

## Effect of pig bodyweight on ileal amino acid endogenous losses after ingestion of a protein-free diet enriched in pea inner fibre isolates

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(Received 30 September 2003; accepted 16 June 2004)

**Abstract** – The present study was conducted to evaluate whether bodyweight and the micronisation of dietary fibre affect the endogenous nitrogen and amino acid losses (ENL and EAAL) in pigs. The effect of the micronising process was tested by providing pigs with 90 g DM·kg<sup>-1</sup> BW<sup>0.75</sup> of a N-free diet supplemented with isolated pea inner fibres, presented in native or micronised form and with a water-holding capacity of 12 and 4 g water·g<sup>-1</sup> DM, respectively. ENL and EAAL were measured on pigs weighing 24, 62 and 105 kg. In all cases, daily ENL increased linearly ( $P < 0.05$ ) with BW, for the majority of the AA and total N. As BW increased, daily ENL, total EAAL and the majority of EAAL increased linearly independently of micronisation ( $P < 0.05$ ). When expressed per kg DMI, total EAAL and the majority of each EAA decreased curvilinearly and reached nadir at around 100 kg BW. For ENL expressed per kg DMI, micronisation resulted in a curvilinear decrease with increasing BW, as compared to a linear decrease for pigs fed the native pea fibre diet (non-micronised). Micronisation of pea inner fibres did not decrease ENL or EAAL daily, except for proline. When the losses were expressed as g·k<sup>-1</sup>g DMI, micronisation did not decrease ENL but decreased ( $P < 0.05$ ) endogenous losses for a majority of AA as well as for total AA. The results suggest that small pigs excrete more endogenous N per kg DMI than large pigs and that pea fibre micronisation reduces EAAL but not ENL when expressed per kg DMI.

**pig / endogenous loss / ileum / amino acid / nitrogen**

### 1. INTRODUCTION

Almost one quarter of the digestive secretions and sloughed epithelial cells released in the lumen of the pig's small intestine is not reabsorbed before reaching the end of the ileum [1]. Part of these endogenous nitrogen losses (ENL) corresponds to the basal losses of the animal, i.e. a constant

amount lost per kg dry matter intake (DMI). The rest is feed specific and varies according to the amount of feed ingested and the composition of the latter [2]. The ENL are usually expressed per kg DMI because they mainly depend on feed intake [3]. However, ENL may also be affected by other factors. In some studies, pig bodyweight (BW) has little or no effect on the ENL [4, 5] whereas

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in others, a significant effect has been found [6]. According to the current data available, ENL are proportional to BW when DMI is lower than  $70 \text{ g DM}\cdot\text{kg}^{-1} \text{ BW}^{0.75}$  [6, 7].

Unabsorbed endogenous proteins are mixed with the indigestible dietary proteins excreted at the end of the small intestine. Therefore, the difference between nitrogen intake and ileal nitrogen output, only provides an “apparent” value of protein digestibility. The determination of the real digestibility of the dietary proteins requires the distinction between the endogenous and dietary proteins excreted [2]. The knowledge of the ENL level is also important because these losses significantly affect N retention in pigs [8].

So far, the range of BW evaluated [4, 6] has been too limited to draw convincing conclusions. Therefore, the analysis of the overall information available is of poor help. Bastianelli [9] compiled 105 literature data and found a poor relationship ( $r = -0.16$ ) between pig BW and ENL, expressed per kg DMI, compared to that obtained between ENL and DMI ( $r = 0.56$ ). Moreover, Stein et al. [5] did not observe any significant difference in ENL between pigs weighing 112 and 215 kg. However, in a recent literature review, the same authors [10] suggested that ENL are higher in young pigs and that the BW effect decreases rapidly as BW increases. This hypothesis is supported by data of ENL obtained on young animals: piglets weighing less than 10 kg and fed a N-free diet excrete from 3.5 to 4.6 g endogenous  $\text{N}\cdot\text{kg}^{-1}$  DMI [11, 12] whereas the average value for pigs weighing from 30 to 100 kg reaches only  $1.7 \text{ g N}\cdot\text{kg}^{-1}$  DMI [13]. Apart of these higher ENL in piglets can be ascribed to a lower DMI. However, when expressed per kg, 10 kg-piglets have an ENL of 0.405 g, compared to 0.057 to 0.017 g in pigs weighing from 30 to 100 kg. Therefore, BW appears to be an important factor in the determination of ENL.

The present study was aimed at quantifying the basal endogenous N and amino acid (AA) losses recovered at the ileum of

pigs across a wide range of BW (24–105 kg). Two diets were formulated to include isolated pea inner fibres, presented in two different forms: native or micronised. In a previous study [14], we demonstrated that native pea inner fibres increased ENL, due to their high water-holding capacity ( $10\text{--}12 \text{ g water}\cdot\text{g}^{-1} \text{ DM}$ ). When the latter was reduced ( $4 \text{ g water}\cdot\text{g}^{-1} \text{ DM}$ ) by micronisation of the fibres, ENL decreased and did not exceed the basal level. Thus, the second aim was to determine whether fibre micronisation decreases ENL and whether there is an interaction with pig BW.

## 2. MATERIALS AND METHODS

### 2.1. Animals

Four piglets ( $20 \pm 1 \text{ kg BW}$ ) of the Seghers Hybrid breed (Buggenhout, Belgium) were fitted with a post-ileal T-cannula [15]. After recovery, the pigs were placed in metabolism cages and, between each experimental period, in larger cages. Their bodyweights during the 3 successive periods were the following:  $24 \pm 0.5 \text{ kg}$ ,  $62 \pm 0.5 \text{ kg}$  and  $105 \pm 4 \text{ kg}$ . Two pigs were discarded after the first period and replaced by others of the same weight and also one after the second period. The BW mentioned corresponds to that measured at the beginning of each period. The experiments were conducted under the guidelines of the Belgian Ministry of Agriculture for animal research.

### 2.2. Diets

Two protein-free diets were formulated (Tab. I) to contain isolated pea inner fibres as the sole fibre source and presented in either native (unprocessed) or micronised form. All the diets were supplemented with  $2 \text{ g Cr}_2\text{O}_3\cdot\text{kg}^{-1} \text{ DM}$  and mixed with water (1:1).

The microniser was composed of a rotor with numerous blades in a vertical cylinder. Micronisation was obtained by the impact

**Table I.** Composition of the diets (g·kg<sup>-1</sup> DM).

	Micronised pea fibre diet	Native pea fibre diet
Ingredients, g·kg <sup>-1</sup> DM		
Maize starch	600	600
Micronised pea inner fibres <sup>1</sup>	200	–
Native pea inner fibres <sup>1</sup>	–	200
Sucrose	100	100
Oil <sup>2</sup>	40	40
Mineral/vitamin premix <sup>3</sup>	60	60
Analyses, g·kg <sup>-1</sup> DM		
Protein (N × 6.25)	24	23
Starch	430	430
Ether extract	45	44
NDF	57	44
Dietary fibres <sup>4</sup>		
insolubles	77	78
solubles	19	1

<sup>1</sup> Provided by Provital (Warcoing, Belgium). The unprocessed fibres contained, on average, 490 g dietary fibres, 430 g starch and 11.8 g N·kg<sup>-1</sup> DM and the micronised fibres: 450 g dietary fibres, 450 g starch and 12 g N·kg<sup>-1</sup>. The nitrogen was mostly in a free form. N-NDF (nitrogen bound to the neutral detergent fibres, i.e. the total insoluble fibres) represented 156 and 37 mg·kg<sup>-1</sup> DMI in the unprocessed and micronised fibre diets, respectively [16]. The AA content of the pea fibre isolates was as follows (g·kg<sup>-1</sup> DM): Arg 6.4; His 2.1; Ile: 3.5; Leu: 5.7; Lys: 6.0; Met: 1.0; Phe: 3.6; Thr: 3.6; Trp: 0.08; Val: 4.3; Ala: 3.7; Asp: 7.9; Cys: 1.5; Glu: 9.5; Gly: 3.9; Pro: 3.4; Ser: 3.6; Tyr: 0.3; Sum: 73.5 g AA.

<sup>2</sup> Oil: maize/groundnut/soyabean (1/1/1).

<sup>3</sup> Premix (g·kg<sup>-1</sup> diet): 10 g dicalcium phosphate, 5 g chalk, 5 g NaCl, 30 g Brichart premix (Sombrefe, Belgium, described by Leterme et al. [14, 16]).

<sup>4</sup> Dietary fibres: AOAC method [17].

of the particles with the blades and the jacketing of the cylinder. After micronisation, 95% of the particles had a size < 60 µm. The isolation process and the composition of the fibre isolates were previously described [14, 16].

### 2.3. Experimental procedure

Four pigs were used successively at 24, 62 and 105 kg BW. They were divided into 2 blocks of 2 pigs. For each BW level, 2 pigs received the N-free diet supplemented with native pea fibres and the 2 others the N-free diet with micronised pea fibres. After ileal digesta collection (see below), the diets were permuted and the digesta were collected so that, at each BW level, each diet was tested on 4 pigs. The pigs received daily, in 2 meals (8–16 h), 90 g DM·kg<sup>-1</sup> W<sup>0.75</sup> of the experimental diet. After 3 days of adaptation, ileal digesta were collected for 3 days from 9 to 17 h, in plastic bags attached to the cannula. During previous experiments, it was established that more than 95% of the digesta from the morning meal are excreted during that collection period. Each collected sample was immediately frozen and the samples were pooled per day before freeze-drying. After the first collection period, the pigs received a balanced diet for 2 days before receiving the second experimental diet, in order to restore their protein status and maintain their appetite, which may decrease over time with prolonged N-free nutrition. Between each experimental period, the pigs rested in large cages (1 × 1.5 m) and received a balanced diet (Brichart, Sombrefe, Belgium).

### 2.4. Chemical analyses

Diet ingredients were analysed for N by the Kjeldahl method on a Kjeltac 1030 analyser (Foss, Denmark). Starch was analysed by the enzyme method of AOAC [17], using amyloglucosidase (EC3.2.1.3; Sigma A7255), glucose oxidase (EC1.1.3.4; Sigma G7773) and peroxidase (EC1.11.1.7; Sigma P8125). The NDF determination was performed on a Fibertec analyser (Foss, Denmark) and, prior to the analyses, the samples were boiled for 1 h in a thermostable α-amylase solution (Termamyl 120L, Novo Nordisk, Denmark). The dietary fibre content was also determined by the enzymatic-gravimetric method of AOAC [18]. The AA were determined, after

acid hydrolysis (6 M HCl + 1% phenol; 24 h at 110 °C in glass tubes under a N atmosphere) by HPLC using the Pico-Tag method of Waters (Millipore Corp., Bedford, MA, USA) with phenyl-thiocarbamyl derivatives. Cysteine and methionine were determined by the same method following oxidation with performic acid. Tryptophan was not analysed. The digesta were analysed for N, AA and chromium contents. The latter were obtained by titration with Mohr salt (Sigma F3754) after nitric acid/perchloric acid digestion [19].

### 2.5. Statistical analyses

The BW effect was tested using the GLM procedure of SAS [20] according to the following model:  $Y_{ijk} = m + BW_i + F_j + (BW \times F)_{ij} + P_k + e_{ijk}$ , where  $Y_{ijk}$  = dependent variable,  $m$  = overall mean,  $BW_i$  = body-weight ( $i = 24, 62$  and  $105$ ),  $F_j$  = fibre ( $j =$  micronised and native),  $BW \times F$  the interaction between BW and fibre processing,  $P_k$  = period ( $k = 1$  and  $2$ ) and  $e_{ijk}$  = residual error. The "Fibre processing  $\times$  BW" interaction was tested against the residual error. Treatment means were separated using the least squares difference test. Least-squares means presented in Tables II and III come from two separate analyses made within the diets. Least-squares means were compared by a protected t-test, i.e., after verifying for significant effects of BW, in the two separate analyses.

The effect of BW with 2 degrees of freedom (DF) was tested for linearity (1DF) and deviation from linearity (2 DF). The regression equations used for each diet were:  $Y_{ij} = \alpha + \beta \cdot BW_i$  when the linear effect only was significant and  $Y_{ij} = \alpha \cdot BW_i^\beta$  when the deviation from linearity was significant.

The possible presence, in the ileal digesta, of undigested proteins coming from the pea dietary fibres was checked through a  $\chi^2$ -test that compared the AA profile of the ileal digesta of the pigs with that of the pea fibre isolates, as described by Le Guen et al. [21].

### 3. RESULTS

The results of the ileal endogenous N and AA losses are presented in Table II (expressed in  $\text{g} \cdot \text{day}^{-1}$ ) and Table III (in  $\text{g} \cdot \text{kg}^{-1}$  DMI). The ileal DM digestibility of the micronised fibre diet also detailed in Table II, was lower ( $P < 0.05$ ) in the piglets weighing 24 kg, compared with the larger pigs.

The daily ileal excretion of the majority of the AA and total N increased linearly ( $P < 0.05$ ) with pig BW (Tab. II and Fig. 1) but no fibre micronisation effect ( $P > 0.05$ ) was observed, except for proline, for which the excretion was increased after micronisation. The linearity of the increase in daily ileal excretion with BW was observed for 12 AA (Arg, His, Leu, Lys, Met, Thr, Val, Ala, Asp, Glu, Gly, Tyr) with the micronised pea fibre diet and 10 AA (Arg, Ile, Leu, Lys, Met, Thr, Val, Ala, Asp, Glu) with the native pea fibre diet.

The N and AA endogenous losses expressed in  $\text{g} \cdot \text{kg}^{-1}$  DMI (Tab. III) decreased ( $P < 0.05$ ) with BW. The decrease was more pronounced between 24 and 62 kg BW. The difference between pigs weighing 62 and 105 kg was significant for proline only and the sum of AA for both diets. In both cases, proline excretion was high in the piglets. Fibre micronisation decreased ENL ( $P < 0.05$ ) for most of the AA and total N. The decrease in ENL with BW increase was quadratic for the sum of AA and total N, with the exception of the total N in pigs fed the diet supplemented with native pea fibres (Fig. 1).

There was an interaction between fibre processing and BW ( $P < 0.05$ ) for 4 AA (Met, Phe, Cys and Pro) when the data of ileal excretion were expressed in g AA/d and also 4 AA (Arg, Met, Phe and Cys) when these were expressed per kg DMI (Tabs. II and III). The animal effect was significant for only 3 AA (Ile, Leu, Phe).

Since the fibre source of the experimental N-free diets, i.e. the pea fibre extracts, contained proteins (see Materials and Methods), possible contamination by residual

**Table II.** Ileal dry matter digestibility (%) and ileal endogenous N and amino acid excretion (g·d<sup>-1</sup>) in pigs of different bodyweights fed a N-free diet containing micronised or native pea isolated fibres.

BW	Micronised pea fibre diet					Native pea fibre diet					Fibre processing effect <sup>b</sup>	BW effect <sup>c</sup>				BW × fibre interaction
	24 kg	62 kg	105 kg	SEM <sup>a</sup>	L/Q <sup>d</sup>	24 kg	62 kg	105 kg	SEM <sup>a</sup>	L/Q <sup>d</sup>		24 kg	62 kg	105 kg	significance	
dDM	82.3a	85.3b	84.9b	0.46**		80.5	82.3	82.4	0.57		*	a	b	b	*	
Essential amino acids																
Arg	0.46	0.48	0.52	0.03	L	0.57	0.59	0.57	0.02	L	ns	–	–	–	ns	ns
His	0.24	0.25	0.29	0.03	L	0.29	0.24	0.28	0.03	Q	ns	–	–	–	ns	ns
Ile	0.36a	0.57ab	0.72b	0.06**	Q	0.37a	0.47a	0.66b	0.04**	L	ns	a	a	b	*	ns
Leu	0.99a	1.13a	1.60b	0.12**	L	1.03a	1.20a	1.52b	0.07**	L	ns	a	a	b	*	ns
Lys	0.67a	0.84ab	1.05b	0.05*	L	0.71a	0.78a	1.03b	0.05**	L	ns	a	a	b	*	ns
Met	0.55	0.50	0.57	0.01	L	0.29a	0.39a	0.53b	0.04**	L	ns	a	a	b	*	*
Phe	0.53a	0.86b	1.15c	0.10**	L	0.55a	1.12b	1.06b	0.09**	Q	ns	a	b	b	*	*
Thr	0.66a	0.81a	1.33b	0.11**	L	0.75a	0.99b	1.21b	0.07**	L	ns	a	a	b	*	ns
Val	0.65a	0.82a	1.22b	0.10**	L	0.71	0.80	1.14	0.07**	L	ns	a	a	b	*	ns
Non-essential amino acids																
Ala	1.01a	1.11a	1.27b	0.08*	L	1.07	1.08	1.17	0.03	L	ns	–	–	–	ns	ns
Asp	0.93a	1.18a	2.14b	0.18**	L	0.97a	1.47a	2.24b	0.16***	L	ns	a	b	c	**	ns
Cys	0.58	0.67	0.73	0.05	Q	0.33a	0.64b	0.81b	0.07**	Q	ns	a	a	b	*	*
Glu	0.98a	1.61a	2.74b	0.27**	L	1.17a	1.62a	2.69b	0.27*	L	ns	a	a	b	*	ns
Gly	1.06	1.32	2.07	0.23	L	1.43	1.66	1.50	0.12	Q	ns	–	–	–	ns	ns
Pro	2.10	2.22	2.26	0.25	Q	2.19	2.85	2.77	0.14	Q	*	a	b	a	*	*
Ser	0.97	1.25	1.32	0.09	Q	1.11	1.55	1.62	0.10	Q	ns	a	b	b	*	ns
Tyr	0.37a	0.62ab	0.96b	0.09*	L	0.40a	0.76b	0.81b	0.06***	Q	ns	a	a	b	*	ns
Σ AA	13.11a	16.54ab	21.93b	1.59*	L	13.94a	18.21b	21.51c	0.98**	L	ns	a	ab	b	*	ns
N	2.46a	3.20ab	3.91b	0.24*	L	2.59a	3.86b	4.90c	0.31***	L	ns	a	b	c	*	ns

<sup>a</sup> Pooled standard-error of the mean and level of significance of the bodyweight effect (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ).

<sup>b</sup> Level of significance of fibre effect (ns: not significant; \*  $P < 0.05$ ).

<sup>c</sup> Bodyweight effect: means within a row lacking a common superscript (a, b, c) differ (ns: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ).

<sup>d</sup> L/Q: linear ( $Y = \alpha + \beta \cdot BW$ ) and quadratic ( $Y = \alpha \cdot BW^2$ ).

**Table III.** Ileal endogenous N and amino acid excretion ( $\text{g}\cdot\text{kg}^{-1}$  DMI) in pigs of different bodyweights fed a N-free diet containing micronised or native pea isolated fibres.

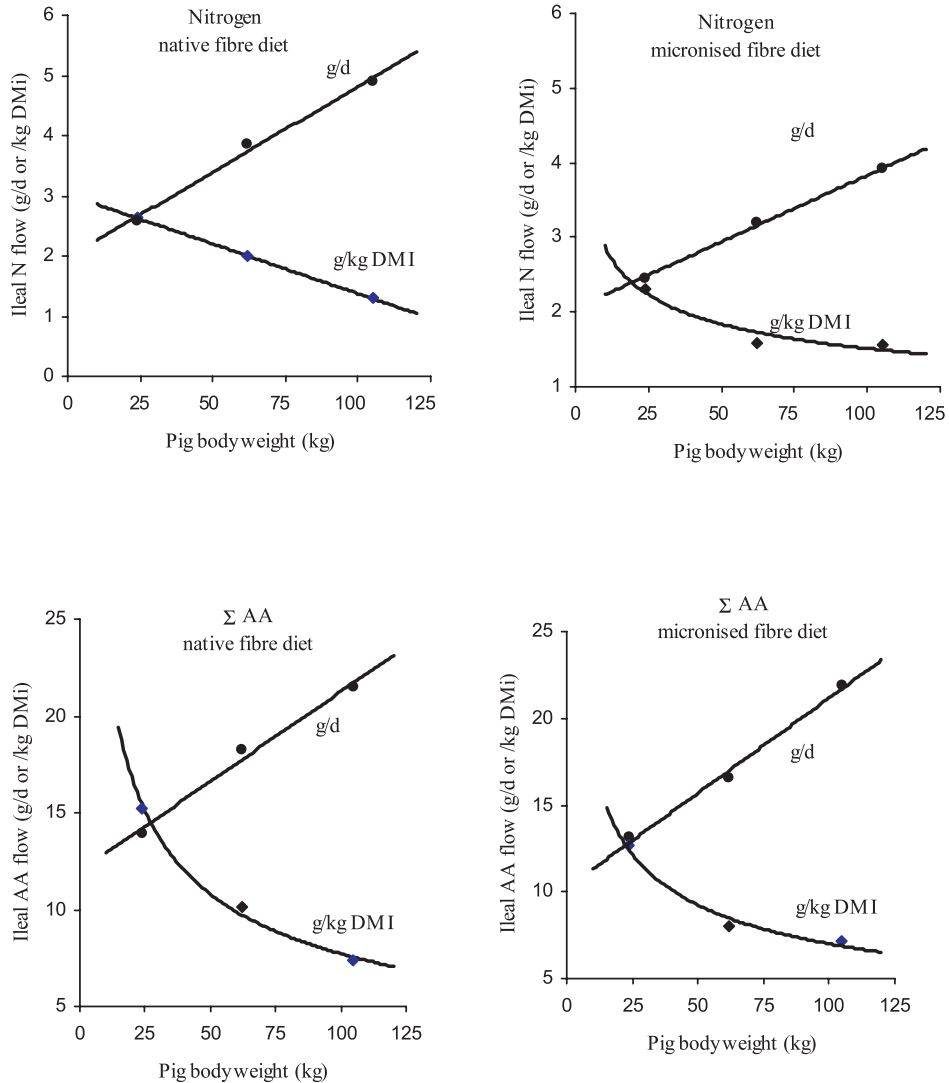
BW	Micronised pea fibre diet					Native pea fibre diet					Fibre processing effect <sup>b</sup>	BW effect <sup>c</sup>				BW × fibre interaction
	24 kg	62 kg	105 kg	SEM <sup>a</sup>	L/Q <sup>d</sup>	24 kg	62 kg	105 kg	SEM <sup>a</sup>	L/Q <sup>d</sup>		24 kg	62 kg	105 kg	significance	
Essential amino acids																
Arg	0.50a	0.21b	0.17b	0.05**	Q	0.59a	0.30b	0.27b	0.05***	Q	*	a	b	c	*	*
His	0.20a	0.09b	0.09b	0.02*	Q	0.30a	0.12b	0.10b	0.03**	Q	ns	a	b	b	*	ns
Ile	0.32	0.19	0.23	0.03	Q	0.39a	0.24b	0.23b	0.03**	Q	ns	a	b	b	*	ns
Leu	0.97a	0.49b	0.52b	0.08**	Q	1.07a	0.61b	0.54b	0.08***	Q	ns	a	b	b	*	ns
Lys	0.62a	0.34b	0.34b	0.04***	Q	0.74a	0.40b	0.37b	0.05***	Q	ns	a	b	b	*	ns
Met	0.64a	0.16b	0.19b	0.07***	Q	0.30a	0.20b	0.20b	0.05***	Q	*	a	b	b	*	**
Phe	0.51	0.44	0.37	0.03	L	0.57a	0.48ab	0.38b	0.04*	L	*	a	a	b	*	*
Thr	0.63a	0.43b	0.44b	0.04*	Q	0.78a	0.60ab	0.43b	0.05**	L	*	a	b	b	*	ns
Val	0.62a	0.32b	0.40b	0.05*	Q	0.73a	0.41b	0.41b	0.05***	Q	*	a	b	c	*	ns
Non-essential amino acids																
Ala	0.93a	0.45b	0.41b	0.08***	Q	1.10a	0.56b	0.42b	0.09***	Q	ns	–	–	–	*	ns
Asp	0.94a	0.58b	0.70ab	0.06*	Q	0.99a	0.75b	0.80b	0.04**	Q	*	a	b	c	*	ns
Cys	0.68a	0.24b	0.24b	0.06***	Q	0.35	0.32	0.32	0.02	Q	*	a	b	b	*	**
Glu	0.97	0.88	0.90	0.03	Q	1.21	1.14	1.12	0.07	Q	*	a	b	b	*	ns
Gly	0.91	0.70	0.64	0.07	Q	1.48a	0.85b	0.54b	0.14**	Q	*	a	b	b	*	ns
Pro	1.95a	1.68a	0.76b	0.26*	L	3.07a	2.23a	0.64b	0.34***	L	*	a	a	b	*	ns
Ser	0.91a	0.65b	0.42b	0.07**	L	1.14a	0.80b	0.40c	0.10***	L	*	a	b	c	*	ns
Tyr	0.36	0.31	0.31	0.04	Q	0.41	0.39	0.29	0.02	Q	ns	–	–	–	ns	ns
Σ AA	12.61a	7.98b	7.14b	0.86**	Q	15.22a	10.31b	7.45c	1.04**	Q	*	a	b	c	*	ns
N	2.45a	1.61b	1.56b	0.13**	Q	2.63a	1.99b	1.29b	0.14**	L	ns	a	b	b	*	ns

<sup>a</sup> Pooled standard-error of the mean and level of significance of the bodyweight effect (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ).

<sup>b</sup> Level of significance of fibre effect (ns: not significant; \*  $P < 0.05$ ).

<sup>c</sup> Bodyweight effect: means within a row lacking a common superscript (a, b, c) differ (ns: not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ).

<sup>d</sup> L/Q: linear ( $Y = \alpha + \beta \cdot \text{BW}$ ) and quadratic ( $Y = \alpha \cdot \text{BW}^{\beta}$ ).



**Figure 1.** Evolution of the ileal flow of nitrogen or the sum of amino acids ( $\Sigma$  AA) with the bodyweight of pigs fed a N-free diet supplemented with either native or micronised pea fibres. Nitrogen – native pea fibre diet: ●  $\text{g}\cdot\text{d}^{-1}$   $y = 1.97 + 0.028x$   $r^2 = 0.99$ ; ◆  $\text{g}\cdot\text{kg}^{-1}$  DMI:  $y = 3.02 - 0.017x$   $r^2 = 1.0$ . Nitrogen – micronised pea fibre diet: ●  $\text{g}\cdot\text{d}^{-1}$   $y = 2.05 + 0.018x$   $r^2 = 0.99$ ; ◆  $\text{g}\cdot\text{kg}^{-1}$  DMI:  $y = 5.49x^{-0.28}$   $r^2 = 0.90$ . Sum of AA – native pea fibre diet: ●  $\text{g}\cdot\text{d}^{-1}$   $y = 12.0 + 0.09x$   $r^2 = 0.99$ ; ◆  $\text{g}\cdot\text{kg}^{-1}$  DMI  $y = 72.3x^{-0.486}$   $r^2 = 0.99$ . Sum of AA – micronised pea fibre diet: ●  $\text{g}\cdot\text{d}^{-1}$   $y = 10.2 + 0.11x$   $r^2 = 0.99$ ; ◆  $\text{g}\cdot\text{kg}^{-1}$  DMI  $y = 43.6x^{-0.397}$   $r^2 = 0.97$ .

undigested dietary proteins in the ileal digesta was verified, using a  $\chi^2$  analysis, as suggested by Le Guen et al. [21]. The AA profile of the pea fibre fraction (see Materials and Methods) was compared with that of the ileal digesta of pigs fed the native pea fibre diet and also to that of pigs fed a N-free diet supplemented with cellulose [22]. No relationship ( $P > 0.05$ ) was found between the AA profile of the ileal digesta and that of the pea fibre fraction. The AA profile of the digesta collected in the present experiment, compared to that of digesta collected from pigs fed a conventional N-free diet, was different ( $P < 0.05$ ).

#### 4. DISCUSSION

In a previous experiment [14], pigs of 45–65 kg fed with diets similar to those used in this study and supplemented with unprocessed pea isolated fibres, presented higher ENL than those reported for micronised fibres: 2.73 vs. 1.64 g N·kg<sup>-1</sup> DMI, respectively. The difference was limited in the present case and not significant for N (Tab. III), despite the fact that the fibres used were identical to those used previously. No satisfying explanation can be provided to explain the difference. At the AA level, the difference comes mainly from the non-essential AA such as proline, which is always excreted in high quantities in N-free conditions [2]. On the contrary, no significant “Fibre processing × BW” interaction was observed, except for a few AA.

The contribution of N coming from the fibre sources on the total N ileal excretion was checked. The nitrogen bound to fibres (N-NDF) contributed to 156 and 37 mg·kg<sup>-1</sup> DMI of the diets containing native and micronised fibres, respectively. The fibre sources also contained proteins, as attested by their AA analysis (see Materials and Methods). Assuming that their real digestibility reached 90% [23] and taking the N-NDF contribution into account, it can be calculated that the indigestible N coming from the pea fibre fraction represented from 11 to 18% of the

total ileal N excreted by pigs fed the micronised fibre-based diet and from 15 to 30% of the native fibre-based diet. However, the data cannot be verified in vivo and our values are within the range of endogenous N and protein excretion usually mentioned in the literature when pigs are fed N-free diets supplemented with fibres: 10.0–12.5 g endogenous proteins·kg<sup>-1</sup> DMI [9, 13].

The  $\chi^2$  test also indicates that the contribution of undigested pea AA in the ileal AA flow measured here did not affect the estimation of the ileal endogenous AA flow.

On a daily basis, Furuya and Kaji [4] found no difference in ENL between pigs of 49 and 92 kg. This may have been due to the fact that the pigs were fed at different levels (respectively 92 and 27 g DM·kg<sup>-1</sup> BW<sup>0.75</sup>·day). Hess and Sève [6] found no effect either, between the pigs of 45 to 77 kg, receiving the same amount of DM·kg<sup>-1</sup> BW<sup>0.75</sup>. In the present experiment and, also with pigs receiving the same amount of diet proportionally to their metabolic weight, we observed a linear increase in the daily loss of N and most of the AA with BW. However, when expressing the ENL per kg BW, both the results of Hess and Sève [6] and ours show a decrease with BW. These authors obtained 40 and 13 mg N·kg<sup>-1</sup> BW or 102 and 38 mg N·kg<sup>-1</sup> BW<sup>0.75</sup> for pigs at 45 and 77 kg BW, respectively, whereas, in the present case, we obtained 105, 57 and 42 mg N·kg<sup>-1</sup> BW or 233, 160 and 134 mg N·kg<sup>-1</sup> BW<sup>0.75</sup>, for pigs at 24, 62 and 105 kg BW, respectively.

We also observed significant ENL expressed per kg DMI, that decreased ( $P < 0.05$ ) when BW increased. Stein et al. [5] found no significant difference between growing pigs (112 kg BW) and lactating sows (215 kg), despite a numeric difference between both ENL (12.4 vs. 9.4 g N·kg<sup>-1</sup> DMI, respectively). However, our results suggest that the BW effect is limited in animals with BW > 50–60 kg (Fig. 1). Hence, the difference between pigs of 62 and 105 kg was significant for proline and the sum of the AA only. This could explain why many



authors did not observe any significant effect of BW on basal ENL in large pigs.

On the contrary, Bastianelli [9] assumed that the negative relationship between ENL and BW was under the effect of DMI since, in his compilation of the literature, he found a high correlation ( $r = 0.70$ ) between the latter and pig BW. This was ascribed to the lower DMI of piglets. In the present case and in the study by Hess and Sève [6], however, DMI was adjusted to the same level of metabolic weight, regardless of BW. Our results confirmed the assumption by Stein and Nyachoti [10] that small pigs excrete, proportionally to DMI, more ENL than larger ones.

Other literature data support this hypothesis. Leibholz [11] and Wilson and Leibholz [12] fed young pigs (27 to 35-d old) a N-free diet and measured ileal ENL ranging from 3.5 to 4.6 g N·kg<sup>-1</sup> DMI. However, the DMI was low (45 g DM·kg<sup>-1</sup> BW<sup>0.75</sup>·day). Schulze et al. [24] fed piglets (14 kg on average) higher intake levels of N-free diets ( $\pm 70$  g DM·kg<sup>-1</sup> BW<sup>0.75</sup> day) and also measured high ENL: from 2.7 to 3.9 g N·kg<sup>-1</sup> DMI, depending on the dietary fibre source. Finally, with slightly heavier piglets (20 kg on average) that received  $\pm 60$  g DM·kg<sup>-1</sup> BW<sup>0.75</sup> day, we measured ENL ranging from 1.8 to 3.3 g N·kg<sup>-1</sup> DMI, depending on the type of fibre (barley hulls or bran) and the dietary fibre content (100 or 200 g·kg<sup>-1</sup>) [25]. In most of the cases, DMI was even higher than what our regression curves predict ( $\pm 2.4$  to 2.7 g N·kg<sup>-1</sup> DMI for piglet weighing 14 kg or 20 kg, respectively; Fig. 1).

The higher ENL in piglets can be partly explained by the fact that the protein turnover of the gut tissues is higher than that in larger pigs [26]. Moreover, both the digestive and absorptive capacities of the intestines increase with age [27, 28]. It can be hypothesized that this lowers the capacity of the piglets to recycle their digestive secretions efficiently.

A higher susceptibility of the small pigs to dietary factors was not evidenced here. The experiment was aimed at studying the

basal ENL, i.e. those which are independent of any dietary factor. However, the effect of native pea inner fibres vs. micronised inner fibres was also studied because in previous experiments, we observed an important effect of native fibres on ENL: 4.8 g N·kg<sup>-1</sup> DMI vs. 1.8 g for 25 kg-pigs receiving a N-free diet supplemented with cellulose [14]. The effect observed here, although significant for part of the AA, was not comparable to those observed previously and no "Fibre processing  $\times$  BW interaction" was observed. Further experiments are necessary to determine whether piglets are more sensitive than large pigs to fibre intake.

Numerous studies have shown the positive effect of fibre intake on ENL in pigs [14, 16, 24, 25, 29]. Their composition and their physico-chemical properties, explain this effect [14, 16, 25, 29]. The presence of fibres and their properties such as viscosity or water-holding capacity increase the turnover of the epithelial cells but also mucus excretion, pancreatic secretions or the microflora [30–33]. A feed-back effect, coming from the volatile fatty acids produced in the colon on the small intestine epithelial cell proliferation is also suspected [34].

In summary, this study showed that the ENL expressed per kg DMI are proportionally higher in young pigs. ENL decrease rapidly as BW increases but appear to plateau when BW exceeds 50–60 kg. Micronisation of the dietary fibres did not affect the total ENL expressed per day or per kg DMI but significantly decreased the endogenous losses, expressed per kg DMI, of some amino acids.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mrs G. Jean, MM L. Givron, T. Monmart and X. Moreaux for their expert technical assistance. They are also indebted to Professor J.E. Muñoz, from the National University of Colombia for his help in the statistical analyses. The research was financed by the Department of Research & Development (DG6) of the Belgian Ministry of Agriculture, Brussels (contract No. D1/2 – 5818A).

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