Influence of source and concentrations of dietary fiber on in vivo nitrogen excretion pathways in pigs as reflected by in vitro fermentation and nitrogen incorporation by fecal bacteria[1,2,3]

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ABSTRACT: The inclusion of dietary fiber (DF) in diets has been suggested as a way to reduce NH_3 emission in pig barns because it contributes to a shift in N excretion from urine to feces owing to enhanced bacterial growth in the intestines. This study compared an in vitro method to measure bacterial protein synthesis during fermentation with an in vivo N excretion shift induced by diets differing in DF concentrations and solubility. The first experiment measured the effect of graded concentrations of sugar beet pulp (SBP; 0, 10, 20, and 30%) in corn- and soybean meal-based diets on in vivo N excretion partitioning between the urine and feces. A second experiment investigated the replacement of SBP, rich in soluble DF, with oat hulls (OH), rich in insoluble DF (20:0, 10.5:10.5, and 0:22%, respectively). In parallel, the fermentation characteristics of the dietary carbohydrates not digested in the small intestine were evaluated in an in vitro gas test, based on their incubation with colonic microbiota, using a mineral buffer solution enriched with ^{15}N. The N originating from the buffer solution incorporated into the bacterial proteins (BNI) was measured when half the final gas volume was produced (8.5 to 14.5 h of fermentation) and after 72 h of fermentation. Short-chain fatty acids were determined in the liquid phase. In the first experiment, the inclusion of SBP linearly decreased urinary N excretion from 0.285 to 0.215 g of N excreted in the urine per gram of N ingested and decreased the urinary-N:fecal-N excretion ratio from 2.171 to 1.177 ($P < 0.01$). In the second experiment, substituting SBP with OH linearly increased the urinary-N:fecal-N excretion ratio ($P = 0.009$). Unlike short-chain fatty acid production, BNI was greater at half-time to asymptotic gas production than at 72 h of fermentation. Sugar beet pulp enhanced BNI linearly ($P < 0.001$), 2.01, 2.06, and 2.35 mg g^{-1} of diet with 10, 20, and 30% SBP, respectively, as compared with 1.51 mg for the control diet. The substitution of SBP with OH decreased BNI ($P < 0.01$). With the exception of final gas production, all in vitro kinetic characteristics and BNI were correlated with in vivo N excretion parameters, and regression equations for the prediction of N excretion pathways from in vitro data were identified. Even if the presence of resistant starch in the diet might alter the composition of the fibrous residue that is fermented, the in vitro method is a possible useful tool for the formulation of diets, reducing the effects of pig production on the environment.

Key words: bacterial protein synthesis, dietary fiber, fermentation, in vitro method, nitrogen excretion, pig

J. Anim. Sci. 2009. 87:583–593
doi:10.2527/jas.2007-0717

[1]This is a corrected version of the original article. Corrections have been made to the math equations in text and in Table 6.

[2]Presented in part at the Midwestern American Society of Animal Science/American Dairy Science Association Annual Meeting, Des Moines, Iowa, March 17 to 19, 2008 ("Fermentable nonstarch polysaccharides increase the excretion of bacterial proteins in the pig's feces and reduce urinary nitrogen excretion").

[3]Research funded by the National Fund for Scientific Research (FNRS, Brussels, Belgium). The authors gratefully acknowledge the personnel of the Faculty of Gembloux and the "Centre wallon de Recherches agronomiques" for their technical assistance and Nicolas Gengler (FUSAGx, Gembloux, Belgium) for his contribution to the statistical analysis of the results.

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Received November 8, 2007.
Accepted September 11, 2008.

INTRODUCTION

Well-formulated diets contribute to a reduction in NH_3 emissions in pig barns. The use of synthetic AA and balanced and highly digestible proteins minimizes N excretion, namely, in the form of urea in urine (Aarnink and Verstegen, 2007). More recently, attention has also been paid to the possible effects of fermentable dietary fiber (**DF**) on N emissions (reviewed by Bindelle et al., 2008). The fermentation of these carbohydrates by gut bacteria induces a shift of N excretion from urea in urine to feces (Zervas and Zijlstra, 2002a), as explained by an increased uptake of NH_3 by bacteria in the large intestine (Mosenthin et al., 1992). The increased production of short-chain fatty acids (**SCFA**) also acidifies feces (Canh et al., 1998). Ammonia emissions during manure storage and handling are thereby reduced (Kreuzer et al., 1998; Nahm, 2003).

The efficiency of fermentable DF to shift N from urine to feces has been highlighted in reports on pigs (Canh et al., 1997; Zervas and Zijlstra, 2002a; Shriver et al., 2003), but little information is available on the amount and type of DF required to obtain the desired effect. Numerous studies have been conducted with sugar beet pulp (**SBP**) or isolated DF fractions, but rarely with whole feedstuffs.

In vitro techniques are of interest because they can provide information on the variables that affect DF fermentability and SCFA production more rapidly than in vivo approaches (Coles et al., 2005). Recently, Bindelle et al. (2007b) demonstrated, using an in vitro technique, that it is possible to correlate N uptake by bacteria isolated from the large intestine of pigs with the rates of fermentation, as well as with the chemical composition of DF. However, these observations require validation in vivo.

The present study aimed to assess whether the influence of fermentable DF in pig diets on N excretion pathways measured in vivo could be reflected by intestinal bacterial protein synthesis and fermentation characteristics measured in vitro. Therefore, this study aimed to correlate in vivo with in vitro characteristics and to seek the limits of the in vitro method as a first validation step before it can be used routinely.

MATERIALS AND METHODS

The animal protocols were approved by the Ethical Committee of the Belgian Council for Laboratory Animal Science.

Two experiments were undertaken to compare the urinary-N:fecal-N excretion ratio in vivo with the results of in vitro DF fermentation. Each experiment consisted of a total-tract in vivo digestibility trial and an in vitro fermentation test. The 2 experiments differed according to the diets that were used. In Exp. 1, increasing the DF content in the diet by increasing the amount of SBP was evaluated. In Exp. 2, the substitution of SBP, rich in soluble DF, with oat hulls (**OH**), rich in insoluble DF, was assessed.

Total-Tract In Vivo Digestibility

Animals. A total of 32 Belgian Landrace × Pietrain castrated pigs were used. Sixteen pigs (41.5 ± 8.2 kg of BW) were used for the digestibility trials corresponding to Exp. 1, and the other 16 pigs (46.7 ± 11.0 kg of BW) were used for the digestibility trials corresponding to Exp. 2.

Diets. In Exp. 1, 4 diets were formulated to contain graded concentrations of total DF by using SBP: a standard diet (**StD**), 0% SBP; a 10% SBP diet (**SBP_10**); a 20% SBP diet (**SBP_20**); and a 30% SBP diet (**SBP_30**) at the expense of corn (*Zea mays* L.; Table 1). In Exp. 2, besides the StD, 3 diets were prepared to contain similar concentrations of total DF but different soluble DF:insoluble DF ratios, by means of SBP as a source of soluble DF, and OH as an insoluble DF source (Table 1): a 20% SBP and 0% OH diet (**SBP_20**), a 10.5% SBP and 10.5% OH diet (**SBP_10:OH_10**), and a 0% SBP and 22% OH diet (**OH_20**). All diets were formulated to contain similar contents of DE, NE, CP, and digestible protein.

Methodology. The same methodology was used for Exp. 1 and 2. The experimental scheme was completely randomized with changeover of pigs between 2 periods of measurements: 4 pigs × 4 diets × 2 periods. After a 2-wk adaptation period to the diets, the pigs (4 per diet) were placed in individual metabolism cages designed to collect urine (using 1 M H_2SO_4) and feces separately. During the 5-d collection period, the pigs received 90 $g \cdot kg^{-0.75} \cdot d^{-1}$, corresponding to 2.7× the maintenance requirements of the pigs (Institut Technique du Porc, 2000) in 2 meals (0800 and 1500 h), mixed with an equal amount of fresh water. All feces and urine were collected every day and weighed. An aliquot representing 10% of the daily excretion was kept at −18°C. The refusals were also collected, weighed, and kept at −18°C. At the end of the experimental period, pigs were randomly assigned to another experimental diet and the procedure was repeated for a second period. A pig could be on the same diet for both periods.

Analyses. Refusals, diets, and feces were freeze-dried, ground to pass through a 1-mm mesh screen by means of a Cyclotec 1093 Sample Mill (Foss Electric A/S, Hillerød, Denmark), and analyzed for their content of DM by drying at 105°C for 24 h (method 967.03; AOAC, 1990), ash by burning at 550°C for 8 h (method 923.03; AOAC, 1990), N by using the Kjeldahl method and calculating the CP content (N × 6.25; method 981.10; AOAC, 1990), ether extract with the Soxhlet method by using diethyl ether (method 920.29; AOAC, 1990), and NDF by the method of Van Soest et al. (1991), using Na_2SO_3 and Termamyl (Novo Nordisk, Bagsværd, Denmark) with the Fibercap system (Foss Electric, Bagsvaerd, Denmark). Urine was analyzed for

Table 1. Composition and analysis of the diets (g·kg^{-1}, as-fed basis)

Item	StD[1,2]	SBP_10[1]	SBP_20[1,2]	SBP_30[1]	SBP_10:OH_10[2]	OH_20[2]
Composition						
Corn	752.0	630.0	500.0	340.0	455.0	375.1
Sugar beet pulp (SBP)	—	100.0	200.0	300.0	105.0	—
Oat hulls (OH)	—	—	—	—	105.0	225.0
Soybean meal	198.7	190.0	185.0	175.0	200.0	195.0
Soybean oil	—	5.0	25.0	42.0	50.0	65.0
Skim milk powder	—	30.0	50.0	100.0	45.0	100.0
Mineral and vitamin premix[3]	20.0	20.0	20.0	20.0	20.0	20.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	6.3	3.0	—	—	—	—
Dicalcium phosphate, dihydrate	19.8	18.8	16.8	20.0	16.8	16.8
L-Tryptophan	0.2	0.2	0.2	0.01	0.02	0.01
Calculated						
DE, MJ·kg^{-1} of diet	13.61	13.44	13.53	13.57	13.59	13.66
NE, MJ·kg^{-1} of diet	9.76	9.55	9.59	9.58	9.66	9.71
Digestible protein	131.6	129.8	127.1	126.0	127.5	129.1
SID lysine[4]	8.6	9.0	9.4	10.5	9.4	10.3
Ca	12.0	11.7	11.2	13.4	10.4	10.0
Available P	3.8	3.8	3.6	4.3	3.6	3.8
Analyzed						
GE, MJ·kg^{-1} of DM	16.3	16.3	17.0	17.3	17.5	18.1
Ash	64.5	68.3	70.0	79.7	67.9	69.4
CP	155.6	156.4	154.6	154.7	153.4	153.6
Fat	34.4	38.8	59.4	76.2	79.2	105.3
Starch	467.7	395.5	325.6	232.8	289.4	253.4
NDF	63.3	92.2	108.6	137.7	141.3	185.0
ADF	26.4	45.5	61.9	83.0	80.5	100.4
ADL	2.1	3.2	5.2	7.0	7.6	13.2
Total dietary fiber	95.9	146.1	194.5	254.3	210.1	212.8
Insoluble dietary fiber	88.3	119.9	151.9	188.1	186.7	205.0
Soluble dietary fiber	7.7	26.2	42.6	66.2	23.4	7.9

[1]Diets used in Exp. 1, where the influence of an increasing concentration of dietary fiber was investigated: StD = standard diet, 0% SBP; SBP_10 = 10% SBP diet; SBP_20 = 20% SBP diet; SBP_30 = 30% SBP diet.

[2]Diets used in Exp. 2, where diets containing similar concentrations of total dietary fiber but different soluble dietary fiber:insoluble dietary fiber ratios were investigated: SBP_20 = 20% SBP and 0% OH diet; SBP_10:OH_10 = a 10.5% SBP and 10.5% OH diet; OH_20 = a 0% SBP and 22% OH diet.

[3]Mineral and vitamin premix [2507 VAPOR 220 LMT GREEN (Trouw Nutrition, Ghent, Belgium)] supplied per kilogram of diet: vitamin A, 8,000 IU as vitamin A acetate; vitamin D_3, 2,000 IU as cholecalciferol; vitamin E, 50 mg as DL-α-tocopherol acetate; vitamin K_3, 0.86 mg as menadione sodium bisulfite; thiamine, 0.86 mg as thiamine mononitrate; betaine, 100 mg as betaine anhydrate; riboflavin, 2.8 mg; niacin, 7 mg; pyridoxine, 1.76 mg as pyridoxine chlorhydrate; cyanocobalamin, 0.015 mg; pantothenic acid, 7 mg as calcium D-pantothenate; folic acid, 0.1 mg; I, 1 mg as potassium iodine; Co, 0.5 mg as cobalt carbonate hydroxide monohydrate; Se, 0.42 mg as sodium selenite; Cu, 12 mg as copper(II) sulfate pentahydrate; Mn, 40 mg as manganese oxide; Zn, 100 mg as zinc oxide; Fe, 150 mg as ferrous sulfate monohydrate, L-lysine-HCl, 1.6 g; DL-methionine, 0.4 g; L-threonine, 0.325 g; Ca, 4.0 g; Na, 1.4 g.

[4]Standardized ileal digestible (SID) lysine; AA supply was calculated to meet the recommended grams of AA per kilogram of diet (Institut Technique du Porc, 2000): SID lysine, 8.6; SID methionine + cystine, 5.1; threonine, 5.6; tryptophan, 1.6; leucine, 6.0; isoleucine, 5.0; valine, 5.5; histidine, 2.0; arginine, 2.5; phenylalanine + tyrosine, 8.0.

N content by the Kjeldahl method. Gross energy was measured by means of an adiabatic oxygen bomb calorimeter (1241 Adiabatic Calorimeter, Parr Instrument Co., IL). The diets were also analyzed for ADF and ADL (Van Soest et al., 1991); starch (Faisant et al., 1995); and total, soluble, and insoluble DF contents (method 991.43; AOAC, 1990) after grinding the samples through a 0.5-mm mesh screen.

Calculations and Statistics. Apparent digestibility and N retention were calculated by the difference between the amount of nutrients ingested and the amount excreted. The individual pig was considered the experimental unit. In Exp. 1, the feed intake, digestibility coefficients, and N retention were analyzed for linear and quadratic effects of the concentrations in SBP by means of the MIXED procedure (SAS Inst. Inc., Cary, NC), using the following regression models:

$$\text{Linear model}: \quad Y = \mu + \alpha \cdot S + P_j + \alpha_{P_j} \cdot S + A_k + \varepsilon,$$

$$\text{Quadratic model}: \quad Y = \mu + \alpha \cdot S + P_j + \alpha_{P_j} \cdot S + \beta \cdot \left(S\right)^2 + \beta_{P_j} \cdot \left(S\right)^2 + A_k + \varepsilon,$$

where Y is the result, μ is the mean, S is the continuous effect of SBP concentration in Exp. 1 (S = 0, 10, 20, 30), α is the linear regression coefficient for S, P_j is the class effect of the period (j = 1, 2), α_{Pj} is the linear regression coefficient for S specific to each period, A_k is

the random effect of the pig ($k = 1,...,16$), β is the quadratic regression coefficient for S^2, β_{Pj} is the quadratic regression coefficient for S specific to each period, and ε is the error term. In Exp. 2, the effect of the amount of soluble DF through SBP content was analyzed by using the same models. However, this time the StD was not included in the analysis ($S = 0$, 10.5, 20), because the total DF content was significantly less compared with the SBP_20, SBP_10:OH_10, and OH_20.

For both experiments, the quadratic model was tested first. When the P-value of the regression coefficient for the quadratic effect of SBP concentration was not significant ($P > 0.05$), the linear model was used. The P-values for the regression coefficients of S specific to a period (α_{Pj} and β_{Pj}) were never significant ($P > 0.05$). Therefore, only the P-values for the linear regression coefficients, quadratic regression coefficients, or both for SBP concentration (α and β) are presented in the tables.

Enzymatic Hydrolysis and In Vitro Fermentation

The diets tested in Exp. 1 and 2 were treated simultaneously during the in vitro part of this study. The method has been described in detail by Bindelle et al. (2007a). Briefly, samples were treated with pepsin and pancreatin according to the method of Boisen and Fernández (1997). The DM disappearance (IVDMD) during enzymatic hydrolysis was calculated. Subsequently, the hydrolyzed residue was incubated in glass syringes by using a bacterial inoculum prepared with a buffer solution (Menke and Steingass, 1988) and a mix of feces from 3 pigs fed the SBP_10:OH_10 diet, containing SBP and OH (Table 1). The ^{15}N-labeled NH_4Cl (2% of enrichment, T85-70216, Isotec, Miamisburg, OH) was used as the sole N source in the buffer solution. In a preliminary step, the incubation lasted for 72 h. The half-time to asymptotic gas production (***T/2***), corresponding to the time required to produce 50% of the total gas production, was then estimated. The procedure was repeated for 3 different periods and the incubation was stopped at $T/2$ (from 8.5 to 14.5 h, depending on the diets; see Results section) and 72 h, according to the syringe.

The experimental scheme was as follows: (9 syringes × 6 diets + 3 blanks) replicated over 3 fermentation runs. Per diet and fermentation run, 6 syringes were stopped at $T/2$ by quenching in an ice-water bath for 20 min. The 3 syringes remaining were stopped after 72 h and their gas volumes were recorded at regular intervals.

When the syringes were stopped, they were subsequently emptied and rinsed with distilled water (2 × 5 mL). The fermentation broths of 3 syringes stopped after $T/2$ hours of fermentation were pooled and freeze-dried for further determination of remaining starch and total DF in the residue. The content of the 3 other syringes stopped at $T/2$ and the 3 syringes stopped after 72 h of fermentation were centrifuged (12,000 × g, 20 min, 4°C), and an aliquot of the supernatant was taken for SCFA analysis. The pellet was suspended in distilled water (30 mL) and centrifuged (12,000 × g, 20 min, 4°C), and the supernatant was discarded. The resulting pellet concentrating the bacterial and undigested substrate was freeze-dried, weighed, and analyzed for total N and ^{15}N enrichment. For each period, 6 samples of the inoculum were also taken for ^{15}N, SCFA, and starch and total DF analysis.

Kinetics of Gas Production. Gas accumulation curves recorded during the 72 h of fermentation were modeled according to France et al. (1993):

$$G = 0, \text{ if } 0 < t < L$$

$$= G_f\left(1 - \exp\left\{-\left\langle \begin{matrix} b(t-L) \\ +c\left(\sqrt{t} - \sqrt{L}\right) \end{matrix} \right\rangle\right\}\right), \text{ if } t \geq L,$$

where G denotes the gas accumulation, G_f (mL·g^{-1} hydrolyzed residue) denotes the maximum gas volume for $t = \infty$, and L (h) denotes the lag time before fermentation starts. 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:

$$\mu = b + \frac{c}{2\sqrt{t}}, \text{ if } t \geq L.$$

The kinetic characteristics [G_f, L, $\mu_{t\,=\,T/2}$ (fractional rate of degradation), and $T/2$] were compared in the statistical analysis (Figure 1). The syringes that suffered an accidental leakage of gas caused by broken clips were discarded.

Measurement of SCFA Production. The supernatants, prepared as described above, were filtered with a 0.2-μm nylon 13-mm HPLC Syringe Filter (No. 2166, Alltech Associates Inc., Deerfield, IL) and analyzed for SCFA with a Waters 2690 HPLC system (Waters, Milford, MA) fitted with a HPX 87 H column (Bio-Rad, Hercules, CA) at 30°C, with isocaproic acid as the internal standard and 0.01 N H_2SO_4 as the mobile phase, combined with a Waters 2487 Dual Wavelength Absorbance Detector operating at a wavelength of 210 nm.

Measurement of N Incorporation into Microbial Cells. Total N and ^{15}N enrichment in the freeze-dried pellets were measured by an elemental analyzer coupled to an isotope-ratio mass spectrometer (Europa Scientific Ltd., Crewe, UK). Bacterial N incorporation (**BNI**) corresponding to N in the pellet incorporated from the buffer solution into the bacteria, per gram of diet, per gram of incubated hydrolyzed residue, and per gram of actually fermented polysaccharides was calculated from total N and ^{15}N content

according to the equations of Bindelle et al. (2007b) and from IVDMD.

The BNI in feces subsequent to fermentation of the fibrous fraction of the diet was estimated as follows:

$$\text{Fecal bacterial N } (\text{g}\cdot\text{g}^{-1}) = \frac{BNI \times 6.25}{\left(1 - dCP\right) \times CP},$$

where BNI ($\text{g}\cdot\text{g}^{-1}$ of DM) denotes the bacterial N incorporation per gram of diet, 6.25 denotes the conversion factor of N into CP, and dCP denotes the apparent fecal digestibility of the CP (content in the diet).

Statistical Analysis. The syringe was the experimental unit. For the statistical analysis, the syringes were allocated to 2 groups according to the diet used in vivo: the Std, SBP_10, SBP_20, and SBP_30 diets for Exp. 1; and the SBP_20, SBP_10:OH_10, and OH_10 diets for Exp. 2. In Exp. 1, the gas production characteristics, SCFA production, and BNI were analyzed for linear and quadratic effects of the concentration in SBP by means of the MIXED procedure of SAS, using the following regression models:

$$\text{Linear model}: \quad Y = \mu + \alpha \cdot S + R_k + \varepsilon,$$

$$\text{Quadratic model}: \quad Y = \mu + \alpha \cdot S + \beta \cdot (S)^2 + R_k + \varepsilon,$$

where Y is the result, μ the mean, S is the continuous effect of the SBP concentration in Exp. 1 ($S = 0$, 10, 20, 30), α is the linear regression coefficient for S, β is the quadratic regression coefficient for $(S)^2$, R_k is the random effect of the fermentation run ($k = 1$, 2, 3), and ε is the error term. The quadratic model was tested first. When the P-value of the regression coefficient for the quadratic effect of SBP concentration was not significant ($P > 0.05$), the linear model was used. In Exp. 2, the effect of the amounts of SBP was analyzed by using the same models, where $S = 0$, 10.5, or 20.

Linear regressions were calculated between in vivo and in vitro data by using the STEPWISE and the REG procedures of SAS. These analysis were performed on all the diets simultaneously (StD, SBP_10, SBP_20, SBP_30, SBP_10:OH_10, and OH_20) by merging the databases of Exp. 1 and 2. The average value was used for the in vivo variables of diets StD and SBP_20 because they appeared in both Exp. 1 and 2.

RESULTS

Total-Tract In Vivo Digestibility

The pigs behaved normally and remained in good health during both experiments, with the exception of 2 pigs that were removed because of leg problems. In Exp. 1, DMI slightly and linearly decreased with increasing concentration of SBP ($P = 0.012$; Table 2). Conversely, no effect of the SBP:OH ratio on DMI was observed in Exp. 2 ($P = 0.358$; Table 3).

The N retention values were not affected by SBP concentration ($P = 0.629$) or the SBP:OH ratio ($P = 0.903$), but the N excretion balance was greatly modified. In Exp. 1, N excretion in urine ($P < 0.001$) and the urinary-N:fecal-N excretion ratio ($P = 0.002$) decreased linearly when graded concentrations of SBP were added to the diets (Table 2). In Exp. 2, the substitution of SBP DF with OH linearly increased the urinary-N:fecal-N excretion ratio ($P = 0.009$) and N excretion in the urine, with a concentration approaching significance ($P = 0.052$; Table 3).

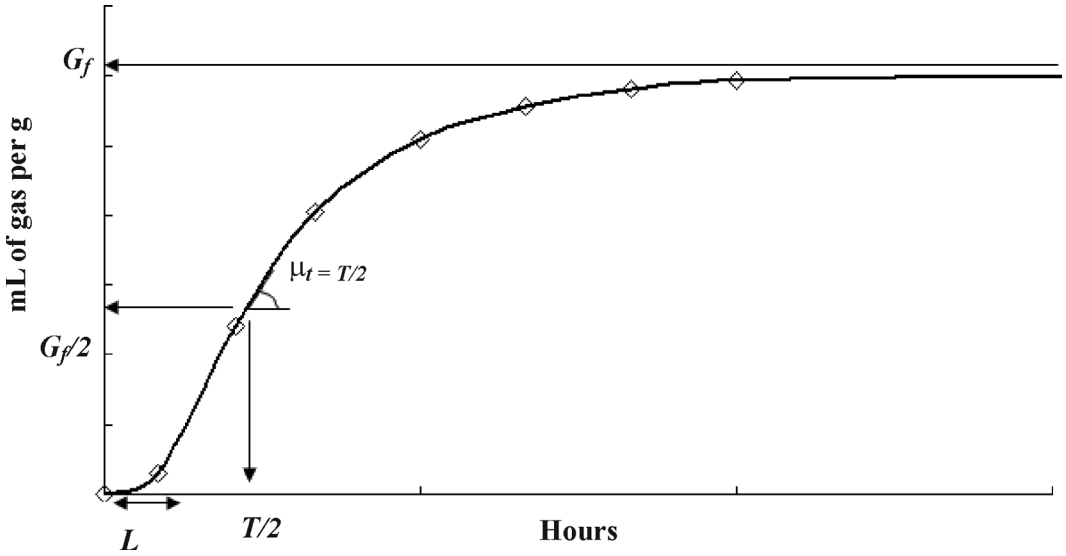

Figure 1. Representation of the kinetic parameters of the gas accumulation curves modeled according to France et al. (1993), where G_f ($\text{mL}\cdot\text{g}^{-1}$ of substrate) denotes the maximum gas volume for $t = \infty$, L (h) denotes denotes the lag time before fermentation starts, $T/2$ denotes 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.

Table 2. Digestibility, and N retention and excretion pathways of the diets with increasing sugar beet pulp (SBP) content (Exp. 1)

	Diet[1]					Regression coefficients for SBP concentration	
Item	StD	SBP_10	SBP_20	SBP_30	SEM	Linear, *P*-value	Quadratic, *P*-value
No. of pigs	8	8	7	8			
Intake, g·kg^{-75}·d^{-1}	98.9	96.8	95.5	92.2	9.15E-1	0.012	NA[2]
Digestibility coefficient							
DM	0.870	0.852	0.842	0.832	4.18E-3	<0.001	NA
OM	0.899	0.882	0.872	0.865	3.57E-3	0.006	NA
NDF	0.509	0.640	0.707	0.789	2.00E-2	<0.001	NA
CP	0.865	0.831	0.818	0.815	6.61E-3	0.004	0.046
N-retention, g of N retained/g of N ingested	0.580	0.571	0.595	0.600	7.70E-3	0.629	NA
Urinary-N, g of N excreted in urine/g of N ingested	0.285	0.261	0.223	0.215	6.79E-3	<0.001	NA
Urinary-N:fecal-N	2.171	1.646	1.243	1.177	9.61E-2	0.002	0.044

[1]StD = standard diet, 0% SBP; SBP_10 = 10% SBP diet; SBP_20 = 20% SBP diet; SBP_30 = 30% SBP diet.

[2]NA = not applicable because the *P*-value of the quadratic regression coefficient for SBP concentration was not significant ($P > 0.05$) and the linear model was used.

The digestibility of the other components of the diets was generally linearly affected by SBP concentration or the SBP:OH ratio ($P < 0.01$) of the diets, except for CP in Exp. 1, which was quadratically affected by the SBP concentration ($P = 0.046$). In Exp. 2, the SBP:OH ratio also quadratically affected the NDF digestibility ($P = 0.034$).

In Vitro Digestion and Fermentation

The IVDMD was linearly and negatively affected by the inclusion of SBP in the diet ($P < 0.001$; Table 4). For a similar concentration in total DF, the substitution of SBP with OH quadratically and negatively influenced IVDMD after pepsin-pancreatin hydrolysis ($P < 0.001$; Table 4).

In Exp. 1, the fermentation rates were positively and quadratically affected by the presence of SBP in the diets, as indicated by the decreasing $T/2$ and increasing fractional rates of degradation ($\boldsymbol{\mu_{t\,=\,T/2}}$) recorded when passing from the StD to the SBP_10, the SBP_20, and finally the SBP_30 diet ($P < 0.01$). These fermentation characteristics were affected by the incorporation of OH; $T/2$ increased and $\mu_{t\,=\,T/2}$ decreased when passing from the SBP_20 to the SBP_10:OH_10 and OH_20 diets. Oat hulls also linearly decreased the final gas production ($P < 0.001$), but the inclusion of SBP had no effect on this variable ($P = 0.322$).

The BNI (Table 5), expressed per gram of incubated residue of the hydrolysis or per gram of diet, was positively and linearly affected by the presence of SBP in the diets ($P < 0.05$). The substitution of SBP with

Table 3. Digestibility, and N retention and excretion pathways of the diets with differing oat hull (OH):sugar beet pulp (SBP) ratios (Exp. 2)

	Diet[1]					Regression coefficients for SBP concentration	
Item	StD	OH_20	SBP_10:OH_10	SBP_20	SEM	Linear, *P*-value	Quadratic, *P*-value
No. of pigs	8	8	7	8			
Intake, g·kg^{-75}·d^{-1}	109.7	106.2	97.9	106.1	2.46E+0	0.358	NA[2]
Digestibility coefficient							
DM	0.867	0.731	0.788	0.842	1.00E-2	<0.001	NA
OM	0.896	0.751	0.813	0.870	1.06E-2	<0.001	NA
NDF	0.507	0.191	0.369	0.708	3.63E-2	0.101	0.034
CP	0.857	0.861	0.829	0.810	5.11E-3	0.002	NA
N-retention, g of N retained/g of N ingested	0.554	0.575	0.579	0.587	1.16E-2	0.903	NA
Urinary-N, g of N excreted in urine/g of N ingested	0.303	0.285	0.250	0.224	9.29E-3	0.052	NA
Urinary-N:fecal-N	2.178	2.076	1.477	1.198	1.05E-1	0.009	NA

[1]StD = standard diet, 0% SBP and 0% OH; OH_20 = 0% SBP and 22% OH diet; SBP_10:OH_10 = 10.5% SBP and 10.5% OH diet; SBP_20 = 20% SBP and 0% OH diet. The effect of the ratio of soluble dietary fiber (DF):insoluble DF was investigated. The StD was not included in the analysis because its total DF content was significantly less compared with the other diets.

[2]NA = not applicable because the *P*-value of the quadratic regression coefficient for SBP concentration was not significant ($P > 0.05$) and the linear model was used.

Table 4. Dry matter disappearance (IVDMD) during the pepsin-pancreatin hydrolysis, and kinetic parameters of the gas accumulation curves recorded during fermentation of the hydrolyzed diets

	Diet							Regression coefficients for SBP concentration			
								Exp. 1		Exp. 2	
Item	StD[1,2]	SBP_10[1]	SBP_20[1,2]	SBP_30[1]	SBP_10:OH_10[2]	OH_10[2]	SEM	Linear, *P*-value	Quadratic, *P*-value	Linear, *P*-value	Quadratic, *P*-value
Pepsin-pancreatin hydrolysis											
No. of replicates	20	20	23	20	22	23					
IVDMD[3]	0.765	0.745	0.752	0.714	0.722	0.719	2.36E-3	<0.001	NA[4]	0.032	<0.001
Gas production kinetics											
No. of syringes	9	9	9	8	9	9					
L,[5] h	1.3	1.1	1.2	3.5	1.7	2.9	1.51E-1	<0.001	<0.001	<0.001	NA
$T/2$,[6] h	10.1	9.2	8.7	8.5	9.7	14.5	3.03E-1	<0.001	0.006	<0.001	<0.001
$\mu_{t=T/2}$,[7] h^{-1}	0.088	0.100	0.122	0.150	0.090	0.047	4.47E-3	0.019	0.003	<0.001	NA
Gf,[8] $mL \cdot g^{-1}$ of hydrolyzed residue	302	304	301	307	238	158	7.77E+0	0.322	NA	<0.001	NA

[1]Diets used in Exp. 1: StD = standard diet, 0% sugar beet pulp (SBP); SBP_10 = 10% SBP diet; SBP_20 = 20% SBP diet; SBP_30 = 30% SBP diet. The effect of increasing SBP content was investigated.

[2]Diets used in Exp. 2: StD = standard diet, 0% SBP and 0% oat hulls (OH); OH_20 = 0% SBP and 22% OH diet; SBP_10:OH_10 = 10.5% SBP and 10.5% OH diet; SBP_20 = 20% SBP and 0% OH diet. The effect of the ratio of soluble dietary fiber (DF):insoluble DF was investigated. The StD was not included in the analysis because its total DF content was significantly less compared with the other diets.

[3]IVDMD = DM disappearance during the enzymatic hydrolysis.

[4]NA = not applicable because the *P*-value of the quadratic effect was not significant ($P > 0.05$) and the linear model was used.

[5]L = lag time before fermentation starts.

[6]$T/2$ = half time to asymptotic gas production.

[7]$\mu_{t=T/2}$ = fractional rate of degradation of the substrate.

[8]G_f = maximum gas volume.

OH had a negative and quadratic effect on BNI ($P < 0.01$). When expressed per gram of fermented polysaccharides, BNI was also positively affected by the presence of SBP ($P = 0.038$). Because of a reduction in the number of replicates caused by the pooling of samples for residual DF analysis after the fermentation, the effect of the substitution of SBP with OH was not significant ($P = 0.073$). The polysaccharide disappearance at $T/2$ was negatively affected by the presence of OH in the diet ($P < 0.001$).

The production of SCFA was affected only by the substitution of SBP with OH in the diets ($P < 0.001$). The SBP concentration in the diets had no effect on this variable. However, graded concentrations of SBP induced a linear decrease in the proportion of butyrate ($P = 0.003$).

Regressions Between In Vitro and In Vivo Data

Bacterial N incorporation, measured in vitro and expressed per gram of diet, was used to calculate the BNI in feces subsequent to the fermentation of the fibrous fraction of the diet (Table 5). As indicated by the regression equation presented in Table 6, in vivo CP apparent digestibility and the urinary-N:fecal-N excretion ratio were negatively affected by the soluble DF content of the diets ($r^2 > 0.72$, $P < 0.05$), the in vitro $\mu_{t = T/2}$ ($r^2 > 0.59$, $P < 0.05$), and the in vitro BNI ($r^2 > 0.65$, $P < 0.05$). Stronger regression equations ($R^2 > 0.89$, $P < 0.017$) were obtained when introducing the butyrate molar ratio (***but***, %) in the equations linking BNI to in vivo CP apparent digestibility and the urinary-N:fecal-N excretion ratio, with BNI not being correlated with *but* ($r = -0.090$, $P = 0.865$).

DISCUSSION

Because similar N retention values were recorded in pigs fed diets differing in DF concentrations and types, especially the ratio between soluble and insoluble DF, the differences in N excretion pathways can be ascribed to DF fermentation in the intestines. In Exp. 1, the increase in NDF digestibility with the fiber content of the diet was due to the high fermentability of SBP NDF, compared with corn NDF, as reported by Chabeauti et al. (1991) and Guillon et al. (1998). Their reports are consistent with the results of Lynch et al. (2008). The effect of SBP on N excretion is also consistent with the results of Canh et al. (1997) and Zervas and Zijlstra (2002a), who observed increased fecal N output and decreased urinary N excretion in pigs fed SBP and soybean hulls. Conversely, the low NDF digestibility of the SBP_10:OH_10 and OH_20 diets in Exp. 2 is consistent with the poor fermentability of OH fiber previously observed by Zervas and Zijlstra (2002b). It also explains why the addition OH favors N excretion in the urine.

The regression equation linking (throughout Exp. 1 and 2) the soluble DF content of the diets to the apparent fecal digestibility of CP or the urinary-N:fecal-N excretion ratio confirms the role of fermentable DF originating from SBP in N excretion, because SBP was the main source of soluble DF in the diets.

Consistency Between In Vivo and In Vitro Observations

Most of the in vitro fermentation characteristics, kinetics of gas production, BNI, and SCFA production values were consistent with the in vivo modifications of N excretion pathways subsequent to the increase in concentration of fermentable SBP fiber. As indicated by the results of Exp. 1 and 2, greater concentrations of fermentable SBP fiber enhanced the rates of fermentation of the fibrous pepsin-pancreatin hydrolyzed residues, as expressed through increased $\mu_{t = T/2}$ and reduced $T/2$. The presence of rapidly fermenting SBP DF (Bindelle et al., 2007b) favors the shift in N excretion from urine to feces. This relationship was highlighted by the linear regression linking the N-urine:N-fecal excretion ratio to $\mu_{t = T/2}$. The in vitro relationships between BNI and $\mu_{t = T/2}$ ($r = 0.84$) or $T/2$ ($r = -0.99$) observed in this study (data not shown) confirm previous observations showing that rapidly fermenting substrates yield N incorporation by isolated porcine colonic bacteria (Bindelle et al., 2007b). Diets with increased in vitro $\mu_{t = T/2}$ induced reductions in CP apparent digestibility, urinary-N excretion, and the urinary-N:fecal-N excretion ratio in pigs. Consequently, the BNI in feces subsequent to the fermentation of the fibrous fraction of the diet could be doubled when using a DF source with high rates of fermentation instead of poorly fermentable DF.

The final gas and total SCFA production of the diets used in Exp. 1 was not affected by the concentration of SBP, the converse of fermentation kinetics. This means that the fibrous residues recovered after the pepsin-pancreatin hydrolysis showed the same final degradability by intestinal bacteria. Nevertheless, their fermentability actually differed because the presence of SBP increased the fermentation rates. Furthermore, gas and SCFA production should take the proportion of the diet that was actually submitted to fermentation into account. This proportion increased with SBP concentration because, in the in vitro method, it is linked to the IVDMD during enzymatic hydrolysis. Indeed, when expressed per gram of diet instead of per gram of fibrous residue, gas and SCFA production was positively affected by increasing the fermentable DF concentration. This was also applicable to Exp. 2.

Limits of the In Vitro Method

The accuracy of the regression equation (Table 6) was greatly enhanced when using the *but* as a second

Table 5. Short-chain fatty acid (SCFA) production, molar ratios, bacterial N incorporation (BNI), and polysaccharide disappearance measured after one-half the final gas volume was produced ($T/2$) and 72 h of fermentation

		Diet							Regression coefficients for SBP concentration		
									Exp. 1	Exp. 2	
Time stop, h	Item	StD[1,2]	SBP_10[1]	SBP_20[1,2]	SBP_30[1]	SBP_10:OH_10[2]	OH_10[2]	SEM	Linear, *P*-value	Linear, *P*-value	Quadratic, *P*-value
$T/2$	No. of syringes	9	8	8	8	9	8				
	BNI, mg·g^{-1} of hydrolyzed residue	6.41	7.89	8.31	8.21	7.80	2.91	4.05E-1	0.027	<0.001	0.001
	BNI, mg·g^{-1} of fed diet	1.51	2.01	2.06	2.35	2.17	0.82	1.09E-1	<0.001	<0.001	<0.001
	SCFA, mg·g^{-1} of hydrolyzed residue	156	161	151	154	139	73	6.91E+0	0.815	<0.001	0.025
	Acetate, %	68	67	72	74	71	68	1.04E+0	0.099	0.498	NA[3]
	Propionate, %	23	26	24	23	23	27	8.31E-1	0.772	0.378	NA
	Butyrate, %	8	7	4	3	6	4	5.32E-1	0.003	0.864	NA
	No. of fermentation runs	3	3	3	3	3	3				
	BNI, mg·g^{-1} of fermented polysaccharide	11.0	14.5	16.4	15.5	17.4	10.8	1.24E+0	0.038	0.073	NA
	SCFA, mg·g^{-1} of fermented polysaccharides	264	289	287	301	326	283	1.28E+1	0.378	0.911	NA
	Polysaccharide disappearance[4]	0.597	0.561	0.539	0.526	0.437	0.265	2.87E-2	0.059	<0.001	NA
	BNI in feces[5]	0.389	0.426	0.404	0.463	0.466	0.217				
72	No. of syringes	8	9	9	8	9	9				
	BNI, mg·g^{-1} of hydrolyzed residue	3.71	3.65	3.60	3.42	3.30	1.44	2.32E-1	0.732	0.002	NA
	BNI, mg·g^{-1} of fed diet	0.87	0.93	0.89	0.98	0.92	0.40	6.08E-2	0.478	0.009	NA
	SCFA, mg·g^{-1} of hydrolyzed residue	295	301	335	318	266	164	1.21E+1	0.358	<0.001	NA

[1]Diets used in Exp. 1: StD = standard diet, 0% sugar beet pulp (SBP); SBP_10 = 10% SBP diet; SBP_20 = 20% SBP diet; SBP_30 = 30% SBP diet. The effect of increasing SBP content was investigated.

[2]Diets used in Exp. 2: StD = standard diet, 0% SBP and 0% oat hulls (OH); OH_20 = 0% SBP and 22% OH diet; SBP_10:OH_10 = 10.5% SBP and 10.5% OH diet; SBP_20 = 20% SBP and 0% OH diet. The effect of the ratio of soluble dietary fiber (DF):insoluble DF was investigated. The StD was not included in the analysis because its total DF content was significantly less compared with the other diets.

[3]NA = not applicable because the *P*-value of the quadratic effect was not significant ($P > 0.05$) and the linear model was used. For Exp. 1, the quadratic model was never significant.

[4]Proportion of polysaccharides (DF and starch) that had disappeared from the syringe content at $T/2$.

[5]Bacterial N incorporation in feces subsequent to fermentation of the fibrous fraction of the diet.

Table 6. Equations for the prediction of in vivo CP digestibility (*dCP*) and the urinary-N:fecal-N excretion ratio from in vitro bacterial N incorporation (BNI, mg·g^{-1} of diet), fractional rate of degradation [$\mu_{t = T2}$ (h^{-1})], proportion of butyrate in the short-chain fatty acid molar ratio (*but*, %), and the soluble dietary fiber (*SDF*) content (g·kg^{-1} of DM) of the 6 experimental diets[1]

Equation	r^2 or R^2	*P*-value	RMSE[2]
$dCP = 0.897 - 0.0332 \times BNI$	0.70	0.023	0.011
$dCP = 0.887 - 0.505 \times \mu_{t=T/2}$	0.59	0.047	0.014
$dCP = 0.861 - 0.000770 \times SDF$	0.72	0.021	0.011
$dCP = 0.870 - 0.0317 \times BNI + 0.00456 \times but$	0.89	0.017	0.007
Urinary-N:fecal-N $= 2.77 - 0.626 \times BNI$	0.65	0.033	0.25
Urinary-N:fecal-N $= 2.62 - 9.90 \times \mu_{t=T/2}$	0.60	0.043	0.26
Urinary-N:fecal-N $= 2.13 - 0.0154 \times SDF$	0.77	0.014	0.20
Urinary-N:fecal-N $= 2.14 - 0.593 \times BNI + 0.106 \times but$	0.94	0.007	0.10

[1]Diets were formulated to contain graded concentrations of sugar beet pulp (SBP), oat hulls (OH), or both at the expense of corn: a standard diet; a 10% SBP diet; a 20% SBP diet, a 30% SBP diet, a 10.5% SBP and 10.5% OH diet, and a 22% OH diet.

[2]RMSE, root mean square error.

predictor. Butyrate accumulation was due to the presence of resistant starch (**RS**) in the corn used in the diets. This assumption was confirmed by measuring the starch content in the hydrolyzed residues, which increased with the amount of corn and decreased with the amount of DF in the diet (data not shown). It is consistent with the results of Martinez-Puig et al. (2003) and Bednar et al. (2001), who also measured significant in vivo and in vitro RS content in corn, approximately 25% of the total starch. Starch is known as a butyrogenic carbohydrate when fermenting in the intestines of simple-stomached animals (Sajilata et al., 2006). Bindelle et al. (2007b) showed that starch supports increased in vitro bacterial growth. The presence of starch in the hydrolyzed diet residues available to bacterial in vitro fermentation certainly enhanced the in vitro BNI values of the diets containing increased amounts of corn (especially reduced-fiber diets such as the StD and SBP_10 diets). In vivo, the effect of RS on bacterial growth and on subsequent N fecal excretion was probably less compared with in vitro data. Indeed, a significant part of RS (between 40 and 80% according to the starch source) is already fermented in the distal small intestine (Martinez-Puig et al., 2003), and this fermentation is less likely to contribute to a N excretion shift, in comparison with fermentation occurring in the large intestine. Furthermore, Bindelle et al. (2007a), comparing the fermentation kinetics when using donors fed diets differing in DF content, showed that SBP enhanced bacterial activity in the feces because of the greater availability of fermentable DF in the large intestine and a reduction in the intestinal transit time. Unlike SBP, RS has a minor effect on water-holding capacity and transit time (Ferguson et al., 2000). With longer transit times, RS is totally fermented before reaching the distal colon (Martinez-Puig et al., 2003); thus, in our study, proteolysis was more likely to occur in the large intestine with low-fiber diets and would thus to give a greater urinary-N:fecal-N excretion ratio.

Indeed, the decrease in BNI between $T/2$ and 72 h of fermentation suggests that the N excretion shift was affected by the intestinal transit time. Unlike the SCFA, the bacterial biomass did not accumulate continuously in the syringes. After the linear phase of gas production, when the fermentation broth becomes depleted in carbohydrates, an autoconsumption phase and a death phase occur (Prescott et al., 1996) and BNI decreases. In animals with long transit times, such as adult sows (Le Goff et al., 2002), the same phenomenon is likely to occur. When the intestinal content passes from the cecum and the proximal colon to the distal colon, it becomes depleted in fermentable carbohydrates. After a phase of bacterial protein synthesis, proteolysis occurs, with a release of NH_3 that will again pass into the blood and will finally be excreted in urine. However, in growing pigs (>35 kg) fed diets with a similar DF content, transit can be too fast to reach the depletion in fermentable carbohydrates (Le Goff et al., 2002; Bindelle et al., 2007a), reducing the proteolysis compared with sows. This means that fermentable carbohydrates might have a greater impact on N excretion pathways in growing pigs than in sows. This is of the utmost importance because growing pigs are responsible for 50 to 64% of NH_3 emissions from intensive pig barns (Hayes et al., 2006), but it also questions the choice of time BNI is measured when using the in vitro method.

It can be concluded that the in vivo N excretion shifts observed with graded concentrations of fermentable DF in pig diets were correlated with enhanced bacterial N uptake in the large intestine, as measured in vitro. The gas test method may help to characterize their contribution to the N excretion shift. Nevertheless, the use of the regression equations to predict N excretion shifts in pigs requires the database to be enlarged to include other DF sources in addition to OH and SBP, because

the latter was nearly the only source of fermentable DF in the experimental diets. Furthermore, a general equation that accurately predicts the N excretion ratio of all ingredients or diets is probably not attainable. It seems more adequate to develop specific equations to address different substrate categories, as this is the case for ruminant studies. The discrepancy between the contribution of RS to in vitro BNI and the N excretion pathway modifications highlights the need for further investigation into the actual site of carbohydrate fermentation in the intestines and the link with the expression of DF functionalities.

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