Animal Feed Science and Technology 163 (2011) 185–193

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

In vitro evaluation of the fermentation characteristics of the carbohydrate fractions of hulless barley and other cereals in the gastrointestinal tract of pigs

R. Jha[a,b], J. Bindelle[a,b,c,d], B. Rossnagel[e], A. Van Kessel[b], P. Leterme[a,*]

[a] *Prairie Swine Centre Inc., 2105 8th St. E., Saskatoon, SK, Canada S7H 5N9*
[b] *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Canada S7N 5A8*
[c] *University of Liège, Gembloux Agro-Bio Tech, Animal Science Unit, 2 Passage des Déportés, 5030 Gembloux, Belgium*
[d] *Fond de la Recherche scientifique-FNRS, 5 Rue d'Egmont, 1000 Brussels, Belgium*
[e] *Crop Development Centre, 51 Campus Drive, University of Saskatchewan, Saskatoon, Canada S7N 5A8*

ARTICLE INFO

Article history:
Received 4 November 2009
Received in revised form 13 October 2010
Accepted 14 October 2010

Keywords:
Hulless barley
NSP
β-Glucan
In vitro fermentation
Pig intestines

ABSTRACT

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

Abbreviations: AA, acetic acid; ADF, acid detergent fibre; AOAC, Association of Official Analytical Chemists; BA, butyric acid; BCFA, branched-chain fatty acids; βG, β-glucan; CDC, Crop Development Centre; CHO, Carbohydrate; CP, crude protein; DF, dietary fibre; DM, dry matter; GC, gas chromatography; hB, hulless barley; HB, hulled barley; iNSP, insoluble non-starch polysaccharides; *IVDMD*, *In vitro* dry matter degradability; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; OG, oat groat; PA, propionic acid; SCFA, short-chain fatty acids; sNSP, soluble non-starch polysaccharides.

* Corresponding author at: Prairie Swine Centre Inc. P.O. Box 21057, 2105 8th St. E., Saskatoon, SK, Canada S7H 5N9. Tel.: +1 306 667 7445; fax: +1 306 955 2510.
E-mail address: pascal.leterme@usask.ca (P. Leterme).

0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2010.10.006

and less ammonia than hulled barley and oat. Thus, hulless barleys have a better potential to be used in pig nutrition to manipulate the fermentation activity in the intestine of pigs.

1. Introduction

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

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

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

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

2. Methodology

2.1. Cereal samples

A total of 15 cereal samples (6 cultivars of hB, 3 of HB, 2 of dehulled oats (*i.e.* oat groats; OG), 3 of oats and wheat (reference)) were tested. Hulless barleys were obtained from the Crop Development Centre (CDC) at the University of Saskatchewan (Saskatoon, SK, Canada) while hulled barleys, oats and wheat were collected from different farms of Saskatchewan in 2005 and 2006. The samples were chosen according to their different fermentable carbohydrate compositions (Table 1).

2.2. Enzymatic hydrolysis

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

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

After hydrolysis, the residues were collected by filtration on a nylon cloth (42 μm), washed with ethanol (2 × 25 ml 95% ethanol) and acetone (2 × 25 ml 99.5% acetone), dried for 24 h at 60 °C and weighed. The enzymatic hydrolysis was repeated from 5 to 11 times, depending on the degradability of each cereal in order to obtain enough samples for the *in vitro* fermentation and their analysis. The CV of *in vitro* DM disappearance during hydrolysis within and between the batches for each ingredient ranged from 0.99 to 3.31%. Hydrolyzed residues from the different replicates and batches of same ingredients were pooled for subsequent analyses (dry matter, DM; crude protein, CP; βG and starch) and *in vitro* fermentation.

Table 1
Chemical composition of the cereal samples and resulting hydrolyzed substrates (g/kg DM).

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

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

[a] H, high amylose (~40% of total starch); N, normal amylose (~25%) and Z, no amylose (~0%).

Source: Rossnagel et al. (2005) and reports of Crop Development Centre, University of Saskatchewan, Saskatoon, Canada.

[b] Feed sample (commercial grower feed, devoid of antibiotics) of pig used as donor of faeces used as inoculum for *in vitro* fermentation.

2.3. In vitro fermentation

The rate of fermentation of the hydrolyzed substrates was assessed *in vitro*, using a cumulative gas-production technique adapted to the pig by Bindelle et al. (2007): 200 mg samples were incubated at 39 °C (in a shaking water-bath with 50 rpm) in a 125 ml-glass bottle with 30 ml buffer solution containing macro- and micro-minerals (Menke and Steingass, 1988) and a faecal inoculum. Three weaned piglets (4–5 weeks age) from the herd of the Prairie Swine Centre (Saskatoon, SK, Canada), fed a standard commercial diet devoid of antibiotics (Table 1), were used as donors for the faecal inoculums. Faecal samples were collected directly from the rectum and immediately placed in air tight plastic syringes and kept in a water-bath at 39 °C until further use, but shorter than 1 h. The inoculum prepared from faeces was diluted 20 times in the buffer solution and filtered through a 250 μm-screen and transferred into the bottle with fermentation substrates. Bottles were sealed with a rubber stopper and placed for incubation. An anaerobic environment was maintained throughout the experiment, from the inoculum preparation until the incubation step by flushing with CO_2 gas. The gas generated by the fermentation process and the CO_2 released by the buffering of the SCFA produced during the fermentation were measured at 0, 2, 5, 8, 12, 18, 24, 36 and 48 h by means of a pressure transducer (GP:50 SIN-54978, Grand Island, NY, USA) (Mauricio et al., 1999), fitted with digital data tracker (Tracker 211, Intertechnology Inc., ON, Canada). The bottles were vented after every measurement. Fermentation was stopped at 48 h of incubation by quenching the bottles in iced water.

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

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

2.4. Chemical analyses

All samples and the diet (of the faeces donor pig) were ground with a laboratory mill (Retsch mill ZM1, Newton, PA, USA) to pass through 1 mm mesh screen. Chemical analyses (Table 1) were performed according to the Association of Official Analytical Chemists standard procedures (AOAC, 2007) with specific methods as follows: DM (135 °C for 2 h, AOAC 930.15), nitrogen (AOAC 968.06; using an elemental analyzer LECO FP528, St Joseph MI, USA; CP = N × 6.25), ether extract using Soxhlet apparatus and petroleum ether (AOAC 920.39), ash (AOAC 942.05), ADF (AOAC 973.18) and NDF (AOAC 2002.04).

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

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

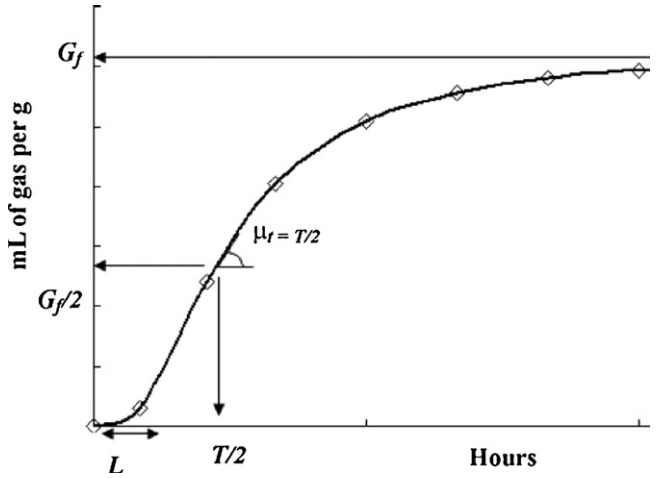

Fig. 1. Representation of the kinetic parameters of the gas accumulation curves modelled according to France et al. (1993), where G_f (mL gas/g of substrate) denotes the maximum gas volume for $t = \infty$, L (h) denotes the lag time before fermentation starts, $T/2$ denotes the half-time to asymptotic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

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

2.5. Calculations and statistical analyses

2.5.1. In vitro degradability

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

$$IVDMD = \frac{\text{dry weight of the sample before hydrolysis} - \text{dry weight of the residue}}{\text{dry weight of the sample before hydrolysis}} \quad (1)$$

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

2.6. Kinetics of gas production

Gas pressure measurements were converted into gas volume (G, g^{-1} DM) using the ideal gas law, assuming an atmospheric pressure of 101,325 Pa and a temperature of 312.15 K. Gas accumulation curves recorded during the 48 h of fermentation were modelled according to France et al. (1993):

$$\begin{aligned} G(\text{ml g}^{-1}\text{ DM}) &= 0, && \text{if } 0 < t < L \\ &= G_f\left(1 - \exp\left\{-\left\langle b(t-L) + c\left(\sqrt{t} - \sqrt{L}\right)\right\rangle\right\}\right), && \text{if } t \geq L \end{aligned} \quad (2)$$

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

$$\mu = b + \frac{c}{2\sqrt{t}}, \quad \text{if } t \geq L \quad (3)$$

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

2.7. Statistical analyses

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

$$Y = \alpha + S_i + B_j + \varepsilon_{ij} \quad (4)$$

Table 2
Degree of enzymatic hydrolysis of the different cereal samples.

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

Abbreviations: hB, hulless barley; HB, hulled barley; OG, oat groat.
Means with different superscripts within the column are significantly different ($P < 0.05$).
[1] Number of replicates of enzymatic hydrolysis.
[2] Values from pooled samples of different replicates of enzymatic hydrolysis, thus statistical analysis could not be conducted.

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

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

3. Results

3.1. Degree of enzymatic hydrolysis of the cereals

Table 2 details the results of degree of hydrolysis of the cereals. Differences in *IVDMD* were observed (P<0.001) between samples. Higher values were observed for OG (0.88–0.90) whereas those obtained for whole oat, hB and HB, ranged from 0.63 to 0.73, 0.68 to 0.80 and 0.69 to 0.71, respectively. No statistical analysis could be performed on the degree of hydrolysis of CP, βG and starch because samples from the different batches of enzymatic hydrolysis were pooled. No specific pattern was noted for protein degradability among cereal types. It ranged from 0.76 to 0.89, the highest value being observed for wheat and the lowest for CDC Bold hulled barley and Morgan oat. In general, βG degradability was higher in oat than in barley, irrespective of the presence or absence of hull, except for CDC Fibar barley, which had similar βG degradability to oat. A similar trend was noted for starch degradability, with 0.92 on average for barley and 0.97 for oat.

3.2. Kinetics of gas production

In general, all fermentation kinetics parameters of gas production varied according to cereal type but also between some cultivars (P<0.05) (Table 3). Hulless barleys were fermented more rapidly and yielded more gas than hulled barleys, oats and wheat. The fractional rate of degradation was similar in hB and OG (0.15/h, on average) and higher than that of HB cultivars (0.12/h, on average). Finally, total gas production was the highest for hB (ranging from 218 to 235 ml/g of DM substrate incubated and from 427 to 621 ml/g CHO fermented) and the lowest for oat (from 48 to 61 ml/g DM substrate incubated and from 170 to 237 ml/g CHO fermented). Due to the very slow rate of fermentation, the gas production curves of the oat cultivars did not fit France's model. Differences of fermentation kinetics were also observed between the hB cultivars. The SH99073 and SH99250 hB started to ferment slowly (lag time of 1.4 and 1.7 h, respectively) but produced more gas than the other hB cultivars while CDC Fibar started to ferment sooner (lag time 0.7 h). The SB90300 hB cultivar degraded rapidly and the CDC Fibar cultivar slowly (fractional rate of degradation being 0.17 and 0.12/h, respectively) but they produced a similar amount of total gas (218 ml/g DM incubated). Expressed per g of CHO fermented, SB90300 hB produced the highest amount of both total gas and SCFA.

3.3. Profile of end-products after cereal fermentation

SCFA production was higher for hB, compared to HB and oat (6.1 *vs.* 4.9 and 1.5 mmol/g DM incubated, respectively; P<0.05), but, conversely to fermentation kinetics, no difference in SCFA production was observed between hB cultivars (Table 4). When SCFA production was expressed per g CHO fermented, similar trends were found among cereal types, and

Table 3
Fitted kinetics parameters (means) of gas accumulation recorded for different hydrolyzed substrates incubated with a faecal inoculum from pigs.

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

Abbreviations: CHO, carbohydrates (non-starch polysaccharides + starch); hB, hulless barley; HB, hulled barley; OG, oat groat.
NA, not available; data not presented as these parameters do not fit in fermentation kinetics model used.
Means with different superscripts within the columns are significantly different (P<0.05).
[1] N, number of observations in fermentation.
[2] L, lag time (h).
[3] $T/2$, half-time to asymptote (h).
[4] μ, fractional rate of degradation (h^{-1}) at $t = T/2$.
[5] G_f, maximum gas volume (ml per g DM incubated/CHO fermented).
[6] N, number of observation in pooled fermented substrates.

differences (P<0.05) were observed between hB cultivars. SB90300 hB yielded the highest SCFA production (16.1 mmol/g CHO fermented) while SH99073 hB had the lowest SCFA production (11.1 mmol/g CHO fermented). In contrast, ammonia concentration was higher (P<0.05) for oat than for barley (4.5 *vs.* 2.3 mmol/g CHO fermented on average, respectively).

SCFA molar ratio profiles differed between ingredients. Oat with higher hull content had higher ratios of acetate (0.57–0.61) and lower of butyrate (0.13–0.19), while hulless barley yielded lower acetate (0.46–0.50) and higher butyrate (0.16–0.20) proportion in the profile.

Table 4
Concentration of metabolites in solution after fermentation in a faecal inoculum (mmol/g DM incubated or carbohydrate fermented).

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

Abbreviations: AA, acetic acid; BA, butyric acid; BCFA, branched-chain fatty acids (sum of iso-butyric and iso-valeric acids); CHO, carbohydrates (non-starch polysaccharides + starch); hB, hulless barley; HB, hulled barley; N, Number of observation in fermentation; NH_3, Ammonia; OG, oat groat; PA, propionic acid; SCFA, short-chain fatty acids.
Means with different superscripts within the columns are significantly different (P<0.05).
[1] Percentage of the individual SCFA.

3.4. Correlations between carbohydrate components and fermentation parameters

Correlations were found between the carbohydrate composition of the ingredients and their fermentation parameters. The βG content measured in the hydrolyzed substrates correlated positively with the fractional rate of degradation ($r = 0.29$, P=0.004), total gas (G_f) and SCFA production ($r = 0.64$ and $r = 0.65$, respectively, P<0.001). There was a strong correlation between the starch content of the hydrolyzed substrates with total gas (G_f) and SCFA production ($r = 0.86$ and $r = 0.83$, respectively, P<0.001).

4. Discussion

This study aimed to investigate how the indigestible carbohydrate fraction of hB could improve fermentation patterns in the large intestine of pigs, as opposed to hulled barley, oat or wheat. Cultivars differing in β-glucan content and amylose/amylopectin ratio were therefore tested. The main difference with the other cereal types in terms of chemical composition lied in their insoluble fibre contents, due to the absence of hulls.

The *IVDMD* after pepsin–pancreatin hydrolysis varied among cereal types and cultivars but, as already observed by Bindelle et al. (2010), comparing hB to HB, it was not influenced by the presence or absence of hulls. Conversely, the amylose/amylopectin ratio possibly influenced differences in enzymatic degradability of the different hB cultivars, as attested by the highest *IVDMD* and the high starch digestibility obtained for CDC Fibar. The starch of the latter is essentially composed of amylopectin (Rossnagel et al., 2005). The starch content of cultivars rich in amylose is less degraded during enzymatic hydrolysis in the small intestine (Pierce and Stevenson, 2008). The undigested starch, called "resistant starch", is further available for microbial fermentation in the large intestine (Topping and Clifton, 2001). The higher βG degradability observed for oat, compared to the barleys, may be attributed to differences in βG structure which induces higher loss of that soluble fibre during the filtration step preceding the fermentation in the *in vitro* model. Lambo et al. (2005) observed that 68% of βG was soluble in oat *vs*. only 15% in barley.

As hypothesized, the differences in IVDMD of the different carbohydrate fractions described above, lead to differences in fermentation kinetics and metabolite production between the cereal types. Differences between cultivars were also observed, especially for hB and ascribed to differences in the soluble fraction of the NSP, including βG (Jha et al., 2010) and the amylose/amylopectin ratio of starch (Li et al., 2001). Some hB contains higher levels of sNSP. The sNSP are fermented faster and more extensively by intestinal microbes than iNSP (Brøbech et al., 1993). As a consequence, they favoured higher SCFA production in hB and lower NH_3 concentration in the fermentation broth, compared to HB and oat (Jha et al., 2010). The βG content measured in the hydrolyzed substrates correlated positively, although weak, with the fractional rate of degradation ($r = 0.29$, P=0.004), total gas (G_f) and SCFA production ($r = 0.64$ and $r = 0.65$, respectively, P<0.001). The reduction in NH_3 concentration indicates lower protein fermentation in the presence of hB indigestible carbohydrates. This could explain why Pieper et al. (2009), in an *in vitro* experiment using the same barley varieties and aiming at testing the effect on Salmonella growth, observed that hB varieties reduced the proportion of Salmonella in the medium.

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

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

As expected, higher butyrate proportion was observed for the hB having high resistant starch content (SH99250, SH99073, among others) and higher acetate was measured in cereal types with high iNSP contents, *i.e.* HB and oats. Butyrate is an important metabolite as it is the principal oxidative fuel for the colonocytes. Pieper et al. (2008) detected *L. sobrius*- and *B. pseudolongum*-like phylotype in the ileum of high butyrate yielding-hulless barleys. Thus, these hB varieties may change the ecosystem of the gastrointestinal tract, as bifidobacteria and lactobacilli exert antimicrobial properties and may modulate the defence system of the host's gastrointestinal tract (Servin, 2004).

There were some differences in both the fermentation kinetics and the end-products between cereal types as well as within the hB cultivars that cannot be explained only by their carbohydrate fractions. This may be due to the interaction between the composition of the cereal samples, the inoculum and the *in vitro* model itself. The extent of fermentation and the profile in SCFA depend on the substrate (Macfarlane and Macfarlane, 2003) and the microbiota available during the fermentation process (Williams et al., 2001). This gives a complex fermentation process.

The observations regarding the fermentation process and the relationships with cereal composition require further *in vivo* confirmation for several reasons. First, the current model does not account for microbes/host interactions during the fermentation process. Moreover, the fine grinding of all cereal samples (1 mm-mesh screen) might not reflect actual differences

in particle size after chewing by the pigs between cereal types (*e.g.* hB *vs*. HB). This might also affect the digestive process in the upper gut and the fermentation process in a way not considered with the model (Anguita et al., 2007). Finally, there is also a limit in the capacity of the *in vitro* model to mimic the digestive process in the pig's gastrointestinal tract. The 1 mm particle size substrate is used for enzymatic hydrolysis and fermentation, which is not the case *in vivo*. A decrease in particle size increases surface area for enzymatic/microbial digestion and fermentation, which influences the digestive process in the gastrointestinal tract (Anguita et al., 2006, 2007). This also suggests that an enzymatic pre-treatment followed by drying could make the DF more susceptible to bacterial degradation than what happens *in vivo,* as freeze-drying and pre-digestion change the micro- and macro-porosity of cell walls (Lebet et al., 1996). The fermentability of the substrates could thus have been overestimated (Wisker et al., 1998). On the other hand, the βG disappearance during the enzymatic hydrolysis represents a fraction that could be available to microbiota in the pig intestines and could also rapidly ferment, already in the distal small intestine. However, the effect of such pre-enzymatic treatment is likely to be less important for highly fermentable substrates. On the other hand, the *in vitro* gas technique uses the total gas produced as an indicator of fermentation rather than of substrate disappearance and the gas produced does not come only from the microbial fermentation of the substrate but also from the bicarbonate buffer used in all *in vitro* fermentation methods (Coles et al., 2005). Moreover, the mathematical model used could not be used for all the substrates. This can be ascribed partly to the fact that, after pepsin–pancreatin hydrolysis, the residual fast-fermenting substrates such as resistant starch are embedded into the whole fibrous matrix, which lowers the actual fermentation rate of these substrates. Moreover, their fermentation might not be complete after 48 h. The average transit time of a substrate in the pig gastrointestinal tract is around 44 h, including in the stomach and small intestine, which leaves more or less 40 h in the large intestine. The time used here for fermentation was slightly longer (48 h) but still comparable to an *in vivo* situation. As an alternate, biphasic models were tested in previous studies by the authors of this study, using the model of Groot et al. (1996). Nevertheless, the monophasic model of France et al. (1993) always gave similar or even better results in terms of fitting the experimental curve to the model.

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

Acknowledgements

Funding of National Pork Board (project # 06-117) and Alberta Barley Commission (project # 60-192) for this study are acknowledged. The continuing core support of the Prairie Swine Centre received from Sask Pork, Manitoba Pork Council, Alberta Pork and the Saskatchewan Agriculture Development Fund is gratefully acknowledged. The technical assistance of Brian Grimmelt and Pam Kish in the laboratory is gratefully acknowledged.

References

Anguita, M., Gasa, M., Martín-Orúe, S.M., Pérez, J.F., 2006. Study of the effect of technological processes on starch hydrolysis, non-starch polysaccharides solubilization and physicochemical properties of different ingredients using a two-step in vitro system. Anim. Feed Sci. Technol. 129, 99–115.

Anguita, M., Gasa, J., Nofrarias, M., Martín-Orúe, S.M., Pérez, J.F., 2007. Effect of coarse ground corn, sugar beet pulp and wheat bran on the voluntary intake and physicochemical characteristics of digesta of growing pigs. Livest. Sci. 107, 182–191.

AOAC, 2007. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Awati, A., Williams, B.A., Bosch, M.W., Gerrits, W.J.J., Verstegen, M.W.A., 2006. Effect of inclusion of fermentable carbohydrates in the diet on fermentation end-product profile in feces of weanling piglets. J. Anim. Sci. 84, 2133–2140.

Bach Knudsen, K.E., 2001. The nutritional significance of "dietary fibre" analysis. Anim. Feed Sci. Technol. 90, 3–20.

Bindelle, J., Buldgen, A., Boudry, C., Leterme, P., 2007. Effect of inoculum and pepsin–pancreatin hydrolysis on fibre fermentation measured by the gas production technique in pigs. Anim. Feed Sci. Technol. 132, 111–122.

Bindelle, J., Pieper, R., Leterme, P., Rossnagel, B., Van Kessel, A.G., 2010. Changes in intestinal microbial ecophysiology as related to the carbohydrate composition of barleys and oats cultivars in an *in vitro* model of the pig gastrointestinal tract. Livest. Sci. 133, 151–153.

Boisen, S., Fernandez, J.A., 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68, 277–286.

Brøbech Mortensen, P., Nordgaard-Andersen, I., 1993. The dependence of the in vitro fermentation of dietary fibre to short-chain fatty acids on the contents of soluble non-starch polysaccharides. Scand. J. Gastroenterol. 28, 418–422.

Charalampopoulos, D., Wang, R., Pandiella, S.S., Webb, C., 2002. Application of cereals and cereal components in functional foods: a review. Int. J. Food Mircobiol. 79, 131–141.

Coles, L.T., Moughan, P.J., Darragh, A.J., 2005. In vitro digestion and fermentation methods, including gas production techniques, as applied to nutritive evaluation of foods in the hindgut of humans and other simple-stomached animals. Anim. Feed Sci. Technol. 123-124, 421–444.

Dongowski, G., Huth, M., Gebhardt, E., Flamme, W., 2002. Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. J. Nutr. 132, 3704–3714.

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

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R., Isac, D., 1993. A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. J. Theor. Biol. 163, 99–111.

Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A., Lantinga, E.A., 1996. Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. Anim. Feed Sci. Technol. 64, 77–89.

Izydorczyk, M.S., Storsley, J., Labossiere, D., MacGregor, A.W., Rossnagel, B.G., 2000. Variation in total and soluble beta-glucan content in hulless barley: effects of thermal, physical, and enzymatic treatments. J. Agr. Food Chem. 48, 982–989.
Jha, R., Rossnagel, B., Pieper, R., Van Kessel, A.G., Leterme, P., 2010. Barley and oat cultivars with diverse carbohydrate composition alter ileal and total tract nutrient digestibility and fermentation metabolites in weaned piglets. Animal 4, 724–731.
Lambo, A.M., Oste, R., Nyman, M.E.G.L., 2005. Dietary fibre in fermented oat and barley beta-glucan rich concentrates. Food Chem. 89, 283–293.
Le, P.D., Aarnink, A.J.A., Ogink, N.W.M., Becker, P.M., Verstegen, M.W.A., 2005. Odour from animal production facilities: its relationship to diet. Nutr. Res. Rev. 18, 3–30.
Lebet, V., Jorger, F., Arrigoni, E., Wenk, C., Amado, R., 1996. Influence of a simulated digestion procedure on in vitro colonic fermentation patterns. In: Malkki, Y., Cummings, J.H. (Eds.), Dietary Fibre and Fermentation in the Human Colon. Proceedings of the COST Action 92 Workshop, Brussels, pp. 101–109.
Li, J.H., Vasanthan, T., Rossnagel, B., Hoover, R., 2001. Starch from hull-less barley: I Granule morphology, composition and amylopectin structure. Food Chem. 74, 395–405.
Macfarlane, S., Macfarlane, G.T., 2003. Regulation of short-chain fatty acid production. P. Nutr. Soc. 62, 67–72.
Mauricio, R.M., Moulda, F.L., Dhanoab, M.S., Owena, E., Channaa, K.S., Theodorou, M.K., 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Technol. 79, 321–330.
Menke, K.H., Steingass, H., 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28, 7–55.
Novozamsky, I., van Eck, R., van Schouwenburg, J.C., Walinga, I., 1974. Total nitrogen determination in plant material by means of the indophenolblue method. Neth. J. Agr. Sci. 22, 3–5.
Pieper, R., Bindelle, J., Rossnagel, B., Van Kessel, A.G., Leterme, P., 2009. Effect of carbohydrate composition in barley and oat cultivars on microbial ecophysiology and proliferation of Salmonella enterica in an in vitro model of the porcine gastrointestinal tract. Appl. Environ. Microbiol. 75, 7006–7016.
Pieper, R., Jha, R., Rossnagel, B., Van Kessel, A.G., Souffrant, W.B., Leterme, P., 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiol. Ecol. 66, 556–566.
Pierce, J., Stevenson, Z., 2008. Nutrition and gut microbiology: redirecting nutrients from the microbes to the host animal with SSF. In: Taylor-Pickard, J.A., Spring, P. (Eds.), Gut Efficiency; The Key Ingredient in Pig and Poultry Production. Wageningen Academic Publishers, Pays-Bas, pp. 167–182.
Rossnagel, B.G., Zatorski, T., Arganosa, G., 2005. Food barley development at the Crop Development Centre, University of Saskatchewan. In: Proceedings of 18th Triennial North American Barley Researchers Workshop and 4th Canadian Barley Symposium, Red Deer, AB, pp. 15–18.
SAS, 2003. SAS User's Guide: Statistics, Version 9.1. SAS Institute Inc., Cary, NC.
Servin, A.L., 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol. Rev. 28, 405–440.
Topping, D.L., Clifton, P.M., 2001. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol. Rev. 81, 1031–1064.
Verstegen, M., Williams, B., 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. Anim. Biotechnol. 13, 113–127.
Wellock, I.J., Houdijk, J.G.M., Kyriazakis, I., 2007. Effect of dietary non-starch polysaccharide solubility and inclusion level on gut health and the risk of post weaning enteric disorders in newly weaned piglets. In: Livest. Sci. 10th International Symposium on Digestive Physiology in Pigs, Denmark 2006, Part 1 108, pp. 186–189.
Williams, B.A., Verstegen, M.W.A., Tamminga, S., 2001. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. Nutr. Res. Rev. 14, 207–227.
Wisker, E., Daniel, M., Rave, G., Feldheim, W., 1998. Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and in vitro. Br. J. Nutr. 80, 253–261.