 15 cereal samples x 2 replicates + 6 blanks (containing the inoculums only) repeated over 4 run (batches).

At the end of the fermentation, samples were collected from the bottles for measurement of SCFA and ammonia. Samples of the inoculum prior to fermentation were also analysed for SCFA and ammonia.

3.2.4 Chemical analyses

All samples and the diet (of the faeces donor pig) were ground with a laboratory mill (Retsch mill ZM1, Newton, PA, USA) to pass through 1 mm mesh screen. Chemical analyses (Table 3.1) were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007) with specific methods as follows: DM (135°C for 2 h, AOAC 930.15), nitrogen (AOAC 968.06; using an elemental analyser LECO FP528, St

Joseph MI, USA; CP = N × 6.25), ether extract using Soxhlet apparatus and petroleum ether (AOAC 920.39), ash (AOAC 942.05), ADF (AOAC 973.18) and NDF (AOAC 2002.04).

Samples were ground to pass through a 0.5 mm-mesh screen and analysed using commercial test kits (Megazyme International Ltd., Ireland) to determine βG (AOAC 995.16) and total starch (AOAC 996.11). Samples were analysed for their NSP content by gas chromatography (GC) along with the individual sugar content (Englyst et al., 1994). Chromatographic analysis was carried out with a GC system (Agilent 6890 system, Germany) equipped with a flame ionization detector and a fused-silica capillary column (DB-17 HT, Agilent Technologies, USA), using 2-Deoxy-D-Glucose as the internal standard.

SCFA were analysed by GC (Agilent 6890 system, Germany) fitted with a flame ionization detector and a fused-silica capillary column (ZB-FFAP, Phenomenex, USA), using crotonic acid as internal standard. Branched-chain fatty acids (BCFA) content was calculated as the sum of the iso-butyric and iso-valeric acids.

Ammonia N concentration was determined by spectrophotometry using a method adapted from Novozamsky et al. (1974). Briefly, ammonia was oxidised by sodium hypochloride in the presence of sodium nitroprusside, which forms a blue colour complex and was measured at 600 nm using a spectrophotometer (Pharmacia LKB- Ultraspec III; Amersham, Freiburg, Germany).

3.2.5 Calculations and statistical analyses

3.2.5.1 *In vitro degradability*

In vitro dry matter degradability (*IVDMD*) during the pepsin and pancreatin hydrolysis was calculated as follows:

$$IVDMD = \frac{\text{dry weight of the sample before hydrolysis - dry weight of the residue}}{\text{dry weight of the sample before hydrolysis}} \quad (3.1)$$

The disappearance of the other nutrients was calculated using the degradability of DM and the relative content of individual nutrients in the ingredients and hydrolysed substrates.

3.2.5.2 *Kinetics of gas production*

Gas pressure measurements were converted into gas volume (G, g^{-1}DM) using the ideal gas law, assuming an atmospheric pressure of 101325 Pa and a temperature of 312.15

K. Gas accumulation curves recorded during the 48 h of fermentation were modelled according to France et al. (1993):

$$G\,(\text{ml g}^{-1}\text{DM}) = 0, \qquad \text{if } 0 < t < L \tag{3.2}$$

$$= G_f\left(1-\exp\left\{-\left\langle b(t-L)+c\left(\sqrt{t}-\sqrt{L}\right)\right\rangle\right\}\right), \qquad \text{if } t \geq L$$

where, G denotes the gas accumulation to time, G_f (ml g^{-1}DM) the maximum gas volume for $t = \infty$ and L (h) the lag time before the fermentation starts. The model is illustrated in Figure 3.1. The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the substrate μ (h^{-1}), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, \qquad \text{if } t \geq L \tag{3.3}$$

Kinetics parameters (G_f, L, $\mu_{t=T/2}$ and $T/2$) were compared in the statistical analysis. $T/2$ is the time to half-asymptote when $G = G_f/2$.

3.2.5.3 *Statistical analyses*

The *IVDMD* during hydrolysis, total gas production, fermentation kinetics parameters and fermentation metabolites production were analysed using the Mixed procedure of SAS 9.1 software (SAS, 2003) with the cereal as a fixed factor and batch as a random factor, using the following general linear model:

$$Y = \alpha + S_i + B_j + \varepsilon_{ij} \tag{3.4}$$

where Y is the parameter to be tested, α the mean, S_i the effect of the cereal (i = 1... 15), B_j the effect of batch (j = 1… 4) and ε_{ij} the error term. Means were separated using Tukey method with a significance level of 0.05.

Pearson's correlation calculations between different variables were performed using CORR procedure of SAS 9.1 software (SAS, 2003).

3.3 Results

3.3.1 Degree of enzymatic hydrolysis of the cereals

Table 3.2 details the results of degree of hydrolysis of the cereals. Differences in *IVDMD* were observed (P<0.001) between samples. Higher values were observed for OG

(0.88 to 0.90) whereas those obtained for whole oat, hB and HB, ranged from 0.63 to 0.73, 0.68 to 0.80 and 0.69 to 0.71, respectively. No statistical analysis could be performed on the degree of hydrolysis of CP, βG and starch because samples from the different batches of enzymatic hydrolysis were pooled. No specific pattern was noted for protein degradability among cereal types. It ranged from 0.76 to 0.89, the highest value being observed for wheat and the lowest for CDC Bold hulled barley and Morgan oat. In general, βG degradability was higher in oat than in barley, irrespective of the presence or absence of hull, except for CDC Fibar barley, which had similar βG degradability to oat. A similar trend was noted for starch degradability, with 0.92 on average for barley and 0.97 for oat.

Table 3.2 Degree of enzymatic hydrolysis of the different cereal samples

Cereal type	Cultivar	No[1]	Dry matter	Crude protein[2]	β-glucan[2]	Starch[2]
hB	SB94893	6	0.72^{efg}	0.86	0.44	0.92
hB	CDC Fibar	6	0.80^{b}	0.86	0.67	0.96
hB	SH99250	5	0.68^{gh}	0.83	0.39	0.91
hB	CDC McGwire	6	0.72^{ef}	0.84	0.47	0.93
hB	SH99073	6	0.73^{def}	0.85	0.49	0.91
hB	SB90300	6	0.75^{cd}	0.83	0.47	0.96
HB	CDC Bold	6	0.69^{gh}	0.76	0.39	0.92
HB	McLeod	6	0.70^{fgh}	0.85	0.43	0.92
HB	CDC Helgason	6	0.71^{efg}	0.81	0.50	0.92
OG	Morgan	6	0.90^{a}	0.85	0.68	0.97
OG	CDC Sol-FI	6	0.88^{a}	0.87	0.62	0.97
Oat	Morgan	5	0.63^{i}	0.78	0.70	0.99
Oat	CDC Sol-FI	5	0.67^{h}	0.84	0.69	0.97
Oat	CDC Baler	6	0.73^{de}	0.87	0.61	0.97
Wheat	Unknown	6	0.77^{bc}	0.89	0.39	0.94
SEM			0.075			
P value			<0.001			

Abbreviations: hB, hulless barley; HB, hulled barley; OG, oat groat

[1] Number of replicates of enzymatic hydrolysis

[2] Values from pooled samples of different replicates of enzymatic hydrolysis

3.3.2 Kinetics of gas production

The kinetics of gas production recorded during *in vitro* fermentation is described in Table 3.3. In general, all fermentation parameters varied according to cereal type but also between some cultivars ($P<0.05$). Hulless barleys degraded more rapidly and yielded more gas than HB, oat and wheat. Expressed per g fermented carbohydrates (NSP + starch), the final gas production, lag time and rate of degradation differed according to hB cultivar. The fractional rate of degradation was similar in hB and OG (0.15/h, on average) and higher than that of HB cultivars (0.12/h, on average). Finally, total gas production was the highest for hB (ranging from 218 to 235 ml/g of DM substrate incubated and from 427 to 621 ml/g CHO fermented) and the lowest for oat (from 48 to 61 ml/g DM substrate incubated from 170 to 237 ml/g CHO fermented). Due to the very slow rate of fermentation, the gas production curves of the oat cultivars did not fit France's model. Differences of fermentation kinetics were also observed between the hB cultivars. The SH99073 and SH99250 hB started to ferment slowly (lag time of 1.4 and 1.7 h respectively) but produced more gas than the other hB cultivars while CDC Fibar started to ferment sooner (lag time 0.7 h). The SB90300 hB cultivar degraded rapidly and the CDC Fibar cultivar slowly (fractional rate of degradation being 0.17 and 0.12/h, respectively) but they produced a similar amount of total gas (218 ml/g DM incubated). Expressed per g of CHO fermented, SB90300 hB produced the highest amount of both total gas and SCFA.

3.3.3 Profile of end-products after cereal fermentation

The values for metabolites produced after fermentation is presented in Table 3.4. SCFA production was higher for hB, compared to HB and oat (6.1 vs 4.9 and 1.5 mM/g DM incubated, respectively; $P<0.05$), but no difference was observed between hB cultivars. When SCFA production was expressed per g CHO fermented, similar trends were found among cereal types, but differences ($P<0.05$) were observed between hB cultivars. SB90300 hB yielded the highest SCFA production (16.1 mM/g CHO fermented) while SH99073 hB had the lowest SCFA production (11.1 mM/g CHO fermented). In contrast, ammonia concentration was higher ($P<0.05$) for oat than for barley (4.5 vs 2.3 mM/g CHO fermented on average, respectively).

SCFA profiles differed between samples. Oat with higher hull content had higher ratios of acetate (0.57 to 0.61) and lower of butyrate (0.13 to 0.19), while hulless barley yielded lower acetate (0.46 to 0.50) and higher butyrate (0.16 to 0.20) ratios.

3.3.4 Correlations between carbohydrate components and fermentation parameters

The βG content measured in the hydrolysed substrates correlated positively with the fractional rate of degradation (r=0.29, P=0.004), total gas (G_f) and SCFA production (r=0.64 and r=0.65 respectively, P<0.001). There was a strong correlation between starch content of the hydrolysed substrates with total gas (G_f) and SCFA production (r=0.86 and r=0.83 respectively, P<0.001).

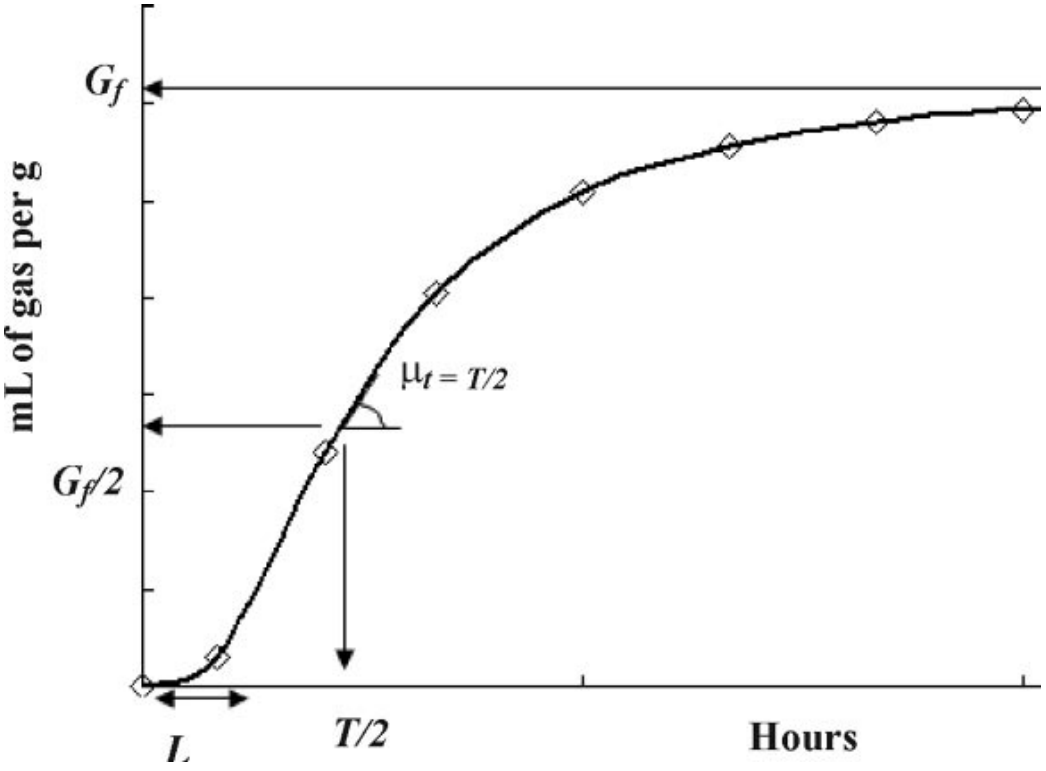

Figure 3.1 Representation of the kinetic parameters of the gas accumulation curves modelled according to France et al. (1993) , where G_f (mL gas/g of substrate) denotes the maximum gas volume for $t = \infty$, L (h) denotes the lag time before fermentation starts, $T/2$ denotes the half-time to asymptotic gas production when $G = G_{f/2}$, and μ (h^{-1}) denotes the fractional rate of degradation of the substrate.

Table 3.3 Fitted kinetics parameters (means) of gas accumulation recorded for different hydrolysed substrates incubated with faecal inoculum from pigs

Cereal type	Cultivar	Per g DM incubated					Per g CHO fermented		
		N[1]	L[2]	T/2[3]	μ[4]	G_f[5]	N[6]	μ[4]	G_f[5]
hB	SB94893	8	1.3^{ab}	7.4^{cde}	0.16^{ab}	225^{ab}	4	0.16^{ab}	503^{bcd}
hB	CDC Fibar	8	0.7^{b}	6.9^{ef}	0.12^{d}	218^{bc}	4	0.12^{de}	456^{de}
hB	SH99250	8	1.7^{a}	8.4^{a}	0.15^{ab}	235^{a}	4	0.15^{ab}	506^{b}
hB	CDC McGwire	8	0.9^{b}	6.8^{f}	0.16^{ab}	225^{ab}	4	0.16^{ab}	542^{b}
hB	SH99073	8	1.4^{ab}	8.0^{ab}	0.15^{bc}	228^{a}	4	0.14^{bcd}	427^{e}
hB	SB90300	8	1.4^{ab}	6.8^{f}	0.17^{a}	218^{bc}	4	0.17^{a}	621^{a}
HB	CDC Bold	8	0.9^{b}	7.1^{def}	0.12^{d}	191^{d}	4	0.12^{e}	505^{bc}
HB	McLeod	8	0.9^{b}	7.5^{bcd}	0.11^{d}	193^{d}	4	0.11^{ef}	470^{cde}
HB	CDC Helgason	8	1.0^{ab}	7.2^{def}	0.13^{cd}	180^{e}	4	0.13^{cde}	434^{e}
OG	Morgan	8	1.1^{ab}	7.0^{def}	0.15^{bc}	184^{de}	4	0.15^{bc}	463^{cde}
OG	CDC Sol-FI	8	0.7^{b}	6.1^{g}	0.15^{bc}	190^{de}	4	0.15^{bc}	508^{bc}
Oat	Morgan	8	NA	NA	NA	48^{g}	4	NA	170^{g}
Oat	CDC Sol-FI	8	NA	NA	NA	56^{fg}	4	0.07^{f}	177^{g}
Oat	CDC Baler	8	NA	NA	NA	61^{f}	4	NA	237^{f}
Wheat	Unknown	8	0.9^{b}	7.9^{abc}	0.12^{d}	213^{c}	4	0.12^{de}	526^{b}
SEM			0.18	0.55	0.004	3.3		0.005	14.2
P value			0.005	<0.001	<0.001	<0.001		<0.001	<0.001

Abbreviations: CHO, carbohydrates (non-starch polysaccharides + starch); hB, hulless barley; HB, hulled barley; OG, oat groat;

[1] N, number of observations in fermentation

[2] L, lag time (h)

[3] T/2, half-time to asymptote (h)

[4] μ, fractional rate of degradation (h^{-1}) at $t = T/2$

[5] G_f, maximum gas volume (ml per g DM incubated/CHO fermented)

[6] N, number of observation in pooled fermented substrates

[*] NA, not available; data not presented as these parameters do not fit in fermentation kinetics model used

[#] Means with different superscripts within the columns are significantly different ($P<0.05$)

Table 3.4 Concentrations of metabolites in solution after fermentation in a faecal inoculum (mM/g DM incubated or carbohydrate fermented)

Cereal type	Cultivar	mM/g DM incubated		mM/g CHO fermented						
		N	SCFA	N	SCFA	AA[1]	PA[1]	BA[1]	BCFA[1]	NH_3
B	SB94893	8	6.3^{a}	4	14.1^{ab}	47^{d}	29^{a}	19^{abc}	1.6	2.2^{fg}
hB	CDC Fibar	8	6.1^{ab}	4	12.6^{bc}	50^{d}	30^{a}	16^{cdef}	1.5	2.3^{fg}
hB	SH99250	8	6.2^{ab}	4	13.3^{bc}	46^{d}	29^{a}	20^{a}	1.7	2.0^{fg}
hB	CDC McGwire	8	6.1^{ab}	4	14.5^{ab}	48^{d}	28^{a}	19^{abc}	1.7	2.5^{fg}
hB	SH99073	8	6.0^{ab}	4	11.1^{c}	47^{d}	29^{a}	19^{ab}	1.6	1.9^{g}
hB	SB90300	8	5.7^{abc}	4	16.1^{a}	48^{d}	27^{a}	19^{abc}	1.6	2.8^{def}
HB	CDC Bold	8	5.1^{cd}	4	13.4^{bc}	50^{d}	27^{a}	18^{abc}	1.5	2.7^{ef}
HB	McLeod	8	5.0^{cd}	4	12.3^{bc}	51^{bcd}	28^{a}	17^{abcd}	1.1	2.6^{efg}
HB	CDC Helgason	8	4.7^{d}	4	11.3^{c}	49^{d}	28^{a}	18^{abc}	1.5	2.5^{fg}
OG	Morgan	8	5.0^{cd}	4	12.5^{bc}	51^{cd}	27^{a}	16^{bcd}	2.2	3.3^{cde}
OG	CDC Sol-FI	8	5.0^{cd}	4	13.2^{bc}	50^{d}	27^{a}	16^{bcde}	2.2	3.6^{bcd}
Oat	Morgan	8	1.3^{e}	4	4.7^{d}	61^{a}	24^{ab}	12^{ef}	1.5	4.6^{ab}
Oat	CDC Sol-FI	8	1.5^{e}	4	6.6^{d}	58^{ab}	26^{ab}	13^{f}	2.1	4.2^{abc}
Oat	CDC Baler	8	1.8^{e}	4	7.5^{c}	57^{abc}	21^{b}	14^{def}	1.8	4.6^{a}
Wheat	Unknown	8	5.5^{bc}	4	13.6^{abc}	49^{d}	27^{a}	19^{abc}	1.5	2.6^{efg}
SEM			0.14		0.82	1.6	1.6	1.2	0.18	0.31
P value			<0.001		<0.001	<0.001	<0.001	<0.001	0.340	<0.001

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); CHO, carbohydrates (non-starch polysaccharides + starch); hB, hulless barley; HB, hulled barley; N, Number of observation in fermentation; NH_3, Ammonia; OG, oat groat; PA, propionic acid; SCFA, short-chain fatty acids;

[1] Percentage of the individual SCFA

[#] Means with different superscripts within the columns are significantly different ($P<0.05$)

3.4 Discussion

This study aimed at exploring the potential of hulless barleys, as opposed to hulled barleys, oat groats and whole oat, to be incorporated in pig diets as sources of fermentable fibre. Cultivars differing in β-glucan content and amylose/amylopectin ratio were tested. The main difference with the other cereal types was in their insoluble fibre content, due to the presence or absence of hulls. However, differences between cereal cultivars of a same category can be ascribed to differences in βG concentration and possibly to starch composition with varying amylose-amylopectin ratios and resistant starch contents. Such variability within the hulless barley categories was similar to those reported by Bird et al. (2004) and Holtekjolen et al. (2006).

The *IVDMD* after pepsin-pancreatin hydrolysis varied among cereal types and cultivars but, surprisingly, it was unrelated to presence or absence of hulls. HB and oats had similar *IVDMD* while OG showed the highest rate of degradability. Besides the indigestibility of the hulls, their presence in HB and whole oats was expected to make starch less accessible to enzymes. It was not the case with hB and OG.

The amylose-to-amylopectin ratio was responsible for the variability in enzymatic degradability, as attested by the highest *IVDMD* and the high starch digestibility obtained for CDC Fibar. The starch of the latter is essentially composed of amylopectin (Rossnagel et al., 2005). The starch content of cultivars rich in amylose is less degraded during enzymatic hydrolysis in the upper gut (Pierce and Stevenson, 2008). The undigested starch, called "resistant starch", is further available for microbial fermentation in the lower gut (Topping and Clifton, 2001). The higher βG degradability observed for oat may be attributed to differences in βG solubility. Lambo et al. (2005) observed that 68% of βG was soluble in oat vs only 15% in barley.

Differences in fermentation kinetics and metabolite production between hB, HB, OG and oat were observed, showing variation among cereal types during fermentation. There was also a difference within the same cereal type, especially for hB, which is attributed to differences in the soluble fraction of the NSP (Jha et al., 2010) and the amylose/amylopectin ratio of starch (Li et al., 2001). Some hB contains higher levels of sNSP. The sNSP are better fermented than iNSP and produce more SCFA with hB, compared to HB and oat. The βG content measured in the hydrolysed substrates correlated positively with the fractional rate of degradation ($r=0.29$, $P=0.004$), the total gas (G_f) and SCFA production ($r=0.64$ and $r=0.65$ respectively, $P<0.001$).

Moreover, there was a strong correlation between starch content of the hydrolysed substrates with total gas (G_f) and SCFA production (r=0.86 and r=0.83 respectively, P<0.001). This observation suggests that the undegradable portion of starch (mostly amylose) of hB can be an important factor to consider while selecting cultivars to increase fermentation in the lower gut of the pigs, since the resistant starch fraction becomes available for microbial fermentation.

Differences in starch structure seem to also influence βG disappearance during hydrolysis. As an example, CDC Fibar had the lowest degradation rate and time to half asymptote gas production among the hB. This can be explained by the high βG loss during the enzymatic hydrolysis (0.67), compared to other hB cultivars (on average, 0.45), which affected the availability of βG for fermentation despite the highest βG in the raw grain. The disappearance of βG during the enzymatic hydrolysis appears thus to be differently influenced by the complex starch-fibre matrix in the grain that varies from one cultivar to another. Interestingly, there was a strong and negative correlation between the NDF content of ingredients with total gas (G_f) and SCFA production (r=-0.91 and r=-0.87, P<0.001). This supports the fact that cellulose and lignin are less fermentable by the microbiota.

Differences in the proportion of individual SFCA reflect the amount and type of substrate fermented and probably the microbial diversity present in the gut. When a substrate is abundant, the proportion of propionate and butyrate increases whereas the proportion of acetate decreases considerably (Macfarlane and Macfarlane, 2003). Higher butyrate and lower acetate from hB cultivars are linked to sNSP contents in the residues while reverse scenario of substrate type available for fermentation explains the higher acetate and lower butyrate from HB cultivars.

As expected, higher butyrate proportion was observed for the hB having high resistant starch content (SH99250, SH99073, among others). Butyrate is an important metabolite as it is the principal oxidative fuel for the colonocytes. It may also have beneficial tropic effects on inflamed caeco-colonic mucosa (Brouns et al., 2002) and on the improvement of the immune surveillance in gut (Macfarlane et al., 1992). High butyrate yielding-hulless barleys can thus contribute to an improvement of gut health.

The higher, although not significant, ammonia concentration observed for oat can be ascribed to a shift of fermentation toward protein, due to higher CP content in the hydrolysed residues as well as to a lower availability of fermentable carbohydrate substrates (Salvador et al., 1993), as indicated by the high ADF content and the slow fermentation pattern that did not fit with the mathematical model used.

There were some differences in both the fermentation kinetics and the end-products between cereal types as well as within the hB cultivars that cannot be explained only by their carbohydrate fractions. This may be due to the interaction between the composition of the cereal samples, the inoculum and the *in vitro* model itself. The extent of fermentation and the profile in SCFA depends on the substrate (Macfarlane and Macfarlane, 1993), the microbiota available during the fermentation process (Williams et al., 2001) and the interaction between the host and the microbiota (Coles et al., 2005). This gives a complex fermentation process.

There is also a limit in the capacity of the *in vitro* model to mimic the digestive process in the pig's gastrointestinal tract. This suggests that an enzymatic pre-treatment followed by drying could make the DF more susceptible to bacterial degradation than what happens *in vivo,* as freeze-drying and pre-digestion change the micro- and macro-porosity of cell walls (Lebet et al., 1996). The fermentability of the substrates could thus have been overestimated (Wisker et al., 1998). But, the βG lost during the enzymatic hydrolysis represents a fraction that could be available to microbiota in the pig's large intestine and could also rapidly ferment, already in the distal small intestine. However, the effect of such pre-enzymatic treatment is likely to be less important for highly fermentable substrates. The *in vitro* gas technique uses the total gas produced as an indicator of fermentation rather than of substrate disappearance and the gas produced does not come only from the microbial fermentation of the substrate but also from the bicarbonate buffer used in all *in vitro* fermentation methods (Coles et al., 2005).

In conclusion, advantage could be taken from the variation in carbohydrate composition of barley, in order to modulate the gut environment of the pig and possibly improve gut health. Irrespective of cultivar type, hulless barleys had higher *in vitro* fermentability and produced more beneficial (SCFA) and less harmful (ammonia) metabolites than hulled barleys and oats. There was variation between the cereal types and within the cultivar of same cereal type due to differences in their carbohydrate fraction. Considering the fermentation kinetics and fermentation metabolites produced, it can be concluded that hB cultivars, especially SH99250 (high amylose, high βG), SB90300 (normal amylose, low βG) and CDC Fibar (zero amylose, high βG) might be more interesting than others in swine nutrition in order to formulate pig diets with desirable gut health-promoting properties.

CHAPTER 4

BARLEY AND OAT CULTIVARS WITH DIVERSE CARBOHYDRATE COMPOSITION ALTER ILEAL AND TOTAL TRACT NUTRIENT DIGESTIBILITY AND FERMENTATION METABOLITES IN WEANED PIGLETS

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Abstract

An experiment was conducted to evaluate the effects of cereal carbohydrate form (isolated vs in cereal matrix) and level, especially mixed linked β-glucan (hereafter referred to as β-glucan) and starch amylose/amylopectin ratio on nutrient digestibility and fermentation parameters in the intestines of weaned pigs. Four hulless barley cultivars containing varying β-glucan levels (41 to 84 g/kg) were compared with hulled barley, supplemented or not with a β-glucan concentrate (BBG; 270 g/kg β-glucan) and two oat cultivars for digestibility and fermentation metabolites. Seventy-two weaned piglets (BW = 12.8 ± 1.9 kg) were assigned to one of nine diets composed of 815 g/kg cereal, 60 g/kg whey, 90 g/kg soy protein isolate and 35 g/kg minerals. After 15 days, the pigs were killed, and digesta collected from ileum and colon were analysed for proximate nutrients, short-chain fatty acids (SCFA), lactic acid (LA) and ammonia. Ileal and total tract digestibility of proximate nutrients and non-starch polysaccharides (NSP) were determined using HCl-insoluble ash as a marker. Organic matter (OM) ileal digestibility was greater ($P<0.05$) for diets based on hulless barley (77 ± 1.1% on average), as compared to hulled barley (64 ± 1.4%) and oat (58 ± 1.5%). Similar trends were found for total tract OM digestibility, varying from 90 ± 0.3% for hulless barley to 67 ± 0.4% for oat, on average. NSP digestibility differed ($P<0.05$) within and between cereal types, ranging from 20% (hulled barley plus 163 g/kg BBG or ~ 40 g/kg β-glucan) to 51% (SB94893 hulless barley cultivar with high β-glucan and high amylose ratio) at the ileum and from 44% (hulled barley) to 84% (SB94893 cultivar) at the total tract level. No dietary effect ($P>0.05$) was found for SCFA concentration in ileal contents, whereas in colonic contents, SCFA was lower in pigs fed oat ($P<0.001$). LA concentration was greater ($P<0.001$) in the colon of pigs fed hulless barley than in pigs fed hulled barley and oat. Expressed per kg carbohydrate (NSP + starch) fermented, the ammonia concentration at the colon was lowest for hulled barley diets (supplemented with β-glucan) and the highest for oat diets. In conclusion, the interaction of both form and level of β-glucan impacted nutrient digestibility and fermentation. Hulless barleys with high soluble NSP like β-glucan and resistant starch yielded, in general higher SCFA and LA and lower ammonia. Hulless barleys may, therefore, have potential for use in feeding strategies designed to improve gut health in pigs.

Key Words: Barley, β-glucan, Digestibility, Fermentation, Piglets

4.1 Introduction

Cereal non-starch polysaccharides (NSP) influence the digestive processes and gut microbiota composition in pigs (Bach Knudsen et al., 1991; Bach Knudsen and Hansen, 1991). Part of the NSP is fermented by distal tract intestinal microbiota, resulting in the formation of short-chain fatty acids (SCFA) (Daniel et al., 1997). Furthermore, NSP stimulate the activity of the microbial population, and some types of NSP may stimulate the growth of health-promoting bacteria, such as *lactobacilli* and *bifidobacteria* (Brown et al., 1997; Charalampopoulos et al., 2002), and thus can contribute to the gut health maintenance (Bouhnik et al., 2004; Wong et al., 2006). β-glucan, the primary soluble NSP in barley and oat, are being evaluated as potential gut ecosystem modulators in human nutrition (Brennan and Cleary, 2005).

NSP such as β-glucan, have mainly been studied in isolated form, whereas swine diets are usually composed of cereals containing large amounts of fermentable carbohydrates (CHO) in the grain matrix. The major components in barley fibres are β-glucan and the arabinoxylans, located in the cell wall of the endosperm and the aleurone layer (Holtekjolen et al., 2006). The fermentation rate of these carbohydrates in the intestinal tract will thus depend on their composition, form and physical properties (Le Goff et al., 2003). As a consequence, cereal NSP in isolated form or within a matrix may act differently in the gastrointestinal tract (GIT) (Topping, 2007).

It was hypothesised that the fermentation of carbohydrates, especially soluble NSP (sNSP) like β-glucan and resistant starch from hulless barley would increase the SCFA and lactic acid (LA) production and decrease ammonia production in the pig's gastrointestinal tract, and if confirmed, this would make it possible, within the same cereal species, to select cultivars with desirable properties to be used in swine nutrition to improve gut health.

Altering the carbohydrate structure and content of cereals such as barley and oat has been a major goal of plant breeders in the past decades and collections of hulless barleys and oat cultivars with a wide content in β-glucan or altered amylose/amylopectin ratio are now available (Izydorczyk et al., 2000). Utilizing some of these cultivars a companion study revealed that hulless barleys differing in their β-glucan (41 to 84 g/kg) and amylose content (70 to 430 g/kg of total starch) have strong potential to alter the intestinal microbial composition of weaned pigs, as compared to hulled barleys and oats (Pieper et al., 2008). The results presented here were obtained from the same study and describe the effect of these

barley and oat cultivars on the ileal and total tract nutrient digestibility and fermentation metabolites in weaned pigs.

4.2 Materials and Methods

The animal experiment was performed in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 1993) as specified in the Guide to the Care and Use of Experimental Animals and the Standard Animal Care Protocol (no. 970019) approved by the University of Saskatchewan Committee on Animal Care and Supply.

4.2.1 Animals and housing

The experiment was carried out at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). Seventy-two piglets (Camborough Plus females x C337 sires, PIC Canada Ltd, Winnipeg, Canada) were used in a completely randomised experiment, where individual pig was the experimental unit. Animals were weaned at 21 days of life and reared for two weeks in group pens. Creep feeding was not employed. At week 5, pigs (12.8 ± 1.9 kg) were moved to individual pens (1.2 x 0.6 m), with free access to water and randomly allocated to one of nine experimental diets with eight piglets per diet. Standard rearing conditions (24^{o}C temperature, ~45% humidity and a 12 to 12 h light/dark lighting program) were maintained during the whole experimental period. No antibiotics, for either prophylactic or therapeutic purpose, were administered to the animals during the study.

4.2.2 Experimental diets

Nine experimental diets were formulated: three diets with hulled barley, supplemented or not with isolated β-glucan concentrate (BBG; isolated by dry fractionation, containing 270 g/kg β-glucan, 170 g/kg CP, 320 g/kg starch, 320 g/kg total dietary fibre, 30 g/kg fat and 20 g/kg ash on dry weight; Parrheim Foods, Saskatoon, SK, Canada), four hulless barley cultivars with β-glucan content from 41 to 84 g/kg and 2 oat cultivars. Their composition is detailed in Table 4.1. In the BBG supplemented diets, 82 g/kg or 163 g/kg (w/w) BBG, containing 270 g/kg β-glucan, was added at the expense of hulled barley. The diet was offered in mash form (110 g/kg $BW^{0.75}$) for 60 min twice daily (0800 and 1600 h) for 15 days and residuals were collected subsequently and stored at -20^{0}C.

4.2.3 Slaughtering and sample collection

After an adaptation period of 12 days to individual cages, faecal samples were collected over 3 consecutive days. On day16, exactly 4h after the last meal, pigs were killed by captive bolt and exsanguination. After killing, the abdomen was opened and the GIT was removed. Digesta samples from the ileum (last quarter of the small intestine, defined as ileum) and the colon (medial colon, 20 cm) were collected and homogenised on ice. The pH of digesta contents was measured immediately by using a digital pH-meter (SymPHony, VWR, Westchester PA, USA). Aliquots for SCFA, LA and ammonia analyses were snap-frozen and stored at -80^{0}C until analysis. Residual digesta were frozen in containers for subsequent analysis of nutrients and acid-insoluble ash (AIA).

4.2.4 Analyses and calculations

4.2.4.1 *Proximate nutrients*

All the ingredients, diets, ileal and faecal samples were ground with a laboratory mill (Restch Mill ZM1, Newton, PA, USA) to pass through a 1 mm screen and chemical analyses were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007) with specific methods as follows: dry matter (DM) (AOAC 930.15), nitrogen (N) (AOAC 968.06 using an elemental analyser LECO FP528, St Joseph MI, USA; CP= N×6.25), ether extract (EE) (AOAC 920.39 using Soxhlet apparatus and petroleum ether), ash (AOAC 942.05), ADF (AOAC 973.18), NDF (AOAC 2002.04) and gross energy (GE; PARR 1281 calorimeter, Meline IL, USA). The composition of the ingredients and diets is presented in Table 4.1 and Table 4.2, respectively.

4.2.4.2 *Carbohydrate composition*

All the ingredients, diets and ileal and faecal samples were ground to pass through a 0.5-mm mesh screen with a laboratory mill (Restch Mill ZM1) for β-glucan, total starch and NSP analysis. Commercial test kits (Megazyme International Ltd., Ireland) were used to determine mixed-linked β-glucan content (AOAC 995.16), total starch (AOAC 996.11) and amylose/amylopectin ratio (Yun and Matheson, 1990). NSP, based on individual monomer contents, were analysed by gas chromatography (GC) after hydrolysis of total and insoluble fractions with 12M H_2SO_4, as described by Englyst et al. (1994). Chromatographic analysis was carried out using a GC system (Agilent 6890 system, Agilent technologies Inc.,

Waldbron, Germany) equipped with a flame ionization detector (FID) and a fused-silica capillary column (DB-17 HT, Agilent Technologies, Wilmington, DE, USA), using 2-deoxy-D-glucose as the internal standard.

Table 4.1 Chemical composition of the barley and oat varieties (g/kg DM)

Diet no	Cereal cultivar/ variety	Cereal type	Dry matter (g/kg)	Ash	Crude protein	Ether extract	Starch	β-glucan
1-3	Common barley	HB	879	24	98	22	624	34
4	SB 90300[1]	hB	888	17	132	24	647	41
5	CDC McGwire	hB	879	18	173	25	601	56
6	SB 94893[1]	hB	889	20	151	28	532	73
7	CDC Fibar	hB	892	19	213	34	534	84
8	CDC Sol-Fi	Oat	886	40	197	30	295	40
9	CDC Baler	oat	899	32	165	40	458	29

Abbreviations: HB, hulled barley; hB, hulless barley

[1]Breeding lines (Crop Development Centre, University of Saskatchewan, SK, Canada)

4.2.4.3 *Fermentation metabolites in intestinal contents*

The SCFA and LA of the ileal and colon samples were analysed using a GC (Agilent 6890 system) fitted with a FID and a fused-silica capillary column (ZB-FFAP, Phenomenex, Torrance, CA, USA), using crotonic acid as the internal standard. Branched-chain fatty acids (BCFAs) were calculated as the sum of iso-butyric and iso-valeric acids. Ammonia concentration was determined as described by Novozamsky et al. (1974) with slight modifications. Briefly, ammonia was oxidised by sodium hypochloride in the presence of sodium nitroprusside and the resulting complex was measured at 600 nm using a spectrophotometer (Pharmacia LKB- Ultraspec III; Amersham, Freiburg, Germany).

4.2.4.4 *Nutrient digestibility*

Nutrients (DM, ash, CP, starch and NSP) in the ileal, colonic and faecal contents were analysed as described above. For determination of ileal and faecal starch and NSP content, two samples of the same treatment but from consecutive replicates were pooled resulting in four samples per treatment. The diets and the ileal and faecal digesta were analysed for their

AIA content by gravimetry, after treatment with 3N HCl (AOAC 971.33). The results are presented in Table 4.4.

The ileal and faecal apparent digestibility (AD) of the different nutrients were calculated for each pig based on the correction of the AIA content, using the equation:

$$AD\ (\%) = \{1 - [(IA_d/IA_f)/(N_d/N_f)]\} \times 100 \quad (4.1)$$

where IA_d and IA_f are the AIA contents in the diets and faeces/ileal digesta, respectively, and Nd and Nf are the nutrient contents in the diets and faeces/ileal digesta, respectively.

4.2.4.5 *Statistical analysis*

Data were analysed using the mixed model procedure of SAS 9.1 software (SAS, 2003) using "diet" as the main effect and with the statistical model:

$$Y = \mu + \alpha_i + \varepsilon_{ij} \quad (4.2)$$

where Y is the parameter to be tested, μ the overall mean, α_i is the effect of diet, and ε_{ij} is the experimental error. Means were separated using the Tukey method. An α level of 0.05 was used to assess significant differences between means, unless otherwise stated.

Table 4.2 Composition and chemical analysis of experimental diets (g/kg DM)

Diet no.	1	2	3	4	5	6	7	8	9
Ingredient	Hulled Barley (HB)	HB + 20 g/kg β-glucan	HB + 40 g/kg β-glucan	SB90300[1] (hB)	CDC McGwire (hB)	SB94893[1] (hB)	CDC Fibar (hB)	CDC Sol-Fi (oat)	CDC Baler (oat)
Composition									
Cereals	815	734	652	815	815	815	815	815	815
BBG[2]	-	82	163	-	-	-	-	-	-
SoyComil®[3]	90	90	90	90	90	90	90	90	90
Whey[4]	60	60	60	60	60	60	60	60	60
Minerals[5]	5	5	5	5	5	5	5	5	5
Vitamins[6]	5	5	5	5	5	5	5	5	5
Salt	5	5	5	5	5	5	5	5	5
Dical phosphate	10	10	10	10	10	10	10	10	10
Limestone	5	5	5	5	5	5	5	5	5
Celite[7]	5	5	5	5	5	5	5	5	5
Analysis									
DM (g/kg)	886	893	892	890	894	897	898	898	908
Ash	61	63	60	45	45	48	47	70	61
CP	173	210	191	199	206	219	171	246	154
Ether extract	19	16	17	17	19	21	24	29	33
NDF	179	187	180	139	121	169	153	324	285
ADF	73	61	58	30	29	37	30	153	129
β-glucan	24	40	53	30	42	65	84	32	23
NSP Total	118	130	128	85	88	120	90	169	190
Insoluble	99	105	80	59	53	53	53	154	179
Soluble	18	25	48	27	35	67	37	16	11
Uronic acids	1.4	1.3	1.7	1.3	1.4	1.2	1.2	1.7	1.9
Total Starch	457	456	444	558	533	497	432	329	316
Amp/Aml	70/30	71/29	70/30	68/32	69/31	59/41	93/7	66/43	72/28
GE (Mcal/kg)	4.28	4.24	4.3	4.34	4.3	4.36	4.36	4.36	4.35

Abbreviations: Amp/Aml, amylopectin and amylose ratio (%) of total starch; BBG, isolated barley mixed-linked β-glucan concentrate; HB, hulled barley; hB, hulless barley; NSP, non-starch polysachharides

[1]Breeding lines (Crop Development Centre, University of Saskatchewan, SK, Canada)

[2]Isolated barley β-glucan concentrate, containing 270 g/kg β-glucan, 170 g/kg crude protein, 320 g/kg starch, 320 g/kg total dietary fibre, maximum 30 g/kg fat and maximum 30 g/kg ash on dry weight basis (Parrheim Foods, Saskatoon, SK, Canada)

[3]SoyComil® (crude protein- 650 g/kg, dry matter- 930 g/kg) – Archer Daniels Midland (ADM) Specialty Ingredients (Europe) B.V., P.O. Box 2 1540 AA, Koog aan de Zaan, The Netherlands

[4]Crino whey powder (crude protein- 90 g/kg, lactose- 800 g/kg, dry matter- 920 g/kg, Ash- 120 g/kg) - Agropur Co-operative Granby, Quebec, Canada

[5] Mineral premix- providing (per kg of diet); Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite

[6] Vitamin premix- providing (per kg of diet), vitamin A, 8,250 IU; vitamin D_3 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; 5 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; vitamin B_{12}, 25 µg

[7]Celite 545, Celite Corporation, Lompoc, CA, USA

4.3 Results

All piglets remained healthy throughout the experiment. Daily feed intake and weight gain (Table 4.3) were similar to pigs of similar age and fed a commercial diet. There was no effect (P>0.05) of either cereal type or variety on these parameters. The DM content of the colonic digesta (Table 4.3) was higher (P<0.05) in pigs fed oat.

Table 4.3 Feed intake, gain and dry matter content of digesta of pigs fed diets containing different barley and oat cultivars

			DM of digesta, %	
Diet	ADFI, g	ADG, g	Ileal	Colon
HB	832	250	11.9^{ab}	24.3^{a}
HB + 20 g/kg β-glucan	855	271	10.0^{b}	23.3^{a}
HB + 40 g/kg β-glucan	862	238	10.6^{b}	24.0^{a}
SB90300 (hB)	840	263	13.0^{ab}	21.8^{a}
CDC McGwire (hB)	864	274	13.6^{ab}	23.1^{a}
SB94893 (hB)	856	291	15.9^{a}	22.4^{a}
CDC Fibar (hB)	866	274	14.8^{ab}	23.0^{a}
CDC Sol-Fi (oat)	843	254	12.5^{ab}	29.3^{b}
CDC Baler (oat)	819	234	11.6^{ab}	29.9^{b}
SEM	34.3	18.4	1.09	0.78
P value	0.986	0.435	0.005	<.001

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; DM, dry matter

*Mean values with different superscript within column differ (P<0.05)

4.3.1 Digestibility

All digestibility coefficients were affected by cereal type, both at ileal and total tract levels (P<0.05, Table 4.4). Ileal organic matter (OM) digestibility was higher (P<0.05) for diets based on hulless barley, as compared to hulled barley and oat but without any difference (P>0.05) within cereal types. Similar trends were found for total tract OM digestibility. Ileal CP digestibility was the highest for diets based on hulless barley, followed by those based on hulled barley and oat. On the contrary, the total tract CP digestibility was lower (P<0.001)

with diets containing the CDC Fibar hulless barley. Hulless barley starch was more completely digested ($P<0.05$) than that of hulled barley and oat. Ileal digestibility of total NSP (tNSP) decreased when β-glucan content increased in hulless barley diets, with the exception of the SB94893 barley. Similar trends were noted for diets supplemented with BBG. Total NSP digestibility for hulless barley was also higher ($P<0.001$) than that of hulled barley. There was a negative flow of NSP in the lower gut of the pigs fed oat. Therefore, it was decided to remove the results of the oat diets from the statistical analysis for tNSP digestibility (as indicated in Table 4.4) to improve the accuracy of the comparison between barley cultivars.

4.3.2 Fermentation metabolites and pH in intestinal contents

The absolute values of fermentation metabolites are presented in Tables 4.5 and 4.6. In addition, as carbohydrates (NSP and starch) are the main sources of fermentation in the large intestine, the relative amount of metabolites per g of fermented carbohydrates was calculated. The pH values of the intestinal contents were, in general, influenced by fermentation metabolite concentration in the gut segments, but not in a direct manner (Tables 4.5 and 4.6). The pH of the ileal content of the pigs fed with CDC Fibar hulless barley was higher than that of the pigs fed the other diets ($P<0.05$). The pH of the colonic digesta of the pigs fed oat was higher ($P<0.05$) than that of the pigs fed with hulless barley. There was no dietary effect ($P>0.05$) on the ileal SCFA content, whereas in the colon, SCFA concentrations were lower in pigs fed oat-based diets. The LA concentration was higher ($P<0.001$) in the colon of pigs fed hulless barley, as compared to the other cereal types. Among hulless barley cultivars, the LA concentration was higher for the SB94893 cultivar, which has a high soluble NSP (sNSP) content, followed by the high β-glucan cultivar CDC Fibar. The ammonia concentration, calculated per kg fermented carbohydrate, was lower in the colonic content of pigs fed 82 g/kg BBG or the SB90300 hB cultivar and higher for the oat based diets. However, when expressed per kg digesta sample, neither the ammonia concentration nor the SCFA to ammonia ratio (data not shown) were different ($P>0.05$) at the ileum or colon level. No dietary effect ($P>0.05$) was detected for the proportion of the individual SCFA in ileal contents. Pigs fed oat with low sNSP content had higher acetic and lower butyric acid concentration in the colon. On the other hand, higher propionic and lower BCFA levels were found for diets containing SB94893, the hulless barley cultivar with high sNSP content.

Table 4.4 Acid-insoluble ash content of diets and digesta samples (g/kg DM, mean ± SD) and ileal and total tract digestibility (%) of different nutrients in pigs fed diets containing different barley and oat cultivars

Diet ID	AIA content			Ileal digestibility				Total tract digestibility		
	Diet	Ileal digesta	Faecal digesta	OM	CP	Starch	tNSP	OM	CP	tNSP
HB	11	34 ± 2.9	61 ± 4.4	69^{ab}	65^{bc}	91^{b}	31^{cd}	83^{a}	73de	44^{e}
HB + 20 g/kg β-glucan	11	25 ± 4.1	61 ± 3.0	61^{bc}	64^{bcd}	91^{b}	28^{d}	84^{a}	79^{abc}	48^{d}
HB + 40 g/kg β-glucan	10	23 ± 3.8	60 ± 2.5	61^{bc}	59^{cd}	96^{a}	20^{e}	84^{a}	76^{bcd}	53^{c}
SB90300 (hB)	5	21 ± 4.1	51 ± 5.4	75^{a}	69^{abc}	94^{a}	40^{b}	91^{b}	80^{ab}	80^{b}
CDC McGwire (hB)	5	23 ± 3.9	50 ± 4.9	77^{a}	77^{ab}	96^{a}	37^{bc}	91^{b}	81^{a}	79^{b}
SB94893 (hB)	5	24 ± 3.4	43 ± 4.1	80^{a}	83^{a}	96^{a}	51^{a}	89^{b}	78^{abc}	84^{a}
CDC Fibar (hB)	5	20 ± 3.6	43 ± 4.9	75^{a}	74^{abc}	95^{a}	29^{d}	89^{b}	71^{e}	78^{b}
CDC Sol-Fi (oat)	22	44 ± 3.6	61 ± 1.6	58^{c}	59^{bcd}	92^{b}	18$^{\#}$	65^{c}	81^{a}	14$^{\#}$
CDC Baler (oat)	18	38 ± 4.0	54 ± 2.0	58^{c}	47^{d}	92^{b}	30$^{\#}$	68^{d}	74^{cde}	31$^{\#}$
SEM				2.2	3.5	0.6	1.8	0.4	1.0	0.9
P value				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: AIA, acid-insoluble ash; HB, hulled barley; hB, hulless barley; OM, organic matter; tNSP, total non-starch polysaccharides

*Mean values with different superscript within column are significantly different (P<0.05);

$^{\#}$removed from the pool for statistical analysis

Table 4.5 pH and fermentation metabolites in the ileal digesta of pigs fed diets containing different barley and oat cultivars

		mM/kg digesta sample			% total SCFA			
Diet ID	pH	SCFA	LA	NH_3	AA	PA	BA	BCFA
HB	6.5[ab]	9	27	7	93.8	2.0	3.4	0.5
HB + 20 g/kg β-glucan	6.2[b]	5	39	7	93.7	4.6	0.9	0.7
HB + 40 g/kg β-glucan	6.4[ab]	7	39	8	95.3	3.1	0.7	0.7
SB90300 (hB)	6.4[ab]	7	39	9	96.2	1.4	1.6	0.5
CDC McGwire (hB)	6.8[ab]	7	22	8	95.6	1.9	1.6	0.6
SB94893 (hB)	6.7[ab]	9	28	8	92.0	4.9	2.0	0.7
CDC Fibar (hB)	7.0[a]	7	23	6	94.7	2.3	1.8	0.6
CDC Sol-Fi (oat)	7.0[a]	11	12	9	94.9	2.9	1.6	0.4
CDC Baler (oat)	6.8[ab]	8	17	8	94.1	1.8	3.1	0.6
SEM	0.15	1.2	8.2	0.7	1.28	1.13	0.60	0.12
P value	0.003	0.130	0.198	0.237	0.519	0.344	0.060	0.553

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); HB, hulled barley; hB, hulless barley; LA, lactic acid; NH_3, ammonia; PA, propionic acid; SCFA, short-chain fatty acids

[*]Mean values with different superscript within column are significantly different ($P<0.05$)

Table 4.6 pH and fermentation metabolites in the colonic digesta of pigs fed diets containing different barley and oat cultivars

Diet ID	pH	mM/kg digesta sample			mM/kg carbohydrate (NSP + starch) fermented			% of total SCFA[+]			
		SCFA	LA	NH_3	SCFA	LA	NH_3	AA	PA	BA	BCFA
HB	6.5^{bc}	96^{a}	1^{d}	26	1851^{a}	9^{c}	446^{ab}	53.7^{bc}	23.5^{bc}	17.9^{a}	2.6^{bc}
HB + 20 g/kg β-glucan	6.2^{cd}	101^{a}	3^{cd}	29	1562^{ab}	8^{bc}	320^{b}	51.0^{c}	25.2^{abc}	17.3^{a}	3.8^{abc}
HB + 40 g/kg β-glucan	6.2^{cd}	101^{a}	2^{cd}	24	1683^{ab}	17^{bc}	418^{b}	52.8^{c}	26.3^{abc}	15.5^{abc}	2.2^{c}
SB90300 (hB)	6.2^{cd}	102^{a}	13^{abc}	24	1630^{ab}	114^{abc}	327^{b}	51.1^{c}	26.2^{abc}	16.0^{ab}	2.5^{bc}
McGwire (hB)	6.2^{cd}	109^{a}	9^{bcd}	27	1783^{ab}	112^{ab}	397^{ab}	53.0^{c}	25.4^{abc}	14.5^{abc}	2.6^{bc}
SB94893 (hB)	5.9^{d}	115^{a}	21^{a}	27	1852^{a}	167^{a}	469^{ab}	47.3^{c}	31.4^{a}	13.9^{abc}	1.8^{c}
Fibar (hB)	6.2^{cd}	112^{a}	17^{ab}	28	1717^{ab}	170^{a}	441^{ab}	47.8^{c}	28.9^{ab}	13.4^{abc}	2.1^{c}
Sol-Fi (oat)	7.2^{a}	49^{b}	2^{cd}	14	1516^{ab}	27^{bc}	605^{ab}	60.5^{ab}	22.5^{bc}	10.0^{c}	4.8^{a}
Baler (oat)	6.9^{ab}	47^{b}	1^{d}	15	1131^{b}	9^{c}	723^{a}	61.9^{a}	21.5^{c}	10.6^{bc}	4.5^{ab}
SEM	0.11	6.1	2.5	3.7	138.4	25.3	70.4	1.49	1.49	1.26	0.73
P value	<0.001	<0.001	<0.001	0.054	<0.049	<0.001	0.018	<0.001	<0.001	<0.001	0.037

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); HB, hulled barley; hB, hulless barley; LA, lactic acid; NH_3, ammonia; NSP, non-starch polysaccharides; PA, propionic acid; SCFA, short-chain fatty acids

[*]Mean values with different superscript within column are significantly different (P<0.05)

[+]based on mM/kg digesta sample

4.4 Discussion

This study was aimed at evaluating the effect of differential carbohydrate composition in barley and oat cultivars on ileal and total tract nutrient digestibility and intestinal fermentation activity in weaned pigs. Overall, differences in digestibility and fermentation activity can be explained by differences in chemical composition of the cereals used in the diets. The lower OM and starch digestibility of the hulled barleys and oats was likely due to greater insoluble fibre content, which negatively affects accessibility and action of endogenous enzymes required for insoluble fibre digestion in the upper gut and microbial fermentation in the lower gut (Bach Knudsen, 2001). Higher ileal CP digestibility in the SB94893 barley cultivar and lower CP total tract digestibility of the CDC Fibar barley cultivar could be explained by the CP and sNSP content in the respective diets. This interaction might be not only due to the negative effect of sNSP on the digestive processes but also to an increase in endogenous N excretion, which causes decreased apparent protein digestibility (De Lange et al., 1989; Leterme et al., 2000). In this study, there was considerable variation in total tract digestibility of tNSP within and between hulled and hulless barley cultivars due to differences in their physical structure and chemical composition. Variation in NSP digestibility can also be explained by the lignin content since the latter is neither digested nor fermented and prevents digestion (Van Soest, 1985). Lignin was not analysed here but variation in lignin content in barley cultivars has been reported (Oscarsson et al., 1996; Bhatty, 1999), and its presence negatively affected NSP disappearance in the pig intestines (Stanogias and Pearce, 1985c).

The results of this study confirm the initial hypothesis that variation in NSP composition of the cereals has similar, if not greater, effect on digestion and fibre fermentation than the addition of isolated NSP (Topping, 2007). Within the hulless barley group, the tNSP content varied from 85 to 120 g/kg with β-glucan content ranging from 30 to 84 g/kg (Table 4.2). This obviously affected ileal digestibility and colonic fermentation (Tables 4.4 and 4.6).

The addition of isolated β-glucan also had a significant influence, but the specific effect of β-glucan cannot be definitely clarified from these results, as the concentrate used here (BBG) contained only 270 g/kg pure β-glucan. Diets 2 and 3 thus contained approximately 20 and 40 g/kg pure β-glucan, respectively, and this might have been insufficient to affect the digestive and fermentative processes. The remaining portion of the BBG contained significant amounts of starch and total dietary fibre (320 g/kg each), but the

nature of latter was not characterised during this study and its contribution in the fermentation process was not determined. Differences in total SCFA, LA and ammonia produced per kilogram carbohydrate (Tables 4.5 and 4.6) are thus ascribable to differences in the type and form of NSP and starch molecules (amylose/amylopectin ratio). This is consistent with other studies (Bach Knudsen and Canibe, 2000; Dongowski et al., 2002). Only slight differences were found between the diets containing 20 to 40 g/kg β-glucan content in this study as well as in the companion study (Pieper et al., 2008).

Supplementation of a diet with isolated dietary fibre normally results in increased fermentation-end products (Awati et al., 2006) and beneficial microbiota (Bouhnik et al., 2004). However, inconsistent effects on metabolite concentrations for cereals supplement with isolated BBG or with high NSP and β-glucan content diets were found in this study. This can be attributed to the complex fermentation process *in vivo*, which is affected not only by the substrate available for fermentation in the gut, but also by the host, its microbiota and interactions between them (Williams et al., 2005a). SCFAs, which are basically the fermentation products of carbohydrates (Bach Knudsen et al., 1993a), did not show concentration variation among treatments at the ileum, whereas in the colon, SCFA concentration was markedly lower in pigs fed oat, a cereal with higher insoluble NSP (iNSP) content. This can be explained by the fermentation characteristics of the fibre fraction as lignified and insoluble fibres, in general, are less fermentable than soluble fibres (Bach Knudsen and Hansen, 1991). On the other hand, a higher variability and inconsistent results were obtained in relation to tNSP and β-glucan content of the diets and resulting fermentation-end products. Similar results were reported by Pluske et al. (2003) studying different DF sources in pig diets. According to Laerke et al. (2007), the change and variation in microbial population in the pig intestines is not specifically limited to dietary composition, which in turn affect the fermentation process in the gut. Moreover, in the dynamic *in vivo* system, absorption of the SCFA produced in the large intestine is an on-going process with varying rates; it is not only affected by the diet composition but also by the host, the microbiota and their interaction in the gut (Macfarlane and Macfarlane, 2003).

LA is the predominant fermentation-end product in the terminal small intestine of weanling pigs, most likely due to the predominance of *lactobacilli* at this site (Pieper et al., 2008). Although not statistically significant, there was more LA in the ileal contents of pigs fed diets supplemented with BBG as compared with those fed with high β-glucan hulless barleys, suggesting higher activity of lactic acid bacteria. This is supported by the result of the companion study (Pieper et al., 2008), where higher numbers of *lactobacilli* were found

in the small intestine of pigs fed hulled barley diets supplemented with BBG isolates. Moreover, it has previously been shown that significant amounts of soluble β-glucan are already fermented in the upper GIT (Johansen et al., 1997). In the large intestine, LA is metabolised to SCFA, mainly to *n*-butyrate by cross feeding between bacterial species in the gut ecosystem (Flint et al., 2008). Among different dietary treatments, higher concentrations of LA and *n*-butyrate were found in the colon of pigs fed hulless barleys SB94893 and CDC Fibar, which confirms higher flow rates and colonic fermentation of sNSP and β-glucan trapped within the grain matrix. Individual SCFAs in the ileum were not affected by dietary treatment, which is in agreement with the lack of bacterial diversity observed in the companion study (Pieper et al., 2008). This can be partially ascribed to the high starch digestibility in the small intestine of pigs in all diets.

Differences in the proportion of individual SCFA reflect the amount and type of substrate fermented and microbial diversity present in the gut. In this study, the oat-based diets contained more iNSP, which increase the passage rate of digesta and provide less available substrate for microbiota fermentation, thus resulting in more acetic and less butyric acid in the colon. The reverse scenario explains the higher ratio of propionic acid found in pigs fed hulless barley (breeding line SB94893), which contained more sNSP. In addition, cross feeding between species might have some influence, like the metabolism of lactic to propionic acid (Flint et al., 2008). According to Macfarlane and Macfarlane (2003), there is greater propionic and butyric acid whereas acetic acid decreases considerably in the presence of diverse microbiota and high substrate availability. Ammonia and BCFA are the products of protein fermentation, which increases when the level of carbohydrate for fermentation decreases (Macfarlane et al., 1992). Differences in ammonia concentration, expressed per kilogram fermented tNSP, and the absence of dietary effect on the ratio of SCFA and ammonia either in the ileum or in the colon, again support the hypothesis that both the amount of NSP and the matrix affect fermentation.

Two oat cultivars were included in this study as references for their high-insoluble fibre content. The reason for the negative digestibility of NSP of some diets is unclear. Other authors have made similar observations (Bach Knudsen et al., 1993a; Bach Knudsen et al., 1993b). This is to be ascribed to methodological problems related to the collection of ileal samples (slaughter method), the use of AIA as a marker (Van Leeuwen et al., 1996) and the analysis of NSP in a complex matrix.

There was no linear effect of β-glucan concentration, neither in isolated form nor in the matrix, which corroborates previous observations on gut microbiota in the companion

study (Pieper et al., 2008). For most of the parameters, the response was also higher when β-glucan was embedded in a matrix. It might be explained by the dose-response pattern of utilization of the β-glucan by the gut microbiota (Bach Knudsen et al., 2008). Moreover, digesta viscosity is affected by the concentration and molecular weight of β-glucan (Gómez et al., 1997), which ultimately affects the physiological effects of β-glucan in the GIT. Finally, the presence of lactose in the experimental diets may have affected β-glucan utilization, as lactose has a negative effect on the utilization of β-glucan (Lynch et al., 2008).

In conclusion, both the ileal and total tract nutrient digestibility of starch and NSP were greater in pigs fed hulless barleys than in pigs receiving hulled barleys or oats, and the difference is likely due to differences in sNSP and amylose content. Similar patterns were found for fermentation metabolites in the large intestine, but with, in general, higher SCFA and LA for hulless barley based diets than those based on hulled barleys or oats. However, there was no marked effect of β-glucan level, either in isolated form or in the cereal matrix. This study gives a broader view on the positive effects of β-glucan and resistant starch embedded in the matrix of specialty hulless barleys for the pig's gut health.

CHAPTER 5

IN VITRO FIBRE FERMENTATION OF FEED INGREDIENTS WITH VARYING FERMENTABLE CARBOHYDRATE AND PROTEIN LEVELS AND THEIR EFFECT ON BACTERIAL PROTEIN SYNTHESIS IN THE PIG INTESTINE

Submitted to *Animal Feed Science and Technology*

Abstract

An *in vitro* experiment was carried out to study the fermentation characteristics of different feed ingredients differing in their fermentable carbohydrate and protein composition using porcine faecal flora. The effect on *in vitro* bacterial protein synthesis was also evaluated. The ingredients used were wheat bran (WB), wood cellulose (Solka-floc®, SF), peas, pea hulls (PH), pea inner fibre (PIF), sugar beet pulp (SBP), flax seed meal (FSM) and corn distillers dried grains with solubles (DDGS). The samples were pre-treated with pepsin and pancreatin and the hydrolysed substrates were then incubated with pig faeces in a buffered mineral solution. The nitrogen source in the buffer solution (NH_4HCO_3) was replaced by an equimolar quantity of ^{15}N-labeled NH_4Cl, used for the determination of the rate of bacterial protein synthesis. Gas production, proportional to the amount of fermented carbohydrate, was recorded for 48 h and modelled. The fermented product was subjected to short-chain fatty acids (SCFA) analysis. The source of fibre affected the *in vitro* dry matter degradability (*IVDMD*), the fermentation kinetics and the gas production profile ($P<0.05$). The highest ($P<0.001$) *IVDMD* values were observed for peas (0.80) and FSM (0.70), whereas SF was essentially undegraded (0.06). The fractional rate of degradation appeared to be lower ($P<0.001$) for WB and DDGS (0.07 and 0.05/h, respectively) and highest for SBP (0.20/h). Peas started to ferment rapidly (lag time 1.3 h). Half gas production (T/2) was achieved sooner for PIF (8.4 h) and was the longest for DDGS (19.8 h). The total gas production was the highest for PH, followed by SF, PIF and peas (276, 266, 264 and 253 ml/g DM incubated, respectively) and lowest for FSM and WB (130 and 124 ml/g DM incubated, respectively). There was no difference ($P>0.05$) in SCFA production after the fermentation of SF, P, PH, PIF and SBP (ranging from 3.8 to 4.5 mM/g DM incubated) while WB and FSM yielded lowest ($P<0.05$) SCFA. The bacterial nitrogen incorporation (BNI), both at T/2 and after 48 h of fermentation was the highest ($P<0.001$) for PIF (18.5 and 15.6 mg/g DM incubated, respectively) and the lowest for DDGS and WB. In conclusion, peas and pea fibres had higher rates of fermentability, produced more SCFA and had high bacterial protein synthesis capacity. They thus have the potential to be included in pig diets as a source of fermentable fibre to modulate the gut environment, possibly extend health-promoting properties and reduce ammonia excretion.

Key words: Bacterial protein synthesis, Fermentation, Fibre, Pig

5.1 Introduction

Swine nutritionists are working on strategies to reduce nitrogenous gaseous emission from pig facilities. To deal with this socio-technical issue, different approaches have been investigated (Aarnink and Verstegen, 2007). One of them consists in increasing fermentable dietary fibre (DF) in the pig diets. The non-starch polysaccharides (NSP) and resistant starch (RS) that escape small intestinal digestion are fermented by the caecal and colonic microbiota (Cummings and Englyst, 1987). Increased intestinal fibre fermentation has been reported to reduce the ammonia emission from manure (Mroz et al., 2000; Nahm, 2003) by shifting nitrogen (N) excretion from urine to faeces (Kirchgessner et al., 1994; Zervas and Zijlstra, 2002; Bindelle et al., 2009). This shift in the N excretion pathway is due to an increase in ammonia uptake by bacteria in the large intestine (Mosenthin et al., 1992). In addition, the fermentation of DF in the pig intestines increases the production of short-chain fatty acids (SCFA) (Houdijk et al., 2002), since fibre is the main substrate for microbial fermentation in gut. Thus, DF inclusion might be an effective approach to improve gut health and, at the same time, to reduce the footprint of pork production on the environment (Verstegen and Williams, 2002). However, most of the functional properties of DF have been obtained with isolated fibres or with wheat bran and sugar beet pulp, while these characteristics are also affected by the source of fibre and the fibrous matrix (Pieper et al., 2008; Jha et al., 2010). Moreover, the inclusion of isolated fibre in pig diets on a regular basis to reduce ammonia emission or improve gut health is not economically justified. Thus, the use of feed ingredients with desirable properties instead of isolated fibre in pig rations to achieve these functional benefits makes more sense. However, many fibrous ingredients have not been investigated in this perspective yet. Many feed ingredients with highly fermentable DF are also rich in indigestible protein (iCP), which impairs the positive effect of DF fermentation on ammonia emission. Again, limited information is available on the interaction of DF and crude protein (CP) fermentation, especially when it resides in their matrix.

Bindelle et al. (2009) recently concluded that *in vivo* N excretion shifts observed with graded concentrations of fermentable DF in pig diets were correlated with enhanced bacterial N uptake in the large intestine, as measured by an *in vitro* fermentation technique. They suggested that the *in vitro* technique can be used to predict the N excretion shift from urine to faeces by measuring the bacterial nitrogen incorporation (BNI). *In vitro* models also provide

information on different fermentation variables for a large number of ingredients in relatively short time. They are also cost-effective, easy to develop and non-invasive (Coles et al., 2005).

The present study was carried out to evaluate the fermentation characteristics of feed ingredients with varying fermentable fibre and indigestible protein content. It also aimed at evaluating their possible influence on the intestinal environment and nitrogen excretion, as indicated by the BNI, using an *in vitro* technique. The investigation was based on the hypotheses that feed ingredients differing in their content and type of fermentable fibre and indigestible protein affect the fermentation kinetics and their end products profile as well as bacterial protein synthesis in the pig's gastrointestinal tract.

5.2 Materials and methods

5.2.1 Ingredients

Eight feed ingredients with potentially high levels of fermentable fibre content were studied: wheat bran (WB), wood cellulose (Solka-floc®, SF), peas, pea hulls (PH), pea inner fibre (PIF), sugar beet pulp (SBP), flax seed meal (FSM) and corn distillers dried grains with solubles (DDGS). They were selected based on their diversity of fibre and protein content. Their chemical composition, along with the feed of the pigs used as faeces donor for the *in vitro* fermentation, is presented in Table 5.1.

5.2.2 Enzymatic hydrolysis

Prior to the fermentation, the substrates, ground to pass a 1 mm screen (Retsch ZM1 mill, Newton PA, USA) underwent an *in vitro* pepsin and pancreatin hydrolysis following the first steps of the protocol of Boisen and Fernandez (1997) in order to mimic digestion occurring in the upper gastro-intestinal tract. Briefly, 2 g sample were weighed in conical flasks. A phosphate buffer solution (100 ml, 0.1 M, pH 6.0) and an HCl solution (40 ml, 0.2 M) were poured into the flasks. The pH was adjusted to 2.0 with 1 M HCl or 1 M NaOH and 2 ml of a chloramphenicol (Sigma C-0378) solution (0.5 g 100/ml ethanol) was added to prevent bacterial growth during hydrolysis. Fresh pepsin solution (4 ml, 20 g/l, porcine

pepsin: Sigma P-0609) was added and the flasks were placed in a water-bath at 39°C for 2 h under gentle agitation (50 rpm).

Afterwards, 40 ml phosphate buffer (0.2 M, pH 6.8) and 20 ml of a 0.6 M NaOH was added into the solution. The pH was adjusted to 6.8 with 1 M HCl or 1 M NaOH. Fresh pancreatin solution (2 ml, 100 g/l pancreatin, Sigma P-1750) was added and hydrolysis was continued for 4 h under the same conditions.

After hydrolysis, the residues were collected by filtration on a nylon cloth (42 µm), washed with ethanol (2 × 25 ml 95% ethanol) and acetone (2 × 25 ml 99.5% acetone), dried for 24 h at 60°C and weighed. The enzymatic hydrolysis was repeated from 6 to 26 times according to the degradability of the ingredients, in order to yield enough hydrolysed residues for the *in vitro* fermentation and their characterisation (N, NSP and starch content). Hydrolysed residues from the different replicates and periods were pooled for subsequent analyses (N, NSP and starch content) and for the *in vitro* fermentation.

Table 5.1 Chemical composition of the ingredients and the hydrolysed substrates used in the experiment (g/kg DM)

Ingredient	Ingredients								Hydrolysed substrates		
	DM	Ash	EE	CP	NDF	ADF	NSP	S	CP	NSP	S
Wheat bran	884	62	57	221	382	125	183	186	103	347	7
Solka-floc®	953	2	4	16	995	901	234	NA	NA	487	NA
Peas	879	31	10	257	214	81	81	441	50	254	80
Pea hulls	922	32	14	176	400	350	228	184	17	380	31
Pea inner fibre	890	16	3	48	213	134	151	540	12	245	71
Sugar beet pulp	913	117	5	97	425	271	196	NA	32	286	NA
Flax seed meal	906	59	107	381	245	165	130	NA	72	222	NA
Corn DDGS	883	56	134	271	366	177	136	59	147	215	21
Feed[#]	887	56	38	220	172	80	NA	384	NA	NA	NA

Abbreviations: DM, dry matter; EE, ether extract; NA, not analysed; NSP, non-starch polysaccharides; S, total starch

[#] Feed sample of pig used as donor of faeces used as inoculum for *in vitro* fermentation

5.2.3 *In vitro* fermentation

The rate of fermentation of the hydrolysed ingredients was assessed *in vitro* using a gas-test adapted to the pig by Bindelle et al. (2007a) as follows. The 200 mg-substrates were incubated at 39°C (in a shaking water-bath with 50 rpm) in a 125 ml-glass bottle with 30 ml buffer solution containing macro- and micro-minerals (Menke and Steingass, 1988) and a faecal inoculum. The N source in the buffer solution (NH_4HCO_3) was replaced by an equimolar quantity of ^{15}N-labeled 2% NH_4Cl (ISOTEC, Miamisburg, Ohio, USA, CAS 39466-62-1), which was used for the determination of BNI. Three growing pigs (6-8 weeks age) from the herd of the Prairie Swine Centre (Saskatoon, SK, Canada), fed a standard commercial diet devoid of antibiotics (Table 5.1), were used as donors for the faecal inoculum. Faecal samples were collected directly from the rectum and placed in an air tight plastic syringe immediately. The inoculum was prepared by diluting faeces 20 times in the buffer solution and filtering through a 250 µm screen. The incolum was transferred in bottles with fermenting substrates and the bottles were sealed with a rubber stopper and placed for incubation. Anaerobic environment was mainained thoroughout, from inoculum preparation till the incubation step by flushing with CO_2 gas. The fermentation gas production and the CO_2 released by buffering of the SCFA produced during the fermentation were measured at 0, 2, 5, 8, 12, 18, 24, 36 and 48 h by means of a pressure transducer (GP:50, Grand Island, NY) (Mauricio et al., 1999), fitted with digital data tracker (Tracker 211, Intertechnology Inc., Ontario, Canada). The bottles were vented after every measurement and fermentation was stopped at 48 h by quenching the bottles in iced water.

The experimental scheme was as follows: 8 ingredients x 6 bottles + 6 blanks (containing only inoculum) repeated over 4 runs (batch).

The incubation of 4 bottles from each ingredient was stopped at half time asymptotic gas production (T/2), time varying according to the ingredient (Table 5.3), by quenching the fermentation bottles in an iced water-bath for 20 min. The remaining 2 bottles of each ingredient were stopped at 48 h. When the bottles were stopped, they were subsequently emptied and rinsed with distilled water (2 x 5 ml). The fermentation broth of 2 bottles stopped after production of half-asymptote gas volume, T/2 h according to the mathematical model of France et al. (1993) described below, were pooled and freeze-dried for further determination of total carbohydrates (CHO, NSP + starch) content in the residue. The content of the 2 other bottles stopped at T/2 and the 2 bottles stopped after 48 h of fermentation were

centrifuged (12,000 g, 20 min, 4°C) and an aliquot of the supernatant was taken for SCFA analysis. The pellet was suspended in distilled water (30 ml), centrifuged (12,000 g, 20 min, 4°C) and the supernatant was discarded. The resulting pellet containing the bacteria and the undigested substrate was freeze dried, weighed and analysed for total N, ^{15}N-enrichment and CHO (NSP + starch) content. For each period, 6 samples of the inoculum were also taken for N, ^{15}N, SCFA and CHO determination.

5.2.4 Chemical analyses

All the ingredients and the diet were ground in a laboratory mill (Restch ZM1, Newton, PA, USA) to pass a 1 mm screen and chemical analysis (Table 5.1) were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007): dry matter (135°C for 2 h, AOAC 930.15), nitrogen (AOAC 968.06; using an elemental analyser LECO FP528, St Joseph MI, USA; crude protein= N × 6.25), ether extract using Soxhlet apparatus and petroleum ether (AOAC 920.39), ash (AOAC 942.05), ADF (AOAC 973.18), NDF (AOAC 2002.04) and gross energy (PARR 1281 calorimeter, Moline IL, USA).

Samples were ground to pass through a 0.5 mm-mesh screen and analysed using commercial test kits (Megazyme International Ltd., Ireland) to determine total starch (AOAC 996.11). Samples were analysed for their CHO (NSP + starch) by gas chromatography (GC) along with individual sugars content (Englyst et al., 1994), without enzymatic digestion to retain the starch content of the sample.

The SCFA were analysed by GC (Agilent 6890 system, Germany) fitted with a flame ionization detector and a fused-silica capillary column (ZB-FFAP, Phenomenex, USA), using trimethyl acetic acid as internal standard. Branched-chain fatty acids (BCFA) were calculated as the sum of the iso-butyric and iso-valeric acids.

Total N and ^{15}N-enrichment in the freeze-dried pellets were measured by means of an elemental analyser coupled with a Tracermass mass spectrometer (Europa Scientific of Crewe, UK) (Bindelle et al., 2007b).

5.2.5 Calculations and statistical analyses

5.2.5.1 In vitro degradability

The *in vitro* dry matter degradability (*IVDMD*) during the pepsin and pancreatin hydrolysis was calculated as follows:

$$IVDMD = \frac{\text{dry weight of the sample before hydrolysis - dry weight of the residue}}{\text{dry weight of the sample before hydrolysis}} \quad (5.1)$$

The disappearance of the other nutrients was calculated using the degradability coefficient of DM and the relative content of individual nutrients in the ingredients and hydrolysed substrates.

5.2.5.2 Kinetics of gas production

Gas pressure measurements were converted into gas volume (G, g^{-1}DM) using to the ideal gas law, assuming an atmospheric pressure of 101325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 48 h of fermentation were modelled according to France et al. (1993):

$$G\,(\text{ml g}^{-1}\text{ DM}) = 0, \qquad \text{if } 0 < t < L \quad (5.2)$$

$$= G_f\left(1 - \exp\left\{-\left\langle b(t-L) + c\left(\sqrt{t} - \sqrt{L}\right)\right\rangle\right\}\right), \qquad \text{if } t \geq L$$

where, G denotes the gas accumulation to time, G_f (ml g^{-1}DM) the maximum gas volume for $t = \infty$ and L (h) the lag time before the fermentation starts. The kinetics parameters of the model are illustrated in Figure 3.1 (Chapter 3). The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the substrate μ (h^{-1}), which is postulated to vary with time as follows:

$$\mu = b + \frac{c}{2\sqrt{t}}, \quad \text{if } t \geq L \quad (5.3)$$

The kinetics parameters (G_f, L, $\mu_{t=T/2}$ and $T/2$) were compared in the statistical analysis. T/2 is the time to half-asymptote when $G = G_f/2$.

5.2.5.3 *Measurement of nitrogen incorporation into microbial cells*

Bacterial nitrogen incorporation (BNI, corresponding to N in the pellet incorporated from the buffer solution into the bacteria) per g of incubated hydrolysed residue (DM) and per g of actually fermented polysaccharides (CHO, NSP + Starch) was calculated from total N and ^{15}N content according to the equations of Bindelle et al. (2007b), as follows:

Bacterial Nitrogen Incorporation (BNI) (mg/g incubated DM) =

$$\left[\left(\frac{15N^{*}N^{*}Mpellet}{0.003663} - N * Mpellet\right) / \left(\left(\frac{0.02}{0.003663} - 1\right)^{*}W * \frac{Vskip}{Qf^{*}2}\right)\right] - BNI\ inoculum * \frac{V0}{W} \qquad (5.4)$$

where N (g/g) denotes the concentration of N in the pellet, Mpellet (mg) the dry weight of the pellet, 0.003663 the natural enrichment in ^{15}N of the substrates and the faeces used to prepare the inoculum, 0.02 the enrichment of the mineral buffer in ^{15}N, ^{15}N (g/g) the concentration of ^{15}N in total N of the pellet, V0 (ml) the volume of inoculum transferred in the bottle at the start of the fermentation, W (gDM) the amount of substrate placed in the bottle and V_{skip} the volume of gas when fermentation was stopped at T/2.

The BNI was measured when half of the final gas volume was produced (T/2) and after 48 h of fermentation.

5.2.5.4 *Statistical analyses*

IVDMD during hydrolysis, gas production kinetics and fermentation metabolites production were analysed using the Mixed procedure of SAS 9.1 software (SAS, 2003) with the ingredient as fixed factor and batch as random factor, using the following general linear model:

$$Y = \alpha + S_i + B_j + \varepsilon_{ij} \qquad (5.5)$$

where Y is the parameter to be tested, α the mean, Si the effect of the ingredient (i = 1... 8), Bj the random effect of the batch (j = 1....4) and ε_{ij} the error term. Means were separated using Tukey method with a significance level of 0.05.

Pearson's correlation calculations between different variables were performed using CORR procedure of SAS 9.1 software (SAS, 2003).

5.3 Results

5.3.1 Degradability of nutrients during enzymatic hydrolysis

The *IVDMD* and degree of enzymatic hydrolysis of the ingredients are presented in Table 5.2. The *IVDMD* varied from one ingredient to another (P<0.001). The highest values were observed for peas (0.80), followed by FSM (0.70), while SF was almost indigestible (0.06). No statistical analysis could be performed on CP and starch degradability, because the samples were pooled from different batches of hydrolysis. Nevertheless, the CP degradability was similar in pattern as *IVDMD*, the highest values being obtained for peas and PH (0.96 each), followed by FSM (0.94), WB, SBP and DDGS (0.82 to 0.83). The results for starch degradability were different: WB had the highest starch degradability (0.99) followed by peas and PIF (0.96 and 0.94 respectively). The latter also showed the highest starch content in the hydrolysed residues (80 and 71 g/kg DM, respectively).

5.3.2 Kinetics of gas production

In general, the fermentation parameters varied according to the ingredient (P<0.05) (Table 5.3). Due to very slow fermentation at the beginning, followed by a sharp increase of the gas production curves, SF did not fit the mathematical model used and was excluded from the pool of statistical analysis for fermentation kinetics parameters. When expressed per g incubated DM, the fractional rate of degradation appeared to be lower (P<0.001) for WB and DDGS (0.07 and 0.05/h, respectively) and higher for SBP (0.20/h), with no difference between the other ingredients (0.10/h on average). The final gas production also differed between ingredients (P<0.001) with the highest values obtained for PH, SF and PIF (276, 266 and 264 ml/g DM incubated, respectively) and the lowest (P<0.05) for DDGS, FSM and WB (158, 130 and 124 ml/g DM incubated, respectively). However, the values changed drastically when expressed per g fermented CHO. In that case, the DDGS accounted for the highest gas volume (1199 ml/g CHO fermented), followed by PIF and peas (1050 and 1040 ml/g CHO fermented, respectively). The lowest value was obtained for SF (608 ml/g CHO fermented). Peas started fermentation sooner (lag time 1.3 h) while the half gas production was achieved sooner for PIF (8.4 h). DDGS took the longest time to reach T/2 for total gas produced (19.8 h) and PH had the highest lag time (3.6 h). Interestingly, SF showed one of

the slowest fermentation but produced one of the highest final gas volumes by the end of fermentation time.

Table 5.2 Degree of enzymatic hydrolysis of the ingredients

Ingredient	N[1]	Degree of hydrolysis			
		IVDMD	Crude protein[2]	NSP[2]	Starch[2]
Wheat bran	13	0.63[d]	0.83	30	0.99
Solka-floc®	6	0.06[g]	NA	NA	NA
Peas	26	0.80[a]	0.96	36	0.96
Pea hulls	12	0.55[e]	0.96	25	0.92
Pea inner fibre	12	0.55[e]	0.89	26	0.94
Sugar beet pulp	10	0.48[f]	0.82	23	NA
Flax seed meal	18	0.70[b]	0.94	48	NA
Corn DDGS	16	0.67[c]	0.82	47	0.88
SEM		0.004			
P value		<0.001			

Abbreviations: *IVDMD*, *in vitro* dry matter degradability; NA, not available; NSP, non-starch polysaccharides

[1] Number of replicates of enzymatic hydrolysis

[2] Values from pooled samples of different replicates of enzymatic hydrolysis

Table 5.3 Fitted kinetics parameters (means) on the gas accumulation recorded for the different hydrolysed substrates incubated with pig faecal inoculums

	Per g DM incubated					Per g CHO fermented		
Ingredient	N[1]	L[2]	T/2[3]	μ[4]	G_f[5]	N[6]	μ[4]	G_f[5]
Wheat bran	8	2.83^{a}	12.4^{b}	0.07^{c}	124^{f}	8	0.07^{d}	639de
Solka-floc®	8	NA	NA	NA	266^{b}	8	NA	608^{e}
Peas	8	1.33^{d}	10.23^{b}	0.10^{b}	253^{c}	8	0.11^{c}	1040^{b}
Pea hulls	8	3.59^{a}	12.85^{a}	0.10^{b}	276^{a}	8	0.11^{c}	713^{cde}
Pea inner fibre	8	1.46^{cd}	8.38^{d}	0.11^{b}	264^{b}	8	0.14^{b}	1050^{b}
Sugar beet pulp	8	2.91^{b}	9.39^{c}	0.20^{a}	237^{d}	8	0.16^{a}	834^{c}
Flax seed meal	8	1.75^{c}	10.66^{b}	0.10^{b}	130^{f}	8	0.11^{c}	753^{cd}
Corn DDGS	8	1.20^{b}	19.75^{a}	0.05^{d}	158^{e}	8	0.05^{e}	1199^{a}
SEM		0.289	0.519	0.006	1.9		0.003	32.6
P value		<0.001	<0.001	<0.001	<0.001		<0.001	<0.001

Abbreviations: CHO, carbohydrates (non-starch polysaccharides + starch); DM, dry matter

[1] N, number of observations in fermentation

[2] L, lag time (h)

[3] T/2, half-time to asymptote (h)

[4] μ, fractional rate of degradation (h^{-1}) at $t = T/2$

[5] G_f, maximum gas volume (ml per g DM incubated or CHO fermented)

[6] N, number of observations in pooled fermented substrates

[*] NA, Data not presented as these parameters did not fit in fermentation kinetics model used

[#] Means with different superscripts within the columns are significantly different (P<0.05)

Table 5.4 Concentrations of metabolites (mM/g DM incubated or CHO fermented) from different fibre sources after 48 h fermentation

Ingredient	mM/g DM incubated						mM/g CHO fermented	
	N[1]	SCFA	AA[2]	PA[2]	BA[2]	BCFA[2]	N[1]	SCFA
Wheat bran	8	2.0[b]	0.65[c]	0.20[cd]	0.11[b]	0.020[ab]	8	17
Solka-floc®	8	3.9[a]	0.80[a]	0.10[e]	0.09[cd]	0.000[e]	8	11
Peas	8	4.0[a]	0.61[de]	0.22[b]	0.13[a]	0.017[bc]	8	35
Pea hulls	8	4.5[a]	0.70[b]	0.19[d]	0.08[d]	0.005[de]	8	19
Pea inner fibre	8	4.4[a]	0.60[e]	0.26[a]	0.11[b]	0.011[cd]	8	34
Sugar beet pulp	8	3.8[a]	0.69[b]	0.21[bc]	0.07[e]	0.010[cd]	8	21
Flax seed meal	8	2.4[b]	0.62[d]	0.27[a]	0.07[e]	0.017[bc]	8	29
Corn DDGS	8	3.3[ab]	0.66[c]	0.20[d]	0.10[c]	0.027[a]	8	37
SEM		0.30	0.004	0.004	0.003	0.0025	8	7.4
P value		<0.001	<0.001	<0.001	<0.001	<0.001		<0.001

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); CHO, carbohydrates (non-starch polysaccharides + starch); DM, dry matter; PA, propionic acid; SCFA, short-chain fatty acids

[1] *N*, number of observations in fermentation

[2] Molar ratio of the individual SCFA

[#] Means with different superscripts within the columns are significantly different (P<0.05)

Table 5.5 Bacterial nitrogen incorporation obtained for different fibre sources measured after one-half the final gas volume was produced (T/2) and 48 h of fermentation

Ingredient	T/2			48 h		
	N[1]	mg/g DM incubated	mg/g CHO fermented	N[1]	mg/g DM incubated	mg/g CHO fermented
Wheat bran	8	6.5[d]	52.9[cd]	4	4.1[d]	22.2[d]
Solka-floc®	8	10.9[c]	30.6[d]	4	12.7[b]	29.1[cd]
Peas	8	11.9[c]	106.0[b]	4	9.5[c]	39.6[bc]
Pea hulls	8	14.4[b]	60.0[c]	4	13.6[ab]	36.0[bc]
Pea inner fibre	8	18.5[a]	133.9[a]	4	15.6[a]	62.6[a]
Sugar beet pulp	8	14.7[b]	79.3[bc]	4	12.2[b]	43.4[b]
Flax seed meal	8	7.6[d]	72.2[c]	4	6.3[d]	37.0[bc]
Corn DDGS	8	5.6[d]	78.3[bc]	4	5.2[d]	40.1[bc]
SEM		0.86	7.53		0.82	2.98
P value		<0.001	<0.001		<0.001	<0.001

Abbreviations: CHO, carbohydrates (non-starch polysaccharides + starch); DM, dry matter

[1] N, number of observations in fermentation

[#] Means with different superscripts within the columns are significantly different (P<0.05)

5.3.3 Profile in fermentation end-products of the different substrates

There was no difference (P>0.05) in SCFA production after fermentation of SF, peas, PH, PIF and SBP (ranging from 3.8 to 4.5 mM/g DM incubated) while less (P<0.05) SCFA was produced with WB and FSM (2 and 2.4 mM/g DM incubated, respectively) (Table 5.4). When expressed per g fermented CHO, no difference (P>0.05) in SCFA production was observed between ingredients.

In terms of SCFA profile, SF resulted in the highest acetic acid (AA) and one of the lowest butyric acid (BA) proportions (0.80 and 0.09, respectively). Peas and PIF had the lowest AA proportion (0.61 and 0.60, respectively) and the highest propionic acid (PA) and BA proportion (0.13 and 0.11, respectively). The highest proportion of BCFA was found in DDGS and WB (0.027 and 0.02, respectively) while no BCFA was detected in the fermented solutions of SF.

5.3.4 Bacterial nitrogen incorporation

Bacterial nitrogen incorporation both at T/2 and after 48 h of fermentation was the highest for PIF (18.5 and 15.6 mg/g DM incubated, respectively) and the lowest for DDGS and WB, both at T/2 and 48 h of fermentation. Similar trend was found when BNI was expressed per g fermented CHO, with the highest value obtained for PIF both at T/2 and 48 h of fermentation. The lowest BNI were obtained for SF at T/2 and for WB at 48 h of fermentation.

5.3.5 Correlations between carbohydrate components, fermentation parameters and bacterial nitrogen incorporation

There was a strong negative correlation observed between the ADF and *IVDMD* (r=-0.94, P<0.001) and between NDF and *IVDMD* (r=-0.93, P<0.001). A similar relationship was found between the NDF and CP degradability (r=-0.54, P<0.001) and between ADF and NDF with starch degradability (r=-0.58 and r=-0.45 respectively, P<0.001). A positive correlation was obtained between the ADF and NDF content of the ingredients and the lag time of fermentation (r=0.62 and r=0.63, respectively, P<0.001).

There was also a positive correlation obtained between the total amount of gas produced and BNI (per g DM incubated), both at T/2 and 48 h of fermentation (r=0.58 and 0.80 respectively, P<0.001), and a similar relationship was found between the SCFA concentration and BNI, both at T/2 and 48 h of fermentation (r=0.45 and r=0.62 respectively, P<0.001).

5.4 Discussion

This study aimed to explore the fermentation characteristics, with a special focus on bacterial nitrogen incorporation of some fibrous ingredients with potential to be incorporated in pig diets using an *in vitro* model. The ingredients varied widely in composition, especially in their fibre fractions and indigestible protein content, which affected the studied parameters like *IVDMD*, fermentation kinetics and metabolites produced, as well as the bacterial protein synthesis.

As expected, while mimicking the upper gut enzymatic digestion *in vitro*, *IVDMD* was largely influenced by the type and amount of fibre, especially the insoluble fibre content of the ingredients. The high *IVDMD* observed for peas and the low *IVDMD* obtained for SF can be ascribed to their respective insoluble fibre content, which is reflected by the strong negative correlation observed between the ADF and *IVDMD* (r=-0.94, P<0.001) and between NDF and *IVDMD* (r=-0.93, P<0.001). A similar relationship was found between the NDF and CP degradability (r=-0.54, P<0.001) and between ADF and NDF with starch degradability (r=-0.58 and r=-0.45 respectively, P<0.001). These negative relationships between fibre content and the degradability of nutrients is probably due to the presence of hulls, which are mainly made of insoluble fibre as well as to the fact that their presence prevents a good accessibility of the enzymes to their substrates (Bach Knudsen, 2001).

These differences in pepsin and pancreatin hydrolysis induced differences in kinetics, metabolite profiles and BNI during fermentation. This variation can be ascribed to their NSP content, as well as to the amount of RS and CP available for fermentation, which might be as important as the NSP fraction. The starch embedded in the fibre matrix might be inaccessible to the pig's digestive enzymes. Amylose is also more resistant to enzymatic hydrolysis. The starch of WB is well digested by the porcine enzymes (Bach Knudsen and Canibe, 2000) while that of peas is less digestible, due to the higher level of amylose (Le Guen et al., 1995; Wiseman, 2006). Thus, more RS is available from peas for microbial fermentation, as evidenced in this study.

Conversely to starch, the CP enzymatic digestibility of the cereal products (WB and DDGS) was lower than that of pea products. This is a consequence of the high proportion of protein entrapped within the aleuronic layer of the grains' bran. In corn DDGS, the high indigestible protein content might also be due to the Maillard reactions occurring during the distillation process, which binds the free sugars that were not fermented to the proteins (Pierce and Stevenson, 2008).

In the *in vitro* method, the rates of fermentation of the indigestible ingredients and the subsequent SCFA and gas production are reflected by the gas production measurements. The pea NSP were previously found to be more fermentable than those of WB (Goodlad and Mathers, 1991). The difference in the fermentation kinetics and products found between ingredients can be ascribed to the nature of their respective fibre fractions. *In vitro* data on fermentation kinetics may inform the location of fibre fermentation *in vivo*. For example, the degradation of the CHO fraction of peas occurs throughout the large intestine, as the soluble and pectic polysaccharides of the cotyledon are readily available to the microbiota present in the distal small intestine and in the proximal segments of the large intestine while slowly degradable fractions are available at the distal segments. On the other hand, acidic xylans and cellulose from pea hulls require longer transit times (Canibe et al., 1997) to undergo fermentation. As shown in this experiment by the lower rates of fermentation, these types of fibre are slowly depolymerised and become available to microbiota for fermentation at a later stage (caecum/colon). This is consistent with the positive correlation obtained here between the ADF and NDF content of the ingredients and the lag time of fermentation ($r=0.62$ and $r=0.63$, respectively, $P<0.001$) as well as with the slow fermentation and lower level of SCFA produced per g CHO fermented for SF.

Differences in the proportion of individual SFCA reflect the type of substrate fermented as well as microbial diversity. According to Macfarlane and Macfarlane (2003), more propionate and less acetate is produced in presence of a diverse microbiota and high substrate availability. Higher butyrate and lower acetate concentration obtained after fermentation suggests that the fibrous residues of pea fibres enhanced bacterial proliferation, as indicated by their BNI (Table 5.5). This might have yielded a more diverse community, probably linked to the level of soluble NSP fractions in the residues (Pieper et al., 2009). A reverse scenario of substrate type available for fermentation explains the higher acetate and lower butyrate production obtained for SF fibre. Thus, higher butyrate yielding peas and PIF have a potential to improve gut health, as butyrate is the principal oxidative fuel for the colonocytes. Butyrate could also have beneficial tropic effects on inflamed caeco-colonic

mucosa (Topping and Clifton, 2001) and could improve the immune surveillance in gut (Brouns et al., 2002).

There was a high bacterial nitrogen uptake found with high SCFA producing fibre sources like peas and pea fibres along with SBP, which is similar to the findings of Bindelle et al. (2009), suggesting that, in presence of higher levels of CHO substrate for fermentation, the resident microbiota in the large intestine retain more N for their own growth. This finding was also supported by the positive correlation obtained between the total amount of gas produced and BNI (per g DM incubated), both at T/2 and 48 h of fermentation ($r=0.58$ and 0.80 respectively, $P<0.001$) and also by the similar relationship obtained between the SCFA concentration and BNI, both at T/2 and 48 h of fermentation ($r=0.45$ and $r=0.62$ respectively, $P<0.001$). However, the efficiency of bacterial protein synthesis is not only affected by the degree of fibre fermentation, but also by the sugar components available for fermentation, as was evidenced by the difference in ranking of the total gas and SCFA produced by different ingredients when expressed per g DM incubated or fermented. This is supported by the findings of Kirchgessner et al. (1994), where an increase in excretion of all the nitrogenous fractions was reported with different levels of NSP degradation, without significant changes in the faecal N composition.

Our results show that DF has a major impact on BNI. However, there was a clear impact of the amount of indigestible protein in the substrates as well. There was higher BNI, both at T/2 and 48 h of fermentation, with the peas, pea fibres and SBP. All these ingredients have a relatively low CP:NSP ratio. However, a high level of fermentable DF will not necessarily result in an increased N uptake by intestinal bacteria: pea fibre fractions had the highest rate of fermentation, they presented lower N excretions (expressed per kg N intake) than cellulose (as evidenced in an *in vivo* study, Chapter 6), which is less fermented in the gut. While, the DDGS were not well fermented but contained high levels of iCP and the latter contributed to the N excretion. This is in the line with previous findings of Canh et al. (1998c) and Sutton et al. (1999) that the source of DF in the diet can significantly affect N excretion. However, there is an interaction effect of DF and iCP, as shown by our study. Some fibrous feedstuffs of this study have a high indigestible CP content. In this case, CP is usually not well digested by the pig since it is partially bound to the fibrous matrix. It also plays a role in N excretion pathways, which is similar with the findings of Le et al. (2008a). Fibrous feed ingredients may thus bring more indigestible N to the diet, which will eliminate the advantage gained by the presence of fermentable DF. Thus, the advantage of fermentable

DF on BNI and ammonia concentration will depend on the overall composition of the feed ingredients, especially the ratio of fermentable DF and iCP.

Ingredients with low fermentable fibre and high CP residues after enzymatic hydrolysis (WB, DDGS) yielded lower BNI and a higher proportion of BCFA, which can be ascribed to the shift of fermentation to protein (Macfarlane and Macfarlane, 2003), when there is depletion of carbohydrate substrate available for microbiota (Piva et al., 1996) or when the ratio between carbohydrate and protein substrates available to microbes as energy source is unbalanced in the medium. Thus, it becomes important to stimulate carbohydrate fermentation to minimise the growth of potential protein fermenting bacteria (Macfarlane and Macfarlane, 1995) which can lead to the production of toxic substances like ammonia, amines, short-chain phenols and indoles (Macfarlane et al., 1992).

When expressed per g CHO fermented, the DDGS ranked in the top for both the total gas and SCFA productions. It is basically due to the artifact that DDGS contain high levels of iCP. The latter is fermented and yields carbon dioxide and SCFA, which account for gas production, but it is not considered in the fermented CHO. The same was true for the high BNI expressed per g CHO fermented found with DDGS. Conversely, there is a decrease in protein fermentation when the level of NSP for microbial fermentation is increased (O'Connell et al., 2005), as was found in the peas and pea fibres during this study. There was higher BNI with these ingredients, containing higher levels of soluble fibre. At the energy balance state, resident bacteria obtain energy from fermentable fibre and N from dietary protein residue as well as endogenous nitrogen to synthesise their biomass in the gut. Thus, not only the fibre fraction but also the ratio of available CHO and protein source become important for bacterial protein synthesis.

In conclusion, the fermentation kinetics, the profile in metabolic products and the bacterial protein synthesis were influenced by the amount and type of fibre fractions and iCP content in the ingredients. Peas and pea fibres were highly fermentable, which produced higher amounts of SCFA and had high bacterial protein synthesis capacity, as indicated by bacterial nitrogen incorporation. They thus have the potential to be included in pig diets as a source of fermentable fibre to modulate the gut environment, to extend health-promoting properties and reduce ammonia excretion.

CHAPTER 6

FEED INGREDIENTS DIFFERING IN FERMENTABLE FIBRE AND INDIGESTIBLE PROTEIN CONTENT AFFECT FERMENTATION METABOLITE AND NITROGEN EXCRETION IN GROWING PIGS

Submitted to *Animal Feed Science and Technology*

Abstract

To study the fermentation characteristics of different non-conventional fibre sources with varying level of indigestible protein content and their effect on gut health and nitrogen (N) excretion, an experiment was conducted with 64 weaned pigs (24 kg) using wheat bran (WB), wood cellulose (Solka-floc®, SF), peas, pea hulls (PH), pea inner fibre (PIF), sugar beet pulp (SBP), flaxseed meal (FSM) or corn distillers dried grains with solubles (DDGS). The diets were balanced for energy and amino acids with soy protein isolate, pea starch, sucrose and a premix. Faecal samples were collected for 3 consecutive days from d10 and pigs were slaughtered on d16. Digesta from the ileum and colon were collected and analysed for their short-chain fatty acids (SCFA) and ammonia content. The coefficient of total tract apparent N digestibility was lowest in diets based on FSM, DDGS and peas (0.72, 0.74 and 0.75, respectively), medium in diets with WB and SBP (0.76 each) and highest ($P<0.001$) in those with SF, PIF and PH (0.78, 0.79 and 0.81, respectively). Expressed per kg fermented non-starch polysaccharides (NSP), faecal N excretion was higher with DDGS, WB and FSM diets (130, 113 and 109 g/kg NSP fermented, respectively) and lowest with peas, PH, PIF and SBP diets. The pea- and pea hulls-based diets had highest ($P<0.05$) SCFA concentrations (39 and 27 mM/kg digesta, respectively) at the ileum level, while no difference ($P>0.05$) in SCFA concentration was observed between diets in the colon. A higher ammonia concentration was also found in the colon of pigs fed with PH, FSM, and peas (132, 129 and 127 mM/kg digesta, respectively). In conclusion, peas and pea fibres enhance bacterial fermentation and SCFA production in the intestine, making peas an interesting ingredient to use in pig diet to improve gut health and reduce N excretion.

Key words: Dietary fibre, Fermentation, Indigestible crude protein, Nitrogen excretion, Pig

6.1 Introduction

Fermentation of both dietary fibre (DF) and protein in the pig intestine is a matter of interest for pig nutritionists due to their possible beneficial or harmful effect on gut health and the environment. Fermentable DF constitute a source of energy for the pig, after their fermentation and transformation into short-chain fatty acids (Houdijk et al., 2002; Awati et al., 2006). DF fermentation can also lead to a decrease in ammonia concentration in the gut, explained by inclusion of nitrogen (N) into the bacterial proteins (Le et al., 2005; Awati et al., 2006). However, fermentation shifts from sacchrolytic to proteoytic when there is depletion of fermentable carbohydrate substrate (Piva et al., 1996), producing harmful metabolites like ammonia and amines (Cone et al., 2005). Increased level of ammonia and amines in the colon is considered to be a predisposing factor for post-weaning diarrhoea in piglets (Gaskins, 2001), which is a major concern for the pig industry.

Inclusion of fermentable DF (Awati et al., 2006) and reduction of protein (Htoo et al., 2007) in weanling diets reduces protein fermentation along the gastrointestinal tract (GIT). Moreover, the presence of fermentable DF in the diet can also reduce the emission of gaseous nitrogenous compounds by shifting N excretion from urine to faeces (Canh et al., 1997; Zervas and Zijlstra, 2002; Bindelle et al., 2009), thus reducing the ammonia emission from piggery (Mroz et al., 2000; Nahm, 2003).

These benefits outweigh the limitations of DF to be used in pig diets. However, most of these functional properties have been observed with isolated fibres. Recent research carried out in our lab (Pieper et al., 2008; Jha et al., 2010) suggests that the fermentation characteristics of fibre depend on how they are presented: in isolated form or embedded as an integrated part of the grain matrix. Moreover, adding DF just for its fermentative properties is not economically viable. A more pragmatic approach would involve using feed ingredients that present desirable functional properties. By choosing barley varieties according to the properties of their fibre fraction, it is, for example, possible to obtain positive effects on pig gut health (Jha et al., 2009a).

Many feed ingredients with high fermentable DF are also rich in indigestible crude protein (iCP), which can annulate the positive effects of the fermented DF. However, there is limited information available on the possible interactions between DF and crude protein (CP).

Thus, this study was carried out with different non-conventional sources of DF with varying levels of iCP to evaluate the interactions between DF and CP and the consequences on N excretion and the concentration of fermentation metabolites in the pig intestine. *An in*

vitro experiment has been conducted on the same feed ingredients, in order to evaluate the fermentation characteristics and rate of bacterial protein synthesis. The results of that study are presented in a companion paper (Jha et al., 2009b).

6.2 Materials and methods

The animal experiment was performed in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 1993), as specified in the Guide to the Care and Use of Experimental Animals and the standard animal care protocol (#970019) approved by the University of Saskatchewan Committee on Animal Care and Supply.

6.2.1 Animals and housing

The experiment was carried out at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). A total of 64 piglets (Camborough Plus females x C337 sires, PIC Canada Ltd, Winnipeg, Canada, both males and females) were used in a completely randomised experiment where one pig was the experimental unit. They were weaned at 21 days and reared for two weeks with their littermates in nursery rooms without any creep feeding. At 6 weeks of age (average body weight 24.1 ± 3.1 kg), the pigs were moved to individual pens (1.2 x 0.6 m), with free access to water and randomly allocated to one of the 8 experimental diets with 8 piglets/diet. Standard rearing conditions (24^{o}C temperature, ~40% humidity and 12-12 h light/dark lighting program) were maintained during the whole experimental period. No antibiotics, neither for prophylactic nor therapeutic purpose, were administered to the animals during the study.

6.2.2 Experimental diets

Altogether eight diets differing in soluble fibre and iCP content were formulated using different non-conventional ingredients: wheat bran (WB), wood cellulose (Solka-floc®, SF), peas; pea hulls (PH), pea inner fibres (PIF), sugar beet pulp (SBP), flax seed meal (FSM) and corn distillers dried grains with solubles (DDGS). WB, peas, SBP and DDGS were received from Co-op Feeds, Saskatoon, SK, Canada while other fibre sources were received from elsewhere, mentioned in the footnote of Table 6.1. The diets were formulated based on the information from the ingredient suppliers in order to be iso-caloric and iso-nitrogenous. The

analysed value of the ingredients and diets used in the experiment is presented in Table 6.1 and 6.2 respectively. The diets were semi-synthetic in nature, where energy and other nutritional requirements were met by soya-protein isolate, pea starch, sucrose (sugar) and vitamin and mineral premixes. Celite® (Celite 545- Celite Corporation, Lompoc CA, USA) was incorporated into the diet (6 g/kg DM) as a source of acid-insoluble ash (AIA), an indigestible marker and used to measure the coefficient of total tract apparent digestibility (CTTAD). Chromic oxide (Cr_2O_3) was used as another marker to measure the coefficients of ileal apparent digestibility (CIAD), as the post-prandial passage rate of N and Cr is more similar than N and AIA (Van Leeuwen et al., 1996). Limitations of AIA in measuring ileal digestibility were shown in our previous study (Jha et al., 2010). The Cr_2O_3 was added to the diet (3 g/kg DM) directly before feeding from d13 for 3 consecutive days. The diet was offered in mash form (110 g/kg $BW^{0.75}$ per day) for 60 min twice daily (0800 h and 1600 h) for 15 d and residuals were collected subsequently and stored at -20°C until analysis.

6.2.3 Slaughtering and sample collection

After an adaptation period of 9 days to the experimental diet, the faeces were collected for 3 days (day 10-12) and stored at -20°C for further analysis. On day-16, pigs were fed at 15 minutes interval and were killed by captive bolt and bled, 4 h after the meal in the order of feeding with the same time interval. After killing of the animals, the abdomen was opened and the complete gastrointestinal tract was removed. Digesta samples from ileum (last 1/4 of the small intestine) and colon (medial 20 cm) was collected and subsequently homogenised on ice. The pH was measured immediately. Aliquots were taken for subsequent analyses of short-chain fatty acids (SCFA), total N and ammonia and stored in Eppendorf tubes at -80°C. The residual digesta were frozen in containers for subsequent analysis of nutrients, AIA and Cr_2O_3.

6.2.4 Analyses, calculations and statistics

6.2.4.1 Proximate nutrients

All the ingredients, diets, ileal and faecal samples were ground in a laboratory mill (Restch Mill ZM1, Newton, PA, USA) to pass through 1 mm-mesh screen. The chemical analysis was performed according to the Association of Official Analytical Chemists (AOAC, 2007) standard procedures with specific methods as follows: dry matter (DM; 135°C for 2 h,

AOAC 930.15), nitrogen (AOAC 968.06 using an elemental analyser LECO FP528, St Joseph MI, USA; CP= N × 6.25), ether extract (EE; AOAC 920.39 using a Soxhlet apparatus and petroleum ether), ash (AOAC 942.05), ADF (AOAC 973.18), NDF (AOAC 2002.04), TDF (AOAC 985.29) and gross energy (GE; PARR 1281 calorimeter, Moline IL, USA).

6.2.4.2 *Carbohydrate composition*

For total starch and NSP analysis, all the samples were ground to pass through a 0.5 mm-mesh screen in a laboratory mill (Restch Mill ZM1, Newton, PA, USA). Commercial test kits (Megazyme International Ltd., Ireland) were used to determine the total starch (AOAC 996.11). NSP were analysed by gas chromatography (GC) using the method described by Theander et al. (1995) (AOAC 994.13 method). Chromatographic analysis was carried out using a GC system (Agilent 6890 system, Germany) equipped with a flame ionization detector and a fused-silica capillary column (DB-17 HT, Agilent Technologies, USA), using 2-Deoxy-D-Glucose as the internal standard.

6.2.4.3 *Fermentation metabolites and pH of intestinal contents*

The pH-value of the ileal and colonic content was measured immediately after collection by means of a digital pH-meter (SymPHony, VWR, USA). The SCFA of the ileal and colonic content were analysed by GC (Agilent 6890 system, Germany) fitted with a flame ionization detector and a fused-silica capillary column (ZB-FFAP, Phenomenex, USA), using trimethyl acetic acid as the internal standard. Branched-chain fatty acids (BCFA) were estimated as the sum of the iso-butyric and iso-valeric acids.

Ammonia concentration was determined according to Novozamsky et al. (1974) with slight modifications. Briefly, ammonia was oxidised by sodium hypochloride in the presence of sodium nitroprusside, which forms a blue colour complex and was measured at 600 nm using a spectrophotometer (Pharmacia LKB- Ultraspec III; Amersham, Freiburg, Germany).

Table 6.1 Chemical composition of the ingredients (g/kg DM)

Diet no.	Ingredients	Dry matter (g/kg)	Ash	Crude protein	Ether extract	NDF	ADF	TDF	Starch
1	Wheat bran	884	62	57	221	382	125	401	186
2	Solka-floc®[1]	953	2	4	16	995	901	989	0
3	Peas	879	31	10	257	214	81	265	441
4	Pea hulls[2]	922	32	14	176	400	350	524	184
5	Pea inner fibre[3]	890	16	3	48	213	134	449	540
6	Sugar beet pulp	913	117	5	97	425	271	563	na[5]
7	Flax seed meal[4]	906	59	107	381	245	165	394	na
8	Corn DDGS	883	56	134	271	366	177	365	59

Abbreviations: ADF, acid detergent fibre; DM, dry matter; NDF, neutral detergent fibre; TDF, total dietary fibre

[1]Solka-floc®- wood cellulose (Canada Colors and Chemicals Ltd, Ontario, Canada

[2]Exlite® - pea hulls (Parrheim Foods, Saskatoon, SK, Canada)

[3]Swelite® - pea inner fibre (Cosucra-Groupe, Rue de la Sucrerie; B-7740 Warcoing, Belgium)

[4]FSM (Vandeputte S.A., 120, Boulevard Industriel, B-7700 Mouscron, Belgium)

[5]Not analysed

Table 6.2 Composition and analysis of the experimental diets (g/kg DM)

Item	WB	SF	Peas	PH	PIF	SBP	FSM	DDGS
Composition								
Wheat bran	374							
Solka-floc®		152						
Peas			577					
Pea hulls				183				
Pea inner fibre					333			
Sugar beet pulp						267		
Flax seed meal							382	
Corn DDGS								412
Pea starch[1]	368	458	323	470	321	459	512	382
Canola oil	60	70	0	50	50	0	0	50
SoyComil®K[2]	97	217	0	194	193	180	7	56
Sucrose[3]	50	50	50	50	50	50	50	50
Minerals premix[4]	5	5	5	5	5	5	5	5
Vitamins premix[5]	5	5	5	5	5	5	5	5
Sodium Chloride	5	5	5	5	5	5	5	5
Dicalcium Phosphate	22	24	22	24	24	20	23	22
Limestone	5	5	4	5	5	0	2	4
Celite[6]	5	5	5	5	5	5	5	5
Lysine HCl	2	2	2	2	2	2	2	2
DL-Methionine	2	2	2	2	2	2	2	2
L-Tryptophan	0.0	0.0	0.2	0.2	0.2	0.1	0.1	0.2
Analysis								
DM (g/kg)	919	929	897	925	916	919	912	922
CP	183	200	169	208	184	192	154	191
Ash	71	58	56	63	59	70	48	65
EE	80	67	7	52	52	4	41	104
NDF	170	198	100	131	142	184	116	218
ADF	85	177	63	102	85	94	58	113
TDF Total	210	238	166	207	244	244	172	260
Insoluble	181	217	142	178	216	167	133	223
Soluble	29	21	24	30	28	77	39	38
Lignin	23	14	22	27	81	77	75	27
Total NSP	111	150	115	125	159	140	87	117
Starch	365	356	486	386	409	353	545	292
GE (MJ/kg)	19.4	16.0	19.1	18.1	17.1	16.3	21.8	21.9

Abbreviations: ADF, acid detergent fibre; CP, crude protein; DM, dry matter; EE, ether extract; GE, gross energy; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; TDF, total dietary fibre

[1]Starlite® - Pea starch (Parrheim Foods, Saskatoon, SK, Canada)

[2]SoyComil®K - ADM Speciality Ingredients (Europe) B.V., P.O. Box 2 1540 AA, Koog aan de Zaan, The Netherlands

[3]White Sugars- Rogers Sugar Ltd, Canada

[4]Minerals- provided (per kg of diet); Zn, 100 mg as zinc sulfate; Fe, 80 mg as ferrous sulfate; Cu, 50 mg as copper sulfate; Mn 25 mg as manganous sulfate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as soldium selenite.

[5]Vitamins- provided (per kg of diet), vitamin A, 8,250 IU; vitamin D_3 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; 5 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; vitamin B_{12}, 25 ug

[6]Celite 545- Celite Corporation, Lompoc CA, USA

6.2.4.4 *Nutrient digestibility*

The nutrients (DM and N) of the ileal and faecal contents of each piglet were analysed as described above. Dietary and faecal samples were analysed for their AIA content by gravimetry, after treatment with 3N HCl (AOAC 971.33) while the Cr_2O_3 content in the diets and ileal samples was determined by colorimetry after nitro-perchloric hydrolysis, as described by Furukawa and Tsukahara (1966). The digestibility values were used to calculate N excretion.

6.2.4.5 *Calculations and Statistical analyses*

The CIAD and CTTAD of the different nutrients were calculated for each pig based on the ratio of the AIA and Cr_2O_3 content using the equation:

$$\text{CIAD or CTTAD (\%)} = \{1 - [(IAd/IAf)/(Nd/Nf)]\} \times 100 \quad (6.1)$$

where IA_d and IA_f are the Cr_2O_3 or AIA contents in the diets and ileal digesta or faeces, respectively, and N_d and N_f are the nutrient contents in the diets and ileal digesta or faeces, respectively.

Data were analysed using the Mixed model procedure of SAS 9.1 software (SAS, 2003) using diet as main effect, with the following statistical model:

$$Y = \mu + \alpha_i + \varepsilon_{ij} \quad (6.2)$$

where Y is the parameter to be tested, μ the overall mean, α_i the effect of diets, and ε_{ij} the experimental error. Means were separated using the Tukey method. An α level of 0.05 was used to assess significant differences among means, unless otherwise stated.

The Pearson's correlation coefficient calculations between different variables were performed using the CORR procedure of SAS 9.1 software (SAS, 2003).

6.3 Results

6.3.1 Pig performances, coefficient of apparent digestibility and nitrogen excretion

All piglets remained healthy throughout the experiment. There was no difference in feed intake between treatments, while higher average daily gain ($P<0.05$) was observed for the diets based on pea hulls and pea inner fibres (Table 6.3).

Table 6.3 Performances of pigs fed diets differing in fibre and protein source

Diet	ADFI, g	ADG, g
Wheat bran	1125	425^{abc}
Solka-floc®	1041	409^{abc}
Peas	1055	351^{c}
Pea hull	1088	568^{a}
Pea inner fibre	1082	520^{ab}
Sugar beet pulp	1108	500^{abc}
Flax seed meal	1066	346^{c}
Corn DDGS	1125	378^{c}
SEM	42.6	37.2
P value	0.801	<0.001

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain

*Mean values with different superscript within column differ ($P<0.05$)

The coefficient of ileal and total tract apparent digestibility (CIAD/CTTAD) of DM and N of the experimental diets and of N excretion in pigs are shown in Table 6.4. No difference ($P>0.05$) in CIAD of DM or N was observed between treatments. However, differences in CTTAD were found ($P<0.001$) both for DM and N. The CTTAD of both DM and N was highest in peas, PH and PIF while it was lowest in FSM and DDGS based diets. The N excretion, expressed both per kg DM intake or N intake, was highest ($P<0.05$) in pigs fed diets containing FSM or DDGS. Similar trend was found when N excretion was

expressed per kg fermented NSP, where FSM and DDGS had highest (P<0.001) N excretion while the peas, pea fibre and SBP-based diets had the lowest N excretion.

6.3.2 Fermentation metabolites and pH of intestinal contents

The results of pH measured in the ileal and colonic contents are presented in Tables 6.5 and 6.6 respectively. In the colonic contents (Table 6.6), the pH-value of the pigs fed PIF, PH and SBP diets was lower while higher (P>0.05) pH-value was found when fed the WB, FSM and DDGS diets. As expected, all diets had lower pH-value at colon except for the WB and FSM diets, which had a lower pH-value in the ileum than in the colon.

The major fermentation end-products along with the proportions of the individual SCFA in the ileal and colonic contents are presented in Table 6.5 and 6.6 respectively. There were higher (P<0.05) SCFA concentrations observed in the ileal digesta of pigs fed peas and pea hulls (39 and 27 mM/kg digesta respectively), while no difference (P>0.05) in SCFA concentration was found at the colonic level, with a tendency to have higher SCFA in PH and PIF based diets and lower in WB and FSM diets. Pigs fed pea-based diets had the lowest acetic acid (AA) proportion at the ileum. The lower AA is associated with the higher proportion of BA for peas. This is the most pronounced effect of all and corresponds to previous findings in the rat (Stark and Madar, 1993), and resulting increased concentrations of BA peripheral blood (Goodlad and Mathers, 1990). Propionic acid (PA) proportion was found highest in the WB and lowest in DDGS based diets at the ileum while the proportion of PA was highest with PIF and the lowest in WB and SF based diets in the colon. Butyric acid (BA) production was significantly higher (P<0.001) at the ileum when peas were offered, as compared to the other fibre sources. However, no such difference was observed at the colonic level of pigs, which was the case for BCFA for both at ileal and colonic level.

The ammonia concentration was higher (P<0.05) in the ileal digesta of the pigs fed pea hulls. In colon, higher (P<0.05) ammonia concentration was found in peas, PH, FSM and DDGS based diets fed pigs.

6.3.3 Correlations between carbohydrate and protein level, N digestion and fermentation metabolites

There was a negative correlation between the CP:NSP ratio content and the CTTAD of nitrogen (r=-0.31, P=0.012). In addition, the CP:NSP ratio in the diets and ammonia concentration were positively correlated (r=0.47, P<0.001) and a similar relationship was observed between the NSP level and the ammonia concentration (r=0.46, P<0.001) in the colon. However, there was a weak but positive correlation between the NSP content of the diets and the SCFA concentration in the colon (r=0.28, P=0.025).

Table 6.4 Apparent digestibility coefficients of nutrients and nitrogen excretion in pigs fed diets differing in fibre and protein source

Diet	CIAD		CTTAD		Nitrogen excretion		
	DM	N	DM	N	g/kg DM intake	g/kg N intake	g/kg NSP fermented
Wheat bran	0.68	0.71	0.78^{bc}	0.76^{abcd}	6.9^{b}	237^{abcd}	113^{ab}
Solka-floc®	0.67	0.77	0.77^{c}	0.78^{abc}	7.1^{ab}	217^{bcd}	102^{b}
Peas	0.77	0.71	0.88^{a}	0.75^{bcd}	7.0^{b}	251^{abc}	64^{c}
Pea hull	0.75	0.78	0.89^{a}	0.81^{a}	6.5^{b}	191^{d}	52^{c}
Pea inner fibre	0.70	0.77	0.88^{a}	0.79^{ab}	6.5^{b}	206^{cd}	42^{c}
Sugar beet pulp	0.73	0.79	0.87^{a}	0.76^{abcd}	7.6^{ab}	236^{abcd}	55^{c}
Flax seed meal	0.65	0.65	0.82^{b}	0.72^{d}	7.4^{ab}	280^{a}	109^{ab}
Corn DDGS	0.72	0.76	0.78^{bc}	0.74^{cd}	8.3^{a}	262^{ab}	130^{a}
SEM	0.322	0.375	0.103	0.123	0.40	12.2	5.5
P value	0.127	0.089	<0.001	<0.001	0.049	<0.001	<0.001

Abbreviations: CIAD/CTTAD, coefficient of ileal/total tract apparent digestibility; DM, dry matter; N, nitrogen

*Mean values with different superscript within column differ (P<0.05)

Table 6.5 Fermentation metabolites in the ileal digesta of pigs fed diets differing in fibre and protein source

Diet	pH	SCFA[+]	NH_3[+]	% of total SCFA[+]			
				AA	PA	BA	BCFA
Wheat bran	6.36[b]	23.0[b]	6.5[ab]	88.3[b]	4.4[a]	7.3[b]	0.03
Solka-floc®	7.00[a]	24.6[b]	7.0[ab]	92.3[ab]	2.1[ab]	5.6[b]	0.06
Peas	6.31[b]	39.3[a]	7.0[ab]	83.4[c]	2.7[ab]	13.6[a]	0.08
Pea hulls	6.34[ab]	26.9[ab]	8.5[a]	89.7[ab]	1.7[ab]	8.4[b]	0.03
Pea inner fibre	7.10[a]	16.6[b]	5.5[b]	92.3[ab]	1.9[ab]	5.7[b]	0
Sugar beet pulp	6.57[ab]	24.3[b]	6.5[ab]	90.1[ab]	2.0[ab]	7.7[b]	0
Flax seed meal	6.35[b]	20.6[b]	6.8[ab]	91.3[ab]	2.3[ab]	6.4[b]	0
Corn DDGS	7.08[a]	19.1[b]	5.6[b]	93.9[a]	1.3[b]	4.7[b]	0
SEM	0.14	3.16	0.64	0.95	0.63	0.89	0.003
P value	<0.001	<0.001	0.056	<0.001	0.042	<0.001	0.500

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (the sum of iso-butyric and iso-valeric acids); NH_3, ammonia; PA, propionic acid; SCFA, short-chain fatty acids

[+]mM/kg digesta sample

[*]Mean values with different superscript within column differ (P<0.05)

Table 6.6 Fermentation metabolites in the colonic digesta of pigs fed diets differing in fibre and protein source

Diet	pH	$SCFA^+$	NH_3^+	% of total $SCFA^+$			
				AA	PA	BA	BCFA
Wheat bran	6.65^{a}	94.1	99.5^{abc}	63.6	19.6^{b}	12.4	2.8
Solka-floc®	6.17^{ab}	112.9	87.6^{abc}	61.8	18.0^{b}	16.3	2.1
Peas	5.97^{b}	113.9	126.5^{ab}	55.9	21.0^{ab}	16.5	2.6
Pea hulls	6.13^{ab}	121.6	132.1^{a}	57.8	21.0^{ab}	15.3	2.6
Pea inner fibre	6.03^{b}	119.0	68.1^{c}	59.9	24.1^{a}	11.9	2.0
Sugar beet pulp	5.96^{b}	112.5	73.3^{bc}	61.1	21.8^{ab}	13.5	2.0
Flax seed meal	6.66^{a}	101.4	129.1^{a}	61.5	21.6^{ab}	11.5	2.8
Corn DDGS	6.37^{ab}	100.0	106.3^{abc}	60.5	22.1^{ab}	11.8	2.8
SEM	0.13	6.97	12.3	1.86	0.95	1.84	0.29
P value	<0.001	0.076	<0.001	0.126	0.003	0.276	0.204

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (the sum of iso-butyric and iso-valeric acids); NH_3, ammonia; PA, propionic acid; SCFA, short-chain fatty acids

$^+$mM/kg digesta sample

*Mean values with different superscript within column differ (P<0.05)

6.4 Discussion

The current study aimed at evaluating the effect of different non-conventional fibre sources with varying levels of indigestible protein content on the intestinal fermentation activity of weaned pigs and their effect on nitrogen excretion. Previous studies (Canh et al., 1997; Zervas and Zijlstra, 2002) have shown that inclusion of isolated dietary fibre in pig diet shifts N from urine to faeces, leading to reduced N excretion in the environment. These studies assume that it is also true when fibres are in their matrix as well.

In the present study, the diets were formulated to be iso-caloric and iso-nitrogenous, based on the information of chemical composition and nutritional value of the fibre sources provided by the suppliers and obtained from the individual ingredients. Unfortunately, there was some variation noted in the energy and protein content between the diets. However, all the diets met or exceeded the minimum requirements of the pigs (NRC, 1998). So no adverse effect of the differences in energy or protein level between the diets was expected on the parameters studied. Moreover, the data were expressed per unit N ingested to eliminate the possible differences related to different nutrient intakes.

Overall, differences in digestibility and fermentation activity were explained by differences in ingredient composition, especially the type and amount of DF and the level of iCP in the matrix. Lower CTTAD of DM for the ingredients rich in insoluble fibre such as WB, SF and DDGS is in accordance with several previous studies (Bach Knudsen and Hansen, 1991; Kreuzer et al., 1991; Hansen et al., 2006). It is ascribed to the higher insoluble fibre content of these diets, which negatively affects the accessibility and action of the endogenous enzymes in the upper gut and microbial fermentation in the lower gut (Bach Knudsen, 2001). There was a negative correlation between the CP:NSP ratio content and the CTTAD of nitrogen ($r=-0.31$, $P=0.012$), suggesting that not only the DF content but also the ratio of CP and DF plays an important role in the protein digestion. Similar interaction was reported by Lynch et al. (2009) while studying the effect of different level of CP and lactose on N digestibility in pig. This is attributed to the role of highly fermentable fibre fractions like hemicellulose and pectin, which stimulate bacterial growth in the hind gut. This, in turn, increases faecal N excretion in the form of bacterial biomass and thus reduces apparent N digestibility (Kreuzer et al., 1991; Hansen et al., 2006). However, this interaction might not only be due to the effect of soluble NSP on the digestive process but also to an increase in endogenous N excretion, which causes decreased apparent total tract protein digestibility (De Lange et al., 1989; Huisman et al., 1992; Leterme et al., 2000).

Decreases in ammonia concentration in the colonic digesta and N excretion, as observed in the present study, are commonly interpreted as a result of an increased incorporation of N into bacterial proteins in presence of fermentable fibre due to growth of microbiota being stimulated by the availability of extra amounts of fermentable energy (Jorgensen et al., 1996; Van Nevel et al., 2006). In this study, there was lower N excretion (expressed as per kg NSP fermented) with peas, the pea fibre fractions and SBP, ingredients with a relatively lower CP:NSP ratio, which tends to suggest the role of both DF and iCP in N excretion, as discussed above. However, a high level of fermentable DF in the diet will not necessarily result in a decrease in N excretion. The pea fibre fractions, for example, have the highest rate of fermentation in the pig large intestine (Jha et al., 2009b). Despite that, they presented lower N excretions (expressed per kg N intake basis) than wood cellulose, which is less fermented in the gut. On the contrary, FSM and DDGS with higher contents of iCP also presented higher levels of N excretion. However, this does not necessarily mean that their DF was highly fermented and that the bacterial biomass produced would be responsible for high N excretion. It is certainly the case for SBP, which is rich in highly fermentable pectin and hemicellulose and has low protein content. DDGS are not well fermented in pig intestine, as evidenced by an *in vitro* method (Jha et al., 2009b) but contain high levels of iCP and the latter contributed to the total N excretion. Our data also show that fibrous feedstuffs often have a high CP content and the protein is usually not well digested by the pig because it is bound in the matrix of fibres. Fibrous feed ingredients may thus bring more indigestible N to the diet, which will eliminate the advantage gained by the presence of fermentable DF. Thus, the advantage of fermentable DF on N excretion will depend on the overall composition of the feed ingredients, especially the ratio of fermentable DF and iCP and how it is incorporated in the matrix of the ingredients.

The concentrations of SCFA, the fermentation products of carbohydrates, did not vary among treatments in the colon, whereas in the ileum, it was markedly higher in pigs fed peas and PH with relatively higher soluble fibre content, which is consistent with other studies (Bach Knudsen and Canibe, 2000; Dongowski et al., 2002). There was a weak but positive correlation between the NSP content of the diets and the SCFA concentration in the colon ($r=0.28$, $P=0.025$), which suggests that not only the level of NSP but also the type of NSP fraction available for microbiota affect SCFA production in the lower gut (Glitsø et al., 1998). The amount and type of substrate fermented and the microbial diversity present in the gut affects the proportion of individual SFCA (Macfarlane and Macfarlane, 2003).

There was a significant effect of diets on ammonia concentration in the colon, indicating differences in carbohydrate and protein fermentation in the intestines. Proteins in excess enhance the growth of N-utilizing bacteria (Reid and Hillman, 1999) that ferment the available protein, leading to increased ammonia and amines concentration in the colon (Macfarlane et al., 1992). Ammonia is normally found in small amounts in a healthy colon (Rasmussen et al., 1988). Ammonia and amines are considered to be detrimental for gut health (Nollet et al., 1999; Cone et al., 2005). It can negatively affect the development of the intestinal mucosa (Visek, 1984) and villus height (Nousiainen, 1991), with negative consequences for digestion and absorption and risks of post-weaning diarrhoea in piglets (Gaskins, 2001). The higher ammonia concentrations in the colon were obtained with the feedstuffs having the highest CP content (peas, FSM, corn DDGS). The CP:NSP ratio in the diets and ammonia concentration were positively correlated here ($r=0.47$, $P<0.001$). The same was observed between the NSP level and the ammonia concentration ($r=0.46$, $P<0.001$) in the colon. This supports our hypothesis that both the NSP content and the CP:NSP ratio in the diets affect the ammonia concentration in the pig intestines, which is in line with the finding of Le et al. (2008b). A lower ammonia concentration obtained for the PIF diet indicates a reduction in bacterial hydrolysis of nitrogenous compounds in presence of highly fermentable fibre in the matrix. This confirms previous observation by Houdijk et al. (1998) that in case of increased supply of fermentable carbohydrate, an excess of indigestible protein is more likely to be incorporated into bacterial protein rather than being fermented to be used as a source of energy. Moreover, the amount of ammonia in the colon also reflects the shift of N excretion pathway from urine to faeces. However, the results must be linked with results of urinary N excretion before any conclusion can be drawn. Although it was not measured here, a shift in N from urine to faeces can be expected in pigs fed sources of highly fermentable DF such as peas or pea hulls.

In conclusion, the results of the present study show that peas and pea fibre based diets enhanced bacterial fermentation and SCFA production in the intestines of pig which had less N excretion as well. The fermentation process can be attributed to both source and level of fibre and indigestible protein content in the diets, in addition to DF and CP interaction. Moreover, the sources of DF and its iCP content had a major affect on accumulation of ammonia in the colon and nitrogen excretion. Thus, peas and pea fibres could be considered for swine nutrition, in order to reduce N excretion and improve gut health of pigs, compared to other ingredients.

CHAPTER 7

GENERAL DISCUSSION

7.1 Overview

Although, dietary fibre (DF) negatively affects the digestibility of nutrients and energy (Just, 1982; Bach Knudsen, 2001), nutritionists are increasingly interested in incorporating fermentable fibre sources in pig diets, due to some desirable functional properties, like the improvement of gut health and reduction of ammonia and malodours from pig production facilities (Verstegen and Williams, 2002). The soluble portion of DF is fermented in the pig intestines by resident microbiota and produces short-chain fatty acids (SCFA), which in turn stimulate the growth of beneficial microbiota. This property of fibre fermentation serves as an alternative to use of antibiotics as growth promoters in swine nutrition, which is a mean to address public health concern with the pig industry. The DF in the pig diet also contributes to reduce nitrogen (N) excretion, which can positively impact on environmental footprints, another main concern of modern piggery. However, these properties are affected by the source of fibre being incorporated in pig diets. So far, most of these functional properties of DF to reduce the negative impact of pork production on public health and on the environment has been suggested (Verstegen and Williams, 2002; Aarnink and Verstegen, 2007) with isolated fibre sources (as reviewed in **Chapter 2**). But, pig diets are formulated basically with whole cereal grains. Thus, including feed ingredients as a source of fermentable fibre will be more practical than including isolated fibre in commercial feeds if these ingredients provide similar functional benefits as isolated fibres. On the other hand, some fibre sources contain significant amounts of indigestible protein, which are fermented in the large intestine, modify the overall fermentation characteristics and negatively affect the gut health of the host animal, apart from contributing to increased ammonia production. But, there is limited information on the interaction of carbohydrate and protein fermentation from different ingredient sources.

With this background, this thesis project was conceptualised and implemented to evaluate different feedstuffs with varying fibre contents for their fermentation characteristics and capacity for bacterial protein synthesis and reduction of N excretion from the pig intestines, using both *in vitro* gas production techniques and animal experiments.

7.2 Effect of fibre fermentation on metabolites production in the gut

The availability of hulless barleys has broadened the opportunity of using barley in pig diets, as these cereals contain variable levels of non-starch polysaccharides (NSP),

especially β-glucan and/or the amylose/amylopectin ratio (Rossnagel et al., 2005). Among the NSP fractions, β-glucan is an easily fermentable energy source for bacteria in the intestines and yields high levels of SCFA (Brennan and Cleary, 2005). NSP can thus modulate the gut environment, which results in several beneficial physiologic effects to the host (Dongowski et al., 2002). The chemical characterisation of some of the hulless and hulled barley and oat varieties/cultivars revealed that there is wide variation in the composition, both within and between these barley and oat varieties/cultivars. More specifically, the β-glucan content of these ingredients ranged from 29 and 43 g/kg DM in the CDC Baler oat and hulled barley to 84 g/kg DM in the CDC Fibar hulless barley, while other hulless barleys had medium to high levels of β-glucan content. Similarly, there was wide variation in starch content, with a significant difference in the amylose/amylopectin ratio, which is one of the main factors to determine the level of resistant starch available for fermentation in the large intestine. Thus, the selected ingredients had the diversity required to study the effect of fermentable fibre components in the intestines of pigs.

Two experiments were conducted to evaluate the effect of the NSP content in the matrix of barley and oat on fermentation metabolites (**Chapters 3 and 4**). It was hypothesised that the fermentation of carbohydrates, especially soluble NSP like β-glucan and resistant starch from hulless barley would increase the SCFA and lactic acid (LA) production and decrease ammonia production in the pig's gastrointestinal tract and if confirmed, this would make it possible, within the same cereal species, to select cultivars with desirable properties to be used in swine nutrition to improve gut health.

The availability of the *in vitro* model in our lab provided the opportunity to screen a large number of barley and oat varieties with varying levels of NSP and β-glucan content for their fermentation characteristics (**Chapter 3**). From the *in vitro* study, it was found that all the fermentation parameters varied according to cereal type and also between some cultivars, in general. Hulless barleys degraded more rapidly and yielded more gas than HB and oat. The final gas production, lag time and rate of degradation differed according to hB cultivar (**Chapter 3**). These fermentation characteristics were related to the amount of soluble NSP fractions of the ingredients, which are easily utilised by the bacteria. The findings also showed that there was a positive relationship between the fermentation end-products and the β-glucan level and with the overall NSP and resistant starch content, suggesting the higher fermentation capacity of these fibre fractions in the hulless barley. However, the low fermenting oat varieties did not fit in the mathematical model used to determine the fermentation kinetics, suggesting the limitation of the model for this kind of feed ingredient.

To confirm these findings, an animal experiment was carried out, where the effect of both forms (isolated vs in matrix of ingredients) and level of β-glucan on the fermentation metabolite production was studied. The results supported the experimental hypothesis that there is an effect of both forms and amount of β-glucan on SCFA production (**Chapter 4**). However, there was no linear effect of β-glucan concentration on metabolite concentration, neither in isolated form nor in the matrix, which corroborates previous observations on gut microbiota in the companion study (Pieper et al., 2008). It might be explained by the dose-response pattern of utilization of the β-glucan by the gut microbiota (Bach Knudsen et al., 2008). In addition, it also supports the statement that fermentation is a complex process, which is affected not only by the substrate available for fermentation in the gut, but also by the host, its microbiota and interactions between them (Williams et al., 2005a). For most of the parameters studied, the response was also higher when β-glucan was embedded in a matrix. Moreover, the supplemented isolated barley β-glucan (BBG) in the diets might not have been sufficient to affect the digestive and fermentative processes since the concentrate used (BBG) in the study contained only 270 g/kg pure β-glucan. Thus, BBG supplemented diets (diets 2 and 3 in Chapter 4) contained ~20 and 40 g/kg pure β-glucan, respectively. The remaining portion of the BBG contained significant amounts of starch and total dietary fibre (320 g/kg each) but the nature of the latter was not characterised during this study and its contribution to the fermentation process was not determined. This also gives indication for future studies that higher level of β-glucan should be tested to see if there is a distinct effect of β-glucans on the fermentation characteristics.

Among the cereals tested, hulless barleys, in general, had higher fermentability with higher SCFA and LA production than hulled barley and oat (**Chapters 3 and 4**). However, some variation was found within the hulless barley cultivars, which can be ascribed to their composition, especially the type and form of NSP and starch molecules (amylose/amylopectin ratio). The soluble NSP (sNSP) fractions are preferred substrates for microbiota (Glitsø et al., 1999), thus hulless barley containing higher sNSP fractions were well fermented by the bacteria in the large intestine. Higher concentrations of LA and *n*-butyrate were found in the colon of pigs fed hulless barleys SB94893 and CDC Fibar, which confirms higher flow rates and colonic fermentation of sNSP and β-glucan trapped within the grain matrix. Moreover, there was a difference in pattern for the individual SCFA production, which can be ascribed to the NSP fraction or monomer source fermented and bacterial population in the intestines (Englyst et al., 1987; Salvador et al., 1993). Differences in fermentability of different sugars in the large intestine could be expected to turn the

metabolism products into specific SCFA. The hulless barley varieties with high amylose content yielded higher levels of butyrate. Thus, hulless barleys, producing more butyrate, might be more interesting in the pig intestines since butyrate is found to have important implications for the metabolism, structure and function of epithelial cells of the large intestine (Sakata and Yajima, 1984; Scheppach, 1994) and promote the overall colon health (Wong et al., 2006).

Although the study focused on testing the fermentation characteristics of hulless barley as a potential source of fermentable fibre in pig diets, two oat cultivars were included in the study as references for their high insoluble fibre content. As opposed to hulless and hulled barleys, the NSP digestibility of the oat diets was negative, which is in agreement with previous reports (Bach Knudsen et al., 1993a; Bach Knudsen et al., 1993b). The reason remained unclear. It might be due to methodological problems related to the collection of ileal samples (as there is backflow of NSP in the slaughter method, used here), the use of acid-insoluble ash as a marker which does not have same passage rate as NSP in the intestine (Van Leeuwen *et al.*, 1996) and the analysis of NSP in a complex matrix.

7.3 Effect of fibre fermentation on gut microbiota and health

There are several reports suggesting beneficial effects of DF on health due to the modulation of the intestinal environment of the host animal (Williams et al., 2001). Although this thesis project did not look directly at the health parameters of the pigs, there was no sign of illness or diarrhoea due to the experimental treatment. The type of fermentation metabolites produced in the gut are considered to be an indicator of gut health (Wong et al., 2006) which was studied in details for this thesis project and are described in **Chapters 3 through 6**. Moreover, parallel studies conducted on the effect of fibre matrix on the gut microbiota revealed that NSP and resistant starch content of hulless barley have significant effects on the diversity of the gut microbiota population (Pieper et al., 2008). Quantitative PCR and a nested PCR-DDGE approach revealed that there was a graded shift in both the ileal and colonic microbial communities in the pigs fed the hulless barley cultivars with normal to high β-glucan content. These hulless barley cultivars had the lowest microbial diversity in the colon, compared to the hulless barleys with low β-glucan and the hulled barleys with or without supplemented β-glucan. The hulless barleys with high β-glucan favoured the growth of *Butyrivibrio fribrisolvens, Eubacterium cellulosolvens* and *E. xylanovorans*-like communities, which are xylan and β-glucan degrading bacteria (Hespell

and Cotta, 1995; Yoda et al., 2005), while the supplementation of β-glucan in hulless barley favoured the growth of *Lactobacilli*. These bacteria, showing xylanase and β-glucanase activity, belong to Clostridial cluster XIVa which are found to contribute to butyrate production in the large intestine, which in turn, contribute to colonic health (Pryde et al., 2002; Wong et al., 2006). The result of the study also showed that there is an effect of both form (purified supplemented versus grain matrix) and quantity of β-glucan on the gut microbial composition in pigs, which corresponds to the findings on fermentation metabolites in this thesis, as presented in **Chapter 4**. Thus, both form and amount of β-glucan should be considered while developing feeding strategies to get optimum benefit from this soluble NSP component on gut health of pigs.

7.4 Interaction of carbohydrate and protein fermentation and its effect on nitrogen excretion

Intensive pig production increases N excretion and ammonia emission from pig barns, which requires attention not only for environmental concerns, but also for the health of the pigs and the people working in the barn. Among different techniques suggested to reduce N excretion and ammonia emission from manure, dietary manipulation seems to be a very effective strategy (Le et al., 2005; Aarnink and Verstegen, 2007). More specifically, fibre inclusion and protein reduction in the diet are beneficial for the reduction of ammonia concentration in the pig intestines.

Thus, to evaluate the interactions between DF and crude protein (CP) and the consequences on N excretion and the fermentation metabolite production in the intestines of pigs, an experiment was carried out with different non-conventional sources of DF with varying levels of indigestible protein using both an *in vitro* (**Chapter 5**) and an *in vivo* (**Chapter 6**) approach.

Moreover, *in vitro* fermentation kinetics data may inform the location of fibre fermentation occurring *in vivo*. Our results showed that the degradation of the fibre fraction of peas occurs throughout the large intestine, as the soluble and pectic polysaccharides of the cotyledon were readily available to the microbiota present in the distal small intestine and in the proximal segments of the large intestine while slowly degradable fractions were available at the distal segments. On the other hand, acidic xylans and cellulose from pea hulls require longer transit times (Canibe et al., 1997) to undergo fermentation. As shown in this thesis (**Chapter 5**) by the lower rates of fermentation, these types of fibre are slowly depolymerised

and become available to microbiota for fermentation at a later stage (caecum/colon). This property of peas and pea fibres are more important for practical application, the use of such fibre source in pig diet might be more promising to improve gut health as substrates become available throughout the gut for microbial fermentation.

The fibre sources used in the study also affected the different individual SCFA proportions. Higher butyrate and lower acetate in peas and PIF suggest that during fermentation, the fibrous residues of pea fibres enhanced bacterial proliferation which was reflected by their bacterial nitrogen uptake (**Chapter 5**). This might have yielded a more diverse community, probably linked to the level of soluble NSP fractions in the residues (Pieper et al., 2009). The higher bacterial nitrogen uptake found with high SCFA producing fibre sources like peas and pea fibres also suggest that, in presence of higher levels of CHO substrate for fermentation, the resident microbiota in the large intestine retain more N for their own growth.

The source of DF in the diet can significantly affect ammonia concentration and N excretion due to their fermentation characteristics, as were reflected in results of our studies. Decreases in ammonia concentration in the colonic digesta and N excretion, as observed in this thesis project (**Chapter 6**), are commonly interpreted as a result of an increased incorporation of N into bacterial proteins, in presence of fermentable fibre, due to the growth of microbiota being stimulated by the availability of extra amounts of fermentable energy (Jorgensen et al., 1996; Van Nevel et al., 2006). However, our data show that fibrous feedstuffs often have high CP contents and the latter are usually not well digested by the pig. Fibrous feed ingredients may thus bring more indigestible N to the diet, which will eliminate the advantage gained by the presence of fermentable DF. The choice of the feedstuff for reduced N excretion is thus of major importance. DF will also contribute to a decrease in ammonia concentration (**Chapters 3, 4 and 6**), a malodorous compound. However, the results observed on faecal ammonia should be compared to the advantage provided by fermentable DF on ammonia emission coming from urine before any conclusion can be drawn on that point, as DF modify the route of N excretion in pigs (Zervas and Zijlstra, 2002; Bindelle et al., 2009). The advantage of fermentable DF on N excretion and odour will thus depend on the overall composition of the feed ingredients, especially their DF and indigestible protein content.

7.5 Scope and limitations of the thesis

The thesis project used both *in vitro* and *in vivo* approaches to study the fermentation characteristics of some feedstuffs. The first project (**Chapters 3 and 4**) studied the fermentation characteristics of different cereal sources for their potential to be used in pig diet to attain some of the functional benefits claimed for the isolated fibres by some workers (reviewed in **Chapter 2**). The *in vitro* approach was used to screen the ingredients while the *in vivo* study confirmed the findings in selected cultivars. The first project attained the fermentation characteristics of some hulless barley cultivars, in comparison to hulled barley and oat, along with supplemented or not with BBG, which is presented in **Chapters 3 and 4**. At the same time, effect of the same experimental diets on the microbial community was also assessed, which is presented in a companion paper (Pieper et al., 2008) of Chapter 4. There was no linear effect of β-glucan levels from either sources (isolated source or from matrix) on fermentation characteristics or microbial population, as the formulated diets did not provide enough concentrated β-glucan to see differences. Moreover, this project was limited to the screening of specific varieties/cultivars of hulless barley for their functional properties as indicated by their fermentation characteristics and end-products produced and were successfully achieved. However, more research is required to determine their inclusion level and their effect on the specific gut health parameters.

In the second animal experiment (**Chapter 5**), the diets were formulated to have same the level of energy and protein supplied from different non-conventional sources. However, some variations were noted in the energy and protein contents of the experimental diets. This was certainly not ideal for the experimental plan but the results were presented per unit of N ingested, which minimises the impact of the differences found in diet composition.

The availability of the *in vitro* gas production technique adopted to the pig intestines (Bindelle et al., 2007a) made it possible to screen large numbers of ingredients after characterising their fermentation parameters (**Chapter 3**). Based on the fermentation characteristics, some cultivars of hulless barley were used in the animal experiment (**Chapter 4**). Those hulless barleys were used as source of fibre, but other ingredients were incorporated to balance diets. Thus, we did not get the exact comparison of same substrates, using both techniques, but the trend was found to be similar in both the *in vitro* and *in vivo* approaches. Thus, it provided an idea about the similarity of both techniques, but cannot be considered as validation of results.

The *in vitro* gas production technique have proven to be useful for the evaluation of NSP on the fibre fermentation and production of metabolites (Barry et al., 1995; Karppinen et al., 2000; Awati et al., 2005), intestinal microbial population (Crittenden et al., 2002; Awati et al., 2005) and of the rate of bacterial protein synthesis (Bindelle et al., 2009) in pigs. However, Williams et al. (2005b) warranted validation of these methods before application in routine feed evaluation as there are several factors that might affect the fermentation process *in vitro* and *in vivo*. Also, there is a lack of validated results from both techniques.

Moreover, another limitation of the *in vitro* gas production technique also lies in their mathematical model of determining fermentation kinetics (France et al., 1993) as it does not fit with very slow fermenting substrates, as evidenced in our studies (**Chapters 3 and 5**). It might be due to the fact that this model was developed for ruminant fermentation kinetics, which has a different fermentation pattern than the one in the large intestine of monogastric animals like pigs. Thus, any work in filling this gap will facilitate the study of diverse types of fibre sources for their fermentation characteristics in the pig intestines.

Due to the limited financial resources and time as well as the interests of the researchers, the thesis project evaluated only limited numbers of fibre sources. But, the studies provided clear message that there is potential for using such ingredients in pig diets. However, there are several ingredients or fibre sources that might be of interest for the pork industry and their utilization in pig diets. Thus, a similar approach can be used to evaluate large numbers of samples, thus broader prospective on utilisation of alternative fibre sources in pig diets can be made and feeding strategy for improved health and reduced ammonia emission from pig can be recommended.

Moreover, the economic interest of using fibrous feedstuffs for the reduction of N excretion or odour emission was also not evaluated in this thesis project, which is an important factor to consider before the application of any finding at a practical level.

7.6 Overall conclusions

- Among the hulless barleys, there is wide variation in the composition of their individual fibre fractions, especially the soluble fibre fractions and the amylose/amylopectin ratio, which has an impact on their fermentation characteristics (**Chapters 3 and 4**).
- Hulless barleys have higher fermentability in general; produce higher amounts of SCFA and less ammonia than hulled barley and oat (**Chapters 3 and 4**).

- Both form (purified supplement versus grain matrix) and amount of fibre have an effect on the fermentation end-products (**Chapters 3 and 4**).
- Among the non-conventional fibres sources studied, peas and pea fibres produce higher SCFA, have a higher capacity for bacterial protein synthesis in the pig intestines and thus reduced N excretion (**Chapters 5 and 6**).
- Peas and pea fibres are fermented throughout the large intestine (**Chapters 5 and 6**), thus have a better potential to be incorporated in pig diets.

It can be summarised that hulless barleys and pea-fibres appear to have the potential to be included in pig diets as a source of fermentable fibre, in order to modulate the gut environment, extend health-promoting properties and reduce nitrogenous gas emission from pig production facilities. However, further research is needed to know the specific health benefit of these fibre sources and amount of the fibre required to achieve these benefits. Moreover, the economic effect of inclusion of these fibre sources should also be considered, so that its practical application can be assured.

7.7 Recommendations for future studies

- Screening specific hulless barley varieties and determining their inclusion level in pig diets to gain the desired functional benefits of fibre fermentation.
- Study on specific health benefits obtained from these fibres.
- More studies on the *in vitro* gas production technique to validate the technique for routine use when evaluating fermentation kinetics in the pig's intestine, especially for feed sources with wide variation in their fermentability.
- Improvement of the mathematical model of France et al. (1993) or development of a new mathematical model that fits to the fermentation kinetics of all types of fibre sources in the pig intestine.
- Economic analysis of incorporation of these potential fibre sources and their impact on the gut health improvement and environmental footprint.

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