Title: Alternative feedstuffs for reducing ammonia and odor emissions in pork production systems and improving the pig's gut health **– NPB #06-117**

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Date submitted: November 30, 2008

Industry summary

Nutrition can help reduce the impact of pork production on the environment and improve pig gut health. The formulation of diets based on highly-digestible feedstuffs and which meet the exact requirements of the pigs, markedly reduces the excretion of minerals such as nitrogen and phosphorus into the environment. The presence of fermentable dietary fiber in the diet can also decrease the emission of gaseous nitrogenous compounds by shifting nitrogen excretion from urine to feces. It is also hypothesized that fermentable dietary fiber can improve gut health by favoring the development of health-promoting bacteria such as Lactobacilli, at the expense of pathogenic ones.

The overall objective of the project was to evaluate alternative feedstuffs for their potential impact on the character of the manure excreted, on the formation of odor-causing compounds and on the gut health of the pig. More specifically, it aimed at screening a series of feedstuffs for their ability to enhance colonic fermentation and thus reduce the emission of ammonia and other odor-causing compounds and improve the gut health of the pig by favoring the development of a beneficial microflora at the expense of a pathogenic one. The evaluation was performed in the lab (in vitro) and directly on pigs (in vivo).

The first experiment focused on gut health. In particular, we wanted to see if it was possible to take advantage of the variation in carbohydrate composition of a specific ingredient in order to improve the pig gut health. Barley was used as a model for two reasons. First, it is widely used in swine nutrition. Second, its carbohydrate composition is extremely variable. It has, for example, hulled and hulless varieties. The latter have very variable contents in soluble dietary fiber (called β-glucans) and starch composition. Oats were used as a reference. The rate of fiber fermentation in the intestines was first studied by means of a lab technique. The results demonstrated that hulless barleys provided the highest rates of fiber fermentation, especially in the colon. An experiment carried out on pigs confirmed that the fermentation of the soluble fiber in the gut led to an increased production of lactic acid, to a lower pH value in the colon (which means a more acidic content) and to a modification of the microbial diversity and of composition of the intestinal bacteria. As a consequence, noxious bacteria such as Enterobacteria and Streptococci were not detected, by molecular techniques, in the small intestine of pigs fed with the hulless barleys having the highest β-glucan contents. This important finding shows that prebiotic effects (i.e. development of health-promoting bacteria in the gut by adding favorable substrates in the diet) can be obtained by a judicious selection of feed ingredients rather than by the addition of antibiotics, of

probiotics (living bacteria) or of prebiotics (purified carbohydrates used by bacteria as a substrate). In other terms, it is possible to improve the pig gut health through feed formulation and not only with additives.

The second experiment was designed to compare the effect of non-conventional feed ingredients differing in their dietary fiber and protein content on nitrogen excretion in pigs. The feedstuffs included wheat bran, cellulose, peas, pea inner fiber, pea hull fiber, sugarbeet pulp, flaxseed meal and corn Distiller's Dried Grains with Solubles (DDGS). Pigs were fed with diets based on these ingredients and the content of their small and large intestines was analyzed for fiber fermentation and the metabolites produced, namely odorous compounds such as ammonia and butyric acid. The results show that diets equally balanced in digestible energy and N content produce very different levels of N excretion, fermentation metabolites, depending on the dietary fiber nature. The sources of dietary fiber thus have a major incidence on nitrogen excretion and the emission of malodorous compounds.

Finally, the economic impact of the incorporation of sources of fermentable dietary fibers for the reduction of ammonia emission in the environment was evaluated. Based on the current feed costs and the properties of some dietary fiber sources, it can be concluded that a reduction in the level of crude protein in the diet is more efficient than the use of fiber sources, with the possible exception of citrus pulp, which has a good nutritional value, is cheap and has demonstrated interesting properties for the reduction in ammonia emission.

Scientific abstract

Nutrition can help reduce the impact of pork production on the environment and improve pig gut health. The formulation of diets based on highly-digestible feedstuffs and using diets that meet the exact requirements of the pigs, considerably reduces the excretion of minerals such as nitrogen and phosphorus into the environment. The presence of fermentable dietary fiber in the diet can also reduce the emission of gaseous nitrogenous compounds by shifting nitrogen excretion from urine to feces. It is also believed that fermentable dietary fiber can improve gut health by favoring the development of health-promoting bacteria such as Lactobacilli, at the expense of pathogenic ones. The present project is aimed at quantifying the effect of dietary fiber composition on both N excretion and gut health.

The overall objective of the project was to evaluate alternative feedstuffs for their potential impact on the character of manure excreted, on the formation of odor-causing compounds and on the gut health of the pig. More specifically, it aimed at screening a series of alternative feedstuffs for their ability to enhance colonic fermentation and thus reduce the emission of ammonia and other odor-causing compounds and to improve the gut health of the pig by favoring the development of a beneficial microflora at the expense of a pathogenic one. The evaluation was conducted using both in vitro and in vivo studies.

The first experiment focused on gut health. In particular, we wanted to see if it was possible to take advantage of the variation in non-starch polysaccharides (NSP) and starch composition of a specific ingredient in order to improve gut health. Barley was used as a model for two reasons. First, it is widely used in swine nutrition. Secondly, its carbohydrate composition is extremely variable. For example, there are hulled and hulless barleys. The latter have extremely variable contents of β-glucan, a soluble polymer of glucose (41 to 84 g β-glucan/kg) and amylose/amylopectin ratios (40/60% to 0/100%). Oats were used as a reference.

Seventy two weaned piglets were allocated to one of nine diets composed of 81.5% cereal, 6% whey, 9% soy protein isolate and 3.5% minerals. The cereals were: hulled barley, hulled barley supplemented with 2.3 or 4.6% β-glucan, 4 hulless barleys with different β-glucan content (from 41 to 84 g/kg) and 2 oat varieties. After 15d, pigs were killed and ileum and colon contents collected for analysis (short-chain fatty acids, ammonia, lactic acid and pH) and DNA extraction. Quantitative polymerase chain reaction (qPCR) and a nested PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) approach were used to evaluate the microbial communities.

The analysis of fermentation metabolites in the intestines showed lower pH values in the colon of pigs fed with hulless barleys. No marked differences between cereals were observed at the ileum level, with the exception of lower contents in lactic acid in the ileum of pigs fed oats ($P < 0.03$) and higher short chain fatty acids (SCFA) content ($P < 0.01$). At the colonic level, lower SCFA and ammonia contents ($P < 0.001$) were observed in pigs fed oats and higher contents in lactic acids were obtained in pigs fed the hulless barleys ($P < 0.001$).

Graded shifts in both, ileal and colon microbial communities were observed with the hulless barley varieties/lines with normal to high β-glucan content. These hulless barley varieties/lines had the lowest ($P < 0.05$) microbial diversity in the colon; whereas oat varieties had intermediate diversity compared with low β-glucan hulless varieties/lines and hulled varieties with or without β-glucan supplementation. DGGE band identification suggested hulless high β-glucan varieties/lines favored xylan and β-glucan degrading bacteria whereas β-glucan supplemented hulled barley favored the growth of lactobacilli. Enumeration by qPCR revealed a decrease of lactobacilli, enterobacteria and streptococci in the ileum with hulless/high β-glucan diets. Our results show that both form (purified supplement versus grain matrix) and quantity of dietary cereal β-glucan are important factors affecting changes in gut microbial composition in the pig.

Pig gut health was improved through feed formulation, an indication of “prebiotic effects” of hulless barleys and high β-glucan content. The results will help to develop future feeding strategies.

The second experiment was designed to compare the effect of non-conventional feed ingredients differing in dietary fiber and protein content on N excretion in pigs. Altogether 64 weaned pigs (average initial weight 24 kg) were fed with one of 8 diets. The feedstuffs included in the experimental diets were wheat bran, cellulose, peas, pea inner fiber, pea hull fiber, sugar beet pulp, flaxseed meal and corn Distiller's Dried Grains with Solubles (DDGS). The diets were balanced in energy and amino acids with soy protein isolate, pea starch, sucrose and a premix. Fecal samples were collected for 3 consecutive days from d10 and pigs were slaughtered on d16. Digesta from ileum and colon were collected at the ileal and colonic

levels and analyzed for their short-chain fatty acids (SCFA) and ammonia content. The digestibility of the nutrients was estimated by means of chromium oxide (Cr_2O_3) at the ileum level and acid insoluble ash at total tract level, which in turn was used to calculate nitrogen excretion from the pigs.

Taking wheat bran as the reference diet, the total tract digestibility of N was lower in pigs fed with flaxseed meal and DDGS (72% and 74% respectively) and higher with pea hulls (81%) and pea inner fiber (79%). This, in turn affected the fecal N excretion. The latter was higher with diets containing flaxseed meal and DDGS (280 and 262 g/kg N intake respectively). Diets based on peas and pea hulls had higher ($P<0.05$) SCFA (39 and 27mMol/kg digesta) at ileum, while no difference ($P > 0.05$) in SCFA concentration was observed among diets in colon. In colon, higher ammonia concentration was found in peas, PHF, FSM and DDGS based diets fed pigs. The results of the study suggest that both the fermentable fiber and protein level in the diet affect the ammonia concentration in the intestines and fecal nitrogen excretion. Although it was not measured here, a shift in nitrogen from urine to feces can be expected in pigs fed sources of highly fermentable dietary fiber such as peas or pea hulls, which reduces ammonia emission. On the contrary, no effect of dietary fiber was observed on the emission of butyric acid, a malodorous compound.

Finally, the economic impact of the incorporation of sources of fermentable dietary fiber for the reduction of ammonia emission in the environment was evaluated. Based on the current feed costs and the properties of some dietary fiber sources, it can be concluded that a reduction in the level of crude protein in the diet is more efficient than the use of fiber sources, with the possible exception of citrus pulp, which has a good nutritional value, is cheap and has demonstrated interesting properties for the reduction in ammonia emission.

Introduction

The pork industry has made efforts to maximize productivity and profitability but, new challenges are coming. The public is increasingly concerned by the impact of the industry on the environment and on the quality and safety of pork. The consumer wants healthy food, namely pork products devoid of antibiotic residues. Technologies to process manure and reduce its environmental impact are costly and affect the competitiveness of the industry.

Other alternatives exist. They are based on a better balance of the diets, technology (processing, enzymes) and the choice of feedstuffs with favorable properties. These feedstuffs have highly fermentable fiber content, which confer them with the capacity to shift nitrogen from urine to feces and to enhance the development of a gut microflora that is beneficial for health. The shift of nitrogen from urine to feces comes from the increase in bacterial mass in the hindgut, consecutive to an increased fiber fermentation rate (Bindelle et al., 2008). Urea, continuously released from blood to the intestines, is used by bacteria for protein synthesis. This increases the amount of nitrogen present in the feces and decreases the nitrogen excreted in urine, in form of urea (Zelvas & Zijlstra, 2002). Consequently, less ammonia is emitted in the air, since the latter comes from the hydrolysis of urea (Nahm, 2003). More nitrogen is also kept in the bacterial proteins and will be incorporated in soil when the manure will be used as fertilizer. However, to our knowledge, all the attempts to shift nitrogen from urine to feces have been obtained with diets supplemented in isolated fiber and little information is available on the effect of feedstuffs with high fermentable fiber content.

The rate of fermentation of dietary fiber in the pig's intestines depends on its composition and physical properties: the soluble fraction and the non-cellulosic polysaccharides are more fermentable than the insoluble fraction and cellulose. However, little information is available on the rate of fiber fermentation of the different feedstuffs used in swine nutrition.

Fiber fermentation has positive consequences on gut health, by favoring the development of beneficial bacteria (*Bifidobacteria, Lactobacilli*), at the expense of pathogenic ones (*Clostridium, Salmonella*) (Brown et al, 1997; Bouhnik et al, 2004). The positive effect is more important in the small intestine and is to be ascribed principally to the soluble fiber fraction. However, the effect varies from one soluble fraction to another. Thus, more information is required on the real effect of non-starch polysaccharides (NSP) on the gut microflora composition.

Finally, a change in the composition of manure (urinary vs fecal compounds) and on gut fermentation has also consequences on the composition of the volatile compounds that are responsible for the odors. Ammonia is one of them but many others, such as butyrate, are also emitted. Attempts have been made to incorporate fermentable fiber to reduce the synthesis of skatole, also responsible for boar taint but, in general, little information is available on the relationship between dietary fiber fermentation in the gut and the emission of volatile compounds responsible for the odors.

Objectives

The **overall objective** of the project was to evaluate alternative feedstuffs for their potential impact on the character of manure excreted, on the formation of odor causing compounds and on the pig gut health.

The **specific objectives** were:

1. Screening a series of alternative feedstuffs, with a novel in vitro technique, for their capacity to:
   i. Enhance colonic fermentation to modify the character of manure excreted
   ii. Reduce the emission of ammonia and other odor causing compounds
   iii. Improve the gut health of the animals by enhancing the development of a beneficial microflora, at the expense of noxious bacteria

2. Confirming, through in vivo studies, that these feedstuffs
   iv. Increase fecal excretion of nitrogen and decrease urinary excretion
   v. Decrease odor emission
   vi. Improve the composition of the gut microflora

3. Evaluating the potential economic impact on producers of utilizing alternative feedstuffs in feeding programs.

Two experiments were carried out. The first aimed to study the effect of different barley varieties differing in their NSP content. By doing this, we wanted to discard possible interactions with other dietary components on the fermentative processes in the pig intestines. We also wanted to see if the choice of specific cereal varieties could be a feed strategy to improve the pig gut health. The second study aimed at comparing the effect of different fiber sources used in swine nutrition on N excretion in the environment. Feedstuffs with high fiber content usually also have high indigestible protein content. It is therefore important to study the interaction between proteins and fiber in the fermentative processes of the pig intestines. Finally, the potential economic impact of using alternative feedstuffs in feeding programs was evaluated.

Material and methods

6.1. Effect of the carbohydrate composition of cereals on the pig's gut bacterial population

The experiment was divided in three parts: the characterization of the chemical composition of the cereal samples, the evaluation of the kinetics of fiber fermentation by means of an in vitro technique and the in vivo study of the intestinal bacteria population in pigs fed different barley varieties.

6.1.1. Chemical composition of the barleys

A total of 8 samples of hulless barleys (high or low in β-glucan, high or low in amylose) and 3 oat varieties (taken as references) were provided by Dr B. Rossnagel, from the Crop Development Centre of the University of Saskatchewan. The samples were selected according to their β-glucan content and starch composition (high/low in amylose). They were analyzed for their content in dry matter (AOAC 930.15), nitrogen (AOAC 968.06 using an elemental analyzer LECO FP528, St Joseph MI, USA), ether extract (AOAC 920.39 using Soxhlet apparatus and petroleum ether), ash (AOAC 942.05), and gross energy (PARR 1281 calorimeter, Moline IL, USA). Neutral (NDF) and Acid Detergent Fiber (ADF) were analysed by the method of Van Soest and Wine (1967), using the Ankom method with nylon bags. β-glucan and total starch test kits (Megazyme International Ltd., Ireland) were used to determine the β-glucan (McCleary & Codd, 1991) and total starch (McCleary et al., 1997). The feedstuffs were analyzed for their soluble and insoluble NSP by gas chromatography (Englyst et al., 1994). Total dietary fiber (TDF) was analyzed by the gravimetric method of AOAC (985.29).

6.1.2. In vitro evaluation of the kinetics of barley fiber fermentation in the pig intestines

6.1.2.1. Description of the method

The whole technique has been described in detail elsewhere (Bindelle et al., 2007a,b). In brief, the in vitro technique includes two steps (see Figure 1 below):

1) A pre-incubation with digestive enzymes to simulate digestion in the stomach and the small intestine. After incubation, most of the protein and starch are removed
2) The fermentation process itself. The residue of the pre-digestion step, corresponding to the undigested fiber fraction, is mixed together with inoculum (= bacteria) coming from pig feces, in a buffer solution. The bottle is incubated at 39 ºC for 72 h and gas production is measured by means of a pressure transducer. The results are used to characterize the kinetics of gas production. The residues are analyzed for short-chain fatty acids, malodorous compounds and bacteria.

6.1.2.2. Protocol

Treatment with pepsin – pancreatin Fifteen cereal samples, comprising 9 hulless barley samples, 1 wheat and 5 oat samples (3 whole grains and 2 oat groats) were used for the study. The samples were finely ground. Eight samples (2 g) of each cereal were incubated. The residues were pooled and analyzed for their content in N, starch and ß-glucan. The rest was used for the fermentation assay.

Fiber fermentation with the gas technique The experimental scheme was as follows: (15 cereal samples + 3 blanks) x 2 replicates x 4 periods. Gas production was measured after 0, 2, 5, 8, 12, 18, 24, 36 and 48h. Fermentation was stopped after 48h by quenching the bottles in iced water. The pH of the final solution was measured and the latter was also analysed for its content in SCFA. Gas accumulation curves recorded during the 48 h of fermentation was modelled according to France et al. (1993):

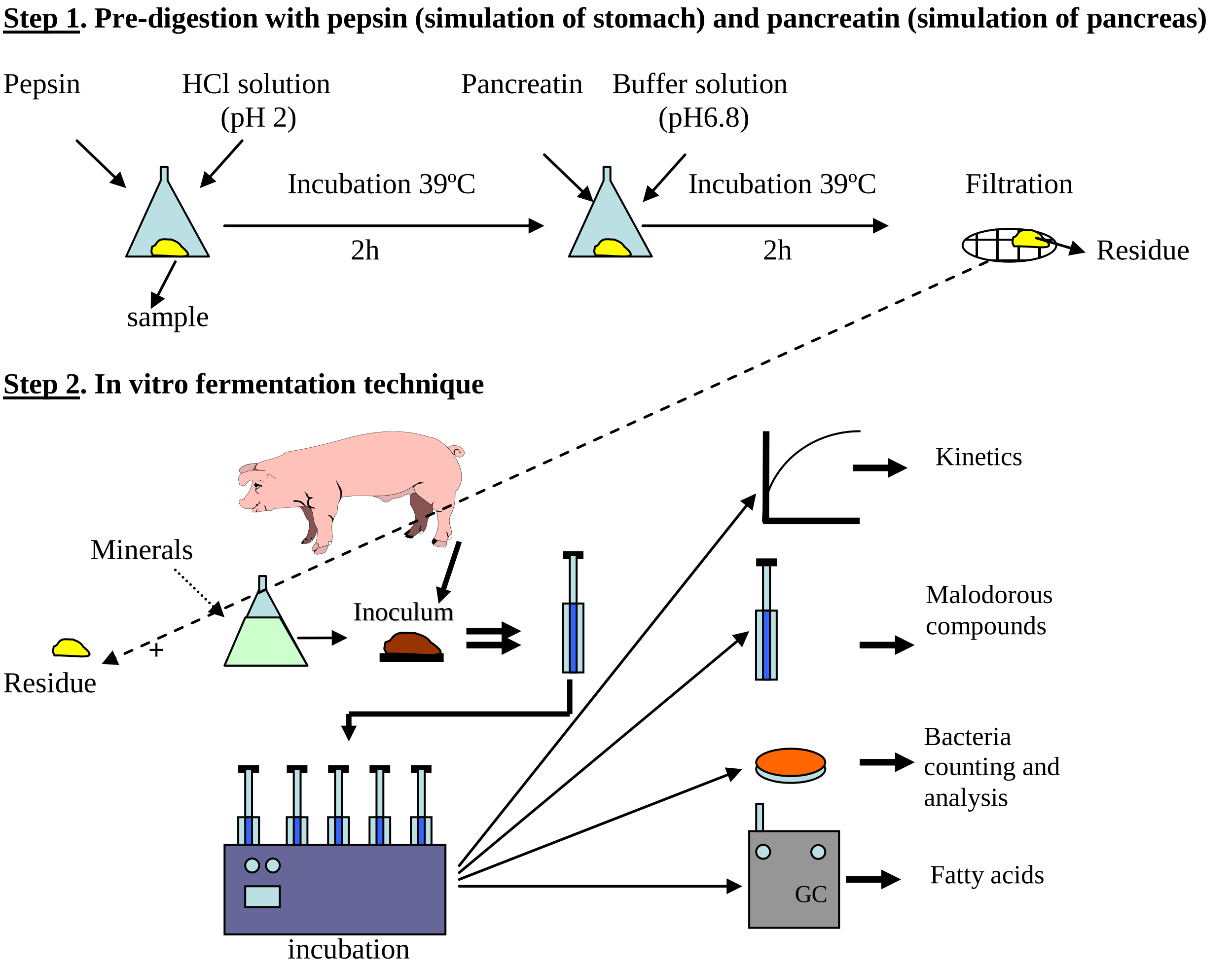

Figure 1. Novel in vitro technique for fiber fermentation studies

6.1.3. Effect of fiber fermentation on the intestinal bacteria population in pigs

6.1.3.1. Protocol

Animals

A total of 72 male piglets weighing from 12 to 20 kg were used for the experiment, during 2 consecutive periods (36/period). Three weeks after weaning, they were selected in the nursery room. They were randomly assigned to a metabolic cage and to one of the 9 experimental diets (see below). The metabolism cages were located in two different rooms and each contained 20 cages. The latter (115 x 55 cm or 0.66 m^2) had fully slatted floors and were equipped with a single feeder and nipple drinker. Eighteen cages were used in each room.

Diets

Nine diets were formulated. They were composed of 79.4% cereal, 7.5 % soybean concentrate, 7.5% whey), 5% minerals (50% limestone, 20% dicalcium phosphate, 10% sodium chloride, 10% PSC mineral premix, 10% PSC vitamin premix) and 0.6% Celite (used as a source of insoluble ash). Diets were offered twice daily (100 g fresh matter/kg metabolic weight) at 8.00 am and 4.00 pm, mixed with an equal amount of water, except for the diets containing β-glucan concentrates (1.5:1).

The hulless barleys and the oat samples were provided by the Crop Development Centre of the University of Saskatchewan (Dr B. Rossnagel). Oats with high hull content were used as a control in order to make sure that the different techniques used to identify bacteria populations could make differences between diets.

Table 1. Cereals used in the in vivo experiment

Diet	Cereal	Characteristics
Diet 1	Common barley	Hulled Barley
Diet 2	Common barley	Hulled Barley + 8.2% ß-glucan concentrate
Diet 3	Common barley	Hulled Barley + 16.3% ß-glucan concentrate
Diet 4	Barley SB 90300	Hulless barley, very low β-glucans content, normal starch
Diet 5	Barley SB 94893	Hulless barley, high β-glucans content, high amylose
Diet 6	Barley CDC Fibar	Hulless barley, high β-glucans content, 100% amylopectin
Diet 7	Barley CDC McGwire	Hulless barley, normal β-glucans content, normal starch
Diet 8	Oat CDC Sol-Fi	high hull and normal β-glucans content
Diet 9	Oat CDC Baler	high hull and low β-glucans content

Table 2. Composition of the experimental diets (g/kg)

Ingredient	diets 1, 4-9	diet 2	diet 3
Cereal	815	734	652
Soya Protein[1]	90	90	90
Whey[2]	60	60	60
Barley β-glucan[3]	-	82	163
Minerals	5	5	5
Vitamins	5	5	5
Salt	5	5	5
Dicalcium Phosphate	10	10	10
Limestone	5	5	5
Celite[4]	5	5	5

[1] SoyComil®K (CP- 65%) (ADM Speciality, Nl) [2] Crino Whey Powder (Agropur Co, Canada) [3] Isolated barley β-glucan (23.5% β-glucan) (Parrheim Foods, Saskatoon) [4] Celite 545, Celite Corp, Lompoc CA, USA

Table 3. Chemical composition of the experimental diets (g/kg)

Ingredients	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9
Dry matter	886	893	892	890	897	898	894	898	908
Ash	61	63	60	45	48	47	45	70	61
Crude protein	173	210	191	199	219	171	206	246	154
Ether extract	19	16	17	17	21	24	19	29	33
ADF	73	61	58	30	37	30	29	153	129
NDF	179	187	180	196	193	177	158	324	285
β-glucan	24	40	53	30	65	84	42	32	23
Total starch	46	46	44	56	50	43	53	33	32
GE (Mcal/kg)	4.28	4.24	4.30	4.34	4.36	4.36	4.30	4.36	4.35

NDF, ADF: neutral and acid detergent fiber, GE: Gross energy

Methodology

After an adaptation period of 10 d in the metabolic cage, fecal samples were collected for 5 d and on day-15, the weaned pigs were killed by captive bolt, followed by exsanguination, 4 h after the last meal. They were fed at different intervals in order to maintain the same period of time between the last meal and the sacrifice. After killing of the animals, the abdomen was opened and the gastrointestinal tract removed. Digesta samples from duodenum, ileum, caecum and medial colon were taken and subsequently homogenized on ice. Aliquots for short-chain fatty acid (SCFA) analysis and DNA-isolation were stored in Eppendorf tubes and in liquid N. The residual digesta were frozen in containers for subsequent analysis of nutrients and insoluble ash.

Analyses

Routine analyses

The SCFA were analyzed by gas chromatography (GC). Ammonia was analyzed by the Na prussiate method, using microplates. Ash was measured after combustion of the organic matter at 500 ºC for 6h. Insoluble ash was obtained by filtration on cellulose filter after treatment with HCl 3N. N was analyzed by the combustion method. NSP were analyzed by the Englyst method, using GC and after derivatization in aldol acetates. Digestibility was calculated by using insoluble ash as an indigestible marker.

DNA extraction, qPCR and DGGE

DNA was isolated from digesta using as described by Hill et al. (2005). To study the effect of varietal differences in the carbohydrate composition of hulled and hulless barleys and oats on the gastrointestinal microbiota in piglets, PCR-DGGE fingerprinting of V6-V8 regions of 16S rRNA genes was used, as described by Hill et al. (2005), Dumonceaux et al (2006) and Janczyk et al. (2007).

6.2. Effect of feed ingredients differing in their fermentable fiber and protein content on excretion of nitrogen and odorous compounds in pigs

Nitrogen excretion, ammonia emissions depend on the rate of fermentation of dietary fiber in the intestines. Since the ingredients used in swine nutrition differ in their fiber content and composition, differences in nitrogenous compounds and odors can be expected according to the fiber source ingested by the animal. A screening of the different ingredients with a potential in swine nutrition for these parameters is thus required.

6.2.1. Protocol

6.2.2.1. Chemical composition of the feed ingredients

The ingredients were analyzed as explained above (Experiment 1). The composition of the feed ingredients and of experimental diets is presented in Tables 4 and 5, respectively.

6.2.1.2. Animals

A total of 64 piglets (average initial weight 24 kg) were used for the experiment, during 2 consecutive periods (32/period). Three weeks after weaning, they were selected in the nursery room. They were randomly assigned to a metabolic cage and to one of the 8 experimental diets (see below). The metabolism cages were located in two different rooms and each contained 20 cages. The latter (115 x 55 cm or 0.66 m^2) had fully slatted floors and were equipped with a single feeder and nipple drinker. Sixteen cages were used in each room.

6.2.1.3. Diets

Eight diets were formulated using non conventional feed ingredient as different source of fiber. The feedstuffs included in the experimental diets were wheat bran (WB), cellulose, peas, pea inner fiber (PIF), pea hull fiber (PHF), sugar beet pulp (SBP), flaxseed meal (FSM) and corn Distiller's Dried Grains with Solubles (DDGS). The diets were balanced in energy and amino acids with soy protein isolate, pea starch, sucrose and a premix. Celite (used as a source of insoluble ash) was used in diet (0.6%) to measure digestibility at total tract level, while chromium oxide (Cr_2O_3) was mixed (0.3%) directly before feeding from d12 for 3 consecutive days to get the better estimation of marker at ileum level. Diets were offered twice daily (100 g fresh matter/kg metabolic weight) at 8.00 am and 4.00 pm, mixed with an equal amount of water.

Table 4. Chemical composition (g/kg DM) of the feedstuffs used in the experimental diets

	Dry matter	Crude protein	NDF	ADF	Total diet. fiber	Ether extract	Starch
Wheat bran	880	220	460	140	400	40	230
Solka-floc®	930	0	-	-	990	0	0
Peas	880	260	140	70	260	10	510
Pea hulls	920	90	700	630	820	10	10
Pea inner fiber	890	50	280	170	450	0	470
Sugar beet pulp	910	100	460	230	560	10	0
Flaxseed meal	910	380	260	160	390	120	0
Corn DDGS	880	270	360	100	360	50	130

DM: dry matter, CP: crude protein, NDF, ADF: neutral and acid detergent fiber; TDF: total dietary fiber; EE: ether extract

Table 5. Composition of the diets used in the experiment (g/kg)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Composition								
Wheat bran	374	-	-	-	-	-	-	-
Solka Floc®[1]	-	152	-	-	-	-	-	-
Peas	-	-	577	-	-	-	-	-
Pea hull fiber	-	-	-	183	-	-	-	-
Sugar beet pulp	-	-	-	-	333	-	-	-
Flaxseed meal	-	-	-	-	-	267	-	-
Corn DDGS	-	-	-	-	-	-	382	-
Pea starch[4]	-	-	-	-	-	-	-	412
Oil	368	458	323	470	321	459	512	382
SoyComil K®K[5]	97	217	0	194	193	180	7	56
Sucrose[6]	50	50	50	50	50	50	50	50
PSC Min[7]	5	5	5	5	5	5	5	5
PSC Vit[8]	5	5	5	5	5	5	5	5
Salt	5	5	5	5	5	5	5	5
DiCal Phosphate	22	24	22	24	24	20	23	22
Limestone	5	5	4	5	5	0	2	4
Celite[9]	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	-	-	0.2	0.2	0.2	0.1	0.1	0.2
Analysis (g/kg DM)								
DM (g/kg)	919	929	897	925	916	919	912	922
Crude protein	180	200	170	210	200	198	162	197
Starch	365	356	486	386	409	353	545	292
Ash	71	58	56	63	59	70	48	65
Ether extract	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
Net Energy (Mcal/kg)	2.20	1.97	2.42	2.24	2.17	2.34	2.56	2.30

[1] SolkaFloc® (TDF- 99%), Canada Colors and Chemicals Ltd, Ontario, Canada) 2 Exlite® Pea hull fiber (Parrheim Foods, Saskatoon, Canada) 3 Swelite® pea fiber (CP-3.76%, Cosucra, B-7740 Warcoing, Belgium) 4 Starlite® Pea starch (Parrheim Foods, Saskatoon, Canada) [5] SoyComil®K (CP- 65%) - ADM Speciality Ingredient, Koog aan de Zaan, The Netherlands [6] White Sugar- Rogers Sugar Ltd, Canada [7] PSC Min- Provided (/kg 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. [8] PSC Vit- Provided (per kg of diet), vit. A, 8,250 IU; vit. D_3 825 IU; vit. 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; vit. B_{12}, 25 ug. [9] Celite 545, Celite Corp., Lompoc CA, USA

6.2.1.4. Methodology

After an adaptation period of 10 d in the metabolic cage, fecal samples were collected for 5 d and on day-16, the weaned pigs were killed by captive bolt, followed by exsanguination, 4 h after the last meal. They were fed at different intervals in order to maintain the same period of time between the last meal and the sacrifice. After killing of the animals, the abdomen was opened and the gastrointestinal tract removed. Digesta samples from ileum and medial colon were taken and subsequently homogenized on ice. Aliquots for short-chain fatty acid (SCFA) and ammonia analysis were stored in Eppendorf tubes and in liquid N. Residual digesta were frozen in containers for subsequent analysis of nutrients, Cr_2O_3 and insoluble ash.

6.2.1.5. Analyses

The SCFA were analyzed by GC. Ammonia was analyzed by the Na prussiate method, using microplates. Ash was measured after combustion of the organic matter at 500 ºC for 6h. Insoluble ash was obtained by filtration on cellulose filter after treatment with 3N HCl. Nitrogen was analyzed by the combustion method. The NSP was analyzed by the Englyst method, using GC and after derivatization in aldol acetates (Englyst et al, 1994). Digestibility was calculated by using Cr_2O_3 as an indigestible marker at ileum level and acid insoluble ash at total tract level. The digestibility values were used to calculate the nitrogen excretion from pigs.

6.3. Evaluating the potential economic impact of utilizing alternative feedstuffs in feeding programs.

The inclusion of sources of fermentable dietary fiber (DF) in balanced diets for pigs is one way to reduce the emission of ammonia in barns or the environment. It is also possible to reduce the protein content of the rations through the use of complementary protein sources (in terms of amino acid profile) or of synthetic amino acids. However, the sources of fermentable DF generally have a poor nutritional value and their contribution to nutrient or energy supply will be limited. It is thus necessary to evaluate the possibility to incorporate them in balanced diets so that 1) the latter still supply the amount of nutrients required for optimal animal growth and 2) the effect of DF on ammonia emission is significant.

Different DF sources available on the US market were selected and balanced diets destined to growing pigs (40-60kg) were formulated by means of software of linear programming for low-cost calculation (Single-Mix, Format Int. Ltd, Woking, England). Their chemical composition and nutritional value came from three databases: NRC (1998) tables, French INRA tables (Sauvant et al., 2004; INRA Ed, Paris) and PSC database (Table 6).

The sources of DF were: soy hulls, citrus pulp, sugarbeet pulp and alfalfa. The price of the different feedstuffs was that mentioned in Feedstuffs (July 21, 2008) for Minneapolis. When not available, we used the price of the feed ingredients at Prairie Swine Centre in July 2008 (Table 6). The pig requirements are those published by NRC (1998) for growing pigs (20-50kg), with a proposed digestible energy (DE) concentration of 3.4 Mcal/kg but with higher requirements for the truly digestible essential amino acids: 9.5 g truly digestible Lys/kg diet, 6.5 g Thr/kg, 5.6 g Met-cys/kg, 1.7 g Trp/kg.

The diets were formulated in agreement with the technical rules used by feed producers: oil inclusion rate limited to 35 g/kg of the total diet and inclusion of synthetic lysine to 3.6 g /kg diet. Two experiments were conducted:

1. The diets were first formulated in order to contain 3.4 Mcal DE/kg, 9.5 g truly digestible Lysine/kg (and the other essential amino acids in constant proportion to Lysine) and 175 g crude protein/kg. The diet ingredients were limited to cereals (wheat, barley, corn) and soybean meal. Other diets were formulated to contain less crude protein (160 g/kg instead of 175 g/kg) but similar amounts of digestible amino acids, by means of some other feedstuffs (peas, canola meal). The fermentable DF sources were then included. Attempts were made to maintain the DE content of the diets (3.4 Mcal DE/kg), with feedstuff inclusion rates of 100 or 200 g/kg diet. When it was not possible to maintain that level of DE, the concentration of the latter was reduced to 3.3 Mcal/kg and then to 3.25 Mcal/kg.
2. Four diets were calculated as described by Hansen et al. (Anim. Feed Sci. Technol., 2007, 134: 326). A control diet was mainly composed of cereals (barley and wheat) and the three others were formulated to contain 150 g/kg of sugarbeet pulp, citrus pulp or soy hulls. The production costs of these diets were calculated and confronted to the results of N excretion and ammonia emission published by the above-mentioned authors and obtained with pigs weighing between 32 and 80 kg.

Table 6. Nutritional value and price of the different feedstuffs used for the study

	Crude protein g/kg	**DE Mcal/kg**	**NE Mcal/kg**	**Price $/t**
Corn	83	3.55	2.55	$267
Barley	100	3.07	2.28	210
Wheat	105	3.31	2.51	270
Soybean meal	433	3.47	1.92	427
Peas	207	3.32	2.32	260
Canola meal	337	2.76	1.51	321
Canola oil	-	8.00	7.10	1150
Soy Hulls	120	2.00	1.00	175
Alfalfa	158	1.66	0.84	240
Sugarbeet Pulp	81	2.60	1.48	540
Citrus pulp pellet	63	2.73	1.70	210

DE and NE: digestible and net energy

Results

7.1. Effect of the carbohydrate composition of cereals on the pig's gut bacterial population

7.1.1. Chemical composition of the oats and barleys

A prospective analysis of different barley and oat samples was conducted in order to select some of them for the in vitro and in vivo studies (Table 7). The ß-glucan (β-G) content of the barley samples ranged from 3.6 to 9.9 % of the total DM whereas in oats, it ranged from 3.3 to 4.8 % in whole oats. The variation in the other components was also high: from 10.8 to 17.6 % of crude protein (CP) and from 10.3 to 17.6 % of NDF in barley.

Table 7. Chemical composition of barley and oat varieties tested (g/kg DM)

Crop	Variety	Dry matter	Ash	ADF	NDF	Crude protein	Ether extract	β-Glucan	Total fiber
Barley	CDC Bold	930	27	64	172	108	19	38	594
Barley	SB94893	932	19	27	150	176	24	81	525
Barley	McLeod	932	25	58	175	139	17	49	561
Barley	CDC Fibar	933	22	17	110	154	30	99	515
Barley	CDC McGwire	930	19	23	113	153	22	52	587
Barley	SH99073	933	22	30	156	144	30	96	476
Barley	SB90300	933	21	21	103	131	19	36	593
Barley	CDC Helgason	935	24	49	145	129	22	47	542
Oat	Morgan groats	938	20	23	317	138	57	51	558
Oat	Morgan WG	946	37	198	375	99	41	31	276
Oat	Sol-FI groats	934	20	29	110	174	64	63	549
Oat	Sol-FI WG	942	38	161	316	127	46	46	324
Oat	CDC Baler	899	32	145	310	165	40	29	458

* WG- Whole grain

7.1.2. In vitro evaluation of fiber fermentation

The results of DM digestibility (dDM) and of the chemical composition of the residues after pepsin/ pancreatin hydrolysis are detailed in Table 8. Digestibility ranged from 61 to 85%. Unlike those of oats, the residues of barleys still contained significant amounts of β-glucan and of total soluble fiber. There was a good relationship between the initial β-glucan content and the content of the residues.

Table 8. DM digestibility and characteristics of the residues obtained after pepsin/pancreatin hydrolysis (g/kg DM)

Crop	Variety	dDM	β-Glucan	Soluble fiber	Crude protein
	CDC Bold	65.7	6.4	13.3	7.5
Barley	SB94893	68.7	13.8	13.0	7.9
Barley	McLeod	66.8	8.1	13.0	6.3
Barley	CDC Fibar	76.8	13.3	9.1	9.1
Barley	SH99250	65.8	15.3	12.3	6.8
Barley	CDC McGwire	69.1	8.5	13.0	8.1
Barley	SH99073	69.9	15.4	13.6	7.2
Barley	SB90300	72.6	6.6	7.5	8.1
Barley	CDC Helgason	68.5	7.2	13.3	7.6
Oat	Morgan groats	86.0	11.1	9.8	14.7
Oat	Morgan	60.7	2.2	0.7	5.4
Oat	CDC Sol-FI groats	84.7	14.5	9.8	14.5
Oat	CDC Sol-FI	64.6	3.8	3.0	5.7
Oat	CDC Baler	70.4	3.0	4.9	7.4
Wheat	Common Wheat	74.6	1.6	12.1	7.2

Figure 2 illustrates the kinetics of gas production after in vitro incubation of the residues with intestinal bacteria inoculum.

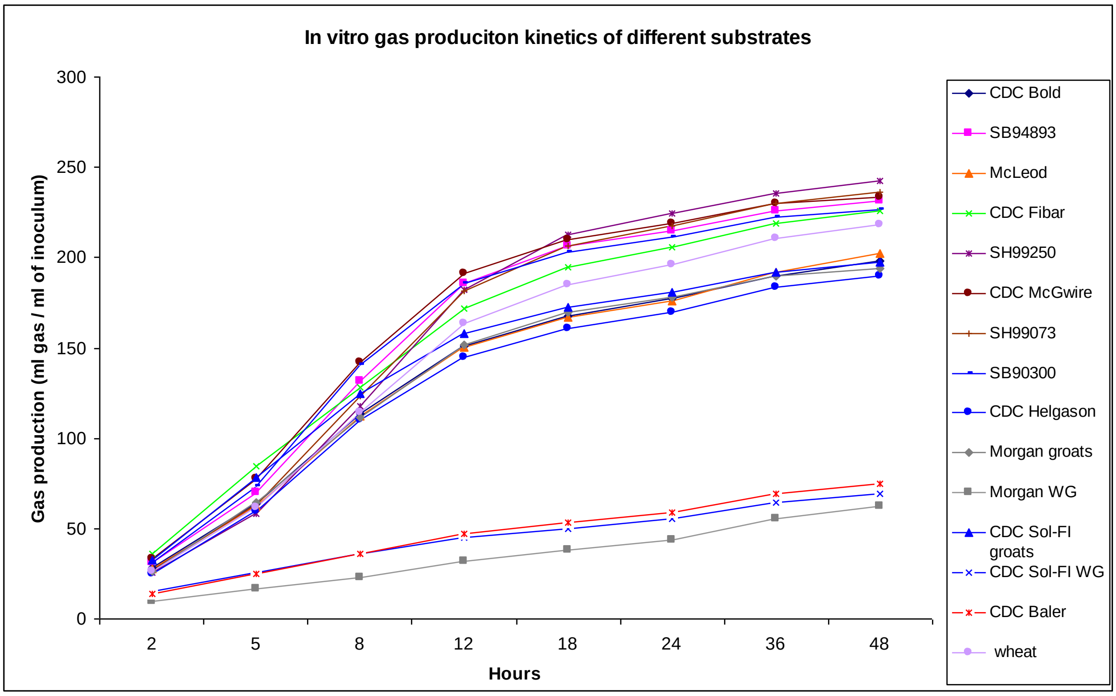

Figure 2. Kinetics of in vitro gas production following cereal carbohydrate fermentation

The final gas production was quite variable between hulless barley varieties and ranged from 170 to 230 ml gas/ml inoculum. However, the results obtained for the whole grains of the oat varieties (Morgan WG, Sol-FI WG and Baler) was considerably lower than that of the hulless barley samples and that of the oat groat (Sol-Fi groats, Morgan groats) and the wheat varieties. The oat hulls thus have a very low fermentation rate in the large intestine of the pig.

The hulless barley varieties with the highest ß-glucan levels (SH99250 - 9.1 %, SH94893 – 8.1%, Fibar 9.9%) were among the varieties with the highest rates of fermentation, whereas varieties with lower values such as Mc Leod (4.9%), Helgason (4.7%) and Sol-Fi (4.6%) present slightly lower values (Figure 2). Bold and Mc Gwire are exceptions since they have low and average values (respectively 3.8 and 5.2%) and high rates of fermentation. The high fermentation rate of these samples is possibly to be ascribed to the composition of the overall dietary fiber fraction.

7.1.3. Effect of fiber fermentation on the intestinal bacteria population in pigs

7.1.3.1. Digestibility of the experimental diets and parameters of fiber fermentation

The results of average daily feed intake and daily gain and the ileal and fecal digestibilities of the experimental diets in pigs are shown in Table 9. No difference in feed intake or daily gain was observed between pigs fed with the different experimental diets ($P > 0.05$). On the contrary, differences were observed for ileum and total DM digestibility ($P < 0.0001$) between hulled barleys, hulless barleys and oats. As predicted by the in vitro fermentation assay, the lowest digestibilities were observed for oat. Hulless barleys were also better digested than hulled barleys.

The results of pH of the ileum and colonic contents of the pigs fed the different experimental diets are given in Figure 3. The addition of β-glucan isolates to a hulled barley-based diet slightly increased the pH of the ileum content and decreased that of the colonic content but the differences were not significant ($P > 0.05$). Hulless barleys and oats had no marked effect on the pH of the ileum content either. On the contrary, all the hulless barley-based diets (diets # 4, 5, 6 and 7) had a lower pH value at the ileum level, suggesting an early fermentation of the indigestible carbohydrates in the small intestine.

Table 9. Average daily feed intake (g), average daily gain (g), digestibility (%) and DM (%) in pig fed with barleys or oats differing in their carbohydrate composition

Diet	ADG	ADFI	F:G	Ileal digest	Fecal digest	Ileal DM	Fecal DM
Diets							
Hulled barley	250	832	3.33	68^{abc}	83^{a}	11.9^{ab}	24.3^{a}
Hulled b.+10% [β-G]	271	855	3.15	56^{cd}	83^{a}	10.0^{b}	23.3^{a}
Hulled b.+20% [β-G]	238	862	3.62	64^{bcd}	83^{a}	10.6^{b}	24.0^{a}
Hulless b. SB90300	263	840	3.19	76^{ab}	90^{b}	13.0^{ab}	21.8^{a}
Hulless b. SB94893	291	856	2.94	81^{a}	90^{b}	15.9^{a}	22.4^{a}
Hulless b. Fibar	274	866	3.16	74^{ab}	89^{b}	14.8^{ab}	23.0^{a}
Hulless b. Mc Gwire	274	864	3.15	73^{cd}	89^{b}	13.6^{ab}	23.1^{a}
Oat Sol-Fi	254	843	3.19	56^{cd}	66^{c}	12.5^{ab}	29.3^{b}
Oat Baler	234	819	3.50	54^{d}	69^{c}	11.6^{ab}	29.9^{b}
SEM	18	34	0.20	3.4	0.3	1.09	0.78
P- value	0.44	0.99	0.26	<0.001	<0.001	0.005	<0.001
Type of cereals							
Hulled barley	253±10	849±19	3.4^{a}±0.2	63^{b} ± 2	83^{a} ±0.2	10.5^{b}±0.7	23.8^{a} ± 0.4
Hulless barley	275 ± 9	857±17	3.1^{b}±0.1	75^{c} ± 2	90^{b}±0.2	14.3^{a} ±0.5	22.6^{a} ± 0.4
Oat	244 ±23	831±13	3.4^{a}±0.2	55^{a} ± 2	68^{c}±0.3	12.1^{b} ±0.8	29.6^{b} ± 0.5
P- value	0.095	0.667	0.03	<.0001	<.0001	0.0001	<.0001

ADFI: av. daily feed intake; ADG av. daily gain; F:G; feed/gain ratio; Ileal and fecal DM: dry matter of ileum content and feces

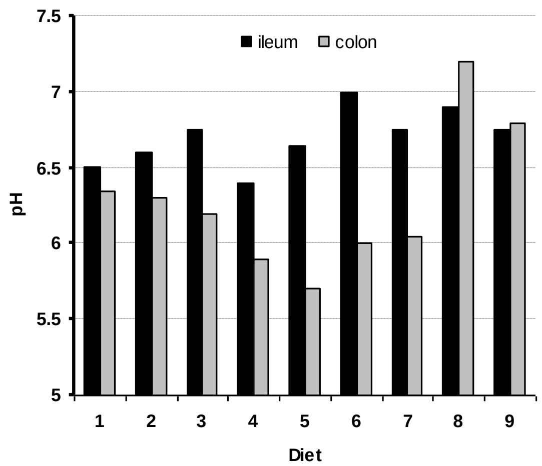

Figure 3. pH of the content of the small and large intestines of pigs fed with different barley- or oat-based diets

1. Control 2. Control + 10 % β-glucan concentrate 3. Common barley + 20 % β-glucan concentrate 4. Hulless barley with very low β-glucan content and normal starch 5. Hulless barley with high β-glucan content and high amylose content (40%) 6. Hulless barley with high β-glucan content and 100% amylopectin starch 7. Hulless barley with normal β-glucan content and normal starch content 8. Oats with high β-glucan content 9. Oats with high hull content

The profile in fermentation end-products of the ileum content of pigs fed the experimental diets is detailed in Table 10 and Table 11. Higher contents in short-chain fatty acids (SCFA) were observed in pigs fed oat-based diets (P < 0.01) whereas higher values of lactic acid were observed in pigs fed barley-based diets (P = 0.03). On the contrary, no difference in ammonia content was observed (P > 0.05).

The profile in fermentation end-products of the colonic content of pigs fed the experimental diets is detailed in Table 10. Lower contents in short-chain fatty acids (SCFA) were observed in pigs fed oat-based diets (P < 0.001) whereas higher values of lactic acid were observed in pigs fed hulless barley-based diets (P < 0.001). Unlike the ileum contents, differences were observed in ammonia content: the pigs fed oat-based diets had less ammonia in their colon, as compared to barley-based diets (P < 0.001). This can be ascribed to a lower rate of fermentation of the carbohydrates. The lower SCFA content with the oat-based diets also led to a higher pH.

Table 10. Fermentation end-product profile and pH of ileal digesta in pigs

Diet	SCFA*	Lactic acid*	Ammonia*	pH
Main effects				
Diets				
Hulled barley	8.5a	26.6a	7.0a	6.5ab
Hulled b. +10% [b-G]	5.0a	38.5a	6.7a	6.2b
Hulled b. +20% [b-G]	6.6a	38.9a	8.1a	6.4ab
Hulless b. SB90300	7.4a	39.0a	8.6a	6.4ab
Hulless b. SB94893	8.7a	27.5a	7.5a	6.6ab
Hulless b. Fibar	6.5a	22.8a	6.3a	7.0a
Hulless b. Mc Gwire	7.0a	22.0a	8.1a	6.8ab
Oat Sol-Fi	10.6a	11.7a	8.6a	6.9a
Oat Baler	7.7a	17.1a	7.9a	6.8ab
SEM	1.21	8.19	0.71	0.14
P- value	0.13	0.2	0.24	0.003
Type of cereals				
Hulled barley	6.7a ± 0.8	34.9a ± 4.9	7.3a ± 0.4	6.4b ± 0.1
Hulless barley	7.4a ± 0.6	27.8a ± 4.0	7.6a ± 0.4	6.7a ± 0.1
Oat	9.1b ± 0.9	14.1b ± 5.7	8.3a ± 0.5	6.9a ± 0.1
P- value	0.012	0.03	0.39	0.002

*mMol/ kg of digesta sample

Table 11: Fermentation end-product profile and pH of colonic contents in pigs

Diet	SCFA*	Lactic acid*	Ammonia*	pH
Main effects				
Diets				
Hulled barley	95.8a	0.6d	25.9a	6.5bc
Hulled b. +10% [b-G]	101.4a	3.3cd	29.4a	6.2cd
Hulled b. +20% [b-G]	101.1a	2.47cd	24.3a	6.2cd
Hulless b. SB90300	102.2a	12.9abc	24.4a	6.2cd
Hulless b. SB94893	115.1a	21.1a	26.8a	5.9d
Hulless b. Fibar	111.9a	16.5ab	28.4a	6.2cd
Hulless b. Mc Gwire	108.9a	8.9bcd	27.4a	6.2cd
Oat Sol-Fi	48.8b	2.2cd	14.3a	7.2a
Oat Baler	47.1b	0.9d	14.8a	6.9ab
SEM	6.08	2.51	3.71	0.11
P- value	<0.0001	<0.0001	0.054	<0.0001
Type of cereals				
Hulled barley	99.4a ± 3.4	2.1b ± 1.5	26.5a ± 2.1	6.3b ± 0.1
Hulless barley	109.5a ± 3.0	14.83a ± 1.3	26.8a ± 1.9	6.1b ± 0.1
Oat	47.9b ± 4.2	1.5b ± 1.9	14.5b ± 2.7	7.1a ± 0.1
P- value	<0.0001	<0.0001	0.0008	<0.0001

*mMol/ kg of digesta sample

7.1.3.2. Effect of fiber fermentation on the gut bacteria population in pigs

To study the effect of varietal differences in the carbohydrate composition of hulled and hulless barleys and oats on the gastrointestinal microbiota in piglets, Polymerase chain reaction-Denaturing Gel Gradient Electrophoresis (PCR-DGGE) fingerprinting of V6-V8 regions of 16S rRNA genes was used. DGGE profiles of ileum (not shown) and colon (Figure 4) samples revealed obvious shifts in the community composition and apparent abundance of species as indicated by band position and intensity. Further analysis of the fingerprints revealed decreasing number of bands and Shannon diversity in ileum of animals fed common barley with 8.2% BBG concentrate, hulless barley SB 94893 and Baler oats,

whereas higher values were found with CDC McGwire barley as compared with the other cereals (Table 12). In the colon, there was a graded decrease in number of bands and Shannon diversity from common barley towards hulless barley with the very high β-glucan content (‘CDC Fibar’).
Although this effect was also observed for the diets containing the isolated barley β-glucan concentrate (BBG), was less pronounced and only significant for the 16.3% BBG diet. Diets, containing the common barley (diets 1-3) had the highest number of bands and Shannon diversity values irrespective of BBG addition as compared with the other diets. The oat varieties had intermediate values. No differences were observed for intra-group Dice similarity index for ileum samples except for CDC Sol-Fi oats which had a significantly higher value and the diet containing 16.3% BBG concentrate which had the lowest value.

In contrast, in the colon, a decrease of intra-group similarity was observed from common to hulless varieties and depending on the level of β-glucans. Diets with common barley had the highest values as compared with the other diets. Interestingly, the diets containing either 10 or 16.3% of the BBG concentrate had similar values as the unsupplemented common barley (diet 1) irrespective of the β-glucan content. The oats showed again intermediate values.

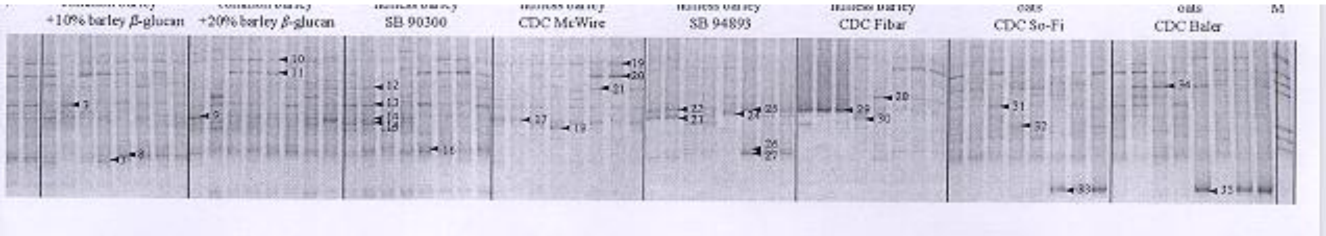

Figure 4. DGGE evaluation of colon microbiota of piglets after feeding common barley, two diets with common barley, supplemented with 15 or 30 g/kg isolated barley β-glucan; two oat varieties (CDC Sol-Fi, CDC Baler) and hulless barleys (SB 90300, CDC McWire, SB 94893, CDC Fibar) with increasing β-glucan content. DGGE of PCR products of V6 to V8 regions of 16SrRNA gene was performed on a 35-55% denaturant gradient. Each lane represents the microbial profile of each piglet per group (8 animals, respectively). M - marker lane. Arrows and numbers indicate excised and reamplified bands for species identification (Table 10).

Table 12. Mean ± SD number of bands, Shannon diversity and intra-group Dice similarity values calculated from DGGE fingerprints of ileum and colon microbial communities

Diet #		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
***β*-glucan**		**HB**[1]	**HB+*β*G**	**HB+*β*G**	**hB**	**hB**	**hB**	**hB**	**oat**	**oat**
(g/kg)		**24**	**40**	**53**	**30**	**42**	**65**	**84**	**32**	**23**
bands	Ileum	8.9 ± 2.5^{a}	6.8 ± 2.6^{b}	7.8 ± 1.8^{abc}	8.6 ± 2.6^{a}	9.9 ± 2.5^{a}	5.9 ± 1.8^{c}	7.6 ± 2.1^{abc}	8.3 ± 2.0^{ab}	6.5 ± 2.5^{bc}
	Colon	17.4 ± 1.9^{a}	17.8 ± 2.4^{a}	14.1 ± 2.6^{b}	11.9 ± 4.0^{b}	11.3 ± 2.8^{b}	8.3 ± 2.7^{c}	5.8 ± 2.1^{c}	13.4 ± 2.1^{b}	12.1 ± 3.0^{b}
Shannon diversity	Ileum	0.73 ± 0.1^{ab}	0.69 ± 0.15^{b}	0.72 ± 0.1^{ab}	0.73 ± 0.1^{ab}	0.80 ± 0.08^{a}	0.54 ± 0.10^{c}	0.68 ± 0.11^{b}	0.66 ± 0.11^{b}	0.64 ± 0.10^{bc}
	Colon	1.15 ± 0.08^{a}	1.12 ± 0.09^{a}	1.02 ± 0.13^{b}	0.91 ± 0.15^{b}	0.86 ± 0.11^{b}	0.80 ± 0.13^{b}	0.52 ± 0.27^{c}	0.96 ± 0.11^{b}	0.86 ± 0.19^{b}
Dice similarity	Ileum	43.4 ± 25^{bc}	42.8 ± 14^{bc}	34.9 ± 17.3^{c}	43.0 ± 23^{bc}	53.4 ± 20.6^{b}	38.8 ± 22^{bc}	47.8 ± 12.^{bc}	69.6 ± 13.3^{a}	52.8 ± 20.0^{b}
	Colon	73.9 ± 5.4^{a}	67.4 ± 5.8^{ab}	67.2 ± 8.0^{ab}	62.5 ± 6.0^{b}	61.6 ± 7.6^{b}	49.5 ± 17.4^{c}	49.0 ± 16.8^{c}	61.9 ± 9.7^{b}	58.0 ± 8.4^{b}

[1] abbreviations: HB - common (hulled) barley, hB - hulless barley, BBG - barley β-glucan concentrate
^{a,b,c} indicate significant ($P < 0.05$) differences

Reamplification and identification of bands from colon samples which were characteristic for each diet revealed a predominance of *Clostridium glycolicum*, *Mogibacterium diversum*, *Butyrivibrio fibrisolvens*, *Syntrophococcus sucromutans*, *Weissella confusa* and *Lactobacillus sobrius*-like phylotypes in animals fed the common barley, common barley supplemented with BBG concentrate and the hulless barley with the lowest β-glucan content SB 90300 (Figure 4, Table 12). Some of these phylotypes such as *Lactobacillus sobrius*, *Butyrivibrio fibrisolvens* were also detected with the other diets, whereas others such as *E. coli*, *Cl. glycolicum* and *M. diversum*-like phylotypes were reduced in abundance as indicated by the relative band intensity. On the other hand, in the other hulless barley diets (CDC McGwire, SB 94893, CDC Fibar), *Eubacterium uniforme*, *Eubacterium cellulosolvens* and *Clostridium xylanovorans*-like phylotypes appeared and represented the predominant bands together with sequences that could not be assigned to any typed bacterium. In the DGGE fingerprints of animals fed the two oat diets, *Eubacterium ramulus*, *Ruminococcus bromii*, *C. glycolicum*, *Mitsoukella jalaludinii* and *Lactobacillus johnsonii*-like phylotypes represented the most dominant bands (Table 13).

Table 13. Affiliation of partial 16S rRNA (V6-V8 region) gene sequences obtained from excised bands of DGGE fingerprints with their closest relatives in GenBank. Closest cultured relatives are given in parentheses

band #	**closest cultured relative (GenBank accession #)**	**ID %**
1, 2, 10, 11, 19, 20	*Clostridium glycolicum*	99
3, 13, 28	*Mogibacterium diversum*	96
4, 9	*Escherichia coli*	99
5	*Weissella confusa*	99
6, 8, 16, 26	*Lactobacillus sobrius*	99
7, 27	*Clostridium xylanovorans*	91
12, 21	uncultured butyrate producing bacterium	99
14, 17	*Butyrivibrio fibrisolvens*	97
15	*Syntrphocoocus sucromutans*	92
18	uncultured butyrate producing bacterium	99
22, 23, 24, 30	*Eubacterium cellulosolvens*	93
23	*Eubacterium uniforme*	87
25, 29	uncultured butyrate producing bacterium	99
31	*Eubacterium ramulus*	93
32	*Mitsoukella jalaludinii*	98
33, 35	*Lactobacillus johnsonii*	99
34	*Ruminococcus bromii*	87

In the ileum fingerprints (figure not shown), *C. glycolicum*, *W. confusa* and *L. sobrius*-like phylotypes were predominant independent of the diet. Interestingly, only in the two hulless barley varieties with high *β*-glucan content (SB 94893, CDC Fibar), some other dominant bacteria such as *E. cellulosolvens* and *C. butyricum* were found. Moreover, a *Bifidobacterium pseudolongum*-like phylotype was detected in three animals fed CDC McGwire and in four animals fed SB94893.

Table 14. Mean ± SD copy numbers (log copies/µl DNA) of 16S rRNA gene of microbial groups in Ileum and Colon genomic DNA. Numbers in parentheses indicate the number of colonized piglets

Diet #		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
		HB[1]	**HB+*β*G**	**HB+*β*G**	**hB**	**hB**	**hB**	**hB**	**oats**	**oats**
***β*-glucan (g/kg)**		**24**	**40**	**53**	**30**	**42**	**65**	**84**	**32**	**23**
lactobacilli	Ileum	5.3 ±1.2	6.1 ±0.7	6.0 ±0.7	5.9 ±0.7	5.3 ±1.2	4.9±1.1[a]	4.6±1.2[a]	6.5±0.3[b]	6.5±0.8[b]
	Colon	7.2 ±0.2	7.3 ±0.5	7.1±0.5	7.1±0.5	6.9±0.4	7.0±0.4	6.9±0.5	7.4±0.4	7.1±0.5
streptococci	Ileum	2.9±1 (4)	2.2 ±1.1 (3)	2.4 ±0.5 (4)	2.4 (1)	2.5 ±0.8 (4)	n.d.	n.d.	2.2 ±0.7	2.5 ± 0.8 (6)
	Colon	8.0 ±0.6	7.8 ±0.6	7.8 ±0.4	7.7 ±0.5	7.7 ±0.2	7.6 ±0.3	7.6 ±0.5	7.9 ±0.2	7.8 ± 0.3
enterobacteria	Ileum	3.6 ± 1 (4)	2.4 ±0.5 (3)	2.8 ±1.1 (4)	2.6 (1)	3.0 ±1.1 (4)	n.d.	n.d.	4.3 ±0.5	3.8 ± 1.4 (6)
	Colon	2.7 ±1.1	2.7 ±0.6	2.7 ±0.9	2.5 ±0.4	2.6 ±0.8	3.3 ±1.0	2.7 ±0.8	3.6 ±1.1	2.9 ± 1.4

[1] abbreviations: HB - common (hulled) barley, hB - hulless barley, BBG - barley *β*-glucan concentrate;
[a,b,c] indicate significant ($P < 0.05$) differences; n.d. not detected

Quantitative real-time PCR was furthermore used to obtain information about 16S rRNA gene copy numbers in the genomic DNA from ileum and colon samples. Results show significant lower numbers of lactobacilli in the ileum of animals fed varieties SB 94893 and CDC Fibar as compared with the oats diet (Table 14). Addition of either 8.2 or 16.3% BBG concentrate increased slightly but not significantly the number of ileal lactobacilli. No differences for all microbial groups were found in the colon samples. Interestingly, no enterobacteria or streptococci could be detected in the ileum of animals fed the high β-glucan diets and were detected only occasionally but still at very low abundance with the other diets.

7.2. Effect of feed ingredients differing in their fermentable fiber and protein content on excretion of nitrogen and odorous compounds in pigs

7.2.1. Digestibility of the experimental diets differing in fermentable fiber and protein content

The results of average daily feed intake and daily gain along with the ileal and fecal digestibilities of DM and nitrogen of the experimental diets in pigs are shown in Table 15. There was no difference ($P > 0.05$) found in feed intake between pigs fed with the different experimental diets, while higher weight gain was observed for the diets based on pea hulls and pea inner fibers. No difference in DM or N digestibility was observed at the ileum level ($P > 0.05$). However, difference is digestibility at total tract was found significant ($P < 0.0001$) for both DM and N. The digestibility of both DM and N was higher in Peas, PHF and PIF while FSM and DDGS were poorly digested.

Table 15. Average daily feed intake (ADFI, g), average daily gain (ADG, g) and digestibility of nutrients in pig fed with different fiber source differing in their carbohydrate composition

Diet	ADFI	ADG	DM digestibility (%)		N digestibility (%)	
			Ileum	Tot. tract	Ileum	Tot. tract
Diet 1 (Wheat bran)	1125	425abc	68	78bc	71	76abcd
Diet 2 (Cellulose)	1041	409abc	67	77c	77	78abc
Diet 3 (Peas)	1055	351c	77	88a	71	75bcd
Diet 4 (Pea hulls)	1088	568a	75	89a	78	81a
Diet 5 (Pea inner fiber)	1082	520ab	70	88a	77	79ab
Diet 6 (Sugar beet pulp)	1108	500abc	73	87a	79	76abcd
Diet 7 (Flaxseed meal)	1066	346c	65	82b	65	72d
Diet 8 (DDGS)	1125	378c	72	78bc	76	74cd
SEM	42.6	37.2	3.2	1.0	3.7	1.2
P- value	0.801	0.0002	0.1272	<.0001	0.0889	<.0001

*DM- Dry matter, DFI- Daily feed intake, DWG- Daily weight gain, N- nitrogen, WB- Wheat Bran, PHF- Pea hull fibers, PIF- Pea inner fibers, SBP- sugar beet pulp, FSM- Flaxseed meals, DDGS- corn Distiller's Dried Grains with Solubles
* Mean values with different superscript within column are significantly different ($P < 0.05$)

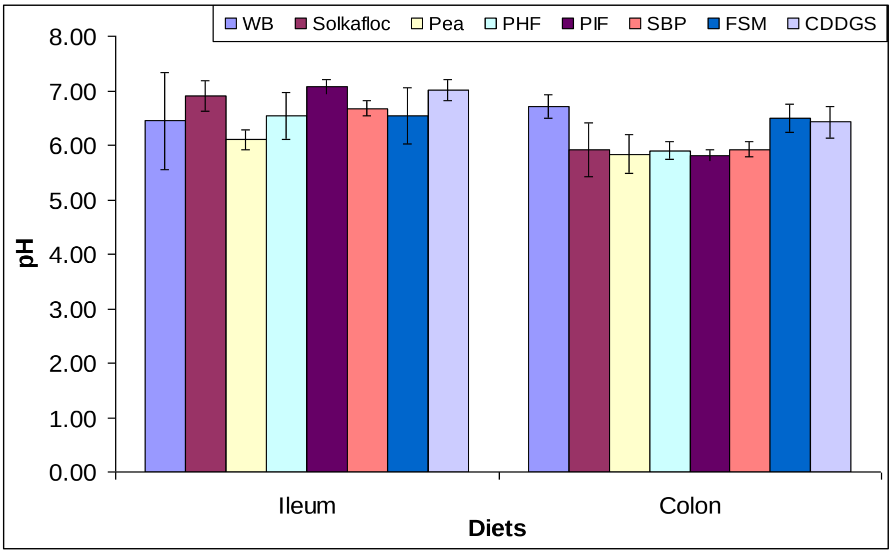

Figure 5: pH of the digesta from ileum and colon of pigs fed different experimental diets

The results of the pH measured in the ileum and colonic contents of the pigs fed the different experimental diets are given in Figure 5. There was no difference between diets, except for the wheat bran and flaxseed meal diets, which had a lower pH value at the ileum level than at colon, suggesting an early fermentation of the indigestible carbohydrates in the small intestine.

Table 16. Dry matter content (g/kg DM) and pH of the digesta of pigs fed different diets

Diet	**Dry matter**		**pH**	
	Ileum	**Colon**	**Ileum**	**Colon**
Diet 1 (Wheat bran)	128bc	190a	6.36b	6.65a
Diet 2 (Cellulose)	114bc	216a	7.00a	6.17ab
Diet 3 (Peas)	194a	226a	6.31b	5.97b
Diet 4 (Pea hulls)	139b	240a	6.34ab	6.13ab
Diet 5 (Pea inner fiber)	71d	114b	7.10a	6.03b
Diet 6 (Sugar beet pulp)	116bc	120b	6.57ab	5.96b
Diet 7 (Flaxseed meal)	106c	195a	6.35b	6.66a
Diet 8 (DDGS)	129bc	235a	7.08a	6.37ab
SEM	7.1	11.4	0.14	0.13
P- value	<.0001	<.0001	<.0001	0.0002

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

7.1.2. Effect of fiber sources and dietary protein content on the metabolic profile and N excretion in pigs

The major fermentation end-products of fiber at the ileum and colon and the total N excretion are presented in Table 17. Similarly, the proportions of the individual SCFA, both at the ileum and colon are presented in Tables 18 and 19. There was higher SCFA concentration observed in the ileal digesta of pigs fed peas and pea hulls, while no difference in SCFA concentration was found at the colon level. There was significantly higher (P <.0001) butyrate produced at the ileum when peas were offered, as compared to the other fiber sources, which was not found at colon level.

Table 17. Concentration of SCFA (mMol/kg digesta) and ammonia (mMol/kg digesta) in the ileum and colon content of pigs fed diets based on different fiber sources and fecal N excretion

Diet	**SCFA***		**Ammonia***		**Fecal N excretion**	
	Ileum	**Colon**	**Ileum**	**Colon**	**g/kg DM intake**	**g/kg N intake**
Diet 1 (Wheat bran)	23.0b	94.1	6.5ab	99.5abc	6.9b	237abcd
Diet 2 (Cellulose)	24.6b	112.9	7.0ab	87.6abc	7.1ab	217bcd
Diet 3 (Peas)	39.3a	113.9	7.0ab	126.5ab	7.0b	251abc
Diet 4 (Pea hulls)	26.9ab	121.6	8.5a	132.1a	6.5b	191d
Diet 5 (Pea inner fiber)	16.6b	119.0	5.5b	68.1c	6.5b	206cd
Diet 6 (Sugar beet pulp)	24.3b	112.5	6.5ab	73.3bc	7.6ab	236abcd
Diet 7 (Flaxseed meal)	20.6b	101.4	6.8ab	129.1a	7.4ab	280a
Diet 8 (DDGS)	19.1b	100.0	5.6b	106.3abc	8.3a	262ab
SEM	3.16	6.97	0.64	12.3	0.40	12.2
P- value	0.001	0.076	0.056	0.001	0.049	<.0001

*mMol/ Kg of digesta sample, N- nitrogen
[#]Mean values with different superscript within column are significantly different (P <0.05)

The ammonia concentration was higher in the ileal and colonic digesta of the pigs fed with pea hulls. The N excretion, expressed per kg DM intake or per kg N intake, was higher in pigs fed diets containing flaxseed meal or corn DDGS.

Table 18**.** Proportion (%) of SCFA concentration in ileum of pigs fed different diets

Diet	Acetic acid	Propionic acid	Butyric acid	Branched-chain FA
Diet 1 (Wheat bran)	88.3b	4.4a	7.3b	0.21
Diet 2 (Cellulose)	92.3ab	2.1ab	5.6b	0.18
Diet 3 (Peas)	83.4c	2.7ab	13.6a	0.23
Diet 4 (Pea hulls)	89.7ab	1.7ab	8.4b	0.06
Diet 5 (Pea inner fiber)	92.3ab	1.9ab	5.7b	0.00
Diet6 (Sugar beet pulp)	90.1ab	2.0ab	7.7b	0.07
Diet 7 (Flaxseed meal)	91.3ab	2.3ab	6.4b	0.06
Diet 8 (DDGS)	93.9a	1.3b	4.7b	0.00
SEM	0.95	0.63	0.89	0.09
P- value	<.0001	0.042	<.0001	0.39

*Ac- acetic acid, Pr- propionic acid, Bu- butyric acid Bc- branched chain fatty acids,
[#]Mean values with different superscript within column are significantly different (P <0.05)

Table 19**.** Proportion (%) of SCFA concentration in colon of pigs fed different diets

Diet	Acetic acid	Propionic acid	Butyric acid	Branched-chain FA
Diet 1 (Wheat bran)	63.6	19.6b	12.4	4.8bcd
Diet 2 (Cellulose)	61.8	18.0b	16.3	3.9d
Diet 3 (Peas)	55.9	21.0ab	16.5	6.6a
Diet 4 (Pea hulls)	57.8	21.0ab	15.3	5.6abc
Diet 5 (Pea inner fiber)	59.9	24.1a	11.9	4.1cd
Diet 6 (Sugar beet pulp)	61.1	21.8ab	13.5	3.9d
Diet 7 (Flaxseed meal)	61.5	21.6ab	11.5	5.0bcd
Diet 8 (DDGS)	60.5	22.1ab	11.8	5.9ab
SEM	1.86	0.95	1.84	0.36
P- value	0.126	0.003	0.276	<.0001

* Ac- acetic acid, Pr- propionic acid, Bu- butyric acid Bc- branched chain fatty acids,
[#]Mean values with different superscript within column are significantly different (P <0.05)

7.1.3. Discussion

Our results show that DF has a major impact on N excretion and the ammonia content of the feces (Table 17). However, a high level of fermentable DF in the diet will not necessarily result in a decrease in N excretion. The pea fiber fractions, for example, have the highest rate of fermentation in the pig large intestine (personal data, not shown here). Despite that, they presented lower N excretions than cellulose, which is virtually not fermented in the gut. On the other contrary, sugarbeet pulp, flaxseed meal and corn DDGS presented the highest levels of N excretion but this doesn’t necessarily means that their DF was highly fermented and that the bacterial biomass produced would be responsible for the high N excretion. It is certainly the case for beet pulp, which is highly fermented and has a low protein content. However, corn DDGS are not well fermented but contain high levels of indigestible protein and the latter contributed to the total N excretion. Flaxseed meal is intermediary: its fiber fraction is highly fermentable but it also contains high levels of non-digestible protein. Our results should be completed by results obtained from N balance studies where urinary N excretion is also considered.

In terms of odour emission, the results show a significant effect on ammonia content in the feces. However, again, the results must be linked to results of urinary N excretion before any conclusion can be drawn. The highest ammonia concentrations in the colon were obtained with the feedstuffs having the highest crude protein content (peas, flaxseed meal, corn DDGS). Thus, again, the ratio protein/fermentable DF is important. Finally, no major impact has been obtained on butyric acid, a highly odorous compound.

7.3. Evaluating the potential economic impact of utilizing alternative feedstuffs in feeding programs.

7.3.1. Experiment 1

The results of the different formulae are detailed in Table 20. Different conclusions can be drawn:

1. Feed costs can be reduced by formulating diets with a lower energy density. It is for example the case between diets 1 and 2 with, respectively 3.4 and 3.3 Mcal/kg. The difference is to be ascribed principally to a lesser use of canola oil. Similar observation can be made between diets 3 and 4.
2. It is possible to reduce the crude protein content of balanced diets by using additional feedstuffs that have a nutrient profile, complementary to the basic feedstuffs (ex. peas), and by increasing the level of synthetic amino acids. The change in feed cost will depend on the price of the additional feed ingredient but an increase in the use of synthetic amino acids will generally lead to higher feed costs, especially when synthetic methionine ($3,600/t) and tryptophan ($50,000/t) are required. A decrease from 175 to 160 g crude protein/kg diet will reduce the total daily N excretion: ±3 g/d for a 35kg-pig, essentially in the form of urinary N (Deng et al., 2007; Livest. Sci. 109, 220). In finishing pigs (80kg), each gram eliminated from the diet will lead to a decrease of 280 mg N per day (Lynch et al., 2007 Livest. Sci. 109, 204). In the present conditions, a decrease in crude protein content from 175 to 160 g/kg diet would raise the cost of the feed ($25/t) and decrease N excretion (3 g/d/pig). Assuming that a 35kg-pig eats 2 kg diet/day, 1 t of feed would thus feed 500 pigs. A change in protein content would thus reduce N excretion as follows: 3 g/d/pig x 500 pigs = 1,500 g/t feed or 60 g N/$ spent.
   However, the same conclusion cannot be drawn with the second example with corn-based diets (Table 2). No difference in feed cost was observed for the diets supplemented with soy hulls and sugarbeet pulp whereas the cost of the diet supplemented with citrus pulp is lower for the diet with the lowest protein content. The economic impact of the inclusion of DF sources in a diet is thus variable and dependent on the composition of the diet and the price of the different feed ingredients.
3. It is possible to incorporate sources of dietary fiber in balanced diets while maintaining low protein contents. By definition, the sources of DF are poor in protein. The proteins are of low quality, i.e. they are not well digested and thus generate higher N excretions. However, it is easy to compensate for their deficiency in essential amino acids by means of high-quality protein sources, such as soybean meal.
4. It is not possible to incorporate sources of DF while maintaining a high energy content in the diet. This aspect of feed formulation is the most challenging one since a low energy density will have to be compensated for by feedstuffs with high energy density and the latter are usually expensive. Moreover, if energy must be supplied by vegetal oil (canola oil, corn oil, etc), the inclusion rate will be limited in order not to exceed the digestive capacity of the pig (maximum 3.0-3.5% of the diet).
5. Based on our study with different DF sources, we conclude that the most interesting source is **citrus pulp**, thanks to its relatively low price ($210/t), its high rate of DF fermentation (it is mainly composed of pectin) and its high DE content (it contains 20% sucrose).
   Sugarbeet pulp has some nutritional interest but is not economic ($540/t).
   Soy hulls are cheap ($175/t) and highly fermentable but their interest is limited by their low energy content (2.0 Mcal DE/kg; 1.0 Mcal NE/kg).
   The interest for alfalfa is nil (low DE content, low fermentable DF content).
6. More fiber means more feces and thus increased manure, as illustrated in the previous experiments. For example, the inclusion of 15% of sugarbeet pulp, citrus pulp or soy hulls will result in a significant increase in DM excretion (> 28-56%; Lynch et al., 2007). Depending on the level of protein, the volume of urine can also increase substantially. This will require a higher capacity of manure storage and will generate higher costs of manure handling.

Table 20. Composition(g/kg diet), nutritional value and cost of balanced diets for growing pigs supplemented or not with sources of fermentable dietary fiber

Diet #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Diet	**Control**	**Control**	**Control**	**Control**	**Control**	**Soy Hulls**	**Alfalfa**	**SB Pulp**	**SB Pulp**	**SB Pulp**	**Citrus**	**Citrus**	**Citrus**
Crude protein (g/kg)	**175**	**175**	**175**	**175**	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**	**160**
DE (Mcal/kg)	**3.40**	**3.30**	**3.40**	**3.30**	**3.40**	**3.25**	**3.25**	**3.30**	**3.25**	**3.25**	**3.30**	**3.30**	**3.25**
NE (Mcal/kg)	**2.43**	**2.34**	**2.45**	**2.44**	**2.48**	**2.32**	**2.35**	**2.37**	**2.30**	**2.32**	**2.31**	**2.31**	**2.26**
Composition (g/kg)													
Barley	200	180	128	587	189	261	76	240	515	-	397	172	387
Wheat	526	572	395	74	497	349	484	338	109	414	210	363	145
Corn	-	-	-	-	-	-	-	-	-	-	-	-	-
Soybean meal	208	200	160	190	136	141	98	110	148	39	171	189	192
Canola meal	-	-	-	-	-	-	-	35	20	91	-	-	-
Peas	-	-	233	84	104	75	-	103	34	182	30	-	-
Canola Oil	30	12	30	30	35	35	35	35	35	35	35	35	35
Fiber source	**-**	**-**	**-**	**-**	**-**	**100**	**100**	**100**	**100**	**200**	**100**	**200**	**200**
HCl-Lysine	2.8	3.0	2.7	2.1	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.7	3.6
DL-Methionine	0.5	0.5	1.1	0.7	1.6	1.2	1.5	1.2	1.1	1.3	1.2	1.2	1.3
L-Threonine	0.9	1.7	1.4	0.9	1.1	1.6	1.7	1.7	1.6	1.9	1.5	1.5	1.5
L-Tryptophan	-	-	-	-	0.1	-	0.2	0.2	0.1	0.4	0.1	0.1	0.1
Limestone	7.5	7.5	7.4	7.6	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
DiCal Phosphate	9.0	8.8	7.7	8.5	9.9	10.8	10.8	10.0	10.0	10.0	10.6	11.8	11.6
Min/Vit/Salt	15	15	15	15	15	15	15	15	15	15	15	15	15
Cost ($/ton)	**334**	**318**	**312**	**304**	**337**	**326**	**341**	**360**	**347**	**398**	**323**	**335**	**323**

Table 21. Composition (g/kg), nutritional value and price of the different feedstuffs used for the study

	Control	Soy hulls		Sugarbeet pulp		Citrus pulp	
Composition							
Fiber source	-	200	200	200	200	200	200
Barley	200	-	-	-	-	-	
Corn	510	543	545	547	495	521	494
Soybean meal	235	183	174	155	233	175	190
Canola meal	-	-	12	50	20	51	8
Canola oil	-	30	35	11	19	11	6
Amino acids	5	6	6	6	5	6	5
Minerals	50	38	38	31	38	36	37
DE (Mcal/kg)	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Crude protein (g/kg)	**160**	**160**	**175**	**160**	**175**	**160**	**175**
Cost ($/ton)	**315**	**340**	**340**	**394**	**398**	**334**	**320**

7.3.2. Experiment 2

The results of the second financial study are detailed in Table 22. Compared to the control diet, it is possible to reduce the feed costs by incorporating cheap feed ingredients with high levels of fermentable DF such as soy hulls and citrus pulp. On the contrary, in U.S. conditions, sugarbeet pulp is not interesting because it raises the feed costs substantially and also causes the highest losses of nitrogen, both at the fecal and urinary levels, and –consequently- the highest emission of ammonia. In the conditions of the study, the diet based on sugarbeet pulp brought more total crude protein than the other diets. This is partly due to the simple formula used by Hansen et al (2007) but also to the fact that only 50% of the protein of that feedstuff is digested (INRA, 2004), which also explains the higher levels of N excretion. Probably, with lower crude protein contents and a better balance in amino acids, N excretion would be lower.

Meanwhile, it is worth considering the case of citrus pulp. It has an astonishingly high DE and NE content, thanks to a high level of sucrose (up to 22% on a DM basis; INRA, 2004). It makes it thus easier to formulate a balanced diet. The fact that it has a low crude protein content is also an advantage in the sense that it is easy to bring other protein sources, balanced in essential amino acids, without increasing the total crude protein content of the diet. Finally, it apparently generates an environmental advantage in the sense that its presence in the diet causes a shift of N excretion from urine to feces with positive consequences on ammonia emission.

Table 22. Composition (g/kg), nutritional value and cost of the different feedstuffs used for the study

	Control	Soy hulls	Sugarbeet pulp	Citrus pulp
Composition				
Fiber source	-	150	150	150
Barley	220	220	220	220
Wheat	550	433	410	410
Soybean meal	180	161	171	181
Canola oil	10	10	10	7
Amino acids	4	4	4	4
Minerals	40	22	35	28
Cost ($/ton)	**305**	**288**	**347**	**300**
N intake and excretion				
N intake (g/d)	63.1^{a}	64.1^{a}	82.6^{b}	60.4^{a}
Fecal N excretion (g/d)	11.2^{a}	16.5^{b}	18.1^{b}	15.4^{b}
Urinary N excretion (g/d)	18.3^{ab}	13.6^{b}	22.1^{a}	12.9^{b}
Total N excretion (g/d)	30.3^{a}	29.8^{a}	41.2^{b}	29.6^{a}
Ammonia emission (mg/h)	**40**	**50**	**60**	**15**

DE, NE: digestible and net energy. Ammonia emission (mg emitted in respiratory chambers, per hour)

7.3.3. Discussion

There are various advantages to reduce N excretion or ammonia emission from pig barns. The atmosphere in the barn will be healthier, animal production will less contribute to acid rains and pollution, N will be used more efficiently, etc. Different techniques have been suggested to reduce ammonia emission from manure: acidifiers for slurry, injection of slurry in the soil, etc.

However, it is possible to substantially reduce ammonia emission by improving feed formulation. Excessive ammonia emission comes mainly from protein excess in the diets. Thus, a reduction in crude protein content in the diet will cause a reduction in N excretion and ammonia emission. Such a reduction is possible through the use of highly digestible protein sources, an optimal combination of protein sources having complementary amino acid profiles and the use of synthetic amino acids.

The first part of the study aimed at evaluating the impact of a reduction in protein content on the production costs of balanced diets commonly used in swine nutrition. No definitive conclusion can be drawn but, in general, a decrease in protein will lead to an increase of the feed costs due to the necessity to rely on highly-digestible feed ingredients and on expensive synthetic amino acids. However, the consequences on N excretion are significant.

Once the reduction in protein content has been achieved, it is possible to consider the addition of some sources of fermentable DF in the diet, in order to shift N excretion from urine to feces and block N within the bacterial proteins present in feces. The second part of the study aimed at evaluating that possibility. Three sources of fermentable DF currently found on the US feed market were considered: soy hulls, sugarbeet pulp and citrus pulp.

The efficiency of these three sources to increase the intestinal carbohydrate fermentations and reduce urinary N excretion has been confirmed, based on the scientific literature.

In Europe, sugarbeet has been used for years in swine nutrition, namely for that purpose. However, its interest is limited in North America, due to its high price. Also, it brings significant amounts of indigestible protein, which leads to increased N excretion.

Soy hulls are cheaper and it is thus worth considering their use. However, their energy value is quite limited, which makes it more difficult for feed producers to formulate balanced diets that will not negatively affect pig's growth. The presence of soy hulls in a diet will lower the energy density. Moreover, the inclusion rate should be limited to 10% and, at that level, the effect on ammonia emission might be limited (Hansen et al, 2007 and Table 22).

The third option might be the best one. Citrus pulp is cheap, has a high energy density (as compared to other DF sources, thanks to its high content in sucrose) and its effect on ammonia emission has been demonstrated (Hansen et al, 2007). Our analysis reveals that its inclusion in balanced diets will not negatively affect quality and will not raise the costs of production. Thus, citrus pulp might be another option.

However, the relevance of using sources of fermentable DF with the objective of reducing ammonia emission or N excretion will depend on specific situations in pig barns. Unlike in Europe, there is no incentive for the moment in North America, to encourage pork producers to use diets that reduce N excretion or ammonia emission. Moreover, due to the development of the ethanol and biodiesel industries, feed producers fear that there will be a shortage in starch or oil sources and that plenty of feed ingredients with high protein content but poor quality (or digestibility) will be available on the market, which will substantially increase the protein content of the diets and thus N excretion and ammonia emission. It is the case of corn DDGS and canola meal. The addition of sources of fermentable DF won't solve the problem since 1) the level of indigestible N will be very high and too high to be corrected with fermentable DF sources and 2) these feed ingredients are also very high in DF and have a low energy density.

It can thus be concluded that, in the current situation, the use of fermentable DF is difficult to consider. However, should a feed producer decide to develop feeds that generate low levels of ammonia emission or N excretion, citrus pulp would be the best option both from nutritional and economic viewpoints.

8. General discussion

The overall objective of the present project was to evaluate alternative feedstuffs for their potential impact on the character of manure excreted, on nitrogen excretion and the formation of odor-causing compounds and on pig gut health. The economic interest of using fibrous feedstuffs for the reduction of N excretion or odor emission was also evaluated

Two experiments were conducted. The first one aimed at studying the impact of cereal NSP fermentation on gut health and the second at studying the effect of feedstuffs differing in their dietary fiber and nitrogen content on the excretion of nitrogen and ammonia emission.

The results of the first study show that both form (purified supplement versus grain matrix) and quantity of barley ß-glucans are important factors affecting changes in gut microbial composition in the pig, which may help to develop future feeding strategies and reduce the utilization of antibiotics used as growth-promoters in feeds for pigs. It is also recognized that improved gut health usually leads to improved growth performances.

The high diversity in carbohydrate composition of hulled and hulless barleys suggest that it is possible to take advantage of that variability to formulate diets that enhance intestinal health in pigs. The fact that hulless barley-based diets reduced drastically the presence of potentially pathogenic bacteria in the pig's small intestine is encouraging and shows that it makes sense to develop feeding strategies based on the functional properties of feedstuffs.

The results of the second study show that the source of DF in the diet can significantly affect N excretion, due to an increase in bacterial fiber fermentation in the large intestine, which means a larger bacterial biomass in the feces, with more fecal nitrogen excreted and less urinary nitrogen excreted in the environment. However, our data show that fibrous feedstuffs often have high crude protein contents and the latter are usually not well digested by the pig. Fibrous feed ingredients may thus bring more indigestible nitrogen to the diet, which will eliminate the advantage gained by the presence of fermentable DF. The choice of the feedstuff for reduced nitrogen excretion is thus of major importance. DF will also contribute to a decrease in ammonia emission, a malodorous compound. However, the results observed here on fecal 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. On the other hand, we analyzed the presence of another malodorous compound, butyric acid, and did not find major changes due to DF for that fatty acid. The advantage of fermentable DF on N excretion and odour will thus depend on the overall composition of the feed ingredients, namely their content in indigestible protein.

The economic impact of using sources of fermentable DF to reduce ammonia emission was also evaluated and did not demonstrate a direct advantage in the current conditions, with the possible exception of the use of citrus pulp, thanks to its high energy content, its low price and its capacity to reduce ammonia emissions. It seems that a reduction in total crude protein content in the diet would have more positive effects and be less expensive than the use of DF sources. However, the feed industry must face a competition with the ethanol industry for the use of starch sources such as wheat and corn. As a consequence, by-products with high levels of poorly-digested proteins are incorporated in rations for swine and their use causes higher losses in N and increased ammonia emission. It is unlikely that a combination between these protein sources and sources of fermentable DF will be efficient.

9. Publications and presentations in congresses

Peer-reviewed journals

Pieper R., Jha R., Rossnagel B., Van Kessel A., Souffrant W., Leterme P. (2008) Denaturing gradient gel electrophoresis analysis of the effect of barley and oat ß-glucans on the intestinal microbial community composition in weaned piglets. *FEMS Microbial Ecology* **66**, 556-566

Jha R., Pieper R., Rossnagel B., Van Kessel A., Leterme P. (2009) Barley and oat varieties with different carbohydrate compositions alter the ileal and total tract nutrient digestibility and fermentation metabolites in weaned pigs. Animal (will be submitted by the end of 2008)

Congresses

Jha R., Rossnagel B., Pieper R., Van Kessel A., Leterme P. (2009) In vitro evaluation of the fermentation characteristics in the pig intestines of hulless barleys differing in beta-glucan content. ASAS/ASDA Midwestern Conference, 18-20 March. *(accepted)*

Jha R., Rossnagel B., Pieper R., Van Kessel A., Leterme P. (2008) Digestibility and fermentation parameters of barleys and oats differing in β-glucan content in the pig intestines. ASAS/ASDA Midwestern Conference, 17-19 March. *J. Anim. Sci.* **86** (2), Suppl., abstract # 96

Pieper R., Jha R., Leterme P., Rossnagel B., Souffrant W., Van Kessel A. (2008) Effect of barley and oat β-glucans on intestinal microbiota in the pig. ASAS/ASDA Midwestern Conference, 17-19 March. *J. Anim. Sci.* **86** (2), Suppl., abstract # 217

Jha R., Pieper R., Rossnagel B., Van Kessel A., Leterme P. (2008) Effect of oats and hulless barleys differing in ß-glucan content on intestinal fermentation parameters in weaned pigs. Proc. Banff Pork Seminar, Jan. 17-19, Abstract #13

Pieper R., Jha R., Leterme P., Rossnagel B., Souffrant W., Van Kessel A. (2008) Effect of barleys differing in β-glucan content on intestinal ecology of weaned pigs. Proc. Banff Pork Seminar, Jan. 17-19, Abstract #12

10. References

Bindelle J., Buldgen A., Wavreille J., Destain J.P., Leterme P. (2008) Influence of source and levels of dietary fiber on *in vivo* nitrogen excretion pathways in pigs and *in vitro* fermentation and protein synthesis by fecal bacteria *Journal* of *Animal Science* (accepted)

Bindelle J., Leterme P. et al (2007a) Effect of pig fecal donor and of pig diet composition on in vitro fermentation of sugarbeet pulp. *Anim. Feed Sci. Technol.* **132**, 212-226

Bindelle J., Leterme P. et al (2007b) The gas production technique in syringes to study fiber fermentation in the large intestine of the pig. *Anim. Feed Sci. Technol.* **132**, 111-122

Bouhnik et al (2004) Capacity of nondigestible carbohydrates to stimulate bifidobacteria in healthy humans. *Am. J. Clin. Nutr*. **80**, 1658

Brown et al (1997) Fecal numbers of Bifidobacteria are higher in pigs fed Bifidobacterium longum with a high amylose cornstarch than with a low amylose cornstarch. *J. Nutr*. **127**, 1822

Dumonceaux T et al. (2006) Enumeration of specific bacterial populations in complex intestinal communities using quantitative PCR based on the *chaperonin*-60 target. *J. Microbiol. Methods* **64**, 46-62

Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119: 1497-1509.

Janczyk P. et al. (2007) Changes in the diversity of pig ileal lactobacilli around weaning determined by means of 16S rRNA gene amplication and denaturing gradient gel electrophoresis. *FEMS Microbiol Ecol* **61**, 132–140

Nahm, KH (2003) Influences of fermentable carbohydrates on shifting nitrogen excretion and reducing ammonia emission of pigs. *Crit Rev Environm Sci Technol* **33**, 165-186

Zervas & Zelstra (2002) Effect of dietary protein and fermentable fiber on N excretion patterns in grower pigs. *J. Anim. Sci.* 80, 3247