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## Original Research Article

Digestive and metabolic bioavailability in healthy humans of  $^{15}\text{N}$ -labeled rapeseed and flaxseed protein incorporated in biscuitsRomain Tessier<sup>1</sup>, Juliane Calvez<sup>1</sup>, Gheorghe Airinei<sup>1</sup>, Nadezda Khodorova<sup>1</sup>, Sylvie Dauguet<sup>2</sup>, Olivier Galet<sup>3</sup>, Martin Chapelais<sup>1</sup>, Daniel Tomé<sup>1</sup>, Robert Benamouzig<sup>1</sup>, Claire Gaudichon<sup>1,\*</sup><sup>1</sup> Université Paris-Saclay, AgroParisTech, INRAE, UMR PNCA, Palaiseau, France; <sup>2</sup> Terres Inovia, Pessac, France; <sup>3</sup> Avril, Paris, France

## A B S T R A C T

**Background:** In the search to diversify protein sources for humans, oilseeds are good candidates due to the high protein content of their coproducts after oil extraction. Among them, rapeseed presents a well-balanced amino acid (AA) profile. Flaxseed is an emerging source but the nutritional value of its protein is not yet documented.

**Objectives:** This study aimed to determine the nitrogen (N) and AA bioavailability of these protein sources.

**Methods:** Nineteen healthy volunteers were intubated with a naso-ileal tube. They ingested 156 g biscuits containing intrinsically labeled  $^{15}\text{N}$  rapeseed ( $n = 10$ ) or flaxseed ( $n = 9$ ) protein over a 4-h period. Ileal digesta, blood, and urine were sampled over 8 h after the first meal ingestion. N and  $^{15}\text{N}$  enrichment and AAs were measured to determine digestive and deamination losses. Ileal digestibility, the digestible indispensable AA score (DIAAS) and net postprandial protein utilization (NPPU) were calculated.

**Results:** Real ileal digestibility was  $80.7 \pm 6.5\%$  for rapeseed protein and  $92.2 \pm 2.0\%$  for flaxseed protein ( $P = 0.0002$ ). Mean indispensable AA (IAA) digestibility reached  $84.1 \pm 6.9\%$  and  $93.3 \pm 6.7\%$  for rapeseed and flaxseed, respectively, lysine being the lowest digestible IAA for both sources. Despite moderate digestibility, the DIAAS was 1.1 for rapeseed but only 0.6 for flaxseed due to lysine insufficiency. Deamination losses accounted for  $20.0 \pm 6.5\%$  of dietary N for flaxseed and  $11.0 \pm 2.8\%$  for rapeseed ( $P = 0.002$ ). The NPPU did not differ between the protein sources, with  $71.3 \pm 6.5\%$  for flaxseed and  $69.7 \pm 7.6\%$  for rapeseed.

**Conclusions:** Despite good digestibility, flaxseed protein cooked in biscuits was penalized by both lysine insufficiency and poor lysine digestibility that decreased its DIAAS and increased deamination. By contrast, rapeseed was moderately digestible but presented no limiting IAA, resulting in an excellent DIAAS and low deamination.

This study was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT04024605.

**Keywords:** stable isotopes, ileal digestibility, intestinal tubes, oilseed protein, digestible indispensable amino acid score, humans

## Introduction

In view of the diversification of plant-based protein foods, the protein quality of these sources needs to be clarified. Oilseeds are good candidates as their oil coproduct, oilcake, is rich in protein. Sunflower and rapeseed represent a significant reservoir of protein as they are among the 10 major crops in France and Europe [1]; in 2021, 1911 Mt of sunflower and 3286 Mt of rapeseed were produced in France [2]. Although a minor crop, flaxseed has been the subject of increased

interest regarding the nutritional quality of its oil, but its protein quality has not been assessed.

We recently determined the ileal digestibility of amino acid (AA) from a sunflower isolate incorporated in a biscuit and found a mean digestibility of indispensable amino acid (IAA) that reached 89% [3]. In a previous study, we had also determined that the ileal nitrogen (N) digestibility of rapeseed protein isolate was only 84% [4], whereas it was around 90%–91% for soy [5] and pea isolates [6] as well as gluten [7]. During these studies, the digestibility of individual AAs was not

*Abbreviations used:* AA, amino acid; Asx, aspartate and asparagine; AP, atom percent; CS, chemical score; DAA, dispensable amino acid; DIAAS, digestible indispensable AA score; DM, dry matter; Glx, glutamate and glutamine; IAA, indispensable amino acid; IRMS, isotope ratio mass spectrometer; N, nitrogen; NPPU, net postprandial utilization; PEG, polyethylene glycol.

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determined. Since the FAO recommended in 2013 that the digestible indispensable AA score (DIAAS) should be the gold standard to assess the capacity of protein to satisfy AA requirements [8], the measurement of AA digestibility has become necessary. However, its determination faces certain methodological barriers. The ileal digestibility of several protein isolates from milk, soy, corn, and sunflower has been measured in humans using a naso-ileal tube to collect digesta [3,9–11].

The aim of our study was to generate data on the ileal digestibility of AAs, the DIAAS, and the metabolic bioavailability of dietary N in 2 oilseed sources: rapeseed, which is known to have moderate protein digestibility but a good biological value [4], and flaxseed, where the quality of its protein has not yet been documented. For this purpose, the proteins were labeled intrinsically with  $^{15}\text{N}$  and extracted from the seeds. They were then incorporated in biscuits that were given as a  $^{15}\text{N}$  test meal to healthy volunteers equipped with a naso-ileal tube.

## Methods

### Subjects

This study formed part of the PRODIGE (Optimized PROcess for sustainable production of highly DIGESTible oilseed proteins for humans) project and was conducted at the Human Nutrition Research Center in Avicenne Hospital (AP-HP, Bobigny). Recruitment started in July 2020 and was completed in May 2021. The study was approved by the Sud Méditerranée IV Ethics Committee and the French Agency for Medicinal and Health Products Safety. It was registered in the [www.clinicaltrials.gov](http://www.clinicaltrials.gov) database (NCT04024605).

The number of volunteers was determined in accordance with previous studies on the AA digestibility of protein sources [9,10,12]. These studies were performed on 6 to 10 volunteers per group. Based on the data on rapeseed protein digestibility [4] and that of other plant protein isolates studied in humans [6,7,9], a difference of  $6 \pm 4\%$  of N digestibility could be expected. In view of the between subject design (2-tailed unpaired Student's *t* test), risks at  $\alpha = 5\%$ , and  $(1 - \beta) = 80\%$ , the sample size thus determined was 9 per group (G\*Power 3.1).

The eligibility criteria were: BMI between 18 and  $30 \text{ kg/m}^2$ , age between 18 and 65 y, negative serology for human immunodeficiency virus, hepatitis C virus antibody, hepatitis B virus surface antigen, and an absence of dietary allergies and digestive disease. All volunteers signed an informed consent form. Thirty-four volunteers were recruited, 13 of whom did not complete the protocol. The causes of these failures included an impossibility to introduce the tube into the nose ( $n = 1$ ), poor tolerance of the tube ( $n = 5$ ), nonmigration of the tube through the pylorus ( $n = 4$ ), and insufficient migration in the small intestine ( $n = 3$ ). Twenty-one volunteers were thus allocated to the rapeseed and flaxseed groups. Randomization was not possible because the flaxseed and rapeseed biscuits could not be produced at the same time. Two batches of each biscuit were produced within a short interval of around a month, thus permitting overlapping of the enrollment of volunteers in each group. One volunteer who completed the protocol had to be excluded because the tube was not positioned in the terminal ileum and another because the tube had migrated in the colon (Supplemental Figure 1).

The final sample size was therefore  $n = 10$  for rapeseed and  $n = 9$  for flaxseed. The characteristics of the subjects were similar in the 2 groups (Table 1).

### Test meals

Rapeseed and flaxseed were labeled by Terres Inovia using  $^{15}\text{N}$  ammonium nitrate enriched at 10% during growth, as detailed in

**TABLE 1**  
Anthropometric characteristics of the subjects<sup>1</sup>

Characteristic	Rapeseed	Flaxseed
Sex (F/M)	4/6	3/6
Weight (kg)	70 $\pm$ 12	68 $\pm$ 10
Height (cm)	172 $\pm$ 12	169 $\pm$ 5
BMI ( $\text{kg/m}^2$ )	23.5 $\pm$ 3	23.7 $\pm$ 3
Age (y)	34 $\pm$ 11	36 $\pm$ 12

<sup>1</sup> Values are means  $\pm$  SDs,  $n = 19$ .

Supplemental Table 1. The seeds were hulled and de-oiled (Olead).  $^{15}\text{N}$  protein was extracted and purified according to the processes described in Supplemental Figure 2. A rapeseed isolate could not be produced because of the small quantity of harvested grains. The protein content ( $\text{N} \times 6.25$ ) in the  $^{15}\text{N}$  labeled protein fractions was 50% for the rapeseed concentrate and 80% for the flaxseed isolate. The enrichment of rapeseed and flaxseed protein was 0.73 atom percent (AP) and 0.51 AP, respectively. The protein fractions were incorporated in the biscuits that were cooked at  $180^\circ\text{C}$  for 21 min (Foodinnov), with a target protein concentration of 15%–16% dry matter (DM). The wheat flour contained a small quantity of gluten (2.8% of the biscuit) (Supplemental Table 2).

The volunteers ingested 156 g of biscuits, divided into 9 portions. The first portion contained 4 biscuits and the other 8 contained 1 biscuit. The protein intake was 18 g and 20 g for rapeseed and flaxseed biscuits, respectively. Alongside the biscuits, the subjects ingested a total of 100 g apple puree, in accordance with our study on sunflower [3]. Overall, energy supply was 670 kcal (rapeseed) to 720 kcal (flaxseed).

Rapeseed protein contained 39% IAA and flaxseed 34%. Rapeseed contained twice as much lysine than flaxseed (Supplemental Table 3).

### Clinical design

The day before the experiment, the subjects were equipped with an intestinal tube as previously described [3,10,13]. The protocol design is presented in Figure 1. After fasting overnight, a solution of polyethylene glycol-4000 (PEG, 20 g/L), a nonabsorbable marker of liquid flow rate, was infused via the intestinal tube at 1 mL/min. Ileal effluent, blood, and urine were collected at baseline. The first meal portion was given to the volunteers, followed by a portion every half hour for 4 h. Volunteers were not informed on the type of protein they had to ingest (single-blind). The digestive contents were collected continuously and pooled by half hour for 8 h after the first meal. Blood was sampled every 30 min for 4 h and then every hour. All urine was collected and pooled by 2-h periods.

### Analytical methods

The concentration of PEG in the digesta was measured using the turbidimetric method [14] to determine the ileal flow rate. Urea concentrations in plasma and urine were measured by means of the Urease-Berthelot method (Urea assay, Randox).

Rapeseed and flaxseed proteins, together with the digestive contents, were hydrolyzed for 24 h with HCl 6N at  $110^\circ\text{C}$  with norvaline as an internal standard. For methionine and cysteine, a performic acid oxidation was done before acid hydrolysis. For tryptophan, proteins were hydrolyzed for 20 h at  $110^\circ\text{C}$  in the presence of barium hydroxide 2N, with 5-methyl-tryptophan as internal standards.

For AA quantification, calibration standards were composed of a mixture of AA (Waters) to which norvaline was added. AA and

standards were derivatized using the AccQ-Tag Ultra Derivatization kit (Waters) according to the manufacturer's protocol. The AA analysis was performed on an Acquity H-class Ultra-HPLC system with a photo diode array detector (Waters), as described previously [11].

Before measuring the  $^{15}\text{N}$  enrichments, AA from digesta and meals were isolated using a hydrogen form of ion exchange resin and derivatized with ethyl chloroformate, as previously described [15].  $^{15}\text{N}$  enrichments were analyzed by gas chromatography (GC 6890N, Agilent Technologies) in constant flow mode, coupled to an isotope ratio mass spectrometer (IRMS) (Isoprime GV Instrument) via the GC5 Isoprime interface [11].

Urinary urea was isolated on a sodium form of cation exchange resin after the isolation of urinary ammonia and treatment with urease. Plasma was split into 3 fractions: proteins, free AAs, and urea, as described previously [7]. Resin-containing urea was eluted with  $\text{KHSO}_4$  (2.5M).

$^{15}\text{N}$  recovery in the digestive contents, urea pools, plasma free AA, biscuits, and protein was measured using an elemental analyzer (Vario Micro Cube, Elementar) coupled with an IRMS (Isoprime, GV Instrument). Atropine (Thermo Electron) and L-glutamic acid (USGS41, Sigma Aldrich) were used as elemental and isotopic standards, respectively.

N, AA digestibility, and DIAAS were the primary outcomes, and the net postprandial protein utilization (NPPU) was the secondary outcome.

## Calculations

The ileal flow rate was evaluated for each period of 30 min (F, mL/30 min):

$$F = \frac{[\text{PEG}]_{\text{solution}}}{[\text{PEG}]_{\text{digesta}}} \times \text{perfusion flow rate} \times 30$$

where [PEG] is the concentration of PEG in the infused solution (1 mL/min) and in the digestive contents.

The total N flow rate ( $N_{\text{tot ileum}}$ , mmol/30 min) in the ileum was assessed for each period:

$$N_{\text{tot ileum}} = \frac{N_s \times DM_s \times F}{14 \times 10}$$

where  $N_s$  is the percentage of N,  $DM_s$  is the DM of the ileal sample (g/100 g), F is the ileal flow rate (mL/30 min), and 14 is the molar mass of N (g/mol).

The dietary N flow rate ( $N_{\text{diet ileum}}$ , mmol/30 min) was calculated as follows:

$$N_{\text{diet ileum}} = N_{\text{tot ileum}} \times \frac{\text{APE}_{\text{ileum}}}{\text{APE}_{\text{meal}}}$$

with APE the enrichment excess compared to  $^{15}\text{N}$  basal enrichment that was measured at  $t = 0$  in each volunteer.

Endogenous N ( $N_{\text{endo ileum}}$  in mmol/30 min) was the difference between total and dietary N recovered in the ileum.

The real ileal digestibility (RID, % of ingested N) was:

$$\text{RID} = \frac{N_{\text{ingested}} - \sum N_{\text{diet ileum}}}{N_{\text{ingested}}} \times 100$$

With  $N_{\text{ingested}}$  the amount of N ingested by the subjects (mmol) and  $\sum N_{\text{diet ileum}}$  the cumulative dietary N recovered in the ileum over 8 h (mmol).

The quantity of dietary AA in digesta ( $\text{AA}_{\text{diet } i}$ , mmol) at each period was:

$$\text{AA}_{\text{diet } i}(t) = [\text{AA}]_{\text{ileum } i}(t) \times \text{DM}(t) \times F(t) \times \frac{\text{APE}_{\text{ileum } i}(t)}{\text{APE}_{\text{protein}}}$$

where  $[\text{AA}]_{\text{ileum } i}(t)$  is the quantity of each AA "i" in the digestive contents at each period "t" (mmol/g), DM the dry matter (g/100 mL), F the ileal flow rate (mL/30 min), APE the  $^{15}\text{N}$  excess enrichment of each AA "i" at each period "t," and  $\text{APE}_{\text{protein}}$  that of rapeseed or flaxseed protein. Basal abundance was measured at  $t = 0$  in each volunteer.

The chemical score (CS) was defined for each IAA:

$$\text{CS} = \frac{\text{mass of dietary IAA}_i \text{ in 1g of dietary protein}}{\text{mass of the same dietary IAA}_i \text{ in 1g of reference protein}}$$

The reference protein profile was the recommended AA scoring pattern for older children, adolescents, and adults [8].

The digestible IAA ratio (DIAA ratio), was calculated for IAA:

$$\text{DIAA ratio} = \text{CS}_i \times \text{AA ileal digestibility}_i$$

The DIAAS was the smallest DIAA ratio calculated. The protein mass was calculated using  $N \times 6.25$  factor, or a more specific factor recommended for plants, which is 5.6 [16].

The dietary N incorporation in plasma proteins, body, and urinary urea pools was calculated using the same dilution principle as for digesta. Plasma protein and body urea pools were calculated as described previously [7].

The NPPU (% of ingested N) was the amount of N retained in the body after 8 h. It corresponded to the N ingested minus metabolic and digestive losses:

$$\text{NPPU} = \frac{N_{\text{ingested}} - (\sum N_{\text{diet ileum}} + \sum N_{\text{diet urinary urea}} + N_{\text{diet body urea}})}{N_{\text{ingested}}} \times 100$$

with  $N_{\text{diet body urea}}$  the remaining dietary N in body urea at 8 h.

## Statistical analysis

Data are expressed as means  $\pm$  SD. Differences between the groups were estimated using an unpaired Student's *t* test after verification that normal distribution of data was not rejected. Kinetic data were analyzed in a mixed model with the group as a fixed factor and time as a repeated factor (SAS 9.4 Software, SAS Institutes). Differences between groups at each time point were determined by contrast analyses when the time  $\times$  group interaction was significant. Differences were considered to be statistically significant for a *P* value lower than 0.05.

## Results

### Digestive flow rates and ileal digestibility

The liquid flow rate (Figure 2A) was similar in the 2 groups during the first 2 h. It increased in the rapeseed group at 3 h but remained stable in the flaxseed group. This flow rate averaged  $139 \pm 61$  mL/h with flaxseed ingestion and  $166 \pm 51$  mL/h in the rapeseed group.

Dietary N (Figure 2B) appeared in the ileum 1 h after ingestion of the first meal, the peak being observed at 5 h in the rapeseed group and with a plateau after 2 h for flaxseed. The amount of dietary N was significantly higher in the rapeseed group than in the flaxseed group from 2.5 h to 7.5 h after the meal. The real ileal protein digestibility of flaxseed protein was therefore  $92.2 \pm 2.0\%$ , but it was only  $80.7 \pm 6.5\%$  for rapeseed ( $P = 0.0002$ ).

The endogenous N ileal flow (Figure 2C) was  $\sim 6.5$  mmol/h in both groups and did not display any marked variations during the postprandial window nor did it differ between the groups.

### Ileal AA digestibility and the DIAAS

Ileal AA digestibility values of rapeseed and flaxseed protein were determined (Table 2). In the flaxseed group, values ranged from 78.2 ± 6.2% for lysine to 96.5 ± 3.3% for methionine. In the rapeseed group, lysine was also the lowest (75.3 ± 10.1%) and methionine the highest digestible AA (92.6 ± 3.6%). For all AAs except leucine and lysine, digestibility was higher with flaxseed than with rapeseed.

Chemical and DIAA ratios were calculated using either the default N-to-protein conversion factor of 6.25 or a specific factor for plant protein of 5.6 [16] (Table 3). Flaxseed was limited in lysine, with a CS ranging from 0.7 to 0.8 depending on the conversion factor. For rapeseed, the CS was higher than 1 whatever the conversion factor. The DIAAS of rapeseed ranged from 1.1 at its lowest (N × 6.25) to 1.2 (N × 5.6), whereas it was only 0.6 to 0.7 for flaxseed, due to lysine insufficiency.

### Deamination losses of dietary N and NPPU

Digestive and deamination losses of dietary N are shown in Figure 3. The transfer of dietary N to urinary urea during the 8 h after the rapeseed meal was 3.4 ± 1.0% of ingested N, whereas it reached 6.4 ± 2.9% in the flaxseed group (P = 0.01). Similarly, the amount of dietary N present in the body urea pool at 8 h was almost doubled in the flaxseed group when compared to rapeseed (P = 0.02). Consequently, total deamination losses were 11.0 ± 2.8% in the rapeseed group but reached 20.0 ± 6.5% in the flaxseed group (P = 0.002). However, this difference was balanced by higher digestive losses reported in the rapeseed group; as a result, NPPU did not differ between the groups (69.8 ± 7.6% for rapeseed compared with 71.5 ± 6.5% for flaxseed).

### Discussion

This study aimed to evaluate the nutritional quality of protein fractions from rapeseed and flaxseed. Plant proteins were labeled intrinsically with <sup>15</sup>N before being purified and incorporated into biscuits. We determined precisely the ileal digestibility of protein and AAs and subsequently the DIAAS, as well as N retention in healthy humans.

### Ileal losses

To our knowledge, our work is the first to have measured the protein digestibility of flaxseed protein isolate, which was good (higher than 92%). Few data are available in the literature regarding the protein quality of flaxseed. When given as a linseed meal to pigs, the standardized ileal digestibility was 76%, whereas it was 72% for a canola meal [17]. Similar values were obtained in rats when flaxseed was given as cereal bars or in an extruded form [18]. The higher

digestibility we obtained could be ascribed to the fact that flaxseed was given as an isolate. This value of 92% was therefore similar to other protein isolates we have assessed using the same methodology, namely soy, pea, and gluten [5–7,11]. By contrast, we had previously found lower protein digestibility values for sunflower (87%) and rapeseed (84%) protein isolates [3,4]. The moderate digestibility of rapeseed is known to be due to its albumin fractions, and particularly napin, which is rich in cysteine and presents some disulfide bonds [19]. The value we determined during the present study was 3% lower than that previously obtained [4]. This result was not surprising because the rapeseed protein fraction we tested was a concentrate rather than an isolate. Further, it was cooked in a biscuit whereas during the study by Bos et al. [4], it had been incorporated in a liquid meal. The degree of purification of plant protein has been identified as a favorable factor in terms of its digestibility because of the removal of antinutritional factors such as the phenolic compounds and phytates that are present in rapeseed [20].

The digestibility of individual AAs was found to be similar to that determined for global protein in both oilseeds, with good homogeneity of AA digestibility values, except for lysine. Indeed, the digestibility of lysine was markedly lower than that of other AAs, as well as leucine in flaxseed only. A lower digestibility of lysine has already been reported in pigs with values 8% to 11% lower than that of mean AA digestibility for flaxseed and canola meal, respectively [17]. However, such a low lysine digestibility of oilseed meals in pigs has not been observed by others [21]. In our study, low lysine digestibility could partly be explained by the fact that the protein was cooked in biscuits, together with sugars, thus altering the lysine through Maillard reactions. Moreover, cooking with gluten-containing products can create aggregates. In particular, methylglyoxal, a product of the Maillard reaction, may react with glutenin, leading to a 30% reduction in *in vitro* digestibility [22]. The loss of lysine digestibility was especially high for flaxseed, the digestibility of which was 20% lower than that of other AAs.

### DIAAS

The determination of AA digestibility enabled us to calculate the DIAAS. Using the default factor for N-to-protein conversion, 6.25, the DIAAS of flaxseed was only 0.6 due to lysine limitation. Although this factor has been acknowledged to be overestimated whatever the protein source, especially from plants [23,24], it is employed universally, and particularly for regulatory and commercial purposes. When using a more specific factor for plant proteins (5.6) [16], the DIAAS of flaxseed rose to 0.7. Rapeseed had no limiting IAA and a DIAAS that revealed a 10% to 23% excess of lysine, depending on the conversion factor.

The AA profile of rapeseed was sufficiently balanced, with an excess of IAAs of ≥42% regarding the CS, so was not penalized by its

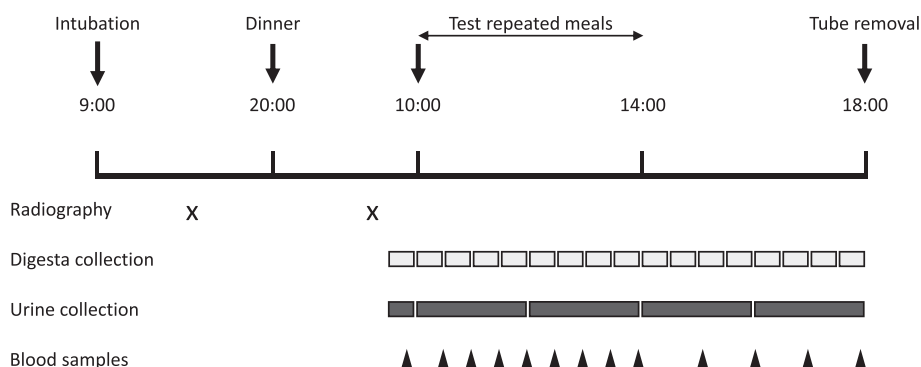
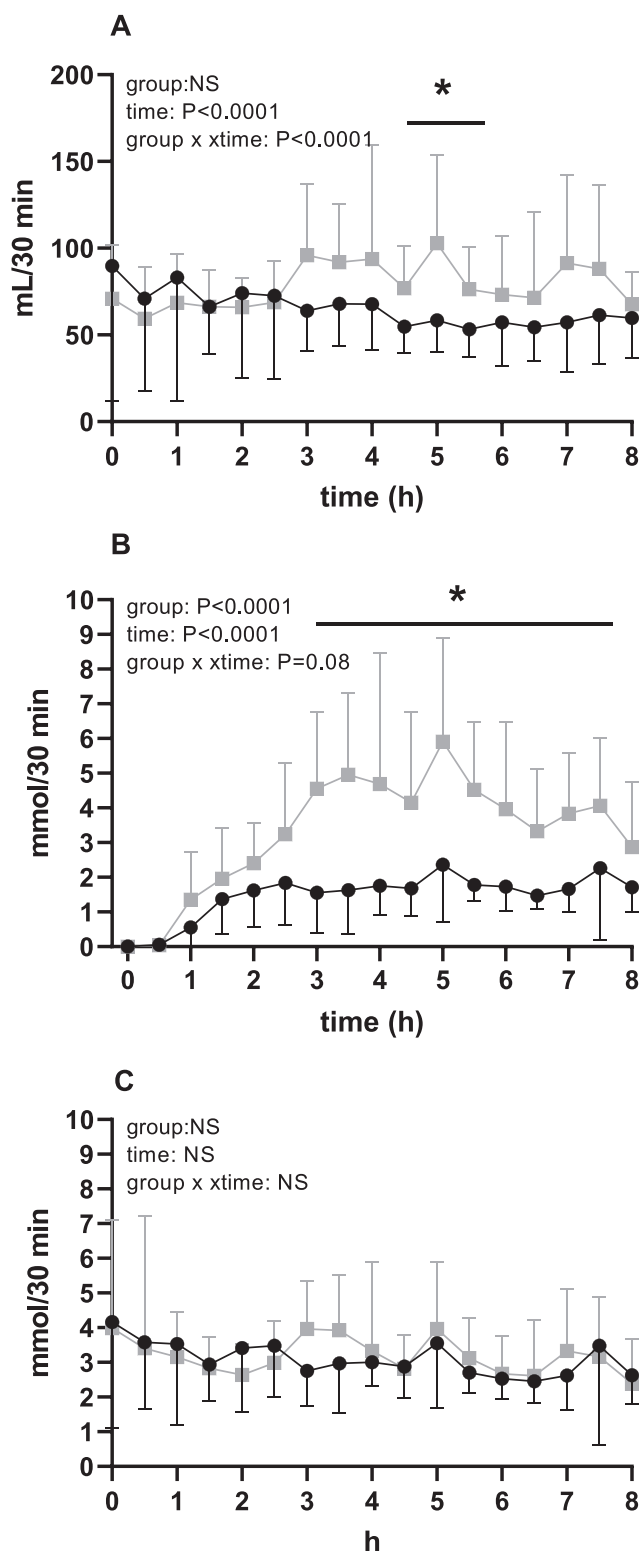


FIGURE 1. Experimental design of the study.



**FIGURE 2.** (A) Liquid ileal flow rate. (B) Dietary N flow in the ileum. (C) Endogenous N flow in the ileum. Values are means  $\pm$  SDs.  $n = 9$  in flaxseed group (black circle) and  $n = 10$  in rapeseed group (gray square). The data were analyzed using a mixed model with repeated measurements (unstructured matrix for the liquid low rate and Toeplitz matrix for dietary and endogenous N). \* indicates a significant difference between groups at a particular time point ( $P < 0.05$ ).

moderate digestibility. By contrast, the low lysine digestibility enhanced lysine deficiency in flaxseed. It would have been interesting to assess the digestibility of the raw flaxseed isolate in order to determine whether the heating process was responsible for this particular effect on lysine and thus lowered the score. As total digestible lysine can be higher than digestible reactive lysine [25], the analysis of reactive lysine would have helped to refine the results, but this would require developing a specific method for gas chromatography combustion IRMS analysis.

### Deamination losses

We found a marked effect for the protein, insofar as  $^{15}\text{N}$  transfer was almost doubled in the urine and residual body urea pool at 8 h in the flaxseed group compared to rapeseed. This resulted in a total deamination rate of around 15% for rapeseed compared with 26% for flaxseed. The low deamination rate observed for rapeseed was similar to what we had observed previously [4], where we found a postprandial transfer of 6% in urine and a similar amount in residual body urea. This low deamination rate could be ascribed to the adequate AA profile of rapeseed, with no limiting AA. By contrast, the high deamination rate of flaxseed could be explained by the low lysine content. Consistent with this, we had reported a deamination rate of 27% for wheat [7] in which lysine was limiting to a concentration of 50% below the requirement. In this latter study, gluten had been incorporated in French biscuits that had been toasted, as during the present study.

Finally, the postprandial retention of both proteins was similar, the high deamination losses of flaxseed being compensated for by low digestive losses, whereas the picture was the reverse for rapeseed.

### Strengths and limitations of the study

Our study was the first to assess the postprandial quality of flaxseed protein and determine the DIAAS of rapeseed and flaxseed in human volunteers. The labeling of plants with  $^{15}\text{N}$  offered a particularly interesting tool because in addition to digestibility and the DIAAS score, it also provides data on N retention. It was thus possible to obtain a complete overview of protein AA and N bioavailability. However, plant labeling in the field involves a degree of risk and in our case, the rapeseed crop yield was not sufficiently good to enable a complete purification of protein, unlike flaxseed. However, this work did reveal that even with a lower degree of purification, rapeseed concentrate was sufficient to meet human AA requirements and even to compensate for lysine deficiency from other sources. In addition,  $^{15}\text{N}$  recycling in the intestine can result in a slight underestimation of digestibility of around 1.5% [26]. In contrast, the repeated meal protocol of this study could have led to an overestimation of digestibility and NPPU. Nevertheless, this is neither supported by our former study, where rapeseed was given as a bolus [4], nor by results obtained for casein and pea given as bolus [6,27] or repeated meals [11]. Lastly, the lack of true randomization could also be considered as a bias [28], but this was limited by the fact that both groups displayed homogenous basal variables and were studied at almost the same time and during successive periods.

In conclusion, we found that flaxseed and rapeseed displayed similar postprandial protein retention, with high levels of digestive losses for rapeseed and of the deamination rate for flaxseed, the latter being associated with the quantity of digestible lysine. Interestingly, despite its moderate AA digestibility, and particularly for lysine, no

**TABLE 2**  
Ileal digestibility of AA from flaxseed and rapeseed protein cooked in biscuits<sup>1</sup>

Amino Acid	Flaxseed	Rapeseed	<i>P</i> <sup>2</sup>
IAA digestibility (%)			
Histidine	93.8 ± 3.4	89.5 ± 5.3	0.03
Isoleucine	96.2 ± 1.9	83.6 ± 5.3	0.0007
Leucine	87.2 ± 1.4	89.0 ± 6.1	0.002
Lysine	78.2 ± 6.2	75.3 ± 10.1	0.3
Methionine	96.5 ± 3.3	92.6 ± 3.6	0.002
Phenylalanine	96.2 ± 2.4	85.2 ± 8.7	0.002
Threonine	93.4 ± 3.4	84.2 ± 7.2	0.002
Valine	95.2 ± 2.5	83.9 ± 8.1	0.001
Mean IAA	<b>93.3 ± 6.7</b>	<b>84.1 ± 6.9</b>	<b>0.0009</b>
DAA digestibility (%)			
Alanine	95.1 ± 2.3	88.5 ± 6.2	0.005
Asx	92.3 ± 4.1	82.4 ± 8.7	0.002
Glycine	91.0 ± 4.0	77.4 ± 10.4	0.003
Glx	95.0 ± 3.4	87.7 ± 7.3	0.007
Proline	92.6 ± 4.0	77.8 ± 9.1	0.0003
Serine	94.7 ± 2.7	82.4 ± 8.9	0.001
Tyrosine	94.8 ± 3.3	87.2 ± 6.3	0.002
Mean DAA	<b>93.6 ± 2.5</b>	<b>82.7 ± 8.1</b>	<b>0.002</b>
Mean of all AA <sup>3</sup>	<b>93.8 ± 3.1</b>	<b>83.9 ± 7.3</b>	<b>0.0008</b>

AA, amino acid; Asx, aspartate and asparagine; DAA, dispensable amino acid; Glx, glutamate and glutamine; IAA, indispensable amino acid.

<sup>1</sup> Values are means ± SDs. *n* = 9 in the flaxseed group and *n* = 10 in the rapeseed group. The digestibility of tryptophan, arginine, and cysteine was not determined.

<sup>2</sup> Differences between groups were estimated using an unpaired Student's *t* test. Considering the number of tests for AA digestibility (*n* = 15), the overall concentration to conclude to a significant difference for each AA was 0.05/15 = 0.0033 (Bonferroni correction). *P* values above this threshold are indicated in italics.

<sup>3</sup> Means of AA digestibility are weighted by the proportion of each AA in the protein.

limiting AAs were revealed in rapeseed by a DIAAS higher than 1, in contrast to flaxseed.

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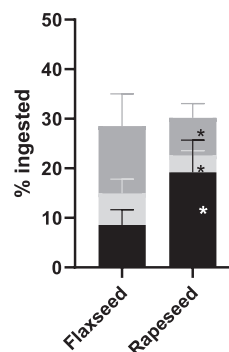
**TABLE 3**  
Chemical and DIAAS for flaxseed and rapeseed protein cooked in biscuits and calculated using 2 different nitrogen-to-protein conversion factors<sup>1</sup>

Amino Acid	Chemical ratio				DIAAS ratio			
	N × 5.6		N × 6.25		N × 5.6		N × 6.25	
	Flaxseed	Rapeseed	Flaxseed	Rapeseed	Flaxseed	Rapeseed	Flaxseed	Rapeseed
Histidine	1.8	2.3	1.6	2.1	1.7	2.1	1.5	1.9
Isoleucine	2.0	1.8	1.8	1.6	2.0	1.5	1.8	1.4
Leucine	1.3	1.6	1.1	1.2	1.2	1.4	1.1	1.3
Lysine	0.8	1.6	0.7	1.5	0.7	1.2	0.6	1.1
SAA	1.7	3.0	1.6	2.7	1.7	2.8	1.5	2.5
AAA	2.5	2.3	2.3	2.0	2.4	1.9	2.2	1.7
Threonine	1.8	2.3	1.6	2.0	1.7	1.9	1.5	1.7
Tryptophan	3.5	3.1	3.1	2.8	—	—	—	—
Valine	1.8	1.7	1.6	1.5	1.7	1.4	1.5	1.3
SCORE <sup>2</sup>	<b>0.8 (Lys)</b>	<b>1.6 (Leu)</b>	<b>0.7 (Lys)</b>	<b>1.2 (Leu)</b>	<b>0.7 (Lys)</b>	<b>1.26 (Lys)</b>	<b>0.6 (Lys)</b>	<b>1.1 (Lys)</b>

AAA, aromatic amino acids (phenylalanine and tyrosine); DIAAS, digestible indispensable amino acid score; Leu, leucine; Lys, lysine; SAA, sulfur amino acids (methionine and cysteine).

<sup>1</sup> Values are calculated based on the mean amino acid composition of protein (Table 2) and mean ileal digestibility for the IAA (Table 3).

<sup>2</sup> The score is the chemical score for columns 1–4 and DIAAS for column 5 to 8. DIAAS corresponds to the lowest DIAAS score, and the IAA concerned is shown in brackets.



**FIGURE 3.** Digestive (black bars) and deamination losses (urinary urea: light gray; body urea: medium gray) of dietary nitrogen 8 h after the ingestion of flaxseed or rapeseed protein cooked in biscuits. Values are means ± SDs. *n* = 9 in flaxseed group and *n* = 10 in rapeseed group. The data were analyzed using Student's *t* test with unequal variances. \* indicates a significant difference between the groups.

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**Author contribution**

The authors' responsibilities were as follows—CG, JC, OG, and DT: designed the research; RT, JC, NK, MC, GA, CG, and RB: conducted the research; SD and OG: provided essential materials; RT and CG: analyzed data; RT, CG, and JC: wrote the article; RT and CG: had primary responsibility for final content; and all authors: read, edited and approved the final manuscript.

**Conflict of interest**

The authors report no conflicts of interest.

**Data availability**

Data described in the manuscript will be made available upon request, pending application and approval.

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## Appendix A. Supplementary data

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