The High Water-Holding Capacity of Pea Inner Fibers Affects the Ileal Flow of Endogenous Amino Acids in Pigs†

Pascal Leterme,* Eric Froidmont, Florence Rossi, and André Théwis

Faculté Universitaire des Sciences Agronomiques, Unité de Zootechnie, 2 Passage des Déportés, B-5030 Gembloux, Belgium

Pigs were fed protein-free diets containing different pea inner fiber (PIF) isolates (from wrinkled or smooth peas, unprocessed or micronized) or different PIF levels (from 50 to 200 g/kg diet), and the flows of endogenous N and amino acids were measured at the ileum level. The flows were better correlated to the water-holding capacity (WHC) of the diet than to fiber intake ($R^2 = 0.996$ vs 0.76, respectively). The relationship with WHC was of the exponential type, with a high increase when the WHC exceeded 3 g of water/g diet. A similar pattern and correlation ($R^2 = 0.98$) was obtained for the ileal flow of nucleobases (markers of epithelial cells and bacteria), whereas the relationships with crude mucus ($R^2 = 0.96$) and diaminopimelic acid (marker of bacteria, $R^2 = 0.55$) were linear. N retention by pigs fed with 0, 30, 60, or 90 g/kg of dry matter (DM) of the same protein isolates did not differ significantly in pigs receiving a 40 or 160 g of PIF/kg diet, despite significant differences in ileal N and amino acid flows between the two fiber levels (2.0 and 3.1 g of N/kg of DM intake, on average). These results and those of the flow of endogenous N compounds suggest an effect of the swollen fibers on the intestinal wall rather than a disturbance of the digestive processes.

Keywords: *Pig; ileum; endogenous N; fiber; water-holding capacity*

INTRODUCTION

The effect of dietary fibers on gastrointestinal function relates to their chemical composition as well as to their physicochemical properties. However, the physiological effects of fiber sources are often overlooked by the feed industry, despite an obvious influence on the animal's performances.

For example, fibers with a high water-holding capacity (WHC) decrease feed intake by the pig (Kyriazakis and Emmans, 1995) and increase ileal excretion of endogenous proteins (Leterme et al., 1996a), with a possible decrease in N retention as a consequence (Sève and Henry, 1996).

In a previous study, we also reported that, at constant NDF fiber intake, pea inner fibers (PIF) markedly increased the ileal outflows of endogenous N in pigs, compared to pea hulls and wood cellulose (Leterme et al., 1996a). PIF are characterized by a very high water-holding capacity (WHC = 10−12 g of water retained/g of matter). Contrary to other fiber sources with a high WHC, they are insoluble. Water is retained in the empty cells. To our knowledge, no data are available to explain how such fibers affect ileal endogenous N losses or protein digestion in pigs.

In a series of experiments on pigs, we aimed at studying the effect of different factors on the ileal endogenous amino acid (AA) losses: (1) fibers isolated from peas differing in carbohydrate composition (smooth and wrinkled peas), (2) fibers differing in physical structure (unprocessed or micronized), (3) fiber level in the diet, and (4) the effect of fiber intake on N retention. The data were used to distinguish between the effect of fiber intake and that of the fiber WHC. Finally, an attempt was made to determine the endogenous origin of the losses by measuring the ileal flow of nucleobases (markers of the epithelial cells and bacteria), diaminopimelic acid (marker of bacteria), and crude mucus.

MATERIALS AND METHODS

Animals. Twelve pigs of the Large White breed were fitted with a postvalve T-cannula as described by van Leeuwen et al. (1991). The cecum was removed and replaced by a large T-cannula. When the cannula was closed, the digesta coming from the ileum flowed into the colon. When it was open, the ileocecal valve protruded in the aperture of the cannula and the digesta were collected in plastic bags. The average body weights of the pigs during the three successive periods were respectively 45, 54, and 64 kg. For the second experiment, eight pigs were used, weighing on average 40 ± 4 kg.

After recovery, the pigs were placed in metabolism cages, and, between each experimental period, they rested in large cages (1 × 1.2 m).

Diets. *Experiment 1.* The diets were based on nearly protein-free components, so that the AA collected at the ileum could be considered of endogenous origin (Table 1). For the smooth and wrinkled pea fiber-based diets, some egg yolk was added as a source of highly digestible protein to stimulate the digestive secretions without increasing the ileal dietary N output. These two diets were also composed of, respectively, smooth and wrinkled pea starch, whereas the others contained corn starch. Their starch and inner fibers were isolated in the pilot-plant facility of Provital (Warcoing, Belgium), whereas those incorporated in the other diets came from its industrial plant. In both cases, the hulls were first mechanically separated from the seeds. The cotyledons were then finely ground and plunged into an alkaline solution (pH = 8) for protein solubilization. The proteins were isolated by ultrafil-

* Author to whom correspondence should be addressed (telephone 32/81 622 117; fax 32/81 622 115; e-mail zootechnie@fsagx.ac.be).

† Results presented in part at the 28th and 29th Days on Pig Research in France, Paris, Jan 30−Feb 1, 1996, and Feb 3−5, 1997.

S0021-8561(97)00955-2 CCC: $15.00
Published on Web 04/09/1998

Table 1. Composition of Diets of Experiment 1

	pea fiber origin		fiber form		PIF content (g of AOAC fibers/kg of DM)			
	smooth	wrinkled	unprocessed	micronized	25	50	75	100
ingredients (g/kg of DM)								
corn starch[a]			660	660	740	710	680	650
pea starch (smooth)[a]	610							
pea starch (wrinkled)[b]		650						
pea fibers (smooth peas)[c]	190		190		50	100	150	200
pea fibers (wrinkled peas)[d]		150						
pea fibers (micronized)[e]				190				
sucrose	60	60	60	60	120	100	80	60
defatted egg yolk[f]	60	60						
oil[g]	20	20	30	30	30	30	30	30
mineral/vitamin premix[h]	60	60	60	60	60	60	60	60
chemical analysis (g/kg of DM)								
crude protein	54	64	17	17	7	10	14	17
starch	605	460	676	685	687	681	676	670
neutral detergent fiber	66	80[i]	42	31	13	27	40	54
acid detergent fiber	22	46[i]	15	15	4	8	12	16
AOAC fiber total	119	257	93	85	24	48	72	96
insoluble	117	245	87	73	23	46	69	92
soluble	2	12	6	12	1	2	3	4
Englyst fiber total	96	208	73	72	19	38	56	75
insoluble	84	167	54	56	14	28	43	57
soluble	12	41	19	16	5	10	13	18
WHC (g of water/g of DM)								
pea fibers	15.8	14.2	12.1	3.5	12.1	12.1	12.1	12.1
diets	4.05	4.15	3.22	1.58	1.63	2.19	2.76	3.33

[a] Smooth pea starch and corn starch, 930 g of starch/kg; WHC, 1.4 and 1.3 g of water/g of DM, respectively. [b] Wrinkled pea starch, 680 g of starch/kg and 160 g of AOAC fibers/kg; WHC, 2.8 g of water/g of DM. [c] Smooth PIF of subexperiment 1, 630 g of AOAC fibers/kg, 250 g of starch/kg, 12.8 g of N/kg; PIF of subexperiment 2, 3, 490 g of AOAC fibers/kg, 430 g of starch/kg, 11.8 g of N/kg. [d] Wrinkled PIF, 830 g of AOAC fibers/kg, 110 g of starch/kg, 15.4 g of N/kg. [e] Micronized PIF, 450 g of AOAC fibers/kg, 450 g of starch/kg, 12.3 g of N/kg.[f] Egg yolk defatted with hexane, 680 g of proteins/kg, 180 g of fat/kg; WHC, 2 g of water/g of DM. [g] Oil: corn−groundnut−soya, 1:1:1. [h] Premix (g/kg diet): as described by Leterme et al. (1996b). [i] The fibers present in the wrinkled pea starch were not considered.

Table 2. Composition of Diets of Experiment 2 (g/kg of DM)

	PIF content = 20 (g of fibers/kg of DM), with various protein contents				PIF content = 80 (g of fibers/kg of DM), with various protein contents			
	0 g/kg of DM	30 g/kg of DM	60 g/kg of DM	90 g/kg of DM	0 g/kg of DM	30 g/kg of DM	60 g/kg of DM	90 g/kg of DM
ingredients								
corn starch	823	787	751	714	721	685	649	612
pea inner fibers[a]	25	25	25	25	100	100	100	100
pea hulls[a]	9	9	9	9	36	36	36	36
casein[b]		22	44	66		22	44	66
defatted egg yolk		18	36	54		18	36	54
sucrose	60	60	60	60	60	60	60	60
oil	20	16	12	9	20	16	12	9
premix	63	63	63	63	63	63	63	63
chemical analyses								
crude proteins	5.4	31.3	65.0	95.6	14.4	41.9	71.9	102.5
AOAC fibers	20.3	20.3	20.3	20.3	81.1	81.1	81.1	81.1

[a] Pea hulls and inner fibers provided by Provital (Warcoing, Belgium). The hull/inner fiber ratio is the same as that in whole pea seeds. [b] Bovine milk casein (Sigma C-5890, St. Louis, MO).

tration, and starch was allowed to settle and decant. The remaining product, composed of fiber and starch, forms the inner fibers used here.

The diet of the second subexperiment had the same raw inner fiber content as that of the first one (190 g of smooth PIF), but the fibers were either unprocessed or micronized. The micronizer was composed of a rotor with numerous blades, in a vertical cylinder. The product was carried by air. Micronization was obtained by the impact of the particles with the blades and the jacketing of the cylinder. After micronization, 95% of the particles had a size < 63 μm. The diets of the third subexperiment were formulated in order to contain increasing amounts of PIF, at the expense of corn starch (Table 1).

Experiment 2. Three dietary protein levels (30, 60, and 90 g/kg of dry matter (DM)) were tested in comparison to a reference diet devoid of protein, at two fiber levels: 20 or 80 g of AOAC fibers/kg of DM (Table 2). The protein was a mixture 2:1 of casein and defatted egg yolk, so that the AA maintenance requirements of the pig were met.

All of the diets were supplemented with 3 g of Cr_2O_3/kg of diet for ileal flow determination. Between the experiments, the pigs were fed a commercial diet, supplemented with 100 g of PIF/kg.

Experimental Procedure. *The first experiment* was carried out in four periods: the first was devoted to the wrinkled vs smooth pea fiber comparison, the second to the unprocessed vs micronized pea fiber comparison, and the last two to the evaluation of the fiber intake level effect. For the first two periods, 8 of the 12 pigs were randomly allocated into two groups. The third subexperiment was conducted in a crossover design. Two pigs were eliminated, and 8 of the 10 remaining pigs were randomly allocated into 4 pairs, each of them testing one of the four intake levels. After the first collection period,

Table 3. Ileal Flow of Water (g/Collection Period), Dry Matter, N, and Amino Acids (g/kg of DM Intake) in Pigs Fed a Pea Fiber-Based Diet[a]

	pea fiber origin			fiber form			PIF content (g of AOAC fiber/kg of DM)					
	smooth	wrinkled	SEM	unprocessed	micronized	SEM	25	50	75	100	SEM	relation[b]
water[c]	2523	2853	101*	1963	852	215**	719	1231	1965	2114	150***	log
DM	254	414	30*	198	179	13	122	140	172	207	11**	lin
Arg	0.83	1.00	0.12	0.64	0.36	0.07**	0.37a	0.38a	0.45a	0.56b	0.02**	exp
His	0.59	0.65	0.08	0.33	0.20	0.03**	0.19a	0.21a	0.24a	0.29b	0.01*	exp
Ile	0.78	0.96	0.12*	0.53	0.39	0.03**	0.33a	0.38a	0.44ab	0.51b	0.02***	lin
Leu	1.04	1.29	0.15*	0.70	0.48	0.05**	0.48a	0.59ab	0.65ab	0.74b	0.04*	lin
Lys	0.81	1.14	0.16*	0.45	0.33	0.03**	0.31a	0.36a	0.50b	0.50b	0.03**	lin
Phe	0.94	1.06	0.12	0.61	0.41	0.05**	0.38a	0.43a	0.48ab	0.55b	0.03*	lin
Thr	0.99	1.15	0.14	0.63	0.40	0.05**	0.41a	0.45a	0.49a	0.61b	0.02***	exp
Val	0.96	1.14	0.14*	0.62	0.38	0.05**	0.39a	0.41a	0.54b	0.62b	0.03***	exp
Ala	0.89	1.14	0.15*	0.59	0.38	0.05**	0.38a	0.41a	0.48ab	0.54b	0.02**	exp
Asp	1.80	2.23	0.31	1.05	0.71	0.10**	0.59a	0.60a	0.76b	0.88b	0.04*	exp
Glu	1.65	2.96	0.46*	1.00	0.69	0.09**	0.53a	0.58a	0.67a	0.88b	0.05*	exp
Gly	1.59	1.83	0.23	1.37	0.54	0.19**	0.65a	0.80ab	0.88ab	1.07b	0.05**	exp
Pro	2.51	2.38	0.26	3.54	1.32	0.55**	1.68a	1.82a	1.97a	2.36b	0.18*	exp
Ser	1.66	1.86	0.27	0.72	0.40	0.07**	0.45	0.50	0.48	0.61	0.02	exp
Tyr	0.67	0.74	0.09	0.46	0.28	0.04**	0.28a	0.33a	0.37a	0.43b	0.002**	lin
∑AA	17.73	21.56	2.62	13.26	7.27	1.34**	7.56a	8.35a	9.36ab	11.10b	0.46**	exp
N	3.67	4.20	0.18	2.73	1.64	0.25**	1.69a	1.85a	1.96a	2.31b	0.10*	exp

[a] Values are means of four pigs + SEM. The asterisks on the SEM give the significance level: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Within a row for the PIF content, values with different asterisks differ significantly. [b] Relationship between fiber intake and the ileal N flows: log, logarithmic type: $y = a \ln(X) - b$, lin, linear: $Y = a + bX$; exp, exponential type, $Y = ae^{bx}$, where Y is the AA flow, X the level of AOAC fiber in the diet, and a, b are constants. [c] The water output was measured directly at the ileum level and corresponds to the amount recovered during the collection period.

the diets were permuted and a second collection period was performed, so that each diet had been tested on four different pigs.

During each period, the pigs received daily, in two meals (8−16 h), 90 g of DM/kg of metabolic weight of the experimental diet, mixed with water (1−1.5 L/kg) for 5 days. The ileal digesta were collected on the last 3 days, from 9 to 17 h, with plastic bags attached to the cannula. The time of collection and the weight of each collected sample were noted, but the samples were pooled per day and immediately frozen.

The second experiment was carried out in a double Latin square experimental scheme. The pigs were randomly allocated into two groups, corresponding to the two fiber levels (20 and 80 g of AOAC fibers/kg of DM). During the four successive periods, they received successively a diet devoid of protein or containing one of the three protein levels (30, 60, or 90 g/kg of DM).

After a 3 day adaptation period, feces and urine were quantitatively collected for 3 days. In the 3 following days, the ileal digesta were collected for 8 h (9−17 h) by means of plastic bags fixed to the cannula.

Chemical Analyses. The diet ingredients were analyzed for nitrogen by the Kjeldahl method, using a Kjeltec 1030 analyzer (Perstorp Analytical, Helsingborg, Sweden). Starch was analyzed by the enzymic method of AOAC (1980) using amyloglucosidase (EC 3.2.1.3; Sigma A7255), glucose oxidase (EC 1.1.3.4; Sigma G7773), and peroxidase (EC 1.11.1.7; Sigma P8125). Fat was extracted with diethyl ether by the Soxhlet method. The NDF and ADF determinations were performed separately on a Fibertec analyzer (Perstorp Analytical, Helsingborg, Sweden), and, prior to the analyses, the samples were boiled for 1 h in a thermostable α-amylase solution (Termamyl 120 L; Novo Nordisk, DK). The dietary fiber content was also determined by the enzymatic−gravimetric method of AOAC (Prosky et al., 1988) and by the chromatographic method of Englyst et al. (1992) where dietary fibers are the sum of the constituent sugars of the nonstarch polysaccharides released by acid hydrolysis.

The digesta were finely ground (1 mm mesh screen) and analyzed for N, AA, chromium, α-diaminopimelic acid (DAPA), and the puric and pyrimidic bases. Chromium was determined by titration with Mohr's salt after nitric acid−perchloric acid digestion, as described by François et al. (1978). The AAs were analyzed after acid hydrolysis (6 M HCl + 1% phenol; 24 h at 110 °C in glass tubes, under N atmosphere) by HPLC, using the Pico-Tag method of Waters (Millipore Corp., Milford, MA) with phenylthiocarbamyl derivatives. Cysteine, methionine, and tryptophan were not analyzed.

DAPA was analyzed by gas chromatography, using the heptafluorobutyryl isobutyl ester amino acid derivatives (MacKenzie and Tenaschuk, 1979a,b), with samples five times as large as those utilized for the analysis of the other AAs. The puric (guanine, adenine) and pyrimidic (cytosine, thymine, uracyl) bases were determined by HPLC after oxidative hydrolysis, as described by Lassalas et al. (1993).

Mucin was isolated by putting 3 g of digesta in 25 mL of a 0.15 mol/L NaCl solution. After centrifugation (12000*g*, 30 min, 4 °C), 15 mL of the supernatant was added to 22 mL of ethanol (99%, 0 °C), kept for one night at −20 °C, and centrifuged (1400*g*, 10 min, 4 °C). The precipitate was recovered in 15 mL of the NaCl solution, treated again, and freeze-dried. It was mainly composed of raw mucus, contaminated by noncovalently bound proteins, as previously observed (Leterme et al., 1996b).

The free AA and oligopeptide fractions isolated from the digesta (experiment 2) were obtained after ultrafiltration of the liquid fraction with Centriprep-3 concentrators (cutoff, 3000; Amicon, Danvers, MA). The freeze-dried digesta were rehydrated and centrifuged (25000*g*; 30 min). The soluble fraction was measured in the supernate.

The WHC was measured as follows: 1 g of DM was placed in a centrifuge tube with 20 mL of distilled water. After 16 h, the tubes were centrifuged (2000*g*, 50 min at 20 °C) and kept 8 min before the supernatant water was discarded and the remaining water weighed.

Statistical Analyses. One-way ANOVA was used to examine the effect of the diets in experiment 1. For the fiber levels of experiment 1, a three-way ANOVA (pig, period, diet) was used, but no significant effect of pig or period was observed. The Student−Newman−Keuls test was applied for significance between each level. A linear and an exponential model were then ajusted between fiber intake and ileal N losses, and the coefficients of determination were used to compare the adjustments.

For experiment 2, we first used two Latin square designs (pig, period, diet) for the protein effect by the GLM procedure of SAS/STAT (1994). The Student−Newman−Keuls test was applied for significance between each level. The data were then combined to study the fiber effect by nesting the "pig" in fiber because the pigs used within each Latin square were

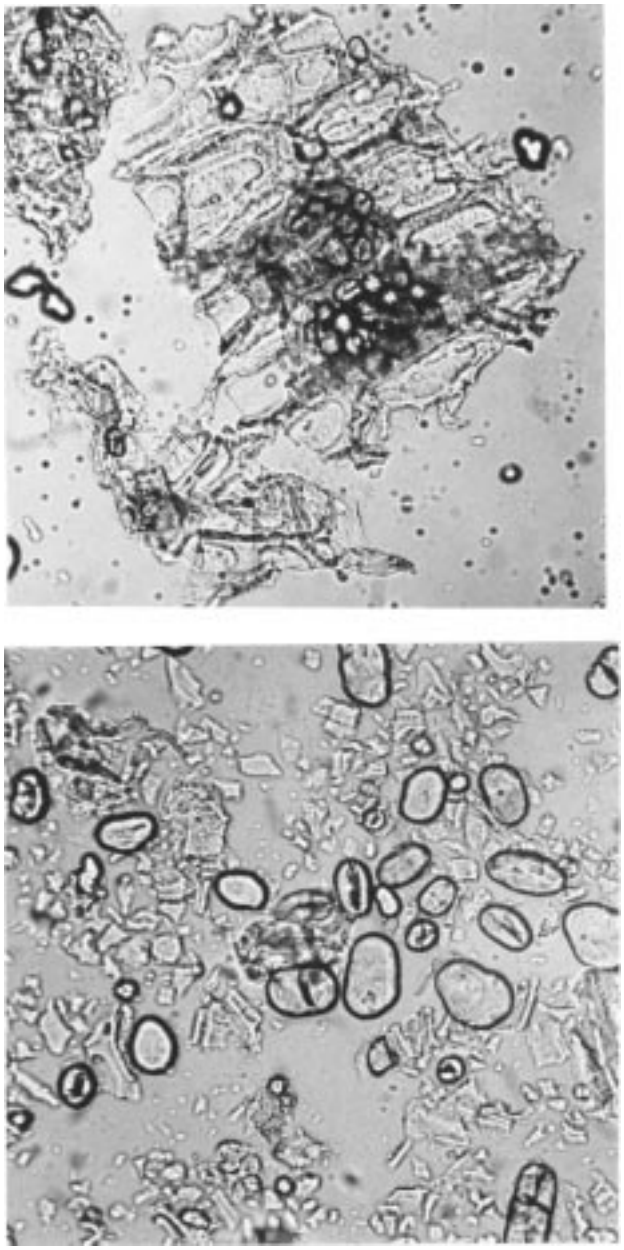

Figure 1. Inner fibers isolated from pea cotyledons. (a, top) Whole fibers: the cell structure and the water vacuoles are visible. Two cells were not emptied and still contained starch granules. Swelling is possible because some cells remain attached. (b, bottom) Micronized fibers: the cell structure is destroyed. Cell debris and starch granules are still visible.

different and by introducing a fiber × protein interaction. The test of linearity was made according to the same model with the partition of the protein and fiber × protein interaction effects (DF3) into the corresponding polynomial, linear, quadratic, and cubic contrasts (DF1 for each).

RESULTS

N and AA Flows. The N and AA outputs measured for smooth and wrinkled pea fiber diets were higher than those observed for the other diets (Table 3). Compared to the others, these diets were supplemented in highly digestible proteins and contained pea starch, instead of corn starch, and fibers isolated on a pilot plant. The dry matter output was higher ($P < 0.01$) for pigs fed wrinkled pea fibers, compared to those fed smooth pea fibers, whereas the difference in AA flow was significant for only six AAs and not for the total N or the sum of the AAs. The difference in DM flow was mainly due to a problem of processing the wrinkled pea seeds in the pilot-plant facility. Due to the difficulty in seed dehulling and to kernel shape and size, some fibers contaminated the isolated starch and their presence was not detected before the experiment (Table 1).

The micronization of the fibers significantly decreased the ileal N, AA, and water flows (Table 3) because the cell wall structure was destroyed and water could no longer be retained in large amounts (Figure 1). The water output was measured directly at the ileum level and corresponds to the amount recovered during the collection period. No marker of the liquid fraction was used because measurements of viscosity were foreseen. It was observed later that such a measurement is not possible on digesta rich in fibers. The water output corresponds to a DM intake of 780, 900, and 1020 g respectively for subexperiments 1−3.

The relationship between ileal outflows and fiber intake was based on the AOAC fiber content of the PIF because we previously observed that the latter is better correlated to ileal N flows than NDF (Leterme et al., 1996a). For most of the AAs, the ileal flow was linear with the increase in fiber intake, at least for the first three levels. For some AAs and the total N, a higher fiber intake (100 g/kg of DM intake) led to a more than proportional increase, and the relationship was then of the exponential type (Table 3). The contrary was observed for water output: beyond 75 g of AOAC fiber/kg of diet, it tended to a plateau, probably because of a limit in intestinal water capacity.

Correlation with WHC. The data of the first experiment were pooled to study the relationship between fiber intake or diet WHC and the ileal N flows. The AOAC fiber intake could not explain all of the results of ileal N excretion ($R^2 = 0.76$), probably because of the different physicochemical properties of the fiber fractions studied (wrinkled or smooth PIF, normal or micronized). Therefore, we also determined the relationship between the WHC of the diets and the ileal flows of N measured on the pigs (Figure 2). The ileal N flow was highly correlated to the diet WHC ($R^2 = 0.996$), and the relationship was of the exponential type, with a high increase in ileal N losses when the WHC exceeded 3 g of water/g of DM.

Flow of Mucus, DAPA, and Nucleobases. The ileal flows of nucleobases (markers of sloughed epithelial cells and bacteria), of mucus, and of DAPA (marker of bacteria) were determined in order to identify the sources of N excreted. The nucleobase value corresponds to the sum of the puric and pyrimidic bases, and it was assumed that no significant amounts of dietary nucleobases reached the ileum.

The relationship between diet WHC and nucleobase outputs presented the same exponential pattern and level of correlation ($R^2 = 0.98$) as that of total N and was also highly influenced by the fibers with the highest WHC (Figure 2). The R^2 between the total N and nucleobase flows reached 0.87. For DAPA and mucus, the relationship was linear but the DAPA flows were very variable ($R^2 = 0.55$ for DAPA vs 0.95 for mucus), probably due to the difficulty in analyzing DAPA in biological samples. By taking the average value of 26.4 mg of DAPA/g of bacterial N determined by Wünsche et al. (1991) for bacteria of pig ileal digesta, we calculated that the ileal bacterial N output ranged from 17

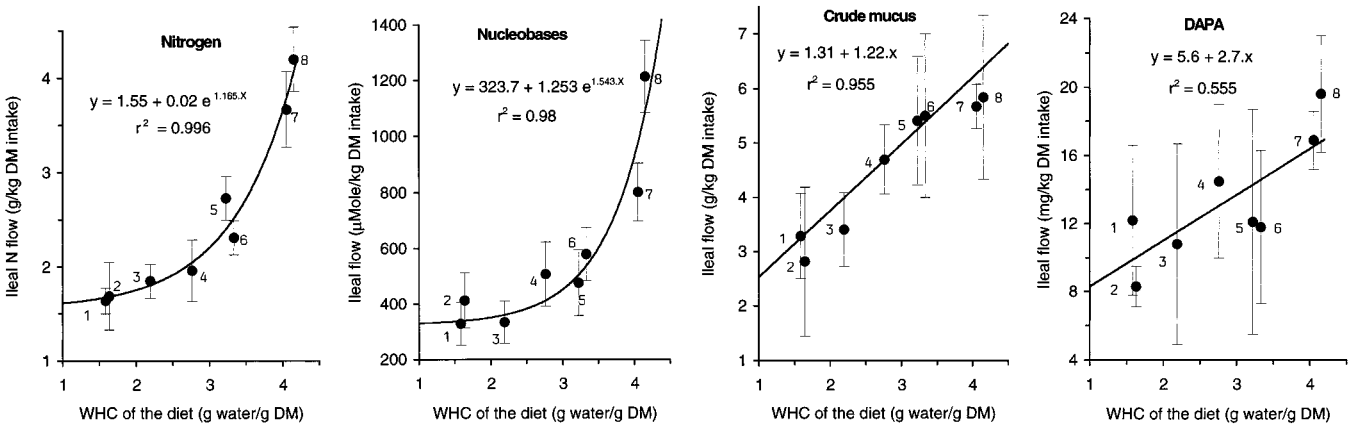

Figure 2. Relationship between the WHC of the diet and the ileal flows of N, nucleobases, mucus, and DAPA in pigs fed PIF-based diets. Values are the means ± SD of four pigs: (1) Micronized fiber diet; (2) 50 g of PIF diet; (3) 100 g of PIF diet; (4) 150 g of PIF diet; (5) unprocessed fiber diet; (6) 200 g of PIF diet; (7) smooth pea fiber diet; (8) wrinkled pea fiber diet.

Table 4. N Balance (g/d) and Ileal Flows of N and Amino Acids (g/kg of DM Intake) in Pigs Fed a Diet Supplemented with Two Levels of Pea Inner Fibers or Three Levels of Isolated Proteins[a]

	fiber = 20 g/kg of DM for given amounts of protein(g/kg DM)					fiber = 80 g/kg of DM for given amounts of protein					fiber effect	
	0 g/kg of DM	30 g/kg of DM	60 g/kg of DM	90 g/kg of DM	SEM	0 g/kg of DM	30 g/kg of DM	60 g/kg of DM	90 g/kg of DM	SEM	significance	SE
N balance												
N intake	1.9	11.3	22.1	29.5	2.8	3.5	12.5	20.9	29.8	2.6		
urinary N	2.8a	3.6ab	4.5bc	5.8c	1.2*	3.3	3.8	4.1	4.7	1.1	NS	0.60
fecal N	2.0	2.5	3.7	2.8	0.8	4.2	4.1	4.6	5.1	0.9	NS	4.85
N retention												
g/d	−2.9a	5.2b	13.9c	20.9d	2.2***	−4.4a	4.6b	12.3c	20.0d	2.5***	NS	3.25
% N intake		45.1a	63.2b	70.4c	3.2***		37.3a	59.1b	67.1b	6.3***	NS	12.0
flows												
sum of AA[b]	7.5	11.8	10.7	11.8	1.8	14.2	17.7	16.4	18.8	4.2	**	17.5
nitrogen	1.70	2.38	2.04	2.07	0.5	3.00	3.13	3.10	3.15	0.62	**	2.72
soluble N	0.86	1.03	0.83	0.75	0.39	1.65	1.57	1.45	1.66	0.35	***	1.79
soluble N < 3000[c]	0.27	0.28	0.20	0.28	0.11	0.51	0.51	0.48	0.40	0.14	**	0.59

[a] Values are presented as means and SEM. Within a row and for each fiber level, values with different asterisks are significantly different (*, $P < 0.05$; ***, $P < 0.001$), according to the Student−Newman−Keuls test. The fiber effect is mentioned as the significance of the P value (NS, not significant; **, $P < 0.01$; ***, $P < 0.001$) and the SE which corresponds to the standard error of comparison and is defined as the square root of means square associated with pigs nested inside fiber effect. [b] The means correspond to the sum of the AA with the exception of Cys, Met, and Trp, not analyzed here. No difference was found at the individual level, except for Ser. The ileal flow of the latter increased significantly with the increase in protein intake. [c] Corresponds to the N of the N molecules present in the soluble fraction that passed through the filter (cutoff 3000) of the Centriprep concentrators.

to 32% of the total N. On the other hand, the ileal flow of mucus N only ranged from 4.5 to 6.5% of the total N. The rest was assumed to be mainly of epithelial origin.

Fiber Intake and N Retention. N retention increased linearly with protein intake but was independent of fiber intake (Table 4). Both fecal and urinary N excretions slightly increased with protein intake, but the difference was significant only for urinary N with the low fiber intake. The latter significantly increased fecal N excretion but not urinary N excretion.

The increase in protein intake had no significant effect on ileal N and AA flows, even if a slight increase was observed when the protein-free diet was supplemented with 30 g of proteins/kg of DM. The flows of the soluble N fraction and that of the fraction containing the soluble N compounds with a MW < 3000, assumed to contain possible remains of the hydrolysis of dietary proteins (oligopeptides, free AAs), were not influenced by protein intake either, whereas these flows were significantly influenced by fiber intake. In all cases, the variability between pigs was very high but comparable within each Latin square (or fiber level).

DISCUSSION

Prevalence of the Influence of WHC. No distinction was made in the digesta between endogenous N and indigestible N from fiber. The latter reached 156 and 37 mg of N−NDF/kg of DM diet in the "unprocessed fiber" and "micronized fiber" diet (subexperiment 2), respectively. This means that the contribution of this N in the total N collected at the ileum ranged from 2.2 to 5.7% of the total N. This could hardly modify the conclusions, all the more because the Σ AA/N ratio of the digesta remained constant and the patterns of excretion of total N and nucleobases were similar, illustrating a major contribution of the organic N sources.

However, the ratio between the flow of the AAs and that of total N remained independent of fiber intake, despite different fiber intakes. This suggests a low contribution of fiber-bound N to the ileal total N flow. This assumption is supported by similar patterns of excretion of total N and nucleobases, illustrating a major contribution of epithelial cells and bacteria in the ileal digesta N.

When investigating the fiber effect, we first tested the relationship between fiber intake and ileal N flow, but the correlation was not very high ($R^2 = 0.76$). This was due to the wrinkled pea fiber and micronized fiber diets because when the latter were discarded, the correlation was highly improved ($R^2 = 0.99$). Therefore, we looked for another parameter that would explain the whole results. We finally demonstrated that the highest correlation with the ileal N flow was obtained with the WHC value of the diets ($R^2 = 0.996$). Decuypere et al. (1994) also found a positive relationship between diet WHC and ileal endogenous or bacterial N losses in pigs and the highest responses with diets having a WHC > 3 g of water/g of DM. For their part, Kyriazakis and Emmans (1995) observed a significant decrease in pig feed intake when the diet WHC exceeded 3.5 g of water/g of DM.

The different composition of the two diets with the highest WHC (smooth, wrinkled PIF) could also have enhanced the ileal N losses. They contained some proteins, pea starch instead of corn starch and PIF isolated on a pilot-plant instead of industrial products. As observed in experiment 2, the addition of proteins in the diet could have slightly increased the secretions. Moreover, pea starch increases the ileal endogenous N losses in pigs, compared to cereal starch (Everts et al., 1996), and the fibers isolated on the pilot-plant had a higher WHC and contained less starch than those used later (Table 1). However, even if these factors may have exaggerated the response of the pig to high WHC (>4 g of water/g of DM), the effect of WHC on endogenous N seems predominant.

Mechanisms. How dietary fibers stimulate the release of endogenous N is poorly understood, and fibers with a high WHC have been scarcely studied. Fibers influence the motility of the intestines, but Decuypere et al. (1994) found no relationship between the mean transit time of the digesta, the WHC of the diets, and ileal endogenous N losses.

Compared to soluble fibers, insoluble types show little or no effect on digestion and absorption (Johansen et al., 1996). A high WHC is often a property of soluble fibers. The effect of PIF seems rather similar to that of insoluble fibers because of the absence of effect on N retention. On the other hand, soluble and viscous fibers also affect endogenous N losses rather than protein digestibilities in rats (Larsen et al., 1993, 1994).

Dietary proteins are the preferred substrate of the digestive enzymes, and this might protect the endogenous proteins from hydrolysis (Matthews, 1991). Würsch et al. (1986) also showed that endogenous proteases have free access to proteins entrapped in cells of legume seeds. Finally, the presence of grain legumes in a diet does not interfere with the digestive processes (Wong and O'Dea, 1983). Since the "barrier" hypothesis did not seem strongly founded, we focused our attention on the effect of swollen fibers on the intestinal wall.

Fibers have important effects on intestinal morphology, namely, on mucosal mass and villus length and width (Cassidy et al., 1981; Jacobs, 1983). PIF intake increased the ileal output of mucus and, supposedly, of epithelial cells. This could also explain the increase in bacterial N output since cells and mucus are the preferred substrates of bacteria (Ratcliffe, 1991).

Bulky diets rich in insoluble fibers increase both the number of goblet cells and the capacity of the latter to secrete mucin in rat colons (Schmidt-Wittig et al., 1996). An increase in ileal mucus excretion was also observed in pigs receiving insoluble fibers (Mariscal-Landín et al., 1995) and in rats fed with soluble fibers (Larsen et al., 1993). Schmidt-Wittig et al. (1996) suggest a mechanical effect on the wall that would affect the integrity of the mucus layer and cause superficial cell lesions. Identical results were obtained with germ-free rats.

Some loss of epithelial cells may occur after a high-fiber diet intake (Moore et al., 1988), but we still do not know if physical abrasion of the intestinal wall increases epithelial cell desquamation or if desquamation stimulates epithelial cell proliferation (Sakata and Miyakozawa, 1997). However, insoluble fiber intake increases intestinal cell turnover by increasing the rate of cell death in pigs (Jin et al., 1994), and a high viscosity helps to stimulate epithelial cell proliferation (Gee et al., 1996). Hara et al. (1996) also suspect an important effect of physical stress on the intestinal wall because when comparing different fiber sources, they obtained the highest mucosa weights as well as DNA, RNA, and protein cell contents in rats receiving fibers with a low fermentability but with a high WHC.

Short-chain fatty acids (SCFA), involved in epithelial cell proliferation in the cecum and colon, cannot be considered in the small intestine because PIFs are not fermented in significant amounts at this level (unpublished data). However, SCFA produced in the large intestine stimulate epithelial cell proliferation in the small intestine (jejunum) by still unexplained mechanisms (Sakata, 1987). This could have occurred here because more than 80% of pea fibers are fermented in the large intestine (Canibe and Bach Knudsen, 1997) and only part of the ileal digesta were collected, allowing a significant fiber fraction to reach the colon.

N Retention. As far as the effect of fibers on the ileal endogenous N losses is concerned, the results of experiment 2 are mainly confirmative in nature. The main objective was rather to study the possible effect on protein digestion and utilization. Since we could not resort to the isotope dilution technique using ^{15}N, necessary to distinguish between endogenous and dietary N (Leterme et al., 1996b), we tested increasing highly digestible protein levels and measured the N retention and the ileal AA flows. An increase in ileal AA flows would have meant an interference of fibers on protein digestion, assuming that the intake of highly digestible proteins does not affect the ileal N losses significantly (Souffrant et al., 1997).

No such increase was observed here at both fiber levels, with the exception at the first dietary protein level that suggests a slight increase in basal endogenous losses associated with satisfaction of the maintenance requirements. The following plateau suggests that the true digestibility was close to 100%, regardless of fiber intake. Therefore, the digestive process of highly digestible protein was apparently not affected by fiber. This assumption is supported by the fact that the N compound fraction with a MW < 3000, which is supposed to contain the unabsorbed residues of dietary protein hydrolysis (Leterme et al., 1996c), did not increase with protein intake. Moreover, the AA profile of this fraction was different from that of egg yolk (Leterme et al., 1997), demonstrating a poor contribution of the dietary AAs.

On the other hand, since no interaction between fiber and protein was observed, the linear effect of protein, without influence of fiber on the slopes, means that the

marginal efficiency of protein for protein deposition was not influenced by fiber. This may be interpreted as an absence of interference of fiber in the availability of the absorbed dietary AAs to tissue metabolism, which could be expected in view of the 100% true digestibility of protein.

No main effect of fiber on N retention was more surprising. This observation is inconsistent with the recent data of Grala et al. (1997) who found a negative relationship between endogenous N losses and N retention, with diets differing in protein source. However, Tetens et al. (1996) found no effect of fiber intake on N retention in rats either. According to Dierick et al. (1983), the effect on N retention becomes significant when NDF intake exceeds 100 g/kg of diet, which was not the case here.

The lack of effect here is not easy to explain, but it must be underlined that the variability observed between pigs within a treatment was unusually high for all of the parameters studied. The collection period (3 days) of feces and urine may have been too short for N retention measurements. For the ileal flows, this period is usually sufficient for accurate determinations, but the variation obtained here is unusual. As a consequence, this could have affected the interpretation of the data because the variation in N retention was higher than the expected effect of fibers on it (−1.02 g of N retained/g of endogenous N lost at the ileum level, according to Sève and Henry (1996)). Moreover, the indigestible N from the PIF was not taken into account here, and this may also be a source of inaccuracy. The poor effect of fiber on the digestibility of a protein isolate can hardly be contested, but no final settlement can be drawn on the effect of PIF on N retention.

CONCLUSION

Our results suggest that a specific effect of the diet WHC on the ileal endogenous N losses can be distinguished from that of fiber intake. This effect was perceptible when WHC was reduced by micronization or when the diet WHC exceeded 3 g of water/g of DM intake. Above the latter value, the endogenous N outflows increased exponentially. The similar pattern observed for the ileal outflow of nucleobases and the linear, highly correlated, relationship with crude mucus output pointed to the sloughed epithelial cells and mucus as major contributors to the ileal endogenous N flow increase. However, the validity of nucleobases as markers of epithelial cells must be checked.

On the other hand, the intake of insoluble fibers with a high WHC do not disrupt protein digestion and absorption significantly. Further experiments are required to determine if the increase in ileal endogenous N losses following PIF intake affect N retention.

ABBREVIATIONS USED

AA, amino acids; ADF, acid detergent fiber; AOAC, Association of Official Analytical Chemists; DAPA, α-diaminopimelic acid; DM, dry matter; MW, molecular weight; NDF, neutral detergent fiber; PIF, pea inner fibers; SCFA, short-chain fatty acids; WHC, water-holding capacity.

ACKNOWLEDGMENT

We thank Provital s.a. (Warcoing, Belgium) for providing the pea products and Geneviève Jean, L. Givron, J. P. Haulotte, and Th. Monmart for their expert technical assistance. We acknowledge Prof. T. Sakata (Ishinomaki Senshu University, Japan) for his thorough review of the manuscript.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 13th ed.; AOAC: Washington, DC, 1980.

Canibe, N.; Bach Knudsen, K. E. Digestibility of dried and toasted peas in pigs. 1. Ileal and total tract digestibilities of carbohydrates. *Anim. Feed Sci. Technol.* **1997**, *64*, 293−310.

Cassidy, M.; Lightfoot, F.; Grau, L.; Story, J.; Kritchevsky, D.; Vahouny, G. Effect of chronic intake of dietary fibres on the ultrastructural topography of rat jejunum and colon: A scanning electron microscropy study. *Am. J. Clin. Nutr.* **1981**, *34*, 218−228.

Decuypere, J.; Spriet, S.; van Gils, L. Influence of the water-holding capacity of the feed on the precaecal and faecal apparent digestibility in pigs. In *Proceedings of 6th International Symposium on Digestive Physiology in Pigs*; Souffrant, W., Hagemeister, H., Eds.; EAAP Publ. 80, Rome, Italy; Pudoc: Wageningen, The Netherlands, 1994; pp 125−128.

Dierick, N.; Vervaeke, I.; Decuypere, J.; Henderickx, H. Influence de la nature et du niveau des fibres brutes sur la digestibilité iléale et fécale apparente de la matière sèche, des protéines et des acides aminés et sur la rétentions azotée chez les porcs (Effect of the nature and level of crude fibre on apparent ileal and faecal digestibility of dry matter, protein and amino acids and on nitrogen retention in pigs). *Rev. Agric.* **1983**, *36*, 1691−1711.

Englyst, H.; Quigley, M.; Hudson, G.; Cummings, J. Determination of dietary fibre as nonstarch polysaccharides by gas-liquid chromatography. *Analyst* **1992**, *117*, 1707−1714.

Everts, H.; Dekker, R.; Smiths, B.; Cone, J. The digestion of corn and native pea starch in the small intestine of pigs. *Proc. Nutr. Soc.* **1996**, *55*, 59A.

François, E.; Thill, N.; Théwis, A. Méthode rapide de dosage de l'oxyde de chrome dans les aliments, les fèces et les contenus digestifs par titrage après oxidation nitro-perchlorique (A rapid volumetric method for the determination of chromium sesquioxide in feeds, faeces and digesta after nitroperchloric digestion). *Ann. Zootech.* **1978**, *27*, 355−361.

Gee, J.; Lee-Finglas, W.; Wortley, G.; Johnson, I. Fermentable carbohydrates elevate plasma enteroglucagon but high viscosity is also necessary to stimulate small bowel mucosal cell proliferation in rats. *J. Nutr.* **1996**, *126*, 373−379.

Grala, W.; Verstegen, M.; van Leeuwen, P.; Huisman, J.; Jansman, A.; Tamminga, S. Nitrogen balance of pigs as affected by feedstuffs causing different endogenous nitrogen flow at the terminal ileum. *Livest. Prod. Sci.* **1997**, *48*, 143−156.

Hara, H.; Suzuki, K.; Kobayashi, S.; Kasai, T. Fermentable property of dietary fibre may not determine cecal and colonic mucosal growth in fibre-fed rats. *J. Nutr. Biochem.* **1996**, *7*, 549−555.

Jacobs, L. Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: A comparison of oat bran, pectin and guar with total fiber deprivation. *Am. J. Clin. Nutr.* **1983**, *37*, 954−960.

Jin, L.; Reynolds, L.; Redmer, D.; Caton, J.; Crenshaw, J. Effects of dietary fiber on intestinal growth, cell proliferation and morphology in growing pigs. *J. Anim. Sci.* **1994**, *72*, 2270−2278.

Johansen, H.; Bach Knudsen, K.; Sandström, B.; Skjøth, F. Effects of varying content of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs. *Br. J. Nutr.* **1996**, *75*, 339−351.

Kyriazakis, I.; Emmans, G. The volontary feed intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *Br. J. Nutr.* **1995**, *73*, 191−208.

Larsen, F.; Moughan, P.; Wilson, M. Dietary fiber viscosity and endogenous protein excretion at the terminal ileum of growing rats. *J. Nutr.* **1993**, *123*, 1898−1904.

Larsen, F.; Wilson, M.; Moughan, P. Dietary fiber viscosity and amino acid digestibility, proteolytic digestive enzyme activity and digestive organ weights in growing rats. *J. Nutr.* **1994**, *124*, 833−841.

Lassalas, B.; Jouany, J.; Broudiscou, L. Dosage des bases puriques et pyrimidiques par chromatographie liquide à haute performance (Determination of the puric and pyrimidic bases by high-performance liquid chromatography). *Ann. Zootech.* **1993**, *42*, 170−171.

Leterme, P.; van Leeuwen, P.; Théwis, A.; Huisman, J. Chemical composition of pea inner fibre isolates and their effect on the endogenous digestive secretions in pigs. *J. Sci. Food Agric.* **1996a**, *72*, 127−134.

Leterme, P.; Théwis, A.; François, E.; van Leeuwen, P.; Wathelet, B.; Huisman, J. The use of ^{15}N-labeled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. *J. Nutr.* **1996b**, *126*, 2188−2199.

Leterme, P.; Monmart, T.; Morandi, P.; Théwis, A. Oral and parenteral N nutrition vs N-free nutrition on the ileal endogenous losses and on the molecular weight distribution of the nitrogenous compounds in the ileal digesta of pigs. *J. Sci. Food Agric.* **1996c**, *71*, 265−271.

Leterme, P.; Rossi, F.; Théwis, A. Effet des protéines et des fibres alimentaires sur les pertes iléales d'acides aminés endogènes chez le porc (Effect of dietary proteins and fibres on the ileal endogenous amino acid losses in pigs). *Journ. Rech. Porcine Fr.* **1997**, *29*, 243−248.

MacKenzie, S.; Tenaschuk, D. Quantitative formation of N (O, S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analyses. I. Esterification. *J. Chromatogr.* **1979a**, *171*, 195−208.

MacKenzie, S.; Tenaschuk, D. Quantitative formation of N (O, S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analyses. II. Acylation. *J. Chromatogr.* **1979b**, *173*, 53−63.

Mariscal-Landín, G.; Sève, B.; Colléaux, Y.; Lebreton, Y. Endogenous amino nitrogen, collected from pigs with end-to-end ileorectal anastomosis, is affected by the method of estimation and altered by dietary fiber. *J. Nutr.* **1995**, *125*, 136−146.

Matthews, D. *Protein absorption: Development and present state of the subject*; Wiley-Liss: New York, 1991.

Moore, R.; Kornegay, E.; Grayson, R.; Lindermann, M. Growth, nutrient utilization and intestinal morphology of pigs fed high-fiber diets. *J. Anim. Sci.* **1988**, *66*, 1570−1579.

Prosky, L.; Asp, N.; Schweizer, T.; De Vries, J.; Furda, I. Determination of insoluble, soluble and total dietary fiber in foods and food products. *J. AOAC Int.* **1988**, *71*, 1017−1023.

Ratcliffe, B. The role of the microflora in digestion. In *In vitro digestion for pigs and poultry*; Fuller, M. F., Ed., CAB: Wallingford, U.K., 1991; pp 19−34.

Sakata, T. Stimulatory effect of short-chain fatty acids on epithelial cell proliferation in the rat intestine: Possible explanation for the trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Br. J. Nutr.* **1987**, *58*, 95−103.

Sakata, T.; Miyakozawa, M. Effects of mucosal abrasion on epithelial cell loss and epithelial cell proliferation of pig distal colon in vitro. In *Digestive physiology in pigs*; Laplace, J. P., Fevrier, C., Barbeau, C., Eds.; EAAP Publ. 88, Rome, Italy; Pudoc: Wageningen, The Netherlands, 1997; pp 558−561.

SAS/STAT. *User's Guide*, version 6, 4th ed.; SAS Institute: Cary, NC, 1994; Vol. 2.

Schmidt-Willig, U.; Enss, M.; Coenen, M.; Gärtner, K.; Hedrich, H. Response of rat colonic mucosa to a high fiber diet. *Ann. Nutr. Metab.* **1996**, *40*, 343−350.

Sève, B.; Henry, Y. Protein utilization in nonruminants. In *Proceedings of the 7th Symposium International on Protein Metabolism and Nutrition*; Nunes, A., Portugal, A., Costa, J., Ribeiro, J., Eds.; EAAP Publ. 81, Rome, Italy; Pudoc: Wageningen, The Netherlands, 1996; pp 59−82.

Souffrant, W.; Février, C.; Laplace, J.; Hennig, U. Comparison of methods to estimate ileal endogenous nitrogen and amino acids in piglets. In *Digestive physiology in pigs*; Laplace, J., Février, C., Barbeau, A., Eds; EAAP Publ. 88, Rome, Italy: Pudoc: Wageningen, The Netherlands, 1997; pp 591−595.

Tetens, I.; Livesey, G.; Eggum, B. Effects of the type and level of dietary fibre supplements on nitrogen retention and excretion patterns. *Br. J. Nutr.* **1996**, *75*, 461−469.

van Leeuwen, P.; van Kleef, D.; van Kempen, G.; Huisman, J.; Verstegen, M. The post-valve T-caecum cannulation technique: an alternative method for chyme collection in pigs. *J. Anim. Physiol. Anim. Nutr.* **1991**, *65*, 183−193.

Wong, S.; O'Dea, K. Importance of physical form rather than viscosity in determinng the rate of starch hydrolysis in legumes. *Am. J. Clin. Nutr.* **1983**, *37*, 66−70.

Wünsche, J.; Völker, T.; Souffrant, W.; Borgmann, E. Bestimmung der Bakterien-N-Anteile im Kot und unterschiedlich gewonnenem Ileumchymus von Schweinen (Assessment of the bacteria N proportions in faeces and differently obtained ileal digesta in pigs). *Arch. Anim. Nutr.* **1991**, *41*, 703−714.

Würsch, P.; del Vedovo, S.; Koellreutter, B. Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *Am. J. Clin. Nutr.* **1986**, *43*, 25−29.

Received for review November 10, 1997. Revised manuscript received February 17, 1998. Accepted February 18, 1998. The project was supported by the Belgian Ministry of Agriculture, Department of Research and Development (DG6), Brussels (Contract No. 5693A).

JF970955+