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Issues and Opinions

Protein Quality Assessment of Follow-up Formula for Young Children and Ready-to-Use Therapeutic Foods: Recommendations by the FAO Expert Working Group in 2017

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ABSTRACT

The FAO of the UN convened an Expert Working Group meeting to provide recommendations related to protein quality evaluation of Follow-up Formula for Young Children (FUF-YC) and Ready-to-Use Therapeutic Foods (RUTFs). The protein and amino acid (AA) scoring patterns for the target age groups were defined and recommendations provided on the use of currently available protein and indispensable AA digestibility data. For FUF-YC, an age category of 1–2.9 y was identified, and a matching protein requirement of 0.86 g · kg^{-1} · d^{-1} with corresponding AA requirements were recommended. For RUTF, the protein requirement recommended was 2.82 g · kg^{-1} · d^{-1}, to achieve a catch-up weight gain of 10 g · kg^{-1} · d^{-1} in children recovering from severe acute malnutrition. The AA requirements were factorially derived based on the adult protein requirement for maintenance and tissue AA composition. A flowchart was proposed for the best available methods to estimate digestibility coefficients (of either protein or AAs), in the following order: human, growing pig, and rat true ileal AA digestibility values. Where this is not possible, fecal protein digestibility values should be used. The Expert Working Group recommends the use of the Protein Digestibility Corrected Amino Acid Score (PDCAAS), with existing protein digestibility values, or the Digestible Indispensable Amino Acid Score provided that individual AA digestibility values are available for protein quality evaluation using the latter score. The Group also recommends the use of ileal digestibility of protein or of AAs for plant-based protein sources, recognizing the possible effects of antinutritional factors and impaired gut function. A PDCAAS score of ≥90% can be considered adequate for these formulations, whereas with a score <90%, the quantity of protein should be increased to meet the requirements. Regardless of the protein quality score, the ability of formulations to support growth in the target population should be evaluated. Future research recommendations are also proposed based on the knowledge gaps identified. *J Nutr* 2020;150:195–201.

Keywords: amino acid score, bioavailability, fecal digestibility, ileal digestibility, protein requirement, protein digestibility, FUF-YC, RUTF

Introduction

The 38th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) identified the need for guidelines to assess the protein quality of Follow-up Formula for Young Children (FUF-YC) and Ready-to-Use Therapeutic Foods (RUTFs). An RUTF is a therapeutic food to be provided under medical supervision to children with uncomplicated severe acute malnutrition (SAM) aged between 6 and 59 mo. Uncomplicated SAM is defined by a retained appetite test with no fever, no signs of infection, nor complicated disease. It is recommended to feed RUTF during the recovery phase, to ensure adequate provision of required macro- and micronutrients for net tissue deposition. FUF-YC is intended to bridge or improve the nutrient gap in children's diets between 12 and 36 mo, in those who are on complementary feeding with or without breastfeeding, but is not intended

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to have the undesired consequence of replacing the natural home-based diet of the child. For RUTF, there are particular concerns related to the recent introduction of plant-based protein sources, which are being tried and tested in different regions, instead of the commonly used milk–peanut-based mix containing milk products as the main source (∼50%) of protein (1–3). Replacing milk protein with plant sources is likely to affect the protein quality of the formulation and this requires quantification.

In relation to this, the CCNFSDU sought scientific advice from the FAO of the UN, which subsequently convened an Expert Working Group at the FAO Headquarters, Rome, Italy, from 6 to 9 November, 2017 to provide scientific advice on setting up guidelines related to protein quality assessment of FUF-YC and RUTF. With the existing background on protein and amino acid (AA) requirements and protein quality assessments, the Expert Working Group discussed questions related to protein and individual indispensable AA (IAA) requirements for the target populations, relevant AA scoring patterns to be used, related methods for protein and AA digestibility, and practical aspects for using the scoring approach for FUF-YC and RUTF (4).

The report aimed to set out practical guidelines and assistance to countries and the industry on how protein quality should be assessed, by defining protein and AA requirements and scoring patterns according to the Protein Digestibility Corrected Amino Acid Score (PDCAAS) or Digestible Indispensable Amino Acid Score (DIAAS) methods. Furthermore, key gaps and issues that emerged through these discussions were recommended for future research. Two important and key limiting questions are to define IAA requirements and the IAA reference pattern, and to recommend reference methods for the measurement of true protein and AA digestibility for food and mixed diet for the target populations of FUF-YC and RUTF, respectively.

Chemical Score and Digestibility-Corrected Score for Protein Quality of FUF-YC and RUTF

Although several methods exist for the assessment of the quality of proteins (5, 6), previous expert consultations conducted on protein quality (6–9) concluded that the preferred method is the chemical scoring approach that relates the AA content of individual foods (or a calculated weighted average in case of a mixed diet) to the AA body requirement. The scoring approach considers the content of IAAs in mixed diet or individual foods which is made available to the body for utilization in anabolic and catabolic pathways (6). The chemical score represents the ratio of an IAA (mg/g protein) in food (mixed diet or an individual food or a formulation) to the reference requirement scoring pattern of the IAA (mg/g protein) for the target population:

$$\text{chemical AA score} = [(\text{mg of IAA in 1 g dietary protein})/(\text{mg of the same IAA in 1 g requirement scoring pattern})] \quad (1)$$

The score of each IAA is then corrected either by the digestibility of the protein in the PDCAAS or more recently by the specific digestibility of each IAA in the DIAAS. From the chemical AA score the PDCAAS and DIAAS are estimated as follows:

$$\text{PDCAAS} = [(\text{mg of AA in 1 g dietary protein})/(\text{mg of the same AA in 1 g requirement scoring pattern})] \times \text{protein digestibility.} \quad (2)$$

In the modified DIAAS, the chemical score of each AA is corrected for its specific digestibility:

$$\text{DIAAS} = [(\text{mg of AA in 1 g dietary protein})/(\text{mg of the same AA in 1 g requirement scoring pattern})] \times \text{same IAA digestibility.} \quad (3)$$

The PDCAAS of a protein is calculated by correcting the chemical score of each AA by a single or weighted average protein digestibility for a single protein source or a mixed diet, respectively. The DIAAS of a protein is calculated by correcting the chemical score of each AA by a single or weighted average digestibility of the same IAA for a single protein source or a mixed diet, respectively. For both the PDCAAS and DIAAS, the score is calculated for individual IAAs, and the IAA with the lowest score (limiting IAA) is used as the score for the protein, expressed as either a fraction or a percentage. For a single food (or a mixed diet) PDCAAS or DIAAS calculation, the scores >100% are truncated, because IAA concentrations in excess do not confer additional nutritional benefit. A PDCAAS or DIAAS value of ≥90% can be considered adequate for the FUF-YC and RUTF formulations, whereas a score <90% is considered deficient and implies that it is necessary either to increase the quantity of protein to meet the AA requirements (5) or to fortify with the limiting IAA.

Although the use of the DIAAS rather than the currently available PDCAAS is preferable, there are still insufficient individual AA digestibility values for foods in humans. Hence, the CCNFSDU noted that at present, the existing PDCAAS and related methods should continue to be used to determine the protein quality of the current products (FUF-YC and RUTF) and to design new formulations (4).

Protein and AA Requirements and AA Reference Patterns in the Target Populations for FUF-YC and RUTF

The Expert Working Group derived protein and IAA requirements for the target population by a factorial approach, which uses the sum of the mean maintenance requirement and the requirement for growth (calculated as protein deposited during growth corrected by efficiency of utilization). Further, the IAA reference pattern was derived from the ratio of IAA requirement to protein requirement (4) (**Table 1**). Owing to the lack of direct

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Abbreviations used: AA, amino acid; ANF, antinutritional factor; CCNFSDU, Codex Committee on Nutrition and Foods for Special Dietary Uses; DIAAS, Digestible Indispensable Amino Acid Score; FUF-YC, Follow-up Formula for Young Children; IAA, indispensable amino acid; IAAO, indicator amino acid oxidation; PDCAAS, Protein Digestibility Corrected Amino Acid Score; RUTF, Ready-to-Use Therapeutic Food; SAM, severe acute malnutrition.

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TABLE 1 Protein and IAA requirements and amino acid reference pattern proposed for FUF-YC (age group 1–2.9 y) and for RUTF (for a target weight gain value of 10 g · kg^{-1} · d^{-1}, age group 0.5–4.9 y)[1]

Requirement	Protein, g · kg^{-1} · d^{-1}	IAA, mg · kg^{-1} · d^{-1}								
		His	Ile	Leu	Lys	SAAs	AAAs	Thr	Trp	Val
1–2.9 y	0.86	15	27	54	45	22	40	23	6.4	36
Catch-up growth	2.82	66	95	198	183	88	177	103	29	130
IAA reference pattern,[2] mg/g protein										
1–2.9 y		18	31	63	52	26	46	27	7.4	42
Catch-up growth		24	34	70	65	31	63	36	10	46

[1] AAA, aromatic amino acid (Phe + Tyr); FUF-YC, Follow-up Formula for Young Children; IAA, indispensable amino acid; RUTF, Ready-to-Use Therapeutic Food; SAA, sulfur amino acid (Met + Cys).

[2] Calculated as amino acid requirement in mg · kg^{-1} · d^{-1} divided by total protein requirement in g · kg^{-1} · d^{-1}.

measurements of protein and IAA requirements in the target populations, the factorial method and the scoring approach were considered as the more accurate to calculate protein and IAA requirements and protein quality by the Expert Working Group.

For FUF-YC, using the age range of 1–2.9 y, the maintenance protein requirement was assumed to be equal to the adult value of 0.66 g · kg^{-1} · d^{-1} (value adopted from 8). The requirement for growth was derived from protein deposition, which was longitudinally estimated in <2-y-old children by whole-body potassium counting, further corrected for an efficiency of utilization of 58% that was derived from studies in children (0.5–12 y of age) on plant- or animal-based diets (8). The sum of the maintenance (0.66 g · kg^{-1} · d^{-1}) and growth (0.20 g · kg^{-1} · d^{-1}, average of 1–3 y) requirements results in a total protein requirement of 0.86 g · kg^{-1} · d^{-1}. The factorial approach based on the maintenance and growth components was also used to estimate the IAA requirements (8). The IAA requirements for maintenance were assumed to be like those of adults and the growth component was estimated using the AA composition of the body tissue proteins with 58% efficiency of protein utilization, which was applied to individual IAAs. The IAA requirements were thus calculated as the sum of the adult maintenance protein requirement of 0.66 g · kg^{-1} · d^{-1} times the maintenance AA pattern (mg/g protein), plus the 0.2 g · kg^{-1} · d^{-1} tissue protein deposition rate times the tissue AA pattern (mg/g protein) adjusted for 58% efficiency of deposition.

For RUTF, protein and AA requirements for catch-up growth were considered. Weight deficits in children with SAM are assessed as thinness or wasting (weight-for-height z score <−2 as per the WHO growth standard) (10) and are attributed to the combined effect of environmental factors and poor nutrition (8). The rate of recovery in these children is assessed as the rate of weight gain. Therefore, the Expert Working Group focused on the weight deficit and agreed a reference rate of weight gain of 10 g · kg^{-1} · d^{-1} in children with SAM (11). The target protein requirement to achieve a rate of weight gain of 10 g · kg^{-1} · d^{-1} was estimated to be 2.82 g · kg^{-1} · d^{-1}, which is the sum of the requirements for maintenance and for net tissue deposition. For this, a safe level of maintenance requirement was used (0.82 g · kg^{-1} · d^{-1}; the 97.5th percentile of the distribution of the adult maintenance requirement). The growth requirement was calculated as the product of the rate of weight gain (10 g · kg^{-1} · d^{-1}), the protein composition of the weight gain (14%), and the efficiency of dietary protein utilization (70%), yielding a value of 2.0 g · kg^{-1} · d^{-1} (values adopted from 8). The IAA requirements for catch-up growth were factorially derived from the maintenance (0.82 g · kg^{-1} · d^{-1}) and growth requirements (2.0 g · kg^{-1} · d^{-1}), related to the adult maintenance AA pattern and tissue AA composition, respectively (Table 1). In addition, the formulations should preferably maintain a phenylalanine to tyrosine, and methionine to cysteine ratio of 1:1 (wt:wt), to induce a sparing effect on the phenylalanine and methionine, and ensure an adequate aromatic AA and sulfur AA supply during catch-up growth (8, 12).

In addition to these recommendations on the amount and quality of proteins for FUF-YC and RUTF, countries and manufacturers of these formulas need to carefully consider not only the impact of the protein content of any new formulation on growth and body composition, but also their long-term consequences for growing children or those recovering from SAM. Some studies have linked higher protein intakes (≥15% of protein energy) and type of protein (particularly animal sources) to an elevated risk of obesity in adulthood and an early adiposity rebound in normal growing children (13, 14). In contrast, other recent studies that have measured body composition in infants, children, and adolescents showed positive association with protein intake and lean mass but no association between protein intake and adiposity (15, 16). Moreover, animal protein sources can serve as important sources of bioavailable minerals as well as high-quality protein, as indicated by numerous studies in less industrialized countries that have observed better nutritional status in children with higher intakes of animal products.

The Digestibility Issue: Fecal Compared with Ileal Digestibility, Protein Compared with AA Digestibility

Protein and AA digestibility has been detailed in a different review but remains a concern (17, 18). The term protein (or AA) digestibility refers to the digestion and disappearance of ingested protein-derived nitrogen (or AA) from the intestinal lumen and is calculated as the difference between nitrogen (or AA) ingestion and nitrogen (or AA) recovery measured in the feces (oro-fecal digestibility) or the terminal ileum (oro-ileal digestibility). Digestibility is referred to as true digestibility when contributions from endogenous protein or AA sources (e.g., enzymes, hormones, and immunoglobulins present in salivary, gastric, pancreatic, biliary, and jejunal secretions along

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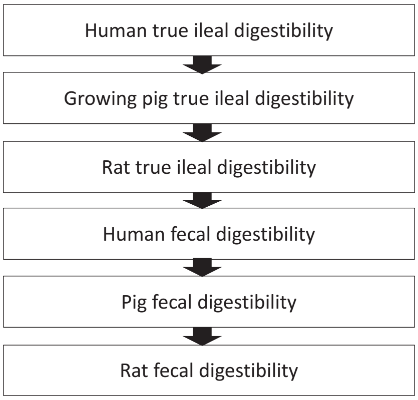

FIGURE 1 Flowchart for the use of available digestibility values for protein quality assessment.

with desquamated intestinal epithelium) are accounted for in the digestibility calculation. True protein digestibility at the terminal ileum is theoretically preferable, because it confines consideration to the loss of AAs from the small intestine only, thereby avoiding the confounding effect of the substantial movement of nitrogen through the large intestine related to the metabolic activities of the resident microbiota. Lastly, protein digestibility refers to the overall digestibility of the protein and considers that the different IAAs have the same digestibility, whereas IAA digestibility refers to the specific digestibility of each AA. Therefore, in a related approach, when the true specific IAA digestibility is determined at the level of the terminal ileum, it is termed as true ileal IAA digestibility (6).

The Expert Working Group agreed that true nitrogen and AA digestibility determined at the ileal level, where available, should be used to correct for protein availability in the formulation of FUF-YC and RUTFs (4; in agreement with previous recommendations of the FAO in 2013 and 2014). Further, a flowchart-based approach was proposed, which selects the best available methods to assess protein digestibility, in the following order: human true ileal AA digestibility values, growing pig true ileal AA digestibility values, and rat true ileal AA digestibility values. When these are not available, human, pig, or rat fecal protein digestibility values should be used (**Figure 1**). The Expert Working Group pragmatically recommends using the PDCAAS and available protein digestibility values to determine the protein quality of FUF-YC and RUTF, in the absence of data on true ileal IAA digestibility (4). However, it should be noted that the preferred metric for protein quality assessment is the DIAAS, using the specific true ileal digestibility of each AA in the food or formulation. There is sufficient evidence from animal models (pigs and rats) that demonstrate the disagreement between the PDCAAS and DIAAS values (over- or underestimated), specifically when PDCAAS overestimates the values for poor-quality protein (mean difference of 59%), which is crucial in populations consuming a predominantly vegetarian diet (19, 20).

The direct determination of true ileal IAA or N digestibility requires the collection of ileal digesta. In humans, this is performed by using naso-ileal intubation methods or collection of digesta from surgically exteriorized ileum (ileostomates). These methods are invasive and not suitable for routine use (8). Alternatively, minimally invasive or noninvasive methods for determining ileal IAA digestibility were discussed. Stable isotope signature–based methods for IAA digestibility were proposed including the indicator amino acid oxidation (IAAO) technique, a noninvasive method, based on comparison with a mixture of free AAs, and the dual stable isotope tracer approach which has the potential to be noninvasive (8, 19). Recently, some data on the true ileal IAA digestibility of animal source proteins (chicken, egg, and meat) and plant proteins (rice, finger millet, and mung bean) have been generated in healthy adults and children (aged <2 y) using the dual stable isotope tracer technique (21–23); and for rice and African cornmeal using the IAAO method in healthy adults (24, 25).

Another proposed alternative for estimating true ileal IAA digestibility coefficients of different foods, owing to difficulties in routine determinations involving humans, was using data generated in animal models. The 2 animal models commonly used are the pig and rat. The possibility of generating prediction equations for true ileal IAA digestibility values should also be obtained through comparisons between pig or rat models and humans, and that would provide a better scope for future research. A further alternative for true ileal IAA availability in humans is data generated using in vitro methods. In the future, owing to ethical considerations with studies involving humans or animal models, in vitro methods are likely to become the preferred methods. At present, there are many different in vitro models which differ in reaction conditions and thus most likely in the digestibility results generated. There is currently no agreement on the models for in vitro determination of true ileal AA digestibility, and too little data are available on digestibility values of human foods to allow the use of these methods at present.

Considerations for Impacts of Poor Environment and Antinutritional Factors

The Expert Working Group recommends considering the influence of malnutrition, poor environments, and infections (including infestations) on digestibility of foods and requirements, in infants and children, when calculating the PDCAAS or DIAAS. One must be aware of the adverse effects of poor environment and infections on intestinal function in children, suggestive of environmental enteric dysfunction, because digestibility values may be altered in such instances, as shown by the reduced absorption of D-xylose sugars (26). Evidence indicates a ∼20% increase in lysine requirement in chronic-malnourished (asymptomatic, with height-for-age <2 SD as per WHO standards) Indian children aged ∼7.5 y owing to gut parasite infestation (27). In addition, a cautionary note should be considered in formulations utilizing plant-based protein sources, owing to the potential effect of antinutritional factors (ANFs), mainly trypsin inhibitors, tannins, and phytates, on digestibility. ANFs may reduce protein digestibility by inhibiting the action of digestive enzymes, blocking intestinal epithelial nutrient transporters, binding with food proteins causing precipitation, or by chelating nutrients, digestive enzymes, and mineral cofactors. A recent study in Indian adults showed that co-ingestion of tea polyphenols with

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meals reduced the mean true ileal IAA digestibility of egg by 17% (28). In addition, processing and storage can also result in the generation of ANFs, such as those formed during the Maillard reaction, racemization, and lysinoalanine. Other factors that limit AA digestibility are as follows: severe heat treatment (130°C for 45 mins), which has been shown to reduce the standardized ileal digestibility of all AAs (both essential and nonessential); methionine and cysteine are susceptible to oxidization during processing; and protein crosslinking between the ε-amino group and acidic AAs (29, 30). Where possible, appropriate processing measures should be adopted to minimize these effects. In such situations it may be necessary to include a correction for the individual IAA digestibility when using the PDCAAS that includes the specific IAA endogenous losses attributable to the type of fiber and ANFs of the protein source (17) or to use values of true ileal IAA digestibility from methods that intrinsically account for the inhibitory effects of ANFs in the food matrix (such as the direct measurement of ileal digestibility, the dual stable isotope tracer approach, or the IAAO method).

Strengths and Limitations of the Recommendations

The strength lies with the consortium of experts using the available data in recommending protein and AA requirements for FUF-YC and RUTF protein quality estimation. Further, the approach to utilizing the best possible methods for digestibility values and the scope for future research have been detailed. The limitations relate to assumptions of the factorial method and catch-up growth protein requirement, specifically on the composition of weight gain and efficiency of utilization (8). For the FUF-YC recommendation, the protein and in turn the IAA requirements may be less than accurate owing to the lack of data on protein deposition between 2 and 3 y, which was interpolated using a quadratic model (8).

Summary of Recommendations

From a public health perspective, there is a need for better understanding on how adjusting for protein quality could change the pattern of protein availability and the adequacy of protein intakes. Furthermore, clear evidence-informed guidelines are needed to ensure that products developed for the purpose of managing specific conditions (e.g., treatment of SAM) and achieving specific nutritional outcomes do meet required standards. In the context of the evaluation of products intended for use as food aid, the adjustment of IAA requirements for physiologic status has been shown to provide a more secure assessment of protein quality (31) and protein quality scores have been reported to be highly correlated with the rate of weight gain in recovery from SAM (32). Computing the PDCAAS or DIAAS scores to assess protein quality is recommended as part of the assessment of the nutritional composition of a new food formulation for use either as a FUF-YC or as an RUTF (**Box 1**).

Box 1

Key points that the Expert Working Group recommends in relation to protein quality assessment in FUF-YC and RUTF:

1) To evaluate protein quality of FUF-YC and RUTF, the DIAAS or PDCAAS should be calculated with available individual ileal IAA or crude nitrogen or protein fecal digestibility values (as per the flowchart in Figure 1).
2) The reference IAA requirements and scoring patterns of children in the 1–2.9 y age group should be used to determine the protein quality of FUF-YC.
3) The reference IAA requirements and scoring patterns to achieve a catch-up growth rate of 10 g · kg^{-1} · d^{-1} should be used to determine the protein quality of RUTF.
4) A high-quality protein source will have a PDCAAS score of 100. However, a PDCAAS score ≥90 can still be considered adequate for these formulations. In formulations with a PDCAAS score <90, the quantity of protein should be increased to meet the IAA requirement.
5) The efficacy of new formulations should not rely on protein quality considerations alone and should be tested for their ability to support catch-up growth in the target population, which in this scenario would be children of 1–2.9 y for FUF-YC and 0.5–4.9 y for RUTF.

The challenges of calculating a PDCAAS or DIAAS relate to the choice of database or literature for sourcing available digestibility values. For example, the PDCAAS of peanut–milk RUTF (with 50% protein from milk) calculated using fecal digestibility values from the 2007 WHO report [represented in the FAO 2018 report (4)] is 88% (limited by lysine), as compared with a DIAAS of 65% or 60% (limited by threonine) when standard ileal digestibility from growing pigs is used from different literature sources: the AmiPig database (33) and other published data (19, 34), respectively. For the same calculation for a plant-based RUTF made of Soy-Maize-Sorghum (2), the PDCAAS is 77% (9) when compared with a DIAAS of 74% (33) or 83% (19, 35, 36), with lysine as the limiting AA. These differences arise from different methods of processing the foods. Therefore, it is advisable to carefully match the protein source to the type of processing when selecting digestibility values. In addition, one needs to be aware of food matrix effects when a combination of ingredients is used in the formulation, which might increase or decrease IAA digestibility.

The Expert Working Group proposes a flowchart that ranks the best available digestibility methods to assess protein quality, depending on data availability. It is recommended that the order to follow should be human, growing pig, followed by rat true ileal AA digestibility values. If these values are not available, human, pig, or rat fecal protein digestibility values should be used, in that order. It recommends considering tested and agreed-upon in vitro methods of protein digestibility that are compared against in vivo methods, once they become available.

The FAO report (8) provides steps for computing the PDCAAS for food formulations used either as a FUF-YC or as an RUTF. The formulations with a PDCAAS score are truncated to 100%. A high-quality protein source will have a PDCAAS

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score of 100%. However, a PDCAAS score of 90% can still be considered adequate for these formulations. In formulations with a PDCAAS score <90%, the quantity of protein should be increased to meet the requirements. Alternatively, fortification of foods with the limiting IAA or IAAs could be used.

Although currently almost all RUTFs available for therapeutic purposes are made of a combination of peanut paste and dried skim milk, efforts are being made toward formulating RUTFs using lower-cost milk products or locally available legumes such as soya bean, chickpea, and cereal flours such as rice, millet, oats, wheat, and sorghum. In this context, the protein quality assessment is of relevance because of the potential effects of ANFs and adverse environmental conditions.

Looking forward (**Box 2**), different steps are required to improve the current knowledge on protein and IAA requirements for the target population, to develop new noninvasive methods for assessing nutritional needs and digestibility values, including in vitro methods, and to provide data sets on values that can be used for food quality evaluation.

Box 2

The way forward.

- A complete data set of individual true ileal IAA digestibility values for different protein sources should be generated.
- The effects of ANFs and impaired gut function in the presence of poor environments and infections on digestibility should be determined.
- In order to allow for an algorithm to be operationalized for protein quality assessment of foods, it is necessary to compare true ileal protein and IAA digestibility values of foods among pigs, rats, and humans, and to generate robust statistical prediction equations.
- An agreed-upon in vitro method to predict true ileal nitrogen and IAA digestibility values needs to be developed.
- At present there are no data to show whether available models (i.e., adult human via naso-ileal intubation, pig ileal model, or rat ileal model) are applicable to children with malnutrition. There is a need for studies comparing true ileal digestibility in children, both healthy and malnourished, with adults and appropriate animal models.
- There is clearly a need to further examine whether the IAA needs for supporting adequate growth and development in malnourished children are increased (above current estimates) when frequent episodes of gut insults occur.
- With introduction of formulations or food preparations that are enriched with single or multiple IAAs, the scoring methods to accommodate the added IAAs need to be considered.
- Currently there is considerable uncertainty in the contribution of AAs generated from the colonic microbiota to the AA pool of the whole body. It is important to assess if this makes a significant contribution to the host's AA economy.

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References

1. Bahwere P, Akomo P, Mwale M, Murakami H, Banda C, Kathumba S, Banda C, Jere S, Sadler K, Collins S. Soya, maize, and sorghum–based ready-to-use therapeutic food with amino acid is as efficacious as the standard milk and peanut paste–based formulation for the treatment of severe acute malnutrition in children: a noninferiority individually randomized controlled efficacy clinical trial in Malawi. Am J Clin Nutr 2017;106(4):1100–12.
2. Bahwere P, Balaluka B, Wells JC, Mbiribindi CN, Sadler K, Akomo P, Dramaix-Wilmet M, Collins S. Cereals and pulse-based ready-to-use therapeutic food as an alternative to the standard milk- and peanut paste–based formulation for treating severe acute malnutrition: a noninferiority, individually randomized controlled efficacy clinical trial. Am J Clin Nutr 2016;103(4):1145–61.
3. Irena AH, Bahwere P, Owino VO, Diop EI, Bachmann MO, Mbwili-Muleya C, Dibari F, Sadler K, Collins S. Comparison of the effectiveness of a milk-free soy-maize-sorghum-based ready-to-use therapeutic food to standard ready-to-use therapeutic food with 25% milk in nutrition management of severely acutely malnourished Zambian children: an equivalence non-blinded cluster randomised controlled trial. Matern Child Nutr 2015;11(S4):105–19.
4. FAO. Protein quality assessment in follow-up formula for young children and ready to use therapeutic foods. Rome: FAO; 2018.
5. Tomé D. Criteria and markers for protein quality assessment – a review. Br J Nutr 2012;108(Suppl 2):S222–9.
6. FAO. Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation. FAO Food and Nutrition Paper No. 92. Rome: FAO; 2013.
7. FAO/WHO. Protein quality evaluation: report of the joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper No. 51. Rome: FAO; 1991.
8. WHO/FAO/UNU Expert Consultation. Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU Expert Consultation. WHO Technical Report Series, No. 935. Geneva: WHO; 2007.
9. FAO. Research approaches and methods for evaluating the protein quality of human foods. Report of a FAO Expert Working Group. Rome: FAO; 2014.
10. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl 2006;450:76–85.
11. WHO. Management of severe malnutrition: a manual for physicians and other senior health workers. Geneva: WHO; 1999.
12. Kurpad AV, Regan MM, Varalakshmi S, Gnanou J, Lingappa A, Young VR. Effect of cystine on the methionine requirement of healthy Indian men determined by using the 24-h indicator amino acid balance approach. Am J Clin Nutr 2004;80(6):1526–35.
13. Koletzko B, Demmelmair H, Grote V, Prell C, Weber M. High protein intake in young children and increased weight gain and obesity risk. Am J Clin Nutr 2016;103(2):303–4.
14. Tang M. Protein intake during the first two years of life and its association with growth and risk of overweight. Int J Environ Res Public Health 2018;15(8):1742.
15. Bell KA, Wagner CL, Feldman HA, Shypailo RJ, Belfort MB. Associations of infant feeding with trajectories of body composition and growth. Am J Clin Nutr 2017;106(2):491–8.
16. Switkowski KM, Jacques PF, Must A, Fleisch A, Oken E. Associations of protein intake in early childhood with body composition, height, and insulin-like growth factor I in mid-childhood and early adolescence. Am J Clin Nutr 2019;109(4):1154–63.
17. Darragh AJ, Hodgkinson SM. Quantifying the digestibility of dietary protein. J Nutr 2000;130(7):1850S–6S.
18. Fuller MF, Tomé D. In vivo determination of amino acid bioavailability in humans and model animals. J AOAC Int 2005;88(3):923–34.

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19. Mathai JK, Liu Y, Stein HH. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). Br J Nutr 2017;117(4):490–9.
20. Rutherfurd SM, Fanning AC, Miller BJ, Moughan PJ. Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats. J Nutr 2014;145(2):372–9.
21. Devi S, Varkey A, Sheshshayee MS, Preston T, Kurpad AV. Measurement of protein digestibility in humans by a dual-tracer method. Am J Clin Nutr 2018;107(6):984–91.
22. Kashyap S, Shivakumar N, Varkey A, Duraisamy R, Thomas T, Preston T, Devi S, Kurpad AV. Ileal digestibility of intrinsically labeled hen's egg and meat protein determined with the dual stable isotope tracer method in Indian adults. Am J Clin Nutr 2018;108(5):980–7.
23. Shivakumar N, Kashyap S, Kishore S, Thomas T, Varkey A, Devi S, Preston T, Jahoor F, Sheshshayee MS, Kurpad AV. Protein-quality evaluation of complementary foods in Indian children. Am J Clin Nutr 2019;109(5):1319–27.
24. Prolla IR, Rafii M, Courtney-Martin G, Elango R, da Silva LP, Ball RO, Pencharz PB. Lysine from cooked white rice consumed by healthy young men is highly metabolically available when assessed using the indicator amino acid oxidation technique. J Nutr 2013;143(3):302–6.
25. Rafii M, Elango R, Ball RO, Pencharz PB, Courtney-Martin G. Metabolic availability of the limiting amino acids lysine and tryptophan in cooked white African cornmeal assessed in healthy young men using the indicator amino acid oxidation technique. J Nutr 2018;148(6): 917–24.
26. Keusch GT, Rosenberg IH, Denno DM, Duggan C, Guerrant RL, Lavery JV, Tarr PI, Ward HD, Black RE, Nataro JP, et al. Implications of acquired environmental enteric dysfunction for growth and stunting in infants and children living in low- and middle-income countries. Food Nutr Bull 2013;34(3):357–64.
27. Pillai RR, Elango R, Ball RO, Kurpad AV, Pencharz PB. Lysine requirements of moderately undernourished school-aged Indian children are reduced by treatment for intestinal parasites as measured by the indicator amino acid oxidation technique. J Nutr 2015;145(5): 954–9.
28. Kashyap S, Shivakumar N, Varkey A, Preston T, Devi S, Kurpad AV. Co-ingestion of black tea reduces the indispensable amino acid digestibility of hens' egg in Indian adults. J Nutr 2019;149(8):1363–8.
29. Gilani GS, Xiao CW, Cockell KA. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br J Nutr 2012;108(S2): S315–32.
30. Almeida FN, Htoo JK, Thomson J, Stein HH. Effects of heat treatment on the apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim Feed Sci Technol 2014;187: 44–52.
31. Callaghan M, Oyama M, Manary M. Sufficient protein quality of food aid varies with the physiologic status of recipients. J Nutr 2017;147(3):277–80.
32. Manary M, Callaghan M, Singh L, Briend A. Protein quality and growth in malnourished children. Food Nutr Bull 2016;37(1_suppl): S29–36.
33. AFZ, Ajinomoto Eurolysine, Aventis Animal Nutrition, INRA, ITCF. AmiPig: ileal standardised digestibility of amino acids in feedstuffs for pigs [Internet]. France: AFZ, Ajinomoto Eurolysine, Aventis Animal Nutrition, INRA, ITCF; 2000. Available from: http://www.feedbase.com/downloads/amipeng.pdf.
34. Casas GA, Jaworski NW, Htoo JK, Stein HH. Ileal digestibility of amino acids in selected feed ingredients fed to young growing pigs. J Anim Sci 2018;96(6):2361–70.
35. Stein HH, Kim SW, Nielsen TT, Easter RA. Standardized ileal protein and amino acid digestibility by growing pigs and sows. J Anim Sci 2001;79(8):2113–22.
36. Pan L, Shang QH, Wu Y, Ma XK, Long SF, Liu L, Li DF, Piao XS. Concentration of digestible and metabolizable energy, standardized ileal digestibility, and growth performance of pigs fed diets containing sorghum produced in the United States or corn produced in China. J Anim Sci 2017;95(11):4880–92.

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