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ABSTRACT

Background: It is necessary to propose plant alternatives to animal proteins that are of good nutritional quality. Pea is a good candidate owing to its high protein content and its well-balanced amino acid (AA) profile.

Objectives: This study aimed to assess the real ileal AA and nitrogen digestibility (RID_{AA} and RID_N) of pea protein isolate as compared to milk casein in humans. It also aimed to evaluate their nutritional quality through calculation of the digestible indispensable amino acid score (DIAAS) and to determine the net postprandial protein utilization (NPPU).

Methods: Fifteen healthy volunteers were included in a randomized, single-blinded, 2-arm, parallel-design trial. They were equipped with a naso-ileal tube. They ingested the test meals, which consisted of 9 successive portions of mashed potatoes containing either pea protein or casein, intrinsically labeled with nitrogen 15. Ileal content, plasma, and urine samples were collected regularly over an 8-h postprandial period.

Results: The mean RID_{AA} values were 93.6% ± 2.9% for pea protein and 96.8% ± 1.0% for casein, with no difference between the sources ($P = 0.22$). Leucine, valine, lysine, and phenylalanine were significantly less digestible in pea than in casein. The RID_N values were 92.0% ± 2.7% and 94.0% ± 1.7% for pea protein and casein, respectively, and were not different ($P = 0.11$). The DIAAS was 1.00 for pea protein and 1.45 for casein. The NPPU was 71.6% ± 6.2% and 71.2% ± 4.9% for pea protein and casein, respectively ($P = 0.88$).

Conclusions: Although some AAs are less digestible in pea protein than in casein, the real ileal digestibility and the NPPU were not different. The DIAAS of 1.00 obtained for pea protein demonstrated its ability to meet all AA requirements. This study shows the potential of pea isolate as a high-quality protein. This study was registered at clinicaltrials.gov as NCT04072770. *Am J Clin Nutr* 2022;115:353–363.

Keywords: ileal digestibility, amino acid, pea protein, casein, human, stable isotopes

Introduction

Adequate protein intake is a subject of major importance in the field of nutrition, as protein is an indispensable nutrient with no body organ exclusively dedicated to storage. For environmental reasons, it has been recommended that Western countries reduce their consumption of food of animal origin in favor of plant products (1). In particular, the legume family offers the additional advantage of reducing nitrogen inputs during cultivation. However, most plant proteins are considered to have poorer digestibility as compared to animal sources (2–6). This can be explained by the presence of antinutritional factors that interact with proteolytic enzymes (7–9). Remarkably, products from legumes (e.g., soy, pea, lupin) have demonstrated good digestibility in humans, pigs, or rats (8, 10–15), particularly when consumed in a purified form, such as protein flour, concentrate, or isolate (16, 17). Among them, pea protein offers a valuable option due to its high protein content [24% (18)] and its relatively balanced amino acid (AA) profile (19), despite the admitted deficiency in sulfur AAs in legumes.

To evaluate protein quality, the FAO recommends use of the digestible indispensable amino acid score (DIAAS), based on the indispensable amino acid (IAA) composition of the proteins and their individual ileal digestibilities (20). This implies

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Abbreviations used: AA, amino acid; AP, atom percent; APE, atom percent excess; DIAAS, digestible indispensable amino acid score; EA, elementary analyzer; F, ileal flow rate; GC-CIRMS, gas chromatography–combustion isotope ratio mass spectrometry; Glx, glutamine + glutamate; IAA, indispensable amino acid; IRMS, isotopic ratio mass spectrometry; N, nitrogen; NPPU, net postprandial protein utilization; PEG, polyethylene glycol; RID, real ileal digestibility; U-HPLC, ultra-high performance liquid chromatography.

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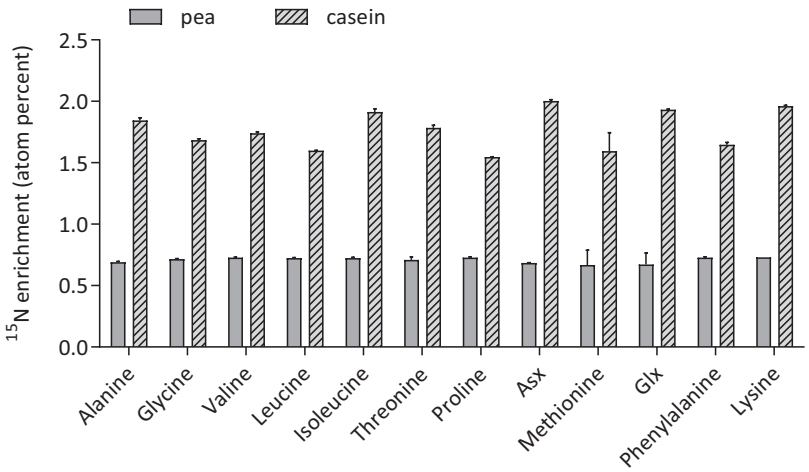

FIGURE 1 ^{15}N enrichment of amino acids in labeled protein isolates. Values are means ± SDs of 3 samples. Abbreviations: Asx, asparagine + aspartate; Glx, glutamine + glutamate.

that digestibility should be assessed individually for each AA in the terminal ileum digesta, instead of in feces, to avoid metabolization of the residual dietary AAs by the colonic microbiota (21). Data on IAA digestibility in humans are limited because measurement of ileal losses is challenging (22, 23). The naso-ileal intubation technique enables collection of digestive content at the end of the small intestine (24). When combined with ^{15}N-labeled proteins, it allows differentiation of the dietary and endogenous fractions among the AA losses, as well as determination of AA real ileal digestibility (RID).

In a previous study on rats, we found that pea protein was highly digestible (94.6% mean true cecal AA digestibility), and we obtained a DIAAS of 0.88 (25). Casein is the main protein in milk, representing 77%–78% of total milk proteins (26). It is considered a reference in terms of nutritional quality, with digestibility ranging from 94% to 100%, depending on the study (14, 15, 27, 28).

The aim of the present study was to evaluate the RID of pea protein in humans in comparison to milk casein, using ^{15}N-labeled dietary proteins and the naso-ileal intubation technique. We calculated the DIAAS and assessed the net postprandial protein utilization (NPPU) as indicators of protein quality.

Methods

Test proteins and experimental meals

We compared 2 purified test proteins that were labeled with nitrogen 15 (^{15}N). Three micro-plots (12 m^2) of yellow peas (*Pisum sativum*) were fertilized with 2 supplies of ^{15}N-labeled ammonium nitrate ($^{15}NH_4{}^{15}NO_3$; 10%; 50 g/supply) at stages 4/6 leaves and floral buds. The labeling protocol was carried out at Unité Expérimentale du Domaine d'Epoisses, National Research Institute for Agriculture, Food, and Environment (INRAE, Bretenière, France). The seeds were harvested at maturity, air dried, and ground to flour. The protein fraction was extracted and purified under pH neutralization, then heat treated and freeze-dried. For casein, a lactating cow was fed ^{15}N-labeled ammonium sulfate [$(^{15}NH_4)_2SO_4$; 99%; 100 g/d] for 4 d at the experimental farm of AgroParisTech. Labeled milk was collected at day 5, skimmed, and pasteurized. The casein fraction was extracted and purified under ultrafiltration and microfiltration, then heat treated and freeze-dried. The protein isolates were tested for microbiological contamination and were both safe for consumption. Nitrogen contents were 12% and 12.5% for the pea protein isolate and casein isolate, respectively. ^{15}N enrichments were 0.72 atom percent (AP) for pea protein isolate and 1.78 AP for casein isolate. ^{15}N enrichments of individual AAs in the protein isolates (**Figure 1**) were uniform among AAs explained by the transaminations, and the mean ^{15}N AA enrichments were 0.71 AP for the pea protein isolate and 1.75 AP for casein. The AA composition of test proteins is given in **Figure 2**. The test meals were composed of mashed potatoes (Picard) containing 1 of the test proteins (pea protein or casein). The meal was split into 12 portions and administered in 9 mini-meals, the first being a loading dose of 4 portions. Each volunteer received a total of 45 g (dry weight) of potato and 4 g of nitrogen from the test protein (35 g of pea protein isolate or 31 g of casein isolate). They were given a glass of water hourly and were not allowed to ingest any other food during the 8 h of the postprandial sampling period.

Study design

This study was approved by the Ethical Committee Sud-Est III (ref. 2019–007 B) and was registered at clinicaltrials.gov as NCT04072770, with nitrogen and AA true ileal digestibility and net postprandial protein utilization of pea proteins and casein as the primary outcomes. The clinical trial was conducted in the Human Nutrition Research Center of Avicenne Hospital (APHP, Bobigny, France) in accordance with the ethical standards of the responsible committee on human experimentation. The protocol was a single-blinded trial using a 2-arm, parallel design, and the volunteers were alternatively allocated to 1 of the 2 groups, pea or casein, in order to avoid any random season effects. Pea and casein proteins mixed with mashed potatoes were prepared in the morning before administration and looked similar. The volunteers were blinded but the study personnel were not.

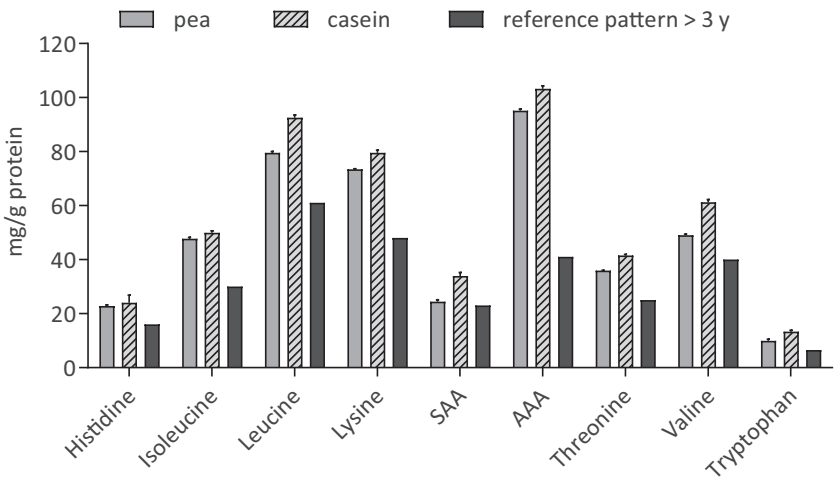

FIGURE 2 Amino acid composition of test protein isolates. Values are means ± SDs of 3 samples. The protein content was calculated from nitrogen content using a 6.25 conversion factor. The reference pattern was the older child, adolescent, adult profile defined in the 2013 FAO report (20). Abbreviations: AAA: aromatic amino acid (phenylalanine + tyrosine), SAA: sulfur amino acid (methionine + cysteine).

Subjects

All volunteers were certified as being in good health. The inclusion criteria were: BMI (in kg/m^2) between 18 and 30, age between 18 and 65 y, no allergies, negative serology for HIV and hepatitis B and C viruses, absence of pregnancy, no abusive drug or alcohol consumption, absence of severe disease, and <7 h of physical activity per week. They received detailed information on the objectives and potential risks of the protocol by the doctor and the nurse in charge of the study and gave written, informed consent for their participation. In studies using the naso-ileal intubation method, the inter-individual variability of nitrogen digestibility ranged from 1.8% to 4.1%, with an average value of 2%, and a difference of digestibility between proteins can be considered as relevant for differences ≥4% (11, 27, 29–33). Hence, according to these data and with a power set at 0.90 and α set at 0.05, the sample size group was calculated to be 7 (G*Power 3.1). Exclusion of a subject a posteriori may occur due to an incorrect position of the tube in the terminal ileum (based on the pH and volume of digesta samples collected); therefore, recruitment stopped when a minimum of 8–10 subjects per group was attained. From June 2019 to March 2020, 26 healthy volunteers participated in the study. Several subjects did not complete the protocol or had to be excluded, and the final sample size was 15 (age, 36 ± 8.3 y; BMI, 23.6 ± 2.4; **Table 1**). The main causes of exclusion during the procedure were failure of migration of the tube through the pylorus, pain or vomiting, and insufficient migration in the small intestine. Two volunteers were removed afterwards because of unusable samples (**Figure 3**).

TABLE 1 Anthropometric data of the volunteers (n = 15)

	Pea protein	Casein	Total
n	8	7	15
Sex, F/M	5/3	5/2	10/5
Age, y	36.0 ± 9.8	36.0 ± 7.0	36.0 ± 8.3
BMI, kg/m^2	23.5 ± 2.7	23.7 ± 2.2	23.6 ± 2.4
TBW,[1] L	33.9 ± 5.8	33.1 ± 7.2	33.5 ± 6.2

[1]TBW indicates total body water assessed by impedancemetry.

Experimental protocol

Volunteers were admitted in the hospital for 2 d (**Figure 4**), with a maximum of 2 volunteers at the same time. The morning of day 1, a triple-lumen tube (polyvinyl chloride tubing; total length 2.5 m) was placed into the participant's stomach through their nose under local anesthesia and migrated along the digestive tract for 24 h with intestinal peristalsis. The progression was facilitated by inflating a terminal balloon through 1 of the 3 lumens once the tube had reached the small intestine. The passage of the tube through the pylorus was checked by radiograph in the early afternoon of day 1. The volunteers ate a standard hospital meal after the radiography and had dinner at 20:00, before fasting overnight. At the beginning of day 2, a second radiograph was performed to check the migration of the tube to the terminal ileum. Correct positioning of the tube was also confirmed by measuring the pH of the intestinal effluent, which was collected by aspiration with a syringe through the second lumen of the tube. Polyethylene glycol 4000 (PEG-4000; 20 g/L) was used as a nonabsorbable marker to calculate the intestinal flow rate. It was integrated into a saline solution and perfused in the ileum at a rate of 1 mL/min through the third lumen, 20 cm above digesta collection. A catheter was placed into the forearm vein of the volunteer for blood sampling. The 9 test meals were given every 30 min from 0 to 4 h, and each meal was consumed in a limited amount of time (5 min). The ileal content was collected continuously from 30 min before the ingestion of the first test meal to the end of the procedure, 8 h later. The digesta were pooled every 30 min, sampled in jars with the addition of a protease inhibitor (diisopropylfluorophosphates), frozen at −20°C, and freeze-dried until analysis. Blood samples were taken every 30 min for the first 4 h, then hourly. Urine was recovered every 2 h during the whole procedure, weighed, and stored at −20°C. A fraction was kept at 4°C with thymol oil and liquid Vaseline and processed the day after for urea extraction.

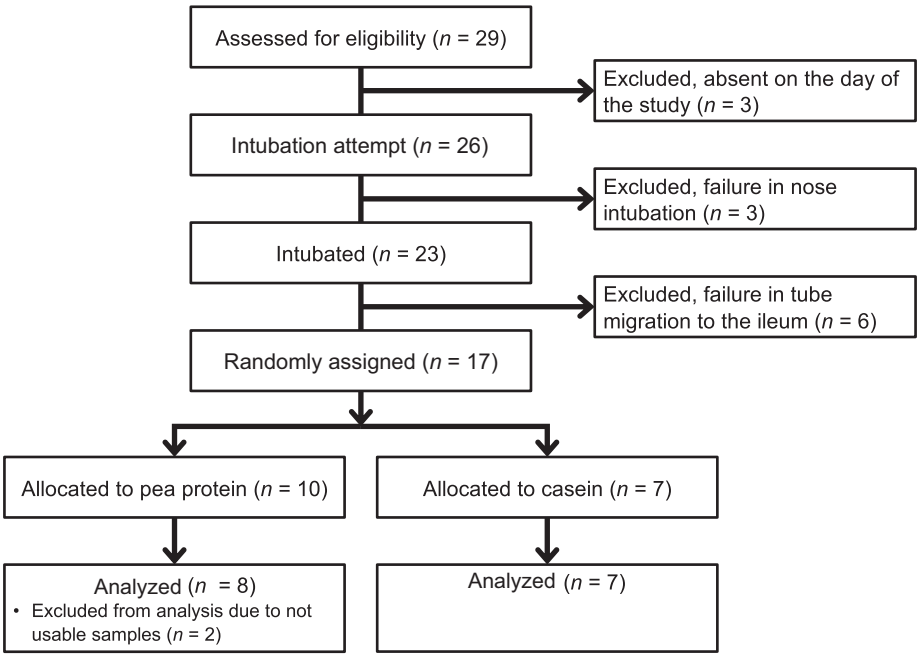

FIGURE 3 Participant flowchart, causes of failure and exclusion criteria.

Analytical procedure

The PEG-4000 content of the digesta was measured by a turbidimetric method (34) to determine the ileal flow rate.

The nitrogen content of digesta and test proteins (pea and casein) was measured with an elementary analyzer (EA) based on the Dumas method (35) (Vario Micro Cube, Elementar). The analyzer was coupled with an isotopic ratio mass spectrometer (IRMS; Isoprime, GV Instrument) to measure ^{15}N enrichment in digesta and test proteins. Atropine (Thermo Electron) was used as the elemental standard, and L-glutamic acid (USGS41; Sigma Aldrich) was used as the isotopic standard.

Nitrogen digestibility and nitrogen utilization were evaluated using ^{15}N recovery in digesta, urea pools (plasma and urine), and test proteins. Urea content in plasma and urine was assessed with the Urease-Berthelot method (36) (Urea assay, Randox). Urea and ammonia were isolated by the method adapted from Preston and McMillan (37). Urine was separated from ammonia and treated by urease (Urease from Jack Bean type III; Sigma-Aldrich). Urinary urea was isolated on a sodium form of cation exchange resin (Dowex 50WX8 sodium form 100–200 mesh; Sigma-Aldrich). The urea fraction of plasma was treated with urease and separated from the AA fraction by use of the sodium form cation exchange resin, like for urinary urea. The resin was eluted by $KHSO_4$ (2.5 M), and the supernatant was assayed for ^{15}N enrichment of plasmatic and urinary urea using EA-IRMS.

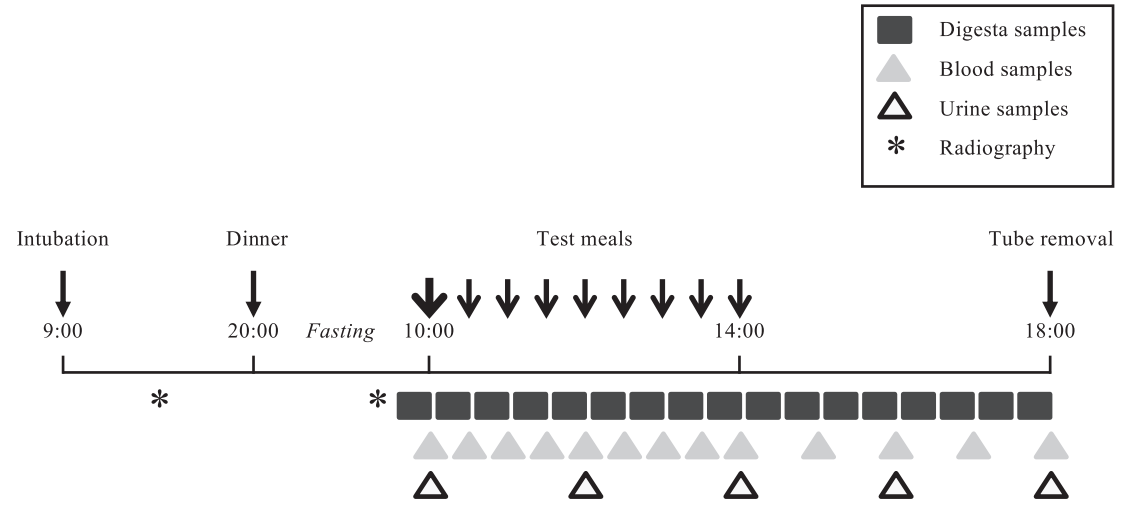

FIGURE 4 Experimental design of the study.

Real ileal digestibility of AAs was assessed from AA content and ^{15}N enrichment of individual AAs in digesta and test proteins. For AA content measurements (other than tryptophan), 10 mg of digesta or protein were hydrolyzed for 24 h with HCl 6N at 110°C. Norvaline was added prior to hydrolysis as an internal standard. For the analysis of sulfur AAs in test proteins (pea and casein), performic acid oxidation was carried out before hydrolysis to convert methionine and cysteine to their acid-stable derivatives of methionine sulfone and cysteic acid, respectively (38). Tryptophan content was determined in protein isolates (pea and casein). A base hydrolysis (barium hydroxide 2N) was carried out on 15 mg of samples for 20 h at 110°C, with 5-methyl-tryptophan as an internal standard. Calibration standards were composed of an AA mixture (Waters), with the addition of specific AAs (norvaline, methionine sulfone, cysteic acid, tryptophan, 5-methyl-tryptophan). Hydrolysates and standards were derivatized using the AccQ-Tag Ultra Derivatization Kit (Waters), according to the manufacturer's protocol. The AA quantification was performed on an Acquity HClass ultra-high performance liquid chromatography (U-HPLC) system with a photodiode array detector (PDA detector; Waters). The AAs were separated with an AccQ-Tag AA C18 column (2.1 × 100 mm; 1.7 μm bead size; Waters) and quantified as mmol/g of dry matter.

For AA isotopic enrichments, 90 mg of digesta and protein samples were hydrolyzed for 24 h with HCl 6N at 110°C. AAs were isolated using a hydrogen form resin (Dowex 50WX8 hydrogen form 100–200 mesh; Sigma-Aldrich) and derivatized with ethyl chloroformate (39). ^{15}N enrichment of isolated AAs was analyzed by gas chromatography (GC 6890N; Agilent Technologies) coupled with an isotope ratio mass spectrometer (Isoprime, GV Instrument) via the GC5 Isoprime interface. The combustion furnace temperature was 950°C. The GC column (RXI-17, 30 m long, 0.25 μm i.d., 0.5 μm film thickness; Restek) temperature program started at 150°C, increased by 4°C per min up to 200°C and by 25°C per min up to 270°C, with the final temperature being maintained for 10 min. The inlet temperature was set at 270°C.

Calculations

The ileal flow rate (F) was evaluated every 30 min as follows:

$$\mathrm{F(mL/30\ min)} = \frac{[\mathrm{PEG}]_{\mathrm{solution}}}{[\mathrm{PEG}]_{\mathrm{digesta}}} \times \text{perfusion flow rate} \times 30 \quad (1)$$

Here, $[PEG]_{solution}$ and $[PEG]_{digesta}$ are the concentrations of PEG-4000 in the perfused solution and in the digestive contents, respectively. The flow rate of the PEG perfusion was 1 mL/min.

The total nitrogen flow rate ($N_{tot\ ileum}$) in the ileum was assessed for each period:

$$N_{\mathrm{tot}\times\mathrm{ileum}}(\mathrm{mmol/30\ min}) = \frac{\mathrm{N} \times \mathrm{DM} \times \mathrm{F}}{14 \times 10} \quad (2)$$

Here, nitrogen was the percentage of nitrogen measured in the ileal sample, DM was the dry matter of the ileal sample (g/100 g), F was the ileal flow rate (mL/30 min), and 14 was the molar mass of nitrogen (g/mol).

The dietary nitrogen flow rate ($N_{diet\ ileum}$) referred to nitrogen from the ingested test protein (pea or casein) remaining in the ileum after absorption:

$$N_{\mathrm{diet}\times\mathrm{ileum}}(\mathrm{mmol/30\ min}) = N_{\mathrm{tot}\times\mathrm{ileum}} \times \frac{\mathrm{APE}_{\mathrm{ileum}}}{\mathrm{APE}_{\mathrm{meal}}} \quad (3)$$

Here, $N_{tot\ ileum}$ was the total nitrogen flow (mmol/30 min), and atom percent excess (APE) was the amount of ^{15}N enrichment gained in the digesta compared to the basal value (in AP). Basal enrichment was defined for each volunteer independently as the enrichment in the t = 0 digesta sample.

The endogenous nitrogen ($N_{endo\ ileum}$, mmol/30 min) was the difference between $N_{tot\ ileum}$ and $N_{diet\ ileum}$.

Real ileal digestibility of nitrogen (RID_N) was calculated as follows:

$$\mathrm{RID_N}(\%\mathrm{N_{ingested}}) = \frac{\mathrm{N_{ingested}} - \Sigma\mathrm{N_{diet\ ileum}}}{\mathrm{N_{ingested}}} \times 100 \quad (4)$$

Here, $N_{ingested}$ was the amount of nitrogen ingested from the test meals and $\Sigma N_{diet\ ileum}$ was the cumulative dietary nitrogen recovered in the ileum over the 8-h protocol (mmol).

Real ileal digestibility was also determined for each AA (RID_{AA}). The quantity of dietary AAs in digesta ($AA_{i\ diet}$) was calculated for each period of time (t):

$$\mathrm{AA_{i\,diet}(t)\,(mmol)} = [\mathrm{AA_i}]_{\mathrm{ileum}}(\mathrm{t}) \times \mathrm{DM(t)} \times \mathrm{F(t)} \times \frac{\mathrm{APE_{i\,ileum}(t)}}{\mathrm{APE_{meal}}} \quad (5)$$

Here, $[AA_i]_{ileum}$ was the quantity of AA_i in the digesta at t period (mmol/g), DM was the dry matter of digesta (g/100 mL), F was the ileal flow rate (mL/30 min), and APE was the amount of ^{15}N enrichment gained in AA_i in the digesta as compared to the basal value (in AP) at t period. Basal enrichment in AA_i was defined for each volunteer independently as AA_i enrichment in the t = 0 digesta sample.

Real ileal digestibility of AAs (RID_{AA}) was calculated for each AA as follows:

$$\mathrm{RID_{AAi}}(\%\mathrm{AA_{i\ ingested}}) = 1 - \frac{\Sigma\mathrm{AA_{i\,diet}}}{\mathrm{AA_{i\ ingested}}} \quad (6)$$

Here, $\Sigma AA_{i\ diet}$ was the sum of dietary AA_i over 8 h (mmol), and $AA_{i\ ingested}$ was the amount of $AA_{i\ ingested}$ by the volunteer (mmol). Average AA digestibility was calculated from the mean of AA digestibilities weighted by the proportion of each AA in the protein. Real ileal digestibility was assessed for 12 AAs and could not be calculated for histidine, tryptophan, cysteine, serine, and tyrosine. The missing AAs were explained by their low recovery or absence in the digesta hydrolysates produced for gas chromatography–combustion IRMS (GC-CIRMS) analyses (for cysteine and tryptophan), coelution with other components in the chromatography column (for serine), or unsatisfactory isolation and/or derivation (for histidine and tyrosine).

The DIAAS was calculated as prescribed by the FAO (20), where DIAAS is the lowest IAA ratio:

$$\mathrm{IAA_i\ ratio} = \frac{\text{mg digestible IAA}_\mathrm{i} \text{ in 1 g of the test protein}}{\text{mg IAA}_\mathrm{i} \text{ in 1 g of the reference protein}} \quad (7)$$

Here, digestible IAA_i content (g/kg protein) equals IAA_i content (g/kg protein) × RID_{AAi} (%). The reference profile used for the DIAAS calculation was the requirement pattern of the older child, adolescent, and adult defined in the 2013 FAO report (20). The N-to-protein conversion factor used was 6.25. The IAA ratios for sulfur AA and aromatic AA were calculated

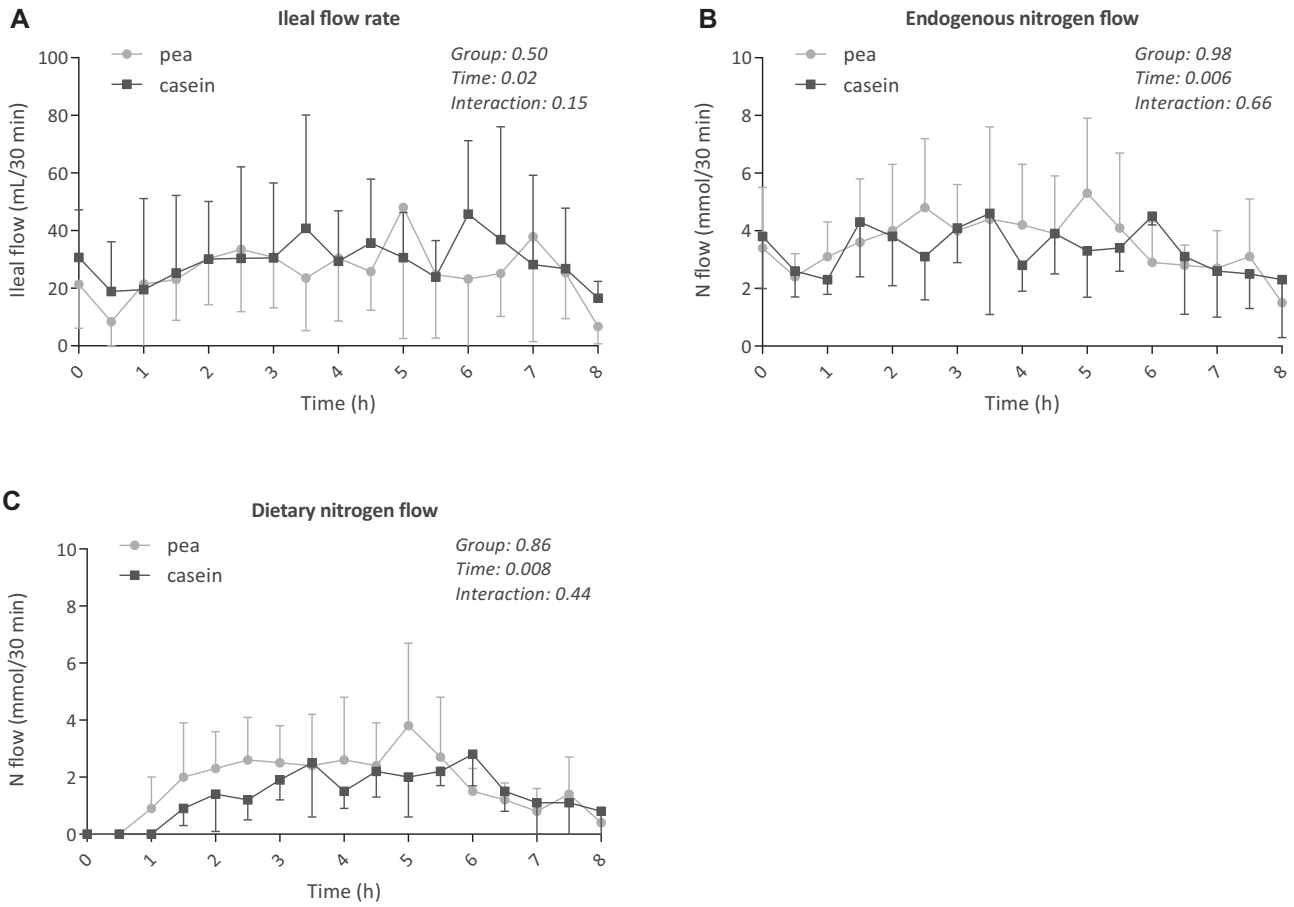

FIGURE 5 (A) Ileal flow rate of digesta, excluding perfusion of PEG-4000 solution. (B) Endogenous nitrogen flow in the ileum. (C) Dietary nitrogen flow in the ileum. Values are means ± SDs; $n = 8$ in pea protein group; $n = 7$ in casein group. Data were analyzed using a mixed model. Abbreviations: N, nitrogen; PEG, polyethylene glycol.

using methionine and phenylalanine digestibility, respectively. The IAA ratio for histidine and tryptophan was calculated using the mean digestibility of all AAs.

The dietary nitrogen incorporation into the different body nitrogen pools (digesta, body urea, urinary urea) was expressed as a percentage of the ingested amount and calculated using the following equation (32):

$$N_{diet}(t)(\%\text{ of ingested N}/30\text{ min}) = N_{tot}(t) \times \frac{APE(t)}{APE_{meal} \times N_{ingested}} \times 100 \quad (8)$$

The NPPU was the amount of nitrogen retained in the body after 8 h and was calculated as follows:

$$NPPU(\%\text{ of } N_{ingested}) = \frac{N_{ingested} - \Sigma N_{diet\ ileum} + \Sigma N_{diet\ urinary\ urea} + N_{diet\ body\ urea}}{N_{ingested}} \times 100 \quad (9)$$

Here, $N_{diet\ urinary\ urea}$ was the nitrogen content in the urinary urea, obtained by multiplying the volume of urine by the urinary urea concentration. $N_{diet\ body\ urea}$ was the remaining dietary nitrogen in the plasma urea, defined as plasma urea nitrogen concentration at 8 h multiplied by total body water, and corrected by the water content of blood (92%). Total body water was measured by bio-impedancemetry (Nutriguard-M; Data Input).

Statistical analysis

The values are expressed as means ± SDs. The data were analyzed using R version 4.0.3. According to quantile compared with quantile plots and Shapiro-Wilk tests, the true ileal digestibility data were assumed to be normally distributed. The difference between pea protein and casein groups was evaluated with an unpaired 2-sided Student *t*-test. Digestive and nitrogen flows were analyzed using a mixed model with a compound symmetry covariance matrix, and with the diet group as a fixed effect and time as a repeated effect. The overall significant difference was set at a P value < 0.05.

Results

Nitrogen flows and RID

The ileal flow rates of digesta were, on average, 25.9 ± 9.7 mL/30 min for the pea protein group and 29.4 ± 7.6 mL/30 min for the casein group (**Figure 5**A). In total, 439.5 and 500.0 mL of digesta flowed through the

terminal ileum over the 8-h protocol in the pea protein and casein groups, respectively. The endogenous nitrogen flows through the terminal ileum over the 8-h protocol were 48.5 ± 9.4 and 36.1 ± 12.7 mmol in the pea protein and casein groups, respectively (Figure 5B). The dietary nitrogen flows were, on average, 1.7 ± 1.1 mmol/30 min and 1.4 ± 0.9 mmol/30 min for the pea protein and casein groups, respectively (Figure 5C). No significant difference of flow (ileal, endogenous, or dietary nitrogen) was found between the 2 groups, but we observed a trend of a higher dietary nitrogen flow in the pea protein group ($P = 0.06$). The ileal and nitrogen flows varied over time (time effect $P = 0.02$, $P = 0.006$, and $P = 0.008$ for ileal, endogenous nitrogen, and dietary nitrogen, respectively), increasing with meal ingestion and decreasing at 6 h, following the ingestion of the last meal (t = 4 h). This time effect was particularly visible for dietary nitrogen flow. Dietary nitrogen appeared in the ileum around 1 h after ingestion of the first meal and increased for 5 to 6 h, before decreasing until the end of the protocol. During the protocol, the volunteers ingested, on average, 292 and 280 mmol of nitrogen in the pea protein and casein groups, respectively. The mean cumulated dietary nitrogen amounts recovered in the ileum were 23.4 ± 7.9 mmol in the pea protein group and 16.8 ± 4.9 mmol in the casein group ($P = 0.08$). Consequently, the RID_N of pea protein was 92.0% ± 2.7% and the RID_N of casein was 94.0% ± 1.7%, with no significant difference between the protein sources ($P = 0.11$).

Ileal AA digestibility and DIAAS

Real ileal digestibility varied depending on the AA. For pea protein, the RID_{AA} ranged from 90.2% ± 3.7% for glycine to 95.8% ± 2.0% for glutamine and glutamate (Glx). For casein, the RID_{AA} ranged from 94.4% ± 2.2% for isoleucine to 98.6% ± 0.4% for leucine (**Table 2**). The RID_{AA} values of leucine, lysine, phenylalanine, valine, asparagine + aspartate, glycine, and proline were significantly higher for casein compared to pea protein. However, the difference between the mean RID_{AA} values of pea protein (93.6% ± 2.9%) and casein (96.8% ± 1.0%) were not significant ($P = 0.22$).

The IAA ratios were all higher for casein compared to pea protein (**Table 3**). The DIAAS values, defined as the lowest IAA ratios, were 1.00 for pea protein and 1.45 for casein, and both corresponded to sulfur AA ratios.

Urea pools and NPPU

The transfer of dietary nitrogen into the urinary urea started immediately after the ingestion of the first meal (**Figure 6**A) and increased until the end of the protocol (t = 8 h) in both groups. No difference was observed over time between proteins ($P = 0.35$). The appearance of nitrogen in body urea also started after the ingestion of the first meal (Figure 6B) and increased until 4–5 h, with the last ingestion at 4 h. No difference was observed over time between proteins ($P = 0.38$).

The cumulative amount of dietary nitrogen excreted in urine was not different between the pea protein and casein groups ($P = 0.30$; **Table 4**). The residual dietary nitrogen in body urea at 8 h was not different between groups, either ($P = 0.13$). The NPPU values were 71.6% ± 6.2% for pea protein and 71.2% ± 4.9% for casein ($P = 0.88$).

TABLE 2 Real ileal digestibility (%) of amino acids obtained for pea protein compared to casein[1]

	Pea protein	Casein	*P* value[2]
n	92.0 ± 2.7	94.0 ± 1.7	0.11
Mean IAA	93.4 ± 3.6	97.0 ± 2.1	0.02
Isoleucine	92.9 ± 3.8	94.4 ± 2.2	0.38
Leucine	94.4 ± 2.8	98.6 ± 0.4	0.002
Lysine	93.9 ± 2.6	98.0 ± 0.5	0.001
Methionine	93.9 ± 5.2	98.3 ± 0.9	0.05
Phenylalanine	94.6 ± 2.8	99.2 ± 0.3	<0.001
Threonine	91.8 ± 4.0	94.6 ± 1.3	0.10
Valine	92.5 ± 3.8	96.1 ± 1.3	0.03
Mean DAA	92.9 ± 3.5	96.3 ± 1.8	0.01
Alanine	92.7 ± 3.9	96.1 ± 1.3	0.05
Asx	93.0 ± 2.9	96.4 ± 1.2	0.01
Glx	95.8 ± 2.0	95.9 ± 1.8	0.92
Glycine	90.2 ± 3.7	95.2 ± 1.4	0.006
Proline	92.3 ± 3.2	97.7 ± 0.5	<0.001
Mean all AA[3]	93.6 ± 2.9	96.8 ± 1.0	0.22

[1] Values are means ± SDs; $n = 8$ in pea protein group; $n = 7$ in casein group. Histidine, tryptophane, cysteine, serine, and tyrosine digestibilities could not be evaluated. Abbreviations: AA, amino acid; Asx, asparagine + aspartate; DAA, dispensable amino acid; Glx, glutamine + glutamate; IAA, indispensable amino acid.

[2] Data were analyzed with an unpaired 2-sided Student *t*-test.

[3] Mean digestibility of all AAs was weighted by the proportion of each AA in the protein isolates.

Discussion

This study aimed to evaluate the nutritional quality of a pea protein isolate as compared to casein through the direct measurement of ileal digestibility and bioavailability of AAs in humans.

The use of naso-ileal intubation allowed determination of the nitrogen flow in the terminal part of the small intestine after the

TABLE 3 IAA ratios and DIAAS of pea protein compared to casein[1]

	Pea protein	Casein	*P* value[2]
Histidine	1.40	1.53	
Isoleucine	1.64 ± 0.07	1.75 ± 0.04	0.004
Leucine	1.30 ± 0.04	1.57 ± 0.01	<0.001
Lysine	1.59 ± 0.04	1.81 ± 0.01	<0.001
SAA	1.00 ± 0.06	1.45 ± 0.01	<0.001
AAA	2.23 ± 0.07	2.54 ± 0.04	<0.001
Threonine	1.47 ± 0.06	1.75 ± 0.02	<0.001
Valine	1.19 ± 0.05	1.55 ± 0.02	<0.001
Tryptophan	1.41	1.95	
DIAAS	1.00 ± 0.06	1.45 ± 0.01	<0.001

[1] Values are means ± SDs; $n = 8$ in pea protein group; $n = 7$ in casein group. The RID value used for histidine and tryptophan ratios was the mean RID value of all AAs, and thus *P* values could not be calculated; the RID value used for SAA was the methionine RID value and the RID value used for AAA was the phenylalanine RID value. Abbreviations: AAA, aromatic amino acids, DIAAS, digestible indispensable amino acid score; IAA, indispensable amino acids; RID, real ileal digestibility; SAA, sulfur amino acids.

[2] Data were analyzed with an unpaired 2-sided Student *t*-test.

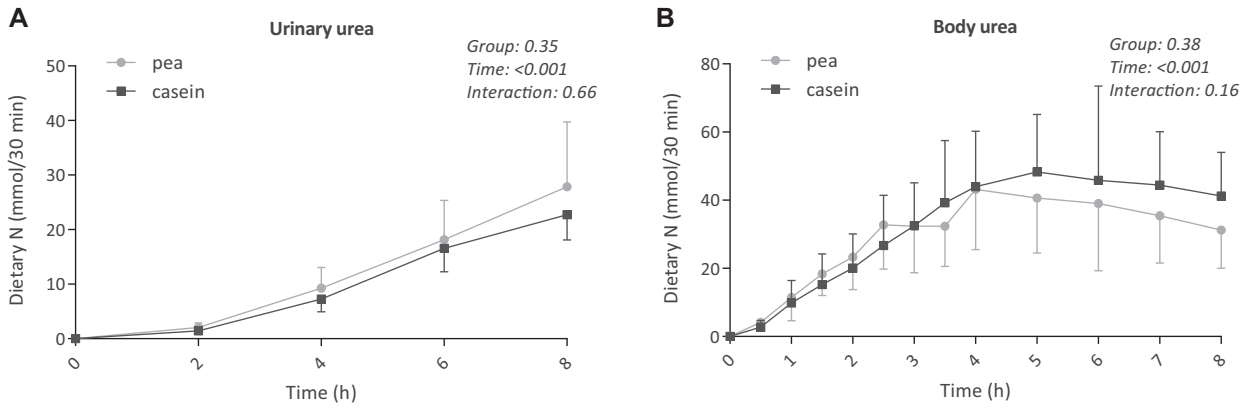

FIGURE 6 (A) Incorporation of dietary nitrogen into urinary urea. (B) Incorporation of dietary nitrogen into body urea. Values are means ± SDs; $n = 8$ in pea protein group, $n = 7$ in casein group. Data were analyzed using a mixed model. Abbreviation: N, nitrogen.

ingestion of test meals. The protein source had no effect on the endogenous nitrogen flow in our study, with no difference in the nitrogen flow rate following either pea (48.5 ± 9.4 mmol nitrogen) or casein ingestion (36.1 ± 12.7 mmol nitrogen). The values of endogenous losses observed in our study were comparable to the values determined after pea protein ingestion in the Mariotti et al. (30) study on pea protein (51.4 ± 15.2 mmol nitrogen collected over their 8-h protocol, compared with 48.5 ± 9.4 mmol nitrogen in our study). Comparable results of endogenous ileal nitrogen losses were obtained by Gaudichon et al. (29) under similar conditions, following ingestion of milk or soy protein isolates stirred into water (39.0 mmol nitrogen over the 8-h protocol for milk, 51.2 mmol nitrogen for soy). However, in the Gaudichon et al. (29) study, the nitrogen endogenous losses were significantly higher for soy protein compared to milk protein. This is in accordance with the literature that described an increase in endogenous nitrogen secretions with legumes and plant proteins in general, notably explained by the presence of antinutritional factors (40–42). In the current study, the difference between protein groups was not significant, probably due to the interindividual variability and the low antinutritional factor concentration in pea protein. The dietary nitrogen kinetics induced by ingestion of either pea protein or casein were not significantly different between the 2 sources, but the decrease seemed to be delayed by 1 h in the casein group. This would be consistent with the characterization of casein as a "slow protein," based on its digestion rate and AA absorption kinetics (43, 44).

TABLE 4 NPPU of pea protein compared to casein[1]

	Pea protein	Casein	*P* value[2]
$\Sigma N_{\text{diet ileum}}$, mmol	23.4 ± 7.9	16.8 ± 4.9	0.08
$\Sigma N_{\text{diet urinary urea}}$, mmol	27.9 ± 11.9	22.7 ± 4.6	0.30
$N_{\text{diet body urea}}$,[3] mmol	31.3 ± 11.2	41.2 ± 12.8	0.13
NPPU, %	71.6 ± 6.2	71.2 ± 4.9	0.88

[1] Values are means ± SDs; $n = 8$ in pea protein group; $n = 7$ in casein group. Abbreviations: N, nitrogen; NPPU, net postprandial protein utilization.

[2] Data were analyzed with an unpaired 2-sided Student *t*-test.

[3] $N_{\text{diet body urea}}$ was the remaining dietary nitrogen in the plasma urea at 8 h.

The slow digestion of casein is explained by its precipitation in the acidic environment of the stomach (45). No significant difference was observed between the dietary nitrogen flows in the ileum after pea and casein ingestion, but a trend of higher dietary nitrogen losses occurred in the pea protein group. The ileal digestibility of nitrogen was not significantly different between the 2 sources, reaching 92.0% and 94.0% for pea protein and casein, respectively. The values reported for pea in the present study are higher than previous results obtained under similar conditions. According to Gausserès et al. (11) and Mariotti et al. (30), ileal nitrogen digestibilities were 89.4% ± 1.1% for pea flour and 89.9% ± 4.0% for pea protein isolate, respectively. In contrast, for milk casein, we obtained an RID_N similar to that obtained by Deglaire et al. (27), which was 94.1%. However, in a study carried out in ileostomates using unlabeled proteins, the nitrogen ileal digestibility of milk proteins was higher but exceeded 100%, and thus can be considered nonphysiological (14). In comparison to other legumes, RID values obtained in this study for pea protein isolate were comparable to the RIDs obtained for soy isolate and lupin flour [91.5 ± 2.0% (46) and 91.0 ± 3.0% (13), respectively] determined in comparable studies.

The mean AA ileal digestibilities (RID_{AA}) were 93.6% ± 2.9% and 96.8% ± 1.0% for pea protein and casein, respectively. Those values were high, demonstrating the potential of pea protein as a high-quality protein. As a comparison, the mean RID_{AA} values determined in studies performed on human subjects using naso-ileal intubation were similar for soy protein isolate [93.8 ± 3.0% (29)] and much lower for zein [corn protein; 63.0 ± 4.6% (47)]. The difference we obtained between pea protein and casein was not significant for the mean RID_{AA}, whereas the RID of casein was higher for many individual AAs, including the mean IAA. As the mean was weighted by the proportion of each AA in the proteins, this result is explained by the high contribution of Glx, for which no difference of digestibility was observed between groups. For both proteins, the average RID_{AA} was higher than the RID_N, with the difference being more pronounced for casein (+2.9% compared with +1.8% for pea protein). This could be explained by the fraction of nonprotein nitrogen contained in the protein isolates that remained in the digestive tract after digestion, leading to an underestimation of RID_N. The nonprotein nitrogen was calculated theoretically by

the difference between the AA composition of proteins measured by U-HPLC and the nitrogen content of proteins measured by EA, and represented 0.1% of total nitrogen for pea protein and 3.3% of total nitrogen for casein (data not shown). According to the literature, the nonprotein nitrogen content of milk represents, on average, 6% of the total nitrogen (48). Lower values, such as 4.5% of total nitrogen, have also been found (49). The nitrogen content in milk is mostly composed of urea, and this amount varies depending on the cow's diet, as well as interindividual factors (50). However, the nonprotein nitrogen fraction of purified proteins is assumed to be negligible, as was the amount we calculated for pea protein. The native form of the casein isolate (micellar casein) used in our study could perhaps explain this higher content compared to pea isolate. It may thereby explain the greater difference between RID_{AA} and RID_N in the casein group. Furthermore, determination of RID_{AA} requires more analytical steps than that of RID_N. Indeed, several steps of sample preparation and 2 different analyses (hydrolysis of digesta samples, extraction and derivatization before ^{15}N enrichment measurement in individual AAs by GC-CIRMS and hydrolysis of digesta samples, derivatization before AA content measurement by UHPLC) are needed to obtain RID_{AA}. In contrast, RID_N relies only on the measurement of nitrogen and ^{15}N enrichment by EA-IRMS in the same sample, without preparation. When RID_N was calculated theoretically from RID_{AA} and the AA composition of proteins, we obtained values of 93.4% and 96.8% for pea protein and casein, respectively. These values were also higher than the experimental values of RID_N (92.0% and 94.0% for pea protein and casein, respectively).

Measurements of RID_{AA} and the AA composition of proteins allowed us to calculate the DIAAS, which was higher than 1 for both proteins (1.00 and 1.45 for pea protein and casein, respectively). This result highlights the adequacy of pea protein in respect to human AA requirements, and thus its relevance to provide enough IAA to ensure protein synthesis. This value of 1 was higher than the DIAAS of our previous animal study (0.88), which was explained by the lower digestibility of sulfur AA (84.2%) obtained in rats (25). In other animal studies, the DIAAS of pea protein concentrate was 0.82 in rats (15) and 0.73 in pigs (4). The higher IAA ratios for casein were explained by the more balanced IAA composition of casein. The lowest IAA ratio was obtained for methionine for both proteins. The limiting content of sulfur AA in legumes is well known (3). Even though pea protein meets the IAA requirements set for individuals aged 3+ (20), methionine is the IAA that carries the highest risk of deficiency in this source. However, the DIAAS was calculated using 6.25 as the protein-to-nitrogen conversion factor. Even if 6.25 is still considered the standard value, it is based on the assumption that proteins contain 16% of nitrogen. This is not accurate for all sources, especially regarding plant proteins. As the conversion factor might have a strong impact on the DIAAS values (51), we also calculated the DIAAS using specific factors: that is, 6.15 for casein and 5.4 for pea protein (52, 53). The DIAAS values we obtained with the specific conversion factors were higher, reaching 1.16 and 1.48 for pea protein and casein, respectively (data not shown).

The kinetics of dietary nitrogen incorporation into body and urinary urea were similar between groups, demonstrating the effectiveness of pea protein utilization after absorption. Over the 8-h protocol, the amounts of incorporation of dietary nitrogen into body urea were 10.7% and 14.7% of ingested nitrogen for pea protein and casein, respectively. The amounts of dietary nitrogen incorporation into urinary urea were 9.6% and 8.1% of ingested nitrogen for pea protein and casein, respectively. These results are consistent with those described in similar studies. For milk casein, values of 12.31% ± 1.95% and 8.76% ± 1.38% of ingested nitrogen were reported for body urea and urinary urea, respectively (28). The NPPU obtained for pea protein (71.6 ± 6.2%) was not significantly different from that of casein (71.2 ± 4.9%), which suggests that the nitrogen utilization following the ingestion of pea protein and casein was not different. The NPPU of pea protein was similar to that found by Mariotti et al. (30) after ingestion of pea protein by naso-ileal intubated volunteers (70.9 ± 6.0%). NPPU results described in the literature for casein in human studies are 78% (54) and 71% (55), which fall within the same range as the values of this study.

The naso-ileal intubation technique has been used in several studies for AA digestibility assessment (27, 29, 47). A limitation of our study is the delay in absorption kinetics induced by our ingestion pattern. Indeed, the ingestion of repeated meals might have disrupted the total quantitative collection of digestive nitrogen and AA losses. The results of nitrogen flows showed that a small amount of dietary nitrogen still remained in the terminal ileum after the 8-h protocol, which might have induced overestimated values of digestibility and NPPU. Dangin et al. (54) evaluated the impact of repeated meals compared to a single meal of an identical AA composition in humans, in the case of whey protein ingestion. They concluded that the ingestion of repeated meals may reduce postprandial AA oxidation and impact postprandial protein deposition. The use of intrinsic labeling of dietary protein may also have limitations. Labeled absorbed AAs are partly recycled into the gut lumen as endogenous proteins (56) and this recycling phenomenon leads to an overestimation of dietary AA losses. However, it was recently shown that this overestimation results in a minor underestimation of protein digestibility of ~1% (57). Additionally, ^{15}N can be exchanged or lost during transamination and deamination, and the use of ^{15}N-labeled protein may be an issue. In our study, small intestinal microbiota might have metabolized some of the dietary AAs lost during the digestion process (21). However, to our knowledge, the impact of transamination due to bacteria on dietary AAs in the intestinal lumen has not been studied and it seems unlikely that it significantly affects RID_{AA}.

The present study highlights the observation that the digestive and metabolic bioavailability of pea protein is high, enabling fulfillment of the IAA requirement as reflected by the DIAAS of 1.00. It emphasizes the potential of pea to be consumed as a source of dietary protein, as it seems to be one of the rare plant proteins with no limiting AAs and high digestibility.

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content; and all authors: read and approved the final manuscript. FMG, LG-D, CL-M are employed by Roquette. All other authors report no conflicts of interest.

Data Availability

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

References

1. Willett W, Rockstrom J, Loken B, Springmann M, Lang T, Vermeulen S, Garnett T, Tilman D, DeClerck F, Wood A, et al. Food in the anthropocene: The EAT-Lancet Commission on healthy diets from sustainable food systems. Lancet North Am Ed 2019;393(10170):447–92.
2. Bos C, Airinei G, Mariotti F, Benamouzig R, Berot S, Evrard J, Fenart E, Tome D, Gaudichon C. The poor digestibility of rapeseed protein is balanced by its very high metabolic utilization in humans. J Nutr 2007;137(3):594–600.
3. Mariotti F. 35-plant protein, animal protein, and protein quality. In: Mariotti F (editor). Vegetarian and plant-based diets in health and disease prevention. Cambridge (MA): Academic Press, Elsevier Science; 2017. p. 621–42.
4. Mathai JK, Liu Y, Stein HH. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br J Nutr 2017;117(4):490–9.
5. Nosworthy MG, Neufeld J, Frohlich P, Young G, Malcolmson L, House JD. Determination of the protein quality of cooked Canadian pulses. Food Sci Nutr 2017;5(4):896–903.
6. Sarwar G, Peace RW, Botting HG, Brule D. Digestibility of protein and amino acids in selected foods as determined by a rat balance method. Plant Foods Hum Nutr 1989;39(1):23–32.
7. Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. J Agric Food Chem 2001;49(3):1069–86.
8. Grala W, Verstegen MWA, Jansman AJM, Huisman J, van Leeuwen P. Apparent protein digestibility and recovery of endogenous nitrogen at the terminal ileum of pigs fed diets containing various soyabean products, peas or rapeseed hulls. Anim Feed Sci Technol 1999;80(3-4):231–45.
9. Sarwar Gilani G, Wu Xiao C, Cockell KA. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br J Nutr 2012;108(S2):S315–32.
10. Boutry C, Fouillet H, Mariotti F, Blachier F, Tome D, Bos C. Rapeseed and milk protein exhibit a similar overall nutritional value but marked difference in postprandial regional nitrogen utilization in rats. Nutr Metab 2011;8(1):52.
11. Gausserès N, Mahe S, Benamouzig R, Luengo C, Ferriere F, Rautureau J, Tome D. [15N]-labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. J Nutr 1997;127(6):1160–5.
12. Katz DL, Doughty KN, Geagan K, Jenkins DA, Gardner CD. Perspective: The public health case for modernizing the definition of protein quality. Adv Nutr 2019;10(5):755–64.
13. Mariotti F, Pueyo ME, Tome D, Mahe S. The bioavailability and postprandial utilisation of sweet lupin (*Lupinus albus*)-flour protein is similar to that of purified soyabean protein in human subjects: A study using intrinsically 15N-labelled proteins. Br J Nutr 2002;87(4):315–23.
14. Moughan PJ, Butts CA, van Wijk H, Rowan AM, Reynolds GW. An acute ileal amino acid digestibility assay is a valid procedure for use in human ileostomates. J Nutr 2005;135(3):404–9.
15. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr 2015;145(2):372–9.
16. Boye J, Wijesinha-Bettoni R, Burlingame B. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. Br J Nutr 2012;108(S2):S183–211.
17. Joint WHO/FAO/UNU Expert Consultation. Protein and amino acid requirements in human nutrition. World Health Organization Technical Report Series. Switzerland: WHO Press; 2007.
18. Lefranc-Millot C, Teichman-Dubois V. Protein from vegetable sources: A focus on pea protein. In: M Hayes. Novel proteins for food, pharmaceuticals and agriculture. United States: Wiley-Blackwell; 2018. p. 197–216.
19. Leterme P, Monmart T, Baudart E. Amino acid composition of pea (*Pisum sativum*) proteins and protein profile of pea flour. J Sci Food Agric 1990;53(1):107–10.
20. FAO. Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. Food and Nutrition Paper n°92. Rome, Italy: FAO; 2013.
21. Davila AM, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, Tome D. Re-print of "Intestinal luminal nitrogen metabolism: Role of the gut microbiota and consequences for the host." Pharmacol Res 2013;69(1):114–26.
22. Fuller M. Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. Br J Nutr 2012;108(S2):S238–46.
23. Gaudichon C, Calvez J. Determinants of amino acid bioavailability from ingested protein in relation to gut health. Curr Opin Clin Nutr Metab Care 2021;24(1):55–61.
24. Mahe S, Huneau JF, Marteau P, Thuillier F, Tome D. Gastroileal nitrogen and electrolyte movements after bovine milk ingestion in humans. Am J Clin Nutr 1992;56(2):410–16.
25. Guillin FM, Gaudichon C, Guerin-Deremaux L, Lefranc-Millot C, Azzout-Marniche D, Khodorova N, Calvez J. Multi-criteria assessment of pea protein quality in rats: A comparison between casein, gluten and pea protein alone or supplemented with methionine. Br J Nutr 2021;125(4):389–97.
26. Tremblay L, Laporte MF, Léonil J, Dupont D, Paquin P. Quantitation of proteins in milk and milk products. In: Fox PF, McSweeney PLH (editors). Advanced dairy chemistry–1 proteins. Part A/Part B. Boston, MA: Springer; 2003. p. 49–138.
27. Deglaire A, Bos C, Tome D, Moughan PJ. Ileal digestibility of dietary protein in the growing pig and adult human. Br J Nutr 2009;102(12):1752–9.
28. Lacroix M, Bos C, Leonil J, Airinei G, Luengo C, Dare S, Benamouzig R, Fouillet H, Fauquant J, Tome D, et al. Compared with casein or total milk protein, digestion of milk soluble proteins is too rapid to sustain the anabolic postprandial amino acid requirement. Am J Clin Nutr 2006;84(5):1070–9.
29. Gaudichon C, Bos C, Morens C, Petzke KJ, Mariotti F, Everwand J, Benamouzig R, Dare S, Tome D, Metges CC. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. Gastroenterology 2002;123(1):50–9.
30. Mariotti F, Pueyo ME, Tome D, Berot S, Benamouzig R, Mahe S. The influence of the albumin fraction on the bioavailability and postprandial utilization of pea protein given selectively to humans. J Nutr 2001;131(6):1706–13.
31. Oberli M, Marsset-Baglieri A, Airinei G, Sante-Lhoutellier V, Khodorova N, Remond D, Foucault-Simonin A, Piedcoq J, Tome D, Fromentin G, et al. High true ileal digestibility but not postprandial utilization of nitrogen from bovine meat protein in humans is moderately decreased by high-temperature, long-duration cooking. J Nutr 2015;145(10):2221–8.
32. Bos C, Juillet B, Fouillet H, Turlan L, Dare S, Luengo C, N'Tounda R, Benamouzig R, Gausserès N, Tome D, et al. Postprandial metabolic utilization of wheat protein in humans. Am J Clin Nutr 2005;81(1):87–94.
33. Mariotti F, Mahe S, Benamouzig R, Luengo C, Dare S, Gaudichon C, Tome D. Nutritional value of [15N]-soy protein isolate assessed from ileal digestibility and postprandial protein utilization in humans. J Nutr 1999;129(11):1992–7.
34. Hyden S. A turbidimetric method for the determination of higher polyethylene glycols in biological materials. Kungliga Lantbrukshogskolans Annaler 1956;22:139–45.
35. Dumas JBA. Procedes de I'analyse organique. Ann Chim Phys 1831;47:198–205.
36. Richterich R, Küffer H. Die bestimmung des harnstoffs in plasma und serum (Urease/BERTHELOT-Methode) mit dem greiner electronic selective analyzer GSA II. Z Klin Chcm Klin Biochem 1973;11(12):553–64.

37. Preston T, McMillan DC. Rapid sample throughput for biomedical stable isotope tracer studies. Biomed Environ Mass Spectrom 1988;16(1–12):229–35.
38. Rutherfurd SM, Gilani GS. Amino acid analysis. Curr Protoc Protein Sci 2009;58:1–37.
39. Fromentin C, Sanders P, Nau F, Anton M, Fromentin G, Tome D, Thibault JN, Gaudichon C. A pilot study for the intrinsic labeling of egg proteins with 15N and 13C. Rapid Commun Mass Spectrom 2012;26(1):43–8.
40. Ansia I, Stein HH, Vermeire DA, Brøkner C, Drackley JK. Ileal digestibility and endogenous protein losses of milk replacers based on whey proteins alone or with an enzyme-treated soybean meal in young dairy calves. J Dairy Sci 2020;103(5):4390–407.
41. Moughan PJ. Amino acid availability: Aspects of chemical analysis and bioassay methodology. Nutr Res Rev 2003;16(2):127–41.
42. Moughan PJ, Rutherfurd SM. Gut luminal endogenous protein: Implications for the determination of ileal amino acid digestibility in humans. Br J Nutr 2012;108(S2):S258–63.
43. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc Natl Acad Sci 1997;94(26):14930–5.
44. Churchward-Venne TA, Snijders T, Linkens AM, Hamer HM, van Kranenburg J, van Loon LJ. Ingestion of casein in a milk matrix modulates dietary protein digestion and absorption kinetics but does not modulate postprandial muscle protein synthesis in older men. J Nutr 2015;145(7):1438–45.
45. Mahe S, Roos N, Benamouzig R, Davin L, Luengo C, Gagnon L, Gausserges N, Rautureau J, Tome D. Gastrojejunal kinetics and the digestion of [15N]beta-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. Am J Clin Nutr 1996;63(4):546–52.
46. Mariotti F, Mahe S, Luengo C, Benamouzig R, Tome D. Postprandial modulation of dietary and whole-body nitrogen utilization by carbohydrates in humans. Am J Clin Nutr 2000;72(4):954–62.
47. Calvez J, Benoit S, Piedcoq J, Khodorova N, Azzout-Marniche D, Tome D, Benamouzig R, Airinei G, Gaudichon C. Very low ileal nitrogen and amino acid digestibility of zein compared to whey protein isolate in healthy volunteers. Am J Clin Nutr 2021;113:70–82.
48. Barbano DM, Lynch JM, Fleming JR. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative study. J Assoc Off Anal Chem 2020;74(2):281–8.
49. Waite R, Castle ME, Watson JN, Drysdale AD. Biuret and urea in concentrates for milking cows. J Dairy Res 1968;35(2): 191–202.
50. Venkatappaiah D, Basu K. Nonprotein nitrogenous constituents of milk. 1. Variation due to species, breed, individuality, season and stage of lactation. Ind J Dairy Sci 1952;5:95–116.
51. Wolfe RR, Rutherfurd SM, Kim I-Y, Moughan PJ. Protein quality as determined by the digestible indispensable amino acid score: Evaluation of factors underlying the calculation. Nutr Rev 2016;74(9):584–99.
52. Sarwar G, Blair R, Friedman M, Gumbmann MR, Hackler LR, Pellett PL, Smith TK. Inter- and intra-laboratory variability in rat growth assays for estimating protein quality of foods. J Assoc Off Anal Chem 1984;67(5):976–81.
53. Sosulski FW, Imafidon GI. Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J Agric Food Chem 1990;38(6):1351–6.
54. Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballevre O, Beaufrere B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. Am J Physiol Endocrinol Metab 2001;280(2):E340–8.
55. AFSSA (Agence française de sécurité sanitaire des aliments). Apport en protéines: Consommation, qualité, besoins et recommandations [Internet]. 2008. Available from: https://www.anses.fr/fr/system/files/NUT-Ra-Proteines.pdf.
56. Leterme P, Thewis A, Francois E, Van Leeuwen P, Wathelet B, Huisman J. The use of 15N-labeled dietary proteins for determining true ileal amino acid digestibilities is limited by their rapid recycling in the endogenous secretions of pigs. J Nutr 1996;126(9):2188–98.
57. Deglaire A, Moughan PJ, Airinei G, Benamouzig R, Tome D. Intact and hydrolyzed casein lead to similar ileal endogenous protein and amino acid flows in adult humans. Am J Clin Nutr 2020;111(1):90–7.