Available online at www.sciencedirect.com
ScienceDirect
Livestock Science 109 (2007) 232–235
LIVESTOCK SCIENCE
www.elsevier.com/locate/livsci

Influence of purified dietary fibre on bacterial protein synthesis in the large intestine of pigs, as measured by the gas production technique ☆

J. Bindelle [a,*], A. Buldgen [a], D. Michaux [a], J. Wavreille [b], J.P. Destain [c], P. Leterme [d]

[a] Faculté universitaire des Sciences agronomiques de Gembloux, Unité de Zootechnie, Passage des Déportés, 2, 5030, Gembloux, Belgium
[b] Centre wallon de Recherches agronomiques, Département Productions et nutrition animales, Rue de Liroux, 8, 5030, Gembloux, Belgium
[c] Centre wallon de Recherches agronomiques, Département Production végétale, Rue du Bordia, 4, 5030, Gembloux, Belgium
[d] Prairie Swine Centre, 2105 - 8th Street East, Saskatoon, SK, Canada S7H 5N9

Abstract

Microbial fermentation of non-digestible carbohydrates in the pig's large intestine induces a shift of N excretion from urea in urine to bacterial protein in faeces. Experiments were carried out to measure the mineral N incorporation by the pig intestinal microflora using 5 purified carbohydrates in a gas-test: starch (S), cellulose (C), inulin (I), pectin (P) and xylan (X). Fermentation kinetics was modelled. N source in the buffer solution was replaced by ^{15}N labelled NH_4Cl. The bacterial N fixation was determined at mid-fermentation, measuring ^{15}N incorporation into the solid phase of the buffer. The bacterial N fixation was higher ($P<0.001$) with I and S (19.9 and 18.1 mg N/g incubated DM), compared to P, C and X (8.7, 5.9 and 5.5 respectively). Inulin and S were fermented also more rapidly, even if I (0.081 h^{-1}) and C (0.074 h^{-1}) showed lower half time fractional rate of degradation than S (0.153 h^{-1}), P (0.133 h^{-1}) and X (0.104 h^{-1}). The insoluble dietary fibre content of the substrates was negatively correlated to bacterial N fixation ($r=-0.957$, $P=0.011$). The high crude protein content of P (32.5 mg g^{-1}DM) might explain the lower impact of this substrate on bacterial N fixation, despite its rapid fermentation. Beside the proportion of insoluble fibre, the N content and the rate of fermentation seem to be the major factors influencing bacterial protein synthesis. Further studies including ingredients with variable content of indigestible protein and mean retention time in the pig's intestines are necessary.

Keywords: Pig; Carbohydrates; Nitrogen excretion; In vitro fermentation

1. Introduction

Non-digestible carbohydrates (NDC) are the major energy source for the bacteria in the pig's large intestine. Their fermentation induces a shift of N excretion from urea in urine to bacterial protein in faeces (Zervas and Zijlstra, 2002), resulting in a decrease of NH_3 emission during the storage of manure and its spreading on the field (Nahm, 2003). This shift may also improve N recycling on farm level, especially when animals are fed

☆ This paper is part of the special issue entitled "Digestive Physiology in Pigs" guest edited by José Adalberto Fernández, Mette Skou Hedemann, Bent Borg Jensen, Henry Jørgensen, Knud Erik Bach Knudsen and Helle Nygaard Lærke.

* Corresponding author. FUSAGx, Unité de Zootechnie, Passage des Déportés, 2, B-5030, Gembloux, Belgium. Tel.: +32 81 62 21 33.
E-mail address: bindelle.j@fsagx.ac.be (J. Bindelle).

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doi:10.1016/j.livsci.2007.01.126

Table 1
Chemical composition of the substrates and the diet (g kg^{-1} DM)

	Starch	Cellulose	Inulin	Pectin	Xylan	Diet
DM (g kg^{-1})	910.7	958.0	948.5	923.8	957.4	897.0
NDF	ND[1]	791.5	ND	ND	115.2	252.6
ADF	ND	881.3	ND	ND	1.1	144.4
ADL	ND	81.9	ND	ND	0.0	28.7
Crude protein	2.2	1.0	ND	32.5	2.1	163.9
Ash	1.9	ND	0.1	47.5	125.4	67.3
Starch	783.0	NA[2]	NA	NA	NA	244.2
Total dietary fibre	ND	1000	34.2	892.9	795.2	311.9
Insoluble dietary fibre	ND	1000	9.1	3.4	328.2	311.9
Soluble dietary fibre	ND	ND	25.1	889.5	467.0	ND

[1]ND, not detected, [2]NA, not analysed.

high-fibre diets (Leterme et al., 2006). The source of NDC, however, influences the extent of this phenomenon (Kreuzer et al., 1998) and the relationship between NDC fermentability and N excretion shift is still unknown.

The aim of the present study was to measure in vitro, the extent of protein synthesis by faecal microbes, when different sources of purified NDC are available as energy source for microbial fermentation.

2. Material and methods

2.1. Substrates

Five purified carbohydrates (Table 1) were chosen according to their soluble and insoluble fibre content, constituent sugars and glucosidic bounds: raw potato starch (Fluka B-5650), fibrous cellulose (Sigma C-6663), inulin (Fibruline, Cosucra, Warcoing, Belgium), citrus pectin (Sigma P-9135) and xylan from oat spelts (Fluka 95590).

2.2. In vitro fermentation

In vitro fermentation was performed using the gas test method described by Menke and Steingass (1988) and adapted to the pig by Bindelle et al. (2007). Briefly, an inoculum was prepared from fresh faeces of 3 Belgian Landrace sows (226 to 257 kg) fed a standard commercial diet (Table 1). Faeces (50 g l^{-1}) were mixed to a buffer solution composed of salts and minerals (Menke and Steingass, 1988). The N source in the buffer solution (NH_4HCO_3) was replaced by an equimolar quantity of ^{15}N-labeled 2% NH_4Cl (ISOTEC n°T85-70216, Miamisburg, Ohio, USA). The fermentation (39±0.5 °C) started introducing 200 mg of the substrates and 30 ml of the inoculum into the glass syringes.

The experimental scheme was as follows: 5 substrates × 6 replicates + 3 blanks (containing only inoculum), repeated over 2 periods. The released gas volumes of 3 syringes per substrate were regularly recorded until 72 h. The 3 remaining syringes were stopped at mid-fermentation by quenching in an iced water bath and their content was centrifuged twice (12,000 ×*g*, 20 min, 4 °C). The final pellet concentrating the bacteria and the undigested substrate was lyophilized, weighed and analysed for total N and ^{15}N enrichment. For each period, 3 samples of the inoculum were also taken and centrifuged for further ^{15}N analysis.

2.3. Kinetics of gas production

Gas accumulation curves were fitted to the model of France et al. (1993) and described by four parameters: final gas production (G_f, ml g^{-1} DM), lag time before the fermentation starts (L, h), mid-fermentation time when gas production is the half of the final gas production ($T/2$, h) and the substrate's fractional rate of degradation at $T/2$ (μ, h^{-1}).

2.4. Measurement of N incorporation into microbial cells

Total N and ^{15}N enrichment in the lyophilized pellets were measured by means of an elemental analyser coupled to an isotope-ratio mass spectrometer (Europa Scientific ltd., Crewe, UK). Bacterial N fixation (N in the pellet incorporated from the buffer solution into the bacteria), per amount of incubated substrate at $T/2$ was calculated as follows:

$$\text{Bacterial N fixation}(\text{mg g}^{-1}\text{DM}) = \left(\frac{\left(\frac{^{15}\text{N} \times \text{N} \times M_{\text{pellet}}}{0.003663}\right) - \text{N} \times M_{\text{pellet}}}{\left(\frac{0.02}{0.003663} - 1\right) \times W \times \frac{V_{\text{stop}}}{G_f/2}} \right) - \text{Bacterial N fixation}_{\text{inoculum}} \times \frac{V_0}{W} \quad (1)$$

where N (g g^{-1}) denotes the concentration of N in the pellet, M_{pellet} (mg) the dry weight of the pellet, 0.003663 the natural enrichment in ^{15}N (of the substrates and the faeces used to prepare the inoculum), 0.02 the enrichment of the mineral buffer in ^{15}N, ^{15}N (g g^{-1}) the concentration of ^{15}N in total N of the pellet, V_0 (ml) the volume of inoculum transferred in the syringes at the start of the fermentation and W (g DM) the amount of substrate placed in the syringe.

Table 2
Kinetic parameters of gas production and bacterial nitrogen fixation at mid-fermentation (means) per amount of substrate incubated with sows faecal inoculum

Substrate	Kinetics					Nitrogen fixation	
	N[a]	Lag time (L, h)	Mid-fermentation time ($T/2$, h)	Fractional rate of degradation[b] (μ, h^{-1})	Final gas production (G_f, ml g^{-1} DM)	N	Bacterial N fixation[c] (mg N g^{-1} DM)
Starch	6	4.5 c[d]	8.5 d	0.153 a	405 b	6	18.1 a
Inulin	6	4.1 cd	11.1 c	0.081 d	393 c	6	19.9 a
Cellulose	6	12.7 a	23.0 a	0.074 d	396 bc	6	5.9 c
Xylan	5	6.5 b	17.3 b	0.104 c	365 d	6	5.5 c
Citrus pectin	6	3.6 d	8.0 d	0.133 b	443 a	6	8.7 b

Source of variation	*df*[e]	*P*-values				*P*-values
Substrate	4	<0.001	<0.001	<0.001	<0.001	<0.001
Variance parameter estimates						
Period		0.77	1.52	0.11^{E-4}	184.0	1.72
Residual		0.39	2.00	1.60^{E-4}	76.2	4.63

[a] N, number of observations.
[b] At mid-fermentation time.
[c] According to Eq. (1).
[d] For one parameter, means followed by different letters in the columns differ at a significance level of 0.05.
[e] *df*, degrees of freedom.

2.5. Chemical and statistical analysis

The substrates and the diet were analysed for their content in dry matter, ash, crude protein (Kjeldahl), ether extract (Soxhlet) following the Weende system, neutral and acid detergent fibres and lignin, using the Fibercap system. Starch was determined according to the amyloglucosidase method and total and soluble dietary fibre contents using the AOAC 991.43 method. The correlation and statistical analysis were performed using the CORR and MIXED procedure of the SAS 8.02 software (SAS, 1999).

3. Results

The bacterial N fixation and the fermentation kinetic parameters of the 5 carbohydrates are shown in Table 2. The bacterial N fixation, expressed per g DM of incubated substrate, was the highest ($P<0.001$) with inulin and starch. Pectin yielded intermediate bacterial N fixation (mg g^{-1} DM), while cellulose and xylan showed the lowest values. With the lowest lag (L) and mid-fermentation times ($T/2$), inulin, starch and pectin were also the most rapidly fermented substrates ($P<0.001$), even if inulin and cellulose showed lower fractional rate of degradation compared to starch, pectin and xylan. The final gas production (G_f) also differed between the substrates ($P<0.001$): pectin yielded the highest production and xylan the lowest.

The bacterial N fixation (Table 2) was also correlated to the insoluble dietary fibre content of the carbohydrate sources (Table 1) (mg g^{-1} DM) ($r=-0.957$, $P=0.011$).

4. Discussion

Despite significant differences in the final gas production (G_f), all substrates tested were probably fermented to the same extent by the faecal flora. The lower G_f value observed for xylan might be explained by the high content in ash. For pectin, the high gas production originates from the specific stoichiometry of the fermentation that yields mostly acetate, at the expense of propionate and butyrate (Drochner et al., 2004). The observed differences in bacterial N fixation between the NDC may not be explained by differences in degradability at the end of the fermentation, but more likely by differences in the kinetics of fermentation.

The NDC with the highest bacterial N fixation were indeed found among the most rapidly fermented (low L and $T/2$ and high μ). However, the high crude protein content of pectin (32.5 mg/g DM) can explain its lower impact on bacterial N fixation compared to starch and inulin, despite its rapid fermentation.

The negative correlation linking the bacterial N fixation to the insoluble dietary fibre content of the NDC indicates also that easily degradable substrates, such as starch or soluble dietary fibre, yield more bacterial protein synthesis compared to insoluble fibre.

It can be concluded that the solubility, the N content and the rate of fermentation of the NDC source seem to be the major factors that affect bacterial protein synthesis. However, all the fermentations were stopped at mid-fermentation ($T/2$) and this value is specific to each NDC source. Further research is thus required, that takes the mean retention time of the dietary fibre in the intestines into account.

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