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PAPER

In vitro fermentation of structural carbohydrate-rich feeds using faecal inoculum from pigs

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Abstract

Seven feeds were tested *in vitro* using faecal inoculum from pigs. Sugar beet pulp, wheat bran, soybean hulls, grapecake, glutamic beet pulp, citrus by-product and fructo-oligo-saccharides (FOS) were fermented for 96 h. Cumulative gas production was measured as indicator of the fermentation kinetics. At the end of incubation organic matter disappearance and fermentation end-products (short-chain fatty acids and NH_3) were also measured. The gas production profiles were fitted with a multi-phasic model. Significant differences were detected between grapecake and FOS: the very low gas production for the first one was probably due to the high lignin and tannins contents of this by-product, while the higher organic matter cumulative gas volume (OMCV) and organic matter disappearance (OMD) values for FOS were due to the high soluble fibre proportion. Soybean hulls and citrus by-product, showed similar values of degradability and gas production and were characterised by different fermentation profiles. Grapecake showed the lower fermentation, while citrus by-product was characterized high gas and short-chain fatty acids production. These characteristics could be particularly useful to optimize the caecum-colon fermentation in order to obtain a high butyrate acid production.

Introduction

Since 2003, when the European Community banned the use of antibiotics as growth promoters [Regulation (EC) No 1831/2003], a lot of studies were carried out in order to find valid alternatives to reduce enteric diseases in piglets. Particular interest was focused on substances capable to promote the bacterial microflora development in the large intestine, such as probiotics and prebiotics. These ingredients were defined functional or nutraceutical feeds, having beneficial effects behind their nutritional characteristics. Prebiotics are the most used functional ingredients in animal nutrition, because of their high shelf-life and resistance to technological procedures, including high temperature. Several fermentable carbohydrates have been classified as prebiotics (Roberfroid, 1993; Sunvold *et al.*, 1995a, 1995b) because they are non-digestible oligosaccharides and promote useful bacterial strains proliferation in the distal part of the small bowel and in the large intestine. In pigs prebiotics have been shown to improve growth performance and to decrease mortality and morbidity (Patterson and Burkholder, 2003; Konstantinov *et al.*, 2004).

In this study, the *in vitro* gas production technique (IVGPT), proposed by Theodorou *et al.* (1994), was performed, using faeces from adult swine as *inoculum,* in order to evaluate the anaerobic fermentation of seven non-structural carbohydrates sources by gut microorganisms thus assessing their potential effects on gut microbiota.

Materials and methods

Seven potential ingredients for pig diets were used as substrates. For each substrate, three different samples collected at different times were analysed in triplicate. Six by-products of different nature were selected: sugar beet pulp (SBP), glutamic acid beet pulp (GBP), wheat bran (WB), soybean hulls (SH), grapecake (GC) and citrus by-product (CBP). The last substrate, fructo-oligosaccarides (FOS), was a purified prebiotic.

Chemical composition

Each sample was milled (1.1 mm) and analysed for crude protein (CP), ether extract (EE) and ash contents according to AOAC (2000) procedures (ID members: 984.13, 920.39 and 942.05, respectively), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) were determined according to Van Soest *et al.* (1991). Starch content was determined with polarimetric detection (Polax L, Atago, Tokyo, Japan) as indicated by Martillotti *et al.* (1987).

In vitro gas production

In order to evaluate the fermentation kinetics into the large intestine, the IVGPT proposed by Theodorou *et al.* (1994) was used. Even if such technique was originally designed for ruminant species, several studies were also performed using as *inoculum* faeces from mono-gastric species (Bauer *et al.*, 2001, 2003; Cutrignelli, 2007; Calabrò *et al.*, 2012b). As reported by Bauer *et al.* (2003), there is a very little change in fermentation as result of enzyme treatment using structural carbohydrate sources as substrates, therefore, the whole samples with no preliminary treatment were used. In order to avoid variability due to different *inocula*, all the samples (three for each raw material) were analysed in a single gas run. Each sample (0.5±0.01 g) was put in 120 mL serum flasks with 82 mL of anaerobic medium (Bauer *et al.*, 2001). Faecal samples, collected *per rectum* from 3 adults neutered finisher pigs (Landrace × Large White) fed a commercial diet (CP: 14.8%; CF: 4.0%), were used as source of *inoculum.* Faeces were pooled, diluted (1:6)

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with NaCl solution, homogenized, filtered through a double thickness of cheesecloth and added to each bottle (5 mL) under anaerobic condition. Bottles were incubated at 39°C for 96 h. Three bottles were incubated without substrate (as blank) in order to correct fermentation parameters. Gas production of fermenting cultures was recorded 26 times (every 2 h at the beginning of the incubation, when the fermentation was more turbulent, and, later, every 4 h) using a manual pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA). The fermentation was stopped and the pH of each flask was measured (Alessandrini Instrument glass electrode, Jenway, Dunmow, UK; model 3030).

Residual dry matter (DM) was determined by drying to a constant weight at 103°C, and organic matter (OM) by difference following incineration (5 h at 550°C). The organic matter disappearance (OMD) was determined by filtering the residues using pre-weighed sintered glass crucibles (Scott Duran, porosity #2) under vacuum. Gas volumes obtained were related to the quantity of incubated organic matter in order to obtain the cumulative gas volume (OMCV). The gas profiles were fitted to the multiphasic model described by Groot *et al.* (1996) as follows:

$$OMCV = \Sigma_n A_n/[1 + (C_n/t)^{Bn}]$$

where:
OMCV = total gas produced (mL/g OM initial substrate weight);
A = asymptotic gas production (mL/g OM initial substrate weight),
B = switching characteristic of the curve;
C = time at which one-half of the asymptote had been reached (h);
t = time (h);
n = number of phases.

The goodness of fit of monophasic and biphasic models was determined using the mean squared prediction error (MSPE) as described by Bibby and Toutenburg (1977) and where the root MSPE was scaled to the observed mean (Mean Prediction Error, MPE). Maximum fermentation rate (R_{max}) and time at which it occurs (T_{max}) were also calculated according to the following formula (Bauer *et al.,* 2001):

$$R_{max} = (A \times B^c) \times C \times [T_{max}^{(-C-1)}]/[(1+B^C) \times (T_{max}^{-C})^2]$$
$$T_{max} = B \times [(C-1)/(C+1)]^{(1/C)}$$

The chemical composition parameters and fermentation characteristics were subjected to analysis of variance to detect the influence of the different substrates and sampling time. The statistical model (GLM procedure, SAS, 2000) was:

$$y_{ijk} = \mu + Sub_i + Time_j + Sub*Time + \varepsilon_{ijk}$$

where:
y = the experimental data;
μ =the general mean;
Sub = the substrates (i = 1, 2, ...7);
Time = the sampling time (j=1, 2, 3);
ε =the error term.

End-products

The fermentation liquor was analysed for ammonia (NH_3) and short-chain fatty acids (SCFAs). Ammonia was determined according to the method described by Searle (1984). Samples were centrifuged twice at 1900 rpm for 10 min at room temperature (about 22°C), diluted 10 times with water, and then 1 mL of the diluent was deproteinized using 10% trichloroacetic acid. Ammonia and phenol were oxidised by sodium hypochlorite in the presence of sodium nitroprusside to form a blue complex. The intensity was measured colorimetrically at a wavelength of 623 nm. Intensity of the blue is proportional to the concentration of ammonia present in the sample. For SCFAs determination the samples were centrifuged twice at 12,000× g for 10 min at 4°C and 1 mL of supernatant was taken and mixed with 1 mL of oxalic acid 0.06 M. The SCFAs were measured by gas chromatography (ThermoQuest Italia SpA, Rodano, MI, Italy; model 8000 top, fused silica capillary column 30m × 0.25mm × 0.25µm film thickness) comparing samples peaks area of each SCFA with the corresponding of an external standard composed by acetate, propionate, iso-butyrate, butyrate, valerate and iso-valerate (Cutrignelli *et al.*, 2009). In order to evaluate protelolysis, branched chain fatty acids (BCFA) were also calculated (iso-valeriate + iso-butyrate\SCFA).

Results

Chemical composition

The chemical composition of the substrates was reported in Table 1. Substrates showed a fat amount from 0.74 to 5.35% DM of SBP and GC, respectively. Concerning carbohydrate composition FOS and citrus by-product were characterized by very high NSC values (98 and 48%, respectively) and were really poor in starch (less than 2%). The other substrates (glutamic acid beet pulp, wheat bran, soybean hulls, sugar beet pulp and grapecake) showed NSC content comprised between 1 and 17% and NDF contents ranged from 45 to 63%. The lignin value of GC was also very high (36%).

In vitro gas production

In Figure 1, the main fermentation characteristics (OM digestibility and gas volume) of the substrates are represented. As a whole, they are well correlated (r values comprised between 0.69 and 0.89). Grapecake showed the lowest fermentation in terms of OM digestibility (OMD 24.2%; P<0.01) and gas production (OMCV 38.5 mL/g OM; P<0.01). On

Table 1. Chemical composition (% DM) of the seven feeds.

	DM	CP	EE	Starch	NSC	TDF	NDF	ADF	ADL	Ash
GC	94.59^{B}	13.91^{C}	5.35^{A}	1.06^{BCa}	4.75^{E}	75.43^{A}	62.32^{A}	59.23^{A}	36.2^{A}	8.26^{B}
FOS	98.46^{A}	-	-	-	97.96^{A}	70.31^{Bb}	0.42^{F}	0.37^{G}	-	0.08^{G}
SH	90.05^{C}	12.45^{D}	3.22^{B}	0.27^{C}	6.70^{D}	74.88^{A}	62.01^{A}	52.52^{B}	2.47^{E}	5.67^{E}
CBP	88.44^{D}	5.27^{F}	1.89^{E}	1.51^{Ba}	48.20^{B}	70.97^{Ba}	23.67^{E}	9.14^{F}	2.71^{DE}	9.41^{A}
GBP	90.27^{C}	34.53^{A}	2.87^{D}	0.94^{BCb}	1.19^{F}	69.45^{Bc}	45.21^{D}	38.74^{C}	3.41^{D}	6.85^{D}
SBP	88.04^{Da}	7.58^{E}	0.74^{F}	1.03^{BCa}	15.61^{C}	65.82^{C}	56.93^{B}	37.50^{D}	9.37^{B}	7.18^{C}
WB	87.08^{Db}	17.79^{B}	2.96^{C}	17.13^{A}	14.64^{C}	47.49^{D}	46.64^{C}	15.12^{E}	4.34^{C}	5.05^{F}
SE	0.496	1.22	0.180	0.776	4.15	1.13	2.71	2.66	1.55	0.359

GC, grapecake; FOS, fructo-oligosaccharides; SH, soybean hulls; CBP, citrus by-product; GBP, glutamic acid beet pulp; SBP, sugar beet pulp; WB, wheat bran; DM, dry matter; CP, crude protein; EE, ether extract; NSC, non-structural carbohydrates; TDF, total dietary fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin. $^{A-C}$P<0.01; $^{a-c}$P<0.05.

the other hand, FOS, soybean hulls and citrus pulp showed the highest OMD (94.0, 87.9 and 90.7%, respectively) and OMCV values (269, 210 and 167 mL/g OM, respectively). The other substrates (sugar beet pulp, wheat bran and glutamic beet pulp) showed intermediate values for both parameters (OMD 72.6, 69.9 and 69.4% and OMCV 161, 136 and 72 ml/g OM, respectively), even if the OMCV values registered for GBP resulted significantly ($P<0.01$) lower.

For brevity and since the bi-phasic model better explains these results, only the parameters obtained with this model are reported in Table 2. Fermentation parameters evidenced specific characteristics for each substrate:

Grapecake and FOS fermentation kinetics showed opposite trends: GC kinetic was characterized by a very low gas production (A_1 12.3 and A_2 32.0 mL/g OM) and reached the A/2 volume very quickly (C_1 3.9 and C_2 3.0 h). The gas production starts very slowly without reaching a peak, so, the curve assumes the shape of a straight line, equally flat appears the trend of the gas production rate over time. Fructo-oligosaccharides showed a more intense fermentative process, characterized by a very fast kinetic (A_1 168.7 and A_2 93.0 mL/g OM; C_1 6.5 and C_2 11.4 h) as reported by other authors (Bauer *et al.*, 2003; Williams *et al.*, 2005).

Glutamic beet pulp produced low gas over all the incubation and its kinetic was quite slow. The adaptation to the bi-phasic model evidenced two phases with similar amount of gas (A_1 40.7 and A_2 36.1 mL/g OM) characterised by different rate (R_{max1} 2.8 and R_{max2} 1.1 mL/h).

Wheat bran and sugar beet pulp showed intermediate and similar gas profiles with slower (A_1 48.1 and 62.0 mL/g OM, respectively) and lower (R_{max1} 2.6 and 0.6 mL/h) gas production during first phase than the second one (A_2 123.2 and 128.8; R_{max2} 3.9 and 6.7 mL /h, respectively).

Soybean hulls and citrus by-products showed, at the end of the incubation, the same gas production with different profiles. The soybean hulls showed a trend that, after 96 h of incubation, seems to go toward the asymptote. Its first phase was slower (R_{max1} 1.9 mL/h) and characterised by little gas (A_1 97.7 mL/g OM), the second one was faster (R_{max2} 4.0 mL/h) with more gas (A_2 106.9 mL/g OM). Citrus by-product kinetics showed a similar gas production during the two phases (A_1 123.0 and A_2 106.9 mL/g OM), but a different production rate (R_{max1} 6.0 and R_{max2} 3.8 mL/h).

End-products

The pH values determined after 96 h of incubation and the products obtained at the end of the gas production trial are reported in Table 3. For all substrates, pH values were included in the physiological range (5.5-7.5) reported by Younes *et al.* (2001). Short chain fatty acids resulted lower in grapecake and higher in FOS (1.31 and 6.58 mM/g OM, respectively), the other samples were fairly uniform, ranging from 2.72 to 3.98 in sugar beet pulp and wheat bran, respectively. For all substrates, the main represented short chain fatty acids were acetate and propionate, whose sum represents more than the 80% except for GBP and FOS (74 and 78% SCFAs, respectively). FOS showed

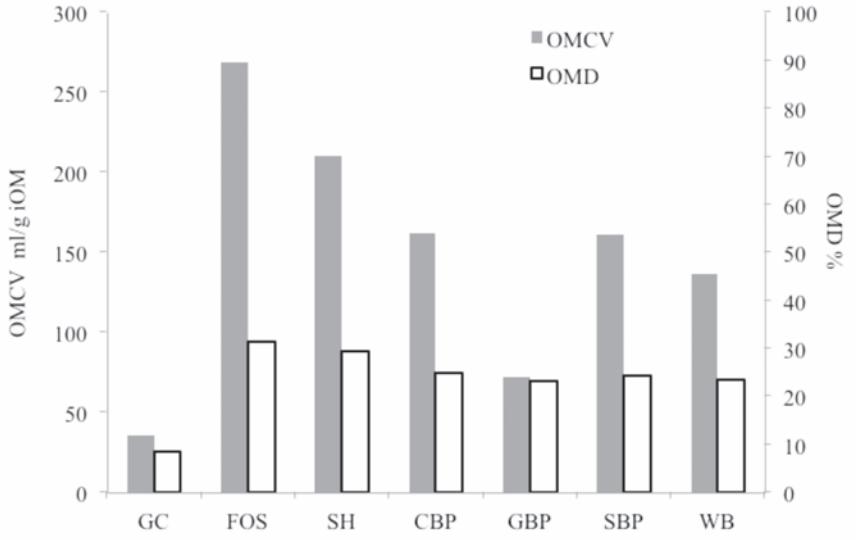

Figure 1. Fermentation characteristics of the seven feed ingredients. GC, grapecake; FOS, fructo-oligosaccharides; SH, soybean hulls; CBP, citrus by-product; GBP, glutamic acid beet pulp; SBP, sugar beet pulp; WB, wheat bran; OMD, organic matter degradability; OMCV, cumulative volume of gas related to incubated organic matter.

Table 2. Model parameters with the bi-phasic model of the seven feeds.

	First phase					Second phase					
	A1, mL/g OM	B1	C1, h	T_{max1}, h	R_{max1}, mL/h	A2, mL/g OM	B2	C2, h	T_{max2}, h	R_{max2}, mL/h	MPE, %
GC	12.3^{F}	21.5^{C}	3.9^{D}	6.3^{B}	-0.3^{E}	32.0^{D}	54.7^{B}	3.0^{C}	42.7^{A}	0.3^{D}	2.95^{B}
FOS	168.7^{A}	16.5^{F}	6.5^{B}	15.7^{A}	17.1^{A}	93.0^{C}	36.2^{D}	11.4^{A}	35.2^{B}	7.0^{A}	3.25^{A}
SH	97.7^{C}	33.5^{A}	5.3^{C}	3.9^{BC}	1.9^{CD}	181.2^{A}	65.6^{A}	1.0^{Db}	31.2^{C}	4.0^{B}	1.58^{D}
CBP	123.0^{B}	19.2^{E}	2.8^{DE}	2.7^{CDa}	6.0^{B}	106.9^{BC}	23.8^{E}	2.6^{CDa}	18.0^{Eb}	3.8^{B}	0.86^{F}
GBP	40.7^{E}	11.0^{G}	1.7^{E}	1.1^{D}	2.8^{C}	36.1^{D}	25.0^{E}	3.5^{B}	21.6^{D}	1.1^{C}	2.02^{C}
SBP	62.0^{D}	22.5^{B}	8.1^{A}	15.4^{A}	0.6^{DE}	128.8^{Ba}	42.2^{C}	1.0^{Db}	20.2^{Ea}	6.7^{Aa}	1.51^{D}
WB	48.1^{E}	20.3^{D}	6.4^{BC}	2.3^{CDb}	2.6^{C}	123.2^{Bb}	39.4^{CD}	1.1^{Db}	19.4^{Eab}	3.9^{B}	1.30^{E}
SE	6.04	0.813	0.275	0.787	0.717	6.39	1.59	0.448	1.10	0.323	0.093

OM, organic matter; GC, grapecake; FOS, fructo-oligosaccharides; SH, soybean hulls; CBP, citrus by-product; GBP, glutamic acid beet pulp; SBP, sugar beet pulp; WB, wheat bran; A, asymptotic gas production; B, switching characteristic of the curve; C, time at which A/2 was reached; R_{max}, maximum rate of gas production; T_{max}, time at which R_{max} occurs; MPE, mean prediction error. $^{A-E}P<0.01$; $^{a-e}P<0.05$.

significantly higher butyrate production (0.926 mM/g OM, P<0.05) than GC (0.095 mM/g OM) and SBP (0.139 mM/g OM). All the other substrates showed intermediate values. The proportion of branched chain fatty acids was significantly (P>0.05) higher for GBP (0.098) and GC (0.071) than the other samples. Regarding nitrogen utilization, the ammonia production was moderate and not affected by the substrate composition, varying from 1.17 mM/g OM (soybean hulls) to 2.69 (wheat bran).

Since sampling time effect did not affect both chemical composition data and fermentation parameters, it was not reported in tables.

Discussion

Chemical composition

The chemical composition of SBP, WB and FOS was similar to that reported in the literature. CBP, SH, GBP and GC showed specific nutritional characteristics, such as a high total dietary fibre (TDF) content (70%). In particular, the high concentration of soluble dietary fibre of CBP and GBP suggests a beneficial effect on gut microbiota. Significant differences were registered between the sugar beet by-products, the main one concerned the crude protein content, which in GBP was more than 5 times higher than SBP. These by-products derived from two different technological processes: SBP from sugar extraction and GBP from glutamic acid extraction. Sugar extraction from beet consists in sugar solubilisation in water, while the extraction of glutamic acid is a hydrolysis of the molasses; these different extraction procedures yield different characteristics.

In vitro gas production and final products

In general, OMD and OMCV values were in agreement with those reported by other authors on *in vitro* studies using faecal *inoculum* from pigs (Sunvold *et al.*, 1995c; DePeters *et al.*, 1997; Bauer *et al.*, 2003; Williams *et al.*, 2005). Few data are available for GBP and GC, in any event, the fermentation parameters and kinetics as well as the relative end-products of all tested by-products are described as follows.

The fermentative process of grapecake, characterized by very low gas production and dry matter disappearance with moderate SCFAs and NH_3 values, could be related to the high lignin content. In turn, the high lignin values could be related to the presence of condensed tannins, as reported by Llobera and Canellas (2007). Indeed, these substances could interfere with ADL determination (Calabrò *et al.*, 2012a). Moreover, the presence of cell wall components such as tannins could negatively affect gas production and dry matter degradability (Guglielmelli *et al.*, 2011; Zicarelli *et al.* 2011). Fructo-oligosaccharides showed intense and fast carbohydrates fermentation due to the high proportion of soluble fibre, which is almost completely fermentable by gut microorganisms. These polysaccharides have the ability to remain almost intact in the first part of the intestine, thus stimulating the growth of beneficial microorganisms as suggested by the high butyrate acid production (Ying *et al.*, 2013), therefore, they are widely used as prebiotics (Manrique and Lajolo, 2001).

Our results confirm that FOS are a high energy source for gut bacteria, which produce butyrate, propionate and lactate acids, thus causing a pH decrease during the fermentation. Soybean hulls showed high values of OMCV. The gas profiles indicated that SH fermentation started after 5 hours of incubation (B_1). The A_1 and A_2 values indicated a continuous gas production along 96 h of incubation, even if the gas production was more intense during the second phase. The kinetic was affected by the high concentration of un-lignified cellulose, moderate soluble fibre and hemicelluloses contents. The fermentation of these polysaccharides was responsible for the obtained gas production and for the moderate SCFAs production. The heterogeneous composition of SH carbohydrates modulates fermentation rate, thus being particularly useful to prevent gastro-intestinal diseases in critical livestock phases (Jensen and Jorgensen, 1994). Since BCFA are produced from the metabolism of branched chain amino acids such as valine, leucine and isoleucine (Mcfarlane and Gibson, 2004), the high values detected for soybean hulls were due to the high crude protein content. Citrus by-product kinetic was very intense, being characterized by high gas production during both phases. This is due to the mix of soluble and insoluble structural carbohydrates present in this by-product. Indeed, soluble fibre, highly represented by pectins, was responsible for the first tumultuous fermentation, while the insoluble hemicelluloses were fermented in the second one. This by-product could be particularly useful in formulating diets for all breeding stages, for its mixture of structural carbohydrates with different physical and chemical properties, characterised by high viscosity and high fermentability. These characteristics lead to a decrease of cholesterol levels both in blood and muscle (Glore *et al.*, 1994) and to an increase in mineral absorption (James *et al.*, 1980). Concerning glutamic beet pulp, despite the high proportion of soluble dietary fibre, GBP showed very low OMCV and low OMD values. Such result was probably due to the high crude protein concentration, which significantly affected short chain fatty acids production, in particular increasing iso-valeriate production thus resulting in high values of BCFA. Sugar beet pulp and wheat bran produced similar fermentation kinetics characterised by higher

Table 3. Fermentation end-products of the seven feed ingredients.

	pH	SCFAs	Acetic	Propionate	Iso-Butyric	Butyric	Iso-valeric	Valeric	NH_3	BCFA
					mM/g OM					
GC	7.30^{A}	1.31^{F}	0.68^{E}	0.39^{E}	0.047^{D}	0.095^{E}	0.044^{Dc}	0.058^{E}	1.66^{C}	0.071^{B}
FOS	6.50^{D}	6.58^{A}	2.86^{A}	2.30^{A}	0.034^{E}	0.926^{A}	0.042^{Dc}	0.338^{A}	1.22^{D}	0.011^{F}
SH	6.73^{C}	3.03^{D}	1.68^{D}	0.88^{B}	0.077^{B}	0.214^{C}	0.083^{C}	0.096^{Cd}	1.17^{E}	0.056^{C}
CBP	6.80^{BC}	3.30^{Cb}	1.92^{Ca}	0.74^{CD}	0.030^{E}	0.423^{Ba}	0.056^{Da}	0.134^{Cc}	1.63^{C}	0.026^{E}
GBP	7.15^{A}	3.36^{Ca}	1.77^{Cb}	0.73^{D}	0.086^{A}	0.294^{Bb}	0.243^{A}	0.242^{Bb}	2.29^{B}	0.098^{A}
SBP	6.87^{BC}	2.72^{E}	1.64^{D}	0.79^{C}	0.031^{E}	0.139^{DE}	0.045^{Dbc}	0.075^{D}	1.63^{C}	0.028^{E}
WB	6.93^{B}	3.98^{B}	2.29^{B}	0.88^{B}	0.070^{C}	0.343^{Bab}	0.145^{B}	0.255^{Ba}	2.69^{A}	0.054^{D}
SE	0.033	0.192	0.079	0.072	0.003	0.034	0.009	0.013	0.065	0.003

OM, organic matter; GC, grapecake; FOS, fructo-oligosaccharides; SH, soybean hulls; CBP, citrus by-product; GBP, glutamic acid beet pulp; SBP, sugar beet pulp; WB, wheat bran; SCFAs, total fatty acids; NH_3, ammonia; BCFA, bran chain proportion. $^{A\text{-}E}P<0.01$; $^{a\text{-}e}P<0.05$.

gas production during the second phase and faster rate during the first phase. These results are probably due to different reasons. Notoriously, dietary fibre of sugar beet pulps is a mixture of soluble and insoluble fibre: the soluble fraction and the residual sugar are fermented rapidly, while the less soluble components were fermented with high gas production during the second phase of incubation. On the contrary, in wheat bran, starch and non-structural carbohydrates represent the more fermentable portion as demonstrated by the high acetate acid production. The comparison between the seven substrates and FOS, whose prebiotic activity is well documented, showed a lower production of gas and SCFAs, probably due to their raw nature. Despite none of the seven substrates was superimposable to FOS in terms of SCFAs, the fermentation characteristics of CBP and SH, mainly regarding OMCV and butyric acid suggest their possible use as prebiotics in swine diet. The high proportion of BCFA in soybean hulls suggests a potential risk for enteric mucosa integrity. Indeed, BCFA are produced by proteolytic fermentation, which can lead to potentially toxic metabolites (NH_3, amines, volatile phenols and indoles) (Williams *et al.*, 2001). Proteolytic fermentation interferes with mucosal development and accelerates villous atrophy in the small intestine, thus predisposing to diarrhoea (Heo *et al.*, 2010). Consequently, their supplementation into the diet should be assessed in relation to the protein amount of the other ingredients.

Further studies are needed in order to evaluate whether CBP and SH should be used in the raw form or as a source of purified soluble fibre. The other five substrates did not show to possess a promising prebiotic activity even if their chemical composition and fermentation characteristics suggest their possible use as of insoluble and unfermentable fibre source.

Conclusions

In general, the results obtained *in vitro* for FOS, wheat bran, soya bean hulls, sugar beet pulp, glutamic beet pulp, agree with those reported by other authors in similar experiments. The relative low variability among samples suggests the uniformity of the production processes. Results on alternative substrates (grapecake and citrus by-products) could be useful to improve swine performance and meat quality. Thereafter, it is important to underline the importance of by-products as an economical and readily available nutrient source. Our results offer good perspectives for the use of these raw materials in the formulation of pig diet.

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