The Poor Digestibility of Rapeseed Protein Is Balanced by Its Very High Metabolic Utilization in Humans

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Abstract

Rapeseed protein (RP, Brassica napus) is used in only animal feed despite its high nutritional potential for human nutrition. We sought to assess the nutritional quality of rapeseed by measuring its real ileal digestibility (RID) and net postprandial protein utilization (NPPU) in humans fed 15N-RP. Volunteers equipped with an intestinal tube at the jejunal (n = 5) or ileal level (n = 7) ingested a mixed meal containing 27.3 g 15N-RP and a total energy content of 700 kcal (2.93 MJ). Dietary N kinetics was quantified in intestinal fluid, urine, and blood sampled at regular intervals during the postprandial period. The RID of RP was 84.0 and the postprandial biological value (PBV) was high at 83.8.

Introduction

Rapeseed (Brassica napus) contains a high proportion of protein (20%) but, to date, this by-product of rapeseed production has been used solely for animal nutrition. To our knowledge, there is no human consumption of rapeseed protein (RP) and no available in vivo evaluation of its nutritional value despite the potential interest in its use.

RP of quantitative importance are storage proteins: cruciferin (12S globulin), a globular protein rich in lysine and methionine, and napin (2S albumin), a soluble protein containing high levels of glutamine, proline, and cysteine. The relative proportions of these proteins differ considerably between cultivars, with albumin levels ranging from 13 to 46% (2). Indices for the nutritional quality of RP in rats or livestock were as high as those of animal protein and far higher than those of other legume or cereal sources (3–6).

In terms of its potential use for human nutrition, RP is of particular interest because of its globally high content in indispensable amino acids (AA) (>400 mg/g protein) and particularly in sulfur AA (40–49 mg/g protein) (7,8). These levels are double the sulfur AA level in the indispensable AA reference pattern established following the last FAO/UNU/WHO consultation of experts in 2001 (9) or the U.S. Dietary Reference Intake for adults (10) and far higher than those usually found in plants, especially legume protein. However, protein quality does not depend only on AA composition but also on other factors related to the kinetics of AA delivery from these proteins (11,12).

There has been no assessment to date of the digestibility or metabolic utilization of RP that provides insights into its suitability and value for human consumption. In this context, our aim was to assess both the bioavailability and metabolic utilization of RP in vivo in humans through the combined use of intestinal tubes and intrinsically and uniformly 15N-labeled RP using the same methodology as that used to measure the nutritional quality of other plant proteins.

Subjects and Methods

Subjects. Twelve 25-y-old subjects (6 female, 6 male) who weighed 71 ± 12 kg and had a BMI of 23.4 ± 3.0 kg/m volunteered for the study. They were included after undergoing a thorough medical examination and routine blood tests. Body composition was determined from isotopic dilution after the oral administration of deuterium oxide (75 mg/kg body...
RP isolate and experimental meal. $^{15}$N-labeled RP was prepared at an experimental scale by growing at the Technical Centre for Oilseed Crops (CETIOM) winter rapeseed that contained very low levels of glucosinolates (Brassica napus L., Goeoil cultivator) in the presence of $^{15}$N-ammonium nitrate. A rapeseed flour was produced from dehulled seeds by extraction with hexane to remove the oil (by CREOL, Pessac, France). The solvent was eliminated at a low temperature and under vacuum to protect protein functionality. A protein isolate was purified by solubilizing the rapeseed flour at pH 11 to eliminate insoluble polysaccharides then adjusted to pH 7 and ultrafiltered at 20°C. The extraction and purification of RP were carried out on a laboratory scale. In this cultivar, the globulin, napin, and lipid transfer protein fractions represent 36.8, 41, and 2.7% of total protein, respectively (13). The final N content of the rapeseed isolate was 14.9%, with $^{15}$N enrichment of 1.16 atom percent. The AA composition (mg/g protein) of the protein isolate and indispensable AA scoring patterns is shown in Table 1.

### Experimental protocol
The subjects were hospitalized for 2 d. On day 1, a 3-m PVC double-lumen tube was inserted via the nose under local anesthesia and then swallowed so as to progress down the gastro-intestinal tract, as verified under X-ray. The tip was halted in either the jejunum (n = 5) or the terminal ileum (n = 7) with mean tube length from the nose reaching 167 and 214 cm, respectively. The subjects were given meals at 1200 and 1900 and then fasted overnight. On the second day, the protocol started at 1000, when a saline solution (NaCl 130 mmol/L, KCl 5 mmol/L, t-mannitol 30 mmol/L) containing 20 g/L polycrylamide glycol (PEG)-4000 was infused continuously through the first lumen of the tube at a constant rate of 1 mL/min. After baseline collections of intestinal fluid, blood, and urine samples, the subjects ingested within 30 min the experimental meal that contained $^{15}$N-RP and 75 mg $^{15}$N-[1-15C]-glycine (98% A), Eurois-top) given as a marker of the gastric emptying rate. Intestinal fluid, expired breath, blood, and urine were sampled at regular intervals over a period of 8 h. Digesta samples were collected on ice and pooled over 30-min periods. Expired breath was sampled every 30 min. Blood was sampled every 30 min for 3 h and then every hour for the next 5 h. Total urine was collected every 2 h throughout the 8-h postprandial period. Urine specimens were weighed and aliquoted. All the samples were stored at −20°C until analysis.

### Analytical methods
Plasma glucose was assayed using a glucose oxidase method (Glucose GOD-POD kit, Kone). The PEG-4000 concentration in digesta samples was determined using a turbidimetric method (16). Urea levels were assayed in both serum and urine using an enzymatic method on a Dimension automat (Dupont de Nemours). Ammonia was measured in the urine by an enzymatic method on a Kone instrument. Amino acid concentrations in deproteinized serum samples were determined by HPLC after separation on cation exchange resin and postcolumn ninhydrin derivatization (Biotek Instruments), as already published (12). Norvaline and amino-guanidopropionic acid were used as internal standards to correct amino acid losses between sampling and preparation for analyses. γ-Amino-butyric acid was added just before analysis to control the injection volume.

For isotopic determinations, urea and ammonia were isolated from urine (17). Serum separation of N fractions (protein N, free N, and urea N) was achieved, as already detailed (18). Protein N and nonprotein N in the ileal samples were fractionated by ethanol delipidation, as previously described (19).

The total N, nonprotein N, and protein N contents of the digesta and serum protein fraction were determined using an elemental nitrogen analyzer (NA 1500 series 2, Fisons Instruments) with atropine as the standard. The $^{15}$N/$^{14}$N isotope ratio was determined by isotope-ratio MS (Optima, Fisons Instruments) in the digesta, urinary urea and ammonia, serum protein, free N, and urea. The $^{15}$N enrichment of CO$_2$ in expired breath was determined using GC-isotope-ratio MS (Multiflow/Isoprime, Micromass).

Rapeseed isolate protein and ileal effluents were analyzed in polyacrylamide gels (4–12%, Invitrogen) in denaturing (SDS) nonreducing conditions to determine the nature of undigested dietary protein. Protein loads were 40 µg per well. A molecular standard (3–62 kDa) was also used (SeeBlue, Invitrogen). Proteins were fixed using trichloroacetic acid and then stained with Coomassie Blue.

### Calculations
The total intestinal flow rate (F, in mL/30 min) was derived for each 30-min period from the dilution of PEG, estimated by F = (PEG$_i$/PEG$_s$) × Fi × t, where PEG$_i$ and PEG$_s$ are the PEG concentrations in the infusion solution and sample, respectively, F is the PEG infusion rate (1 mL/min), and t is the duration of the collection period (30 min). The actual intestinal flow rate (total flow measured corrected for the infusion flow) was calculated as F minus the infusion flow rate (1 mL/min) for each 30-min period.

The jejunal total N flow and the ileal total, nonprotein, and protein N flows (mmol N/30 min) were derived from the following formula: N$_{\text{jej}-\text{digesta}}$ = (N$_i$ × DM$_i$ × F)/140, where N$_i$ is the N percent measured (g/100 g) in the freeze-dried sample and DM$_i$ is the dry matter of the sample (g/100 mL).

The time course of dietary N incorporation (expressed as a percentage of the ingested amount) into the different body N pools monitored (digesta, serum protein, and free AA, body urea, urinary urea, and ammonia) was evaluated using classical dilution equations, as previously detailed (18).

The cumulated recovery of dietary N in ileal samples ($\Sigma$N$_{\text{diet-ileal}}$) served to calculate the real ileal digestibility (RID, percent of ingested N) of RP: RID = (N$_{\text{injected}}$ − $\Sigma$N$_{\text{diet-ileal}}$)/N$_{\text{injected}}$ × 100.
The excretion of $^{13}$C in expired breath after the ingestion of an oral dose of $^{13}$C-glycine in the meal was used to determine the half-time of gastric emptying (20). Total CO$_2$ production was estimated at 300 mmol/m body surface $^{-2}$ · h$^{-1}$ using an estimation of the body surface area (21).

Total, dietary, and endogenous urea production levels (mmol N · kg body weight$^{-1}$ · 2 h$^{-1}$) were evaluated, as previously detailed (18) for the 4 2-h periods following meal ingestion. At the end of the 8-h experimental period, the amount of dietary N retained in the body, or net postprandial protein utilization (NPPU), was calculated as follows: NPPU (% of ingested N) = (meal N intake × RID/100 − dietary urea production)/meal N intake. NPPU at 8 h represents an overall assessment of dietary N retention in the body, i.e., dietary N absorbed but not transferred to the urea pool. This includes dietary N used for protein synthesis, for nonprotein pathways but also dietary N transferred to new AA via transaminations and dietary N in free AA pools. The postprandial biological value (PBV) was calculated as the relative amount of dietary N absorbed that was not deaminated during the postprandial period: PBV (percent of ingested N) = NPPU/RID × 100. Because our method is based on the follow-up of N as a proxy to assess the metabolism of AA, it does not allow us to measure the fate of the carbon skeleton, although ideally, both components of AA metabolism should be assessed concomitantly.

**Statistics.** Data are expressed as means ± SD. Changes over time of variables above the baseline value were tested using contrast analysis under a mixed model with time as a repeated factor (SAS 9.1, SAS Institute). A value of $P < 0.05$ was considered significant.

**Results**

**Intestinal kinetics and RID of RP.** The intestinal liquid flow rates did not change significantly over time (Fig. 1A). Both endogenous and dietary N fluxes changed after meal in the jejunum ($P < 0.005$) but not in the ileum (Fig. 1B). Over the 8-h period, dietary N represented 42 ± 6% of total N in the jejunum and 37 ± 7% in the ileum. The cumulated recovery of dietary N was 29.9 ± 4.8 and 16.0 ± 8.8% of the ingested amount at the jejunal and ileal levels, respectively (Fig. 1C). The RID of RP was 84.0 ± 8.8%.

In subjects with the tube at the ileal site, the flow rate of dietary nonprotein N was stable throughout the postprandial period (0.1–0.3 mmol N/30 min), whereas dietary N in the form of protein reached high, variable amounts, which accounted for >80% of the total ileal dietary N flux between 2 and 5 h after the meal (Fig. 2A). For ileal endogenous N flow, a high (mean: 79 ± 3%), consistent proportion of endogenous N was made up of protein (Fig. 2B).

The electrophoretic profiles of the ileal contents of the 2 individuals with the lowest (65.2%; Fig. 3A) and highest degree of digestibility (90.5%; Fig. 3B) both had a band at 50–55 kDa before and after the meal, presumably corresponding to the heavy chain of secretory Immunoglobulin G, and a group of bands at 30 kDa between 1 and 6 h after the meal, which were probably partly due to the passage of pancreatic enzymes: chymotrypsin, trypsin, elastase. In the first subject, (Fig. 3A), peak levels of ileal dietary N (at 1.5 and 2 h) in the ileum resembled bands of the RP isolate [at ~50 kDa and ~14 kDa, possibly due to the presence of undigested cruciferin (Ca8) and napin (N1), respectively]. These bands were less intense in the subject with the highest degree of digestibility (Fig. 3B).

**Kinetics of circulating glucose, dietary AA, and dietary N-carrying protein.** Plasma glucose concentrations rose after ingestion of the mixed meal and peaked at 1 h (Fig. 4). Plasma total AA concentrations did not change and ranged from 2975 ± 273 μmol/L at baseline to 3326 ± 761 μmol/L at 2 h.

![Figure 1](https://academic.oup.com/jn/article/137/3/594/4664656)

**Figure 1** Postprandial kinetics of intestinal flow rate (A), endogenous and dietary N fluxes (B), and cumulative recovery of dietary N (C) in the jejunum (n = 5, left-hand graphs) and ileum (n = 7, right-hand graphs) of subjects equipped with intestinal tubes after the ingestion of a mixed meal containing $^{15}$N-RP. Time effects are indicated on graphs. Values are means ± SD. *Values significantly different from baseline for dietary N, #, endogenous N ($P < 0.05$, contrast analysis).

$^{13}$C excretion reached its maximum 3.5 h after the meal and represented 42 ± 3% of the dose at the end of the 8-h period (Fig. 5A). The half-asymptotic excretion time, a proxy for the gastric emptying half-time, was 266 ± 24 min. Dietary N in plasma AA reached its maximum 3 h after the meal. Dietary N

![Figure 2](https://academic.oup.com/jn/article/137/3/594/4664656)

**Figure 2** Postprandial kinetics of ileal dietary N (A) and endogenous N (B) in the form of protein N or nonprotein N in subjects after the ingestion of a mixed meal containing $^{15}$N-RP in humans. Values are means ± SD (n = 7).
incorporation into the plasma protein pool followed a sigmoid curve and reached 7.7 ± 2.0% of the N ingested at 8 h postprandially (Fig. 5B).

Dietary N deamination, postprandial retention, and postprandial biological values of RP. The transfer of dietary N to body urea increased during the first 3 h to reach a plateau at 6.7 ± 2.5% of ingested N (Fig. 6). Ammonia and urea levels were low in cumulative dietary N urinary excretion (0.23 ± 0.12% and 5.4 ± 1.8% of ingested N at 8 h, respectively). Endogenous urea production remained steady throughout the postprandial period and was highly variable among subjects (Table 2). Urea production from dietary AA was at its maximum for the first 2 h following the meal but was negligible during the last 4 h. The sum of ileal and deamination losses, representing the amount of dietary N not retained 8 h after the meal, was 29.5 ± 9.6% of the meal content. As a result, the NPPU value was 70.5 ± 9.6%.

Discussion

This work constitutes the first determination of the nutritional value of RP in humans. Using an intestinal tube to quantify ileal N flow rates and 15N-labeled protein to specifically measure the

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Figure 3  Electrophoretic analyses of ileal digesta from 2 individual subjects with the lowest (65.2%; A) and the highest (90.5%; B) levels of digestibility in the study group and the corresponding ileal fluxes of dietary N (mmol N/30 min) during the 8 h following meal ingestion. The first lane (S) contains a molecular weight standard, the second and last lanes (RP) contain the RP isolate, lanes 0–8 contain the ileal samples after the meal. The RP are dimers of α and β cruciferin (Caβ), or individual α or β cruciferin subunits (Ca and Cβ), N1, or the 10-kDa subunit of napin (N2) and 4-kDa subunit of napin (N3). Arrows indicate the bands present in both digesta and the RP isolate.

Figure 4  Postprandial glycemia after the ingestion of a mixed meal containing 15N-RP in humans. Values are means ± SD (n = 7). There was a significant time effect with respect to glycemia. *Values that differed significantly from baseline (P < 0.05, contrast analysis).

Figure 5  13C excretion in expired air and percentage of dietary N in the plasma amino acid nitrogen pool (A) and proportion of ingested N incorporated into plasma protein (B) after the ingestion of a mixed meal containing 15N-RP and an oral dose of 75 mg 13C-glycine in humans. Values are means ± SD (n = 12).

Figure 6  Transfer of dietary N into the deamination pool expressed as percentages of ingested N, after the ingestion of a mixed meal containing 15N-RP. Values are means ± SD (n = 12).

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TABLE 2 Endogenous and dietary urea production rates in humans after the ingestion of a mixed meal containing $^{15}$N-RP

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Endogenous AA</th>
<th>Dietary AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol N/kg body weight</td>
<td>mmol N/kg body weight</td>
</tr>
<tr>
<td>0–2</td>
<td>0.41 ± 0.59</td>
<td>0.20 ± 0.14</td>
</tr>
<tr>
<td>2–4</td>
<td>0.47 ± 0.60</td>
<td>0.17 ± 0.15</td>
</tr>
<tr>
<td>4–6</td>
<td>0.49 ± 0.61</td>
<td>0.07 ± 0.07</td>
</tr>
<tr>
<td>6–8</td>
<td>0.50 ± 0.57</td>
<td>0.03 ± 0.13</td>
</tr>
<tr>
<td>Total (0–8 h)</td>
<td>1.88 ± 1.41</td>
<td>0.47 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SD, n = 12.

Intestinal kinetics of dietary and endogenous N and rapeseed N digestibility. The RID of the RP isolate reached 84%, a low value when compared with the RID of other plant proteins measured using the same methodology [which are all in the range of 89–91% (18,22–25) and to that of milk protein (95%) (26,27) or egg protein using $^{13}$C-protein (94%) (28). The RID of rapeseed was associated with larger variation among subjects than the aforementioned protein sources. This was due in particular to 1 subject with an extremely low RID of 65% whose data were not excluded, because all indicators were in favor of accurate measurements in the ileum (adequate tube length and position, typical ileal high pH, and low liquid flow rate). If this subject had been excluded, the RID of RP would have been 87.1 ± 3.1%, which is still lower than any other protein source already studied in humans.

We took particular care in the preparation of the RP isolate to avoid any drastic heat or alkaline treatment; thus, the low RID measured could not be linked to any technological treatment. In fact, our results agreed with pig data showing the lower apparent fecal digestibility of RP than soy, and the lower true RID (80–88%) of RP than other plant proteins such as wheat gluten, soy, or pea (8,29–33). RP has also demonstrated its poor digestibility in poultry (34) but not in rats (4). It is noteworthy that most of the animal studies used non-dehulled rapeseed rich in lignin, which could partly explain the low level of digestibility observed. However, in dehulled rapeseed, proteins were still less digestible than soy protein (35).

To enable a thorough study of the reasons for the low digestibility of RP, we also determined ileal N fluxes in terms of their chemical form (protein or nonprotein). On average, 68% and 79% of ileal dietary and endogenous N fluxes comprised protein, respectively. This proportion was up to 80% at the peak of dietary N flux through the ileum, which is far higher than previously quantified after the ingestion of pea protein (25) or heated milk (C. Bos, unpublished data), where only 43 to 57% of ileal dietary N was still in the form of protein. In the jejunum, only 5% of casein is still in a protein form 4 h after its ingestion in humans (36). Our findings confirm the hypothesis that rapeseed contains protein fractions particularly resistant to hydrolysis. It has been hypothesized that pepsin may fail to hydrolyze the highly compacted structure of RP (37). Our comparison of the electrophoretic profiles of ileal samples and the corresponding ileal dietary N flux suggested that both the metabolic fate of the dietary N absorbed, we showed that RP have a poor RID in humans (84%). This low bioavailability is compensated for by an excellent PBV (84%). Taken together, these results indicate a postprandial retention of RP of 70.5%, comparable to that of other plant proteins (18,22–24).

Postprandial metabolic utilization of RP. Ingestion of the RP isolate resulted in remarkably little deamination of dietary N (14% of the ingested dose 8 h after the meal), indicating that once absorbed, the catabolism of AA derived from the diet was minimal. This value is the lowest ever observed when studying the nutritional value of protein sources in humans; the deamination of dietary AA ranges from 16 (lupin) to 24% (wheat) (18,22–24,38).

The kinetics of digestive protein digestion and absorption have a marked impact on their metabolic utilization (12,39). The RP meal had a gastric emptying rate comparable to that of a soy protein meal and more rapid than that of a pea protein meal (22,23). The peak of dietary N appearance at the ileal site occurred 1 h 30 min after the meal, which is early compared with soy (22). Under these conditions, it does not appear that digestion kinetics of RP could explain the high rate of dietary N utilization.

It is more likely that the high rapeseed content in indispensable AA was responsible for its excellent postprandial biological value. Indeed, methionine and cysteine levels are as high as 19 and 20 mg/g of RP, respectively, which is 80% higher than the limiting value for sulfur AA (methionine + cysteine = 23 mg/g protein) (10). This content is particularly high for legume proteins, which are usually limiting or sub-limiting sources of sulfur AA. Of particular interest is the high rapeseed cysteine content and the uncommon cysteine to methionine ratio of at least 1:1, comparable to that observed in egg protein. In growing rats with high sulfur AA requirements, rapeseed is particularly appropriate as a protein source (3). RPs are thus promising, high-biological value proteins as a source of sulfur AA, which play a key role in health, notably as a precursor of glutathione (40–42).

Overall, the NPPU of rapeseed was 70.5%, a score comparable to the lower range of other legume proteins and higher than that of wheat protein (Table 3), a finding consistent with pig studies (6,43). Interestingly, a soybean diet produces lower fecal N losses but higher urinary N losses than a rapeseed diet, leading to the same overall N balance in pigs (44), a finding in close agreement with our observations in man. In rat assays, RP generated some of the highest scores for plant proteins, being similar to beef and higher than casein (3,4).

In conclusion, our study provides the first estimate of the nutritional quality of RP in humans, achieved by determining the RID (84%) and NPPU (70%) of a RP isolate given in a mixed meal.
meal to healthy subjects. Our findings show that this protein source could be of great interest to human nutrition. In particular, the high PBV of RP was remarkable, presumably due to the high levels of indispensable AA and partially sulfur AA. An improvement in rapeseed digestibility, or the use of hydrolyzed or partially hydrolyzed RP, is a development that might enhance the value of this protein source for human consumption.

Acknowledgments
We acknowledge the assistance of Dr. Rufin N’Tounda. We are indebted to Catherine Luengo for her skilful assistance with spectrometry analyses and to Sophie Daré for biochemical analyses.

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