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Atoms for Peace and Development

Construction of a database on ileal digestibility of protein and amino acids in foods consumed by humans

Report of a joint FAO/IAEA meeting in Paris
26–29 November 2024



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Food and Agriculture Organization of the United Nations
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Contents

Abbreviations.....	v
Participants.....	vii
1. Introduction.....	1
Opening remarks.....	1
2. Bringing the database into operation.....	3
Database structure.....	3
Key variables to be considered.....	3
Database update and data collection process.....	4
Consensus on data architecture.....	4
Food classification, and protein and amino acid content.....	5
Challenges of protein content quantification.....	5
Protein quality variables to be included in the database.....	6
3. Models and data to be included in the database.....	8
Oro ileal digestibility in humans.....	8
Indirect isotope methods in humans.....	8
Digestibility determined in animal models.....	9
Digestibility determined <i>in vitro</i>	10
4. Roadmap for database construction and next steps.....	12
Calculation of protein quality scores.....	12
Data collection: update on systematic reviews (<i>in vivo</i> data).....	12
Data collection: update on systematic reviews (<i>in vitro</i> data).....	13
Data collection: next steps.....	13
Discussion to coordinate a call for data.....	14
Discussion on other data collection.....	14
Definition of next steps and action plan.....	15
Summary of roadmap for database construction.....	15

Abbreviations

AA	amino acid
DIAAR	digestible indispensable amino acid ratio
DIAAS	digestible indispensable amino acid score
FAO	Food and Agriculture Organization of the United Nations
IAAO	indicator amino acid oxidation
IAEA	International Atomic Energy Agency
INFOODS	International Network of Food Data Systems
PDCAAS	protein digestibility-corrected amino acid score

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1. Introduction

The Food and Nutrition Division of the Food and Agriculture Organization of the United Nations (FAO) and the Division of Human Health of the International Atomic Energy Agency (IAEA) jointly organized a technical meeting on the development of a protein database and the way forward for reviewing protein requirements in Vienna in October 2022. During the meeting, a framework for the development of a protein digestibility database on foods consumed by humans to accurately measure protein quality in foods and diets was discussed and agreed upon. As a follow-up, a joint FAO/IAEA technical advisory group on protein digestibility was established and a virtual meeting was held in April 2024 to mark the official start of the database construction. The virtual meeting's main outcome was a workplan which included timelines for the development of the database template, the publication of calls for data and an agreement on the list of variables to be included in the database. A joint FAO/IAEA technical meeting was held in Paris from 26 to 29 November 2024. At the meeting, the level of progress regarding the construction and validation of the database on ileal digestibility of protein and individual amino acids in foods consumed by humans was discussed. Up-to-date information was also provided on the protein quality from food sources – according to the appropriate scoring method – and actions required to finalize the database were evaluated. In addition, the meeting outlined steps for the formulation and publication of calls for data to populate the database and established a framework for its validation.

Opening remarks

Welcome and meeting framework

Mr Victor Owino of IAEA and Ms Maria Xipsiti of FAO moderated the opening session. Mr Owino welcomed the participants and underlined the importance of this cross-agency collaboration in delivering an expertly curated database on protein and amino acid (AA) ileal digestibility. He emphasized IAEA's interest in promoting the role of stable isotopes in the fight against all forms of malnutrition, and in advancing sustainable dietary habits. He thanked the experts for their commitment to the project and for the work

completed since their last meeting in November 2022 and reminded them of pertinent timelines. After welcoming the participants, Ms Xipsiti noted the importance of the two agencies coming together to co-organize a meeting of this significance. She explained the context of the FAO and IAEA collaboration and together with Mr Owino, they gave a brief introduction to the Atoms4Food flagship initiative. Atoms4Food, a joint FAO/IAEA initiative, aims to help countries effectively use nuclear science and related technologies for food and agricultural development.

Following the introductions Ms Xipsiti took the opportunity to outline the need for a protein digestibility database as part of the actions undertaken by the United Nations (UN) agencies to achieve the Sustainable Development Goals. She highlighted the importance of factors such as sustainably feeding the world's population, the increasing impact of climate change and war conflicts, and how the shift to more sustainable protein sources in this context could deliver better environmental and nutritional outcomes. A key factor in promoting specific protein sources is the evidence-based assessment of their nutritional quality and more specifically their capacity to provide an adequate profile of indispensable AA after considering their composition and digestibility.

Ms Xipsiti reminded the participants that nutrition is at the centre of FAO's strategic framework, with ending hunger, achieving food security, promoting nutritious foods and increasing access to healthy diets all collectively forming one of the four main pillars in FAO's strategy. While the development and hosting of this database falls within the Organization's core function to "assemble, analyse, monitor and improve access to data and information".

FAO has been working on issuing nutritional guidelines and setting global human energy and nutrient requirements for nearly 70 years. The topic of protein requirements has been an FAO priority since October 1955. Between 1963 and 2002, in collaboration with the World Health Organization and/or the United Nations University, FAO has conducted expert consultations on energy and protein requirements for humans. As well as providing reference values of protein requirements, FAO has worked on protein quality evaluation since December 1989, using protein quality criteria and metrics, as well as guidance on research approaches

and methods to evaluate protein quality in foods consumed by humans.

Overview of actions leading to the present meeting

In 2022, the Food and Nutrition Division of FAO and the Division of Human Health of IAEA jointly organized a technical meeting held in hybrid format in Vienna from 10 to 13 October. This meeting discussed FAO's and IAEA's commitment towards the creation of a protein digestibility database and its application as the way forward for reviewing protein quality. The experts at this meeting indicated to the agencies that research was mature enough to be compiled in an expertly curated database to be used by multiple audiences. The main issue of AA availability from protein sources had penetrated multiple expert meetings, and data on AA digestibility of foods and diets from various regions had become available. Already in 2014, the FAO expert meeting on protein quality highlighted the need to establish a robust database of protein digestibility of foods and diets commonly consumed worldwide, including those consumed in low-income countries. The 2022 joint FAO/IAEA meeting was the first step towards this database.

Ms Xipsiti presented the key outcomes and recommendations from the 2022 meeting and the intended use of the database. Several key points were discussed regarding the validation and public availability of nutritional data and the global adoption of the digestible indispensable amino acid score (DIAAS). It was emphasized that published data should be validated by FAO and other international organizations to ensure reliability. Additionally, the importance of enhancing public access to such data was highlighted, allowing both experts and non-experts, including research and technical professionals, food manufacturers, public health professionals, policymakers and regulatory authorities, to make informed decisions. The database will be freely accessible to facilitate broader utilization and informed decision making. The use of the database by public health professionals was recognized as playing a vital role in translating nutritional requirements into practical dietary guidance. This included tailoring recommendations to the dietary patterns of individuals or specific population subgroups. Furthermore, the assessment of the complementarity of protein sources was discussed, focusing on the benefits of combining different foods to provide an adequate quantity of each of the nine indispensable amino acids (IAAs) in bioavailable form as part of a balanced diet. The database is envisaged to inform protein quality estimates used as a basis for policies and programmes that aim to improve nutrition throughout the world, including dietary assessment, food labelling and

claims, school food and food security programmes, food based dietary guidelines and compositional standards for special meals and specialized nutrition.

Ms Xipsiti presented the first set of criteria proposed to inform data selection and curation in the database. She also outlined the structure and the aims of the protein quality database technical advisory group, as a subgroup of the expert committee, tasked to provide advice on the construction of the joint FAO/IAEA database on ileal digestibility of protein and individual AA in foods consumed by humans. The eight members of this group:

- i. provide technical input and recommendations for technical reports, calls for data, data obtained and related technical products;
- ii. promote coherence and complementarity with existing and planned normative products in protein quality assessment; and
- iii. actively participate (as appropriate and relevant) in meetings and communications organized by FAO and IAEA.

The opening remarks concluded with the presentation of the meeting's objectives, which included discussing the progress of the database construction and the appropriate protein quality scoring methods, finalizing technical discussions and evaluating the actions required to complete construction of the database. The meeting was therefore split into three sections:

- i. bringing the database into operation;
- ii. models and data to be included in the database; and
- iii. roadmap for database construction and next steps.

2. Bringing the database into operation

Database structure

Mr Hans Stein chaired the session on database structure in which Ms Fernanda Grande of FAO offered a virtual presentation on practical aspects related to FAO/International Network of Food Data Systems (INFOODS) food composition databases, and Mr Daniel Tomé and Mr Antonis Vlassopoulos presented the proposed database structure and elements regarding data quality assessment. Ms Grande set out the definition and key characteristics of the food composition tables or databases. She then introduced INFOODS and explained its role in enhancing food composition data quality and availability globally. The International Network of Food Data Systems (INFOODS) helps to develop international standards, guidelines and compilation tools, publish new or updated food composition tables, as well as organize expert meetings, conferences and capacity building activities. After presenting the FAO/INFOODS website and databases, Ms Grande gave an overview of the process required to prepare and publish a food composition table or database on the FAO/INFOODS website. Key elements mentioned related to the database format – which can only be a Microsoft Excel spreadsheet – and the need for bibliographical information acknowledging original data sources. Furthermore, she highlighted the importance of assigning unique data codes to each database entry and adhering to standardized units and food description elements to maintain consistency across food composition data. An overview of all the elements needed for the appropriate food descriptions was presented and experts discussed which of those elements were important for the database and which were available in the literature.

Mr Vlassopoulos spoke on the topic of data quality assessment. Existing frameworks as presented by EuroFIR were used as a basis for developing the methodology to assess data quality. The proposal for assessing data quality considered the following factors:

- i. the food description;
- ii. the experimental method used to assess protein digestibility;
- iii. the method of quantification of AA and protein content;
- iv. the sampling plan; and
- v. the sample handling and the number of samples analysed per experiment.

These factors could be evaluated individually, or they could be included in an overall data quality score.

Key variables to be considered

Mr Tomé outlined a proposal for the database architecture. Beyond the necessary product description, Mr Tomé highlighted the variables linked to protein quality that should be included in the database. The database could include ileal digestibility values for 11 AAs, 9 indispensable AAs (lysine, threonine, methionine, tryptophan, valine, isoleucine, leucine, phenylalanine and histidine) and 2 conditionally indispensable AAs (cysteine and tyrosine). The database should also contain data on protein content calculated using the nitrogen to protein conversion factor, as well as the protein content derived by the sum of the 20 anhydrous AA. In terms of bioavailability, the database should include values of ileal digestibility for each individual AA and protein nitrogen digestibility. A database architecture proposal was presented alongside a discussion on the preferred data sources. Mr Tomé highlighted the ranking of digestibility measurement methods, starting with humans as the gold standard. This was followed by pig models, rat models and finally the potential to include data from *in vitro* protocols. Mr Tomé presented the algorithms to calculate the chemical AA score, the protein digestibility corrected amino acid score (PDCAAS) and the digestible indispensable amino acid score (DIAAS). The user guide should include the PDCAAS and DIAAS algorithms, along with clear instructions on how they should be calculated – offering examples of PDCAAS and DIAAS

calculation in ingredient mixes as well as information on using the appropriate reference patterns per age group.

Based on the discussions, it was agreed that the first version of the database could include both feed and food, with a variable specifying which one an entry was. It was also agreed that items could include both single foods/feeds or mixtures. In the case of mixtures, the database must describe the proportions used in the food/feed mix, with different entries being created for different recipes. Also of interest was the degree to which food was processed and the method used. It was agreed that as much information as possible should be extracted from the literature. The experts highlighted that it is important to document the processing of the food itself (cooked, boiled, oven-dried) as well as any previous processing of the ingredients, e.g. corn dehulled, fermented or dried. In this sense, it is important for the food description to provide information both on the processing of the ingredients before and during storage, as well as the processing carried out during the food/feed preparation. Other elements such as country of origin, species and seasonality should be entered in the database as provided in the published report. If any data is missing, experts may contact the authors to obtain such information.

Based on Mr Tomé's proposal the database could include a variable indicating the quality of the data, or all the necessary information to allow the user to carry out their own evaluation of the data quality. Meanwhile, the user guide should explain the principles of data collection and curation, and also provide information on the model and methods used to assess *in vivo* and *in vitro* digestibility, as well as the factors affecting data quality.

Database update and data collection process

Ms Grande informed the experts on the process of data checking before publication and the need for two independent compilers to check all data entries. She also explained that the publishing process includes comprehensive documentation in the form of a user guide and other relevant manuals for the correct use of the data, as well as data submission guidelines. A co-publishing agreement between FAO and IAEA will be required to publish the database, and an editor and designer will also need to be hired. In addition, the database will need to be submitted to the FAO publications workflow system. Examples of all the necessary documentation was provided. The experts

were also informed that databases are usually updated approximately every seven years, depending on the needs of the database in question, and the updates can be carried out either independently by FAO or via the expert panel. The experts were informed by Ms Grande, Ms Xipsiti and Mr Owino that alongside the database publication, a call for data should be published. The call for data serves as a structured format for collaborators and interested parties to submit their own data in the dataset and/or submit data corrections for data already published within the database.

The experts discussed the purpose of the call for data and it was agreed that it should include a Microsoft Excel spreadsheet – identical to the database – for compilers to submit their own data, alongside a document explaining what types of data are eligible for inclusion. The inclusion of unpublished data from private companies and laboratories was discussed among the experts, with different views presented. It was mentioned that private companies and laboratories may have proprietary data on digestibility of foods of interest and that these could be considered for inclusion in the database. After careful consideration, the experts concluded that data quality could be jeopardized if non-peer reviewed information was added to the database. Given that this database will be verified by FAO, it was agreed that only the highest quality data should be included. Companies and private laboratories should be encouraged to publish their analyses and in the spirit of science and public health promotion it was agreed that the call for data should specify that only peer reviewed data can be submitted to the database. The call for data will also include information on which protocols and methods are considered high quality, so that both the database documentation (user guide) and the call for data act as a capacity building activity.

Consensus on data architecture

The experts agreed with Mr Tomé's proposed database architecture and agreed that the database should include all digestibility data available in the literature and that it should be detailed enough for users to easily identify the digestibility and DIAAS values as needed. A discussion was held on the ability of the database to include PDCAAS values as the literature reviews planned for data collection only focus on ileal digestibility studies. The experts also agreed that the database should have all the variables completed for each entry. To achieve this, either manually or automatically through numerical functions, compilers should calculate AA and nitrogen digestibility, the

content of available AA and protein, as well as PDCAAS and DIAAS values for every age group. The AA composition should also be reported both per 100 g of food and per gram of protein. As part of the food description, it is important to include dry matter. When data on human trials are presented, it is important for the database to include information on the country, the ethnicity and the age of the population studied as there can be differences in the digestibility values among populations with different demographic characteristics.

As far as the data quality scoring was concerned, the experts agreed that the user guide should include information that enables users to evaluate data quality. However, it was mentioned that the first published version of the database should only include high quality data. As such, data produced by experiments with limited sample size, inappropriate chemical analysis or poor food descriptions would be excluded from the database. The reason for this exclusion could be attributed to the peer review process which minimizes the likelihood of low-quality data being published and to the literature review screening process in which low-quality data also leads to the exclusion of low-quality reports. The experts mentioned that as the database expands and new data are submitted to FAO for inclusion, a data quality assessment matrix should be developed and used by FAO for data inclusion assessment. Once the matrix is developed there would be an opportunity to assess whether lower quality, but useful data should be added to the database with a variable indicating their quality. At this stage, this cannot be agreed upon, but it is important to consider. A key point raised was the inclusion of digestibility data for novel protein sources or traditional protein sources from lower-middle-income countries which might have value in terms of innovation and policy planning, but might be lacking in high-quality data. The experts agreed to revisit the topic after the first stable version of the database is available and once the literature reviews have highlighted the volume of papers excluded from the analysis based on their quality.

Food classification, and protein and amino acid content

Ms Isidra Recio chaired the session in which Ms Maria Haye presented food protein sources classification and Mr Jurriaan J Mes presented food protein (nitrogen) and AA content. Ms Haye discussed protein digestibility and hydrolysate generation, focusing on marine animals and seaweed as an example of novel and sustainable protein sources. The presentation

started by highlighting the importance of seaweed as a source of bioactive polyphenols and phlorotannins, before going on to highlight the increased recognition of seaweed as a protein source containing between 7 percent and 45 percent protein per dry weight. In her presentation, Ms Haye highlighted the different methods of protein extraction and quantification, and the impact on protein yields. The presentation highlighted that protein quantification in the case of seaweed has higher accuracy using the total AA quantification method than the method used by the Association of Official Agricultural Chemists. The main issue with the nitrogen methods used by the Association of Official Agricultural Chemists was the lack of seaweed specific nitrogen conversion factors, and examples were presented to highlight the over- and underestimation of protein content linked to this issue. Other marine animals, as well as marine animal processing by-products were discussed for their potential to be used as protein sources through extraction and/or hydrolysis. Beyond their protein content, seaweeds are also a source of peptides with potential functions as health-promoting bioactives. Examples of the impact on collagen synthesis, heart health, hypertension, weight management, gut health, inflammation and pain were presented. Other functional properties in food development linked to bitterness and sensory elements were also presented alongside the potential of marine protein being used in the treatment of allergies. The presentation raised issues on the amount of existing research evaluating protein quality in such novel foods, given that human and animal data are limited and most papers employ *in vitro* models. An issue was also raised on whether a database that does not include *in vitro* data for novel protein sources could miss the potential to promote their utilization within the food industry.

Challenges of protein content quantification

Mr Mes presented the issues related to nitrogen-based protein quantification and its difficulties to exclude nitrogen derived from urea, creatine, creatinine, nitrite, nitrate, ammonia, nucleotides, amino-sugar oligosaccharides, polyamines and amino alcohols of phospholipids. Despite being easy to apply and low in cost the nitrogen-based protein quantification methods are still suboptimal compared to total AA quantification methods, which remain the gold standard. A list of advantages and disadvantages for each method was presented, as well as the impact of extraction methods and buffers used on the agreement between the two methods.

As an example, the choice of extraction buffer was linked to a 36 to 104 percent higher protein content estimation when nitrogen-based methods were used. A discussion took place on the need for specific nitrogen conversion factors which account for the non-protein nitrogen. However, even when specific factors are applied, they may need to vary to account for seasonality or extraction method. The discussion highlighted that changing the protein quantification methods to include multiple conversion factors is unlikely to solve the issues, what's more a transversal use of a 6.25 conversion factor (the current approach) is linked to significant overestimation of protein content. In parallel, more accurate protein quantification methods are costly and a change of reference methods would cause the food industry to incur costs, both through the higher analytical costs and the need to update all nutritional declarations. Examples of tables of species-specific nitrogen conversion factors were presented with reference made to the need to guide users in their application if the database was to employ this approach. Regarding the inclusion of AA data in the database, Mr Mes highlighted that published reports might not include data for all 11 AAs of interest for the database, or they might even only report content and digestibility on the total protein level and not on the AA level. He also underlined issues related to the reference unit used, as this can be, for example, per 100 g of food, per 100 g of dry matter or per 100 g of protein. His final point was linked to the knowledge of whether AA values refer to hydrous or anhydrous forms, a question that is linked to ~14 percent differences in values. A proposal was made for the database to include raw data as reported in the publications and recalculated to standard units. Protein content should be reported as grams per 100 g of food, as well as the sum of AA (anhydrous) and individual AAs. An example of a similar database from Wageningen University and Research was presented alongside its potential uses, while names and examples of other databases of AA content for different foods were also given to both inspire the experts and raise potential concerns on connectivity with other similar initiatives/databases. The presentation concluded with an overview of the ASReview open-source software to be used in systematic reviews and its potential to automate data subtraction from the literature, as well as the limitations that such technologies can present in data extraction. After a discussion on the value of a protein digestibility database and its potential applications, a proposed database structure was presented.

Protein quality variables to be included in the database

The experts collectively discussed the protein and AA values that needed to be included in the database. The first point of agreement was that although the inclusion of bioactive components (even peptides) was of high potential interest, it remained out of scope for the current database which proposed to cover only digestibility and quality values. The experts agreed that the database should include total protein content and that it should indicate whether the value was derived using a nitrogen-conversion factor or the sum of all AAs. When a nitrogen-conversion factor is used all data should be back calculated using a common factor of 6.25 for data harmonization. The data should all be expressed in the same unit and the proposal was made for gram per 100 g of food and gram per 100 g of protein with the database providing a value for dry matter (most likely in the food description). The PDCAAS, digestible indispensable amino acid ratio (DIAAR) and DIAAS calculations should be performed by the compilers and a common reference value should be used. In this context the DIAAS and PDCAAS values as reported in the publication will not be used and will instead be calculated based on the protein and AA content, and digestibility values. This will ensure uniformity of the dataset, as well as the capacity to adjust the protein quality calculations according to the latest guidelines. The experts discussed whether the database could be automatically linked to other databases, and the process for data inclusion and quality checking. Ms Xipsiti, Mr Owino and Mr Vlassopoulos explained that FAO will be responsible both for including data collected from the call for data, and for subsequent database updates. In this regard, automatic connections and continuous expansions are not an option. The experts will need to provide FAO with a matrix to assess data quality and the tools to populate the database correctly, both of which will most probably be presented in the user guide. FAO can then plan the database update and expansion. If there is a need for a follow-up literature review or specific tasks linked to the update and expansion, the expert group could be involved in the update, but this will be discussed at the time of the update. Until then, the user guide and the call for data need to be detailed enough for FAO compilers to act independently.

Table 1. Data architecture and variables in and out of scope**Variables to be included**

- Twenty amino acids as mg/g protein
- Protein content based on nitrogen measurement as g/100 g food
- Protein content based on the sum of amino acid content as g/100 g food
- Amino acid and protein digestibility
- Digestible indispensable amino acid ratio
- Protein digestibility corrected amino acid score and digestible indispensable amino acid score values for ages: *up to 6 months, 6 months to 3 years and >3 years up to adulthood*
- Food description based on standards of International Network of Food Data Systems
- Food composition especially in terms of dry matter and water content
- Details on the study population (particularly country, age and ethnicity)

Variables out of scope

- Bioactive component content
- Peptide content
- Indicator amino acid oxidation derived amino acid values (which will be compiled in a separate database)

Source: Authors' own elaboration.

3. Models and data to be included in the database

Oro-ileal digestibility in humans

In a section chaired by Mr Kurpad, Ms Gaudichon and Ms Hodgkinson presented the methodological aspects of collecting oro-ileal digestibility data in humans. The presentations included the issues linked to the methodology of intestinal tubing. A detailed description was given of the apparatus and how an intestinal tube is inserted, while other methods that can be used to collect ileal fluid were also explained. Ms Gaudichon described examples of malfunction of the tube and tube obstruction which leads to inability to collect samples. Ms Hodgkinson then outlined the requirements for ileostomates to participate in digestibility studies in terms of health, anthropometry and gastro-intestinal tract functionality and anatomy. The methodology used to determine the digestibility of food/a meal through ileal fluid collection on the day of the experiment was presented by both speakers. The details of the test meals and controls commonly applied, as well as the indigestible markers used were also presented.

This section focused on a discussion on whether ileostomates or intestinal tubing should be preferred as a method, and whether the two approaches could be considered equivalent. The speakers presented data on the agreement between the two methods, concluding that the methods show very limited differences. Given the difficulty in identifying potential ileostomates able to participate and the fact that such participants can only take part in a limited number of trials, ileostomates were proposed as suitable for research and validation protocol, but not for routine digestibility measurements. Both ileostomates and intestinal tubing are invasive protocols, however intestinal tubing shows some advantages as it can be performed in larger samples and is easier to perform on individuals with good overall health. Nonetheless, although both protocols are recognized as the gold standard, they are equally difficult to implement with high volume digestibility screening and are linked to ethical considerations. Hence, they should be used in moderation and as a validation step for other protocols. For the database, data obtained by the two methods will be included as

equivalent in terms of data quality and the database will include information on the ileal fluid collection method, the population and the indigestible marker used for information and transparency.

Indirect isotope methods in humans

During the section chaired by Ms Gaudichon, Mr Kurpad presented the dual isotope true digestibility and Ms Courtney-Martin the indicator amino acid oxidation (IAAO) method. In his presentation Mr Kurpad explained the principles of the dual isotope method, which is based on the ingestion of an ^2H -labelled test protein together with a ^{13}C -reference protein with known digestibility. For digestibility calculation, the ratio of plasma enrichment in indispensable AA relative to the meal is calculated for the test and the reference protein and the digestibility of the test meal is calculated compared to the reference protein. The method is not impacted by liver and/or splanchnic extraction. Methods for transamination correction were presented as well as examples of intrinsic labelling of grains and goat milk via D_2O (deuterium) water use in plants. The use of ^2H -labelled AA mix orally in hens was presented for the labelling of egg protein. The presentation continued by discussing whether the use of a reference protein or a labelled AA mix would impact the digestibility assessment. Although in 'normal' individuals the two methods show no difference, it is unclear whether the results would be the same in very young children (7–9 years old).

Ms Courtney-Martin presented the principles of the IAAO method. The method assumes that when the test AA is below requirement, all other indispensable AAs are in relative excess and hence can be oxidized. The method then tracks the L-[1- ^{13}C] phenylalanine oxidation in the breath as an indicator to determine whether the subject is in a state of protein synthesis or oxidation. By comparing IAAO in response to a test food protein with IAAO response to a reference food or AA mix the method offers an estimation of protein quality. Examples were given for the use of

the methods in animals and humans alongside the presentation of the methodological protocols.

The experts discussed the two methods and concluded that despite its value, the IAAO method does not provide a direct measure of digestibility which is the focus of the database currently under construction. So, while data on the IAAO will not be added in the same dataset, a secondary dataset will be created with data solely obtained through this method and a separate guide will be created for the use of these data.

Digestibility determined in animal models

In a section chaired by Ms Hodgkinson, Mr Stein presented the protocols, applications and benefits of the digestibility models in pigs and Ms Calvez presented the methodology for ileal digestibility measurement in rat models.

Mr Stein explained how an ileal cannula is installed in pigs and addressed issues related to animal welfare. The presentation included examples of ileal digestibility values measured in pig models for a number of foods and the impact of processing on digestibility. At this point, the experts discussed how the database should include information on the processing of the foods used in the protocols to explain potential differences. Mr Stein showed that ileal digestibility measured ~93 percent agreement in human and pig models, with higher agreement in higher digestibility coefficients. The similarities between the digestive tracts of pigs and humans were discussed, with attention drawn to the capacity of pigs to consume foods/ingredients and mixes that are uncooked or prepared in a form that would make them ingestible for humans. In this sense, published data on pigs would need to be classified as feed and although they might provide an indication of digestibility, these data could not be used for the design of human foods. Examples of DIAAS and PDCAAS calculation in various foods were presented alongside comparison data between plant and animal-based protein sources, combination of protein sources etc.

Ms Calvez presented the same protocols for rat models, in which although the upper gastrointestinal tract mimics that of humans, their eating habits (small and multiple meals, coprophagia) and the inability to install a canula present challenges. Ms Calvez presented the ileal fluid collection method in rat models and described ways to reduce the impact of rat eating habits on the digestibility estimation. Overall, although there is no direct comparison of digestibility estimates in humans and in rats, indirect comparisons show good correlations up to 80 percent for mean AA digestibility. Ms Calvez also showed that ileal and caecal digestibility values in rats are comparable with >98 percent agreement. During her presentation Ms Calvez also updated the experts on the status of the literature review on the digestibility of foods using rat models. Out of 477 articles identified, 33 were considered eligible for inclusion with the majority (23 articles) reporting ileal digestibility and the remainder reporting caecal digestibility. The articles included data for 27 foods and 8 pet foods, while they also included different processing methods and investigated the effect of factors like fibres and tannins on digestibility. The protocols used both chromium oxide and titanium dioxide as markers, continuous hourly meals or single meal feeding, while various methods were employed to determine endogenous AA losses.

The experts agreed that both data sources should be included in the database. Data should be presented separately for each study and each model used for each food. Ileal digestibility will be prioritized for rat data, but when ileal digestibility data are not available caecal digestibility may be considered. In any case, when the same food in the database has digestibility data from multiple methods, then the data should: i) clearly indicate the method used; ii) state whether it is ileal or caecal digestibility; and iii) present the data on the same food in a grouped manner (in consecutive rows) and in descending order from humans to pigs to rats.

Digestibility determined *in vitro*

The section on digestibility determined *in vitro* was chaired by Ms Hayes. Ms Recio presented an overview of the different *in vitro* systems and Ms Fei Han the measurement of digestibility *in vitro*. *In vitro* methods are an important option given it is likely that they will be used in the future due to ethical considerations with studies involving humans or animal models.

There are many different types of *in vitro* methods and it was concluded that they must be further developed and validated. Currently, there are several different *in vitro* models, differing in the reaction conditions and most likely in the results which are generated. Static *in vitro* methods are very simple, low-cost methods with good reproducibility, but they do not mimic the gastric emptying or absorption and physical processes such as gastric movements which are difficult to simulate. These static methods use enzymes of animal origin, and the pH is generally fixed compared to *in vivo* where there is a pH gradient. Other methods include semi-dynamic and dynamic methods which also use animal enzymes. Some of them overcome the limitations of the fixed pH, allow kinetic studies including gastric emptying and in some more complex and more expensive devices, absorption and colonic fermentation are included. Different instruments are available from different laboratories, meaning it can be difficult to compare equipment between laboratories. More recently, there are *in silico* methods using artificial intelligence and machine learning which are cheap and animal-free but are still under development.

Static methods are preferred. In these methods, the foods are digested in different batches that sequentially reproduce gastric and duodenal intestinal digestion using pepsin and pancreatic enzymes, respectively. There are different ways of measuring protein digestibility. Some methods measure digestibility based on pH measurement, like the pH drop and pH stat methods – these are simple and very low cost. In the literature, good reproducibility is reported and acceptable *in vivo/in vitro* correlations have been found for some substrates. However, their limitations are that the digestion is just based on pH measurement, which is influenced by the food buffering capacity and the food composition such as the mineral content. Many factors affect the pH measurement and there are significant differences between the protein sources. The correlation with *in vivo* methods is better for plant proteins than for animal proteins and it was therefore proposed to use different correlation factors for different types of samples.

There are static methods based on the separation of a digestible and non-digestible fraction by membrane filtration or by using a dialysis cell. The dialysis cell allows the continued removal of low molecular weight products. The digestion products can be analysed in the same ways as for *in vivo* digest – by nitrogen, peptides and AA analysis. The reproducibility of the dialysis membrane filtration in these methods is poor because part of the digest can bind to the membrane material. In addition, there are no harmonized protocols or devices. Other static methods use precipitation of the digestates to separate digestible and non-digestible fractions. There are several methods based on precipitation with different agents including trichloroacetic acid, sulfosalicylic acid and methanol. These are more easily reproduced than the use of membranes. The *in vitro* approach often assumes that all soluble material is digestible, however, some soluble protein may avoid precipitation and the soluble material can contain high levels of small limit peptides which may not be absorbed *in vivo*. Heat-treated proteins are more susceptible to this being a problem. The products can be analysed as with *in vivo* methods. These methods have shown good comparability with ileal digestibility values but more comparative studies with a wide range of substrates and foods are needed. The methods that define the digestible fraction by precipitation are in general more easily reproduced than other static methods. However, currently none of the *in vitro* methods have been sufficiently validated and there must be additional studies to validate the methods in comparison to *in vivo* data and, if needed, adapt the digestion conditions to specific foods.

There is currently no agreed model for the *in vitro* determination of true ileal digestibility. These methods should be validated in comparison to *in vivo* methods and correction factors proposed. For instance, the use of similar food products with known *in vivo* digestibility could be included in order to have a validated control.

Table 2. Experimental methods of protein digestibility measurement to be included in the database and those out of scope

Digestibility experimental methods to be included

- Ileal digestibility methods in humans using either ileostomates or intestinal tubing
- Dual-isotope methods in humans
- Ileal digestibility methods in pigs using T-cannula
- Ileal digestibility methods in rats
- Caecal digestibility methods in rats
- *In vitro* methods from protocols validated against *in vivo methods* in humans or pigs

Note: the *in vitro* data will be compiled during a second stage following the completion of the relevant literature review

Methods out of scope

- Indicator amino acid oxidation values (which will be compiled in a separate database)
- *In vitro* values from protocols non-validated against *in vivo methods* (validation of the *in vitro* protocols should be carried out using a relevant reference protein e.g. plant-based foods with known ileal digestibility for other similar plant-based foods, and not casein or whey protein isolate as a reference for plant proteins)

Source: Authors' own elaboration.

4. Roadmap for database construction and next steps

This session addressed the information and data to include in the user guide and database. Its aim was to define the strategy for collecting and selecting data from published and non-published sources, for transferring data to the database and user guide, and for organizing bibliographic references and data sources.

Calculation of protein quality scores

During a section chaired by Mr Ferriolli on the calculation of protein quality scores, Mr Elango presented the reference AA patterns and Mr Tomé presented the procedure for PDCAAS and DIAAS calculation. The discussion included the choice of reference patterns for score calculations and their inclusion either in the user guide or the database. Mr Elango traced the history of the AA scoring patterns as presented in World Health Organization/FAO/United Nations University publications. He highlighted that although the 2007 report included six reference age groups (0.5 years, 1–2 years, 3–10 years, 11–14 years, 15–18 years and >18 years), the updated 2013 report reduces them down to three AA scoring patterns for ages of up to 6 months, 6 months to 3 years and above 3 years of age through to adulthood. As in the previous report, the AA reference pattern for the first six months of life corresponds to that of the human breast milk. In the over 6-month age bracket, the AA reference pattern is calculated by combining the AA requirements for protein maintenance and tissue growth AA requirements. The merging of the age groups 3–10 years, 11–14 years, 15–18 years and >18 years present some challenges in the assessment of protein quality. A long discussion took place on which were the most scientifically appropriate reference patterns to be used in the database. To avoid ambiguity given that different reference patterns can be found in the literature, the database will include DIAAS values for each age group for all foods included. Although the use of the older 2007 reference patterns would offer increased granularity and might allow for better tailoring of food design for each age group, it was agreed that it should follow the latest FAO guidance

since it is FAO that will host the database. As such, the use of the three reference patterns from the 2013 FAO report was agreed. Mr Elango then highlighted cases where the reference patterns might be failing to highlight the actual AA needs. The examples included the AA needs of children diagnosed with stunting and with active gut parasite infections (21 percent increase in lysine needs), the different AA needs during pregnancy per trimester, and also the different AA needs for males and females aged 65 and over. Those examples highlighted potential future research needs to inform the formulation of AA reference patterns for more age groups and/or life stages. However, for the time being the database will be aligned with the official FAO guidance.

Data collection: update on systematic reviews (*in vivo* data)

In another section chaired by Ms Calvez, data collection and updates by systematic reviews were discussed. Ms Recio and Ms Shertukde presented the status of the *in vitro* and *in vivo* literature reviews respectively. The discussions surrounding these updates remained at the technical level, mainly considering the timeline for completion, potential help needed and the next steps. Ms Shertukde reminded the experts of the previously agreed inclusion and exclusion criteria for the literature review on the *in vivo* trials. In terms of outcomes, faecal digestibility, apparent digestibility and protein efficiency ratio studies were excluded. Similarly for this review all *in vitro* data were excluded as were oro-faecal digestibility values and any plasma AAs measured without the use of an isotope tracer in the study protocol. As far as animal models were concerned, only pigs and rats were included and in the case of rat models oro-caecal digestibility values were also included in the review. The complete search strategy was presented along with the stages of the literature review. The search retrieved 5 300 abstracts which were identified by six screeners following a double independent review which resulted in 1 089 full text papers being carried forward. A total of ten

screeners were employed in the full text screening stage which resulted in the inclusion of 477 full articles (identified by two screeners) and an additional 86 full articles which were flagged for inclusion by just one screener and require verification by a second screener before inclusion. The majority of the articles (n=417) were results from pig models, while 29 studies were carried out on humans. Standardized ileal digestibility was the most common outcome studied in pig models (n=344), ileal digestibility through oro-ileal balance and true digestibility via dual stable isotope methods were the most commonly used in humans (n=11 and n=8, respectively). In the rat models oro-caecal digestibility was the most common outcome studied (n=14 out of a total of 29 rat model studies).

Data collection: update on systematic reviews (*in vitro* data)

Ms Recio outlined the search strategy for the *in vitro* literature review and explained the current stage of the process. To date, the search protocol has retrieved 9 210 articles from 1900 to 2023 which will be screened by two independent reviewers for inclusion. The original exclusion criteria proposed for the review were to reject any studies that report outcomes that are not protein digestibility (e.g. solubility, degree of hydrolysis or AA characterization). Studies using non-enzymatic methods to assess digestibility or enzymes which do not mimic the gastrointestinal tracts of humans, pigs or rats were also excluded. Dynamic, static and other *in vitro* digestion models will be included, and digestion methods like the pH-drop, pH-stat, multi-enzyme, two step digestion and the INFOGEST method will all be included in the review. All substrates will be analysed from food ingredients (isolates, concentrates), single foods and mixed foods to whole diets. In order to prioritize the screening process Ms Recio proposed three potential approaches to the experts. The first approach would be to include in the literature review reports that in the same study provide both *in vitro* and *in vivo* digestibility values for the exact same substrate. The second option would, in

addition to the above, be to also include *in vitro* trials which do not report any *in vivo* digestibility values, but these values exist in the literature and hence can be used for validation. The third option would be to include all studies.

Data collection: next steps

The experts agreed that there is quite a considerable volume of articles for the *in vitro* literature review and that including them all would be counterproductive and potentially damaging for the scientific robustness of the method. After discussion, the experts agreed that *in vitro* data included in the database should be validated against *in vivo* studies, ideally in humans or pigs. They also agreed that the *in vitro* method would need to be validated more than once for the data to be accepted. The reason being that the *in vitro* protocol might be suitable for mimicking the digestibility of certain proteins, but that this might not be true for other proteins. The experts discussed the importance of choosing the appropriate reference protein to validate *in vitro* methods, offering examples in which seaweed proteins or marine animal by-product proteins might be analysed *in vitro*, but the reference protein used for validation might be soya or wheat. In such cases, the reference protein should be considered unsuitable and the study could be classed as lacking validation. Following this discussion, the experts agreed that the *in vitro* literature review should focus firstly on studies providing direct comparisons between *in vivo* and *in vitro* values, while assessing the quality of the study based on the reference protein used. Once the database is populated with digestibility data from the *in vivo* literature review, the second phase could be activated to further populate the database. Nevertheless, the database should not include any data for which there are no means to validate the *in vitro* digestibility values against *in vivo* values. Ms Recio and Ms Shertukde also discussed how the data extraction template should be aligned with the database architecture, and working groups were set up to propose a list of variables to be included in the database. Other matters relating to data collection were discussed in a section chaired

by Mr Rajavel Elango, including a call for data and collaborative multidisciplinary research initiatives to generate new data on protein digestibility.

Discussion to coordinate a call for data

Mr Elango chaired a session on the future of the database and the ways to expand data collection beyond the literature reviews.

Ms Xipsiti and Mr Owino explained to the experts that typically a call for data is published alongside the database publication on the FAO website. The call for data is intended to act as a guide for entities interested in adding their data to the database. In the call for data, a template is created for each interested party to curate their data based on the database template. Alongside the template, the call for data includes data specifications, which detail the additional information required by FAO to evaluate whether the data submitted fulfil the database inclusion criteria.

The experts discussed article inclusion and exclusion criteria, as this would be relevant to the call for data. They also revisited the discussion on appropriate data sources and confirmed that although private companies and laboratories may have valuable data, they should be encouraged to subject their studies to the peer review process before submitting data to FAO. The rationale behind this decision was that the information required to assess the quality of the data from a private entity is very similar to the information that might be requested by reviewers during the publication process. It is therefore likely that private entities may try to submit data that will not meet the quality criteria set for the database by experts. Companies and entities that have research-grade digestibility data should be encouraged to publish externally before sending the publication to FAO for inclusion in the next round of updates.

Discussion on other data collection

As far as future data collection is concerned, Mr Owino presented the opportunities that exist from IAEA-supported coordinated research programmes. The coordinated research programmes bring together at least six countries (from both lower-middle-income countries and high-income countries) to focus on a specific research question aimed at methods development/optimization or testing of a hypothesis. Countries receive seed funding from IAEA and participate in meetings convened to facilitate networking and knowledge exchange over a 4–5-year period. As a response to the recommendations from

the 2022 technical meeting, IAEA intends to create interest in generation of protein digestibility from novel foods such as neglected foods (e.g. pulses, cereals, edible insects) through a coordinated research programme on this topic. This was highlighted in the 2022 meeting as one of the least covered areas of protein digestibility. As Ms Haye mentioned on the first day of the meeting, alternative and circular economy protein sources are understudied in terms of their digestibility; yet this group of foods are thought to be climate-smart and highly nutritious. Moreover, the quality of the existing data for these proteins was also discussed as a potential issue as it is likely the reports which are currently published might not fulfil the inclusion criteria set by the experts. Mr Owino spoke of how IAEA is considering focusing on the use of stable isotope techniques and other methods in assessment of protein digestibility from these alternative foods as a topic for the biennium, but it can only offer seed money which mostly covers the costs related to purchasing isotopes and related consumables. As additional funding is needed to cover staff salaries and field logistics for data collection, the effort would be to use the database to highlight the need for coordinated multi-agency research across multiple disciplines to raise resources to expand the database. This will not only enhance data representativeness but will also allow data collection in settings such as lower-middle-income countries where protein digestibility data is still minimal – as underscored at the 2022 technical meeting.

In relation to the call for data, experts mentioned that it should be treated as an opportunity to promote the creation of higher quality digestibility data. As the call for data will detail the scientific methods considered acceptable for inclusion in the FAO database, it would also be valuable to inform the research community on those. Researchers and scientists from across the globe could use the database and accompanying user guide, as well as the call for data and the peer reviewed publications as tools to guide future research. The call for data could also be used by reviewers and journals as a tool to assess the quality of manuscripts in the field of protein digestibility. The systematic reviews will also help in identifying the extent of research resources invested in the study of protein digestibility using lower quality research methods.

In this context the experts agreed that the database construction methodology should be presented in various scientific fora to promote awareness and uptake. Namely, it was agreed that an abstract will be prepared for the International Congress of Nutrition of the International Union of Nutritional Sciences Paris 2025 meeting, the Nutrition 2025 Florida meeting and the 14th International Food Data Conference in Rome.

Definition of next steps and action plan

It was agreed that the database will be structured as a Microsoft Excel spreadsheet with multiple tabs. One tab will be dedicated to the food/feed description, another to the nutritional composition including humidity/water values per 100 g of food, and another for the protein and AA content for all AAs (when available). A separate tab will include digestibility values for all AAs and for total protein, while another will include digestible protein and AA content. Finally, three tabs will calculate ileal PDCAAS and DIAAS. The DIAAS will be calculated for the three age groups detailed in this report. The food description tab will include all data pertinent to the food's categorization as per FoodEx2 and other additional elements like

seasonality, country of origin and processing. The nutritional composition tab will include all data linked to the macro-and micro-nutrient content of the food as described in this report, but most importantly it will include dry weight and/or humidity/water content. The total protein and AA content will be included in a separate tab which will also detail information on the protein calculation method used, such as direct AA measurement or protein content. The tab including the digestibility data will also indicate whether the data are human, animal or *in vitro*, the protocol used and the number of samples analysed.

All experts will be involved in writing the user guide and individual chapters will be assigned based on expertise. It was reaffirmed that only data on oro-ileal digestibility will be included in the database and the IAAO data will be compiled in a separate Microsoft Excel spreadsheet.

Summary of roadmap for database construction

Data collection for protein and AA digestibility

- Literature search and systematic reviews
- Call for data
- Other sources

Preparation of protein and AA digestibility and quality documents

- A Microsoft Excel template including required information on foods, methods, AA composition and conversion of units, digestibility values for nitrogen and AA

Preparation of the user guide

- Including how the database was compiled, reference to the original sources, the methodologies for establishing protein digestibility, the calculation of protein quality scores and the bibliography

Publication on INFOODS FAO website

- Co-publishing agreement with FAO and IAEA
- Editing and design of documents
- Submission to the FAO publication workflow system

