



PAPER

Fermentation characteristics of several carbohydrate sources for dog diets using the *in vitro* gas production technique

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Abstract

Fermentable carbohydrates are an important part of the canine diet. They can improve gastrointestinal health by modifying gut microbial population and metabolic activity. The present study compared the fermentation characteristics and kinetic patterns of 10 carbohydrate sources using the *in vitro* gas production technique (IVGPT) with dog faecal *inoculum*. The substrates tested were: pure cellulose (PC), carboxymethylcellulose (CMC), sugar-cane fibre (SCF), beet pulp (BP), wheat bran (WB), fructooligosaccharides (FOS), inulin, yeast cell wall (YCW), ground psyllium seed (PS), pea hulls (PH). All substrates were incubated at 39°C under anaerobic conditions with faeces collected from dogs as microbial *inoculum*. Gas production of fermenting cultures was recorded and after 48 h, pH, short-chain fatty acids (SCFA) and organic matter disappearance (OMD) were determined. The results confirm high fermentation by dog faecal bacteria of FOS and inulin that produced high amounts of propionate and that underwent very rapid fermentation. Three substrates (SCF, CMC and PC) were not able to support bacterial growth, with low gas and SCFA production, and high BCFA formation. The PH and BP showed moderate OMD and SCFA production. Wheat bran B underwent rapid fermentation and generated a high proportion of butyrate. Psyllium seeds underwent slow fermentation with delayed gas production, supporting a high formation of SCFA, with an adequate amount of butyrate for bacterial growth while YCW, which showed a delayed fermentation, gave moderate SCFA production. The fermentation characteristics of PS and YCW suggest their potential use in promoting a more distal fermentation on intestinal tract.

Introduction

Fermentable carbohydrates are important components of dog diets, including some fibres, starch, non-starch polysaccharides, such as mannanooligosaccharides, fructooligosaccharides, stachyose and raffinose, and non-absorbed sugars. These nutrients reach the colon and are suitable for bacterial fermentation (Gibson and Roberfroid, 2008). They provide an adequate supply of organic matter for the large intestine. Bacterial fermentation of these compounds results in short-chain fatty acid (SCFA) production and pH reduction, which could modify the composition and metabolic activity of the intestinal bacteria (Campbell *et al.*, 1997). The short chain fatty acids, especially butyric acid, are important energy sources for the colonocytes (Roediger, 1982). They lead to suitable ion absorption, and act in intestinal blood flow and peristalsis, helping the animal by increasing the nutrients available for the colonocytes (James, 1980). Some of these fermentable carbohydrates can be classified as prebiotics, defined as non-digestible ingredients that beneficially affect the host by selectively stimulating the growth and/or metabolic activity of a limited number of desirable bacterial species already resident in the colon (Gibson and Roberfroid, 2008). On the other hand, protein fermentation by gut microbiota results in the production of putrefactive compounds, including phenols, indoles, ammonia, branched-chain fatty acids (BCFA) and biogenic amines increasing the quantity of nitrogenous waste materials entering the bloodstream (Smith and Macfarlane, 1997).

The *in vitro* gas production technique (IVGPT) proposed by Theodorou *et al.* (1994) could be useful to assess the fermentation pattern and kinetics of a carbohydrate source by dog intestinal microbiota. The IVGPT is based on the fact that the anaerobic fermentation by gut microorganisms produces many compounds, including gas (CO₂, CH₄ and traces of H₂), SCFA (acetate, propionate, butyrate, valerate, iso-valerate and iso-butyrate) and ammonia. The technique has already been used to evaluate feedstuffs with *inocula* from rumen liquor (Calabrò *et al.*, 2008; Cutrignelli *et al.*, 2005, 2007), content of gastrointestinal tract sections of pigs (Williams *et al.*, 2001), caecal content of rabbits (Bovera *et al.*, 2008), poultry (Williams *et al.*, 1997), and dog faeces (Bosch *et al.*, 2008; Cutrignelli, 2007; Cutrignelli *et al.*, 2009).

The extent of fermentation and the SCFA profile are related to the interaction between

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Key words: Dog, Fibre, Prebiotic, Digestibility, Butyrate.

Acknowledgments: the authors would like to thank Maria Ferrara for her technical support, and Dilumix Industrial Ltda., Leme, Brazil (PH, PS, PB, SCB) and Biorigin S.A., Lencois Paulista, Brazil (YCW) for donating ingredients.

Received for publication: 26 September 2012.
Revision received: 12 November 2012.
Accepted for publication: 28 November 2012.

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Italian Journal of Animal Science 2013; 11:e4
doi:10.4081/ijas.2013.e4

substrate and microbiota. The structure of the carbohydrate (e.g. different sugar compositions and molecular size), the bacterial species present in the ecosystem and their metabolic collaboration are probably important factors in controlling fermentation (Cummings and MacFarlane, 2002). Furthermore, in estimating the fermentation patterns among animal species, the morphological and functional characteristics of the hindgut should be considered. In particular, carnivores have a short and relatively simple large intestine where the undigested food resides for approximately 12 h (Maskell and Johnson, 1993). In spite of the fact that the results of *in vitro* tests are not directly transferable *in vivo*, several characteristics (e.g. standardized fermentation conditions, defined fermentation times, ability to measure fermentation end products, low cost) mean that these methods are also useful to study the fermentation capacity of intestinal microflora of dog. The use of faeces as *inocula* source has been the subject of intense discussion. However, Bosch *et al.* (2008) concluded that because the ranking remained the same for the large intestinal *inocula*, the use of faeces for *inoculum* preparation would be suitable for *in vitro* screening.

Therefore, the aim of this study was to use

IVGPT to compare fermentation characteristics, kinetics and end products of different carbohydrate sources using dog faecal *inocula*, in order to better understand the potential effects of their inclusion in dog diets.

Materials and methods

Substrates

Ten carbohydrate substrates were used: purified cellulose (PC) (CPKelco Limeira, SP, Brazil), a soluble sodium salt of cellulose, carboxymethylcellulose (CMC) (FINNFIX® 700 CPKelco Limeira, SP, Brazil), a purified by-product of sugar cane (*Saccharum officinarum*) namely sugar-cane fibre (SCF), beet (*Beta vulgaris*) pulp (BP), wheat bran (WB), a residue of wheat (*Triticum* spp.) flour production, two commonly used prebiotics, purified fructooligosaccharide (FOS; Actilight Beghin Meiji, France), inulin (Prebiofeed 95, Socode, Belgium), spray-dried yeast cell wall (YCW) (ActiveMOS, Biorigin, Brazil) (consisting of a purified *Saccharomyces cerevisiae* cell wall, a common source of mannanooligosaccharides in dog diets), and two natural sources of dietary fibre (Dilumix Industrial Ltda., Brazil), namely ground whole seed of psyllium (*Plantago psyllium*, PS) and pea hulls (*Pisum sativum*, PH), obtained after peeling and grinding.

Chemical analyses of substrates were carried out using AOAC (2005) procedures: crude protein (ID 954.01), ether extract (ID 920.39C), ash (ID 942.05) and dry matter (ID 934.01). Total dietary fibre (TDF), and soluble and insoluble fibre were measured according to Prosky *et al.* (1988).

Protocol for IVGPT

The IVGPT was performed according to Bosch *et al.* (2008). Each substrate was accurately weighed (mean±SD: 0.502±0.008 g) in triplicate into 120 mL serum flasks and 75 mL of an anaerobic medium was added as described by Williams *et al.* (2005). It was decided to use the whole materials without preliminary treatment because, as reported by Bauer *et al.* (2003), there is very little change in fermentation capacity as a result of enzyme treatment using carbohydrate sources as substrates.

The medium contains nitrogen in several forms suitable for microbial use because the substrates were mainly a source of energy for the microbial population. Contextually, three

flasks were prepared without substrates (blank) to correct degradability, gas and short chain fatty acid (SCFA) productions. All the flasks were immediately sealed with butyl rubber stoppers and aluminium crimp.

Faecal inoculum

Faeces were collected *per rectum* from 2 healthy adult (4-year old) German Shepherd dogs (mean body weight 32 kg) fed a commercial dry food without prebiotics (chemical composition, g/kg on an *as fed* basis: crude protein 258, total dietary fibre 66, acid hydrolyzed fat 175, ash 70) for two weeks prior to faeces collection. Faecal samples were immediately transported to the laboratory of the Department of Animal Science and Food Control (Naples, Italy) and processed within 40 min of collection. Faecal samples were pooled, diluted (1:10, v/v) with 0.9% NaCl sterile solution, homogenized and filtered through six layers of gauze. In each prepared flask (including a blank), 5 mL of the faecal solution were injected. After inoculation, flasks were placed in a PI400 incubator (Carbolite, Sheffield, UK) at 39°C for 48 h under anaerobic conditions.

Gas production and fermentation end-product measurement

According to other *in vitro* studies with inoculum from dogs (Sunvold *et al.*, 1995b; Bosch *et al.*, 2008) the fermentation was stopped after 48 h of incubation, considering the high transit rate of digesta in dog hindgut. Gas production of fermenting cultures was recorded 18 times (at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 26, 30, 32, 34, 42, 46 and 48 h) using a manual pressure transducer (Cole and Parmer Instrument Co., Vernon Hills, IL, USA).

Gas volumes obtained for each sample were related to the quantity of incubated organic matter (organic matter cumulative gas volume, OMCV). Fermentation was stopped after 48 h by cooling at 4°C. At the end of incubation, fermenting liquor was analysed for pH (pH meter model 3030; Alessandrini Instrument SpA glass electrode Jenway, Dunmow, UK) and 10 mL were collected and frozen at -15°C for SCFA analysis. The organic matter disappearance (OMD) was measured by filtering the flask residues using pre-weighed sintered glass crucibles (porosity #2, DURAN Group GmbH Mainz, Germany) under vacuum. Residual dry matter was determined by drying the sample to a constant weight at 103°C, residual organic matter was calculated by difference following burning (5 h at 550°C).

For SCFA determinations, fermenting

liquors were thawed and centrifuged twice at 12,000 g for 10 min at 4°C (Universal 32R, Hettich FurnTech Division DIY, Germany). One mL of supernatant was then mixed with 1 mL of oxalic acid (0.06 mol). Short chain fatty acids were measured by gas chromatography (ThermoQuest Italia SpA, Rodano, Milan, Italy; model 8000^{top}, fused silica capillary column 30 m x 0.25 mm x 0.25 µm film thickness), using as external standard a solution composed of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids, as described by Calabrò *et al.* (2006). In order to evaluate whether proteolysis occurs during the fermentation, the branched chain fatty acid was calculated according to the following equation:

$$BCFA = [(isobutyric + isovaleric)/SCFA].$$

Calculations and statistical analysis

For each flask, the gas production profiles were fitted to the sigmoidal model described by Groot *et al.* (1996):

$$G \text{ (mL/g)} = A/(1+B/t)^C$$

where G is the total gas produced (mL/g of organic matter) at t (h) time, A is the asymptotic gas production (mL/g of incubated organic matter), B is the time at which one-half of the asymptote is reached (h), and C is the switching characteristic of the curve. The NONLIN package (Sherrod, 1995) was used to fit the data to this equation. Maximum fermentation rate (R_{max}) and the time at which it occurred (T_{max}) were calculated according to the following formulas (Bauer *et al.*, 2001):

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

$$T_{max} \text{ (h)} = C [(B-1)/(B+1)]^{(1/B)}$$

The fermentation characteristics (OMCV, cumulative volume per g of incubated OM, OMD, SCFA, BCFA, pH) and the fitted parameters (A, B, T_{max} and R_{max}) were subjected to analysis of variance to detect the influence of the different substrates. The statistical model (GLM procedure, SAS, 2000) was:

$$y_{ij} = \mu + \text{Sub}_i + \epsilon$$

where y is the experimental data, μ the general mean, Sub the substrates ($i = 1, 2, \dots, 10$), ϵ the error term. When significant differences among substrates were found in the analysis of variance, means were compared using the Tukey's test.

Results

Chemical composition of the studied carbohydrate sources is shown in Table 1. Substrates may be classified according to their TDF amount in: very high TDF content (TDF ≥ 850 g/kg; PC, SCF and PH), high TDF content (TDF ≥ 600 g/kg; CMC, FOS, inulin and BP), moderate TDF content (TDF ≤ 400 g/kg; YCW, PS and WB). CMC, FOS and inulin were completely soluble while on the contrary, PC, PH and SCF were completely insoluble. The other substrates were intermediate, most with a higher content of insoluble fibre.

Kinetic parameters were: asymptotic gas production (A), time at which one-half of the asymptote was reached (B), maximum fermentation rate (R_{max}) and time at which it occurred (T_{max}). Kinetic parameters and fermentation characteristics (OMD and OMCV) varied among the studied ingredients (Table 2). As expected, both the cellulose substrates (PC, CMC) and sugar-cane fibre showed very low OMCV values ($P < 0.01$). On the contrary, a significant difference was found between the OMD values of these substrates ($P < 0.01$). Pure cellulose and sugar-cane fibre showed the lowest OMD (2.20% and 5.41%, respectively) and the CMC organic matter disappearance was one of the highest (96.3%). Inulin, FOS and YCW presented high OMD and OMCV, even with the different kinetic parameters shown by those substrates, YCW resulting in lower A (mL/g) and R_{max} (mL/h) and higher T_{max} (h) than inulin and FOS ($P < 0.01$). The other natural fibre sources evaluated (WB, BP, PH and PS) produced moderate (WB and BP) or high (PH and PS) amounts of gas (OMCV); only PS gas production (214 mL/g) did not correspond to the high OM degraded (28.8%). Only PH did not fit the gas profile model used in the study. The potential gas (A, mL/g) and maximum rate (R_{max} , mL/h) were highest ($P < 0.01$) for inulin, FOS and WB, intermediate for BP, YCW and PS, and lowest ($P < 0.01$) for SCF, CMC and PC. The PS and YCW needed more time to reach R_{max} ($P < 0.01$) than all the other substrates, with BP presenting intermediate results.

The *in vitro* gas production and fermentation rate over time of WB, FOS, inulin, YCW, BP and PS are illustrated in Figures 1 and 2, respectively. For PC, CMC, PH and SCF, the curves were almost flat (*data not shown*). Wheat bran, fructooligosaccharides and inulin presented similar gas profiles, which differ from the YCW, BP and PS curves. The first three samples showed their highest values at the beginning of fermentation, while BP, YCW

and PS showed a flatter curve with maximum values between 12 to 24 h (Figure 2).

Values of pH, determined after 48 h of incubation, were significantly higher ($P < 0.01$) for the low fermentable substrates (PC, CMC, and SCF) compared to those that fermented more, as presented in Table 3. The pH values of inulin and FOS were particularly low. High amounts of total SCFA (Mmol/g OM) were found for BP, PS, inulin and WB ($P < 0.05$) while PC, CMC and FOS showed lower values ($P < 0.05$) and the remaining substrates presented intermediate values. In particular, PS showed the highest value (41.24 Mmol/g OM) and PC and CMC the lowest values (8.84 and 7.19 Mmol/g OM, respectively). Most of the tested substrates yielded an SCFA production

with a high proportion of acetate and propionate, which together accounted for more than 50% of SCFA production. In particular, for FOS and inulin, acetic and propionic acids account for more than 85% of produced SCFA. The proportion for butyric acid production was significantly higher for WB (4.49 Mmol/g OM) compared to all the other substrates ($P < 0.01$). As expected for the substrates tested, and in accordance with Bosch *et al.* (2008), the branched chain fatty acid proportion was quite low for all the tested substrates. However, the cellulose samples (PC and CMC) and SCF produced higher proportions of isobutyric, isovaleric and valeric acids than the other substrates ($P < 0.05$). Consequently, these substrates showed the highest proportion of

Table 1. Chemical composition of the evaluated carbohydrate sources (g/kg as fed basis).

	DM	CP	EE	Ash	TDF	Insoluble fibre	Soluble fibre
Carbohydrate							
PC	936	-	0.4	2.0	920	914	15
CMC	937	-	-	195	740	-	740
SCF	982	17	13	56	908	908	-
BP	905	75	5.5	64	620	371	245
WB	883	152	19.8	43	389	229	151
FOS	959	0.3	0.6	-	705	-	705
Inulin	977	-	0.4	-	683	-	683
YCW	941	288	8	57	550	328	207
PS	931	219	101	27	513	401	102
PH	943	55	13	23	853	830	26

DM, dry matter; CP, crude protein; EE, ether extract; TDF, total dietary fibre; PC, purified cellulose; CMC, carboxymethylcellulose; SCF, sugar-cane fibre; BP, beet pulp; WB, wheat bran; FOS, fructooligosaccharide; YCW, spray-dried yeast cell wall; PS, ground whole seed of psyllium; PH, pea hulls.

Table 2. *In vitro* fermentation characteristics of the tested substrates.

	OMD, %	OMCV, mL/g	A, mL/g	B, h	T_{max} , h	R_{max} , mL/h
Carbohydrate						
PC	2.20 ^F	3.55 ^E	3.43 ^D	5.67 ^B	2.83 ^D	0.42 ^C
CMC	96.3 ^A	8.34 ^E	3.83 ^D	3.64 ^C	3.28 ^D	0.69 ^C
SCF	5.41 ^F	16.6 ^E	7.63 ^D	8.36 ^B	7.40 ^{Cc}	1.02 ^C
WB	64.0 ^C	152 ^C	158 ^A	5.83 ^B	2.17 ^D	18.4 ^{ab}
BP	49.8 ^D	116 ^D	137 ^B	19.9 ^{ab}	11.8 ^B	4.54 ^B
PH	81.2 ^B	256 ^A	nd	nd	nd	nd
PS	28.8 ^E	214 ^B	113 ^C	23.1 ^{aa}	20.6 ^{aa}	5.64 ^B
FOS	99.4 ^A	165 ^C	159 ^A	8.39 ^B	5.63 ^D	12.2 ^{Ab}
Inulin	93.8 ^A	185 ^B	188 ^A	8.88 ^B	6.23 ^{Cd}	15.4 ^{aa}
YCW	99.1 ^A	199 ^B	99.1 ^C	19.6 ^{ab}	16.7 ^{Ab}	5.02 ^B
MSE	54.6	191.07	106.83	3.29	3.63	3.88

OMD, organic matter disappearance; OMCV, cumulative gas production related to incubated organic matter; A, asymptotic value for gas production; B, time at which one-half of the asymptote is reached; T_{max} , time at which the maximum rate of gas production is reached; R_{max} , maximum fermentation rate. PC, purified cellulose; CMC, carboxymethylcellulose; SCF, sugar-cane fibre; WB, wheat bran; BP, beet pulp; PH, pea hulls; PS, ground whole psyllium seed; FOS, fructooligosaccharides; YCW, spray-dried yeast cell wall; MSE, mean square error; nd, not detected. ^{a-d}Differences statistically significant for $P < 0.01$; ^{ab}differences statistically significant for $P < 0.05$.

branched chain fatty acids; (isobutyric + isovaleric)/SCFA values were 0.33, 0.31 and 0.32 for PC, CMC and SCF, respectively.

Discussion

Common methods of fibre analysis, including total dietary fibre, and soluble and insoluble fibre provide information on the carbohydrate sources in terms of amount of fibre and physical characteristics (e.g. solubility), but do not provide any information about their suitability for bacterial fermentation (de-Oliveira et al., 2011). Therefore, the combination of the

chemical composition analysis with the *in vitro* gas production technique provides interesting complementary data about the ingredients.

The gas production technique was shown to be useful for an *in vitro* screening of non-digestible carbohydrate for dogs, separating them according to fermentation amount as well as characteristics and SCFA production. The short chain fatty acids are the major end products of bacterial fermentation reactions on carbohydrates in the colon in all mammals. All SCFA are rapidly absorbed and then metabolized by the gut epithelium, liver and muscle. One of the most important SCFA properties is their trophic effect on the intestinal epitheli-

um, maintaining the mucosal defence barrier against invading organisms. Acetate, propionate and butyrate are trophic when infused into the large intestine although butyrate seems to be the most effective and least propionate. Butyrate is the most interesting of the SCFA, also because it is an important energy source for the colonic epithelium, and also regulates cell growth and differentiation (Salminen et al., 1998).

The *in vitro* data obtained for FOS, PC, inulin and BP are in broad agreement with previous canine studies (Cutrignelli, 2007; Cutrignelli et al., 2009).

For PC, CMC and sugar-cane fibre, the high values of BCFA associated with a very low gas

Table 3. *In vitro* fermentation end products after 48 h of incubation.

	pH	SCFA mMol/g	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	BCFA
					% of total SCFA				
Carbohydrate									
PC	7.76 ^A	8.84 ^{Bd}	32.96 ^B	10.77 ^B	17.40 ^{Aa}	2.00 ^B	15.85 ^{Aa}	21.03 ^{Aa}	0.33 ^A
CMC	7.74 ^A	7.19 ^{Bd}	34.93 ^B	10.83 ^B	16.92 ^{Aa}	2.00 ^B	14.15 ^{Aa}	21.17 ^{Aa}	0.31 ^{Ab}
SCF	7.70 ^A	25.73 ^{Bb}	37.72 ^B	10.90 ^B	16.02 ^{Aa}	1.72 ^B	15.75 ^{Aa}	17.89 ^{Aa}	0.32 ^{Ab}
WB	6.28 ^B	34.07 ^{Ba}	48.43 ^A	17.58 ^B	6.94 ^{Bb}	13.18 ^A	4.20 ^{Bd}	9.67 ^{Ab}	0.11 ^{Bc}
BP	6.22 ^B	37.04 ^{Aa}	56.15 ^A	25.31 ^B	4.84 ^{Bb}	4.46 ^B	3.17 ^{Bd}	6.08 ^{Bc}	0.08 ^C
PH	6.67 ^B	26.00 ^{Bb}	57.90 ^{Aa}	12.89 ^B	8.98 ^{Ab}	1.64 ^B	8.18 ^{Bc}	10.42 ^{Bb}	0.17 ^{Abc}
PS	6.74 ^B	41.24 ^{Aa}	44.74 ^A	21.39 ^B	9.68 ^{Ab}	5.52 ^B	7.02 ^{Bc}	11.65 ^{Bb}	0.17 ^{Abc}
FOS	4.72 ^C	11.72 ^{Bc}	52.16 ^A	36.01 ^{Ab}	4.59 ^{Bb}	2.51 ^B	2.74 ^{Bd}	2.00 ^{Bd}	0.07 ^C
Inulin	4.86 ^C	35.99 ^{Aa}	47.35 ^A	41.52 ^{Aa}	6.28 ^{Bb}	1.47 ^B	2.01 ^{Be}	1.38 ^{Bd}	0.08 ^C
YCW	6.80 ^B	17.89 ^B	37.32 ^B	26.77 ^B	12.44 ^{Aa}	2.13 ^B	7.44 ^{Bc}	13.90 ^{Ab}	0.20 ^{Abc}
MSE	0.0104	64.17	76.7	12.7	18.2	10.9	6.32	16.7	0.004

SCFA, sort chain fatty acids; BCFA, branched chain fatty acid proportion [(isobutyric+isovaleric)/SCFA]. PC, purified cellulose; CMC, carboxymethylcellulose; SCF, sugar-cane fibre; WB, wheat bran; BP, beet pulp; PH, pea hulls; PS, ground whole psyllium seed; FOS, fructooligosaccharides; YCW, spray-dried yeast cell wall; MSE, mean square error. ^{A-D}Differences statistically significant for P<0.01; ^{a-d}differences statistically significant for P<0.05.

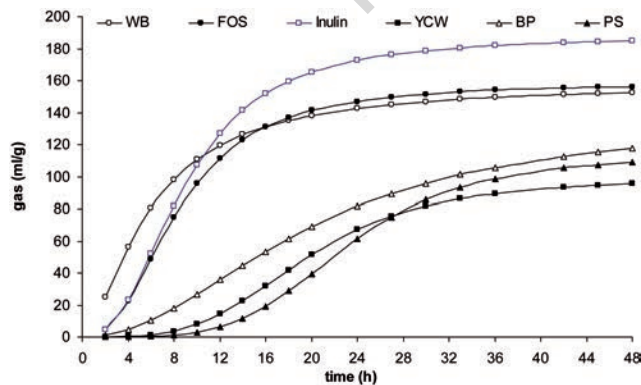


Figure 1. *In vitro* gas production of the tested substrates over time. WB, wheat bran; FOS, fructooligosaccharides; YCW, spray-dried yeast cell wall; BP, beet pulp; PS, ground whole psyllium seed.

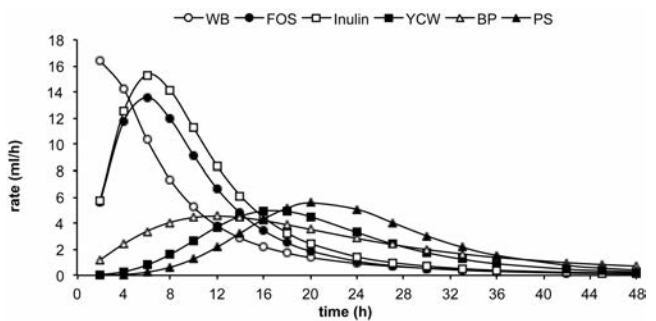


Figure 2. *In vitro* fermentation rate of the tested substrates over time. WB, wheat bran; FOS, fructooligosaccharides; YCW, spray-dried yeast cell wall; BP, beet pulp; PS, ground whole psyllium seed.

production suggest a low availability of nutrients, depressing bacteria fermentation and growth. This situation resulted in bacterial autolysis and the fermentation of dead bacteria by the remaining microorganisms. Fermentation of aminoacids (valine and leucine) released during bacterial death produces isobutyric and isovaleric acids, explaining the higher BCFA values of these substrates (McDonald *et al.*, 2002). The higher values of pH for these substrates can probably be justified by a higher accumulation of ammonia due to protein degradation.

Carboxymethylcellulose is characterized by high solubility and viscosity in water. No previous information about its fermentation had been available. The very low fermentation found in this study suggests the advisability of using CMC in low-energy dog diets formulated for weight maintenance or weight loss, which require the addition of high fibre. In particular, the high viscosity of CMC could allow delayed gastric emptying and nutrient absorption, increasing the animal's sensation of satiety (Slavin and Green, 2007); this could be explored in future studies. The high OM disappearance of CMC could be related to the extremely high solubility of the ingredient. Carboxymethylcellulose is a sodium salt of cellulose that is completely soluble in water. At the end of incubation, the filter did not retain the incubated OM that results in *apparent* OMD, whereas it was only solubilized and not fermented, as the gas data demonstrated. Filtration problems must also be considered during the interpretation of OMD results of other high soluble carbohydrates evaluated, including FOS, inulin and YCW.

The very low fermentation of SCF (comparable with that of PC) suggests the high potential of this insoluble fibre source. In contrast, the association of a poorly fermentable substrate with high insoluble fibre content (91% of insoluble dietary fibre) suggests that SCF could be a cheaper substitute for purified cellulose in formulating low-energy diets. In these diets, the addition of high fibre is necessary to reduce energy digestibility, making it necessary to use insoluble fibre sources with low fermentation properties. Such fibre sources interfere less with protein and fat digestibility, and improve faeces formation compared to those with high fermentation characteristics (Fahey *et al.*, 1990; Sunvold, *et al.*, 1995a).

Little information was previously available on fermentation of YCW by gut microbiota of dog and that was not always consistent. The IVGPT results for this substrate showed a moderate gas production (OMCV) and slow fermentation (large T_{max}) with a fair SCFA pro-

file. Gomes *et al.* (2008) found *in vivo* an increase in dog faecal SCFA concentration when the diet was supplemented with YCW. Yeast cell wall is a source of mannanooligosaccharides and has been considered a useful prebiotic for dogs, potentially favouring gut health (Swanson and Fahey, 2006). The prebiotic effect of YCW is largely attributed to the ability of mannanooligosaccharides to attach to type A fimbriae of *Salmonellae* and *Clostridia* avoiding adhesion to enterocytes (Vernazza *et al.*, 2006). Our findings obtained here suggest that at least the benefits could also be attributed to its intestinal fermentation and SCFA production in dog colon. The delayed maximum rate of gas production suggests that YCW is probably more fermented in the distal part of gastrointestinal tract, and this might favour SCFA production in the colon.

The oligosaccharides FOS and inulin are readily available energy sources for gut bacteria. They produced a high proportion of propionic acid and significantly lower pH values than the other substrates ($P < 0.01$). This was probably due to lactic acid production (Böhmer *et al.*, 2005). Previous studies already demonstrated high fermentation of these substrates by canine faecal *inoculum* (Sunvold *et al.*, 1995b). Besides the high gas production, FOS and inulin presented a short T_{max} , suggesting that these substrates are fermented more cranially in the intestinal tract. These compounds could be considered for diets able to promote the fermentation activities also in small intestine (*e.g.* to treat small intestinal diseases). Wheat bran and pea hulls showed particular characteristics of organic matter fermentability and short chain fatty acid production. Wheat bran was readily fermented and produced a high proportion of butyric acid, contrary to its classification by NRC (2006) as a low fermentable fibre source. Also Bosch *et al.* (2008) found that wheat middling is readily fermented by dog faecal *inoculum*, with a fermentation pattern very close to that found in the present paper. Pea hulls are a high fibre ingredient with 830 g/kg of insoluble and 26 g/kg of soluble fibre. Pea hulls have good fermentation properties and SCFA production. Bosch *et al.* (2008) also studied pea fibre that, in the present study, presented similar fermentation characteristics to PH. However, SCFA production differed and could not be compared because of differences in chemical composition and incubation time.

Beet pulp and wheat bran are the most used fibre source for dogs nutrition. Results from the present study, together with those of Sunvold *et al.* (1995a) and of Bosch *et al.* (2008), suggest that the *in vitro* fermentation

of WB is comparable with that of BP, classifying them also as moderate fermentable fibre sources for dog diets. However, the fermentation pattern of BP and WB differed; WB presented a very high maximum fermentation rate and a very short maximum fermentation time, suggesting a more cranial and intense gas production on dog gut than BP. Furthermore, even if the total SCFA production was similar (34.07 *vs* 37.04 mMol/g for WB and BP, respectively), the amount of main SCFA differed (acetate 16.5 *vs* 20.8, propionate 5.99 *vs* 9.37, butyrate 4.49 *vs* 1.65 mMol/g for WB and BP, respectively) due to the great structural complexity (*i.e.* sugar, starch, insoluble fibres and pectins) of both substrates. Psyllium seed showed low OMD (28.8%) but moderate gas (214 mL/g OM) and very high SCFA (41.24 Mmol/g OM) productions, and a particularly high proportion of butyrate (>5% of total SCFA). Psyllium gum has been widely used in veterinary medicine to prevent constipation, obesity and diabetes mellitus (Leib, 2000; Tortola *et al.*, 2009). However, most pet-food companies use ground psyllium seeds instead of psyllium gum because of their lower cost and greater availability. The difference in gum and seed chemical composition must be considered during ingredient evaluation and more data on psyllium seeds are required to improve their use in diet formulation. Psyllium seeds were composed not only by mucilage (approximately 20%; Anderson and Fereman, 1935) as psyllium gum, but also by starch, triglycerides and protein which alter the substrate fermentation pattern and explain the higher gas production. These differences in chemical composition could explain the disagreements with results of Sunvold *et al.* (1995b). The seeds of psyllium also have a low maximum rate of gas production and a delayed T_{max} , which could indicate that greater fermentation takes place in the distal part of the gastrointestinal tract.

Conclusions

The *in vitro* gas production technique was useful for an *in vitro* screening of non-digestible carbohydrates for dogs, separating them according to fermentation kinetics, organic matter disappearance and SCFA production. Non-digestible carbohydrates could differ widely from almost non-fermentable (PC, CMC and SCF) to highly fermentable (inulin, FOS and WB), fast fermented (inulin, FOS and WB) or slowly fermented (YCW, BP and PS), and with different molar ratios of

SCFA and BCFA profiles. All these outcomes have different consequences on hindgut and dog health. Therefore, ingredients should be chosen which result in higher production of SCFA (particularly of butyric acid) and lower BCFA as an indicator of less branched-chain fatty acid production, which suggest reduced protein fermentation. A selection of several ingredients with different kinetic and end-product characteristics can be made to design diets to stimulate carbohydrate fermentation along the entire tract. However, all considerations on fermentability and SCFA production of the tested substrates should be evaluated in relation to gas production. Indeed, control of intestinal gas production is an important goal in selecting feed ingredients and diet formulations in order to avoid undesirable effects such as flatulence and soft faeces.

References

- Anderson, E., Fireman, M., 1935. The mucilage from psyllium seed, plantago psyllium. *J. Biol. Chem.* 109: 437-442.
- AOAC, 2005. Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Bauer, E., Williams, B.A., Voigt, C., Mosenthin, R., Verstegen, M.W.A., 2001. Microbial activities of faeces from unweaned and adult pigs, in relation to selected fermentable carbohydrates. *J. Anim. Sci.* 73:313-322.
- Bauer, E., Williams, B.A., Voigt, C., Mosenthin, R., Verstegen, M.W.A., 2003. Impact of enzyme pre-treatment on the fermentability of carbohydrate-rich feedstuffs. *J. Sci. Food Agr.* 83:207-214.
- Böhmer, B. M., Branner, G. R., Roth-Maier, D. A., 2005. Precaecal and faecal digestibility of inulin (DP 10-12) or an inulin/Enterococcus faecium mix and effects on nutrient digestibility and microbial gut flora. *J. Anim. Physiol. An. N.* 89:388-396.
- Bosch, G., Pellikaan, W.F., Rutten, P.G., van der Poel, A.F., Verstegen, M.W., Hendriks, W.H., 2008. Comparative in vitro fermentation activity in the canine distal gastrointestinal tract and fermentation kinetics of fibre sources. *J. Anim. Sci.* 86:2979-2989.
- Bovera, F., Calabrò, S., Cutrignelli, M. I., Infascelli, F., Piccolo, G., Nizza, S., Tudisco, R., Nizza, A., 2008. Prediction of rabbit caecal fermentation characteristics from faeces by in vitro gas production technique. *J. Anim. Physiol. An. N.* 92:260-271.
- Calabrò, S., Carone, F., Cutrignelli, M.I., D'Urso, S., Piccolo, G., Tudisco, R., Angelino, G., Infascelli, F., 2006. The effect of haymaking on the neutral detergent soluble fraction of two intercropped forages cut at different growth stages. *Ital. J. Anim. Sci.* 4:327-339.
- Calabrò, S., Moniello, G., Piccolo, V., Bovera, F., Infascelli, F., Tudisco, R., Cutrignelli, M.I., 2008. Rumen fermentation and degradability in buffalo and cattle using the in vitro gas production technique. *J. Anim. Physiol. An. N.* 92:356-362.
- Campbell, J.M., Fahey Jr., G.C., 1997. Psyllium and methylcellulose fermentation properties in relation to insoluble and soluble fibre standards. *Nutr. Res.* 17:619-629.
- Cutrignelli, M.I., 2007. In vitro assessments of pre- and probiotics. *A Supplement to Compendium 29:2A.*
- Cutrignelli, M.I., Bovera, F., Tudisco, R., D'Urso, S., Marono, S., Piccolo, G., Calabrò, S., 2009. In vitro fermentation characteristics of different carbohydrate sources in two dog breeds (German shepherd and Neapolitan mastiff). *J. Anim. Physiol. An. N.* 93:305-312.
- Cutrignelli, M.I., Calabrò, S., Tudisco, R., Zicarelli, F., Gazaneo, M.P., Piccolo, V., 2005. Comparison of buffalo rumen liquor and buffalo faeces as inoculum for the in vitro gas production technique. *Ital. J. Anim. Sci.* 4:319-321.
- Cutrignelli, M.I., D'Urso, S., Tudisco, R., Grossi, M., Piccolo, V., 2007. Effect of ruminant species (bovine *vs* buffalo) and source of inoculum (rumen liquor *vs* faeces) on in vitro fermentation. *Ital. J. Anim. Sci.* 6(Suppl.1):295-297.
- de Oliveira, L.D., Takakura, F.S., Kienzle, E., Brunetto, M.A., Teshima, E., Pereira, G.T., Vasconcelos, R.S., Carciofi, A.C., 2011. Fibre analysis and fibre digestibility in pet foods - a comparison of total dietary fibre, neutral and acid detergent fibre and crude fibre. *J. Anim. Physiol. An. N.* 96:895-906.
- Fahey Jr., G.C., Merchen, N.R., Corbin, J.E., Hamilton, A.K., Serbe, K.A., Hirakawa, D.A., 1990. Dietary fibre for dogs: 2 - Iso-total dietary fibre (TDF) additions of divergent fibre sources to dogs diets and their effects on nutrient intake, digestibility, metabolizable energy and digesta mean retention time. *J. Anim. Sci.* 68:4229-4235.
- Gibson, R., Roberfroid, M.B., 2008. Handbook of probiotics. CRC Press, New York, NY, USA.
- Gomes, M.O.S., Kawachi, I.M., Beraldo, M.C., Schocken-Inturrino, R.P., Coleta, F.E.D., Glória, M.B.A., Brunetto, M.A., Carciofi, A.C., 2008. Mannan oligosaccharides effects on nutrient digestibility, faecal microbiota, fermentation end-products, and immunological parameters of dogs. Paper 25 in Proc. 12th Int. Congr. ESCVN, Vienna, Austria.
- 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. Tech.* 64:77-89.
- James, W.P.T., 1980. Dietary fibre and mineral absorption. In: G.A. Spiller and R.M. Kay (eds.) *Medical aspects of dietary fibre.* New York Plenum Publ., NY, USA, pp 239-259.
- Leib, M.S., 2000. Treatment of chronic idiopathic large-bowel diarrhea in dogs with a highly digestible diet and soluble fibre: a retrospective review of 37 cases. *J. Vet. Intern. Med.* 14:27-32.
- Lowe, S.E., Theodorou M.K., Trinci, A.P.J., Hespell, R.B., 1985. Growth of anaerobic rumen fungi on defined and semi-defined media lacking rumen fluid. *J. Gen. Microbiol.* 131:2225-2229.
- Maskell, I.E., Johnson, J.V., 1993. Digestion and absorption. In: I. Burger (ed.) *The Waltham book of companion animal nutrition.* Pergamon Press, Oxford, UK, pp 25-44.
- McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., 2002. *Animal nutrition*, 6th ed. Pearson Education Ltd. Publ., Essex, UK.
- National Research Council, 2006. *Nutrient requirements of dogs and cats.* National Academy Press, Washington, DC, USA.
- Prosky, L., Asp, G.N., Schweizer, T.F., de Vries, J.W., Furda, I., 1988. Determination of insoluble, soluble, and total dietary fibre in foods and food products: interlaboratory study. *J. Assoc. Off. Ana. Chem.* 71:1017-1023.
- Roediger, W.E.W., 1982. Utilization of nutrients by isolated of epithelial cells of the rat colon. *Gastroenterology* 83:424-429.
- Salminen, S., Bouley, C., Boutron-Ruault, M.C., Cummings, J.H., Franck, A., Gibson, G.R., Isolauri, E., Moreau, M.C., Roberfroid, M., Rowland, I., 1998. Functional food science and gastrointestinal physiology and function. *Brit. J. Nutr.* 80(Suppl. 1): S147-S171.
- SAS, 2000. *User's Guide Statistics.* Version 8.2. SAS Institute, Inc. Cary, NC, USA.
- Sherrod, P.H., 1995. *NONLIN, Nonlinear Regression Analysis Program.* Available from: <http://archives.math.utk.edu/software/msdos/statistics/nonlin25/htm>
- Slavin, J., Green, H., 2007. *Dietary fibre and satiety.* British nutrition foundation. *Nutrition Bulletin* 32: 32-42.
- Smith, E. A., Macfarlane, G. T., 1997. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large

- intestine. *Microb. Ecol.* 33:180-188.
- Sunvold, G.D., Fahey Jr., G.C., Merchen, N.R., Reinhart, G.A., 1995a. In vitro fermentation of selected fibrous substrates by dog and cat fecal inoculum: influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. *J. Anim. Sci.* 73:1110-1122.
- Sunvold, G.D., Fahey Jr., G.C., Merchen, N.R., Titgemeyer, E.C., Bourquin, L.D., Bauer, L.L., Reinhart, G.A., 1995b. Dietary fibre for dogs: IV. In vitro fermentation of selected fibre sources by dog fecal inoculum and in vivo digestion and metabolism of fiber-supplemented diets. *J. Anim. Sci.* 73:1099-1109.
- Swanson, K.S., Fahey Jr., G.C., 2006. Potential role of yeast and yeast by-products in pet foods. In: L.A. Tucker (ed.) *Recent advances in pet nutrition*. Nottingham University Press, UK, pp 19-35.
- Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Tech.* 48:185-197.
- Tortola, L., Brunetto, M.A., Zainei, L., Souza, V.R., de Camargo, O.M.C., Prudente, N.S., Cavalieri, C.A., 2009. The use of psyllium to control constipation in dogs. *Cienc. Rural* 39:2638-2641.
- Vernazza, C.L., Rabi, B.A., Gibson, G.R., 2006. Human colonic microbiology and the role of dietary intervention: introduction to prebiotics. In: G.R. Gibson and R.A. Rastall (eds.) *Prebiotics: development and application*. John Wiley & Sons Ltd Publ., Chichester, UK, pp 1-28.
- Williams, B.A., Bosch, M.W., Boer, H., Verstegen, M.W.A., Tamminga, S., 2005. An in vitro batch culture method to assess potential fermentability of feed ingredients for monogastric diets. *Anim. Feed Sci. Tech.* 124:445-462.
- Williams, B.A., Van Osch, L.J.M., Kwakkel, R.P., 1997. Fermentation characteristics of the caecal contents of broiler chickens fed fine- and coarse particle diets. *Brit. Poultry Sci.* 38:41-42.
- 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.

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