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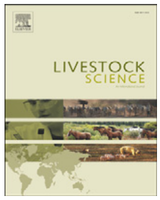

In vitro fermentation characteristics for pigs of hulless barleys differing in β-glucan content☆

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ABSTRACT

Isolated non-starch polysaccharides (NSP), especially isolated β-glucan, are reported to have prebiotic effects in pigs. However, little information is available on their possible functional properties when they are still present in the fibrous matrix of whole cereals. Hulless barleys (hB) are rich but variable sources of β-glucan. In order to evaluate their potential as functional feeds, an *in vitro* experiment was carried out to study the fermentation characteristics of 6 hB varieties and breeding lines varying in their β-glucan content (36–99 g/kg DM) in comparison to three hulled barleys (HB), two oat groats, three oats and one wheat, taken as reference. After pepsin–pancreatin hydrolysis, the ingredients were incubated in a buffered mineral solution and pig faeces (inoculum). The accumulated gas production, proportional to the amount of fibre fermented, was measured for 48 h and modelled. Short-chain fatty acid (SCFA) and ammonia concentrations were measured in the fermented solutions. A cereal type effect ($P<0.05$) was observed on the fermentation kinetics parameters. Rates of degradation and total gas productions were higher with hB than with oat ($P<0.05$). Differences were also found between hB for total gas production, lag time and rate of degradation ($P<0.01$). The production of SCFA was also higher with hB (6.1 mMol/g DM incubated; $P<0.05$) than with hulled barley and oat (4.9 and 2.9 mMol/g DM incubated respectively). In contrast, oat generated higher ammonia ($P<0.05$) production (1.4 mMol/g DM incubated, on average) than both hB and HB (1.0 mMol/g). In conclusion, hB are better fermented, produce more beneficial (SCFA) and less harmful (ammonia) metabolites and have a better potential than other cereal species to modulate gut microbiota and improve gut health.

1. Introduction

The intestinal fermentation of some non-starch polysaccharides (NSP) such as β-glucan (βG) results in the formation of short-chain fatty acids (SCFA), selected growth of enteric bacteria and reduced protein fermentation. This might positively affect gut health. Isolated βG were reported to have prebiotic effects in pigs, improving beneficial bacteria such as lactobacilli and bifidobacteria (Charalampopoulos et al., 2002). However, little information is available on the fermentation characteristics of βG when the latter are present in the fibrous matrix of whole cereals. As pig diets are formulated with whole grains, hB cultivars containing highly-fermentable NSP could possibly provide health-promoting beneficial effects to these animals.

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An experiment was conducted to compare the fermentation characteristics of different hB cultivars varying in their β-glucan content vs hulled barley, oat and wheat as a first step to screen their potential functionalities related to gut health.

2. Methodology

2.1. Ingredients

Six varieties and breeding lines of hulless barley (hB), 3 hulled barleys (HB), 3 oats as well as 2 oat groats (i.e. dehulled oats; OG) and 1 of wheat were chosen according to their carbohydrate composition (Table 1).

2.2. In vitro hydrolysis and fermentation

The ingredients underwent an *in vitro* pepsin–pancreatin hydrolysis (Boisen and Fernandez, 1997). Residues were filtered and used subsequently for an *in vitro* gas fermentation test using pig faeces as inoculum (Bindelle et al., 2007). The experiment was conducted according to the following scheme: (15 varieties/lines × 2 replicates + 6 blanks) × 4 runs yielding 8 observations per variety or breeding line. Gas production was recorded at regular intervals for 48 h. Subsequently, fermentation residues were sampled for SCFA and ammonia analysis.

2.3. Kinetics of gas production

Gas accumulation was modelled according to France et al. (1993) and described with the following parameters: final gas production (*Gf*, ml g^{-1}DM), lag time prior to fermentation start (*L*, h), half-time to asymptote when gas production is half of *Gf* (*T*/2, h) and fractional rate of degradation of the substrate at *T*/2 (μ, h^{-1}).

2.4. Chemical and statistical analyses

Chemical analysis of the ingredients was done as follows: dry matter (DM) (AOAC 930.15), nitrogen (AOAC 968.06 using a LECO FP528 elemental analyzer), NDF (AOAC 2002.04) and ADF (AOAC 973.18). Commercial test kits (Megazyme Int. Ltd., Ireland) were used to determine βG (AOAC 995.16) and total starch (AOAC 996.11) content. SCFA were analysed by GC (Agilent 6890 system, Germany) using crotonic acid as internal standard. Ammonia N concentration was determined by oxidizing with sodium hypochloride in the presence of sodium nitroprusside and reading the absorbance at 600 nm using a spectrophotometer (Novozamsky et al., 1974).

Fermentation characteristics were compared using the mixed model procedure of SAS (SAS, 2003) with the ingredient as fixed factor and occasion as random factor. Means were separated using the Tukey method with a significance level of 0.05.

Table 1
Chemical composition of the cereal samples (g/kg DM).

Cereal type	Cultivar	Dry matter	Crude protein	NDF	ADF	β-Glucan	Starch
hB	SB94893	932	176	150	27	81	525
hB	CDC Fibar	933	154	110	17	99	515
hB	SH99250	935	134	155	21	91	513
hB	CDC McGwire	930	153	113	23	52	587
hB	SH99073	933	144	156	30	96	476
hB	SB90300	933	131	103	21	36	593
HB	CDC Bold	930	108	172	64	38	594
HB	McLeod	932	139	175	58	49	561
HB	CDC Helgason	935	129	145	49	47	542
OG	Morgan	938	138	89	23	51	558
OG	CDC Sol-FI	934	174	117	29	63	549
Oat	Morgan	946	99	390	198	31	276
Oat	CDC Sol-FI	942	127	324	161	46	324
Oat	CDC Baler	899	165	310	145	29	458
Wheat	Common wheat	915	160	134	36	7	513

hB, hulless barley; HB, hulled barley; OG, oat groat.

3. Results

All fermentation parameters varied according to the cereal type but also between some cultivars ($P<0.05$) (Table 2). In general, hulless barley degraded more rapidly and yielded higher total gas production than wheat, HB and oat cultivars. Final gas production and kinetics of fermentation as measured through the lag time and the rate of degradation also differed according to the hB cultivar. SCFA production was higher from hB than from HB and oat (6.1 vs 4.9 and 2.9 mMol/g DM incubated respectively; $P<0.05$), but no difference between hB cultivars was observed. In contrast, ammonia production was higher ($P<0.05$) in oat than in barley (1.4 vs 1.0 mMol/g DM incubated on average), with no difference ($P>0.05$) between hulled and hulless barley. Due to very slow fermentation, the gas production curves of oat cultivars did not fit France's model.

4. Discussion

The overall differences in fermentation characteristics observed between grain types and cultivars, can be attributed to their chemical composition, especially their carbohydrate fraction. Hulless barleys, which contain higher levels of soluble NSP, are favoured substrates for fermentation by microbiota (Glitsø et al., 1999). This was confirmed by the correlations observed between the βG content of the hydrolyzed substrates (data not shown) and the fractional rate of degradation ($r=0.29$, $P=0.0036$) or the final gas production (*Gf*) ($r=0.64$, $P<0.0001$). They degrade rapidly and are better fermented than insoluble NSP, which explains the higher production of SCFA with hB, compared to HB and oats. However, CDC Fibar had the lowest degradation rate and time to half asymptote gas production among the hB. This can be explained by the high solubilisation of βG during the enzymatic hydrolysis (67%), compared to the other hB cultivars (45% on average). It is not retained during the filtration and is excluded from the fermentation step. The solubilisation of βG during the enzymatic hydrolysis appears thus to be differently influenced by the complex fibre matrix in the grain, depending on the cultivar. The loss of βG might also be regarded as a limitation of the *in vitro* method since *in vivo*, the whole βG fraction of the grain is expected to undergo the fermentation. Moreover, almost all the starch of the CDC Fibar cultivar is composed of amylopectin, which is solubilised during hydrolysis, while the other cultivars contain

Table 2
Fitted kinetics parameters (means) on the gas production and fermentation metabolites (mMol/g DM incubated) of different substrates incubated with pig faeces as inoculum.

Cereal type	Cultivars	*N*	L	T/2	μ	*Gf*	SCFA	NH_3
hB	SB94893	8	1.3^{ab}	7.4^{cde}	0.16^{ab}	225^{ab}	6.3^{a}	1.0^{c}
hB	CDC Fibar	8	0.7^{b}	6.9^{ef}	0.12^{d}	218^{bc}	6.1^{ab}	1.1^{bc}
hB	SH99250	8	1.7^{a}	8.4^{a}	0.15^{ab}	235^{a}	6.2^{ab}	1.0^{c}
hB	CDC McGwire	8	0.9^{b}	6.8^{f}	0.16^{ab}	225^{ab}	6.1^{ab}	1.1^{c}
hB	SH99073	8	1.4^{ab}	8.0^{ab}	0.15^{bc}	228^{a}	6.0^{ab}	1.0^{c}
hB	SB90300	8	1.4^{ab}	6.8^{f}	0.17^{a}	218^{bc}	5.7^{abc}	1.0^{c}
HB	CDC Bold	8	0.9^{b}	7.1^{def}	0.12^{d}	191^{d}	5.1^{cd}	1.1^{c}
HB	McLeod	8	0.9^{b}	7.5^{bcd}	0.11^{d}	193^{d}	5.0^{cd}	1.1^{c}
HB	CDC Helgason	8	1.0^{ab}	7.2^{def}	0.13^{cd}	180^{e}	4.7^{d}	1.0^{c}
OG	Morgan	8	1.1^{ab}	7.0^{def}	0.15^{bc}	184^{de}	5.0^{cd}	1.3^{ab}
OG	CDC Sol-FI	8	0.7^{b}	6.1^{g}	0.15^{bc}	190^{de}	5.0^{cd}	1.4^{ab}
Oat	Morgan	8	NA	NA	NA	48^{g}	1.3^{e}	1.5^{a}
Oat	CDC Sol-FI	8	NA	NA	NA	56^{fg}	1.5^{e}	1.5^{a}
Oat	CDC Baler	8	NA	NA	NA	61^{f}	1.8^{e}	1.4^{ab}
Wheat	Common wheat	8	0.9^{b}	7.9^{abc}	0.12^{d}	213^{c}	5.5^{bc}	1.0^{c}
SEM			0.18	0.55	0.004	3.3	0.14	0.92
P value			0.005	<.0001	<.0001	<.0001	<.0001	<.0001

N, number of observations in fermentation; *L*, lag time (h); *T*/2, half-time to asymptote (h); μ, fractional rate of degradation at $t = T/2$ (h^{-1}); *Gf*, maximum gas volume (ml per g DM incubated); SCFA, short-chain fatty acids; NH_3, ammonia; hB, hulless barley; HB, hulled barley; OG, oat groat.
NA, not available as these parameters did not fit in the model (France et al., 1993) used.
Means with different superscripts within the columns are significantly different ($P<0.05$).

varying ratios of amylose/amylopectin (data not shown). Thus, more substrate is available for microbial fermentation.

The higher ammonia concentration observed for the oat cultivars was probably the result of a shift of fermentation toward protein, due to a lower availability of fermentable carbohydrate substrate (Macfarlane et al., 1992), as indicated by the high ADF content and the slow fermentation patterns that did not fit with the model.

In can be concluded that hulless barleys are better fermented *in vitro* and produce more beneficial (SCFA) and less harmful (ammonia) metabolites. Thus, hulless barleys have a better potential to be used in pig nutrition in order to modulate the gut environment and to improve the pig's gut health. Furthermore, the differences observed between hB cultivars indicate that some hB might also be more interesting than others.

Conflict of interest

The authors declare no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three (3) years of beginning the work submitted that could inappropriately influence (bias) their work.

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