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Effect of Barley Fibres and Barley Intake on the Ileal Endogenous Nitrogen Losses in Piglets

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Abstract

Ileal endogenous N losses (ENL) were measured, using the ^{15}N isotope dilution technique, in piglets (17 kg) fed different barley genotypes (naked, spring, winter with low/high *beta*-glucan content) or diets containing 330, 530, 730 or 930 g of a blend of barleys/kg diet. The apparent protein and amino acid digestibilities of the naked variety and the winter variety with a high *beta*-glucan content were, on average, significantly higher than those for the other two varieties. The ENL were inversely correlated ($p<0.01$) with the apparent digestibilities but the difference between each of them was not significant ($p>0.05$). The ENL increased linearly with the inclusion level of barley in a N-free basal diet (2 mg endogenous N/g barley). Isolated hulls added to a N-free diet at the rate of 100 or 200 g/ kg diet exerted no significant effect on the ENL (1.80 g endogenous N/kg diet in both cases *vs.* 1.76 g for the basal level). On the contrary, the effect of isolated bran, measured under similar conditions, was significantly higher and dependent on fibre intake (2.59 and 3.31 g N/kg diet, respectively). It is concluded that the ENL are affected by the insoluble bran fibre but not by the hulls, nor by the level of *beta*-glucan.

Keywords : barley ; pig ; fibre ; endogenous N.

Abbreviations used : AA = amino acids ; ADF = acid detergent fibre ; ENL = ileal endogenous nitrogen losses ; NDF = neutral detergent fibre.

INTRODUCTION

Cereals are the most important ingredients of pig diets in Europe. They supply more than half the feed protein, making the quality of the latter a crucial parameter in the economics of pig feeding. Paradoxically, their nutritional value is not well defined. Feed producers use amino acid (AA) digestibility measured at the ileum level, to formulate their diets. However, digestibility measured in this way is apparent because ileal digesta are composed not only of undigested dietary AA but also of endogenous AA arising from non-reabsorbed digestive secretions or sloughed epithelial cells. True protein digestibilities of cereals, such as barley, are usually estimated *in vitro*[1], *in sacco*[2] or *in vivo* after subtraction, from the ileal digesta, of an endogenous fraction measured indirectly on a N-free diet.

The *in vitro* and *in sacco* approaches are only indicative and are used to screen different batches of cereals. Moreover, the current *in vivo* approach is not satisfactory. Firstly, it assumes that the ileal endogenous N losses (ENL) are constant, whereas these vary according to the presence of dietary factors, such as fibres or antinutritional factors, that either stimulate digestive secretions and/or prevent their reabsorption by the intestine. Secondly, in nutritional terms, the variable fraction of the ENL cannot be included with the undigestible dietary protein fraction. The latter is an exogenous source of protein whereas the ENL have been synthetised by the animal. A negative correlation has been established between the ENL and N retention in pigs fed diets with identical apparent digestible protein content[3]. This is ascribed to a lower marginal efficiency of dietary digestible protein for non-re-absorbed endogenous protein, compared with that for endogenous proteins: 0.55 *vs.* 0.88, respectively. Thus, the protein value of cereals should be characterised both by their true digestibility and the specific ENL they induce.

The true protein digestibility of barley depends on its fibre content[1,4]. The fibre fraction of cereals also affects the ENL[5]. Due to the low protein content of barley, the ENL could explain a great part of the variation observed in

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its apparent protein digestibility.

A distinction between endogenous and dietary N in ileal digesta is possible only by using the ^{15}N isotope dilution technique. In this procedure one of the two N sources (dietary proteins or digestive secretions) is labelled with the stable isotope. The isotope dilution, measured in the ileal digesta, gives an estimate of the respective proportion of the two protein fractions[5,7]. The total ENL measured includes both the basal losses and those specific to the diet. Therefore, protein digestibilities calculated from these values are referred to as 'real' digestibilities. However, the isotope dilution technique is not applied routinely because it is intricate and expensive.

Few data are available on the effects of barley fibre on the ENL. On the other hand, feed producers classify their cereals according to type or variety rather than to chemical composition. They would like to know if type or variety influences the prediction of the nutritional value of the cereals. A series of experiments was undertaken using the ^{15}N dilution technique, to compare four genotypes of barley differing in type or fibre content (naked, spring and winter, with a low or high barley *beta*-glucan content) and at four intake levels. In addition N-free diets were used to study the effect of the two main fibre fractions of barley (hulls and bran) on ENL.

EXPERIMENTAL

Animals

During five successive periods, five series of 12 German hybrid male piglets (17 ± 2 kg) were surgically prepared, under general anaesthesia, with an end-to-end ileo-rectal anastomosis[8]. Four series were used for the ^{15}N studies and the fifth for the experiment with N-free diets and fibre isolates. During the convalescence period (7 d), the piglets received increasing amounts of the test diet. In each series, eight piglets, having good recoveries, were selected for the experiment. They were fitted with two catheters at the carotid artery and jugular vein, for ^{15}N-leucine infusion and blood sample collection, respectively. These animals were treated with antibiotics for 3 days and the ^{15}N-leucine infusion started the day following insertion of the catheters. The animals were held in metabolism cages that allowed total collection of the digesta. The experiments were conducted under the guidelines for animal research of the German Ministry of Agriculture and received the approval of the Ministry of Agriculture of Mecklemburg-Vorpommern (ref. SV5/96).

Diets

The barleys used in the experiments were a blend of barleys from the Faculty of Agronomy of the University of Rostock (Germany), a naked variety (Taïga, Germany), a spring variety (Volga, France), a winter variety (Krimhild, Germany) and a winter variety with a high content of *beta*-glucan (Grete, Germany). Their chemical compositions are detailed in Table I.

Two main fractions of barley fibres, i.e. the hulls (outer fibrous covering of the grain) and the bran (outer layers of the seed composing pericarp, testa and aleurone layer), were also isolated from barley seed meal (variety Krimhild) by first separating 14 flour fractions on a pilot flour mill (Multomat, MIAG, Braunschweig, Germany). The first fraction was composed mainly of hull and the contaminating flour was separated by sifting and discarded. The next two fractions comprised small pieces of hull and coarse flour (bran). The remaining hull material was separated by sifting and discarded, and the two bran fractions were pooled. The pooled bran fraction was suspended, at a concentration of 100 g/L, in a solution of thermostable *alpha*-amylase (1%, Termamyl L-100, Novo Nordisk, Denmark) and heated at 100 °C for 1 h. The residue was then treated (250 g/L) with a solution of 0.01 M HCl + 12.5 g/L of pepsin A (2000 IU/g; Merck; Darmstadt, Germany). After filtration, the residue was placed in distilled water (250 g/L), the pH of the solution adjusted at 7.4 with NaOH and 12.5g/L of pancreatin (Sigma P1500, St Louis, U.S.A.; 1 IU/mg) and 12.5 g/ L of amyloglucosidase (Sigma A7255, St Louis. U.S.A.; 5 IU/mg) added in order to remove the residual starch and proteins. The composition of the hulls and bran is given in Table I.

In the first experiment, a blend of barleys was used: four diets were formulated to contain increasing amounts of the barley blend (330, 530, 730 and 930 g/kg dry matter), at the expense of starch (Table II). In the second experiment, the four varieties were investigated. The diets were composed of barley (930 g/kg), supplemented with minerals and vitamins. The semi-synthetic diets containing the isolated fibres were protein-free, so that the AA collected at the ileum would be of endogenous origin only. Two fibre levels were tested for each fibre source: 100 and 200 g/kg dry matter (Table II).

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Table I: *Chemical composition of the barleys and the barley fibre isolates*

	Barleys					**Fibre isolates**	
	Taiga ***naked***	**Volga** ***spring***	**Krimhild** ***winter***	**Crete** ***winter***	**Blend**	**Hulls**	**Bran**
Protein (N x 6.25}	131	116	93	121	119	28	78
Starch	519	507	494	487	463	22	6
Ether extract	28	31	36	29	25	8	71
Ash	20	26	21	21	22	58	51
Crude fibre	16	49	44	48	52	380	189
NDF	129	164	193	209	204	847	666
ADF	16	53	58	52	54	434	213
Dietary fibre							
—total	153	192	187	209	207	868	777
—insoluble	93	154	121	130	143	838	726
beta-Glucan	51	36	54	63	48	5	67
N-NDF	2.1	1.5	1.8	1.9	2.2	1.4	12.4

Values expressed on a g/kg dry matter basis.

Table II: *Composition of the experimental diets*

	Barley-based diets[a]				**Fibre isolates**			
	930	**730**	**530**	**330**	**Hull**		**Bran**	
					100	**200**	**100**	**200**
Barley	930	730	530	330	—	—	—	—
Maize starch	—	87	174	261	350	300	350	300
Potato starch	—	87	174	261	350	300	350	300
Bran	—	—	—	—	—	—	100	200
Hull	—	—	—	—	100	200	—	—
Sucrose	—	20	40	60	100	100	100	100
Oil[b]	—	6	12	18	30	30	30	30
Premix[c]	70	70	70	70	70	70	70	70

Values are given as g/kg diet.
[a] individual varieties were only studied with diets containing 930 g barley/kg diet.
[b] sunflower oil.
[c]premix: (/kg diet): 20 g dicalcium phosphate, 10 g chalk, 5 g NaCl; 35 g 'King' premix (Brichart, Sombreffe, Belgium).

^{15}N-leucine infusion and blood sampling

The infusion of ^{15}N-leucine into the jugular vein began two days after catheter placement in the jugular vein. The day before the ^{15}N-leucine infusion started, blank samples of blood and digesta were collected to estimate the natural ^{15}N-enrichment. L-leucine-^{15}N (95% of enrichment: Ghemotrade, Leipzig, Germany) was dissolved aseptically in a sterile saline solution and auto-claved. The infusion was performed for 10 days, with a syringe pump (Harvard 22, Harvard Apparatus, Holliston, U.S.A.), at the rate of 2 mL/h, corresponding to 15 mg leucine/kg bodyweight/d. Two blood samples (2 x 10 mL) were collected daily (08·00 h and 18·00 h) from the carotid artery, into ice-cooled, heparinised tubes and immediately centrifuged at 2000 *g* for 15 min. The plasma was removed and treated twice with an equal volume of a 10% (w/v)-trichloroacetic solution and centrifuged (5500 *g*; 15 min). The supernatant was used for ^{15}N analysis.

Experimental procedure

Comparison of barley varieties and intake levels

In the comparison of barley varieties, eight selected piglets were randomly allocated to one of the four diets. After an adaptation period and collection of the ileal digesta, they were killed and replaced by the next series of eight piglets. The latter were also randomly allocated to one of the four diets, so that each diet was tested on four piglets. The two following series were used for the comparison of the barley intake levels, according to the same

experimental scheme.

The piglets received daily, in two meals (08.00h-16.00h), 600 g (± 540 g dry matter) of the experimental diet. Collection of the digesta was started on the 6th day of infusion and continued for 5 days. The total digesta were collected in beakers containing 300 mL ethanol, to inhibit microbial action. Each morning, the digesta were weighed and dried in a fume cupboard for ethanol evaporation, freeze-dried and pooled for analysis. At the end of the experiments, the piglets were killed by intravenous infusion of a barbiturate (2 g Brevinarcon®) and a blood sample collected from the portal vein. The sample was treated as described.

Hull and bran comparison

Due to the limited amounts of hull and bran available and health problems for some animals during the experiment, the number of piglets used was limited to six. During the first period, three piglets received 600 g of the diet containing 100 g hulls/kg diet and the three other piglets, the diet with 200 g bran/kg. After a 4-day adaptation period, the total digesta were collected for 3 days as described above. The piglets then received a balanced diet for one week before receiving one of the two other diets, i.e. containing either 200 g hulls or 100 g bran/kg. After 4 days of adaptation to the diet, the ileal digesta were collected for 3 days. The three-day digesta were pooled for analysis.

Chemical analyses

The dietary ingredients were finely ground through a 0·5 mM mesh-screen. (Pulverisette 14, Fritsch, Idar-Oberstein, Germany) and analysed for nitrogen by the Kjeldahl method, using a Kjeltec 1030 analyser (Perstorp Analytical, Helsingborg, Sweden). Starch was analysed by the enzymic method of AOAC[9] using amylo-glucosidase (EG 3.2.1.3; Sigma A7255), glucose oxidase (EG 1.1.3.4., Sigma G7773) and peroxidase (EG 1.11.1.7, Sigma P8125). Fat was extracted with diethyl ether by the Soxhlet method. The neutral and acid detergent fibre (NDF and ADF) determinations were performed separately on a Fibertec analyser (Perstorp Analytical, Helsingborg, Sweden) and, prior to the analyses, the samples were heated at 100 °C for 1 h in a thermostable *alpha*-amylase solution (1 g/200 mL of a 1 % (w/v)-Termamyl 120 L solution, Novo Nor-disk, Copenhagen, Denmark). The dietary fibre content was determined by the enzymic-gravimetric method of AOAC[10]. The nitrogen bound to the NDF fibres (N-NDF) was determined by the Kjeldahl method on the residues obtained after treatment with neutral detergents. Residues of 12 samples were pooled for the N analysis. The *beta*-glucan content was estimated by the enzymic procedure of McCleary and Glennie-Holmes[11], using the Megazyme (Sydney, Australia) kit. The AA were analysed, after acid hydrolysis (6 M HCl; 2h at 134 °C in an autoclave) by ion-exchange chromatography using a Biochrom 20 analyser (Pharmacia LKB, Cambridge, U.K.). Cysteine and methionine were determined by the same method after oxidation with performic acid. Tryptophan was analysed after alkaline hydrolysis with 4 M NaOH.

The digesta were analysed for ^{15}N, N and AA. After freeze-drying, 0.5 g of digesta were treated for Kjeldahl digestion, distillation and titration to determine the N content. A second distillation was performed and NH_3 recovered in HC1 as NH_4Cl. The latter was placed in small tin cups, dried and analysed using an elemental analyser (Carlo Erba. Milano, Italy) coupled to an isotope-ratio mass spectrometer (Finnigan delta S, Bremen, Germany). The fraction of plasma soluble in trichloroacetic acid solution was directly treated for Kjeldahl digestion and NH_3 directly distilled in HCl to obtain NH_4Cl, which was analysed for ^{15}N-enrichment.

Calculations and statistical analyses

The proportion of endogenous N in the ileal digesta was calculated as follows:
Endogenous N (% total N) = $(^{15}N_{digesta} \times 100)/^{15}N_{deproteinised\ plasma}$
where $^{15}N_{digesta}$ and $^{15}N_{deproteinised\ plasma}$ are the ^{15}N-enrichments measured in the ileal digesta and in deproteinised plasma, respectively. The latter contains the plasma free AA, assumed to be the main precursor pool of the endogenous secretions. The determination was performed on the digesta and plasma samples collected over the last 3 days of the feeding trials with the barley-based diets (days 8 to 10), i.e. when a plateau of ^{15}N-enrichment of the deproteinised plasma was reached. The ENL were calculated by multiplying the proportion of endogenous N in the digesta by the total ileal N flow.

The N (or AA) digestibilities were calculated as follows:
Apparent N digestibility = $(N_i - \text{total } N_f) \times 100/N_i$
True N digestibility = $(N_f-(\text{total } N_f-\text{basal ENL}))\times 100/N_i$
Real N Digestibility = $(N_i -(\text{total } N_f-\text{total ENL}))\times 100/N_i$

where N_i is the N ingested and N_f the N flow at the ileal level. Only the digesta collected over the last 3 days of the feeding trials with the barley-based diets were considered for the calculations. The total ENL were measured by the ^{15}N dilution technique for pigs receiving barley-based diets. The basal ENL, which are constant and independent of the composition of the diet, were measured by the regression technique. A regression was established between the ENL and barley intake. The basal ENL correspond to the ENL for a zero-barley intake level. All the ENL are calculated on a per kg diet intake basis because the flows are proportional to feed intake. Moreover, thus permits data to be used any circumstances (pig weight, feed intake, etc).

A two-way ANOVA (period, diet) was used to examine the effect of the varieties and of the intake levels on the ENL and a three-way ANOVA (pig, period, diet) for the effect of fibre isolates using the ANOVA statistical software package: Stat-istica[12]. No significant effect of the period or of the pig was observed (p>0.05). The Student-Newman-Keuls test was used for significance between the diets.

RESULTS

The apparent ileal AA digestibilities of the naked variety Taiga and the winter variety Grete were, on average, significantly higher than those of the winter variety Krimhild and the spring variety Volga (Table III). The dry matter digestibility was also higher for Taïga but not for Grete and it varied inversely with the fibre content. On average, the differences between Taïga, Grete and the two other varieties were higher for the non-essential AA but significant differences were also observed for essential AA, such as threonine and tryptophan.

The apparent AA digestibilities of the barley blend decreased with the decrease in intake level (Table III). The decrease was linear up to an intake of 530 g barley/kg diet. Thereafter, the values decreased more sharply. At identical inclusion levels (930 g barley/kg), the apparent N and AA digestibilities of the blend were higher than those for the individual varieties.

The ileal N and AA flows increased linearly with barley intake (Table IV). The extrapolation for a zero-barley intake provided the basal value for the ileal endogenous N and AA flows.

By distinguishing between the endogenous and dietary N in the ileal digesta using the ^{15}N dilution technique, it was possible to calculate the ENL and the 'true' and 'real' barley N digestibilities (Table V). No data were obtained for the diet containing 330 g barley/kg because the ^{15}N-enrichment in the ileal digesta was higher than that of deproteinized plasma, assumed to be the precursor pool of the secretions. At the other levels, the ileal flow of endogenous N decreased significantly (p = 0.01) with barley intake. The higher apparent N digestibility of Taiga was partly explained by a lower endogenous N flow in pigs fed this variety. Owing to high inter-animal variation, however, no significant statistical difference was observed between the endogenous flows and between the 'true' or 'real' digestibilities for the four varieties (Table V).

The N and AA flows measured on pigs fed N-free diets supplemented with either isolated hulls or bran are presented in Table VI. The flows measured for the two levels of hulls were almost identical to the basal endogenous AA losses calculated by regression (Table IV). On the contrary, the ENL were higher with isolated bran and the effect tended to be proportional to fibre intake (Table V). The intake of 200 g fibres/kg nearly corresponded to the level of fibre ingested with diets containing 930 g barley/kg.

Table III: *Apparent ileal amino acid digestibilities of different barley varieties or of a barley blend at different inclusion levels in pigs (%)*

	Varieties					**Blend**				
						Level of barley (g/kg diet)				
	Taïga	**Volga**	**Krimhild**	**Grete**	**SEM**	**930**	**730**	**530**	**330**	**SEM**
N	73·4^{a}	67·7^{bc}	65·9^{b}	71·2^{ac}	0·90*	77·1^{a}	73·2^{b}	69·4^{C}	59·3^{d}	1·55***
Dry matter	73·1^{a}	69·0^{b}	68·0^{b}	67·5^{b}	0·66**	70·5^{a}	73·2^{a}	77·6^{b}	84·6^{C}	1·39**
Essential amino acids										
Arginine	80·9	82·9	80·2	83·6	0·48	84·0^{a}	80·8^{ab}	76·6^{b}	60·9^{c}	2·44***
Histidine	81·3^{a}	78·6^{ab}	72·4^{b}	75·3^{ab}	1·07*	84·9^{a}	79·9^{a}	78·3^{a}	67·9^{b}	1.77***
Isoleucine	73·9	71·2	71·1	76·5	0·87	79·8^{a}	77·2^{ab}	70·8^{b}	60·9^{C}	2·08***
Leucine	79·4	76·0	73·2	79·0	0·84	82·7^{a}	78·7^{ab}	74·3^{b}	66·1^{c}	1.76***
Lysine	74·1^{a}	67·1^{b}	72·3^{a}	73·8^{a}	1·16*	77·3^{a}	74.9*	71·4^{b}	57·3^{C}	0.01 ***

Methionine	79·5	79·2	77·3	78·0	0·53	84·3^{a}	80·9^{ab}	77·4^{b}	70·9^{C}	1.48***
Phenylalanine	82·0	81·2	78·1	83·3	0·64	83·8^{a}	81·6^{a}	77·4^{a}	70·2^{b}	1-53***
Threonine	63·3^{a}	56·7^{b}	56·5^{b}	63·3^{a}	1·15*	69·6^{a}	63·7^{a}	55·5^{b}	37·3^{C}	3.43***
Tryptohan	74.4^{a}	61·9^{b}	59·2^{b}	69·6^{a}	1·83**	76·3^{a}	73·2^{a}	67·7^{ab}	42·9^{C}	3.74***
Valine	75·6	70·5	68·8	75·3	0·98	79·2^{a}	75·8^{ab}	70·l^{b}	59·9^{C}	0.11 ***
Non-essential amino acids										
Alanine	65·8^{a}	57·2^{b}	61·2^{ab}	66·l^{a}	1·34*	72·0^{a}	65·4^{ab}	59·4^{b}	45·2^{C}	2·81 ***
Aspartic acid	70·8	61·5	67·4	67·3	1·24	74·7^{a}	69·6^{a}	63·6^{b}	52·0^{C}	2·40***
Cysteine	73·8^{a}	66·1^{b}	67·7^{b}	72·8^{a}	1·07*	78·2^{a}	73·5^{a}	70·9^{ab}	62·3^{C}	1·74***
Glutamic acid	86·5^{a}	83·7^{ab}	81·0^{b}	84·l^{ab}	0·61*	89·1^{a}	87·6^{a}	85·9^{a}	82·l^{b}	0·81***
Glycine	64·9^{a}	56·2^{b}	54·0^{b}	65·7^{a}	1·54*	70·5^{a}	66·2^{a}	57·5^{b}	41·6^{C}	3·55***
Proline	84·0	82·4	80·0	83·3	0·66	87·4^{a}	82·7^{ab}	78·7^{b}	64·5^{C}	1·60***
Serine	76·2^{a}	68·6^{b}	68·4^{b}	74·5^{a}	1·08*	78·0^{a}	71·6^{b}	67·2^{C}	54·0^{d}	2·43***
Tyrosine	74·1^{a}	78·0^{ab}	77·l^{b}	84·8^{C}	1·15*	81·0^{a}	73·2^{b}	61·0^{C}	nd	3·40***
Mean	78·9^{a}	74·7^{b}	73·2^{b}	78·3^{a}	0·76*	82·4^{a}	78·9^{ab}	74·6^{b}	64·3^{c}	1·93***

Values are means + SEM, n = 4. Within a row and for each experiment, values with different superscripts are significantly different (*: p<0.05; **: p<0.01; ***: p<0.001) according to the Newman-Keuls test, nd: not determined

Table IV: *Ileal flow of N and amino acids (g/kg dry matter intake) in pigs fed with increasing amounts of barley and values for terms in the regression equation between barley inclusion level and the ileal N and amino acid flows*

	330	**530**	**730**	**930**	**a**	**b**	r^2
Essential amino acids							
Arginine	0.47	0.52	0.60	0.66	0.36	0.33	0.993
Histidine	0.27	0.32	0.39	0.40	0.20	0.23	0.936
Isoleucine	0.46	0.56	0.60	0.72	0.33	0.41	0.969
Leucine	0.88	1.03	1.15	1.29	0.66	0.68	0.998
Lysine	0.54	0.65	0.75	0.87	0.36	0.55	0.999
Methionine	0.17	0.19	0.22	0.25	0.12	0.135	0.992
Phenylalanine	0.50	0.62	0.70	0.83	0.33	0.535	0.993
Threonine	0.77	0.88	0.96	1.11	0.58	0.55	0.985
Valine	0.70	0.84	0.92	1.09	0.49	0.625	0.983
Non-essential amino acids							
Alanine	0.88	1.02	1.14	1.28	0.66	0.66	0.999
Aspartic acid	1.03	1.21	1.35	1.55	0.75	0.85	0.996
Cysteine	0.30	0.36	0.44	0.49	0.19	0.325	0.993
Glutamic acid	1.65	2.09	2.48	2.96	0.93	2.16	0.999
Glycine	0.84	0.98	1.06	1.25	0.62	0.655	0.976
Proline	1.42	1.34	1.57	1.56	1.27	0.325	0.564
Serine	0.69	0.79	0.90	0.96	0.55	0.46	0.986
Tyrosine	0.29	0.2	0.35	0.41	0.22	0.195	0.966
∑ AA	11.39	13.16	14.92	16.95	8.30	9.22	0.999
Nitrogen[a]	2.60	3.05	3.48	4.09	1.76	2.45	0.993

Y = a + b. X with Y = the ileal AA or N flow (g/kg dry matter intake), X = the inclusion level of a barley blend in the diet Jkg barley/kg diet), a = the intercept (g AA/kg dry matter intake) and b = the slope (g AA/kg dry matter intake). [a] including N-NDF.

DISCUSSION

The present study, based on the ^{15}N dilution technique, demonstrated that the total ENL in piglets fed barley-based diets can reach more than twice the basal level of the ileal losses (Table V). As a consequence, the true protein digestibilities, currently calculated with basal ENL, underestimate the real protein digestibilities of cereals.

The total ENL increased linearly with barley intake, at the rate of 2 mg N/g barley (Table V). It was difficult to distinguish between the effect of barley as a whole or that of its fibre fraction. This may have been due to a low intake: <70g dry matter/kg metabolic weight ($W^{0.75}$). At low feed intake and when the latter is expressed per kg dry matter intake, the specific ENL may appear marginal compared with the basal losses[13], resulting in biasing

differences between diets. The low appetite of the piglets could be ascribed to the anastomosis and to the short recovery period after surgery (10 days), all the more because the implantation of two blood catheters just before the experiment required full anaesthesia of the animals.

The possible difference between covered and naked barleys in term of ileal protein digestibility has not been thoroughly investigated as yet. At similar total fibre contents, they have been observed to have similar digestibility values[14]. However, differences in experimental conditions between the two experiments may have occurred because the barley blend was one of the two samples having the highest fibre content, the other being the variety Grete, whereas the blend also had the highest apparent N digestibility. The intake of naked barley by piglets in the present experiment resulted in slightly lower ENL, compared with the other varieties, but the difference was not significant (Table V). This observation was confirmed and extended in the experiments with the N-free diets supplemented with hulls: the ENL did not exceed the basal level, whatever the hull level in the diet (Table VI). Other fibre sources are also ineffective in either stimulating secretion or preventing their reabsorption by the intestine[15]. The physiological effect of dietary fibres is related to physicochemical properties such as bulk, water-holding capacity or viscosity[16], but hulls are inert and present none of the characters that could affect the ENL. The influence of hulls associated with the grains could be different from that of isolated hulls. Physical interactions with the bran fibres are possible but this could not be demonstrated here.

It is also unlikely that *beta*-glucan level affected the ENL. Although our data are too limited to be conclusive, the comparison between the varieties Grete and Volga provide valuable indications. Both had a similar total dietary fibre content but the former contained 75% more *beta*-glucan (Table I). Despite this, the average apparent AA digestibility for Grete was significantly higher than that for Volga (Table III) and the induced ENL were slightly, but not significantly, lower than those obtained for Volga (Table V). Soluble barley *beta*-glucans have only a limited effect on the digesta viscosity of piglets and even this small effect is limited to the upper part of the gastrointestinal tract[17]. Moreover, from 49 to 90% of the total *beta*-glucan is hydrolysed in the small intestine by the microbial and endogenous barley *beta*-glu-canases[17,18], depending on the variety studied or the animal weight. Pig digesta also have a high water content, precluding any marked depressive effect of viscous *beta*-glucan[19]. Besides, no significant effect of digesta viscosity, caused by soluble barley *beta*-glucan, has been observed either on the pancreatic activity or on the ileal protein digestibility in piglets fed a barley-based diet[17]. Therefore, *beta*-glucan may be considered as a rather unimportant dietary factor in respect to induction of ENL or preventing protein digestion, at least at levels such as those encountered here.

On the basis of the results of the isolated fibre study (Table VI), the insoluble fibre fraction of the kernel is thus likely to be the main factor affecting the ENL. This would explain the high specific (total-basal) ENL observed with the naked seed, although the latter had neither an identical composition to the isolated fibres nor the physicochemical properties of those fibres. The increment in ENL (7.6mg N/g raw fibre) was comparable to that obtained with isolated wheat bran fibres (8 mg N/g NDF fibre)[5]. No distinction was made here between the endogenous N and the N-NDF because the estimation of the latter in the digesta is hazardous. Moreover, part of the N-NDF is digestible: 60% on average for wheat bran, for example[20]. In the present case, the piglets fed with the bran-based diets ingested 1.24 and 2.48 g N-NDF/kg diet, for 100 and 200 g bran/kg diet, respectively. Assuming that 60% of the dietary N was digested, it may be considered that bran fibres still had a positive effect on the endogenous N losses.

Table V: *Proportion of endogenous N in the ileal digesta (% total N), ileal endogenous N loss (ENL; g N/kg dry matter intake), apparent true and real ileal digestibilities of dietary N (%) in pigs fed different varieties or different levels of barley*

	n	Endogenous N		Ileal digestibilities of dietary N		
		Proportion (%) of total N	Ileal ENL	Apparent	True	Real
Varieties						
Taiga	4	73.5 ± 8.5	3.76 ± 0.62	73.4 ± l.2[a]	82.1 ± 1.4	91.9 ± 0.3
Volga	4	79.6 ± 7.3	4.45 ± 0.24	67.7 ± 3.7[b]	77.8 ± 4.2	93.2 ± 0.3
Krimhild	4	85.7 ± 13.6	4.62 ± 0.93	65.9 ± 1·4[b]	78.3 ± 1.7	95.3 ± 0.4
Grete	4	81.4 ± 8.8	4.09 ± 0.51	71.2 ± 2.3[a]	81.0 ± 2.6	92.3 ± 0.6
P		0.073 ns	0.10 ns	0.008**	0.13 ns	0.77 ns
Barley intake level						
930 g/kg	3	72.5 ± 8.4	3.27 ± 0.38[a]	77.1 ± 2.5*	84.4 ± 5.0	93.0 ± 2.3
730 g/kg	3	78.6 ± 2.3	2.92 ± 0.09[a]	73.32 ± 1.2[ab]	86.0 ± 1.4	94.2 ± 0.9
530 g/kg	3	81.2 ± 7.3	2.44 ± 0.12[b]	69.4 ± 3.8[b]	86.0 ± 4.5	94.5 ± 2.3
P		0.52 ns	0.014*	0.02*	0.81 ns	0.79 ns

Values are means + SD. Within a column and for each experiment, values with different superscripts are significantly different (*p:* level of significance, with ns: not significant: *: $p<0.05$; **: $p<0.01$). For each barley intake level, one pig was discarded because of catheter blockage.

Table VI: *Ileal N and amino acid flows in piglets fed with protein-free diets supplemented with two levels of barley hulls or bran (g/kg dry matter intake)*

	Hull		**Bran**		**SEM**
	100 g/kg	**200g/kg**	**100 g/kg**	**200 g/kg**	
Essential amino acids					
Arginine	0.24[a]	0.30[a]	0.48[b]	0.50[b]	0.06
Histidine	0.18[a]	0.19 [a]	0.28[b]	0.31[b]	0.03
Isoleucine	0.30[a]	0.31[a]	0.48[b]	0.57[b]	0.06
Leucine	0.52[a]	0.54[a]	0.85[b]	0.97[b]	0.11
Lysine	0.27[a]	0.27[a]	0.43[b]	0.46[b]	0.05
Methionine	0.08[a]	0.09[a]	0.14[b]	0.13[b]	0.01
Phenylalanine	0.29[a]	0.31[a]	0.50[b]	0.55[b]	0.06
Threonine	0.53[a]	0.54[a]	0.69[ab]	0.91[b]	0.09
Tryptophan	0.12[a]	0.11[a]	0.17[b]	0.23[b]	0.03
Valine	0.45[a]	0.47[a]	0·72[b]	0.87[b]	0.10
Non-essential amino acids					
Alanine	0.49[a]	0.49[a]	0.77[b]	0.95[b]	0.11
Aspartic acid	0.61[a]	0.68[a]	l.01[b]	l.10[b]	0.11
Cysteine	0.20[a]	0.22[a]	0.29[b]	0.35[b]	0.04
Glutamic acid	0.83[a]	0.84[a]	1.27[ab]	l.51[b]	0.15
Glycine	0.71[a]	0.75[a]	l.09[ab]	l.49[b]	0.16
Proline	l.20[a]	l.15[a]	2.06[ab]	3.05[b]	0.38
Serine	0.41[a]	0.45[a]	0.61[b]	0.71[b]	0.07
Tyrosine	0.15[a]	0.20[a]	0.29[b]	0.31[b]	0.04
∑ AA	7.56[a]	7.89[a]	12.10[b]	14.97[b]	1.60
Nitrogen[a]	l.80[a]	l.79[a]	2.59[ab]	3.31[b]	0.36

Values are means + SEM, $n = 3$ (except the diet with 200 g bran/kg: $n = 2$). Within a row, values with different superscripts are significantly different ($p<0.05$) according to the Newman-Keuls test. [a] including N-NDF

Dietary fibre as an entity includes a wide range of components differing in physicochemical properties and physiological effects and it is difficult to ascertain which properties are responsible for the observed effect on ENL. However, insoluble fibres can stimulate the pancreatic secretions and the proliferation of epithelial cells, increase the desquamation of these cells, the production of mucus, the oro-rectal transit time and have important effects on intestinal morphology, namely on mucosal mass, villus length and width[16,21-24]. As far as we are aware, no data are available for barley fibres, but many studies have dealt with wheat bran. Compared with other fibre sources, wheat bran does not significantly affect the desquamation of the epithelial cells of the small intestine[21], oro-ileal transit time[25] or the protein digestion of other components of the diet.

No ideal method is available for ENL determination. The ^{15}N dilution technique is the most appropriate because it allows a distinction to be made between endogenous and dietary N under normal feeding conditions and provides ENL specific for each feedstuff. The animal protein labelling approach was preferred because it provided the total ENL. Labelling of the dietary proteins allows a distinction to be made between the two sources of AA but only during a limited period of time, because of the fast recycling of the dietary ^{15}N into the digestive secretions[6].

However, the validity of the animal labelling approach remains to be demonstrated. The plasma free AA pool is not the real precursor pool of the endogenous secretions. Moreover, the pattern of N, in terms of non-protein N and AA, may be very different in the plasma and in the protein. The ^{15}N-enrichment of some secretions can even be higher than that of the assumed precursor pool[26]. This was observed here with the lowest barley level (330 g barley/kg, i.e. 40 g proteins/ kg dry diet), preventing us from estimating the proportion of endogenous N in the digesta.

A series of other fundamental and technical aspects of the method remain to be examined but it is acknowledged that the current approach, i.e. the labelling of the animal problem, leads to an overestimation of the ENL and, consequently, of the real digestibilities.[7] Moreover, the variability among pigs is often very high but the number of subjects must be limited, due to the extremely high cost of the labelled leucine and the ^{15}N analyses. This may have affected the absolute values of the results but the experiments with isolated fibres confirm the predominant

effect of the insoluble fibre fraction.

CONCLUSIONS

The present results provide evidence that the ENL in pigs may not have the constant value given by the N-free diet (basal ENL) but may vary according to the intake level of barley and presumably to their fibre content. The assumption that the insoluble fraction of the kernel fibres exerts the main effect on the ENL is supported by the data obtained with N-free diets supplemented with insoluble kernel fibres. On the contrary, hulls and *beta*-glucan appear not to affect ENL. These data illustrate the fact that barley may be considered not only for the nutrients it provides, but also for the physiological effects it induces, because the latter can significantly influence the growth or production of pigs fed with barley. Real values for ileal protein digestibilities are preferred to apparent digestibilities in formulating diets but information is also necessary on the specific ENL because their influence on whole protein metabolism of the animal is significant[3]. Further experiments are required to study more satisfactorily the effect of cereal fibre intake on ENL and on the real protein digestibilities with a wide range of barleys differing in fibre content or fibre type.

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