Rapeseed protein exhibit a poor digestibility but a very high metabolic utilization in humans

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Abstract

Rapeseed protein (*Brassica napus* L.) is only used in 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 and postprandial net protein utilization in humans fed ^{15}N-rapeseed protein. Volunteers equipped with an intestinal tube at the jejunal (n=5) or ileal level (n=7) ingested a meal containing 27.3 g ^{15}N-rapeseed protein and a total energy content of 2.93 MJ. Dietary N levels were quantified in intestinal fluid, urine and blood sampled at regular intervals during the postprandial period. The real ileal digestibility of rapeseed protein was 84.0 ± 8.8%. Dietary N at the ileal level was mostly in the form of undigested protein. Both cruciferin and napin fractions appeared to be resistant to proteolysis. Endogenous ileal N losses equalled 2.2 g/d. Aminoacidemia was not significantly increased by meal ingestion. The postprandial distribution of dietary N was 5.4 ± 1.8% in urinary urea and ammonia, 8.2 ± 3.4% in body urea and 7.7 ± 2.0% in plasma protein. The net postprandial protein utilization (NPPU) of rapeseed protein amounted to 70.5 ± 9.6% and the postprandial biological value was high: 83.8 ± 4.6%. Rapeseed protein has a low real ileal digestibility in humans compared to other plant proteins, but also exhibits a very low deamination rate. Thus postprandial nitrogen retention from rapeseed is excellent in humans, being as high as that of milk protein. We conclude that rapeseed protein has a high nutritional potential for human nutrition.

Key words: protein quality, rapeseed protein, ileal digestibility, biological value, humans

Introduction

Rapeseed (*Brassica napus*) contains a high proportion of protein (~ 20%) but, to our knowledge, there is no human consumption of rapeseed protein. Rapeseed proteins 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% (*1*). In rat assays, indices for the nutritional quality of rapeseed protein were seen to be as high as those of animal protein and far higher than those of other legume or cereal sources (*2-4*). Studies of the nutritional quality of rapeseed proteins for livestock feed have shown that rapeseed protein is as efficient as soy protein in terms of animal growth and maintenance (*5*). In terms of its potential use for human nutrition, rapeseed protein is of particular interest because of its globally high content in indispensable amino acids (>400 mg/g protein) and particularly in sulfur amino acids (AAs) (40 to 49 mg/g protein) (*6, 7*). These levels are double the requirement for sulfur AA established following the last FAO/UNO/WHO consultation of experts in 2001 (*8*), and far higher than those usually found in plant, and especially, legume protein. However, protein quality does not only depend on AA composition but also on other factors related to the kinetics of AA delivery from these proteins. For instance, soluble milk proteins exhibit a high chemical score but because of the rapid supply of dietary AA from these proteins, their postprandial oxidation is enhanced when compared to proteins released at a slower rate (*9*). There has been no assessment to date of the digestibility or metabolic utilization of rapeseed protein which 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 rapeseed protein *in vivo* in humans through the combined use of intestinal tubes and intrinsically and uniformly ^{15}N-labeled rapeseed protein.

Subjects and methods

Twelve subjects (6F, 6M) volunteered for the study. They were included after undergoing a thorough medical examination and routine blood tests. The subjects had a mean age of 25 years, weighed 71 ± 12 kg and their BMI was 23.4 ± 3.0 kg.m^{-2}. Body composition was determined from isotopic dilution after the oral administration of deuterium oxide (75 mg/kg body weight): total body water was 40.6 ± 5.8 L, fat-free mass 55.2 ± 7.9 kg and percentage fat mass was 23.1 ± 9.0%. All subjects received detailed information on the protocol and gave their written informed consent to

participation in the study. The protocol was approved by the Institutional Review Board for St-Germain-en-Laye Hospital, France.

^{15}N-labeled rapeseed protein was prepared at an experimental scale by growing at the Technical Centre for Oilseed Crops (CETIOM) winter rapeseed containing very low levels of glucosinolates (*Brassica napus* L., Goëland cultivar) 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 rapeseed protein were carried out on a laboratory scale. In this cultivar, the globulin, napin and lipid transfer protein (LTP) fractions represent 36.8, 41, and 2.7% of total protein, respectively (*10*). The final N content of the rapeseed isolate was 14.9%, with ^{15}N enrichment of 1.16 atom percent (AP). The test meal consisted of 30 g of ^{15}N-labeled rapeseed protein isolate (312 mmol N or 27.3 g protein, N x 6.25), mixed with 96 g carbohydrate (75% as maltodextrin and 25% as sucrose), 23 g canola oil and water to reach a final volume of 500 mL. The total energy content of the experimental meal was 700 kcal.

The subjects were hospitalized for two days. On the first day, a 3-m PVC double-lumen tube was inserted via the nose under local anaesthesia and then swallowed so as to progress down the gastro-intestinal tract under the action of gravity and peristaltic contractions. Tube progression was verified under X-ray and the tip was halted in either the jejunum (n=5, mean tube length from the nose = 167 cm) or the ileum (n=7, mean tube length from the nose = 214 cm). The subjects were given meals at noon and 19h00 and then fasted overnight. On the second day, the protocol started at 10h00, when a saline solution containing 20 g/L polyethylene glycol 4000 (PEG-4000) was infused continuously through the first lumen of the tube. At t=0, the subjects ingested the experimental meal containing ^{15}N-rapeseed protein and 75 mg L-[1-^{13}C]-glycine 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 8h.

Plasma and/or urine urea and ammonia were assayed using enzymatic methods. The PEG-4000 concentration in digesta samples was determined using a turbidimetric method. Amino acid concentrations in deproteinized serum samples were determined by HPLC after separation on cation exchange resin and post-column ninhydrin derivatization. For isotopic determinations, urea and ammonia were isolated from urine, using an Na/K form of the cation exchange resin. Serum separation of N fractions (protein N, free N and urea N) was performed. Protein N and non protein N in the ileal samples were fractionated by ethanol precipitation after hexane delipidation.The total N, non protein N and protein N contents of the digesta and serum protein fraction were determined using an elemental nitrogen analyzer. The ^{15}N/^{14}N isotope ratio was determined by isotope-ratio mass spectrometry in the digesta, urinary urea and ammonia, serum protein, free N and urea. The ^{13}C enrichment of CO_2 in expired breath was determined using GC-IRMS. The atom percent excess (APE) of the samples was calculated by subtracting the baseline value from the atom percent determined at each time point. Rapeseed isolate protein and ileal effluents were analyzed in polyacrylamide gels in denaturing (SDS) non-reducing conditions in order to determine the nature of undigested dietary protein.

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 Inc., NC, USA). A value of $P < 0.05$ was considered as significant.

Results

Intestinal kinetics and real ileal digestibility of rapeseed protein

The flow of dietary N peaked 1h after the meal in the jejunum (14.9 ± 7.8 mmolN/30 min) and 30 min later in the ileum (8.7 ± 12.6 mmolN/30min). On average, over the 8-hour 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. The real ileal digestibility of rapeseed protein was 84.0 ± 8.8%. In subjects with the tube at the ileal site, analysis of the ileal N form (protein or non-protein) revealed constant levels of dietary non-protein throughout the postprandial period (0.1-0.3 mmolN/30 min) and high, variable amounts of dietary N in the form of protein, which accounted for more than 80% of the total ileal dietary N flux between 2 and 5h after the meal, reaching an average of 68% over the 8-h study period.As for ileal endogenous N flow, a high (mean: 79%), consistent proportion of endogenous N was made up of protein. Electrophoretic analyses were performed on the ileal contents in individual subjects and compared to the ileal dietary N flux. The profiles of the two individuals with the lowest degree of digestibility (65.2%) and highest degree of digestibility (90.5%) showed different profiles. In the first subject, peak levels of ileal dietary N (at 1.5 and 2h) in the ileum were associated with SDS-PAGE bands similar to those of the rapeseed protein (RP) isolate (at ~50 kDa and ~14 kDa, possibly due to the presence of undigested cruciferin (Cαβ) and napin (N1), respectively). These bands had a lower intensity in the subject with the highest degree of digestibility.

Kinetics of circulating glucose, dietary amino acids and dietary N-carrying protein

Plasma glucose levels rose after ingestion of the mixed meal, peaking at 8.4 ± 2.7 mmol/L at 1h. Plasma total amino acid concentrations ranged from 2975 ± 273 µmol/L at baseline to 3326 ± 761 µmol/L at 2 h (NS). There was no time effect regarding total, indispensable or dispensable AA levels after the meal.

Oxidation of the oral dose of ^{13}C-glycine and the incorporation of dietary N into the plasma amino acid N pool followed similar time-courses after the meal. ^{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. 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 3h after the meal (6.0 ± 2.0% of the pool). Dietary N incorporation into the plasma protein pool followed a sigmoid curve and reached 7.7 ± 2.0% of the N ingested, 8h postprandially.

Dietary nitrogen deamination, postprandial retention and postprandial biological values concerning rapeseed protein

The transfer of dietary N to body urea increased during the first 3h to reach a plateau with a final value of 6.7 ± 2.5% of ingested N in this pool. Low levels of ammonia were found in cumulative dietary N urinary excretion (0.23 ± 0.12% of ingested dose) but they increased regularly in urinary urea to reach 5.4 ± 1.8% of ingested N at 8h. Endogenous urea production remained steady (0.4 to 0.5 mmolN/kg body weight/2h) throughout the postprandial period and highly variable between subjects (Table 2). Urea production from dietary AA was at its maximum for the first two hours following the meal but almost insignificant during the last four hours. Total endogenous and dietary urea production amounted to 1.88 ± 1.41 and 0.47 ± 0.13 mmolN/kgBW over the entire postprandial period. The sum of ileal and deamination losses, representing the amount of dietary N not retained 8h after the meal, was 29.5 ± 9.6% of the meal content. As a result, the NPPU value was 70.5 ± 9.6%. The postprandial biological value, representing the retention of absorbed N, reached 83.8 ± 4.6%.

Discussion

This work constitutes the first determination of the nutritional value of rapeseed protein in humans. Using an intestinal tube to quantify ileal N flow rates and ^{15}N-labeled protein to specifically measure the metabolic fate of the dietary N absorbed, we showed that rapeseed proteins present a poor real ileal digestibility in humans (84%). This low bioavailability is compensated for by an excellent postprandial biological value (84%), higher than that of milk protein, indicating that the proportion of rapeseed derived-AA absorbed were only catabolized to a very limited extent. Taken together, these results indicate a postprandial retention of rapeseed protein of 70.5%, comparable to that of other plant proteins.

The real ileal digestibility (RID) of the rapeseed protein isolate reached 84%, a low value when compared to the RID of other plant proteins measured using the same methodology, which are all in the range of 89-91% (*11-15*), and to that of milk protein (95%) (*16, 17*) or egg protein using ^{13}C-protein (94%) (*18*). The RID of rapeseed was associated with broader variations between subjects than the aforementioned protein sources. This was due in particular to one subject with an extremely low RID of 65%.

Particular care was taken over preparation of the rapeseed protein isolate to avoid any drastic heat or alkaline treatment, thus the low RID measured could not be linked to any drastic technological treatment. In fact, our results agreed with pig data showing the lower apparent fecal digestibility of rapeseed protein than soy, and the lower true or real ileal digestibility (80-88%) of rapeseed protein than other plant proteins such as gluten, soy or pea (*7, 19-23*). Rapeseed protein has also demonstrated its poor digestibility in poultry (*24*), but not in rats (*3*). It is noteworthy that most of the animal studies used non-dehulled, rich in lignin rapeseed, which could partly explain the low level of digestibility observed. However, in dehulled rapeseed, proteins were still less digestible than soy protein (*25*). Thus, the low digestibility reported here probably resulted from the presence of hydrolysis-resistant sequences in the rapeseed proteins, the nature of which is unknown.

Our findings confirm the hypothesis that rapeseed contains protein fractions particularly resistant to hydrolysis. Based on the lower *in vitro* digestion of rapeseed protein vs. casein, it was hypothesized that pepsin may be less efficient on the highly compacted structure of rapeseed protein (*26*). Our comparison of the electrophoretic profiles of ileal samples and the corresponding ileal dietary N flux suggested that both the cruciferin fraction (α or β subunits, or their assembly into dimers) and napin were resistant to proteolysis. However, it was difficult to interpret some bands because rapeseed protein and endogenous protein, such as secretory IgG or pancreatic proteases have the same molecular weight. Our results therefore need to be confirmed using an accurate, quantitative method to determine the nature of indigestible dietary protein fractions in the ileum.

As a general rule, the globulin fraction of legume protein is seen to be more digestible than the albumin fraction (*14, 27-29*). Our results indicate that the difference is probably less marked for rapeseed protein, the 12S and 2S fractions of which may be resistant to digestion because of their very compact tertiary structure containing several disulfide bridges. An improvement in rapeseed protein digestibility may be achieved by heating to increase protein denaturation and its susceptibility to proteases, although this has not proved to be efficient in pigs (*25*).

Ingestion of the rapeseed protein isolate resulted in remarkably little deamination of dietary N, 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) (*12-15, 30*). The lack of plasma AA increase and the moderate incorporation of dietary N into both plasma AA and plasma protein after the rapeseed meal were consistent with the low deamination rate, as previously observed with milk protein compared to soy protein (*31*).

From our results, it does not appear that digestion kinetics of rapeseed protein 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 rapeseed protein, respectively, which is 80% higher than the limiting value for sulfur AA (met + cys = 22 mg/g protein) (*8*). 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 cys:met ratio of at least 1:1, comparable to that observed in egg protein. In growing rats, with high sulfur AA requirements, rapeseed is consistently particularly appropriate as a protein source (*2*). Rapeseed proteins

are thus promising, high-biological value proteins as a source of sulfur AA, which play a key-role for health, and notably cysteine as a precursor of glutathione (*32-34*).

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, a finding consistent with studies in the pig where rapeseed and soybean diets produce the same range of N retention (*5*) and exhibit no differences in terms of weight gain and lean carcass (*35*). 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 (*36*), a finding in close agreement with our observations in man. In rat assays, rapeseed protein generated some of the highest scores for plant proteins, being similar to beef and higher than casein (*2, 3*).

In conclusion, our study provides the first estimate of the nutritional quality of rapeseed protein in humans, achieved by determining the real ileal digestibility (84%) and NPPU (70%) of a rapeseed protein isolate given in a mixed meal to healthy subjects. Our findings show that this protein source could be of great interest to human nutrition. In particular, the high postprandial biological value of rapeseed protein was remarkable, presumably due to the high levels of indispensable AA and particularly sulfur AA, which exceeded those of all other legume proteins. An improvement in rapeseed digestibility, or the use of hydrolyzed or partially hydrolyzed rapeseed protein, are developments which might enhance the value of this protein source for human consumption.

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