True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAASs) of plant-based protein foods

Yohan Reynaud, Caroline Buffière, Benoît Cohade, Mélissa Vauris, Kevin Liebermann, Noureddine Hafnaoui, Michel Lopez, Isabelle Souchon, Didier Dupont, Didier Remond

True ileal amino acid digestibility and digestible indispensable amino acid scores (DIAASs) of plant-based protein foods

Yohan Reynaud[a,b], Caroline Buffière[c], Benoît Cohade[c], Mélissa Vauris[c], Kevin Liebermann[c], Noureddine Hafnaoui[c], Michel Lopez[a], Isabelle Souchon[d], Didier Dupont[b], Didier Rémond[c*]

[a] IMPROVE SAS, F-80480, Dury, France

[b] STLO, INRAE, INSTITUT AGRO, F-35042, Rennes, France

[c] Université Clermont Auvergne, INRAE, UNH, Unité de Nutrition Humaine, F-63000 Clermont-Ferrand, France

[d] Université Paris-Saclay, INRAE, AgroParisTech, SayFood, F-78850 Thiverval-Grignon, France

Corresponding author: Didier Rémond, Unité de Nutrition Humaine, INRAE de Clermont-Ferrand/Theix, 63122 St Genès-Champanelle, France. Tel: 33 473 624 074. Fax: 33 473 624 755. e-mail: didier.remond@inrae.fr

Authors' e-mail addresses: yohan.reynaud@improve-innov.com, caroline.buffiere@inrae.fr, benoit.cohade@inrae.fr, melissa.vauris@inrae.fr, kevin.lieberman.pro@gmail.com, noureddine.hafnaoui@inrae.fr, michel.lopez@improve-innov.com, isabelle.souchon@inrae.fr, didier.dupont@inrae.fr, didier.remond@inrae.fr

Conflict of interest: The authors declare no conflicts of interest.

Funding: The project was funded by IMPROVE SAS and INRAE. A PhD grant was funded by IMPROVE SAS through a CIFRE procedure (CIFRE n°2016/0719)
Role of the funding source: IMPROVE SAS provided foods and INRAE performed the experimental work.

Running title: DIAAS of plant-based protein foods

Abstract

Plant-based protein foods are increasingly common, but data on their nutritional protein quality are scarce. This study evaluated it for seitan (wheat-based food), tofu (soya-based food), soya milk, and a pea emulsion. The true ileal digestibility (TID) of their amino acids was determined in minipigs, to calculate the digestible indispensable amino acid score (DIAAS). The TID of the proteins was high and not significantly different between the foods tested: 97% for seitan, 95% for tofu, 92% for soya milk and 94% for pea emulsion. There were only minor differences in individual amino acid TIDs. DIAAS ranking was thus essentially driven by the amino acid composition of the food: soya-based food > pea emulsion > seitan. Nevertheless, the lower TID of sulphur-containing amino acids in tofu than in soya milk induced a significant decrease in DIAAS (from 117% to 97%), highlighting the importance of the matrix effect on nutritional protein quality.

Keywords: plant protein, pea, soya, wheat, food matrix, digestibility, DIAAS

1 Introduction

Gross domestic product (GDP) per capita is positively correlated with dietary energy intake. Since it does not influence the contribution of protein to dietary energy (i.e., protein provides 5–15% of total energy) (Gerbens-Leenes, Nonhebel, and Krol 2010), demand for protein may increase worldwide as global per-capita GDP climbs. Indeed, research has found that, as standards of living improve, so does the percentage of energy provided by animal-based products. However, in general, the production of animal protein has a greater environmental impact than does the production of plant protein, as measured via the quantity of greenhouse gases emitted per gram of protein (González, Frostell and Carlsson-Kanyama, 2011). Since 2004, the availability of commercial plant-protein-based foods has increased dramatically in France; the source of protein in these foods is most often soya, wheat, and pea (GEPV, 2019). However, there are two major concerns regarding protein quality in these products: 1) the products may contain antinutritional factors, such as trypsin inhibitors or tannins that limit protein digestion (Gilani, Xiao and Cockell, 2012), and 2) the products may lack certain indispensable amino acids (IAA). Assessing the nutritional quality of the protein in plant-based products remains a significant challenge, complicating efforts to design innovative foods that will respond to the growing demand for protein.

The Food and Agriculture Organisation (FAO) has defined an index for assessing nutritional protein quality that integrates the notion of amino acid bioavailability, namely the digestible indispensable amino acid score (DIAAS) (FAO, 2013). The DIAAS compares the digestibility of individual amino acids at the end of the small intestine (*i.e.*, true ileal digestibility, which is a better proxy for bioavailability than whole-tract digestibility) to a standard of reference. Protein digestibility has largely been studied using protein isolates: the true ileal digestibility of soya, wheat, and pea proteins has been reported to be 91.5% (Gaudichon *et al.*, 2002; Bos *et al.*, 2003), 93.4% (De Vrese *et al.*, 2000), and 89.4–91.5%

(Gausserès *et al.*, 1996, 1997; Mariotti *et al.*, 2001), respectively. However, little is known about the digestibility of these proteins when they are in foods that are traditionally consumed by humans, such as tofu and seitan. However, it is now well known that the food matrix and its transformation during processing affects the digestibility and use of the nutrients (Thorning *et al.*, 2017). Heat treatments, for instance, may inactivate antitrypsin factors in foods, thus increasing protein digestibility, as illustrated by autoclaved and non-autoclaved soybean flour (Li, Sauer and Caine, 1998). But these treatments may also impact protein structure (German, Damodaran and Kinsella, 1982) and food structure (Le Feunteun *et al.*, 2014), which influence such factors as enzyme diffusion, substrate accessibility and, subsequently, protein digestibility. It is thus crucial to estimate the digestibility of proteins in foods as they are actually consumed. We hypothesized that protein digestibility in commonly consumed plant-based food could be different that observed in protein isolates, because of the manufacturing processes involved (heat treatment, coagulation), as previously observed for animal products (Bax *et al.*, 2012)(Barbé *et al.*, 2013).

The aim of this study was to determine the nutritional quality of protein in four plant-based foods (seitan, tofu, soya milk, and a pea emulsion) that differed in their structure (gel vs. liquid) and protein source (wheat, soya and pea). Seitan and tofu were chosen because of the growing interest of French consumers towards these plant-based protein foods. Protein digestibility of the tested foods was evaluated in minipigs, and the DIAAS was calculated.

2 Materials and methods

2.1 Animal handling and surgery

All the procedures were carried out in accordance with European guidelines (Directive 2010/63/EU) and approved by the Auvergne Animal Experimentation Ethics Committee (CEMEAA) and the French government (APAFIS#11001-2017082312525562v2). For the experiment, we used six adult Yucatan miniature pigs (mean mass at 8 months = 20.2 ± 1.5 kg).

Three weeks before the experiment began, the pigs were surgically fitted with two permanent catheters, which were placed in the hepatic portal vein and the upper hepatic vein. They were also fitted with a T-cannula (made of silicone rubber; ID: 12 mm, OD: 17 mm) placed 10 cm upstream from the ileocaecal valve.

To differentiate endogenous and dietary proteins, the pigs were given a continuous perfusion of ^{13}C-leucine solution through the upper hepatic vein catheter (Hess *et al.*, 2000) for five days before the experiment. They received a loading dose of 1 mg.kg^{-1} and then a continuous dose of 1 mg.kg^{-1}.j^{-1} at a rate of 1.5 ml.h^{-1}. Prior to initiating the perfusion, a blood sample was taken to determine the basal level of ^{13}C-leucine enrichment.

The pigs were housed in individual pens in a ventilated room kept at a constant temperature (21°C). Between the experimental trials, the pigs were given 500 g/d of a concentrate containing 16% protein, 1% fat, 4% cellulose, and 5% ash (Porcyprima; Sanders Centre Auvergne, France) distributed in equal portions at 8.00 and 16.00. The pigs had *ad libitum* access to water.

2.2 Test meals

We tested two solid and two liquid foods: seitan (wheat-based protein), tofu (soya-based protein), soya milk (soya-based protein), and a pea emulsion (pea-based protein; a soybean oil-in-water emulsion containing a pea isolate). The seitan, tofu, and soya milk were of commercial origin. The seitan and tofu were ground (the final median particle sizes were 5.1 ± 0.1 mm for the tofu and 3.5 ± 0.1 mm for the seitan (n = 4 trials)) and then freeze dried. The soya milk had undergone ultra-high temperature (UHT) processing. We created the emulsion using a commercial pea isolate (Pisane M9, Lot: N16231O04, Cosucra, Belgium) and commercial soybean oil (Emile Noël, France). A pre-emulsion was generated using a homogeniser (T-50 Ultra-Turrax, IKA, Germany) equipped with a 15 G dispersing tool (IKA, Germany) run at 10,000 rpm for 1 min. This pre-emulsion was then homogenised twice using a bench-top homogeniser (PandaPLUS 2000, GEA, USA) run at 1,000 bar. We added maltodextrine, sugar, and soybean oil to all the foods to ensure they had equal levels of protein (30.0 g), fat (23.1 g), and calories (980 kcal), in each test-meal (Table S1, supplementary information 1). The test-meals containing seitan, tofu, soya milk, and pea emulsion weighed 290 g, 435 g, 1,162 g, and 1,175 g, respectively. To estimate the basal flows of endogenous amino acids in the ileum, pigs were also given a protein-free test-meal, in which the protein source was replaced by 30 g of a mixture of free amino acids (in proportions resembling those found in meat), assumed to be completely absorbed from the small intestine. An indigestible transit marker, chromium oxide (Cr_2O_3), was added (0.3% of the food's dry matter content). The characteristics of the different foods tested are described in **Erreur ! Source du renvoi introuvable.**.

2.3 Sampling and chemical analysis

Each pig was given each of the food types in a randomly determined order. Experimental trials were separated by at least two days. During each trial, the pigs had *ad libitum* access to water. All food types were ingested in less than 15 min.

2.3.1 Portal-vein blood samples

Blood samples (3 ml) were taken from the portal vein before food ingestion and 60, 210, and 360 min after ingestion. The samples were immediately centrifuged (1,500 G, 10 min, 4°C). The plasma was removed, frozen in liquid nitrogen, and stored at -80°C. Deproteinisation was carried out using trichloroacetic acid precipitation. The supernatant was purified using cation exchange chromatography and free amino acids were then converted into their N-acetyl-propyl derivatives. It was then possible to measure the ^{13}C-enrichment of free leucine in the plasma ($^{13}C\text{-Leu}_{plasma\ pv}$) using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; IsoPrime, GV Instruments).

2.3.2 Ileal effluent samples

Between 60 min and 540 min post meal ingestion, ileal effluent was collected continuously in plastic bags (Sachet Whirl-Pak bags; volume: 120 ml, size: 7.5 x 18.5 cm) attached to the ileal cannula. The bags were regularly renewed based on bursts of digestion. The effluent samples were immediately transferred to pre-weighed aluminium dishes kept at -20°C. We created pools corresponding to 1 h of sampling, and stored them at -20°C.

The resulting samples were freeze dried (Cryotec AQ 1460). Dry matter (DM) content was determined after the samples were dried for an additional 24 h in a 60°C oven. The digesta were finely ground.

To estimate levels of chromium oxide (the indigestible transit marker), the samples were first subjected to a step of mineralisation (550°C, 6 h), followed by nitric acid dissolution (3 min of boiling in 5% $HN0_3^-$ [w/w]) and filtration (Whatman filter paper: 4–7 µm). They were then analysed using microwave plasma-atomic emission spectrometry (4210 MP-AES, Agilent).

Next, we created a sample representative of the entire postprandial period for each animal for each food type. These samples contained a fixed percentage (based on DM) of each of the hourly effluent pools, starting from the first-time chromium oxide appeared.

Total nitrogen content ($N_{content}$) was determined using an elemental analyser (vario ISOTOPE cube, Elementar). To estimate the amino acid content of food and digesta, we first performed four separate standardised hydrolysis procedures (AOAC, 2000): sample placement in 6N HCl for 24 h at 110°C, sample placement in 6N HCl for 48 h at 110°C (for branched-chain amino acids), sample placement in 6N HCl for 24 h at 110°C after peroxidation with H_2O_2 (for sulphur-containing amino acids); and sample placement in 4N $Ba(OH)_2$ for 16 h at 110°C (for tryptophan). A fraction of each hydrolysate was dried and then resuspended in a dilution buffer to which D-glucosaminic acid had been added as the injection standard. The levels of the various amino acids ($AA_{content}$) were determined using ion exchange chromatography and ninhydrin post-column detection (L-8900 high-speed amino acid analyser, Hitachi).

The level of ^{13}C-leucine enrichment was measured for the hydrolysed samples using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS; IsoPrime, GV Instruments), as described for the plasma samples.

2.4 Calculations

Flows are expressed as g of AA or N over the 9-h postprandial period. The digestibility of crude protein (CP = N x 6.25) is of no great relevance. Whereas the factor 6.25 used to convert N to CP in food is not perfect but close to reality (Mariotti, Tomé and Mirand, 2008), it does not apply to ileal contents in which about 30% of N is in non-protein form (urea, ammonia, creatinine, etc.) (Miner-Williams, Moughan and Fuller, 2009). The CP flows in the present study were indeed about twice as high as the total AA flows, illustrating the inconsistency of using the factor of 6.25 for evaluating protein content in ileal samples. Such discrepancies between CP ant total AA have been observed previously in pigs and minipigs (Hennig *et al.*, 2004). Thus, the digestibility calculations in this study were performed only for AAs, and the digestibility of proteins in the text refers to digestibility of total AAs.

2.4.1 Total flows of N and amino acids in the ileum

The flows of N and amino acids (AA) were determined using the chromium oxide data and the following equation: $X_i = X_{content} \times DM \times Cr_{intake}/Cr_{content}$, where X is N or an AA, DM is the total dry matter in the ileal sample, $X_{content}$ is the amount of nitrogen or a specific amino acid in the ileal sample, and Cr_{intake} and $Cr_{content}$ are the quantity of chromium oxide in the food type and ileal sample, respectively.

2.4.2 Flows of endogenous crude protein and amino acids in the ileum

The flow of endogenous leucine in the ileum (Leu_{endo}) was calculated using the following equation: $Leu_{endo} = Leu_i \times (^{13}C\text{-}Leu_{content} - {}^{13}C\text{-}Leu_{food})/(^{13}C\text{-}Leu_{plasma\ pv} - {}^{13}C\text{-}Leu_{food})$, where Leu_i is the quantity of leucine in the ileal sample and $^{13}C\text{-}Leu_{content}$, $^{13}C\text{-}Leu_{food}$, and $^{13}C\text{-}Leu_{plasma\ pv}$ are the levels of ^{13}C-leucine enrichment in the ileal sample, food type, and portal vein plasma, respectively.

The flows of other endogenous amino acids in the ileum (AA_{endo}) were determined using the following equation: $AA_{endo} = Leu_{endo} \times ([AA]/[Leu])_{PFcontent}$, where $([AA]/[Leu])_{PFcontent}$ is the ratio between the levels of a given AA and leucine in the ileal samples from the trial in which the pigs were fed the protein-free meal.

2.4.3 Ileal digestibilities of crude protein and amino acids

2.4.3.1 Apparent ileal digestibility of crude protein and amino acids

The apparent ileal digestibilities (AIDs) of nitrogen and the amino acids were expressed as percentages and determined with the equation AID(X) = 100 x ([Xmeal – Xi]/ Xmeal), where X is N or an AA.

2.4.3.2 Standardised ileal digestibility of crude protein and amino acids

Basal flows of endogenous nitrogen and amino acids ($X_{basal\ endo}$) were defined as the flows of N and AAs in the ileum following the ingestion of the protein-free meal (PF).

The standardised ileal digestibilities (SID) of nitrogen and amino acids were expressed as percentages and determined with the equation: $SID(AA) = 100 \times (X_{meal} – [X_i – X_{basal\ endo}]/X_{meal})$, where X is N or an AA.

2.4.3.3 True ileal digestibility of amino acids

The true ileal digestibilities (TID) of amino acids were expressed as percentages and determined with the equation $TID(AA) = 100 \times (AA_{intake} – [AA_i – AA_{endo}]/AA_{intake})$.

2.5 Data and statistical analysis

Data were analysed using an analysis of variance (ANOVA) using protein source and animal as fixed effects (GLM procedure of SAS®). Comparisons of the means were performed using Duncan’s multiple range test at a 95% confidence level. The paired t-test was used for certain specific comparisons between tofu and soya milk. Data are presented as means ± SEM.

3 Results

3.1 Amino acid profiles of the foods

The amino acid profiles of the different foods tested are presented in **Erreur ! Source du renvoi introuvable.**. As expected, the legume-based foods—the tofu, pea emulsion, and soya milk—were relatively rich in lysine. Their lysine content accounted for 5.6 to 7.0 % of CP content. In contrast, for seitan, the cereal-based food, Lysine accounted for only 1.4 % of CP content. Conversely, seitan contained higher levels of sulphur-containing amino acids (SAAs) than did the other foods (3.8 vs 1.4 to 2.9 % of CP content, for the other foods). Compared with the reference amino acid profile for adults (FAO, 2013), seitan had lower than recommended levels of lysine and threonine, and pea emulsion had lower than recommended levels of SAAs. The soya-based foods (tofu and soya milk) had similar, well-balanced amino acid profiles; individual IAA content met or exceeded the reference amino acid profile.

3.2 Apparent ileal digestibility

Although we observed significant differences between the foods tested regarding the AIDs of some of the amino acids (Lys, Met, Asp/Asn, Cys, Glu/Gln) (Table 2), the AIDs for the total AAs were very similar (77–84%). The AID of lysine was lower for seitan (54%) than for the other food types (range: 83–87%). The AID of methionine (49%) was lower for the pea emulsion than for soya milk and seitan (90%), while tofu had an intermediate value (69%). The AID of cysteine was lower for tofu and pea emulsion (36 and 44%, respectively) than for seitan and soya milk (74 and 79%, respectively).

3.3 Standard ileal digestibility

The protein free test meal showed that the contribution of the basal endogenous flow to the total amino acid flow at the ileum averaged 3.7 ± 1.0 g over the postprandial period studied. The amino acid pattern of this endogenous flow is given in Table S2 (supplementary information 2). Standard ileal digestibility (SID; Table 3) takes into account this basal endogenous flow. It is the value of ileal digestibility most often reported in the literature. The SIDs of the total AAs were 91, 92, 89, and 87% for the seitan, pea emulsion, soya milk, and tofu, respectively.

3.4 True ileal digestibility

Based on leucine enrichments, the contribution of endogenous protein secretions to total protein flow at the ileum was about 2/3 for seitan, pea emulsion and tofu, but it was only 1/2 for soya milk (Table 4). Although numerically greater for the solids than for the liquids, endogenous leucine flow was not significantly different between the 4 foods tested. The use of ^{13}C-leucine enrichments allowed taking into account not only basal endogenous flow, but also endogenous losses specific to the food tested. Thus, whereas basal endogenous leucine flow was estimated to be 0.23 ± 0.06 g, total endogenous leucine flows were 0.37 ± 0.11, 0.48 ± 0.09, 0.31 ± 0.03, and 0.26 ± 0.06 for seitan, tofu, soya milk and pea emulsion respectively. The difference of specific endogenous leucine flow between tofu and soya milk was particularly noteworthy (0.25 ± 0.09 vs 0.08 ± 0.03, respectively, $P = 0.05$). In our calculations the basal and total endogenous flows of the other AA relied on the same profile of endogenous AA (that observed with the PF meal), as the same differences were observed for them.

True ileal digestibility (TID; Table 4) takes into account total endogenous flows (basal + specific). The TID of the total AAs were very high, and did not differ significantly between the foods tested (92–97%). The TIDs for all the individual amino acids were also very high. Although the soya milk and the tofu had the same protein source, they displayed small differences in the TIDs of their amino acids: the TID of methionine was lower for tofu, and, conversely, the TID of leucine and isoleucine was lower for soya milk.

3.5 Nutritional protein quality of the foods

The digestible indispensable amino acid content of the food is presented in Table 5. DIAAS (FAO and WHO, 1991; FAO, 2013) of the four foods tested was calculated for the three age groups (< 6 months; 6 months to 36 months; older child, adolescent, and adult) (Table 6). The digestible amino acids that were limiting did not differ between score types or age groups, except for the soya milk, for which leucine was the limiting amino acid for newborns, whereas it was lysine for children below 3 years old, and valine above 3 years old.

4 Discussion

TID is considered as the best predictor of the bioavailability of dietary protein amino acids. However, it is difficult to measure. In this study, we used a miniature pig model as an animal model for humans, pig being considered as the animal model best adapted for the study of digestion in the upper part of the gut (stomach + small intestine) (Rowan *et al.*, 1994). We used the approach of a single test meal in animals otherwise receiving a standard diet. This means that the animals were not subjected to a period of continuous adaptation to the meal being tested. They were exposed to the meals just once before the TID measurement (at least one week before), to check the palatability of the meals. The TID obtained with this standardized approach reflects more what happens in humans, who are characterized by the diversity of their diet. Indeed, it does not exacerbate the potential negative effect of chronic exposure to the potential presence of antinutritional factors, such as lectins that may significantly affect endogenous secretions and gut health (Vasconcelos and Oliveira, 2004).

For the TID measurement, it is necessary to distinguish between residual dietary proteins and endogenous proteins within the chyme collected at the end of the small intestine. Since targeted foods with isotopic intrinsic labelling of proteins were not at our disposal, we opted for the ^{13}C-leucine labelling of endogenous proteins. This method is less subject to the biases associated with rapid amino acid recycling than is the labelled dietary protein method. However, it does not allow the direct measurement of the endogenous flow of each amino acid, in contrast with the use of labelled proteins (Hess *et al.*, 2000). In the present study, the endogenous flow of leucine was extrapolated to the other amino acids, using the amino acid pattern of the ileal chyme collected after a protein free meal (thus containing only endogenous secretion). Endogenous secretions can be separated into basal secretions (those observed with the protein free meal) and the specific endogenous losses in response to the food tested. For the TID calculation in the present study, we postulated a similar pattern of amino acids between basal and specific endogenous secretions. This is an approximation; in fact, the amino acid composition of the specific losses can slightly differ from that of the basal secretions. For instance, some foods containing polyphenols can specifically increase salivary proline-rich proteins (PRP) secretion (Jansman, Frohlich and Marquardt, 1994), which are low in indispensable amino acids and hardly digested by digestive enzymes. In addition, some peptides released during digestion can specifically increase mucin secretions (threonine-rich proteins) (Plaisancié *et al.*, 2013).

There were no data in the literature on the digestibility of plant-based protein foods. Common understanding is that proteins in plant food feature lower digestibility than that in animal products, but evidence is lacking to support this for protein-rich plant foods that are commercially available. Indeed, most digestibility measurements have been performed on plant protein isolates, or raw materials. This study is the first to characterise the true ileal digestibility (TID) of proteins in seitan, tofu, and soya milk; foods commonly consumed by humans. We found that their TIDs were high: 98, 95, and 92%, respectively. Although slightly higher, these values are consistent with those seen for wheat protein isolates (93%) (De Vrese *et al.*, 2000) and soybean protein isolates (91.5–96%) (Bos *et al.*, 2003; Rutherfurd *et al.*, 2015). The TID of the protein in pea emulsion (94%) resembled the TIDs previously observed for pea isolates (90–98%) (Mariotti *et al.*, 2001; Rutherfurd *et al.*, 2015). Furthermore, the TIDs of the protein of the plant food we tested are comparable to those of animal proteins: 91–98% for meat (Oberli *et al.*, 2015; Hodgkinson *et al.*, 2018; Kashyap *et al.*, 2018) and 94–95% for milk (Bos *et al.*, 1999; Rutherfurd *et al.*, 2015).

Whatever the structure of the food, solid or liquid, the endogenous secretions were only slightly affected and the protein digestibility remained high. The differences between soy milk and tofu were however especially noteworthy, because they clearly illustrated the so-called matrix effect (Thorning *et al.*, 2017): different digestion of food products deriving from the same raw material. Indeed, based on leucine flows, it can be estimated that the coagulation process of soya milk in tofu production leads to an increase in specific endogenous losses. In a previous study with the same soya milk and tofu (Reynaud *et al.*, 2020), we observed a very different evolution of gastric pH over the postprandial period. Apart from the potential difference in gastric emptying due to difference in nutrient solubility of the two protein sources (Schop *et al.*, 2019) or to the difference in test-meal volume, this difference may have an impact on the gastric pre-digestion of soya proteins and modify the nature of the peptides released during intestinal digestion, which in turn could affect endogenous ileal losses (Hodgkinson *et al.*, 2000). On the other hand, tofu making slightly increased branched chain amino acids and nearly all amino acids, whereas it decreased the digestibility of sulphur-containing amino acids. Whereas the heat treatment applied during tofu making (70-85°C) may have decreased antiprotease activity and increased protein digestibility, the coagulation process could explain the negative effect on sulphur-containing amino acids. Indeed, it seems that sulphur-containing amino acids mainly occur inside the molecular aggregates of the gels and are surrounded by hydrophilic subunits (Peng, Ren and Guo, 2016), which could make them less accessible to digestive enzymes, and explain their lower digestibility.

Seitan had the lowest nutritional protein quality (DIAAS range: 20–31%) due to its low lysine content. This result concurs with what has been seen for wheat protein in bread (DIAAS: 20%) (Han *et al.*, 2018). Lysine has also been found to be a limiting amino acid in whole-grain wheat, but the DIAASs calculated for whole-grain wheat are generally higher (30–60%) (Cervantes-Pahm, Liu and Stein, 2014; Mathai, Liu and Stein, 2017). This difference may be attributable to seitan's lower lysine levels—they were only half the lysine levels of whole-grain wheat. The DIAASs of the pea emulsion were intermediate (42–64%), and SAAs were limiting across all age groups. These values are consistent with those observed for a pea isolate (45–84%) (Mathai, Liu and Stein, 2017).

Because the seitan and the pea emulsion were almost completely digested, the nutritional protein quality of these two food types is essentially defined by their amino acid profiles, which were unbalanced. In contrast, the tofu and soya milk had similar, well-balanced profiles. Thus, the differences in the nutritional quality of their proteins is essentially determined by the digestibility of their amino acids. Indeed, because soya milk has a well-balanced amino acid composition, with high digestibility, its DIAAS is high (78% to 116%), and the limiting amino acid evolves according to the age-related reference profile, whereas for tofu, the reduced digestibility of the sulphur-containing amino acids makes them limiting, whatever the reference profile, and leads to a 15% decrease in DIAAS values in comparison to soya milk. Whatever the case, in agreement with the results previously reported for soya isolates (Mathai, Liu and Stein, 2017)(Rutherfurd *et al.*, 2015), the DIAAS of soya milk and tofu proteins, for the adult population, are close to those reported for milk and meat (Mathai, Liu and Stein, 2017)(Hodgkinson *et al.*, 2018), indicating the good nutritional quality of the proteins of these products.

4.1 Conclusions

The four plant-based protein food studied here—seitan, pea emulsion, soya milk, and tofu—displayed similar, high values of true ileal digestibility of amino acids. For the tofu and soya milk (both soya-based foods), the difference in nutritional protein quality was primarily driven by differences in AA digestibility, because the foods' AA profiles were well balanced. For the seitan and the pea emulsion, the lower nutritional protein quality was essentially explained by the degree of amino acid imbalance and the amounts of limiting amino acids relative to standard profiles.

Acknowledgements

We would like to thank Julien Hermet, the head of the animal care department, and Philippe Lhoste and Yohan Delorme, both animal care workers. We are also grateful to Jessica Pearce for correcting the English of the manuscript and to Denys Durand for the help he provided in performing the surgery with Didier Rémond.

Author Contributions

DR designed the study. CB, BC, MV, YR, and DR performed the experiments, and NH conducted the amino acid dosages. DR, CB, and YR analysed the data. DR and YR drafted the manuscript, and all the authors helped revise it.

References

AOAC (2000) *Official Methods of Analysis, 17th edn. Association of Official Analytical Chemists, Washington, DC.*

Barbé, F. *et al.* (2013) 'The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids', *Food Chemistry*. Elsevier Ltd, 136(3–4), pp. 1203–1212. doi: 10.1016/j.foodchem.2012.09.022.

Bax, M. L. *et al.* (2012) 'Cooking temperature is a key determinant of in vitro meat protein digestion rate: Investigation of underlying mechanisms', *Journal of Agricultural and Food Chemistry*, 60(10), pp. 2569–2576. doi: 10.1021/jf205280y.

Bos, C. *et al.* (1999) 'Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects', *British Journal of Nutrition*, 81(3), pp. 221–226.

Bos, C. *et al.* (2003) 'Human Nutrition and Metabolism Postprandial Kinetics of Dietary Amino Acids Are the Main Determinant of Their Metabolism after Soy or Milk Protein Ingestion in Humans', *Journal of nutrition*, 133(5), pp. 1308–1315.

Cervantes-Pahm, S. K., Liu, Y. and Stein, H. H. (2014) 'Digestible indispensable amino acid score and digestible amino acids in eight cereal grains', *Brit. J. Nutr.*, 111, pp. 1663–1672. doi: 10.1017/S0007114513004273.

FAO (2013) *Report of an FAO Expert Consultation: Dietary protein quality evaluation in human nutrition*.

FAO and WHO (1991) *Joint FAO/WHO Expert Consultation on Protein Quality Evaluation (1989: Bethesda, Maryland).*

Le Feunteun, S. *et al.* (2014) 'Impact of the Dairy Matrix Structure on Milk Protein Digestion Kinetics: Mechanistic Modelling Based on Mini-pig In Vivo Data', *Food and Bioprocess Technology*, 7(4), pp. 1099–1113. doi: 10.1007/s11947-013-1116-6.

Gaudichon, C. *et al.* (2002) 'Ileal Losses of Nitrogen and Amino Acids in Humans and

Their Improtance to the Assessment of Amino Acid Requirements', *Gastroenterology*, 123, pp. 50–59. doi: 10.1053/gast.2002.34233.

Gausserès, N. *et al.* (1996) 'The gastro-ileal digestion of 15N-labelled pea nitrogen in adult humans', *British Journal of Nutrition*, 76, pp. 75–85. doi: 10.1079/BJN19960010.

Gausserès, N. *et al.* (1997) '[15 N]-Labeled Pea Flour Protein Nitrogen Exhibits Good Ileal Digestibility and Postprandial Retention in Humans', *Nutrient Metabolism*, 127(6), pp. 1160–1165.

GEPV (2019) *Les protéines végétales en plein essor !*

Gerbens-leenes, P. W., Nonhebel, S. and Krol, M. S. (2010) 'Food consumption patterns and economic growth . Increasing affluence and the use of natural resources', *Appetite*, 55, pp. 597–608. doi: 10.1016/j.appet.2010.09.013.

German, B., Damodaran, S. and Kinsella, J. E. (1982) 'Thermal Dissociation and Association Behavior of Soy Proteins', *Journal of Agricultural and Food Chemistry*, 30(5), pp. 807–811. doi: 10.1021/jf00113a002.

Gilani, G. S., Xiao, C. X. and Cockell, K. A. (2012) 'Impact of Antinutritional Factors in Food Proteins on the Digestibility of Protein and the Bioavailability of Amino Acids and on Protein Quality', *British Journal of Nutrition*, 108(S2), pp. S315–S332. doi: 10.1017/S0007114512002371.

González, A. D., Frostell, B. and Carlsson-Kanyama, A. (2011) 'Protein efficiency per unit energy and per unit greenhouse gas emissions : Potential contribution of diet choices to climate change mitigation', *Food Policy*, 36, pp. 562–570. doi: 10.1016/j.foodpol.2011.07.003.

Han, Fei *et al.* (2018) 'Digestible indispensable amino acid scores of nine cooked cereal grains', *Brit. J. Nutr.* doi: 10.1017/S0007114518003033.

Hennig, U. *et al.* (2004) 'Relative ileal amino acid flows and microbial counts in intestinal effluents of Goettingen Minipigs and Saddleback pigs are not different', *Journal of animal* *science*, 82(7), pp. 1976–1985.

Hess, V. *et al.* (2000) ‘Comparison of the isotope dilution method for determination of the ileal endogenous amino acid losses with labelled diet and labelled pigs’, *British Journal of Nutrition*, 83(2), pp. 123–130.

Hodgkinson, S. M. *et al.* (2000) ‘The effect of dietary peptide concentration on endogenous ileal amino acid loss in the growing pig’, *Brit. J. Nutr.*, 83, pp. 421–430.

Hodgkinson, S. M. *et al.* (2018) ‘Cooking Conditions Affect the True Ileal Digestible Amino Acid Content and Digestible Indispensable Amino Acid Score (DIAAS) of Bovine Meat as Determined in Pigs’, *The Journal of Nutrition*, 148, pp. 1564–1569. doi: 10.1093/jn/nxy153.

Jansman, A. J., Frohlich, A. A. and Marquardt, R. R. (1994) ‘Production of proline-rich proteins by the parotid glands of rats is enhanced by feeding diets containing tannins from faba beans (Vicia faba L.)’, *The Journal of Nutrition*, 124(2), pp. 249–258.

Kashyap, S. *et al.* (2018) ‘Ileal digestibility of intrinsically labeled hen’s egg and meat protein determined with the dual stable isotope tracer method in Indian adults’, *The American Journal of Clinical Nutrition*. Oxford University Press, 108(5), pp. 980–987.

Li, S., Sauer, W. C. and Caine, W. R. (1998) ‘Response of Nutrient Digestibilities to Feeding Diets with Low and High Levels of Soybean Trypsin Inhibitors in Growing Pigs’, *J Sci Food Agric*, 76, pp. 357–363.

Mariotti, F. *et al.* (2001) ‘The Influence of the Albumin Fraction on the Bioavailability and Postprandial Utilization of Pea Protein Given Selectively to Humans’, *The journal of nutrition*, 131(6), pp. 1706–1713.

Mariotti, F., Tomé, D. and Mirand, P. P. (2008) ‘Converting nitrogen into protein - Beyond 6.25 and Jones’ factors’, *Critical Reviews in Food Science and Nutrition*, 48(2), pp. 177–184. doi: 10.1080/10408390701279749.

Mathai, J. K., Liu, Y. and Stein, H. H. (2017) ‘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)', *Brit. J. Nutr.*, 117, pp. 490–499. doi: 10.1017/S0007114517000125.

Miner-Williams, W., Moughan, P. J. and Fuller, M. F. (2009) 'Endogenous Components of Digesta Protein from the Terminal Ileum of Pigs Fed a Casein-Based Diet', *Journal of Agricultural and Food Chemistry*, 57(5), pp. 2072–2078.

Oberli, M. *et al.* (2015) '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', *The Journal of nutrition*, 145(10), pp. 2221–2228. doi: 10.3945/jn.115.216838.contrast.

Peng, X., Ren, C. and Guo, S. (2016) 'Particle formation and gelation of soymilk: Effect of heat', *Trends in Food Science and Technology*. Elsevier Ltd, 54, pp. 138–147. doi: 10.1016/j.tifs.2016.06.005.

Plaisancié, P. *et al.* (2013) 'A novel bioactive peptide from yoghurts modulates expression of the gel-forming MUC2 mucin as well as population of goblet cells and Paneth cells along the small intestine ☆', *The Journal of Nutritional Biochemistry*. Elsevier Inc., 24(1), pp. 213–221. doi: 10.1016/j.jnutbio.2012.05.004.

Reynaud, Y. *et al.* (2020) 'Temporal changes in postprandial intragastric pH: comparing measurement methods, food structure effects, and kinetic modelling', *Food Research International*. Elsevier Ltd. doi: 10.1016/j.foodres.2019.108784.

Rowan, A. M. *et al.* (1994) 'Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man', *Br. J. Nutr.*, 71, pp. 29–42.

Rutherfurd, S. M. *et al.* (2015) 'Protein Digestibility-Corrected Amino Acid Scores and Digestible Indispensable Amino Acid Scores Differentially Describe Protein Quality in

Growing Male Rats', *The Journal of Nutrition*, 145(2), pp. 372–379. doi: 10.3945/jn.114.195438.fecal.

Schop, M. *et al.* (2019) 'Increasing intake of dietary soluble nutrients affects digesta passage rate in the stomach of growing pigs', *British Journal of Nutrition*, 121(5), pp. 529–537.

Thorning, T. K. *et al.* (2017) 'Whole dairy matrix or single nutrients in assessment of health effects: current evidence and knowledge gaps', *The American journal of clinical nutrition*, 105(5), pp. 1033–1045. doi: 10.3945/ajcn.116.151548.Am.

Vasconcelos, I. M. and Oliveira, T. A. (2004) 'Antinutritional properties of plant lectins', *Toxicon*, 44, pp. 385–403. doi: 10.1016/j.toxicon.2004.05.005.

De Vrese, M. *et al.* (2000) 'Protein-Bound D -Amino Acids , and to a Lesser Extent Lysinoalanine, Decrease True Ileal Protein Digestibility in Minipigs as Determined with 15N-labeling', *The journal of nutrition*, 130(8), pp. 2026–2031.

Table 1 Dry matter (DM), crude protein (CP), and amino acid (AA) contents in seitan, tofu, soya milk and pea emulsion.

	Seitan	Tofu	Soya milk	Pea emulsion
Dry matter (%; as-fed basis)	76.72	46.03	17.49	15.99
Crude protein (N x 6.25) (% DM)	20.93	23.71	21.83	16.79
Indispensable amino acids (% DM)				
Histidine	0.47	0.63	0.61	0.53
Isoleucine	0.76	1.13	1.07	0.80
Leucine	1.42	1.85	1.69	1.44
Lysine	0.29	1.34	1.22	1.18
Methionine	0.39	0.27	0.29	0.08
Phenylalanine	1.03	1.24	1.12	0.94
Threonine	0.46	0.85	0.82	0.65
Tryptophan	0.57	0.55	0.57	0.51
Valine	0.78	1.12	1.04	0.81
Total indispensable AAs (% DM)	6.16	8.97	8.42	6.95
Dispensable amino acids (% DM)				
Arginine	0.26	0.51	0.55	0.45
Alanine	0.54	0.93	0.83	0.71
Aspartic acid/Asparagine	0.61	2.52	2.36	1.91
Cysteine	0.40	0.30	0.35	0.15
Glutamic acid/Glutamine	6.76	4.09	3.81	2.55
Glycine	0.63	0.92	0.85	0.66
Proline	2.48	1.31	1.28	0.90
Serine	0.83	1.06	0.97	0.79
Tyrosine	0.49	0.66	0.60	0.53
Total dispensable AAs (% DM)	13.00	12.29	11.60	8.65
Total AAs (% DM)	19.16	21.26	20.02	15.60
Lysine (% CP)	1.39	5.65	5.59	7.03
Methionine + cysteine (% CP)	3.78	2.40	2.93	1.37

Table 2 Apparent ileal digestibility (AID) of amino acids in seitan, tofu, soya milk and pea emulsion in minipigs. Values are means ± SEM, n = 6.[a]

	Seitan	Tofu	Soya milk	Pea emulsion	*P*
N, %	74.1 ± 4.6	56.5 ± 6.4	71.3 ± 2.5	68.0 ± 3.5	NS
Indispensable amino acids, %					
Histidine	82.1 ± 4.7	81.3 ± 2.9	82.7 ± 3.7	81.8 ± 2.5	NS
Isoleucine	83.6 ± 3.4	81.0 ± 2.4	81.0 ± 3.4	84.7 ± 2.0	NS
Leucine	84.7 ± 3.2	80.4 ± 3.0	79.3 ± 3.1	84.8 ± 2.0	NS
Lysine	54.3[b] ± 8.9	83.0[a] ± 3.1	84.8[a] ± 2.9	86.6[a] ± 2.0	0.001
Methionine	90.1[a] ± 1.9	69.4[c] ± 5.6	89.7[a] ± 2.8	49.3[b] ± 10.2	0.001
Phenylalanine	85.2 ± 3.0	81.1 ± 3.0	82.3 ± 2.9	84.7 ± 1.7	NS
Threonine	49.8 ± 10.6	58.5 ± 6.4	67.9 ± 4.8	66.0 ± 3.7	NS
Tryptophan	75.3 ± 8.5	77.4 ± 2.4	84.3 ± 2.4	83.6 ± 2.6	NS
Valine	78.9 ± 4.3	75.4 ± 3.3	78.1 ± 3.5	80.3 ± 3.2	NS
Dispensable amino acids, %					
Arginine	55.7 ± 9.4	61.9 ± 6.0	67.1 ± 8.2	75.4 ± 3.0	NS
Alanine	69.6 ± 6.9	72.4 ± 4.6	74.6 ± 3.8	79.1 ± 2.6	NS
Aspartic acid/Asparagine	55.1[b] ± 9.9	78.0[a] ± 3.8	80.1[a] ± 3.9	75.7[a] ± 3.2	0.024
Cysteine	74.1[a] ± 3.9	35.8[b] ± 10.2	78.5[a] ± 3.8	44.4[b] ± 11.3	0.004
Glutamic acid/Glutamine	94.9[a] ± 1.0	86.6[b] ± 2.0	85.8[b] ± 3.6	84.3[b] ± 2.5	0.019
Glycine	45.0 ± 9.7	52.6 ± 5.5	68.5 ± 5.2	56.9 ± 6.5	NS
Proline	89.6 ± 3.2	79.7 ± 4.4	81.9 ± 4.0	82.7 ± 2.8	NS
Serine	80.4 ± 3.9	74.1 ± 4.4	77.6 ± 3.6	78.8 ± 2.4	NS
Tyrosine	79.4 ± 4.9	76.1 ± 3.8	79.0 ± 3.6	86.0 ± 3.1	NS
Total Amino Acids	83.7 ± 3.4	77.1 ± 3.3	80.2 ± 3.6	79.9 ± 2.5	NS

[a] Different uppercase letter in the same line show significant differences ($P < 0.05$) among the different food types for nireogen (N) and amino acids.

Table 3 Standard ileal digestibility (SID) of amino acids in seitan, tofu, soya milk and pea emulsion in minipigs. Values are means ± SEM, n = 6. [a]

	Seitan	Tofu	Soya milk	Pea emulsion	*P*
N x 6.25, %	94.0 ± 4.6	76.0 ± 6.4	92.2 ± 2.5	97.4 ± 3.5	NS
Indispensable amino acids, %					
Histidine	90.1 ± 4.7	87.9 ± 2.9	89.5 ± 3.7	90.2 ± 2.5	NS
Isoleucine	91.2 ± 3.4	86.6 ± 2.4	86.9 ± 3.4	93.1 ± 2.0	NS
Leucine	91.9 ± 3.2	86.6 ± 3.0	86.0 ± 3.1	93.2 ± 2.0	NS
Lysine	76.4 ± 8.9	88.2 ± 3.1	90.5 ± 2.9	93.0 ± 2.0	NS
Methionine	94.0^{a} ± 1.9	75.7^{b} ± 5.5	95.5 ± 2.8^{a}	71.3^{b} ± 10.2	0.013
Phenylalanine	93.8 ± 3.0	89.0 ± 3.0	91.0 ± 2.9	95.8 ± 1.7	NS
Threonine	74.7 ± 10.6	73.5 ± 6.4	83.1 ± 4.8	86.9 ± 3.7	NS
Tryptophan	87.3 ± 8.5	91.3 ± 2.4	97.5 ± 2.4	99.7 ± 2.6	NS
Valine	89.7 ± 4.3	83.8 ± 3.3	87.0 ± 3.5	92.5 ± 3.2	NS
Dispensable amino acids, %					
Arginine	80.9 ± 9.4	76.1 ± 6.0	80.2 ± 8.2	92.5 ± 3.0	NS
Alanine	85.7 ± 6.9	82.7 ± 4.6	86.0 ± 3.8	93.5 ± 2.6	NS
Aspartic acid/Asparagine	78.3 ± 9.9	84.3 ± 3.8	86.7 ± 3.9	84.5 ± 3.2	NS
Cysteine	92.1^{a} ± 3.9	62.8^{b} ± 10.2	101.2^{a} ± 3.8	101.9^{a} ± 11.3	0.011
Glutamic acid/Glutamine	97.8 ± 1.0	92.0 ± 2.0	91.6 ± 3.6	93.5 ± 2.5	0.019
Glycine	74.8 ± 9.7	75.0 ± 5.5	92.6 ± 5.2	90.5 ± 6.5	NS
Proline	94.2 ± 3.2	89.5 ± 4.4	91.7 ± 4.0	97.8 ± 2.8	NS
Serine	91.4 ± 3.9	83.7 ± 4.4	88.0 ± 3.6	92.5 ± 2.4	NS
Tyrosine	88.5 ± 4.9	83.6 ± 3.8	87.0 ± 3.6	95.9 ± 3.1	NS
Total Amino Acids	92.3 ± 3.4	86.0 ± 3.3	89.3 ± 3.6	92.6 ± 2.5	NS

[a] Different uppercase letter in the same line show significant differences (*P* < 0.05) among the different food types for protein (N x 6.25) and amino acids.

Table 4 True ileal digestibility (TID) of amino acids in seitan, tofu, soya milk and pea emulsion in minipigs. Values are means ± SEM, n = 6. [a]

	Seitan	Tofu	Soya milk	Pea emulsion	*P*
$Leu_{endo}/Leut_{otal}$ ileum (%)[b]	71.6^{a} ± 7.7	67.0^{a} ± 7.0	46.1^{c} ± 4.8	62.4^{a,b} ± 5.3	0.025
Leu_{endo}/Leu_{intake} (%)[c]	11.7 ± 3.4	12.8 ± 2.0	9.0 ± 0.8	9.7 ± 1.8	NS
TID, %					
Indispensable amino acids, %					
Histidine	95.6 ± 1.1	95.7 ± 2.1	92.2 ± 3.2	91.8 ± 1.2	NS
Isoleucine	96.1^{a} ± 1.3	92.9^{a,b} ± 1.7	89.1^{b} ± 2.9	94.6^{a} ± 1.0	0.035
Leucine	96.4^{a} ± 1.0	93.3^{a} ± 1.9	88.3^{b} ± 2.6	94.5^{a} ± 0.9	0.009
Lysine	90.1 ± 5.3	93.9 ± 2.1	92.5 ± 2.4	93.9 ± 1.2	NS
Methionine	97.8^{a} ± 1.1	85.6^{b} ± 6.0	99.3^{a} ± 2.5	80.3^{b} ± 7.7	0.034
Phenylalanine	99.9 ± 2.0	98.6 ± 2.2	94.7 ± 2.3	98.3 ± 1.3	NS
Threonine	94.1 ± 5.3	93.0 ± 4.5	90.6 ± 3.9	92.4 ± 2.7	NS
Tryptophan	92.2 ± 4.6	102.6 ± 2.4	99.8 ± 2.1	99.6 ± 3.5	NS
Valine	96.8 ± 1.9	93.3 ± 2.2	90.4 ± 2.9	94.8 ± 1.4	NS
Dispensable amino acids, %					
Arginine	94.7 ± 4.8	90.4 ± 4.3	84.0 ± 7.1	94.2 ± 1.5	NS
Alanine	96.7 ± 3.1	94.7 ± 3.2	90.6 ± 3.1	96.3 ± 1.3	NS
Aspartic acid/Asparagine	92.3 ± 3.4	91.0 ± 2.9	88.9 ± 3.5	85.7 ± 2.0	NS
Cysteine	99.0 ± 5.6	83.8 ± 11.7	104.6 ± 4.2	100.8 ± 9.9	NS
Glutamic acid/Glutamine	99.6 ± 0.6	97.7 ± 1.5	93.4 ± 3.2	94.8 ± 1.0	NS
Glycine	87.9 ± 10.2	94.3 ± 4.6	97.4 ± 4.3	91.3 ± 7.1	NS
Proline	98.2 ± 1.1	103.2 ± 3.4	97.1 ± 3.2	102.7 ± 3.0	NS
Serine	99.8 ± 2.5	95.9 ± 3.4	92.8 ± 3.0	96.0 ± 1.6	NS
Tyrosine	94.4 ± 1.5	92.0 ± 2.8	90.1 ± 3.0	97.7 ± 2.5	NS
Total Amino Acids	97.5 ± 1.5	95.0 ± 2.3	92.3 ± 3.0	94.2 ± 1.3	NS

[a] Different uppercase letter in the same line show significant differences ($P < 0.05$) among the different food types for amino acids.

[b] Percent of leucine of endogenous origin in leucine flowing at the ileum

[c] Endogenous leucine flowing at the ileum relative to ingested leucine

Table 5 Digestible indispensable amino acids (g/ 100 AA) in seitan, tofu, soya milk and pea emulsion in minipigs. Values are means ± SEM, n = 6. [a]

	Seitan	Tofu	Soya milk	Pea emulsion	*P*
Histidine	1.34^{c} ± 0.03	5.91^{b} ± 0.06	5.65^{b} ± 0.10	7.10^{a} ± 0.04	0.001
Isoleucine	3.79^{b} ± 0.05	4.94^{a} ± 0.09	4.74^{a} ± 0.16	4.85^{a} ± 0.05	0.001
Leucine	7.15^{c} ± 0.07	8.13^{b} ± 0.17	7.46^{c} ± 0.22	8.74^{a} ± 0.08	0.001
Lysine	1.34^{c} ± 0.08	5.91^{b} ± 0.13	5.65^{b} ± 0.15	7.10^{a} ± 0.09	0.001
Methionine	2.00^{a} ± 0.02	1.07^{c} ± 0.07	1.42^{b} ± 0.04	0.42^{d} ± 0.04	0.001
Cysteine	2.08^{a} ± 0.12	1.17^{b} ± 0.16	1.82^{a} ± 0.07	0.96^{b} ± 0.09	0.001
Phenylalanine	5.37^{b} ± 0.11	5.76^{a} ± 0.13	5.30^{b} ± 0.13	5.95^{a} ± 0.08	0.001
Tyrosine	2.40^{c} ± 0.04	2.84^{b} ± 0.09	2.70^{b} ± 0.09	3.30^{a} ± 0.08	0.001
Threonine	2.27^{b} ± 0.13	3.71^{a} ± 0.18	3.73^{a} ± 0.16	3.86^{a} ± 0.11	0.001
Tryptophan	2.73^{b} ± 0.13	2.63^{b} ± 0.06	2.82^{b} ± 0.06	3.23^{a} ± 0.11	0.002
Valine	3.95^{b} ± 0.08	4.90^{a} ± 0.12	4.70^{a} ± 0.15	4.95^{a} ± 0.07	0.001

[a] Different uppercase letter in the same line show significant differences ($P < 0.05$) among the different food types for amino acids.

Table 6 Digestible indispensable amino acid scores (DIAAS, %), and limiting amino acid for seitan, tofu, soya milk and pea emulsion.

	Seitan	Tofu	Soya milk	Pea emulsion
Infant (birth to 6 months)	19	68	78	42
	(Lys)	(SAAs)	(Leu)	(SAAs)
Child (6 months to 3 years)	24	83	99	51
	(Lys)	(SAAs)	(Lys)	(SAAs)
Older child, adolescent, adult	28	97	117	60
	(Lys)	(SAAs)	(Val)	(SAAs)

Scores were calculated using the recommended amino acid scoring patterns for three age groups (FAO, 2013).

Table S1 Composition of test-meals (g, as fed)

	Seitan meal	Tofu meal	Soya milk meal	Pea emulsion meal
Seitan (dehydrated)	42.1	-	-	-
Tofu (dehaydrated)	-	68.1	-	-
Soya milk	-	-	1010.1	-
Pea emulsion	-	-	-	1010.1
Maltodextrine	129.6	132.6	120.0	133.0
Sugar	30	30	30	30
Soybean oil	20.8		2.1	2.1
Water	67.4	204.6	-	-

Table S2 N and amino acids ileal flows observed in minipigs (n = 6) receiving a protein-free diet. Values are means ± SEM.

	Ileal flow, g
N (Dumas method)	1.82 ± 0.51
Indispensable amino acids	
Histidine	0.09 ± 0.02
Isoleucine	0.13 ± 0.04
Leucine	0.24 ± 0.06
Lysine	0.14 ± 0.04
Methionine	0.04 ± 0.01
Phenylalanine	0.22 ± 0.06
Threonine	0.29 ± 0.09
Tryptophan	0.14 ±0.04
Valine	0.20 ± 0.05
Dispensable amino acids	
Arginine	0.14 ± 0.04
Alanine	0.38 ± 0.10
Aspartic acid/Asparagine	0.31 ± 0.09
Cysteine	0.14 ± 0.04
Glutamic acid/Glutamine	0.45 ± 0.12
Glycine	0.38 ± 0.10
Proline	0.30 ± 0.08
Serine	0.23 ± 0.06
Tyrosine	0.10 ± 0.03
Total amino acids	3.72 ± 1.01