RESEARCH ARTICLE

Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets

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Abstract

This experiment was aimed at comparing the intestinal microbial community composition in pigs fed hulled common barley supplemented with isolated barley mixed-linked β-glucan, four hulless barley varieties and breeding lines with mixed-linked β-glucan contents ranging from 41 to 84 g kg^{-1} and different amylose/amylopectin ratios as well as two oat varieties. 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. After 15 days, pigs were sacrificed and ileum and colon contents were collected for quantitative real-time PCR (qPCR) and denaturing gradient gel electrophoresis to evaluate microbial communities. Shifts in intestinal microbial communities were observed with the hulless barley cultivars with a normal to high β-glucan content and from normal starch toward either high-amylopectin or high-amylose starch. These hulless barleys had the lowest ($P < 0.05$) microbial diversity, whereas oats had intermediate diversity compared with low-β-glucan hulless cultivars and hulled varieties. Furthermore, hulless varieties favoured xylan- and β-glucan-degrading bacteria whereas mixed-linked β-glucan-supplemented hulled barley favoured lactobacilli. Numbers of lactobacilli decreased in the ileum of pigs fed hulless/high mixed-linked β-glucan barley-based diets. Thus, cultivar differences in both the form and the quantity of carbohydrates affect gut microbiota in pigs, which provides information for future feeding strategies.

Introduction

The gastrointestinal tract (GIT) of pigs is colonized with a highly diverse microbiota, comprising > 400 different phylotypes (Hill *et al.*, 2002; Leser *et al.*, 2002), having a considerable impact on GIT development, physiology and immunology (Chowdhury *et al.*, 2007; Danielsen *et al.*, 2007; Willing & Van Kessel, 2007). Furthermore, they provide the host organism with nutrients from fermentation processes, stimulate the gut mucosal immune system and protect the intestine from pathogen overgrowth, adhesion to gut mucosa and lumen colonization (Berg, 1996; Isolauri *et al.*, 2001). There has been growing interest in the inclusion of dietary fibre in the diet of weaning pigs due to its potential prebiotic effects and promotion of beneficial bacteria such as lactobacilli (Verstegen & Williams, 2002). Carbohydrates are the predominant fraction of cereals such as barley and oat. Because cereals are the major component in pig diets and given the fact that enzymatically indigestible carbohydrates are the main substrate for bacterial fermentation (Bach Knudsen & Hansen, 1991), there might be opportunities for manipulating the composition of the GIT microbiota by selecting cereal grains based on the type and amount of dietary fibre. However, the β-glucan content and starch amylose/amylopectin ratio of barley and oat can differ markedly between single varieties. For example, the

β-glucan content ranges from *c.* 1% in wheat to 3–7% in oat and 5–11% in barley (Brennan & Cleary, 2005). Environmental and, especially, genetic factors have an impact on the β-glucan content (Fox *et al.*, 2007; Gamlath *et al.*, 2008). Although dietary β-glucans attenuate the postprandial glycaemic and insulinaemic response in humans (Wood, 2007), little is known about their effects on the intestinal fermentation processes and microbiota in general. In farm animals, there have traditionally been concerns about the β-glucan content of cereals and its negative effect on digestibility and weight gain. Enzymes (i.e. β-glucanases and xylanases) have been used in pig diets to decrease viscosity and enhance the digestibility of nutrients. However, it has been shown that barley β-glucans in the diet of weaning piglets can promote lactobacilli that are capable of degrading β-glucans, thus suggesting prebiotic properties (Jonsson & Hemmingsson, 1991).

The aim of the present experiment was to investigate the effect of a hulled barley, either alone or supplemented with an isolated barley mixed-linked β-glucan concentrate, and four hulless barley cultivars with increasing mixed-linked β-glucan content (41–84 g kg^{-1}) and different starch compositions on the composition of the small and large intestinal microbial community of weaned piglets by means of PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR) of the 16S rRNA gene. Two oat varieties with 29 and 40 g kg^{-1} mixed-linked β-glucan content, respectively, were also included in the study for comparison.

Materials and methods

Animals and diets

Seventy-two (Commercial breed-PIC Camborough®Plus; PIC Canada Ltd) piglets, weaned at 21 days of age, were used in this study. After weaning, they were kept for 2 weeks in group pens to allow adaptation of the gastrointestinal microbiota to the newly introduced weaning diet in order to prevent weaning bias in the feeding trial (Janczyk *et al.*, 2007; Pieper *et al.*, 2008). No antibiotics, either for prophylactic or for therapeutic purposes, were administered to the animals. Standard commercial rearing conditions (photoperiod, humidity and temperature) were used at Prairie Swine Centre Inc. (Saskatoon, SK, Canada) facilities. Piglets with a mean body weight of 12.8 ± 1.9 kg were then allocated randomly into nine feeding groups with eight piglets each. They were housed in individual cages (1.2×0.6 m). Common hulled barley, four hulless barley varieties and breeding lines and two oat varieties CDC Baler and CDC Sol-Fi (high β-glucan) were used. Two diets were also formulated with 8.2% or 16.3% (w/w) of a commercial barley mixed-linked β-glucan (BBG) concentrate (Parrheim Foods, Saskatoon, SK, Canada), containing 23.5% barley mixed-linked β-glucan, added at the expense of common barley. The experimental diets (Tables 1 and 2) were fed for 15 days according to the metabolic body weight (110 g feed kg$^{-0.75}$ BW). The nutritional composition of the diets was analysed as follows: dry matter (AOAC 930.15), nitrogen (AOAC 968.06 using an elemental analyser LECO FP528, St Joseph, MI), ether extract (AOAC 920.39 using Soxhlet apparatus and petroleum ether), ash (AOAC 942.05), starch and mixed-linked β-glucans (Megazyme International, Ireland) and gross energy (PARR 1281 calorimeter, Moline, IL). The feed was offered in mashed form twice daily (8:00 and 16:00 hours), and animals had *ad libitum* access to water. On the last experimental day, all animals received the last meal 4 h before killing in order to provide approximately equal amounts of bacterial fermentative nutrients in the small intestine.

All procedures involving animal handling and treatment were approved by the Committee of Animal Care and

Table 1. Chemical composition (g kg^{-1} DM) of the barley and oat varieties used in this study

Diet #	Cereal	Dry matter	Ash	Crude protein	Ether extract	Starch	β-glucan
1	Common barley	879	24	98	22	624	34
4	Low β-glucan hulless barley breeding line SB 90300	888	17	132	24	647	41
5	Hulless barley CDC McGwire	879	18	173	25	601	56
6	High amylose starch hulless barley breeding line SB 94893	889	20	151	28	532	73
7	Waxy hulless barley CDC Fibar	892	19	213	34	534	84
8	High β-glucan oat CDC Sol-Fi	886	40	197	30	295	40
9	Oat CDC Baler	899	32	165	40	458	29

Table 2. Composition (g kg^{-1} DM) of the experimental diets

Ingredient	Diets 1, 4–9	Diet 2	Diet 3
Cereal	815	734	652
Soya protein*	90	90	90
Whey†	60	60	60
Barley β-glucan‡		82	163
Premixes			
PSC minerals§	5	5	5
PSC vitamins¶	5	5	5
Salt	5	5	5
Dicalcium phosphate	10	10	10
Limestone	5	5	5
Celite®‖	5	5	5
	100	100	100

*SoyComil®K (CP, 65%; moisture, 7%) (ADM speciality ingredients, Koog aan de Zaan, the Netherlands).

†Crino whey powder (CP, 9%; lactose, 80%; moisture, 8%; ash, 12%) (Agropur Co. Granby, QC, Canada).

‡Isolated barley β-glucan concentrate (Parrheim Foods, Saskatoon, SK, Canada), containing 23.3% mixed-linked β-glucan, 17.3% protein, 35.0% starch, 2.6% fat and 2.0% ash.

§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 sodium selenite.

¶Provided (per kg of diet): vitamin A, 8250 IU; vitamin D_3, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; vitamin B_{12}, 25 μg.

‖Celite 545, Celite Corporation, Lompoc, CA.

Supply of the University of Saskatchewan and were performed in accordance with the recommendations of the Canadian Council on Animal Care (1993).

Slaughtering and sampling

Animals were sacrificed by stunning using a captive bolt, followed by immediate exsanguination. The abdomen was opened and the entire GIT was removed. The small and large intestines were separated and digesta samples were taken from the last quarter of the small intestine (defined as the ileum) and from 20 cm of the medial colon. Approximately 2 g of mixed digesta was subsampled and immediately stored at −20 °C until extraction of genomic DNA (within 12 h).

DNA isolation

DNA was isolated from digesta using a series of chemical, physical and enzymatic treatments, as described previously (Hill *et al*., 2005), with slight modifications. Briefly, 0.3 g of digesta was placed in a bead-beating tube (Mo-Bio Laboratories, Solano Beach, Canada). Cells were lysed by incubation at 37 °C for 30 min in the presence of RNAse A (75 μg), lysozyme (750 μg) and proteinase K (400 μg) in 365 μL of lysing buffer A (containing per litre: 50 mM Tris-HCl, 50 mM EDTA with 0.5% Tween 20% and 0.5% Triton X-100, 18.61 g Na_2EDTA and 6.06 g Tris base). After addition of 135 μL of lysing buffer B (containing per litre: 3 M guanidine-HCl and 20% Tween 20), the mixture was incubated for another 30 min at 50 °C and subsequently frozen at −70 °C for 20 min. After thawing, 700 μL of phenol/chloroform/isoamyl (25 : 24 : 1) was added to each tube, and samples were then processed three times in a FastPrep Instrument (Bio101 ThermoSavant FP120) at 5 m s^{-1} for 20 s to release and purify the genomic DNA. After centrifugation at 14 000 ***g*** for 15 min, the supernatant was transferred to a new tube and 70 μL of 3 M sodium acetate and 700 μL isopropanol were added and the precipitated DNA was pelleted for 15 min at 14 000 ***g***. The pellet was washed with 70% ethanol, air dried and finally redissolved in 100 μL MilliQ water. The DNA concentration was measured using a picoGreen assay (Molecular Probes, Eugene, OR).

Table 3. List of oligonucleotide probes used in this study

Name	Sequence 5′-3′	Reference
S-D-Bact-0011-a-A-17	AGA GTT TGA T(C/T)(A/C) TGG CTC AG	Leser *et al*. (2002)
S-D-Bact-1492-a-A-19	GGT TAC CTT GTT ACG ACT T	Leser *et al*. (2002)
S-D-Bact-0968-a-S-GC	CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC	Nübel *et al*. (1996)
S-D-Bact-1401-a-A-17	CGG TGT GTA CAA GAC CC	Nübel *et al*. (1996)
S-D-Bact-0008-a-S-20	AGA GTT TGA TCC TGG CTC AG	
S-G-Lab-0677-a-A-17	CAC CGC TAC ACA TGG AG	Heilig *et al*. (2002)
S-G-Strc-0493-a-A-19	GTT AGC CGT CCC TTT CTG G	Franks *et al*. (1998)
S-G-Enter-1419-a-A-23	CCT ACT TCTTTT GCA ACC CAC TC	Castillo *et al*. (2006)
S-D-Bact-1055-a-A-19	ATG GCT GTC GTC AGC TCG T	Castillo *et al*. (2006)

PCR and DGGE

The V6–V8 variable regions of the bacterial 16S rRNA gene from ileal and colon contents were amplified by a nested PCR approach using forward primer S-D-Bact-0011-a-A-17 and reverse primer S-D-Bact-1492-a-A-19 (Table 3), followed by a second amplification using forward primer S-D-Bact-0968-a-S-GC and reverse primer S-D-Bact-1401-a-A-17 (Table 3). PCR reactions contained 5 μL of 10 × incubation buffer, 3 μL of 50 mM $MgCl_2$, 1 μL of each of the four primers (10 μM), 1 μL of each dNTP (10 mM) and 0.25 μL of *Taq*-Polymerase (5 U μL^{-1}; '*Taq*-CORE Kit 10',

MPBiomedicals, Eschwege, Germany), and UV-sterilized millipore water was added till 49 μL according to Konstantinov *et al.* (2004). One microlitre of template (adjusted to 1 ng μL^{-1} DNA) was added to each reaction mix. Both PCR amplifications were accomplished as follows: 5 min at 94 °C, 35 cycles of 94 °C for 30 s, 56 °C for 20 s and 68 °C for 40 s and a final extension at 68 °C for 7 min. The size and yield of PCR products were checked by electrophoresis in 1.5% agarose gel after staining with ethidium bromide (0.4 μg EtBr mL^{-1} agarose).

DGGE was performed for separation of PCR products. Eighteen microlitres of each PCR product was loaded on a polyacrylamide gel with a vertical gradient of denaturants of 35–55% (42.16% urea and 40% formamide in 100% denaturant). The electrophoresis was conducted at 85 V for 16 h in 0.5 × TAE buffer (containing per litre: 242 g Tris, 57.1 mL glacial acetic acid and 100 mL 0.5 M EDTA) and a constant temperature of 60 °C in a DCode Universal Mutation Detection System (BioRad, Munich, Germany). DGGE gels were stained with SYBR® Gold Nucleic Acid Gel Stain (Molecular Probes) in 0.5 × TAE buffer (pH 7.5) for 10 min at room temperature, subsequently exposed to UV light (AlphaDigiDoc® RT, Alpha Innotech Corporation, San Leandro, CA) for 2 s and photographed with a digital camera SP-500 UZ (Olympus, Hamburg, Germany) using the ALPHAEASEFC™ 4.0 software (Alpha Innotech Corporation).

Identification of dominant bands

Dominant bands or bands that were characteristic for the majority of the animals in one group were picked from DGGE gels with a sterile needle and reamplified by PCR using the primers S-D-Bact-0968-a-S-18 (without GC clamp) and S-D-Bact-1401-a-A-17 as described above. The PCR products were purified with the Qiagen® PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturers' protocol and sequenced using the DYEnamic™ ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway), followed by automatic sequencing on a MegaBACE™ Sequencing System (Amersham Biosciences). The sequences obtained were compared with public entries at the NCBI website (Altschul *et al.*, 1997) and deposited under GenBank accession numbers EU381235–EU381274.

qPCR

For quantification of 16S rRNA gene copy numbers of some bacterial groups in the DNA isolates of SI and colon contents, previously published group-specific primers (Table 3) were used. The reverse primers S-G-Lab-0677-a-A-17 (lactobacilli) and S-G-Strc-0493-a-A-19 (streptococci) were used in conjunction with forward primer S-D-Bact-0008-a-S-20 yielding PCR products of *c.* 670 and 485 bp. Primers S-D-Bact-1055-a-A-19 and S-G-Enter-1419-a-A-23 were used for amplification of a 370-bp fragment of the enterobacterial 16S rRNA gene. Amplification was accomplished using Platinum SYBR green qPCR SuperMix-UDG (Invitrogen) in the presence of 3 mM $MgCl_2$ and 500 nM of each primer as described by Dumonceaux *et al.* (2006). One microlitre of diluted genomic DNA was used as a template. Amplification conditions for lactobacilli were 50 °C for 2 min (uracil DNA glycosylase activation) and then 95 °C for 5 min (well factor collection), followed by 40 cycles for 95 °C for 30 s, 56 °C for 40 s and 72 °C for 40 s. Identical conditions were used for streptococci and enterobacteria, except that the annealing temperature was 60 °C. The amplifications were performed on an iCycler iQ Real-Time PCR detection system (BioRad), with the data collection set at the annealing/extension step. Standard curves were generated using serial dilutions of the purified and quantified (PicoGreen, Molecular Probes) PCR product generated by standard PCR using appropriate primers and genomic DNA template extracted from pig intestinal contents (Dumonceaux *et al.*, 2006). The detection limit was 10^2 copy numbers g^{-1} digesta. Melting curves were checked after amplification in order to assure correct amplification results. Results are reported as 16S rRNA gene copy number g^{-1} digesta.

Statistical analysis

The DGGE images were analysed using BIONUMERICS 5.0 (Applied Maths, St-Martens-Latem, Belgium). Based on the densitometric curves of each lane, the patterns were normalized, adjusting the distance between bands where marker lanes were used as reference lanes, and using automatic band detection with manual correction. Bands with an area of $\geq 1\%$ of the total area were retained for subsequent analysis. Similarity between lanes (samples) was detected using the Dice correlation coefficient based on the presence/absence of bands. The diversity of bands in individual lanes (single animal/lane) was calculated applying the Shannon index (H′) (Janczyk *et al.*, 2007).

The dietary effects on the number of bands, Shannon index and Dice similarity values calculated from DGGE fingerprints were determined by one-way ANOVA, followed by the least significant difference test using SPSS (SPSS Inc., Chicago). Analysis of the mean differences between bacterial 16S rRNA gene copy numbers was similar, except that the Spjotvoll–Stoline test (Spjotvoll & Stoline, 1973) was used as a nonparametric *post hoc* test because some microbial groups could not be detected in all ileum samples. Generally, differences at $P < 0.05$ were considered significant.

Results

Throughout the study, all animals were in good condition and no incidence of diarrhoea or other disease symptoms

were observed. There were no significant differences in daily weight gain and body weight at the end of the study.

The mixed-linked β-glucan content of the barley varieties used in the present study ranged from 34 to 84 g kg^{-1} and from 29 to 40 g kg^{-1} in the oat. The hulled barley, hulless barley breeding line SB 90300 and hulless variety CDC McGwire and the two oat varieties used in this study had a normal starch composition with a 75% amylopectin/25% amylose (approximate values) ratio, whereas CDC Fibar was a hulless 'waxy' starch barley with 100% amylopectin and the hulless breeding line SB94893 had a 60% amylopectin/40% amylose ratio. The addition of the BBG concentrate increased the mixed-linked β-glucan content in diets 2 and 3 from 24 to 40 and 53 g kg^{-1}, respectively.

To study the effect of varietal differences in the carbohydrate composition of hulled and hulless barleys and oat on the gastrointestinal microbiota in piglets, PCR-DGGE fingerprinting of the V6-V8 regions of the 16S rRNA genes was used. The DGGE profiles of ileum (not shown) and colon (Fig. 1) 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 a lower number of bands and Shannon diversity in the ileal contents of animals fed common barley supplemented with the lowest level (8.2%) of BBG concentrate, hulless barley SB 94893 and CDC Baler oat, whereas higher values were found with CDC McGwire hulless barley as compared with the other cereals (Table 4). In the colon, there was a decrease in the number of bands and Shannon diversity with addition of BBG to common barley. Further, significant ($P < 0.05$) decreases were observed in hulless barleys with increasing mixed-linked β-glucan content as compared with nonsupplemented common barley diets. The lowest values for the band number and the Shannon diversity index occurred for hulless barley with the highest β-glucan content and waxy starch (CDC Fibar). The oat varieties had intermediate values. No difference was observed for the intragroup Dice similarity index for ileum samples, except for the high-β-glucan CDC Sol-Fi oat variety, 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 intragroup similarity was observed from common barley to hulless varieties and breeding lines, and depending on the level of β-glucans and starch composition. Diets with common barley had the highest values, and diets containing either 8.2% or 16.3% of the BBG concentrate had similar values as the nonsupplemented common barley, irrespective of the β-glucan content. The oats again showed intermediate values.

To illustrate the differences between treatment groups, the clustering of all colon samples, based on the inter- and intragroup Dice coefficient of similarity, is shown in Fig. 2. Profiles of piglets fed the common barley and the diet with

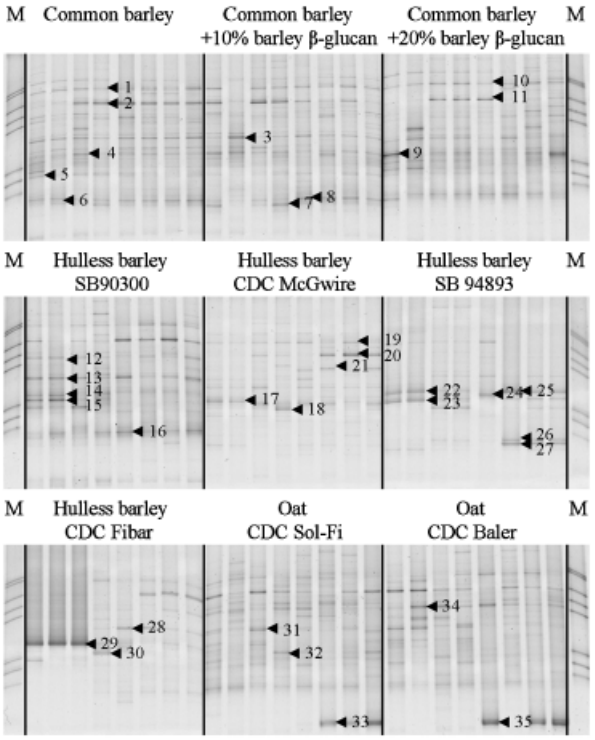

Fig. 1. DGGE evaluation of colon microbiota of piglets after feeding common barley; two diets with common barley, supplemented with 82 or 163 g kg^{-1} isolated barley mixed-linked β-glucan; two oat varieties (CDC Sol-Fi, CDC Baler) and hulless barley varieties (SB 90300, CDC McWire, SB 94893, CDC Fibar) with increasing mixed-linked 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 (eight animals, respectively). M, marker lane. The arrows and numbers indicate excised and reamplified bands for species identification (Table 5).

8.2% BBG concentrate formed one cluster, whereas the profiles of the 16.3% BBG diet formed two clusters with SB 90300 hulless barley (low β-glucan content and normal starch) and four oat profiles. The profiles from the other oat-fed animals formed three smaller clusters. Interestingly, all the samples from animals fed the CDC McGwire hulless barley formed one separate cluster, although the β-glucan content and starch composition were similar to the hulless breeding line SB 90300. Most profiles from colon samples of animals fed the hulless breeding line SB 94893 and the hulless CDC Fibar barley (high β-glucan content) were more similar to each other than to the other treatment groups but generally showed the lowest similarity and formed only small clusters.

The reamplification and identification of bands from colon samples, which were characteristic for the majority of the profiles within each diet, revealed a predominance of *Clostridium glycolicum*, *Mogibacterium diversum*, *Butyrivibrio fibrisolvens*, *Syntrophococcus sucromutans*, *Weissella*

Table 4. Mean ± SD number of bands, Shannon diversity and intragroup Dice similarity values calculated from DGGE fingerprints of ileum and colon microbial communities

	1	2	3	4	5	6	7	8	9
Diet #	HB	HB+BBG	HB+BBG	hB	hB	hB	hB	oat	oat
β-glucan ($g\,kg^{-1}$ DM)	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 ± 0.07^{ab}	0.69 ± 0.15^{b}	0.72 ± 0.10^{ab}	0.73 ± 0.11^{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 ± 24.6^{bc}	42.8 ± 14.2^{bc}	34.9 ± 17.3^{c}	43.0 ± 23.8^{bc}	53.4 ± 20.6^{b}	38.8 ± 22.1^{bc}	47.8 ± 12.0^{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}

[a,b,c]Significant ($P < 0.05$) differences within rows.

HB, common (hulled) barley; hB, hulless barley; BBG, barley mixed-linked β-glucan concentrate.

confusae and *Lactobacillus sobrius*-like phylotypes in animals fed the common barley, common barley supplemented with BBG concentrate and the hulless barley SB 90300 (Fig. 1, Table 5). Some of these phylotypes such as *L. sobrius*, *B. fibrisolvens* were also detected with the other diets, whereas others such as *Escherichia coli*, *C. 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 and 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 5).

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

Furthermore, qPCR was used to obtain information about 16S rRNA gene copy numbers in the genomic DNA from ileum and colon samples. Results show lower numbers of lactobacilli ($P < 0.05$) in the ileum of animals fed the SB 94893 and CDC Fibar varieties, as compared with the oat diet (Table 6). The addition of either 8.2% or 16.3% BBG concentrate increased the number of ileal lactobacilli slightly, but not significantly. The abundance of these microbial groups was not different in the colon samples. Interestingly, no enterobacteria or streptococci could be detected in the ileum of animals fed the high mixed-linked β-glucan diets compared with occasional detection with very low abundance in the other diets.

Discussion

Cereal grains such as barley and oat are interesting sources of potential prebiotic carbohydrates, especially mixed-linked β-glucan and resistant starch (Topping, 2007). Besides the fact that these carbohydrate fractions are coming more to the attention of human nutritionists due to their functional properties, they have been neglected up to now in prebiotic research for farm animals. In the present study, we therefore used a PCR-DGGE approach to examine the potential prebiotic effect of barley and oat β-glucans in weaned piglets either as a 'purified' supplement or as it occurs naturally in the grain in combination with other compounds. Here, we showed that barley and oat varieties, differing in their carbohydrate composition, namely mixed-linked β-glucan content and starch structure, affect the gastrointestinal microbial community composition significantly, as evidenced from analysis of DGGE banding patterns of both ileal and colonic microbial communities. DGGE banding pattern responses were most pronounced in the colon of pigs fed the hulless varieties with a high β-glucan content compared with common barley as a control diet. At this site of the GIT and within the hulless barley varieties, microbial diversity appeared to decline with increasing β-glucan content, a trend also observed, to a lesser extent, when common barley diets were supplemented with a mixed-

linked β-glucan concentrate. Only a few significant differences were observed in ileal microbial profiles. Because the high β-glucan-containing hulless barley varieties had either a high amylose (diet 6) or amylopectin (diet 7) content, these carbohydrates could also have contributed to the diversity response. On the other hand, as shown by Dice coefficient-based cluster analysis, the microbial profiles for these varieties formed a common separate cluster supporting a β-glucan-mediated, rather than starch-mediated, response.

β-glucans and arabinoxylans are the predominant components of cell walls of barleys and oats, respectively (Bach Knudsen, 2001). Soluble nonstarch polysaccharides such as mixed-linked β-glucans can increase the digesta retention time in the GIT and endogenous nitrogen excretion (Leterme *et al.*, 2000; Charalampopoulos *et al.*, 2002), thereby affecting the digestibility of other nutrients such as protein and starch and increasing their flow to the large intestine. Increased viscosity in turn could positively affect the growth of bacteria in the upper GIT. However, we observed significantly lower numbers of lactobacilli and no streptococci and enterobacteria in the small intestine of piglets fed the high mixed-linked β-glucan diets. The reasons

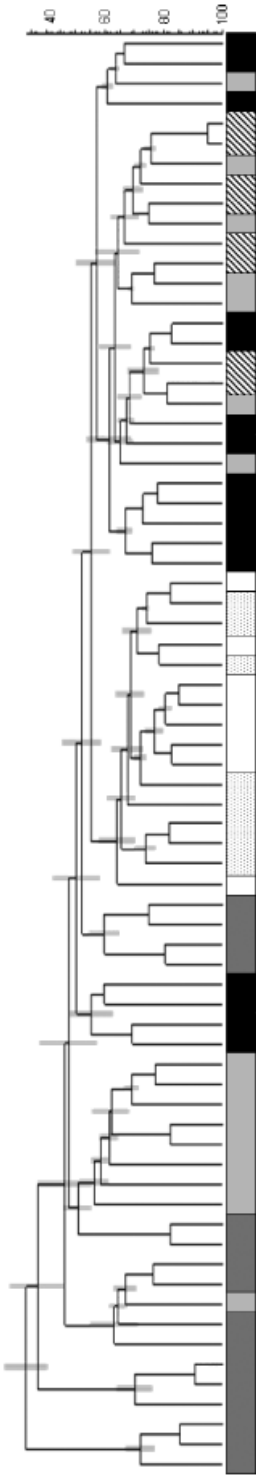

Table 5. Affiliation of partial 16S rRNA (V6–V8 region) gene sequences obtained from excised bands of DGGE fingerprints with their closest relatives in GenBank

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 confusae*	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	*Syntrophocoocus 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

*No sequence from cultivated bacterial species available in GenBank.

Fig. 2. Cluster analysis of DGGE banding patterns of universal microbial profiles in the colon of piglets fed diets containing common barley □, two diets with common barley, supplemented with 82 or 163 g kg^{-1} isolated barley mixed-linked β-glucan (⁝⁝, ▨); two oat varieties (CDC Sol-Fi, CDC Baler, ■) and hulless barley varieties with low/normal (SB 90300, CDC McWire, ▭) and high (SB 94893, CDC Fibar, ▬) mixed-linked β-glucan content. The cluster was generated using the unweighted pair group method with an averaging algorithm based on the Dice coefficient of similarity. The scale bar indicates the percentage of similarity. Error flags are shown on each node.

Table 6. Mean ± SD copy numbers (log copies g^{-1} digesta) of 16S rRNA gene of microbial groups in ileum and colon genomic DNA

	1	2	3	4	5	6	7	8	9
Diet #	HB	HB+BBG	HB+BBG	hB	hB	hB	hB	Oat	Oat
β-Glucan ($g\,kg^{-1}$ DM)	24	40	53	30	42	65	84	32	23
Lactobacilli									
Ileum	5.3 ± 1.2^{ab}	6.1 ± 0.7^{ab}	6.0 ± 0.7^{ab}	5.9 ± 0.7^{ab}	5.3 ± 1.2^{ab}	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^{a}	7.3 ± 0.5^{a}	7.1 ± 0.5^{a}	7.1 ± 0.5^{a}	6.9 ± 0.4^{a}	7.0 ± 0.4^{a}	6.9 ± 0.5^{a}	7.4 ± 0.4^{a}	7.1 ± 0.5
Streptococci									
Ileum	2.9 ± 1.2 (4)^{a}	2.2 ± 1.1 (3)^{a}	2.4 ± 0.5 (4)^{a}	2.4 (1)^{a}	2.5 ± 0.8 (4)^{a}	ND	ND	2.2 ± 0.7^{a}	2.5 ± 0.8 (6)^{a}
Colon	8.0 ± 0.6^{a}	7.8 ± 0.6^{a}	7.8 ± 0.4^{a}	7.7 ± 0.5^{a}	7.7 ± 0.2^{a}	7.6 ± 0.3^{a}	7.6 ± 0.5^{a}	7.9 ± 0.2^{a}	7.8 ± 0.3^{a}
Enterobacteria									
Ileum	3.6 ± 1.3 (4)^{a}	2.4 ± 0.5 (3)^{a}	2.8 ± 1.1 (4)^{a}	2.6 (1)^{a}	3.0 ± 1.1 (4)^{a}	ND	ND	4.3 ± 0.5^{a}	3.8 ± 1.4 (6)^{a}
Colon	2.7 ± 1.1^{a}	2.7 ± 0.6^{a}	2.7 ± 0.9^{a}	2.5 ± 0.4^{a}	2.6 ± 0.8^{a}	3.3 ± 1.0^{a}	2.7 ± 0.8^{a}	3.6 ± 1.1^{a}	2.9 ± 1.4^{a}

Numbers in parentheses indicate the number of colonized piglets.

[a,b,c]Significant ($P < 0.05$) differences.

HB, common (hulled) barley; hB, hulless barley; BBG, barley mixed-linked β-glucan concentrate; ND, not detected.

for this effect are not clear. We did not observe inhibitory effects of β-glucans on DNA extraction efficiency. Although the genomic DNA was diluted to $1\,ng\,\mu L^{-1}$, we cannot exclude the inhibitory effects of β-glucans on the PCR efficiency. Although speculative at this point, it might be possible that other bacteria such as enterococci were favoured in the high mixed-linked β-glucan diets because some strains display β-glucanase activity (Beckmann *et al.*, 2006). In the colon, we observed a decrease in diversity ($P < 0.05$) with cereals high in mixed-linked β-glucan. This suggests favouring of only a few bacterial phylotypes that are able to use β-glucans as a substrate, thereby outcompeting other bacteria.

On the other hand, the inclusion of a mixed-linked β-glucan as a purified supplement did not decrease the microbial diversity in the same way and, in turn, slightly increased the number of ileal lactobacilli, which confirms previous findings in the rat (Snart *et al.*, 2006). Therefore, not only the content of mixed-linked β-glucan in the diet but also the physical form and possible interactions with other nutrients in the matrix of the grain (i.e. starch and proteins) seem to be an important factor influencing the intestinal microbiota. However, animal-related factors such as the gastric emptying rate or altered production of bile acids can also play an important role.

Despite some well-known limitations of 16S rRNA gene-based DGGE in microbial ecology (Muyzer & Smalla, 1998), the method has been introduced and used successfully to study the general or taxonomic group-specific alterations of microbial communities in the porcine GIT during the weaning period or after dietary interventions (Konstantinov *et al.*, 2006a; Janczyk *et al.*, 2007). One limitation to consider is the heterogeneity of *rrn* operon copy number and sequence among bacterial species (Klappenbach *et al.*, 2000; Makarova & Koonin, 2007) that could lead to the misinterpretation of DGGE fingerprints because one bacterial species could produce more than one single band in the banding pattern (Crosby & Criddle, 2003). In our study, this was particularly true for *C. glycolicum,* which yielded two dominant bands. On the other hand, species with near-identical sequences can migrate to the same position, which would lead to an underestimation of species diversity (Simpson *et al.*, 1999). Recognizing these limitations, we excised and sequenced DNA from bands of each group to determine the identity of bacterial species associated with common and unique bands among cereals.

Up to now, only little information exists about the effect of cereal β-glucans on specific microorganisms that inhabit the GIT. Of specific interest in the concept of prebiotics is their potential to promote the growth of beneficial bacteria such as bifidobacteria and lactobacilli or to enhance the large intestinal production of microbial metabolites such as *n*-butyrate (Louis *et al.*, 2007). It has been shown that bifidobacteria and lactobacilli can utilize isolated cereal β-glucans depending on the degree of polymerization (Jonsson & Hemmingsson, 1991). Recently, it has been shown that β-glucans and resistant starch can enhance faecal numbers of lactobacilli and decrease coliforms (Bird *et al.*, 2007). Furthermore, the supplementation of high-viscosity barley β-glucans to a casein-based diet favoured the growth of lactobacilli in the caecum of rats (Snart *et al.*, 2006). In the present study, we detected an *L. sobrius*-like phylotype, which was described recently as an abundant resident of the porcine GIT (Konstantinov *et al.*, 2006b). DGGE bands associated with *L. sobrius* were found in nearly all ileum samples but only in the colon of piglets fed the common barley diet, one diet containing hulless barley with normal mixed-linked β-glucan content and the two oat diets. We

also detected a *B. pseudolongum*-like phylotype in the ileum of piglets fed the hulless breeding line SB 94893 and the waxy starch hulless variety CDC Fibar. Although bifidobacteria play only a minor role in the GIT of pigs (Leser *et al.*, 2002), this species has been found in the caecum of adult pigs (Simpson *et al.*, 2003). Bifidobacteria and lactobacilli are assumed to exert antimicrobial activities that participate in the host's gastrointestinal defence system (Servin, 2004). Although a lower 16S rRNA gene copy number for lactobacilli was detected in the ileum of piglets fed the hulless high-β-glucan barleys, enterobacteria or streptococci were consistently below detection limits in the ileum of these animals. This might be of specific importance, especially in young and susceptible animals such as weaning piglets, and is of specific interest in the postantibiotic era.

Nucleotide sequences associated with DGGE bands suggested that diets containing SB 94893 and CDC Fibar barleys also promoted the growth of *E. uniforme*-, *E. cellulosolvens*-, *B. fibrisolvens*- and *C. xylanovorans*-like phylotypes, which were previously identified as dominant xylan- and arabinoxylan-degrading species inhabiting the rumen of herbivores (Hespell & Cotta, 1995; Yoda *et al.*, 2005). It should be noted here that the sequence similarity of DGGE bands varied between 87% and 99% identities with cultured relatives, indicating that they might also represent novel phylotypes with yet unknown metabolic properties. However, it is well known that fibrous diets can promote the growth of cellulolytic bacteria in the GIT of pigs (Varel & Yen, 1997). The xylan-degrading species identified here could reflect a higher xylan and/or arabinoxylan content in these varieties (not measured). Alternatively, these species may also be capable of degrading β-glucans and thus supported by the higher mixed-linked β-glucan content. The species, identified here on the basis of their partial 16S rRNA gene sequence, seem to belong to the *Clostridium coccoides–Eubacterium rectale* cluster XIVa, which includes many species that produce *n*-butyrate (Barcenilla *et al.*, 2000; Pryde *et al.*, 2002). This might be of specific interest because *n*-butyrate is used as the primary energy source by the colonocytes and reduces the risk of human colon cancer (Wong & Jenkins, 2007).

Little is known about other species such as *C. glycolicum*, *M. diversum* and *S. sucromutans* that were identified in profiles of animals fed common barley and the Sb 90300 and CDC McGwire hulless barleys. *Clostridium glycolicum*-like sequences formed a dominant band in almost all ileum profiles and most colon samples. Because species were identified only based on partial 16S rRNA gene similarities, they might also represent novel phylotypes. For example, *C. glycolicum* has not yet been described to inhabit the porcine intestinal tract. Attempts to obtain more insight into the role of *C. glycolicum* in the porcine intestinal tract are currently underway in our institute.

Conclusion

In the present study, we used a DGGE approach to evaluate the effect of barley and oat varieties and breeding lines, mainly differing in the mixed-linked β-glucan content and starch composition, on the gastrointestinal microbial communities. By analysis of banding patterns and species identification, we could clearly show a varietal-dependent response of microbiota, especially in the colon. Increased levels of β-glucans and altered amylopectin/amylose ratios in the diet seemed to selectively favour butyrate-producing bacteria, possibly capable of degrading complex carbohydrates, which would have beneficial effects on the host. Furthermore, other beneficial microorganisms such as bifidobacteria and lactobacilli were influenced by the choice of cereal variety. However, the exact effect of each carbohydrate fraction still remains to be assessed and will be addressed in future studies. Taken together, our results provide a first comprehensive view of the interaction between varietal differences in barleys and oats and the porcine gastrointestinal microbiota, thus providing further information for future feeding strategies.

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