Effect of carbohydrate composition in barley and oat cultivars on microbial ecophysiology and the proliferation of *Salmonella enterica* in an *in vitro* model of the porcine gastrointestinal tract*

Running Title: Cereal carbohydrates and intestinal microbial ecology

Robert Pieper[1,2,6,7], Jérôme Bindelle[1,2,4,5,7], Brian Rossnagel[3], Andrew Van Kessel[1], Pascal Leterme[2,#]

[1]Department of Poultry and Animal Science, University of Saskatchewan, 51 Campus Dr., Saskatoon, Saskatchewan S7N 5A8, Canada

[2]Prairie Swine Centre inc., 2105 – 8th Street East, Saskatoon, SK, S7H 5N9, Canada

[3]Department of Plant Science, University of Saskatchewan, 51 Campus Dr., Saskatoon, Saskatchewan S7N 5A8, Canada

[4]Animal Science Unit, Gembloux Agricultural University, Passage des Déportés 2, 5030, Gembloux , Belgium

[5]Fonds de la Recherche scientifique-FNRS, Rue d'Egmont 5, 1000, Brussels, Belgium

[6]Current address: Institute of Animal Nutrition, Department of Veterinary Medicine, Free University of Berlin, Brümmerstrasse 34, D-14195 Berlin, Germany

[7] Both authors contributed equally to this work

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Corresponding author:

Dr. Pascal Leterme

Prairie Swine Centre Inc.

2105 – 8th Street East, Saskatoon, SK, S7H 5N9, Canada

phone: +1 306 667 7445

fax: +1 306 955 2510

e-mail: pascal.leterme@usask.ca

Abstract

The influence of carbohydrate (CHO) composition of cereal cultivars on microbial ecophysiology was studied using an *in vitro* model of the porcine gastrointestinal tract. Ten hulless (HLB), 6 hulled barleys (HB), 6 oats (O) and 6 oat groats (OG) differing in β-glucan, non-starch polysaccharides (NSP), starch contents and starch type, were hydrolyzed enzymatically and incubated for 72h with pig feces. Fermentation kinetics were modeled, and microbial composition and short-chain fatty acid (SCFA) profiles analyzed using terminal restriction fragment length polymorphism (TRFLP) and gas chromatography. Cluster analysis and canonical ordination revealed differently affected fermentation and microbial ecology according to type and cultivar. Firstly, in HB and O, cellulose and insoluble NSP content increased (1) *Ruminococcus flavefaciens*-like and *Clostridium xylanolyticum*-like phylotypes, (2) acetate production and (3) decreased fermentation activity. Secondly, in HLB β-glucan, amylose, amylopectin, crude protein and soluble NSP contents determined microbial community composition and activity: (1) amylose contents of the HLB varieties increased butyrate production and abundance of *Cl. butyricum*-like phylotypes, (2) β-glucan content determined the total SCFA amounts and (3) amylopectin and starch content affected abundance of *Cl. ramosum*-like phylotypes, members of *Clostridium* cluster XIVa and *Bacteroides*-like bacteria. Finally, the effect of CHO on proliferation of *Salmonella enterica* in the model was determined. *Salmonella* cell counts were not affected but the relative proportion of *Salmonella* decreased with HLB and increased with O as revealed by quantitative PCR. Our results shed light into the complex interactions of cereal CHO on intestinal bacterial ecophysiology and the possible impact on host health.

Abbreviations used in the manuscript

BCFA - branched chain fatty acids, **CHO** - carbohydrates, **CP** - crude protein, **DM** - dry matter, **dDM** - digestible dry matter, **GIT** - gastrointestinal tract, **lag t** - lag time, **Nal** - Nalidixic acid, **Nov** - Novobiocin**, (i/s/t)NSP** - (insoluble/soluble/total) non-starch polysaccharides, **(q)PCR** - (quantitative) polymerase chain reaction, **RDA** - redundancy analysis, **SCFA** - short chain fatty acids, **TRFLP** - terminal restriction fragment length polymorphism, **TRF** - terminal restriction fragment, **TSB** - Tryptone Soya Broth, **T1/2** - half-time to asymptotic gas production, **UPGMA** - unweighed pair group method with algorithmic means, **Vf** - final gas accumulation per g cereal

Introduction

The gastrointestinal tract (GIT) of pigs is colonized by a highly diverse microbial community, which can be affected by various factors including diet and environmental factors (15, 38). Manipulating the composition and metabolic activity of the gut microbiota through the diet to improve gut health is an increasing focus of nutritionists in the post-antibiotic era. Different strategies including the use of pre-, probiotics, organic acids or zinc have been applied to manipulate the intestinal ecosystem (31, 36). Surprisingly, the fact that compounds in the basal diet, such as cereal indigestible carbohydrates (CHO), can also affect the intestinal microbial ecophysiology is often neglected by nutritionists. Since cereals are a major component in the diets of pigs and other monogastric species, and since intestinal bacteria vary in their genetic potential for substrate utilization, there is great potential to beneficially manipulate microbial ecology in the GIT by choice of cereal cultivars with specific CHO composition.

The cereal CHO composition can vary markedly between cultivars (17, 19), and CHO fractions such as β-glucans could be used as functional food ingredients (6). For example, Pieper et al. (32) recently showed that the intestinal microbial community composition can be modified using the variability in β-glucan content within barley and oat cultivars. The specific mode of action of the other CHO fractions that are present in these cultivars, such as cellulose, soluble and insoluble non-starch polysaccharides (NSP) and starch (e.g. content and the amylose/ amylopectin ratio) on the microbial communities and their activity still remains unclear.

In addition to direct effects on intestinal physiology, changes in intestinal microbial composition may enhance or suppress the growth of specific pathogenic

92 microorganisms by competitive exclusion. For example, *Salmonella* infections are among
93 the most frequent and widespread zoonoses in the world and there might be opportunities
94 to influence *Salmonella* colonization via nutritional strategies (30). Studying the
95 interactions of intestinal bacteria in an *in vitro* simulation of the porcine GIT could help
96 to rapidly screen and evaluate promising strategies for *Salmonella* reduction in pigs
97 without the use of animal infection models.

98 The aim of the present study was to determine the influence of variation in CHO
99 composition found in 10 hulless barleys, 6 common barleys, 6 oat cultivars and their
100 respective oat groats (dehulled oats) on large intestinal fermentation characteristics and
101 microbial community composition. An *in vitro* model of the porcine GIT as described by
102 Bindelle et al. (5) was employed with or without co-inoculation with *Salmonella enterica*
103 and using multivariate canonical analysis.

104

105 ***Materials and Methods***

106 *Substrates and chemical characterization*

107 Ten hulless, 6 hulled barleys, 6 oat and the respective groats (dehulled kernels) of
108 these oat cultivars were chosen according to typical characteristics, but especially
109 according to the content of β-glucan, starch, as well as the amylose/amylopectin ratio
110 (Table S1). Most of the cultivars were developed and provided by the Crop Development
111 Centre (CDC) and grown between 2004 and 2006 at the University of Saskatchewan. The
112 selection was completed by some commercially available cultivars of cereals (McLeod,
113 AC Metcalfe).

Samples were analyzed for dry matter (DM) content (method 967.03; AOAC, 1990), crude protein (CP, method 981.10; AOAC, 1990), ash (method 923.03; AOAC, 1990) and ether extract (method 920.29; AOAC, 1990). Starch, amylose/amylopectin ratio and water-soluble β-glucan contents were analyzed colorimetrically after enzymatic hydrolysis according to standard procedures (Megazyme Ltd, Ireland). The total (t-), soluble (s-) and insoluble (i-) non-starch polysaccharide (NSP) fractions were determined by gas chromatography (Varian Star 3400 GC) equipped with a 30m fused silica capillary column and a gas flow rate of 36.15 cm/sec after the samples were hydrolyzed with 12M H_2SO_4 according to Englyst and Hudson (12).

In vitro *hydrolysis and fermentation*

In vitro hydrolysis and fermentation were performed using the procedure described by Bindelle et al. (5). Briefly, cereal samples were hydrolyzed with porcine pepsin (pH 2, 39°C, 2h) and porcine pancreatin (pH 6.8, 39°C, 4h), and residues filtered through a 42 µm Nylon cloth, washed twice with 96 % ethanol and 99.5 % acetone and dried at 60°C. Dry matter digestibility after hydrolysis (dDM) was recorded. Residues from different hydrolysis replicates of one cultivar were pooled and incubated in an inoculum prepared from fresh faeces of 3 growing pigs that were fed a non-medicated diet, and mixed with a buffer solution (29). Fermentation proceeded at 39 ± 0.5°C using 200 mg of the hydrolyzed residues and 30 ml of the inoculum placed in 140 ml glass bottles equipped with a rubber stopper. The experimental scheme was as follows: 28 ingredients x 3 replicates + 3 blanks (containing only inoculum). Gas pressure in the bottles was regularly recorded over 72h. After 72h, the fermentation broth was

centrifuged (12.000 x g, 5 min) and the supernatant removed for analysis of short chain fatty acids (SCFA). The pellet was further used for extraction of genomic DNA.

Analysis of SCFA

Supernatant of centrifuged samples (1ml) was acidified to pH 2.5 with metaphosphoric acid and the internal standard (crotonic acid solution, 2mg/ml in ddH_2O) was added. SCFA were analyzed by gas chromatography on a 30m x 320µm x 0.25µm fused-silica capillary (ZB-FFAP, Phenomenex, Torrance , CA, USA) in an Agilent 6890 GC system equipped with a flame ionization detector (Agilent, Böblingen, Germany). Helium was the carrier gas at a flow rate of 1.9 ml/min. The flow rate of hydrogen and air were 35 and 350ml/min, respectively. The initial oven temperature was 100°C followed by a ramp of 8°C/min and final temperature 200°C for 13min.

DNA extraction and TRFLP analysis

Genomic DNA of each sample was extracted using the FastDNA® Kit (Qiagen, Mississauga, Ontario, Canada) according to the manufacturer's instructions.

For analysis of the microbial communities, a partial fragment of the bacterial 16SrRNA gene was amplified by polymerase chain reaction (PCR) using universal forward primer S-D-Bact-0008-a-S-20 (AGA GTT TGA TCM TGG CTC AG), labelled with 6-carboxyflourescein (6-FAM) and reverse primer S-D-Bact-0926-a-S-20 (CCG TCA ATT CAT TTG AGT TT) (23). PCR reactions contained 5 µl of 10 x Incubation Buffer, 1.5 µl of 50 mM $MgCl_2$, 1.5 µl of each primer (10µM), 1.5 µl of each dNTP (10mM) and 0.2 µl of *Taq*-Polymerase (5 U/µl) and UV-sterilised millipore water, added

until 50 µl. PCR was performed in a Thermolyne Amplitron II temperature cycler (Barnstead/Thermolyne, Dubuque, IA, USA) and the program set as follows: 5 min at 95°C, 30 cycles of 95°C for 40 s, 55°C for 40 s and 72°C for 60 s, final extension at 72°C for 10 min. Size and yield of PCR products were checked by electrophoresis in 1.5% agarose gel after staining with ethidium bromide (0.5 µg EtBr/ml agarose). The PCR product was subsequently extracted from the gel using the Qiagen® PCR Purification Kit (Qiagen, Mississauga, Ontario, Canada) according to manufacturer's protocol and the DNA concentration was measured on a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

For terminal restriction fragment length polymorphism (TRFLP) analysis, 200 ng of the PCR product were digested at 37°C for 6 h using 15U of MspI (Fermentas, Burlington, Canada) in 2 µl reaction buffer and UV-sterilized Millipore water, made up to 20 µl. Two µl of the digestion solution were subsequently mixed with 9 µl of formamide and 0.5 µl of an internal size standard (ABI GeneScan™ 600 LIZ® Size Standard) and denatured at 95°C for 5 min followed by immediately cooling down on ice for 2 min. Fragment sizes were analyzed using an ABI 3130xl Genetic Analyzer in gene scan mode and GeneMapper v3.7 software. Fragments that were different in less than + /- 3bp were considered to be identical as binning criteria.

To identify the dominant bacteria species, a small clone library (n = 96) was constructed from purified PCR products of pooled DNA isolates using above mentioned primers (without 6-FAM) and standard cloning procedures according to manufacturer's protocol (pGEM®-T Easy Vector System, Promega, Madison, USA). Forward sequences were obtained on an ABI 3730 capillary sequencer and aligned using greengenes

183 (http://greengenes.lbl.gov/; 10). Sequences of closest cultured relatives were retrieved
184 and incorporated into ARB phylogenetic software (www.arb-home.de; 24) to assign
185 bacterial species or at least bacterial groups to individual TRF's using the TRFcut tool
186 (http://www.mpi-marburg.mpg.de/downloads/; 34). The theoretical fragments sizes were
187 calculated for the enzyme MspI and fragment sizes having +/- 1bp similarity with
188 obtained TRF's were considered a match. Sequences were deposited in GenBank and are
189 available under accession numbers GQ214260-GQ214312.
190
191 In vitro *fermentation and proliferation of* Salmonella enterica
192 According to the results of the first experiment, 10 barleys cultivars and 6 oats
193 and groats were selected for study using a co-inoculation (fecal inoculum + *Salmonella*)
194 approach. Citrus pectin (Sigma P-9135) was used as a negative control (7) and Tryptone
195 Soya Broth (TSB) medium was used as growth substrate for enterobacteria.
196 A double antibiotic (Novobiocin and Nalidixic acid, Nov^+/Nal^+) resistant strain of
197 *Salmonella enterica* subsp. *enterica* serotype Typhimurium var. Copenhagen, which was
198 obtained in a grow-finish herd during a survey in Western Canadian swine herds by the
199 Western College of Veterinary Medicine (Saskatoon, SK, Canada) and the Alberta
200 Research Council (Edmonton, AB, Canada) was selected for this study. *Salmonella* was
201 usually cultivated aerobically at 37°C in either TSB or on Brilliant Green agar plates,
202 containing 25µg/ml of each of the two antibiotics.
203 For the co-inoculation approach, the microbial communities in the fermentation
204 bottles were allowed to adapt to the substrate for an initial time of 6h before inoculation
205 with *Salmonella*. The strain was then inoculated with a syringe after gas pressure

206 measurement and gas release to a total concentration of log 3.20 ± 0.2 cfu/ml.
207 Fermentation proceeded for 24 h. Samples of mixed fermentation broth (0.1ml) were
208 taken at 6, 12 and 24 h and immediately plated onto Brilliant Green agar plates
209 (Nov^+/Nal^+) and plates incubated as described above. *Salmonella* colonies were counted
210 and reported as log cfu/ml incubation broth. After 24h, fermentation broth was
211 centrifuged (12.000 x g, 5 min) and the pellet was further used for extraction of genomic
212 DNA as described above.
213
214 *Quantitative real-time PCR analysis of bacterial communities*
215 Total genomic DNA from samples of the second experiment was extracted as
216 described above. Quantitative real time PCR (qPCR) was performed using previously
217 published primer sets and annealing temperatures (Table 1). The total bacterial counts,
218 counts for *Salmonella*, enterobacteria, *Clostridium* cluster XIVa, *Clostridium* cluster IV,
219 *Clostridium* cluster I, lactobacilli and *Bacteroides* were obtained. Amplification was
220 accomplished using an iQ™ SYBR® Green Supermix (BioRad, Guénette, Canada).
221 Amplification conditions were 95°C for 10 min, followed by 40 cycles with 95°C for 30
222 sec, 50-60°C (depending on bacterial species, Table 1) for 40 sec and 72°C for 40 sec.
223 The amplifications were performed using a CFX96 Real-Time PCR detection system on a
224 C1000 thermal cycler (BioRad, Guénette, Canada) with the data collection set at the
225 annealing/extension step. Standard curves were generated using serial dilutions of
226 purified genomic DNA of *Salmonella enterica* (for *Salmonella* quantification). For the
227 quantification of total bacteria, *Clostridium* cluster XIVa, IV and I, enterobacteria,
228 lactobacilli and *Bacteroides*, purified PCR products were used that were obtained by

229 standard PCR using the primers given in Table 1. The detection limit was 10^2 copy
230 numbers/ml fermentation broth. Melting curves were checked after amplification in order
231 to assure correct amplification results. Results of total counts were reported as log gene
232 copy numbers/ml fermentation broth, whereas the values for the other bacterial groups
233 were reported as relative numbers compared to total bacteria.
234
235 *Statistical analysis and calculations*
236 The *in vitro* digestibility of cereal dry matter after pepsin and pancreatin
237 hydrolysis was calculated. Gas accumulation curves during fermentation of hydrolyzed
238 cereals were modeled according to France et al. (14):

239 $$V = 0, \qquad \text{if } 0 < t < lag$$

240 $$= V_f\left(1 - \exp\left\{-\left\langle b(t - lag) + c\left(\sqrt{t} - lag\right)\right\rangle\right\}\right), \qquad \text{if } t \geq L$$

241 where $\boldsymbol{V}$ denotes the gas accumulation, $\boldsymbol{V_f}$ (ml x g^{-1} initial amount of cereal) the
242 maximum gas volume for $t = \infty$ and ***lag t*** (h) the lag time before the fermentation starts.
243 The constants b (h^{-1}) and c ($h^{-1/2}$) determine the fractional rate of degradation of the
244 substrate μ (h^{-1}), which is postulated to vary with time as follows:

245 $$\mu = b + \frac{c}{2\sqrt{t}}, \qquad \text{if } t \geq lag$$

246 The half-time to asymptotic gas production when $V = V_f/2$ was symbolized by ***T1/2***.
247 For analysis of bacterial communites, TRFLP profiles were normalized be
248 calculation of the relative peak area of each individual peak, only fragments with a
249 relative peak area ratio of $P_i \geq 1\%$ and fragments larger than 80bp were considered to
250 perform a cluster analysis using Pearson correlation and unweighed pair group method

251 with algorithmic means (UPGMA) with Statistica software (version 6.0, Statsoft, Tulsa,
252 OK, USA).

253 To analyze the complex interactions of cereal carbohydrate fractions on *in vitro*
254 digestibility, fermentation kinetics parameters, SCFA profiles and relative abundance of
255 bacterial groups and species, we applied a multivariate analysis using CANOCO
256 statistical package version 4.5 (37). The values for starch, amylose, amylopectin, β-
257 glucan, cellulose, tNSP, sNSP, iNSP and CP contents in the cereal cultivars were
258 imported as explanatory variables. Square root transformed values for relative abundance
259 of TRF's, SCFA production and molar ratio, parameters of fermentation kinetics and
260 dDM values were used as response variables. Explanatory and response data were used
261 for constrained linear ordination analysis (redundancy analysis, RDA), Whereas
262 unconstrained ordinations such as principle component analysis, are methods seeking one
263 or more gradients representing predictors that best explain response variable composition,
264 in constrained ordinations such as RDA, these predictors are further restricted and
265 ordination axes must be generated from linear combinations of weighed environmental
266 variables. The explanation of response variables with synthetic variables (ordination
267 axes) can therefore be further defined using values of the explanatory characteristics (37).
268 Significance of the overall ordination model as well as the effect of explanatory variables
269 during development of the ordination model was tested using Monte Carlo permutation
270 test (n=499).

271 Finally, statistical analysis of dry matter digestibility (dDM) during pepsin-
272 pancreatin hydrolysis, *in vitro* gas production kinetics parameters (L, T1/2, Vf), total
273 short-chain fatty acid (SCFA) production and molar ratios of individual SCFA after 72 h

of *in vitro* fermentation as well as qPCR results from the second co-inoculation run was performed by ANOVA followed by Tukey-HSD test using SPSS (version 17.0, Chicago, IL, USA). *P*-values of <0.05 were considered significant.

Results

Chemical composition of cereals

The chemical composition of the cereals used in this study is presented in Table S1. There was a high variation between cereal types and cultivars. The β-glucan content ranged from 4.6 (CDC McGwire) to 12.7% (CDC Fibar) in hulless barleys and from 4.1 to 5.9% in common barleys, whereas slightly lower values where found in the oat cultivars (2.9 to 5.1%). The 'waxy' hulless barleys CDC Rattan, SR93139, CDC Fibar, SB94917 and HB393 showed low levels of amylose starch whereas the 'high amylose' cultivars SH99250 and SB94893 had amylose concentrations of 38.9 and 46.1% of total starch respectively. The tNSP values varied from 7.7 to 15.3% in hulless barleys, 11.9 to 17.2% in common barley, and 16.6 to 26.1% in oats, likely due to the higher content of cellulose and lignified hulls.

In vitro fermentation parameters

The dDM after enzymatic hydrolysis and the fermentation parameters are presented in summarized form for the different cereal types in Table 2. Values for individual cereals and statistical comparison of means between cultivars are presented as supplemental material (Table S2). To illustrate the variability, the mean values with standard deviation and the range are presented for each grain type. The *in vitro* dry matter

297 digestibility varied between grain types and cultivars and was partly related to the
298 amylose/amylopectin ratio in hulless barley cultivars. Common barley types and oats
299 showed almost similar values with lower variability, whereas the highest digestibility was
300 found for oat groats (up to 89.7%). The fermentation characteristics also showed
301 considerable variation within and between grain types. For example, the lag t was
302 increased with high amylose hulless barleys. Most differences were observed for final gas
303 volume, which was expressed per g of original non-hydrolyzed cereal. There was an
304 apparent effect of starch type and β-glucan content on gas production. High amylose
305 barleys SB94893 and SH99250 had the highest values followed by the high β-glucan
306 cultivars. Total SCFA production was highest with hulless barleys and oat groats but
307 ranged from 374 (SB90300) to 535 mg/g substrate (CDC Fibar) in hulless barleys and
308 from 365 (HiFi) to 459 mg/g substrate (CDC ProFi) with oat groats. The lowest values
309 (except for CDC SO-I) were found for oats whereas the common barleys had
310 intermediate values. The high hull containing oats had higher molar ratios of acetate (59.7
311 to 71.2%) and lower values for butyrate (7.6 to 12.0%) as compared to the other varieties.
312 The highest values for propionate were found with oat groats with 24.6 to 26.6% for
313 Morgan and CDC ProFi, respectively. Branched chain fatty acids as indicators of protein
314 breakdown were found to be lowest with oats (0.7 to 4.2% for CDC SO-I and CDC ProFi,
315 respectively), whereas higher values were observed with hulless barleys (5.3 to 6.1% for
316 SB90354 and SB90300, respectively) and oat groats (5.2 to 5.7% for Morgan and CDC
317 ProFi/CDC Sol-Fi, respectively).
318
319 *Bacterial community composition*

320 The cluster analysis based on the TRF's (Figure 1) revealed distinct bacterial
321 profiles based on the cereal type (except for CDC Clyde and SB90300). Two main
322 clusters were formed by either hull containing common barleys and oats or by hulless
323 barleys and oat groats. CDC SO-I clustered different from alls cultivars. However,
324 between cultivars of hulless barleys, there was a very high variability and small
325 subclusters as compared to the very similar clusters with common barleys and oats.
326 To assign bacterial phylotypes to individual TRF's an *in silico* TRF cut tool was
327 implemented into ARB phylogenetic software. The results (Figure 2) revealed that cereal
328 types and cultivars mainly influenced the abundance of members of clostridial clusters IV
329 and XVIa but also clostridial clusters I, XVIII and members of *Bacteroides*. For example
330 *Ruminococcus flavefaciens*-like (TRF 20) and *Clostridium xylanolyticum*-like phylotypes
331 (TRF 16) were enhanced in high cellulose/hull containing cereals whereas other bacteria,
332 belonging to clostridial clusters XIVa and I were enhanced with hulless barleys (data not
333 shown). Unfortunately TRF's # 7, 8, 9, 10, 19, 21 could not be identified by this
334 approach. However, they were included in the analyses since they represented >1% of the
335 total bacterial communities.
336
337 *Multivariate canonical analysis*
338 Results of the RDA analyses for interaction between cereal nutrient composition
339 and digestibility and fermentation responses for all cereals, and barleys, oats and hulless
340 barleys separately are presented in Figure 3a-d. The RDA of the four grain types together
341 (Figure 3a, n = 28) revealed the major influence of cellulose (P=0.002), β-glucan
342 (P=0.006), CP (P=0.030) and a trend for amylose (P=0.058) contents on the overall

343 ordination model. Cellulose, iNSP and tNSP were highly correlated to the first
344 ordinational axis as a consequence of the clustering of the oats with high hulls and
345 cellulose contents along this axis, whereas the other axis of the model covered only
346 12.1% of the variance. As an example how to read and interpret the data in the RDA
347 graphs: the acetate molar ratio, T1/2, *Ruminococcus flavefaciens*-like and *Clostridium*
348 *xylanolyticum*-like phylotypes (TRF16 and 20) were positively correlated to cellulose,
349 iNSP and tNSP as indicated by the small ankle between the arrows for these variables
350 (<90°). An ankle of >90° would indicate a negative correlation (i.e. between amylopectin
351 and cellulose in Figure 2a, r = -0.56). In comparison to cellulolytic materials, the sNSP
352 and CP values were positively correlated with propionate molar ratio and members of the
353 clostridial cluster XIVa (TRF12). Formation of BCFA was associated with *Clostridium*
354 *ramosum*-like bacteria (TRF17), amylopectin and starch contents. The concentrations of
355 β-glucan were associated with high fermentation activity (Vf), high amounts of SCFA
356 and butyrate molar ratio, and members of clostridial cluster XIVa (TRF11).
357 RDA of barleys (Figure 3b, n=16) highlighted the strong influence of β-glucan
358 (*P*=0.002), tNSP (*P*=0.002) and amylopectin (*P*=0.002) on the model. The β-glucan and
359 CP content were positively correlated with the production of SCFA and BCFA As
360 already observed by the analysis of the 4 grains together, acetate molar ratio, half-time to
361 asymptote (T1/2) and *R. flavefaciens* and *C. xylanolyticum*-like phylotypes (TRF16 and
362 20) were positively correlated with cellulose, tNSP and iNSP, but negatively with β-
363 glucan content of the barleys. There was also a positive correlation of amylopectin with
364 propionate molar ratio and abundance of *Bacteroides/Cytophaga*-like phylotypes (TRF6),
365 whereas amylopectin was negatively correlated to butyrate molar ratio with the barleys.

366 However, butyrate molar ratio was positively correlated with lag t, Vf and *C. butyricum*-
367 like phylotypes (TRF23).

368 RDA of only oats and oat groats together (Figure 3c, n=12) highlighted the strong
369 impact of the cellulose contents of the oats on the ordination model (P=0.002). Similar to
370 the overall model, cellulose, iNSP and tNSP contents were associated with elevated
371 acetate molar ratio, high abundance of *R. flavefaciens* and *C. xylanolyticum*-like
372 phylotypes (TRF16 and 20), and fermentation characteristics. In contrast, the oat groats
373 were associated with most of the other response variables.

374 Finally, a RDA analysis was performed for hulless barley varieties only (Figure
375 3d, n=10) since they displayed the highest variation in nutrient composition between
376 cultivars. As already revealed by the analysis of all barleys together, amylopectin was
377 positively correlated with propionate molar ratio, digestibility of dry matter, the
378 production of BCFA and *Bacteroides/Cytophaga*-like phylotypes (TRF6). In contrast, the
379 β-glucan content was positively correlated *C. ramosum*-like (TRF17) phylotypes, other
380 members of clostridial clusters IV and XIVa (TRF11 and 12) and the production of
381 SCFA, whereas it was negatively correlated to *R. flavefaciens*-like an *C. xylanolyticum*-
382 like phylotypes (TRF16 and 20). Higher molar ratios of butyrate were also associated
383 with *C. butyricum*-like bacteria (TRF23), and positively correlated to amylose
384 concentration. Other bacterial phylotypes reacted differentially and showed no clear
385 association with cereal factors.

386

387 *Impact of cereal cultivars on Salmonella proliferation*

388 After the inoculation of the *Salmonella enterica* strain during the second
389 experiment, no significant changes were observed numbers of double resistant
390 (Nal^+/Nov^+) *Salmonella enterica* during the fermentation suggesting no effects of CHO
391 (data not shown). However, qPCR results using genomic DNA extracts after 24h,
392 revealed significant ($P<0.05$) differences for the relative amount of *Salmonella*/total
393 bacterial 16S rRNA gene copy numbers in hulless barley cultivars CDC McGwire, CDC
394 Fibar, SH99250 and SB94893 (Table 3). These four cereal types had similar values as the
395 negative control pectin. In contrast, the relative proportion was highest in the blank and
396 with the oat cultivars. The relative contribution of enterobacteria and lactobacilli was
397 generally low, whereas *Clostrium* cluster XIVa, IV, I and *Bacteroides* dominated the
398 bacterial communities. Overall, 77% of the total bacterial communities were detected
399 with the current qPCR approach ranging from 61% (TSB) to 93% (pectin). Similar to the
400 TRFLP results, there were significant differences according to the grain type with
401 *Clostridium* clusters IV and I. Almost no differences were observed for cluster XIVa,
402 whereas *Bacteroides* showed no clear response according to grain type or cultivar.
403
404 ***Discussion***
405 Although, the results presented here are based on an *in vitro* system of the porcine
406 GIT, they confirm the great potential for manipulating intestinal microbial composition
407 through the use of cereal sources containing carbohydrates with prebiotic properties. Due
408 to the use of an *in vitro* fermentation model, results are limited to the porcine large
409 intestine but as an addition when compared to other *in vitro* models, we simulated the
410 nutrient digestion in the upper GIT using a pepsin-pancreatin pre-treatment, aiming to

411 obtain the indigestible fraction of the ingredients, which is likely to undergo fermentation
412 in the large intestine *in vivo* (5). Both experiments presented here, support the hypothesis
413 of complex CHO interaction with the microbial ecosystem. It is already well known that
414 contrasting sources of CHO can affect the fermentation characteristics *in vitro*, causing
415 differences in lag t, slope of gas production curves and final gas volume (2, 4, 5).
416 Furthermore, it has been shown that these contrasting CHO sources affect the profiles of
417 the fermentation end-products (2). Similar to these results, in the present study,
418 differences in fermentation characteristics and bacterial metabolites were observed
419 between cereal types, hulless barley, common barley, oat and oat groats. However, the
420 effect of contrasting CHO composition between cultivars of a same cereal species or type
421 on these parameters *in vitro* was still unknown. Results indicate that, especially within the
422 hulless barleys, there was a considerable variation of these parameters depending on
423 cultivars. Parameters also partially overlapped with other cereal types. These results were
424 confirmed by cluster analysis of TRFLP profiles, showing very little variation between
425 common barley types and their close relation to high cellulose containing oat cultivars,
426 whereas greater differences where found for hulless barleys and oat groats. This suggests
427 a dominant effect of fibrous materials in the hulls (cellulose, lignin, iNSP) on the
428 fermentation patterns. The fact that CDC SO-I was not included in the oat-common-
429 barley-cluster could be explained with its differential fermentation behaviour, starting a
430 high activity towards the end of the fermentation (Table S2). This cultivar is
431 characterized by a low lignin content, suggesting increased cellulose availability for
432 fermentation. The high variability with hulless barleys confirms the complex interaction
433 of CHO in these cultivars, suggesting that other carbohydrate fractions such as iNSP,

434 sNSP or amylose/amylopectin ratio of the starch fraction had a strong influence on
435 bacterial community composition *in vitro*.

436 Despite the great advantage of molecular microbiological tools, such as TRFLP,
437 to study bacterial communities without the need of cultivation, it has to be noted that
438 there are some drawbacks including primer and PCR bias or formation of pseudo-TRF's
439 (9, 11). Furthermore, it is difficult to assign bacterial species to individual TRF's since
440 different phylotypes could give similar TRF's after restriction digest. In the present study,
441 there were some TRF's that could not be assigned (e.g. TRF's 7, 8, 9, 10, 19, 21), likely
442 due to the size of the clone library. On the other hand, some TRF's (e.g. *R. flavefaciens,*
443 *Cl. xylanolyticum, Cl. butyricum*) could be better assigned to species-like phylotypes due
444 to their frequent occurrence in clone library analysis. Furthermore, since samples in the
445 present study were generated based on the same biases, it is possible to compare between
446 samples on a relative basis.

447 To our knowledge, this is the first time that the interactions of nutrient source and
448 intestinal microbial activity were studied using redundancy analysis. Using this approach,
449 the effect of cereal CHO included in the grain matrix on the microbial composition and
450 activity can be visualized. When analysing the data for all cereal types and varieties
451 together (Figure 3a), high cellulose and i-NSP containing oats and common barleys
452 showed slow fermentation and favoured cellulolytic, acetate producing *R. flavefaciens*-
453 and *Cl. xylanolyticum*-like bacteria (TRF16 and 20). These species have specifically
454 adapted to the breakdown of fibrous (cellulolytic, xylanolytic) material during
455 evolutionary co-existence with the host organism (13). They belong to clostridial clusters
456 IV and XIVa, respectively and are typical colonizers of in the distal gastrointestinal tract

457 of monogastric and forestomach of ruminant species (13, 20, 22, 39). Corresponding to
458 the present data, the breakdown of cellulolytic materials usually resulted in acetate
459 formation. On the other hand, β-glucans favored *Cl. ramosum*-like species (cluster XVIII,
460 TRF17), members of clostridial cluster IV and XIVa (TRF11 and 12) and the overall
461 SCFA production. In contrast to these results, isolated β-glucans favored the growth of
462 *Cl. histolyticum*-like bacteria and increased the propionate molar ratio in another recent *in*
463 *vitro* study using human fecal microbiota (18). This is in contrast to the present findings
464 and might either indicate differences in the general *in vitro* model or whether isolated β-
465 glucans or whole cereals containing the β-glucans in the grain matrix are used (32). RDA
466 on HLB (Figure 3d) revealed that the high amylose content of some hulless barley
467 cultivars favoured the contribution of butyrate producing members of clostridial cluster I
468 (*Cl. butyricum*-like phylotypes) but not members of clostridial cluster XIVa which are
469 commonly involved in butyrate formation (3, 33). Although butyrate production might be
470 a beneficial effect for maintaining intestinal health, this result has to be handled with
471 care, since other members of clostridial cluster I such as *Cl. botulinum*, or *Cl. perfringens*
472 may be harmful for the host organism. Finally, the starch and amylopectin values were
473 positively correlated with propionate molar ratio and *Cytophaga/Bacteroides*-like
474 bacteria, likely due to their ability for starch utilization (42).
475 The differential response of clostridia to the available substrates with hulless and
476 common barleys, oats and oat groats was confirmed by qPCR of dominant bacterial
477 groups in the second experiment. However, the *in vivo* contribution of these bacterial
478 species might be overestimated with in the current *in vitro* method as we used a buffered
479 medium. For example, bacteria such as *Ruminococcus sp.* were shown to exert less

480 metabolic activity below pH 6.3 (16). Furthermore, the abundances of *Bacteroides*
481 increase and *Roseburia*-like species decrease with a shift from pH 5.5 to 6.5 (40).
482 *Salmonella* infections are among the most frequent and widespread zoonotic
483 diseases in the world. Since there might be opportunities to reduce the prevalence of
484 *Salmonella* using feeding strategies, a co-inoculation model was developed to study the
485 effect of cereal CHO on *Salmonella* proliferation *in vitro*. There was no reduction of
486 *Salmonella* counts due to different fermentable substrates indicating that *Salmonella* was
487 able to survive in the buffered system and occupy an ecological niche and without and
488 challenge trough a hosts' immune response. In a recent study by Martin-Peláez et al.,
489 (25), *Salmonella* counts were significantly reduced with lactulose as a substrate.
490 However, in their study Martin-Peláez et al. (25) used very high numbers of *Salmonella*
491 (>log 7.0 cfu), which would not naturally occur during a normal *Salmonella* infection.
492 Callaway et al. (7), by using an *in vitro* simulation technique of ruminal fermentation,
493 revealed that pectin could significantly reduce the prevalence of *Salmonella*. This was in
494 part confirmed by our results, but only the relative proportion of *Salmonella* was reduced
495 with pectin. Although this might represent a beneficial effect, when interpreted as
496 competitive exclusion and reduction in relative *Salmonella* abundance, it appears that *in*
497 *vitro* system have to many limitations to study nutritional effects on pathogen
498 proliferation (26). However, it is not clear to which extent such results could be
499 transferred to *in vivo* conditions and actually reduce *Salmonella* colonization and or the
500 transmission among animals.
501
502 ***Conclusions***

503 The current study reveals that differences in CHO composition between cereal
504 cultivars of the same grain type, can affect the pig intestinal microbial ecophysiology.
505 These effects were furthermore revealed by multivariate canonical analysis showing the
506 usefulness of this approach when studying the intestinal microbial ecophysiology and
507 nutrient-microbe interactions. However, irrespective of grain type, positive correlations
508 were found between acetate production, cellulolytic bacteria and cellulose content;
509 butyrate production and either amylose and/or β-glucan contents; and propionate
510 production and amylopectin. This indicates typical ecophysiological signatures of CHO
511 fractions, namely amylose/amylopection ratio and β-glucan content in the pig intestinal
512 tract. Effects between cultivars not only result in different microbial ecological response,
513 but could affect the susceptibility of the host to opportunistic pathogens such as
514 *Salmonella enterica* due to trend of relative amounts in some hulless barley varieties.
515

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525

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Table 1. List of primers used for quantitative real-time PCR

target organism	sequence (5'-3')	amplicon size (bp)	annealing Temp. (ºC)	reference
total bacteria	CGGYCCAGACTCCTACGGG	200	60	21
	TTACCGCGGCTGCTGGCAC			
Clostridium	AAATGACGGTACCTGACTAA	440	50	28
cluster XIVa	CTTTGAGTTTCATTCTTGCGAA			
Clostridium	GCACAAGCAGTGGAGT	239	50	27
cluster IV	CTTCCTCCGTTTTGTCAA			
Clostridium	TACCHRAGGAGGAAGCCAC	346	63	34
cluster I	GTTCTTCCTAATCTCTACGCAT			
Bacteroidetes	CTTCCTCCGTTTTGTCAA	212	60	27
	GRCCTTCCTCTCAGAACCC			
Lactobacilli	GCAGCAGTAGGGAATCTTCCA	346	55	41
	GCATTYCACCGCTACACATG			
Enterobacteria	CCTACTTCTTTTGCAACCCACTC	364	60	8
	ATGGCTGTCGTCAGCTCGT			
Salmonella	AGCCAACCATTGCTAAATTGGCGCA	430	60	1
	GGTAGAAATTCCCAGCGGGTACTG			

Table 2. Mean ± SD and range (minimum - maximum) of the *in vitro* dry matter digestibility, fermentation kinetics parameters and bacterial metabolite profiles of the 4 cereals.

Cereal type	Hulless barleys		Hulled barleys		Oats		Oat groats	
	n=10		n=6		n=6		n=6	
fermentation parameters								
dDM[1] (%)	64.7 ± 10.4	45.0 - 75.3	66.4 ± 5.9	55.1 - 71.1	64.0 ± 3.5	59.3 - 68.9	87.9 ± 1.1	86.3 - 89.7
lag t (h)	1.8 ± 1.3	0.9 - 4.2	1.9 ± 0.8	1.0 - 2.3	1.1 ± 1.2	0.4 - 3.6	1.3 ± 0.2	1.1 - 1.4
T1/2 (h)	9.4 ± 0.7	8.3 - 10.5	10.5 ± 0.2	10.2 - 10.8	18.8 ± 6.4	11.6 - 30.0	8.6 ± 0.5	8.0 - 9.1
Vf (ml g^{-1}DM)	77 ± 25	47 - 124	62 ± 15	48 - 88	34 ± 17	25 - 67	22 ± 2	19 - 25
short chain fatty acids								
Acetate (%)[2]	53.3 ± 0.5	52.5 - 54.4	56.8 ± 0.5	55.9 - 57.4	66.2 ± 4.0	59.7 - 71.2	55.3 ± 1.1	53.8 - 56.7
Propionate (%)	23.2 ± 0.9	22.0 - 24.5	21.6 ± 0.7	20.3 - 22.1	20.4 ± 1.5	19.1 - 23.3	25.7 ± 0.7	24.6 - 26.6
Butyrate (%)	16.3 ± 0.7	15.1 - 17.2	15.8 ± 0.7	14.9 - 16.9	10.2 ± 1.5	7.6 - 12.0	12.9 ± 0.4	12.3 - 13.3
BCFA (%)	5.7 ± 0.7	5.3 - 6.1	4.4 ± 0.7	4.1 - 5.0	2.5 ± 1.5	0.7 - 4.2	5.5 ± 0.4	5.2 - 5.7
total SCFA (mg/g)	478 ± 51	374 - 535	378 ± 23	354 - 418	198 ± 94	131 - 380	414 ± 33	365 - 459

[1] dDM = digestible dry matter, T1/2 = half time to asymptotic gas production, lag t = lag time, Vf = amount of gas produced/g cereal before hydrolysis, SCFA = short chain fatty acids, BCFA = branched chain fatty acids

[2] results are presented as % of total SCFA

Table 3. Quantitative real-time PCR analysis (Mean±SD) of total bacterial counts (log 16S rDNA gene copy numbers/ml of fermentation broth) and the relative contribution (%) of six bacterial groups and *Salmonella* to the overall bacterial community after 24h of *in vitro* fermentation of hydrolyzed cereal varieties, pectin and Tryptone Soya Broth (TSB) using pig feces as inoculum and co-inoculation of *Salmonella enterica* after 6h. Different superscripts (^{abcd}) within a column indicate significant (P<0.05) differences.

cereal cultivar/ growth medium	Total bacteria log copies/ml	Cl. cluster XIVa %	Cl. cluster IV %	Cl. cluster I %	*Bacteroides* %	Lactobacilli %	Enterobacteria % x 10^{-2}	*Salmonella* % x 10^{-4}
Hulless barleys								
SB90354	10.4^{a}	39.5^{a}	26.0^{cd}	11.0^{a}	7.6^{bc}	0.2^{bc}	2.7^{a}	0.5^{cd}
SB90300	10.1^{ab}	23.4^{b}	35.6^{bc}	12.1^{a}	4.4^{d}	0.3^{bc}	1.3	0.6^{cd}
SR93139	10.1^{abc}	28.2^{b}	30.8^{bcd}	10.5^{a}	6.7^{bcd}	0.2^{bc}	0.4^{c}	0.4^{cd}
CDC McGwire	10.3^{ab}	23.1^{b}	33.6^{bcd}	9.3^{a}	4.2^{d}	0.2^{bc}	0.4^{c}	0.3^{d}
CDC Fibar	10.1^{ab}	25.7^{b}	37.2^{b}	10.4^{a}	8.7^{ab}	0.2^{bc}	0.3^{c}	0.2^{d}
SH99250	10.3^{a}	24.2^{b}	24.8^{d}	9.9^{a}	5.3^{bcd}	0.1^{c}	0.2^{c}	0.2^{d}
SB94893	10.2^{ab}	26.4^{b}	31.8^{bcd}	11.4^{a}	3.6^{d}	0.2^{bc}	0.3^{c}	0.2^{d}
Common barleys								

Mc Leod	10.1^{ab}	24.6^{b}	24.1^{d}	10.0^{a}	10.9^{a}	0.2^{b}	0.8^{bc}	0.9^{bcd}
AC Metcalfe	9.9^{bc}	25.6^{b}	40.4^{b}	14.3^{a}	5.7^{bcd}	0.4^{b}	0.4^{c}	0.5^{cd}
Oats								
CDC Dancer	9.9^{bc}	20.1^{b}	39.4^{b}	4.1^{b}	8.3^{abc}	0.3^{bc}	1.2^{abc}	1.6^{ab}
CDC Sol-Fi	9.7^{c}	24.4^{b}	53.9^{a}	5.2^{b}	6.2^{bcd}	1.0^{a}	0.9^{bc}	0.7^{cd}
CDC SO-I	9.8^{c}	21.0^{b}	57.3^{a}	4.4^{b}	8.3^{ab}	0.6^{a}	0.7^{c}	1.2^{abc}
Oat groats								
CDC Dancer groats	10.2^{ab}	26.7^{b}	30.5^{bcd}	2.1^{b}	5.9^{bcd}	0.4^{bc}	1.1^{ab}	0.8^{cd}
CDC Sol-Fi groats	9.9^{bc}	28.3^{b}	37.5^{b}	3.5^{b}	7.1^{bcd}	0.4^{bc}	0.7^{c}	0.7^{cd}
CDC SO-I groats	10.1^{ab}	25.8^{b}	34.0^{bcd}	3.2^{b}	4.6^{cd}	0.3^{bc}	0.5^{c}	0.4^{cd}
pectin	10.3^{a}	41.1^{a}	43.4^{a}	2.5^{b}	6.4^{bcd}	0.2^{bc}	0.6^{c}	0.3^{d}
TSB	10.1^{ab}	21.5^{b}	30.1^{bcd}	4.6^{b}	3.7^{d}	0.7^{a}	1.6^{abc}	0.7^{cd}
blank	9.7^{cd}	19.4^{b}	48.1^{a}	10.5^{a}	11.6^{a}	0.8^{a}	2.3^{a}	2.0^{a}
SEM	0.042	29.661	43.486	8.054	5.322	0.038	0.009	0.000

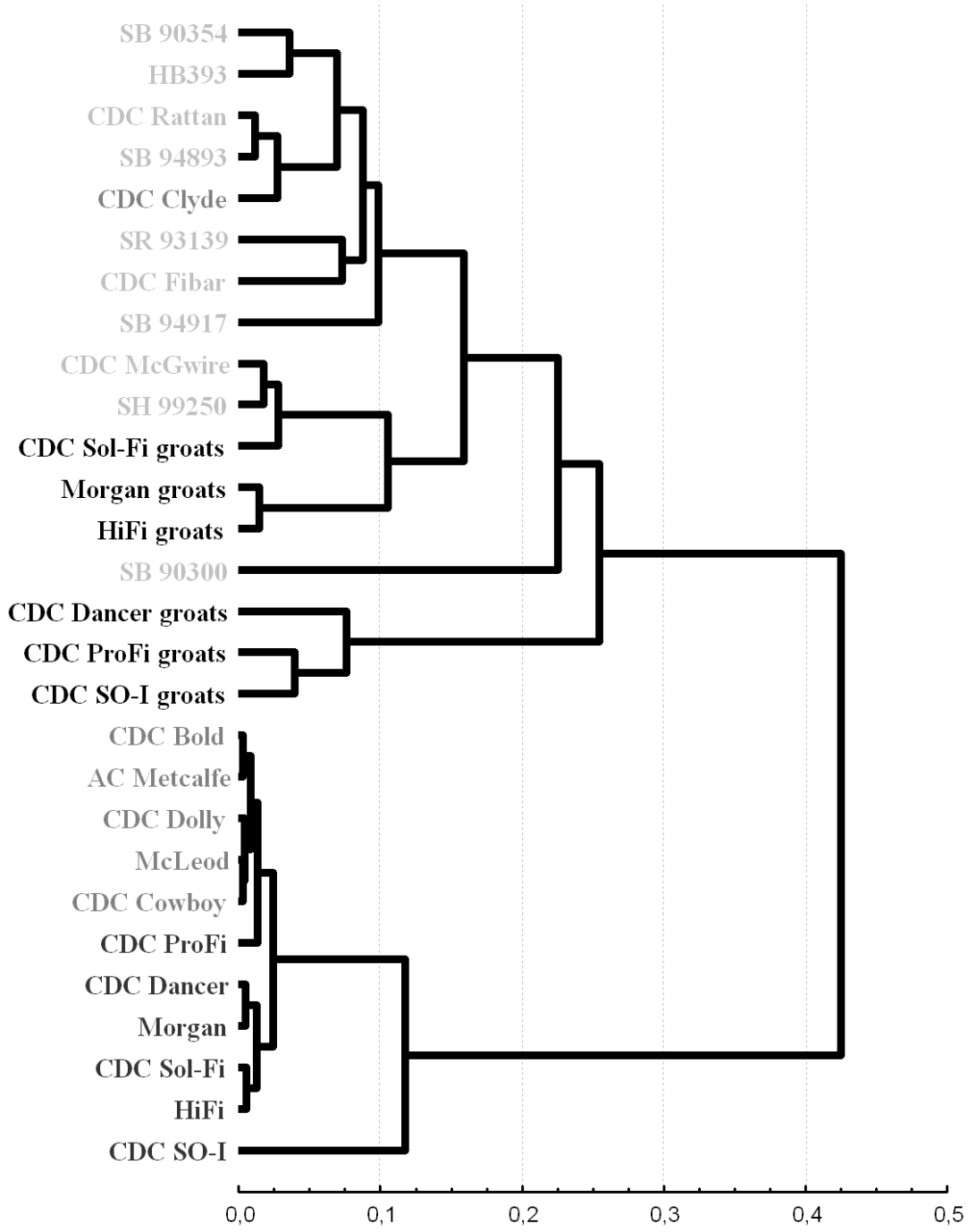


Figure 1. Cluster analysis based on the relative TRF abundances in bacterial TRFLP profiles after 72h fermentation of 10 varieties of **hulless barleys**, 6 **common barleys**, 6 **oats** and 6 **oat groats**. The cluster was constructed based on Pearson correlation and UPGMA algorithm using Statistica software. The scale bar represents the relative similarity of profiles.

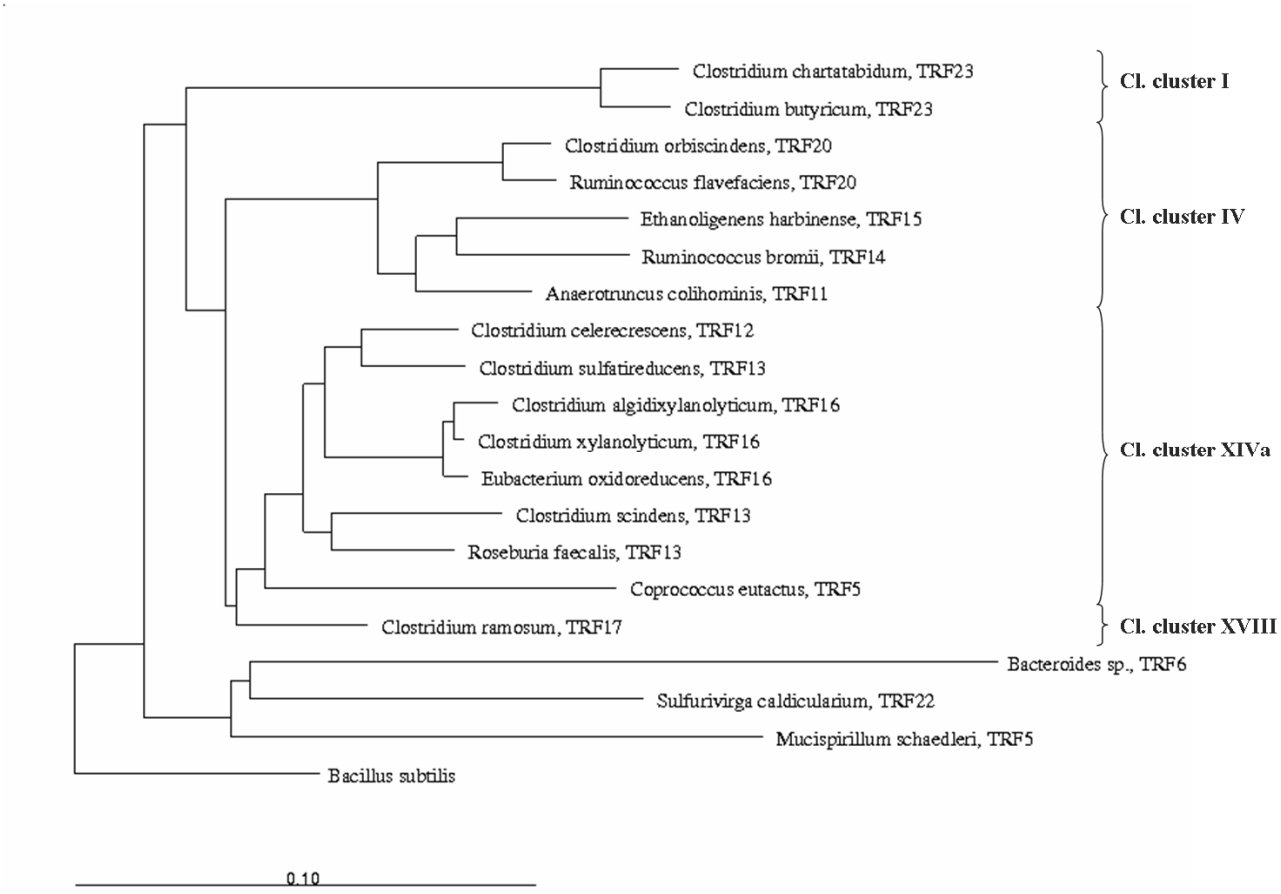
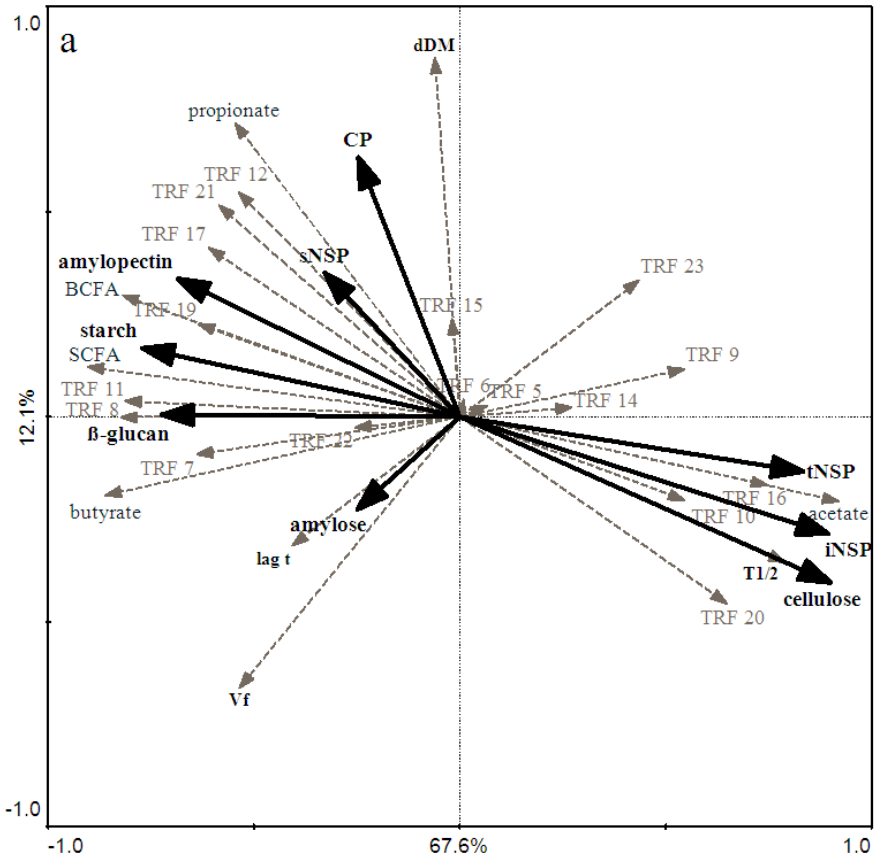


Figure 2. Neighbour joining tree showing the phylogenetic relationships of partial 16S rRNA gene sequences obtained in the study. TRF peak numbers from TRFLP analysis are indicated behind the species name and refer to the RDA analysis in Figure 3. *Bacillus subtilis* was used to root the tree. The bar indicates the calculated evolutionary distance of 10%.


a
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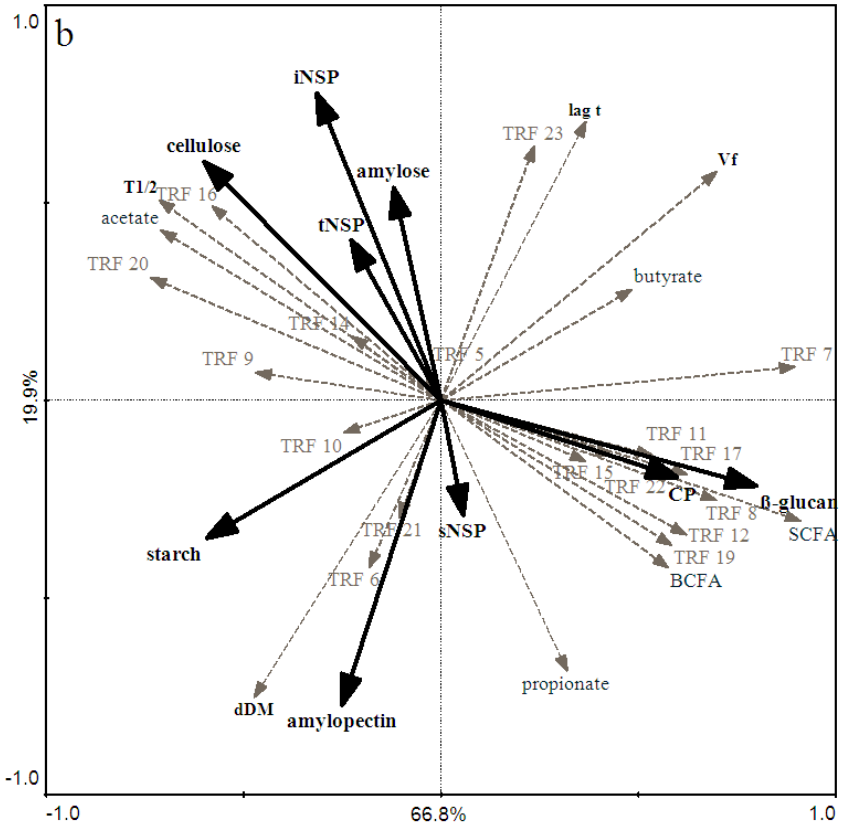
-1.0
12.1%
-1.0
67.6%
1.0
dDM
propionate
CP
TRF 12
TRF 21
TRF 17
amylopectin
sNSP
BCFA
TRF 19
starch
SCFA
TRF 11
TRF 8
ß-glucan
TRF 7
butyrate
amylose
lag t
Vf
TRF 23
TRF 15
TRF 9
TRF 6
TRF 5
TRF 14
TRF 22
tNSP
TRF 16
TRF 10
acetate
iNSP
T1/2
cellulose
TRF 20


b
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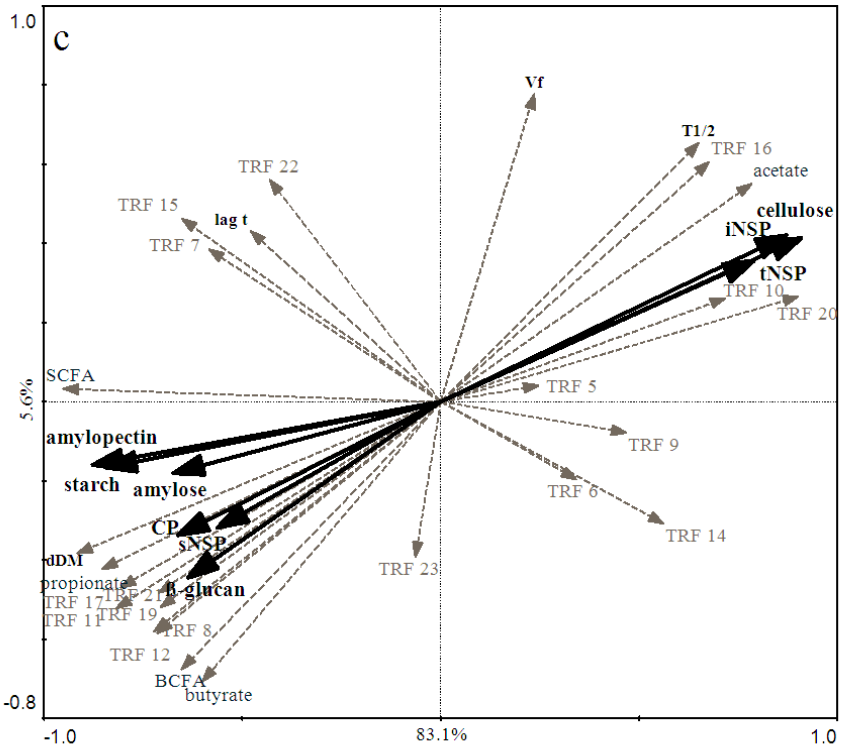
-1.0
19.9%
-1.0
66.8%
1.0
iNSP
lag t
TRF 23
cellulose
amylose
Vf
T1/2
TRF 16
acetate
tNSP
TRF 20
butyrate
TRF 14
TRF 9
TRF 5
TRF 7
TRF 10
TRF 11
TRF 17
TRF 15
TRF 22
CP
ß-glucan
TRF 8
SCFA
TRF 12
TRF 19
BCFA
starch
sNSP
TRF 21
TRF 6
propionate
dDM
amylopectin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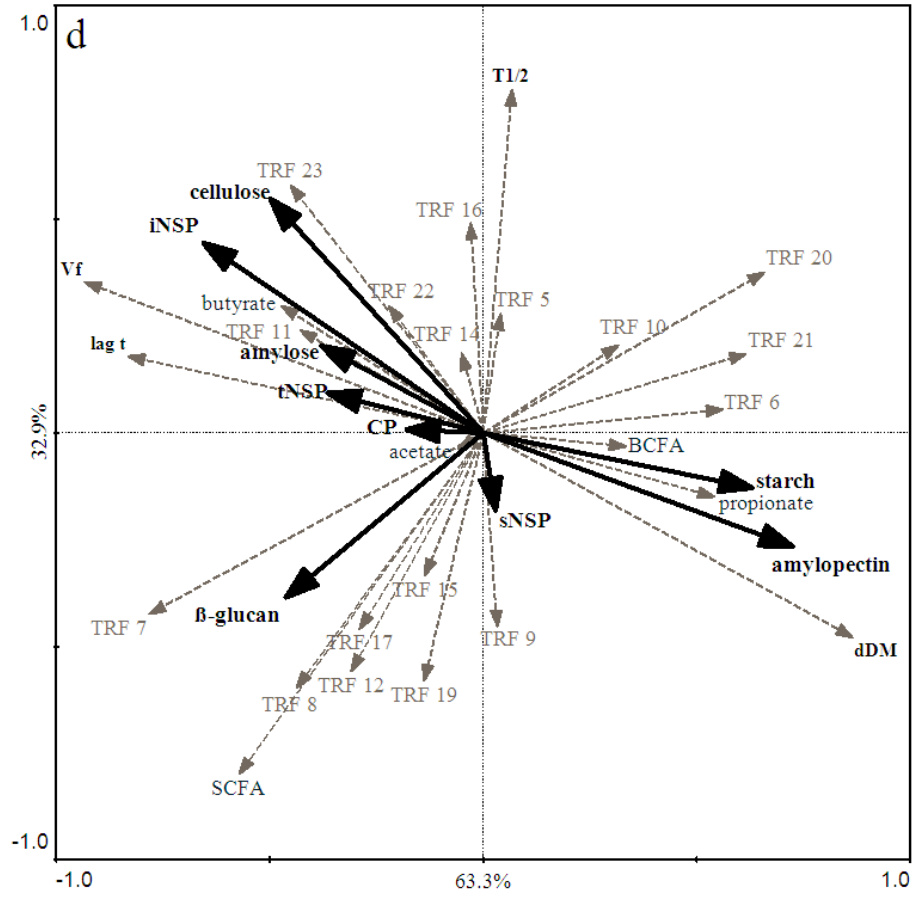
5.6%
-1.0
83.1%
1.0
Vf
T1/2
TRF 16
acetate
cellulose
iNSP
tNSP
TRF 10
TRF 20
TRF 22
TRF 15
lag t
TRF 7
SCFA
TRF 5
TRF 9
TRF 6
TRF 14
amylopectin
starch
amylose
CP
sNSP
dDM
propionate
ß-glucan
TRF 17
TRF 11
TRF 19
TRF 8
TRF 12
BCFA
butyrate
TRF 23


d
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-1.0
32.9%
-1.0
63.3%
1.0
T1/2
TRF 23
cellulose
TRF 16
iNSP
Vf
TRF 20
TRF 22
TRF 5
butyrate
TRF 11
TRF 14
TRF 10
TRF 21
lag t
amylose
tNSP
TRF 6
CP
acetate
BCFA
starch
propionate
sNSP
amylopectin
TRF 15
ß-glucan
TRF 7
TRF 17
TRF 9
dDM
TRF 12
TRF 19
TRF 8
SCFA

Figure 3a-d. Redundancy analysis (RDA) of the effect of nutrient composition (**black arrows**) on response variables (dashed arrows) including *in vitro* fermentation characteristics (**dDM, T1/2, Vf, lag t**), dominant bacterial phylotypes (TRF #, referring to species in Figure 2) and bacterial metabolite molar ratios (SCFA, BCFA, acetate, propionate, butyrate). RDA was performed for all cereals together (n=28, **a**), barleys only (n=16, **b**), oat and oat groats only (n=12, **c**), and hulless barleys only (n=10, **d**). Within the RDA analysis, the length, direction and the ankle between arrows are a direct measure of correlations between variables or variables and canonical axes (e.g. $\alpha = 0°/r = 1$; $\alpha = 90°/r = 0$; $\alpha = 180°/r = -1$). Percentage values on axis 1 and 2 (e.g. 67.6% and 12.1% in Figure 3a) indicate the proportions of variability of data that are described with the respective canonical axis in the model. Significance of overall model and the effect of nutrients were tested using Monte Carlo permutation test (n=499)

Table S1. Chemical composition of cereal varieties

Name	DM[1]	OM	CP	EE	cellulose	lignin	starch	amylose	amylo-pectin	β-glucan	NSP		
											total	insoluble	soluble
	%	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	% of total starch		g/kg	g/kg	g/kg	g/kg
Hulless barleys													
SB 90354	90.5	984	161	25	21	6	604	27	73	77	117	52	66
SB 90300	90.7	983	174	23	25	6	604	26	74	46	93	40	53
CDC Rattan	90.6	979	164	33	20	5	534	10	91	87	123	57	66
SR 93139	91.0	982	179	31	19	6	544	11	89	112	132	47	85
CDC McGwire	90.4	983	152	28	29	6	589	26	74	60	77	54	23
CDC Fibar	91.0	981	197	35	25	12	525	7	93	127	153	52	101
SH 99250	90.9	979	177	19	31	08	495	39	61	91	114	57	58
SB 94893	91.4	977	183	21	46	11	507	46	54	87	125	89	36
SB 94917	92.3	975	178	26	45	14	519	10	90	93	149	81	68

HB393	91.2	979	193	26	31	10	488	10	90	86	91	59	32
Common barleys													
CDC Bold	92.3	975	141	16	68	21	598	28	72	47	119	93	26
CDC Dolly	90.4	973	154	16	50	12	559	28	72	59	172	98	74
McLeod	91.0	973	142	20	65	15	570	32	68	51	137	123	14
AC Metcalfe	90.4	976	141	23	59	17	582	28	72	53	151	74	77
CDC Cowboy	90.8	975	161	18	64	16	549	27	73	41	135	71	64
CDC Clyde	89.8	974	145	21	53	13	565	27	73	50	139	71	68
Oats													
CDC Dancer	91.4	963	156	51	115	37	459	24	76	30	166	142	23
Morgan	92.4	970	117	41	170	50	377	25	75	31	261	204	57
CDC ProFi	92.2	966	166	51	123	43	403	27	73	49	258	190	68
CDC Sol-Fi	91.5	963	186	40	157	59	345	23	77	38	215	184	31
HiFi	91.7	970	162	52	143	44	322	23	77	51	226	197	29

CDC SO-I	91.5	963	153	66	150	23	418	23	77	29	218	194	25
Oat groats													
CDC Dancer	90.9	981	195	54	17	9	597	24	76	48	132	76	56
Morgan	91.8	979	164	57	28	12	600	24	76	55	153	92	61
CDC ProFi	91.7	982	227	73	17	8	510	22	78	84	150	63	87
CDC Sol-Fi	90.8	981	255	58	17	11	533	22	78	68	148	45	104
HiFi	91.1	982	226	76	30	16	442	22	78	92	123	78	45
CDC SO-I	90.9	980	197	85	25	15	462	21	79	72	132	83	59

[1] DM = dry matter, OM = organic matter, EE = ether extract, CP = crude protein, NSP = non-starch polysaccharides

Table S2. Dry matter digestibility (dDM) during pepsin-pancreatin hydrolysis, *in vitro* gas production kinetics parameters (L, T1/2, Vf), total short-chain fatty acid (SCFA) production and molar ratios of individual SCFA after 72 h of *in vitro* fermentation with a fecal inoculum from growing pigs of the hydrolyzed residues of the different barley and oat varieties.

Name	dDM	L	T1/2	Vf	total SCFA	Molar ratio of SCFA			
						Acetate	Propionate	Butyrate	BCFA
	g/kg	h	h	ml/gDM cereal	mg/g	% of total SCFA			
SB 90354	0.713 ^{f}	2.1 ^{bcd}	9.1 ^{ef}	64.2 ^{g}	488.8 ^{abcde}	53.2 ^{hi}	23.1 ^{gh}	16.8 ^{ab}	5.3 ^{abcd}
SB 90300	0.753 ^{d}	1.0 ^{d}	10.5 ^{ef}	46.6 ^{k}	373.5 ^{ghi}	52.8 ^{i}	23.9 ^{efg}	16.1 ^{ab}	6.1 ^{a}
CDC Rattan	0.666 ^{i}	1.1 ^{d}	9.4 ^{ef}	74.0 ^{e}	455.6 ^{abcdefgh}	53.0 ^{hi}	23.9 ^{efg}	16.2 ^{ab}	5.4 ^{abc}
SR 93139	0.690 ^{gh}	1.2 ^{d}	8.3 ^{ef}	70.3 ^{f}	514.2 ^{ab}	52.5 ^{i}	24.3 ^{def}	16.0 ^{ab}	5.6 ^{abc}
CDC McGwire	0.734 ^{e}	1.0 ^{d}	9.6 ^{ef}	59.3 ^{h}	518.2 ^{ab}	53.7 ^{ghi}	22.1 ^{hi}	17.0 ^{ab}	5.6 ^{abc}
CDC Fibar	0.706 ^{fg}	1.2 ^{d}	8.5 ^{ef}	64.2 ^{g}	535.5 ^{a}	53.1 ^{hi}	24.5 ^{de}	15.1 ^{abcd}	5.9 ^{ab}
SH 99250	0.512 ^{n}	4.0 ^{a}	9.6 ^{ef}	114.6 ^{b}	505.8 ^{abc}	53.2 ^{hi}	22.2 ^{hi}	17.2 ^{a}	5.5 ^{abc}
SB 94893	0.450 ^{o}	4.2 ^{a}	10.1 ^{ef}	123.5 ^{a}	499.7 ^{abcd}	53.4 ^{hi}	22.0 ^{ij}	17.0 ^{ab}	5.8 ^{abc}

SB 94917	0.549 ^{m}	1.1 ^{d}	9.9 ^{ef}	94.3 ^{c}	413.3 ^{defghi}	53.3 ^{hi}	23.4 ^{fg}	16.5 ^{ab}	5.4 ^{abc}
HB393	0.697 ^{gh}	0.9 ^{d}	9.2 ^{ef}	62.3 ^{g}	473.0 ^{abcdef}	54.4 ^{fghi}	23.1 ^{gh}	15.3 ^{abc}	5.9 ^{ab}
CDC Bold	0.711 ^{gf}	1.0 ^{d}	10.5 ^{ef}	48.3 ^{jk}	368.9 ^{hi}	57.4 ^{e}	22.1 ^{hi}	15.4 ^{abc}	4.1 ^{f}
CDC Dolly	0.696 ^{fgh}	1.4 ^{cd}	10.2 ^{ef}	55.4 ^{i}	359.5 ^{i}	56.8 ^{ef}	22.1 ^{hi}	15.7 ^{ab}	4.2 ^{f}
McLeod	0.551 ^{m}	3.2 ^{abc}	10.5 ^{ef}	88.2 ^{d}	382.9 ^{ghi}	56.6 ^{ef}	20.3 ^{lm}	16.9 ^{ab}	4.3 ^{ef}
AC Metcalfe	0.682 ^{h}	2.0 ^{bcd}	10.4 ^{ef}	57.3 ^{hi}	380.9 ^{ghi}	57.0 ^{e}	21.4 ^{ijk}	16.0 ^{ab}	4.1 ^{f}
CDC Cowboy	0.693 ^{gh}	1.6 ^{cd}	10.8 ^{ef}	51.2 ^{j}	354.9 ^{i}	57.2 ^{e}	21.8 ^{ij}	14.9 ^{bde}	5.0 ^{cde}
CDC Clyde	0.651 ^{i}	2.3 ^{bcd}	10.8 ^{ef}	68.6 ^{f}	417.6 ^{cdefghi}	55.9 ^{efg}	22.1 ^{hi}	16.0 ^{ab}	4.6 ^{def}
CDC Dancer	0.689 ^{h}	0.7 ^{d}	17.3 ^{c}	24.5 ^{mno}	177.4 ^{j}	65.1 ^{c}	20.4 ^{kl}	10.9 ^{ghij}	2.8 ^{gh}
Morgan	0.593 ^{l}	0.7 ^{d}	20.5 ^{b}	27.5 ^{m}	147.8 ^{j}	68.2 ^{b}	19.1 ^{n}	10.1 ^{ij}	2.0 ^{h}
CDC ProFi	0.669 ^{i}	1.0 ^{d}	11.6 ^{e}	32.6 ^{l}	212.1 ^{j}	59.7 ^{d}	23.3 ^{g}	12.0 ^{fghi}	4.2 ^{ef}
CDC Sol-Fi	0.628 ^{jk}	0.4 ^{d}	14.2 ^{d}	24.9 ^{mno}	130.9 ^{j}	64.8 ^{c}	20.7 ^{jkl}	10.7 ^{hij}	3.1 ^{g}
HiFi	0.619 ^{k}	0.1 ^{d}	19.3 ^{b}	25.2 ^{mn}	140.1 ^{j}	68.4 ^{b}	19.3 ^{mn}	9.7 ^{jk}	2.1 ^{h}
CDC SO-I	0.639 ^{j}	3.6 ^{ab}	30.0 ^{a}	67.2 ^{f}	379.9 ^{ghi}	71.2 ^{a}	20.0 ^{lmn}	7.6 ^{k}	0.7 ^{i}

CDC Dancer	0.897 [a]	1.1 [d]	8.4 [ef]	19.3 [p]	437.9 [bcdefghi]	54.9 [efghi]	25.8 [ab]	13.1 [def]	5.5 [abc]
Morgan	0.877 [bc]	1.1 [d]	9.1 [ef]	19.7 [p]	397.4 [fghi]	56.5 [ef]	24.6 [cde]	13.1 [def]	5.2 [bcd]
CDC ProFi	0.876 [bc]	1.1 [d]	8.1 [f]	24.6 [mno]	459.4 [abcdefg]	53.8 [ghi]	26.6 [a]	13.0 [efg]	5.7 [abc]
CDC Sol-Fi	0.879 [bc]	1.5 [cd]	8.0 [f]	23.0 [nop]	421.2 [cdefghi]	55.5 [efgh]	25.6 [abc]	12.6 [fgh]	5.7 [abc]
HiFi	0.863 [c]	1.4 [cd]	8.9 [ef]	21.1 [op]	364.5 [i]	56.7 [ef]	25.2 [bcd]	12.3 [fgh]	5.3 [abcd]
CDC SO-I	0.884 [b]	1.3 [d]	9.0 [ef]	21.8 [nop]	404.9 [efghi]	54.4 [fghi]	26.3 [a]	13.3 [cdef]	5.3 [abcd]
SEM	0.00667	0.13	0.54	2.86	13.0	0.575	0.227	0.290	0.147

Different superscripts within a column indicate significant (P<0.05) differences.